POLITÉCNICO DE LEIRIA | ESCOLA SUPERIOR DE TURISMO E TECNOLOGIA DO MAR

Marine heatwave effects on Pacific oyster (*Magallana gigas* Thunberg, 1793) growth, condition, soft body colour, and texture

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Dissertation to obtain a master's degree in Aquaculture.

Peniche, April of 2026

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This dissertation is original, prepared solely for this purpose, and all authors whose studies and publications contributed to its preparation have been duly cited.

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"Joy in looking and comprehending is

nature's most beautiful gift."

- **Albert Einstein**

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Resumo

A ostra do Pacífico, *Magallana gigas,* é um marisco altamente procurado, considerado mundialmente um superalimento e uma iguaria. A maior parte da oferta presente no mercado é proveniente de ostricultura. Mesmo assim, este setor enfrenta cada vez mais desafios por parte das alterações climáticas. As ondas de calor marinhas têm vindo a tornar-se cada vez mais recorrentes, causando episódios generalizados de mortalidade em massa. Mesmo quando este fenómeno não é grave o suficiente para causar impactos letais, podem ocorrer efeitos subletais, especificamente no desenvolvimento e na qualidade. O principal objetivo deste estudo foi avaliar os efeitos de uma onda de calor simulada no crescimento, condição, cor e textura da carne da *M. gigas*. Além disso, um ensaio complementar foi realizado para avaliar os efeitos das práticas tradicionais de manuseamento, em relação aos mesmos parâmetros. Em ambos os estudos, as ostras foram obtidas na empresa Exporsado e mantidas em dois Sistemas de Aquacultura de Recirculação (RAS) idênticos, de forma a separar os grupos de controlo e experimental. Na experiência em que o manuseamento foi avaliado, foi aplicada uma agitação leve em conjunto com períodos de dessecação, periodicamente ao longo de 30 dias. Na experiência com a onda de calor, o grupo experimental foi exposto durante 10 dias a uma onda de calor simulada de 24ºC, com um aumento gradual a partir de 18ºC e subsequente regresso à mesma temperatura. Para avaliar os efeitos, foram recolhidos dados morfométricos, de condição, colorimétricos e texturométricos em ambos os tratamentos durante os dois ensaios. Na experiência de práticas de manuseamento, observou-se uma melhoria da condição das ostras submetidas estas práticas em comparação com ao grupo controlo (11,03% mais elevado), a par de uma tendência para um melhor desenvolvimento da concha, embora não tenha sido observada significância estatística. Observou-se uma ligeira diminuição nas propriedades texturais no grupo que foi manuseado, enquanto a análise colorimétrica revelou uma mudança subtil, mas consistente, resultando num tom de carne mais escuro (10,31% mais baixo em luminosidade). As ostras expostas à simulação de onda de calor apresentaram uma redução no crescimento (menos 5,42 % em altura e 8,45 % em peso) e índices de condição mais baixos, particularmente no índice AFNOR, em comparação com o tratamento de controlo (menos 28,72 %). As propriedades dos tecidos moles também foram afetadas, observando-se padrões colorimétricos alterados e valores de textura mais baixos no tratamento com onda de calor. Os resultados demonstram que tanto as práticas de manuseamento como as ondas de calor marinhas influenciam o crescimento, a condição e a cor e textura da carne de *M. gigas*. Além disso, estas conclusões destacam que as práticas de manuseamento podem melhorar o desempenho, enquanto sublinham a importância dos efeitos subletais associados aos eventos de onda de calor marinha na ostricultura.

Palavras-chave: aquacultura, condição, AFNOR, mudanças climáticas, *Crassostrea*, colorimetria, TPA.

Abstract

The Pacific oyster *Magallana gigas* is a highly sought-after seafood, considered a superfood and delicacy worldwide. Most of the oyster market supply comes from aquaculture, despite that, this sector faces increasing threats due to climate change. Marine heatwave events have become more frequent, causing widespread mass mortality events. Even when marine heatwaves aren't severe enough to cause such a deadly impact, sublethal effects may be noticed, specifically in growth performance and quality. The main aim of this study was to evaluate the effects of a simulated heatwave on M. *gigas* growth, condition, and flesh colour and texture. Additionally, a complementary trial was conducted to evaluate the effects of traditional handling practices on the same parameters. In both studies, oysters were obtained at Exporsado and maintained in two identical RAS, separating the control and experimental groups. In oysters subjected to handling practices, a higher condition index was observed compared to the control (11.03% higher), alongside a tendency for improved shell growth, although no statistical significance was observed. A slight decrease in textural properties was observed under handling conditions, while colourimetry analysis revealed a subtle yet consistent shift, resulting in a darker flesh tone (10.31% lower in Luminosity). In the heatwave experiment, the experimental group was exposed for 10 days to a simulated heatwave event of 24ºC, with a gradual increase from and subsequent return to 18ºC. In the experiment where handling was evaluated, light tumbling was applied alongside desiccation periods, periodically throughout the course of 30 days. To evaluate effects, morphological, conditioning, colourimetric and texturometrical data were collected in both groups during the two trials. Oysters exposed to the simulated heatwave showed reduced growth (less 5.42% in height and less 8.45% in weight) and lower condition indices, particularly in the AFNOR index, compared to the control treatment (less 28,72%). Soft tissues' properties were also affected, with altered colourimetric patterns and lower texture values observed in the heatwave treatment. These results demonstrate that both handling practices and marine heatwave events influence *M. gigas* growth, condition, and soft tissue colour and texture. Furthermore, these findings highlight that handling practices may enhance performance, while also highlighting the importance of sublethal effects associated with Marine heatwave events in oyster aquaculture.

Keywords: Aquaculture, condition, AFNOR, Climate change, *Crassostrea*, Colourimetry, TPA.

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1. Introduction

1.1. Evolution and global expansion of modern aquaculture

Aquaculture has been present throughout mankind's history and in many societies around the world. The earliest record of fish rearing dates to 6000 B.C., in Henan Province, China, with the production of common carp (Nakajima et al., 2019). Regarding bivalves' aquaculture, historical records show evidence of intentional and systematic cultivation of oysters for human consumption since ancient Rome, Japan, and China (Rogers, 2023). During the 20th century, *M. gigas* was intentionally introduced in Europe and North America, becoming the most produced oyster species worldwide (Ruesink et al., 2005; FAO, 2022), and today, oyster aquaculture is a widespread practice worldwide.

Modern aquaculture concerns the cultivation of aquatic organisms such as fish, molluscs, crustaceans, and aquatic plants in controlled environments using a wide range of practices and production systems. These systems can adopt different sizes, from small-scale operations run by individuals or communities to large commercial enterprises. They may employ various production methods depending on species requirements, market demand, available resources, and environmental considerations (Giri et al., 2022). With the adoption of the blue economy framework, aquaculture has been perceived as a solution for food security, environmental restoration, and climate adaptation, especially in countries like Portugal, with extensive coastlines and rich maritime resources (Croft et al., 2024; Pradeepkiran, 2019). The capacity to produce low-carbon and low-input nutrient-rich food sources while increasing coastal and marine resilience places aquaculture as one of the most promising sectors of food production (Troell et al., 2014; van der Meer et al., 2023).

The contribution of aquaculture to the global fish supply has increased at a gradual pace since the 1970s, and in global terms, aquaculture production now surpasses fisheries, supplying over 50% of the seafood consumed worldwide (FAO, 2024a). This remarkable growth emerged as a response to the growing global demand for aquatic food, the decline in wild fish stocks, and the need for sustainable food production, economic development, and environmental conservation (Duarte, 2016; FAO, 2026). This expansion reflects the paradigmatic shift in how societies think about the ocean as a source of food, ecosystem services, and long-term economic stability (Costello et al., 2020; Croft et al., 2024).

1.1.1. Bivalve Aquaculture

The production of bivalves, like clams, oysters, scallops and mussels, accounts for approximately 20% of all aquaculture production worldwide (Zhang et al., 2025a), representing around 90% of all bivalves consumed worldwide (Wijsman et al., 2018). Just in the first half of 2025, approximately 479 thousand tons of bivalves were traded around the world (FAO, 2025). This million-dollar market moved 22.7 billion dollars (USD), and the market is expected to increase significantly to 39.8 billion dollars by 2030-2034 (around 33.8 billion euros) (Goel, 2024). According to national aquaculture statistics (DGRM, 2024; INE, 2025), bivalve production contributes 11000 tonnes to Portugal's production, which represents 57% of the Portuguese marine aquaculture (around 21 000 tonnes).

Even though the Bivalvia group gathers a wide variety of species with different habitat requirements, many of these species show enormous potential for aquaculture. Bivalve aquaculture, when practised in its traditional non-invasive forms, tends to have a positive impact on the ecosystems (NOAA, 2022). Multiple studies found that bivalve mariculture can mitigate environmental impacts in coastal areas, particularly by reducing nutrient enrichment caused by intensive fish farming (Martin et al., 2025). By filtering microalgae and removing excess nutrients from the water, bivalves help control algal blooms, including toxic species (Martin et al., 2025;

NOAA, 2022). Additionally, mussel cultures may contribute to restoring eutrophic or polluted enclosed water bodies, offering a cost-effective and self-sustaining strategy for environmental remediation (Zhao & Wu, 2024), and even when the bivalves produced are not suitable for human consumption, their shell-building process helps to carbon fixation (Feng et al., 2023; Tan et al., 2024). The production infrastructures offer shelter to other species, while their shells form a reef-like structure that increases substrate complexity, providing shelter for epifauna and local ecological enrichment (Dame, 2012; Jørgensen, 1990; Ward, 1996). When localised on coastal shoreline areas, they can also provide coastal protection against floods and storms, reducing wave energy and stabilising sediments (Chowdhury et al., 2019; Van Prooijen et al., 2023).

As bivalves are naturally filter feeders, they harvest their food, the phytoplankton, from the surrounding water column by filtering it through their lamellae. This feeding strategy is an advantage for the farmers, as there is no need for investment in feed for the grow-out phase of bivalves in traditional bivalve aquaculture techniques. After the initial investment to start a traditional bivalve production, the only ongoing costs are for maintaining the infrastructure, harvesting the animals, and obtaining new spat (Wijsman et al., 2018). Even though bivalve production is possible in closed systems, with the aid of microalgae feed. It tends to be used only for the initial phases of the life cycle and breeding, as they allow a more controlled environment and feed, but are also more expensive to maintain.

1.2. Pacific oyster *Magallana gigas* (Thunberg, 1793)

The Pacific oyster, *Magallana gigas,* was originally described and named *Ostrea gigas* by Thunberg in 1793 and is included in the vast Mollusca phylum (Margulis & Schwartz, 1998), Bivalvia class (Linnaeus,1758), and the Ostreidae family (Rafinesque, 1815). Until very recently was known as *Crassostrea gigas,* but *a*fter molecular phylogenetic analyses, this species was reassigned to the *Magallana* genus (Salvi & Mariottini, 2016).

Like all bivalves, *M. gigas* has its soft body covered by two valves, commonly denominated as a shell. The colouration of the individuals of *M. gigas* ranges from a chalky white to grey, very often with brown or purple streaks and patches on the shell/valve surface. *Magallana gigas* has an irregular shell growth and asymmetrical valves (Huyghe et al., 2019). The left valve has a concave shape, and naturally, it serves as an anchoring point for the oyster in early life stages. Internally, the colouration of the soft body part can vary from a very light green to white. Although there are records of individuals reaching shell lengths of 450 mm, most large specimens are around 200 – 250 mm. Even though its growth rate is highly dependent on environmental conditions and genetics, they tend to reach their market size of 70 mm – 100 mm in the span of 1.5 to 2.5 years (FAO, 2024b). The maximum life span of this species is believed to be approximately 50 years (Boris & Konstantin, 2024).

If not disturbed, the oyster will remain anchored by the left valve for all life cycle. For aquaculture purposes, this process is disturbed to obtain the spat, which consists of oysters released from the substrate they settled on (NOAA, 2024). Due to the concavity, the left valve harbours the oyster's soft body. The right valve acts as a lid, closing and opening on demand. The two valves connect at the hinge, close to the umbo, by a flexible ligament composed of tanned proteins and calcium carbonate. Both valves act together to protect the animal from external environments. When the oyster senses any disturbances in its surroundings, it closes instantly, protecting itself from any imminent danger (Checa et al., 2018; Suzuki et al., 2019). The shell also allows the oyster to remain closed during the low tide period and possibly harmful changes in water physico-chemical parameters. With the remaining water inside the oyster's valves, it has the capacity to survive for approximately 48 hours outside of water, but this capacity is heavily hindered in warmer environments (Heo et al., 2023). The shells of oysters are mainly composed of calcium carbonate, which is secreted through the mantle and arranged in foliated layers. This

allows shell growth to accompany soft body growth, accommodating the space needed for its sustenance (Checa et al., 2018).

As it is represented in Figure 1, the oyster's soft body is mainly composed of the mantle, ctenidia (gills), adductor muscle, and visceral mass. The adductor muscle connects to both valves and is responsible for the closing and opening action. The mantle is a thin membrane that surrounds the soft body in direct contact with the inner layer of both valves. As mentioned before, the mantle secretes calcium carbonate, forming the oyster's valves from the inside, layer by layer (Cannuel & Beninger, 2006a; Cannuel & Beninger, 2006b; Otegui et al., 2023). The ctenidia, commonly known as gills, are responsible for the feeding and respiratory functions. Due to their filter-feeding habits, oysters can filter up to 200 L per day (Gray & Langdon, 2019), hence their positive impact on water quality in coastal ecosystems. During this process, suspended organic matter gets caught on the gill, which is coated with mucus. Each gill surface contains a vast number of cilia that sort the trapped organic matter and direct it to the labial palps for ingestion.

Magallana gigas' digestive system is composed of a mouth, near the labial palps. The mouth connects to the oesophagus, followed by the stomach, which connects to the digestive gland and crystalline style sac. After passing through the digestive gland, the filtered food moves to the intestines and rectum, which terminate near the adductor muscle (Gosling, 2015). Like all bivalves, the circulatory system is open, with the heart located inside the pericardial cavity. The nitrogenous waste and gas exchange process occurs primarily on the gill surfaces (Bayne, 2017). All internal organs are surrounded by connective tissue, which, during reproductive development, the gonadal follicles grow on (Barillé, 1997; Otegui et al., 2023). The reproductive system is not composed of a distinct organ, but of a vast network of interconnected follicles situated within the connective tissues (Franco et al., 2008; Wang et al., 2023).

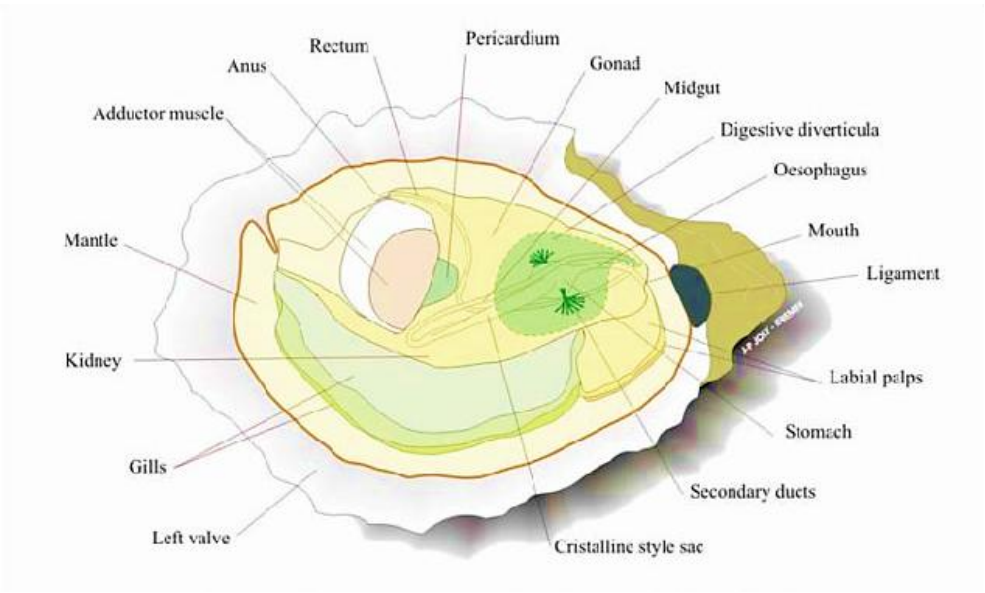

Figure 1 - Schematic anatomic representation of *Magallana gigas* soft body attached to the left valve, viewed from its right side, after the removal of the right valve. (Source: Miossec et al., 2009)

1.2.1. Distribution and Ecology

Magallana gigas is endemic to the coastal and estuarine areas of the Pacific coast of Asia, and due to its compatibility with a temperate climate, it was widely introduced in Europe, North and South America, and Oceania as an aquaculture species. Because of aquaculture breakouts and uncontrolled spawning, wild populations have developed in these areas. Although

they can withstand temperatures near 0 ºC and as high as 35 ºC, the ideal temperature range for this species is between 18 ºC and 24 ºC (Gray & Langdon, 2017; Li et al., 2021). This species normally grows at salinities of 23-28 ppm, but can withstand salinities as low as 5 ppm, and as high as 38 ppm (Gray & Langdon, 2017; Nell & Holliday 1988). It is also believed that these values are heavily influenced by genetic adaptations of each population to the surrounding conditions (Zhao et al., 2012).

It is mainly an intertidal species, but it is also found in the shallow subtidal zone. The maximum depth of a recorded population is 40 m depth, but they are more commonly found above 15 m depth (King et al., 2020). As mentioned before, after larval settlement, the oyster anchors itself to a surface, becoming sessile. During this process, they release a protein mesh that connects to the surface and secrete calcium to solidify the link between the shell and the settling surface. In the case of the oyster loosening itself from the substrate, it becomes a “free-roaming oyster”, but this process can only happen after settlement (MacDonald et al., 2010). The preferred substrates for this species are usually rocks, wood, and shells, but they can also use anthropogenic structures of cement, iron, glass, and plastic as substrates. This species develops very dense populations, which may become numerous to the point that some individuals attach to other living oysters, forming sharp reefs (Varley et al., 2026). Although wild oysters can be equal to aquaculture-produced ones, flavour-wise and nutritionally, they tend to be more impractical to process due to their rough and sharp shell shape (Chinzei, 2013).

1.2.2. Feeding Habits and Nutrition

As oysters become sessile filter feeders after settlement, their main food sources directly depend on the organic matter present in the water column on which they live. Several studies show that dinoflagellates and diatoms are the primary components of their diet (Akter et al., 2025). However, protists and other phytoplanktonic organisms are also ingested, confirming a diverse diet (Tran et al., 2022). Due to their feeding habits, seasonal shifts in the phytoplanktonic communities can influence the available food for oysters. Studies found changes in fatty acid profiles and growth, indicating a direct correlation between nutritional value and abundance of the phytoplanktonic groups present (Pan et al., 2025).

As quality nutrition is critical during gonadal development and larval growth to ensure great quality spat (Kheder et al., 2010), commercial spat facilities are the only oyster production stage that justifies the costs required to sustain fully grown oysters with high-quality live feed. For facilities that focus on oyster reproduction and commercialisation of spat, a highly strict controlled diet is a key factor to their success, as it improves the overall quality of the product. (Marshall et al., 2010). A more precise, controlled diet during gonadal development supports healthier development, which can directly enhance egg quality and fertilisation rates (Li et al.,2025; Lovegrove et al., 2025). Combining multiple microalgae on a mixed diet, alongside supplements such as macroalgae or protists, is a strategy that tends to increase broodstock egg quality (Anjos, 2014; López-Marcos et al., 2025). As for the larvae, a controlled and clean diet plays a critical role in ensuring high survival rates and high-quality spat (Gibbs et al., 2020; Kim et al., 2021; Li et al., 2025; Sühnel et al., 2024). Overall, the vast range of microalgae used to feed *M. gigas* illustrates the resilience of this species and its capacity to thrive under variable dietary and environmental conditions. Although *Magallana gigas* has an exceptional capacity to adjust its feeding behaviour, the feed quality directly affects oyster overall fitness (Tran et al., 2022).

1.2.3. Gonadal development

The Pacific oyster *M. gigas* is a sequential protandric hermaphrodite, although simultaneously hermaphroditism is possible (Broquard et al., 2020). This species exhibits an annual gametogenic cycle. Although they normally mature as males in their first breeding season, environmental factors, such as temperature and food availability, play a key role in the sex

transition to female. This species has no sexual dimorphism, but females tend to be bigger than males (Baghurst & Mitchell, 2002; Broquard et al., 2020). In the wild, *M. gigas* can take up to 20 months to be sexually mature, but in ideal conditions, it can be sexually mature in as young as 6 months, this being influenced by temperature. (Cho et al., 2023; Gomes et al., 2014; Kasmini et al., 2019).

When comparing subtidal populations to intertidal populations, Ngo et al., (2006) found that subtidal populations have a larger energy allocation to gonad development, as they have more access to food and cooler temperatures. The intertidal population tends to be under constant risk of desiccation and temperature changes. This stress may lead to a smaller allocation of energy to gonadal development, resulting in a smaller gonadal mass and consequently a smaller spawning capacity.

Differences in the reproductive cycle behaviour are found between populations around the globe (Barman et al., 2024; Cho et al., 2023). A study from Cardoso et al. (2013) compared *M. gigas* populations of Ria Formosa (Portugal) with those of Ría de Ribadeo (Spain), finding differences in spawning performance. Ría de Ribadeo populations have larger gonadal mass, but due to suboptimal spawning temperatures, they don't fully empty their gonads during spawning. In the Ria Formosa populations, higher winter temperatures lead to reduced investment in gonad development, but they completely empty their gonads during spawning. The Northern Europe *M. gigas* populations have a smaller spawning period with a higher female ratio, when compared to the Portuguese and Spanish populations. This maximises recruitment in colder environments. This other study shows that water temperature seemed to be the main factor affecting gonad development and the main trigger for spawning. While chlorophyll a, which is a proxy for food (phytoplankton) availability, had a more pronounced impact on the size of oocytes during development than on gonad development stages (Balić et al., 2020). In the case of triploid oysters, they are sterile, which means that their energy is mainly channelled to growth and higher meat quality without investing in gonadal development.

1.2.4. Gametogenesis and Spawning

Through *M. gigas* annual reproductive cycle, their gonads have significant structural changes associated with gamete production and later, spawning (da Silva et al., 2015; Enríquez-Díaz et al., 2016). As mentioned above, adult oysters don't have a specific reproductive organ, but rather a branching system of gonadal follicles diffused within their visceral mass, which disappears after spawning. Each follicle starts as a small cluster of self-renewed stem cells (Fabioux et al., 2005). Through mitosis, they start to differentiate into many gonia, the precursors to gametes. This cellular growth leads to the expansion of tubules that surround the digestive system, where germ cells will mature and be stored (Franco et al., 2010). This growth will invade the connective tissue, consuming the energy stored. The gonadal tubules will continue to enlarge with the accumulation of mature gametes, which will lead to the confluence of the gonadal tubes (Dridi et al., 2007). When mature, the gonads give the oyster's visceral area a milky white colour. Before the release of eggs, the oyster concentrates elevated levels of glycogen, fatty acids, and other nutrients (Cho et al., 2023).

As temperature is the main trigger for spawning, it tends to occur during spring and summer in a temperate climate, like Portugal (Azeredo et al., 2018). During spawning, the gametes are released into the mantle chamber and expelled into the water column via contractile valve movements (Broquard et al., 2020). After spawning, the empty gonadal follicles start to diffuse, giving space once again to the storage of energy in the connective tissue during the winter and autumn (Berthelin et al., 2001; Dridi et al., 2007).

1.2.5. Fertilisation, Larval Development, and Settlement

Fertilisation happens externally during a conjoined release of gametes, normally triggered by temperature rises. As it is schematized in Figure 2, after spawning, both unfertilized eggs and sperm are released into the water column. Post-fertilisation, the embryo starts to divide, becoming a morula, blastula, and later a gastrula (Gavery et al., 2025; Huang et al., 2017). About 12 hours after fertilisation, it becomes a trochophore larva, already capable of swimming and feeding (Hou et al., 2025). After 27 hours post-fertilisation, the trochophore larvae enter the characteristic bivalve larval phase, the D-larvae, which already have differentiated valves with a shape resembling a “D”. After 15 days post-fertilisation, the D-larvae have transformed into their veliger stage, and later their pediveliger stage, the last planktonic free-swimming stage of their life (Summa et al., 2023). After 30 days, the larvae undergo a metamorphosis where its foot becomes residual, attaching to the substrate, turning into a sessile organism. After settlement, the oyster grows around its surroundings, never relocating itself.

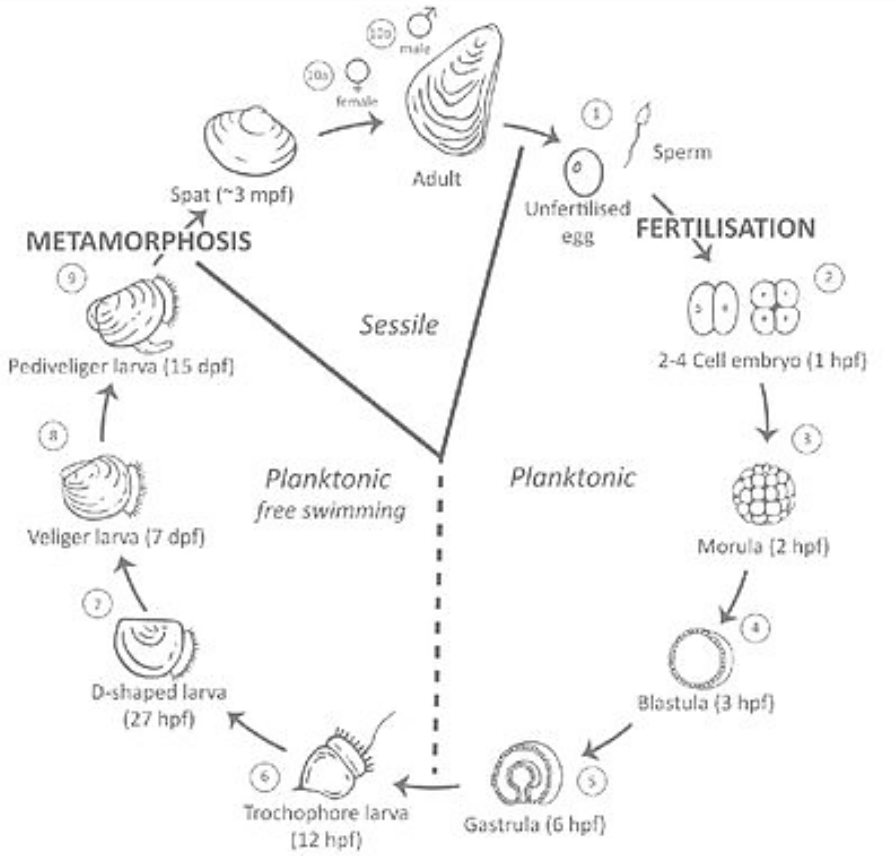

Figure 2 - Life cycle of the Pacific oyster, *Magallana gigas*. Numbers 1-10 represent different life stages. Legend: hpf - hours post fertilisation; dpf - days post fertilisation; mpf - months post fertilisation. (source: Vogeler et al., 2016)

1.3. Oyster Aquaculture and Global Market

Oysters are among the most valuable bivalves in global aquaculture and are widely consumed on all continents. Their market price varies considerably depending on species, location of production, size, shape, weight and quality, which is then translated to category, class, quality grade and even certified PDO (Protected designation of origin) brands. According to FAO (2022), in the past decade, global oyster production has remained above 5 million tons annually.

More than 95% of the market's supply is sourced from aquaculture production, with China dominating global production, followed by the Republic of Korea, Japan, the United States, and France. Nowadays, the Pacific oyster, *M. gigas,* is the second most cultivated species worldwide, only being surpassed by *M. angulata* (Lamarck, 1819). Overall, global production of oysters is approximately 5 485 037 tonnes, with China accounting for 85% of this value (4 661 121 tonnes) (Jung et al., 2025).

Worldwide, *M. angulata* leads production with approximately 1 920 311 tonnes (35%), followed by *M. gigas* with around 1 705 847 tonnes (31%). Both these species have high environmental tolerance, resilience, rapid growth, and strong market demand (Jung et al., 2025). However, mortality events in *M. angulata* related to diseases have been noticed in Europe, leading to wider regional implementation of *M. gigas* (Fabioux et al., 2002). *Magallana angulata* is also more fragile, making it more susceptible to damage during production and transport.

In Portugal, total production reached 3 632 tonnes in 2020. *M. gigas* accounted for 69% of production (2 500 tonnes), followed by *Ostrea edulis* (29%, 1 050 tonnes) and *M. angulata* (2%, 72 tonnes). More than 60% of production is exported, mainly to France (Dias et al., 2025). Oyster production in Portugal has grown exponentially over the last decade, particularly in estuarine and lagoon systems, such as Ria Formosa and Estuário do Sado (Pereira, 2026). The Pacific oyster is the dominant species due to its fast growth rate and market demand, while interest in the Portuguese oyster (*M. angulata)* is increasing because of its higher market value and biodiversity restoration purposes (Pereira, 2026). In European markets, depending on the quality, fresh live oyster prices tend to range from 6 to 15 € per kilogram, but premium labelled oysters can sell for much higher prices. In France, one of Europe's leading markets, oysters are a culturally significant seafood product, with some regions having over 2 kg of annual consumption per capita (EUMOFA, 2023; FAO, 2024c).

Normally, oysters are marketed live, kept alive until preparation and consumed raw or lightly cooked. Their quality is one of the key factors in determining an oyster's market value. Quality is determined by shell shape, glycogen content, texture, meat yield, and overall sensory profile. Many of these indicators are combined in formulas to determine Condition Indices (CI) able to quantitatively score the quality of oysters (Azeredo et al., 2018). Condition indices and quality of the soft body are heavily influenced by the reproductive stage. As gametogenesis evolves, the glycogen reserves decrease, reducing quality through maturation. At this stage, the oyster's flavour is considered less desirable for most consumers, though at this stage, the nutritional value reaches its peak. After spawning, glycogen reserves start to be renewed, and alongside, oyster quality and commercial value increase (Syvret et al., 2021).

From a nutritional perspective, oysters are recognised as a great source of lean protein, rich in glycogen, omega-3 polyunsaturated fatty acids (EPA and DHA), vitamins (mainly B12), and essential minerals like zinc, iron, and selenium (Raut et al., 2025; Wright et al., 2018; Zhang et al., 2025b). Due to their high nutritional value, the consumption of bivalves will promote cognitive function, immune function, cardiovascular, brain, and bone health (Raut et al., 2025; Wright et al., 2018). These attributes support their position in the global market as a health-associated and premium seafood product (FAO, 2024c).

Oyster farming is considered a promising strategic activity for Portugal's Blue Economy sector, due to its ecosystem services, low trophic level, and strong export potential (OECD, 2025). Nevertheless, this sector remains highly dependent on environmental factors, challenged by mortality events, poor water quality, harmful algal blooms, and increasing temperature fluctuations (Pereira, 2026). To strengthen Portugal's position and resilience in the oyster production sector, investments are being promoted for the establishment of hatcheries and sustainable aquaculture farms (European Commission, 2022; Portugal.gov.pt., 2023). As part of a product valorisation strategy, certification schemes and product differentiation, including origin-based branding, are being implemented (DRGM, 2024).

Given the rising global search for high-quality seafood protein and the biological efficiency of bivalve aquaculture, it is anticipated that oyster farming will remain a valuable sector for blue

economy strategies (Goel, 2024). To increase productivity, resilience, and year-round supply, innovative hatchery technologies, offshore cultivation systems, and selective breeding for productivity and disease resistance are being developed (FAO, 2024c; EUMOFA, 2023).

Magallana gigas spat can be obtained in two ways: naturally using cultch techniques or obtained from a hatchery (zu Ermgassen et al., 2020). However, since the development of commercial hatcheries, reliance on natural spat collection has become less desirable, as controlled production allows for selective breeding programs target improved growth rates, disease resistance, and shell profile (Dégremont et al., 2015; Helm et al., 2004). Hatcheries were also particularly important in the widespread implementation of triploid variants. This sterile variant has total energy investment into somatic growth, presenting better yield, improving market quality and extending harvest periods (Allen & Downing, 1986; Guo et al., 2009).

As for the grow-out phase, many techniques can be applied, allowing for greater adaptation to diverse hydrodynamic and ecological conditions. The most common grow-out methods for *M. gigas* are longline cultivation, bottom culture, and suspended rack and back systems (FAO, 2022). Off-bottom (suspended) systems are the most adopted for intertidal areas, as they reduce predation and are easier to maintain, thus improving growth rates (Azeredo et al., 2018).

Longline systems are the most common in subtidal environments, as they optimise water flow and food availability (Azeredo et al., 2018). If oysters are left unattended throughout their growth, fouling, predators, and density become problems, hindering productivity and increasing mortality rates (Park et al.,1998). Given that, regular clean up, shell maintenance (oyster thumbling), and stock redistribution are beneficial practices with a positive influence on growth performance and shell quality (Helm et al., 2004; Azeredo et al., 2018).

Once oysters reach the desired market size, post-harvest practices play a critical role in determining the final product's quality and value. After harvest, oysters are usually subjected to a thorough cleaning process, eliminating sediments, loose shell flakes, and epiphytic organisms biovarious strict sanitary and environmental regulations to ensure sustainability and safety for the consumer. In Europe, harvest areas are classified based on microbiological quality, depuration standards, and traceability requirements, ensuring public health. To reduce microbial contamination risks, oysters go through a depuration process that eliminates possible harmful microbial contamination (Campbell et al., 2022). This process consists of placing the oysters in a controlled condition system, with filtered seawater under monitored conditions, giving time for their natural filtration process to purge out any contaminants (Laing & Bopp, 2019). These depuration systems are mandatory in many places and fall under strict regulation to ensure public health and safety norms (Canadian Food Inspection Agency, 2018; Azeredo et al., 2018).

After depuration, oysters are sorted and graded by size and shell quality, then packed and placed under refrigerated conditions. Through the distribution process, a strict cold environment-chain and a traceability system must be followed, guaranteeing product safety, extended shelf life, and, more importantly, compliance with international food safety regulations (Azeredo et al., 2018).

1.4. Main threats to oyster aquaculture

Despite its successful worldwide implementation and stable flow, the oyster sector still faces significant challenges. As oysters filter larger volumes of water daily for feeding, they tend to be at risk of bioaccumulation of toxins and harmful bacteria (Fiori et al., 2024; Sol Dourdin et al., 2024). Due to its filter-feeding habits, it is also highly prone to microplastic accumulation, and this accumulation impacts oysters through chemical leaching, which has been found to decrease their heart rates (Bernardini et al., 2024). Heavy metal accumulation is also a problem, as they can absorb considerable amounts of cadmium, lead, copper, zinc, mercury, and other metals. This causes tissue damage, physiological and reproductive stress, reducing fertilisation success

(Sol Dourdin et al., 2024). In areas where constant underwater noise pollution is present, metabolic rates, feeding activity, and consequently growth is reduced. On the other hand, chronic noise pollution limits the bioaccumulation of heavy metals (Charifi et al., 2018). Even though the adult life stage of this species is very resilient to contaminants, the larval stages present direct toxicity to many pollutants, which can critically hinder survival rates (Sol Dourdin et al., 2024).

Diseases and pests are also a constant threat in most aquaculture productions, as production sites are subject to the quality of the surrounding water masses. *Perkinsus marinus* (Guiry & Guiry, 2026) and Ostreid herpesvirus are two of the main diseases that cause high mortality rates (EFSA, 2015; Renault et al., 2014). Climate change-related stressors, such as algal blooms, ocean acidification, and warming, increase the already existing production risks in oyster production, forcing farmers to develop strategies to mitigate their effects.

1.4.1. Marine Heatwaves in Oyster Farming

Climate change is one of the major threats to aquaculture production. It directly affects ecosystems, biodiversity, and animal behaviour, leading to chronic physiological stress. For many animals, food availability, breeding conditions, and natural habitat have become harder to find, leading to changes in distribution patterns (Haris et al., 2025). Considering the current climate fluctuation, studies show increased heavy rainfall in tropical areas and rising storm intensity (WMO, 2025). Temperature rise, including ocean warming, has also led to record-breaking marine heatwaves in many areas (Fischer & Knutti, 2015; Neto et al., 2025). In subpolar regions, the more frequent heatwaves and overall warming are increasing seawater temperature, disrupting natural processes of native marine species (Menary et al., 2025; NASA, 2024).

Specifically in Portugal, over the last few decades, there has been an increase in storm intensity. This increase leads to unexpected floods in many areas, and salinity drops in coastal marine habitats (Antunes et al., 2019). During summer, heatwaves and droughts have become more intense and frequent, creating ideal conditions for wildfires to spread (Parente et al., 2018). While in coastal areas, specifically in the Algarve, sea temperatures have risen above average, threatening coastal ecosystems (IPMA, 2026; DGRM, 2024).

Climate change also directly impacts human activities, many related to the food production systems, like agriculture (Bibi & Rahman, 2023). The increase of precipitation patterns, water temperatures, and frequency of extreme climatic events already impacts many aquatic organisms globally, wild and cultured (Global Seafood Alliance, 2025). As for aquaculture, if farms don’t have total control over their water quality, as is the case for most coastal aquaculture productions, they are more vulnerable to changes in the environmental conditions (FAO, 2016).

Water temperature plays a key role in marine invertebrates’ physiology. Extreme climatic events can trigger mass mortality in aquaculture productions. Marine heatwaves and, in general, abrupt water temperature shifts can lead many species to the limit of their temperature tolerance. On its own turn, the increase in water temperatures directly affects many biological processes, such as metabolism, energy allocation, and feeding frequency of marine organisms. The chronic stress generated by these factors leads to high levels of physiological stress, which results in sudden, large-scale deaths for more sensitive species (Fernández et al., 2022; Khalid, 2022; Mugwanya et al., 2022). Salinity can also be the cause of high levels of physiological stress, mainly in events of heavy rainfall. Estuaries don’t have the capacity that oceans do to counterbalance salinity drops caused by heavy rain events, making them more vulnerable to rain events. Violent episodes of rain mainly impact the salinity level in estuarine areas. When a sudden input of freshwater arrives from the river mouth, salinity levels decrease significantly, resulting in copious amounts of environmental stress for endemic species (Alosairi et al., 2019).

On Portuguese aquacultures, the main impact is derived from temperature, salinity, and consequent environmental changes. Marine heatwaves are becoming more intense and frequent during summer, contributing to rising mean water temperatures. In winter, storms have increased

in severity, resulting in more frequent storms and larger rain events. As most farms are based in estuarine zones, many face the challenge of rising temperatures, which threatens the sustainability and efficiency of this sector (CESAM, 2023; Monteiro et al., 2025; Pereira, 2026).

Among all climate-related stressors, marine heatwaves (MHW) are one of the most disruptive phenomena affecting marine ecosystems. Defined as prolonged periods of anomalously high sea surface temperatures (Hobday et al., 2016), marine heatwaves have been longer, more intense, and more frequent over the last century, with a noticeable acceleration since the 1980s (Oliver et al., 2018; Pinto et al., 2024). These events lead to the disruption of energy allocation, physiological stress, hindered immune response, and increased mortality in marine invertebrates, including commercially important bivalves (Smale et al., 2019).

The impacts of marine heat waves have already been felt in oyster production worldwide. In the years 2010 and 2011, a marine heatwave in Australia led to the mortality of many aquaculture species, including oysters (Wernberg et al., 2013). In the northeast Pacific, an extreme marine heatwave known as “the blob” causes a significant ecological disruption, affecting bivalve growth and the survival of many marine species (Cavole et al., 2016). European oyster producers have also reported an increase in mortality events related to elevated temperatures and pathogen proliferation, such as ostreid herpesvirus, which thrives in warmer environments (Pernet et al., 2012; Petton et al., 2013).

Marine heatwaves affect Portugal’s oyster aquacultures through many direct and indirect mechanisms. Higher temperatures lead to energy allocation from natural growth and reproductive processes, as more energy is shifted to combat metabolic stress. Prolonged thermal stress also enhances vulnerability to opportunistic pathogens by hindering the immune system's defences. Additionally, rising sea temperatures alter the composition of the phytoplankton community, consequently affecting the availability and quality of food. In estuarine environments, this cumulative stress problem is further exacerbated by salinity fluctuations derived from intense rainfall events (Alosairi et al., 2019).

Recently, Europe started its documentation process of oyster’s biological responses to temperature increase and extreme climatic variations. Field and Laboratory studies have reported decreased physiological performance under exposure to high temperatures, including filtration rates, oxidative stress, and higher mortality rates during anomalously warm periods (Funesto, 2023; Mosqueira et al., 2022; Malhotra, 2025). Despite the growing recognition of marine heatwaves because of contemporary climate change, region-specific assessments of the impact on *M. gigas*’ aquaculture in Portugal remain limited, particularly under real farming conditions.

Understanding the impact of extreme heatwave events on oyster physiology, survival, and production efficiency is essential due to the economic and social significance of oyster farming in Portugal and the concerning intensification of these thermal events (IPCC, 2023). Further addressing this knowledge gap is critical for the development of adaptive management strategies and resilient measures combating the threat this sector faces against the accelerating climate variability (DGRM, 2024).

1.5. Objectives

This study focuses on evaluating the effects of marine heatwaves on *M. gigas* growth, condition indices, and quality indicators during the early grow-out phase by comparing a heatwave simulation group with a control group at stable temperature (18 ºC). Additionally, in a different trial, an undisturbed grow-out strategy was compared to traditional grow-out handling practices (weekly dissection periods and light thumbling) in growth, condition indices, and quality indicators. In both trials, the effects of the disturbing factor, handling and marine heatwave, were evaluated by:

- Compare the development of morphometric characteristics of oysters in two different conditions (1º Trial: Handling *vs* Control; 2º Trial: Heatwave *vs* Control) by evaluating the changes in length, height, width, and weight.
- Compare the development of the AFNOR index and condition index of each treatment (1º Trial: Handling *vs* Control; 2º Trial: Heatwave *vs* Control) during the trial.
- Evaluate the total colour difference between oyster soft body samples of the distinct studied treatments.
- Compare the oyster's soft body texture expressed by the different parameters (Springiness, cohesion, hardness, resilience, chewiness, and gumminess) by performing a texture analysis profile (TPA).

2. Materials and Methods

2.1. Experimental Recirculating Aquatic System

Two recirculating aquatic systems (RAS) were assembled for the planned experiments. Each RAS included a 250 L SUMP, divided into three sections (Figure 3a). The middle section served as the biological filter, where plastic Bioballs ® and aeration stones were added. A water pump (Reef Pump 2000, Tropical Marine Centre Group, Hertfordshire, UK) drew water from the SUMP to three 50 L glass tanks. Each tank contained a mesh basket to hold the oysters at midwater, helping position them more effectively for filtering the microalgae used as feed (Figures 3b and 3c). Each tank was equipped with an airlift aeration system that improved circulation inside the tank, promoting better aeration and even distribution of the microalgae. The water outlet on the tanks was a surface exit linked to a collector pipe that combined the flow from all three tanks into the SUMP reservoir.

To ensure a consistent feed source throughout the day, two gravity feeding systems were developed using drip irrigation valves and a 5 L jug. The system could split the feed among the three tanks and deliver it gradually throughout the day, reducing feed loss by gravity.

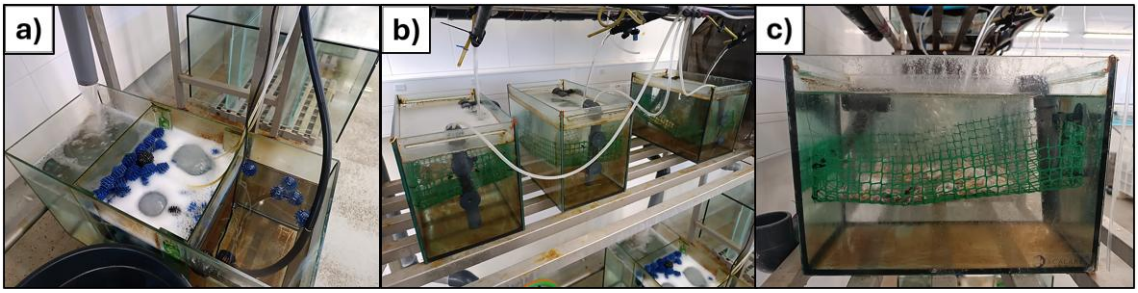

Figure 3 - Experimental Recirculatory Aquatic System (RAS) assembled for the oyster rearing experiments: (a) A SUMP including a biofilter section with aeration; (b) The three 50 L glass tanks equipped with an airlift; and (c) the mesh basket positioned in the middle of each tank.

2.2. Microalgae Culture

During acclimation and trials, the oysters were fed a mixture of three microalgae, namely *Tetraselmis chui* (Butcher, 1959), *Chaetoceros gracilis* (Schütt, 1895) and *Skeletonema costatum* (Cleve, 1873). The microalgae stocks were maintained in 250 mL round-bottom flasks with strong aeration, under a light cycle of 10 hours of light and 14 hours of darkness at 19 °C (Figure 4a). Each week, the stocks were renewed with sterilised seawater enriched with a commercial f/2 culture medium and vitamins (NUTRIBLOOM®). The composition of this nutritive solution is presented in Table I. For the diatom species, such as *S. costatum* and *C. gracilis,* a sodium silicate solution with a concentration of 45 g/L was also added in the proportion of 1mL per 1 L of culture to fulfil the nutrient requirements of the diatom algae's dietary needs.

Table I - Chemical composition of the commercial microalgae culture medium Nutribloom®. Compositional percentage (%w/w) used as feed for microalgae cultures.

Composition:	%w/w
Nitrates (N)	2.800
Phosphate (P)	0.310
Iron (Fe)	0.112
Zinc (Zn)	0.014
Molybdenum (Mo)	0.010
Manganese (Mn)	0.006
Magnesium (Mg)	0.005
Cobalt (Co)	0.001
Copper (Cu)	0.001
Thiamine	0.0035
Biotin	0.0005
B12	0.0003
Ratio N:P	9:1

To obtain the volumes and densities of microalgae required to feed the oysters, a culture scale-up was conducted, achieving a maximum volume of 15 L for each microalgae species using previously sterilised seawater. To initiate, a portion of 250 mL of the initial stock was diluted with sterilised seawater and nutrients in a 1 L round-bottom flask. The scale-up was performed by increasing the culture volume weekly until achieving the 15 L culture volume. For the initial culture volumes (up to 2 L), the seawater was sterilised at 120 ºC for 20 min, while for larger volumes it was treated with sodium hypochlorite. Briefly, the 15 L water jugs were scrubbed and washed, and an aeration hose and stone were added to them. Then, filtered seawater was poured into the jugs and sodium hypochlorite at a ratio of 0.1 mL per litre, the water was aerated for 24 hours. A solution of potassium thiosulfate was added at ratio of 1 mL to eliminate the remaining sodium hypochlorite. After this process the water jugs are then ready to be inoculated. The water jugs then sat under aeration for a period of around 7 to 10 days before they began approaching their maximum concentration (Figure 4b).

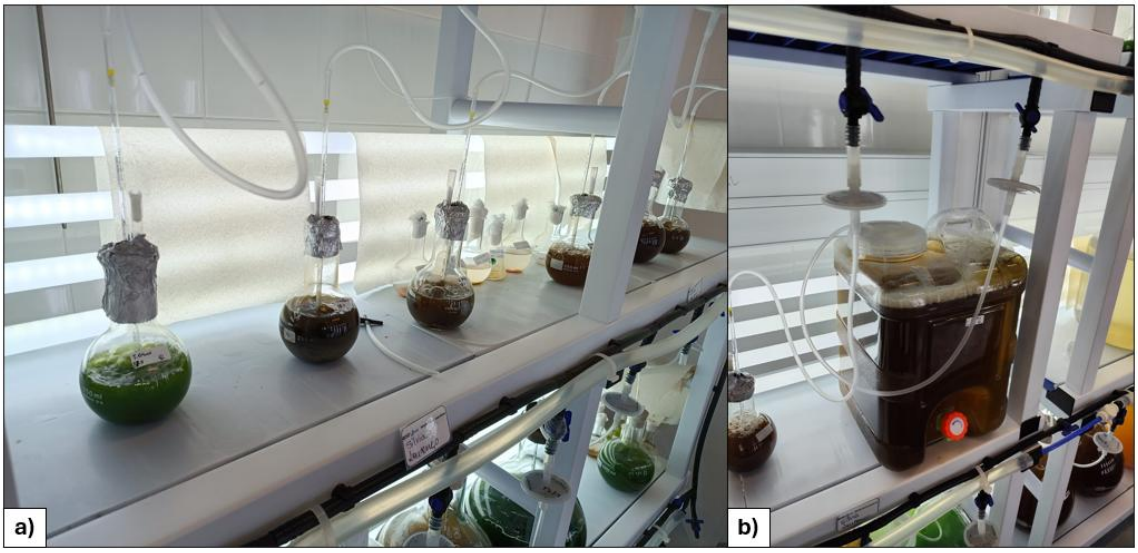

Figure 4 - Microalgae stocks in a 250 mL round-bottom flask (a). 15 L water jug inoculated with *Chaetoceros gracilis* (b).

2.2.1. Determination of the microalgae growth curve and biomass weight

To achieve the maximum yield of each species, a growth curve analysis was conducted to identify the microalgae density peak, defined as the best moment to be used to feed the oysters. To determine the growth curve, cell counts, culture optical density, and biomass were determined daily in 14-day cycles. For each microalgae species, culture biomass was also determined daily by microalgae dry weight. Culture cell density was determined by cell counts at a microscope (Zeiss Axio Lab.A1, Carl Zeiss Microscopy GmbH, Göttingen, Germany) using a Neubauer® chamber. A previous count was conducted to assess if a sample dilution was needed. If the preliminary count was above 300 cells, a known volume of distilled water was added to the sample to achieve a cell concentration between 30 and 300 cells observed. After dilution, at least three independent cell counts were conducted.

To determine the optical density of microalgae cultures, microalgae samples of 10 mL were collected from the stock, and their absorbance was determined in a wavelength range between 550 and 850 nm with a UV-Vis spectrophotometer (Thermo-Fisher Scientific Evolution 201, Massachusetts, USA). Triplicate readings were conducted using distilled water as a blank. For each microalga, the wavelength with the absorbance peak was selected as the reference to determine optical density.

To determine microalgae biomass dry weight, triplicate samples of 10 ml were filtered using a pre-dried 70 µm Glass microfiber filter (grade 698, Avantor Sciences, Frankfurt, Germany). The filters used were previously dried in a laboratory oven (UF 110, Memmert, Schwabach, Germany) for 24 hours at 60°C and weighed. After filtration, the filter was placed in the lab's oven at 60 °C for 24 hours to dry. After reaching room temperature, the filter was weighed.

The microalgae culture growth was monitored until a decrease in cell number was noted, then a graph was built to determine the best days to start algae harvest, achieving the best amount of feed per culture.

2.3. Oyster sourcing and acclimation

The oysters were acquired from EXPORSADO, an aquaculture company located in the Sado estuary. In the production site, the oysters were grown in bags placed on tables in the riverbed. When collected, the oysters were placed in a bag inside a Styrofoam cooler filled with ice, avoiding direct contact with it, and transported by car to MARE – IPLeiria aquaculture laboratory (Peniche). Upon arrival, the oysters were left on a tray until they reached room temperature. After that, they were placed in a glass tank with seawater at room temperature. The oysters were suspended at midwater level using mesh baskets. At the beginning of the experiment, the temperature inside the aquaculture laboratory was similar to the temperature at the production site in the Sado Estuary. As a result, only a small acclimatisation of 2 days was necessary before the experiment and was only needed for the new environment after the stress of transportation.

2.4. Oyster handling experiment

In this first experiment, traditional handling practices were simulated by removing the oyster baskets from the water for 12 hours and thumbling them before placing them back in the water. This process was performed on Tuesday and Thursday each week. The stimulus was applied to oysters in system 2, with system 1 serving as a control. During the five weeks of the experiment, the oysters of both treatments were fed with a mixture of *T. chuii*, *S. costatum,* and *C. gracillis* in a ratio of 20%:40%:40% standardized by microalgae dry weight in a proportion of 10 % of the oysters' average dry weight as proposed by Ronquillo et al (2012), as optimal dietary conditions for juvenile oysters.

Water quality was also measured regularly to ensure proper conditions for the oysters. Temperature, pH, salinity, and dissolved oxygen were measured using the YSI Professional Plus handheld multiparameter meter (YSI Inc., Yellow Springs, OH, USA). Nitrates, nitrites, and ammonia were measured using the colour test kits (Tropic Marin, Wartenberg, Germany).

2.5. Marine heatwave simulation

The second experiment simulated the temperature conditions of a marine heatwave. The heatwave profile used for this essay aimed to mimic a natural heatwave in the estuarine areas of Portugal (Figure 5a), where it is common to experience periods of seven to ten days with a significant temperature increase (Pereira et al., 2023; Hobday et al., 2016). A new set of oysters was acclimated to the RAS systems, with the addition of a water temperature regulation system. A water chiller (Hailea HC-150A, China) (Figure 5b) with a water pump and a thermostat (EHEIM 125 watt, Germany) was added in the SUMP of each RAS. This equipment allowed the simulation of a marine heatwave in system 2 and maintained the seawater temperature steady in the control system. Initially, both systems started at the same temperature, but the temperature of system 2 increased gradually over five days by 6°C and then maintained at this level for 10 more days. Afterwards, a gradual decrease in temperature followed by stabilisation was performed, as shown in Figure 5a.

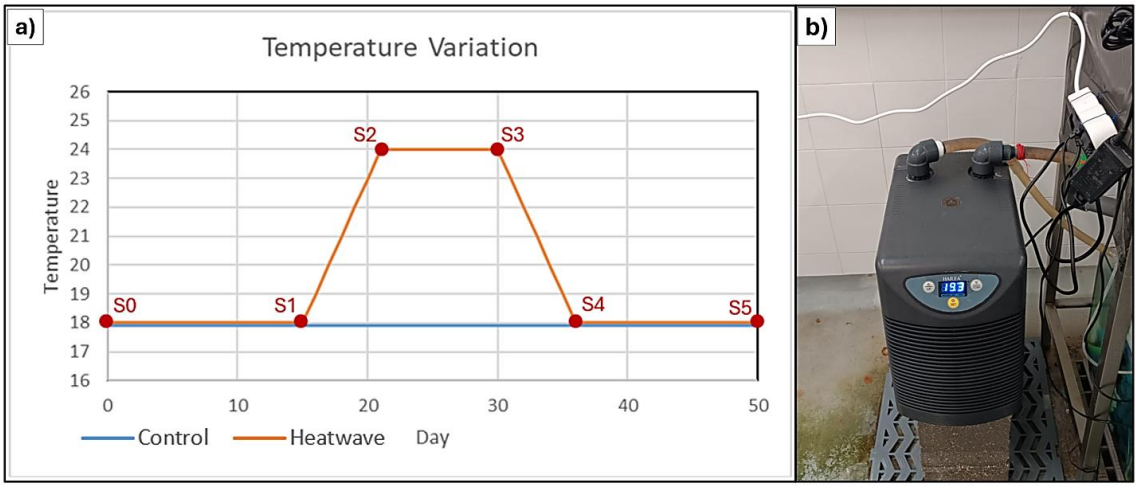

Figure 5 - (a) Graphical representation of the marine heatwave profile designed for this experiment, red dots (S0 to S5) indicate days when samplings were performed (a); (b) Temperature control equipment.

In this second experiment, for logistical convenience, the oysters were fed with a microalgae mixture of 33.3 % *T. chuii*, 33.3% *S. costatum,* and 33.3% *C. gracilis* in a proportion of 10 % of the oysters' average dry weight (Ronquillo et al., 2012). To supply quality feed for the oysters, fresh microalgae were used and grown every week, as in the first experiment. Oyster sampling was conducted at each critical point of the heatwave temperature profile (S0-S5), as shown in Figure 5a.

The water quality parameters (Dissolved oxygen, temperature, pH, and salinity were measured daily with the multi-parametric probe. Nitrates, nitrites, and ammonia were measured with colour test kits, as in the handling experiment.

2.6. Oyster sampling procedure

As summarised in Table II, before the start of each experiment, 20 oysters were randomly selected for the initial baseline sampling (S0). At all sampling points, all oysters were measured and weighed, separated by tank and treatment. Subsequently, 5 oysters per tank were sacrificed (N = 30) to determine condition indices, colour, and texture parameters. In the handling experiment, sampling was performed weekly (7-day interval). As for the heatwave experiment, sampling was conducted at every critical point of the trial as identified in Figure 5a.

Table II - Summary of the number of oysters sampled for morphometric analysis, condition indices, colour, and texture parameters in each sampling event during the two experiments.

Sampling event	Handling experiment		Heatwave experiment	
	Morphometric analysis	Condition, colour, and texture parameters	Morphometric analysis	Condition, colour, and texture parameters
S0	30 per tank (180 in total)	20 oysters in total	35 per tank (210 in total)	20 oysters in total
S1	30 per tank (180 in total)	5 per tank (30 in total)	35 per tank (210 in total)	5 per tank (30 in total)
S2	25 per tank (150 in total)	5 per tank (30 in total)	30 per tank (180 in total)	5 per tank (30 in total)
S3	20 per tank (120 in total)	5 per tank (30 in total)	25 per tank (150 in total)	5 per tank (30 in total)
S4	15 per tank (90 in total)	5 per tank (30 in total)	20 per tank (120 in total)	5 per tank (30 in total)
S5			15 per tank (90 in total)	5 per tank (30 in total)

2.6.1. Morphometric parameters

After collecting all oysters, they were dried on the outside using a paper towel and placed on Petri dishes labelled with their identifying numbers (Figure 6a). Each oyster was then measured for width, length, and height using a calliper (Insize CO., LTD, China) (Figure 6b), and then weighed on a scale (Ohaus Navigator, New Jersey, USA). After completing the morphometric sampling, five oysters were randomly selected from each tank's petri dish for the determination of the condition indices. All the remaining oysters were returned to their tanks, and their routine feeding and cleaning cycles resumed. All data collected during sampling were recorded in an Excel sheet for later analysis.

2.6.2. Determination of condition indices

To determine the condition indices, the five oysters previously selected were carefully opened by applying pressure on the hinge area (Figures 6c and 6d). Once opened, the internal liquid is drained, and the muscle is separated from the shell. The soft body was weighed and then placed on a microscope slide, labelled with the same number attributed to its respective valves. Both shells are weighed together and then placed in a Petri dish labelled with a number for subsequent sampling steps.

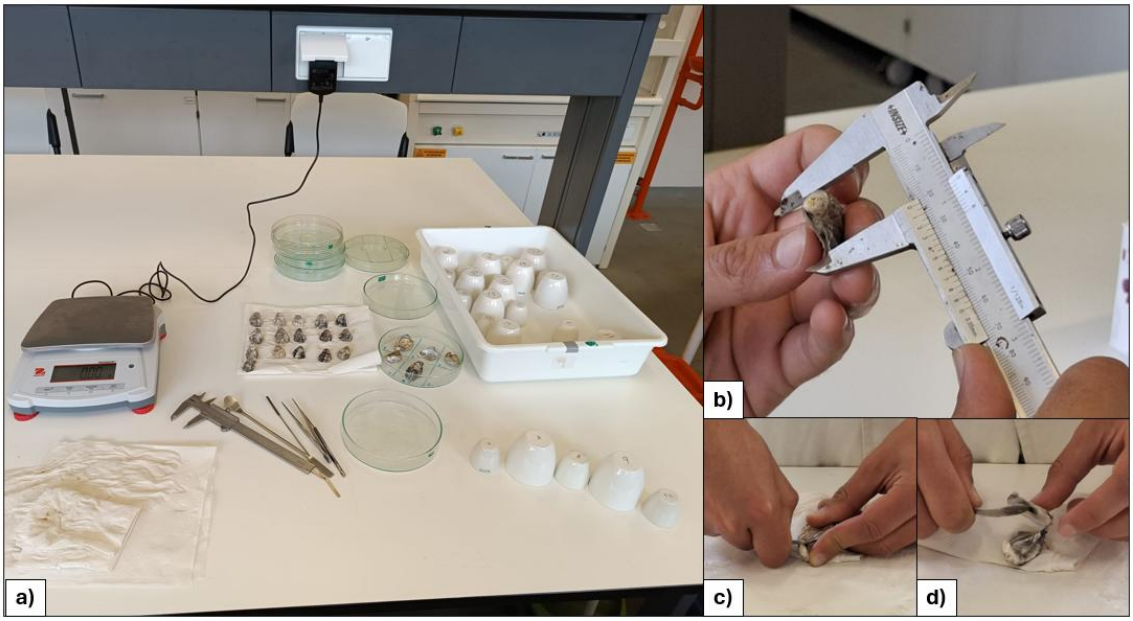

Figure 6 - Sampling setup for oyster morphometric, condition and quality analysis (a), separating the oyster soft body from the shell (a). An oyster being measured using a calliper (b). Process of opening the oyster, initially opening the oyster (c), and then separating both shells (d)

.

To calculate the AFNOR and Condition indices, it was necessary to obtain the dry weights of the oyster shell and soft body, as well as the ash weight of the soft body. The soft body tissues and shells were dried in the laboratory oven at 100 °C for 24 hours. After drying, the samples were immediately transferred to a desiccator to cool overnight. After attaining room temperature, both shells and the dried soft body were weighed.

To determine soft body ash content, the dried soft body samples were burned in a muffle furnace (Nabertherm B 170 GmbH, Lilienthal, Germany) at 500 °C for five hours. This process was conducted overnight to allow the samples to cool gradually. The next morning, the crucibles were removed from the furnace, placed in a desiccator to reach room temperature, and then weighed.

Once all measurements were obtained, the AFNOR (I_{AFNOR}) and Condition (CI) indices were calculated using the following formulas (*Azeredo et al., 2018*):

$$I_{AFNOR}(\%) = \frac{Wet\ muscle\ weight(g)}{Total\ weight(g)} X100 \qquad \text{(eq.1)}$$

$$CI\ (\%) = \frac{[Dry\ muscle\ weight(g) - Muscle\ ash\ weight(g)]}{Dry\ shell\ weight(g)} X100 \qquad \text{(eq.2)}$$

After the calculation of the percentages obtained from the AFNOR index, based on the I_{AFNOR}, the oysters were classified according to different commercial categories defined in Table III.

Table III - Commercial classification of oysters, according to AFNOR quality index (Azeredo et al., 2018).

AFNOR Index (%)	Classification	Algarve Classification
< 6.5	Unclassified	Normal
6.5--9	Fine	Special
>9	Special	Extra

2.6.3. Determination of oyster soft body colour

The colour of the oyster soft body was measured with a Minolta Chroma Meter 400 colorimeter (Konica Minolta, CR-400, Tokyo, Japan), following the International Commission on Illumination (CIE) Lab colour space, where L* represents the luminosity from black to white (0-100), a* indicates the variation of green to red (-60 to +60) and b* the variation of blue to yellow (-60 to +60) (Figure 7). Firstly, the equipment was calibrated against a standard white tile, and the oyster soft body colour was measured at standard illuminant C and a 2° observer, using the SpectraMagicTM NX Colour Data Software (Konica Minolta, Tokyo, Japan).

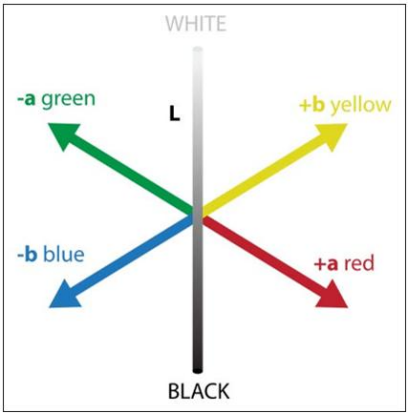

Figure 7 - Graphical representation of the CIELAB colour system, in an XYZ graph. (Source: Datacolor, 2024)

Since the oyster's soft body has a naturally slimy texture and variable shape, the collection of colour parameters was obtained by putting the soft body between two glass slides and approximating the colourimeter from those slides (Figure 8a). This approach standardises the colour surface and allows for precise colour measurements (Figure 8b). The colour of the oyster's soft body was expressed as a means of two measurements per individual oyster of each replicate system.

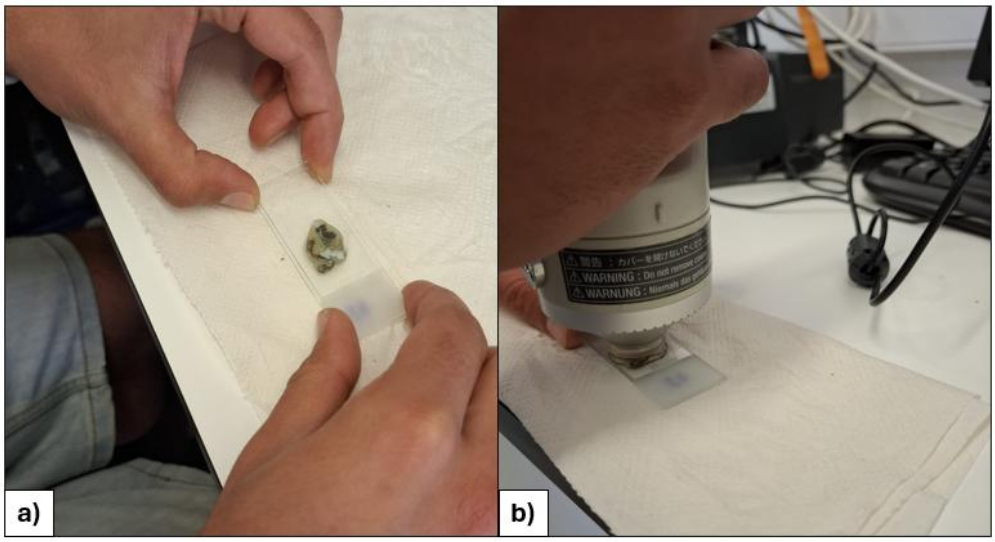

Figure 8 - Preparation to measure the colour parameters of the oyster soft body (a). Oyster colourimetric analysis, performed over the microscope slide (b).

Additionally, the total colour difference was also calculated using equation 3 and classified accordingly with the criteria defined in Table IV (Drlange, 1994).

$$Total\ Colour\ Difference(TCD) = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad \text{(eq. 3)}$$

Where:

$\Delta L = L_1 - L_2$, with L_1= L* value from 1º sample and L_2 = L* value from 2º sample.

$\Delta a = a_1 - a_2$, with a_1 = a* value from 1º sample and a_2 = a* value from 2º sample.

$\Delta b = b_1 - b_2$, with b_1 = b* value from 1º sample and b_2 = b* value from 2º sample.

Table IV - Colour difference classification criteria according to Drlange (1994*)*.

Total color difference	Classification
0.0-0.2	Imperceptible difference
0.2-0.5	Very small difference
0.5-1.5	Small difference
1.5-3.0	Distinct difference
3.0-6.0	Very distinct difference
6.0-12.0	Big difference
>12.0	Very big difference

2.6.4. Determination of oyster flesh texture parameters

The texture of the oyster's soft body was assessed using the TA. XT plusC texturometer (Stable Micro Systems, Godalming, Surrey, United Kingdom), equipped with a load cell of 5 kg, and the Texture Profile Analysis (TPA) was conducted to evaluate the mechanical and sensory-related properties of the oyster. The samples were subjected to a double compression cycle, simulating mastication, at a velocity of 5 $mm.sec^{-1}$, with a 5-second interval between compressions. The oyster soft body was placed under a 5 mm stainless spherical probe (Stable Micro Systems, Godalming, Surrey, United Kingdom), subject to a penetration distance of 2 mm, representing 80% of oyster compression. The resulting force-time curve (Figure 9), which

originated from a texture profile analysis, was used to determine several texture parameters, such as the hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness, and resilience (Table V). For the actual testing procedure, the oyster muscle was placed under the texturometer probe, and a TPA was performed (Figure 10) with the specific predefinition mentioned above.

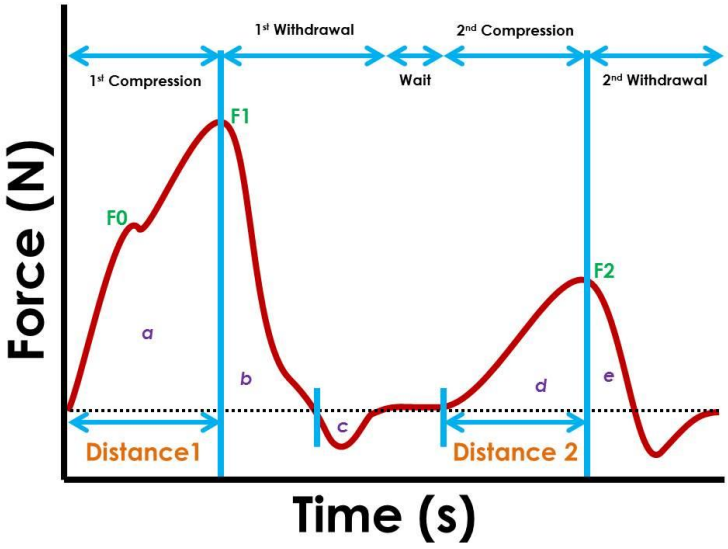

Figure 9 - Force–time curve obtained from a Texture Profile Analysis (TPA) double-compression test, illustrating the first and second compression cycles and the parameters derived from peak forces, areas under the curve, and distance measurements (Source: The Centre for Industrial Rheology, 2026).

Table V - Summary of the principal Texture Profile Analysis (TPA) parameters, including their definitions and mechanical significance as derived from the double-compression test.

Parameter	Definition
Hardness	The height of the first peak represents the maximum force required to compress the sample (Newtons or grams/force).
Resilience	This measures how well the sample recovers from the first compression. It's calculated by dividing the area under the curve after the first peak by the area under the curve before the first peak (%).
Cohesiveness	Measures how well the material breaks apart during the second bite. It is calculated as the ratio of the area under the second compression curve to the area under the first compression curve (%).
Springiness	Describes the product's ability to "spring back" after the first bite. It is often expressed as a percentage of the original height or distance (%).
Gumminess	Calculated as a product of hardness, cohesiveness, and springiness, it is a measure of the energy required to masticate semi-solid food (Newtons or grams).
Chewiness	A measure of how much work is required to chew solid food until it is ready to swallow (Newtons).

Source: Johnson, 2023

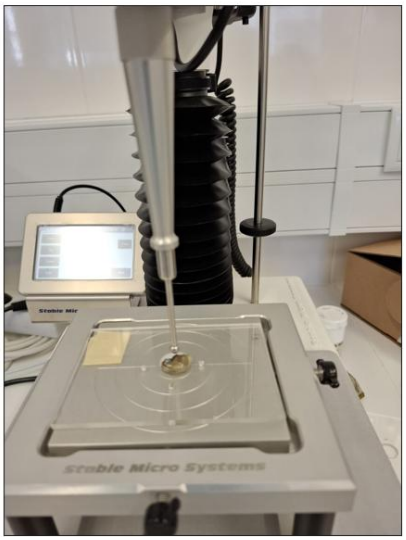

Figure 10 - Texture profile analysis (TPA) of an oyster muscle sample, using a texturometer.

2.7. Statistical Analysis

Statistical analysis was performed using IBM SPSS® Statistics 29 Windows version (IBM Corporation, New York, USA). Results were expressed as mean ± standard deviation (SD). In all cases, statistically significant differences were examined using a significance level of p - value < 0.05. Data were initially assessed for the assumptions of normal distribution, using the Shapiro-Wilk test, and for homogeneity of variances by Levene's test.

For both handling and heatwave experiments, all parameters (morphometry, quality indexes, colour, and texture) were compared between treatment groups, Control *vs* Handling and Control *vs* Heatwave, respectively, using an independent *t*-test (*t*, df, p - value). Data was also compared to verify homogeneity between groups.

Additional analysis was performed for the marine heatwave experiment to evaluate the evolution of the parameters analysed through time. For each treatment, one-way ANOVA (F, df, *p-value*) was used to compare parameters between sampling events as factor levels (S1 to S5). If ANOVA assumptions were not confirmed, the nonparametric Kruskal-Wallis test (H, df, p - value) was used. When statistically significant differences were found, multiple pairwise comparisons were performed using the post-Hoc honestly significant difference (HSD) test (p – value < 0.05).

3. Results

3.1. Oyster handling experiment

3.1.1. Oyster morphometry

The mean length (L), width (W), height (H), and weight (Wt) of *M. gigas* oysters from both treatments increased over 30 days, except for the mean length of the handled group. Regarding oyster mean length, the oysters from the control group attained the highest mean length, while the handling group showed a decrease in length. (Figure 11a). The highest mean width and mean weight were also observed in the control group (Figure 11b and d). The mean height was the only morphometric parameter that was higher in the handled group at the end of the experiment. However, when the means were compared using the *t*-test, no significant differences were noticed between treatments in any of the morphometric parameters. (**L**: $t = 1.116$, df = 87, p-value = 0.267; **W**: $t = -0.301$, df = 87, p-value = 0.764; **H**: $t = -1.040$, df = 87, p-value = 0.301; **Wt**: $t = 0.145$, df = 87, p-value = 0.885).

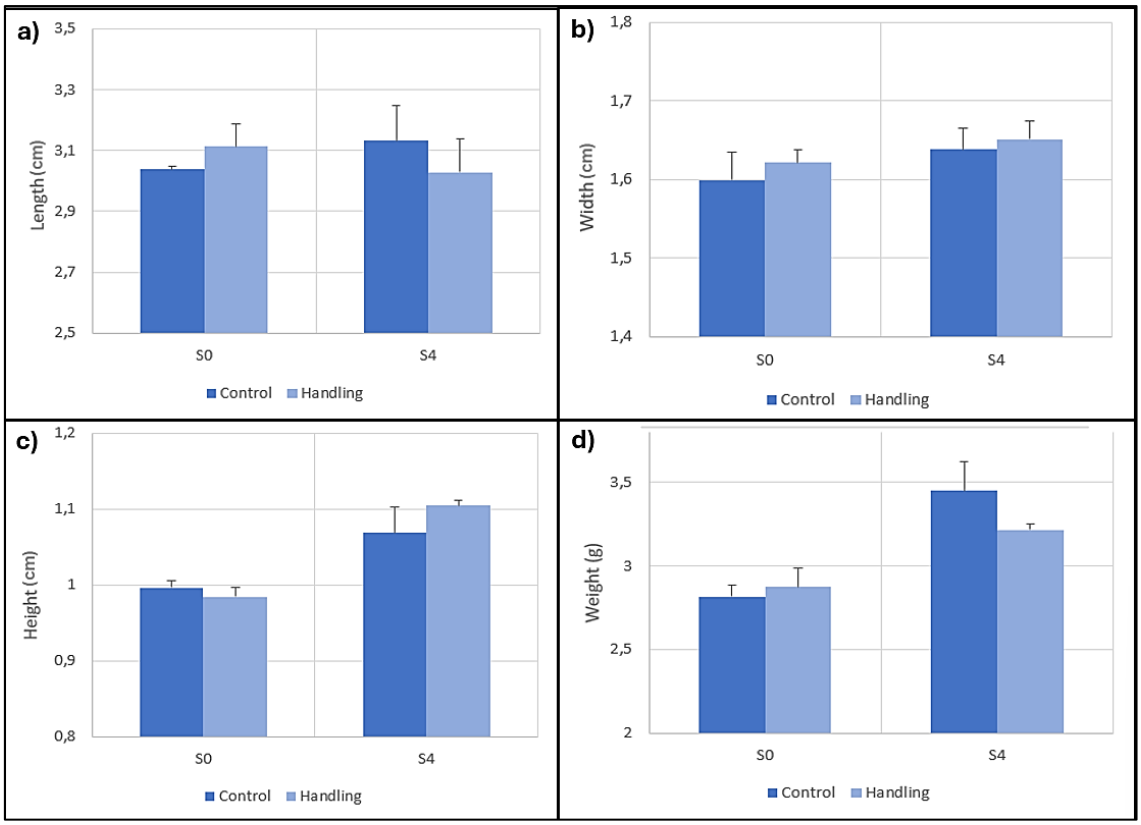

Figure 11 - Evolution of *Magallana gigas* morphometric parameters during handling experiment: (a) Mean Length (cm); (b) Mean Width (cm); (c) Mean Height (cm); (d) Mean Weight (g). Legend: S0 – beginning and S4 – end of the experiment at the fourth week. Data is expressed as mean ± SD.

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3.1.2. Condition indices

A generalised increase in the condition of *M. gigas* oysters was observed across the experiment. Analysing the condition index, it was observed that while the control (3.63 ± 0.09%) and handled (4.08 ± 0.22%) group present statistically different CI ($t = -2.302$, df = 27, p-value = 0.029) (Figure 12a), their I_{AFNOR} in the end of the experiment was similar (control: 20.17 ± 1.32%; handled: 20.01 ± 1.57%) ($t = 0.25$, df = 27, p-value = 0.804) (Figure 12a). Based on Table III, a mean value of AFNOR index superior to 9 is considered a special quality of oyster, and both treatments showed a mean higher than 20.

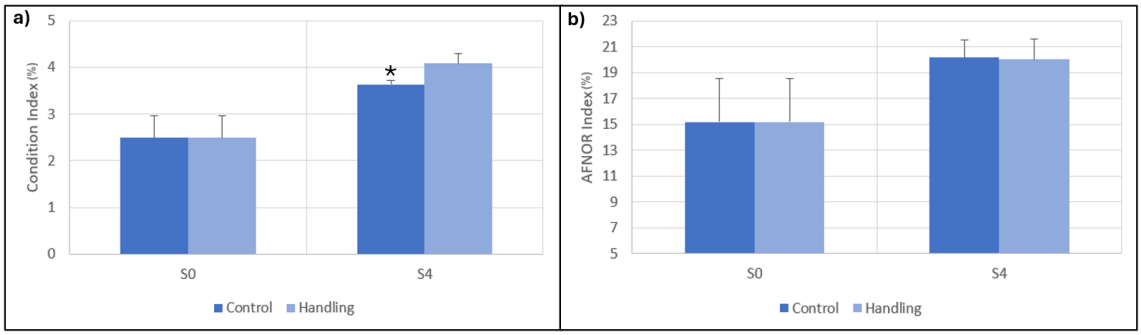

Figure 12- Evolution of *Magallana gigas* condition through the handling experiment: (a) Mean condition index of both control and handling treatments at the beginning (S0) and at the end (S4); (b) Mean AFNOR index of both control and handling treatments at the beginning (S0) and at the end (S4). Data is expressed as mean ± SD. * represents statistically significant differences between the control group and handling group.

3.1.3. Colour analysis

Table VI summarises the mean L*, a*, and b* parameters of *M. gigas* soft body obtained at the handling experiment. The b* value remained similar between groups (t = 0.319, df = 28; p-value = 0.752). On the other hand, statistical differences were observed in the luminosity (L*) and a* colour parameter, between treatments at the end of the trial (**L***:t = 2.185, df = 20.59, p-value = 0.041; **a***: t = -2.698, df = 28, p-value = 0.012). A higher L* value was observed in the control group (48.41 ± 7.90), implying a darker soft body tone in the handling group. A higher a* value was observed in the handled group (-0.22 ± 0.46), indicating less green soft body tonality than the control group.

Table VI - Mean L*, a* and b* values obtained in the colour analysis of *Magallana gigas* soft body, at day 0 (S0) and after day 30 (S4), for both treatments (Control and Handling). Data is expressed as mean ± SD. * represents statistically significant differences between the control group and handling group.

Color parameter	Treatment	Sampling	Mean Values
L* black (0) -white (100)	Control	S0	40.8 ± 4.49
		S4	48.41 ± 1.53*
	Handling	S0	40.8 ± 4.49
		S4	43.42 ± 1.43
a* green (-60) to red (+60)	Control	S0	-0.26 ± 0.48
		S4	-0.7 ± 0.15
	Handling	S0	-0.26 ± 0.48
		S4	-0.22 ± 0.08*
b* blue (-60) to yellow (+60)	Control	S0	6.73 ± 1.86
		S4	6.27 ± 0.73
	Handling	S0	6.73 ± 1.86
		S4	6.03 ± 1.03

The total colour difference calculated from the L*, a*, and b* values was 5.02. According to the colour difference classification criteria by Drlange (1994), this corresponds to a very distinct difference. The representative colours of each treatment at the end of the trial, obtained from the L*, a*, and b* values, are represented in Figure 13.

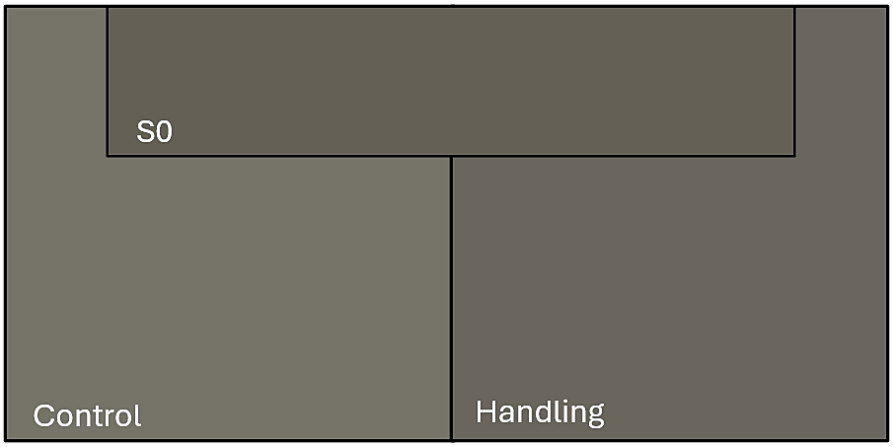

Figure 13 - On the left side, the representative image of the colourimetry values of the control treatment (L*: 48.41; a*: -0.7; b*: 6.7). On the right side, the colour corresponding to the mean colourimetry values of the handled treatment (L*: 43.42; a*: -0.22; b*: 6.03). On top, the colour corresponding to the mean colourimetry values of the initial sampling (S0), serving as a baseline for both treatments (L*: 40.8; a*: -0.26; b*: 6.73). All colours were transcribed using the CIELAB colour space.

3.1.4. Texture analysis

After the end of the trial, it was noticed a generalised decrease in hardness, resilience, gumminess, and chewiness on the oysters' soft body of both treatments, although more marked in the handling group (Figure 14). Cohesion and springiness also had a slight decrease during the 30-day trial. When the mean parameters of the texture profile were compared between treatments, no statistical differences were recognized in any of the parameters measured (**Hardness:** t = -0.071, df = 25, p-value = 0.944; **Resilience:** t = -0.576, df = 25, p-value > 0.576; **Cohesion:** t = 0.634, df = 17.347, p-value = 0.535; **Springiness:** t = 0.607, df = 14.824, p-value = 0.553; **Gumminess:** t = 0.390, df = 25, p-value = 0.700, **Chewiness:** t = 0.898, df = 25, p-value = 0.378).

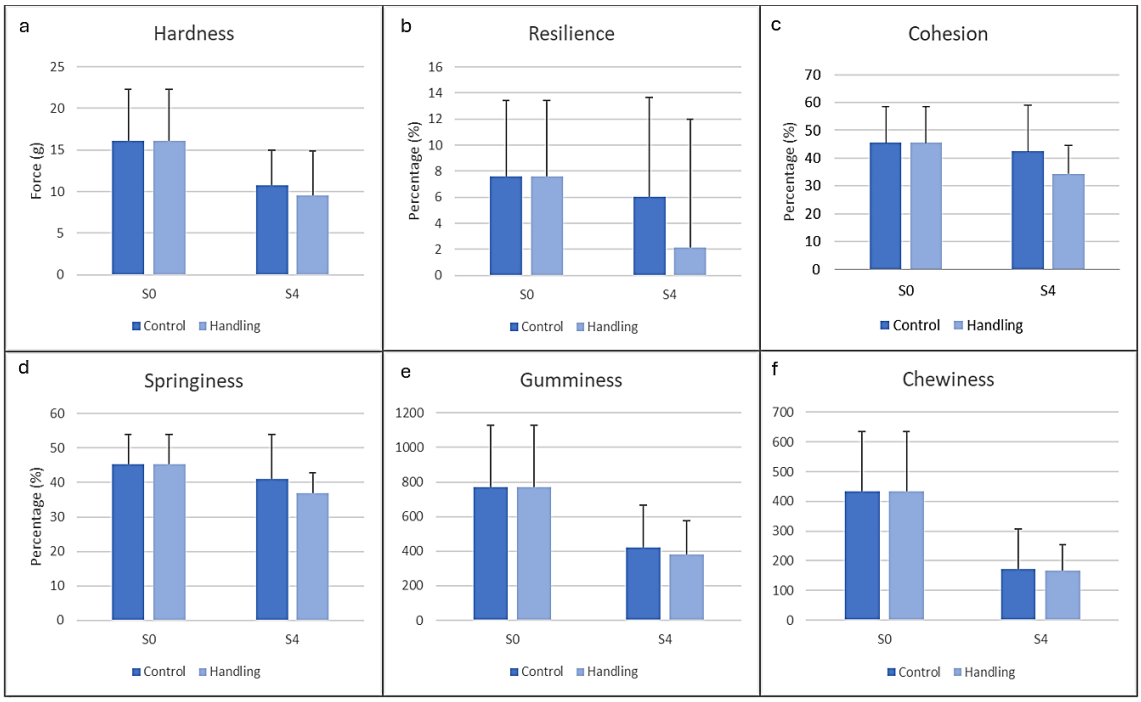

Figure 14 - Texture parameters obtained and calculated from the texture analysis profile, obtained on the soft body of *Magallana gigas* from both control and handling treatments at the beginning of the trial (S0) and at the end, on day 30 (S4): (a) Hardness (g); (b) Resilience (%); (c) Cohesion (%); (d) Springiness (%); (e) Gumminess; (f) Chewiness. Data is expressed as mean ± SD.

3.2. Marine heatwave experiment

3.2.1. Oyster morphometry

During the heatwave experiment, the mean length of oysters of both groups (control and heatwave) did not change across sampling events (Figure 15a), with no statistical differences found in either treatment throughout the trial course (**Control:** F = 0.344, df = 5, *p*-value = 0.886; **Heatwave**: *F* = 0.190, df = 5, *p*-value = 0.966).

Regarding the oyster width, while no changes were observed in the control group throughout the experiment (*F* = 1.034, df = 5, *p*-value = 0.397), the width of the oysters subject to heatwave conditions increased with increasing temperature (*F* = 4.450, df = 5, *p*-value = 0.001). At S4, the oysters subject to heatwave are significantly larger than in S1 (Figure 15b). When comparing the control group with the heatwave group at each sampling, the control group presented a higher mean width (21 ± 2 mm) than the heatwave group (20.2 ± 2 mm) in S1 (*t* = 2.832, df = 219, *p*-value = 0.005).

Concerning the oyster height, the opposite trend was observed. The height of the control group increased with the progression of the experiment (*F* = 2.532, df = 5, *p*-value = 0.028), with the oysters of first sampling (S1) being significantly smaller than those measured in S3, S4, and S5. On the other hand, the mean height of oysters of the heatwave treatment showed no statistical difference when compared across the experiment (*F* = 0.424, df = 5, *p*-value = 0.832). These differences in progression trends between control and heatwave treatments resulted in statistically significant differences between the control group and heatwave group in S5, the control group presented a higher height (12.9 ± 1.4 mm) than the heatwave group (12.2 ± 1.6 mm) (*t* = 2.418, df = 99, *p*-value = 0.017) (Figure 15c).

In relation to oysters' weight, a progressive increase was noticed in both treatments' mean weight throughout the trial. A larger increase was noticed in the control treatment, with the oysters analysed in S5 being significantly heavier than oysters at S1 and S2 (*F* = 7.394, df = 5, *p*-value<0.001).

As for the heatwave treatment, the largest mean weight was found on S5, and differences were found when compared with S1 and S2 (F = 2.847, df = 5, p-value = 0.015). Oysters' mean weight obtained in the other sampling points (S1, S2, S3, and S4) were statistically identical to the mean weight observed in S5. However, when comparing the mean weight between the control and heatwave group at each specific sampling point, no statistical differences were observed.

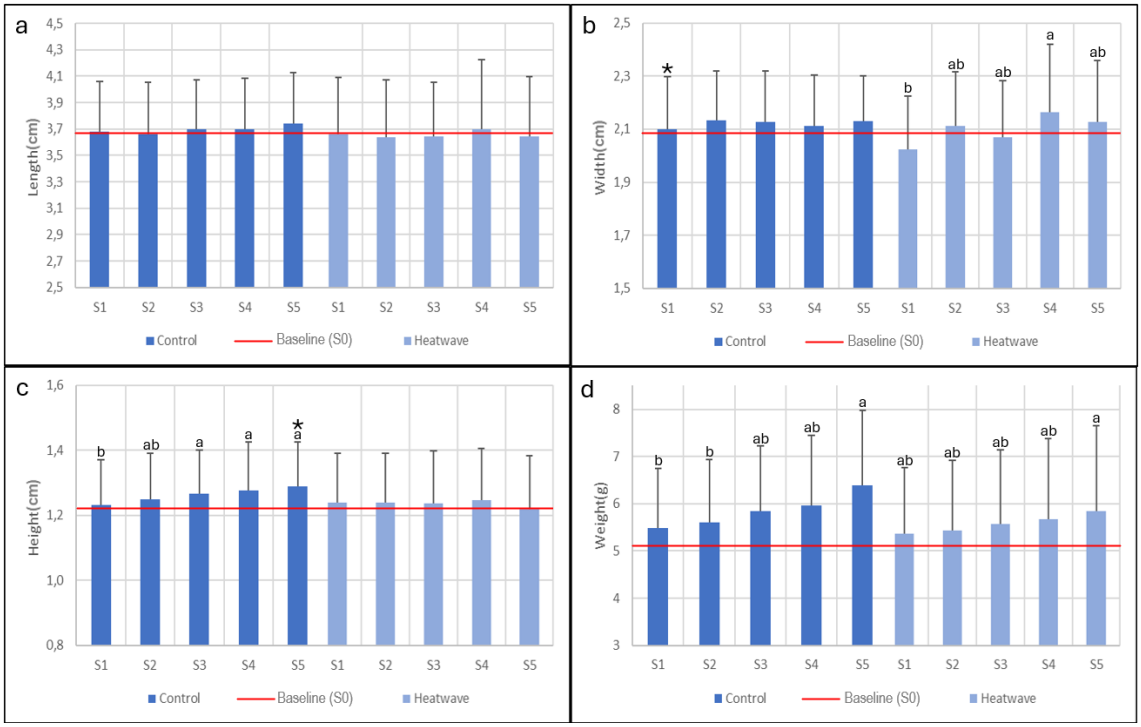

Figure 15 - Mean length (a, in cm), mean width (b, in cm), mean height (c, in cm) and mean weight (d, in g) of *Magallana gigas* oysters of control and marine heatwave groups during their course (S1 to S5) of the handling experiment. The red line refers to the mean baseline parameter obtained at S0, identical for both treatments. Data is expressed as mean ± SD. The subscripts ([a]) and ([b]) indicate statistically significant differences in the control group and heatwave group between sampling events; * indicates statistically significant differences between the control group and marine heatwave group.

3.2.2. Oysters condition

Both the indices used to follow the oyster's condition showed a decreasing trend across the heatwave experiment (Figure 16). Namely, the CI of both control and heatwave presented statistically significant differences along the sampling events (**control:** F = 4.215, df = 4, p-value = 0.004; **heatwave**: H = 4.962, df = 4, p-value = 0.001). The control group CI in S1 was 2.71 ± 0.68% and decreased in S5 to 2.44 ± 0.29%, while in the heatwave group, a more significant decrease was observed, decreasing from 3.12 ± 0.90% (S1) to 2.33 ± 0.43%. On the other hand, when comparing the mean I_{AFNOR} of each treatment throughout time (Figure 16b), there was no evident reduction of condition in the control group (H = 4.962, df = 4, p-value = 0.001). While in the heatwave group, in S1, the oysters presented a significantly higher I_{AFNOR} (16.77 ± 2.81%) than those in S5 (11.91 ± 1.72 %) (F = 5.722, df = 4, p-value<0.01).

When comparing the control group with the heatwave group in each sampling event, statistical differences between the control and exposed groups were noticed in S1, where the control group presented a higher mean CI (2.71 ± 0.68%) in relation to the heatwave group (3.12 ± 0.90%) (t = -2.448, df = 24.56, p-value = 0.024). In return, the oyster I_{AFNOR} index showed an increasing trend without significant differences between control and heatwave groups (Figure 16b). Only in S5, the control group presented a higher mean I_{AFNOR} (16.71 ± 1.39%) than the heatwave group (11.91 ± 1.72%) (t = 7.935, df = 27, p-value < 0.001).

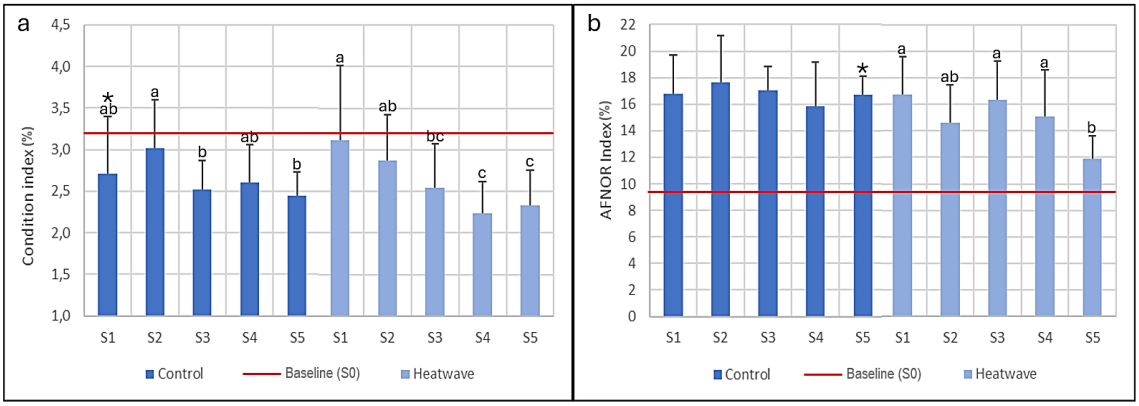

Figure 16 – *Magallana gigas* oysters mean Condition Index (a, in %) and mean AFNOR index (b, in %) of both control and marine heatwave groups (b) during the marine heatwave experiment (S0 to S5): (B) Mean AFNOR index of both treatments of the heatwave experiment during its course (S0-S5). The red line refers to the mean baseline parameter obtained at S0, identical for both treatments. Data is expressed as mean ± SD. The subscripts [a] and [b] indicate statistically significant differences in the control group and heatwave group between sampling events; * represents statistically significant differences between the control group and handling group.

3.2.3. Colour analysis

When analysing the colour parameters of the control treatment over time, statistically significant differences were found in L*, a*, and b* (Table VII). The lowest L*(Luminosity) values were registered on S2 (43.90 ± 4.61), being significantly lower than S1, S3, S4, and S5 show no differences when compared (H = 15.923, df = 4, p-value = 0.004). In the remaining samplings, the mean L* values were similar. On the a* colour parameter (green to red), mean values fluctuated through sampling, although always remaining negative. The highest mean value was registered in S1(-0.14 ± 0.62) and S4 (-0.14 ± 0.79). Both these values show statistical differences when compared to S2 and S3 (F = 5.635, df = 4, p-value < 0.01), with the lowest mean values registered through the trial (-0.68 ± 0.63; -0.37 ± 0.71). As for the b* colour parameter (blue to yellow), the highest value registered was observed on S5 (8.12 ± 2.08), which shows statistically significant differences to any of the remaining average b* across the experiment (F = 9.787, df = 4, p-value < 0.01). Although differences were registered between samplings, all values registered were positive side of the b* axis (Yellow).

When comparing the mean colorimetry values of the heatwave treatment, no statistically significant differences were found when comparing separately mean L* and mean a*, over time (**L***: H = 3.094, df = 4, p-value = 0.61; **a***: H = 6.171, df = 4, p-value = 0.07), However, statistically significant differences were observed in the mean b* parameter across experiment (H = 5.979, df = 4, p-value < 0.01). Highlighting S5 as the sampling point with the highest b* (8.12 ± 2.08), which shows statistical differences, specifically to S3, the lowest mean value registered (6.25 ± 2.45).

Table VII - L* (luminosity: black (0) -white (100)), a* (green (-60) to red (+60)) and b*(blue (-60) to yellow (+60)) values obtained in the colour analysis performed on *M. gigas* soft body, across marine heatwave experiment (S1-S5), for both treatments (Control and Heatwave).Baseline refer to the mean value obtained at S0, identical for both treatments. Data is expressed as Mean ± SD. The subscripts [a] and [b] indicate statistically significant differences in the control group and heatwave group between sampling events; * represents statistically significant differences between the control group and handling group.

Treatment	Sampling	Colour parameter		
		L* black (0) - white (100)	a* green (-60) to red (+60)	b* blue (-60) to yellow (+60)
Both treatments	Baseline	42.82 ± 3.36	0.28 ± 0.61	4.48 ± 1.54
Control	S1	47.55 ± 9.14[a]	-0.14 ±0.62[a]	6.44 ± 2.68[b]
	S2	43.90 ± 4.61*[b]	-0.68 ± 0.63[b]	5.48 ± 1.96[b]
	S3	47.25 ± 2.73*[a]	-0.37 ± 0.71[b]	6.25 ± 2.45*[b]
	S4	46.35 ± 3.05[a]	-0.14 ± 0.79*[a]	6.63 ±2.02[b]
	S5	46.55 ± 4.65[a]	-0.36 ± 0.63[ab]	8.12 ± 2.08[a]
Heatwave	S1	45.84 ± 4.97	-0.41 ± 0.74	6.57 ± 3.17[ab]
	S2	45.74 ± 5.81	-0.28 ± 0.85	6.38 ± 2.00[ab]
	S3	45.93 ± 1.75	-0.63 ± 0.37	4.34 ± 1.12[b]
	S4	45.48 ± 4.01	-0.47 ± 0.65	6.59 ± 2. 67[ab]
	S5	45.66 ± 3.7	-0.04 ± 0.64	9.14 ± 2.65[a]

Throughout the trial, the brightness parameter L* did not show significant variation between control and heatwave groups. However, punctual statistically significant differences were identified in S2 and S3. In S2, the heatwave group was significantly brighter than the control group (t = -2.893, df = 58, p-value = 0.005). In S3, the control group was significantly brighter than the heatwave group (t = 2.584, df = 58, p-value = 0.012).

Regarding the a* colour parameter, this tended to be negative for the two groups from week S1 until the end of the trial, indicating that oyster flesh was becoming greener throughout the experiment. This was particularly evident in S4, when the oyster group subjected to a heatwave presented a greener value (negative a* value) than the control group (t = 2.584, df = 58, p-value = 0.012).

As for the b* parameter, inconsistent shifts were found from sampling point to sampling point, on both treatments. Statistically significant differences were identified in S3, with the control group presenting a higher b* parameter than the heatwave group (t = -2.893, df = 40.699, p-value = 0.006), indicating a yellower tonality.

After the experiment, the total colour difference was calculated (Table VIII), and a certain level of difference was found in all samplings between the two treatments (Figure 17). Apart from S2, which was classified as a “Very distinct difference”, all other samplings (S1, S3, S4, and S5) were classified as a “Distinct difference”.

Table VIII - Total colour difference (TCD) between both treatments at the different sample points (S1-S5).

Sampling	TCD	Classification
S1	2.81	Distinct difference
S2	4.02	Very distinct difference
S3	1.90	Distinct difference
S4	1.73	Distinct difference
S5	1.88	Distinct difference

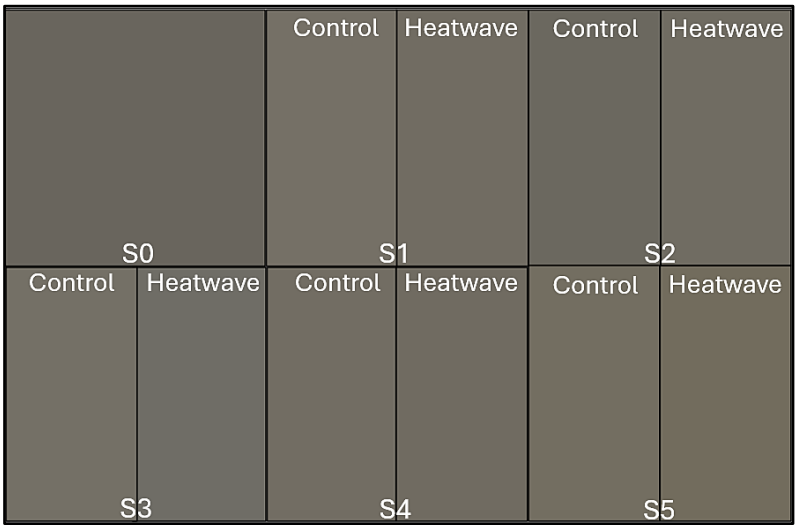

Figure 17 - Representative image of the mean colour parameters of each treatment (control and heatwave), at sampling points S0, S1, S2, S3, S4 and S5. All colours were transcribed using the CIELAB Colour space.

3.2.4. Texture profile analysis

For the TPA, when comparing the sampling points of control treatment across the experiment, no statistically significant differences were observed in the control treatments' mean hardness (F = 1,864, df = 4, p-value = 0.120; Figure 18a). When comparing the mean resilience values of the control group obtained at different sampling points, statistically significant differences were found (Fig 18b; F = 3.237, df = 4, p-value = 0.014). The lowest mean value was obtained at S4 (22.04 ± 6.99%), and when compared with the highest mean value recorded at S3 or S2, the second highest mean value (26.03 ± 8.21%, 25.61 ± 6.03%, respectively), which also shows no statistical significance when compared. In relation to the cohesion or springiness values of the control group through time (Figure 18c and d), no statistically significant differences were found between (cohesion: F = 1.392, df = 4, p-value = 0.240 & springiness: H = 2.881, df = 4, p-value = 0.120). The gumminess values of control showed statistically significant differences when comparing the multiple sampling points across the trial (Figure 18e; F = 2.688, df = 4, p-value = 0.034). The lowest mean values were recorded at the end of the trial, in S5 (758.50 ± 238.26). This value shows no statistical differences when compared to S3 and S4. The highest mean value was recorded in S2 (1037.19 ± 258.80), and when compared to S4 or S5, statistically significant differences are observed (P-value < 0.01). Regarding the mean chewiness values (Figure 18f), no statistically significant differences were observed when comparing the sampling points of the control treatment (F = 1.878, df = 4, p-value = 0.117).

As for the heatwave treatment, the mean values of S2 and S3 were the highest values recorded and show statistical differences when compared to S4, the lowest value observed (H = 4.856, df = 4, p-value = 0.015). As for the heatwave treatment resilience, no statistically significant differences were detected when comparing sampling points (F = 3.351, df = 4, p-value = 0.012). In relation to the cohesion or springiness values of the heatwave group through time (Figure 18c and d), no statistically significant differences were found between samplings (cohesion: F = 4.380, df = 4, p-value = 0.059; springiness: H = 4.467, df = 4, p-value = 0.205). The lowest gumminess value recorded was on S4 (651.52 ± 256.10), followed by S5, which shows no significant differences when compared to each other. The highest value recorded was observed in S2 (1057.47 ± 361.61), when this sampling is compared to all samplings of this treatment, but statistically significant differences are found when compared to S4 or S5 (H = 3.489, df = 4, p-value < 0.001). Regarding chewiness, the highest mean value observed was found on S2 (627.55 ± 234.35). When S2 is compared to the last two samplings performed, S4 and S5, statistically significant differences were found (F = 3.351, df = 4, p-value = 0.012). The lowest value registered

was S5 (379.45 ± 246.78), but it only presented statistical differences to S2, and similarities to all other sampling points (S1, S3, and S4).

During this trial, a tendency for lower mean texture was noted in the heatwave treatments, when comparing treatments (Figure 18c, e, and f). However, in most of these cases, they did not present statistically significant differences. On the samplings before the increase in temperature (S0 and S1), no statistical differences were observed in any of the texture parameters between the control and MHW group. On S2, the sampling performed at the end of the temperature rise, statistical differences were observed in Cohesion between the two treatments (t = 2.102, df = 58, p-value = 0.040). At the end of the heatwave period (S3), statistically significant differences were found in springiness when comparing treatments (t = 2.595, df = 58, p-value = 0.012). At the sampling performed at the end of the temperature decrease of the heatwave group (S4), statistical differences were identified between treatments in mean gumminess values (t = 2.206, df = 58, p-value = 0.031). On the sampling, when the trial finished (S5), Statistical differences were observed in mean springiness values between treatments (t = 2.668, df = 58, p-value = 0.010).

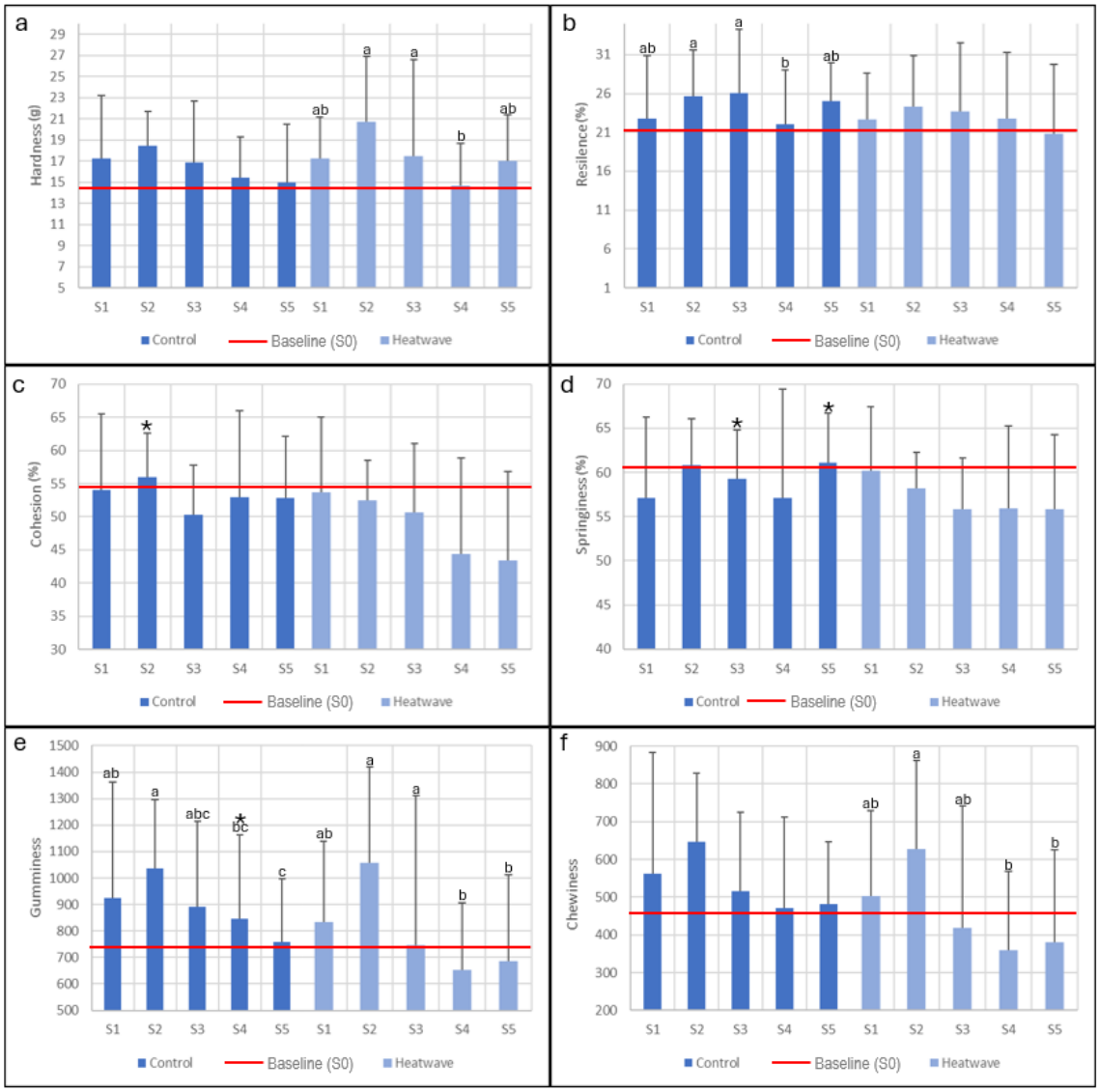

Figure 18-Texture analysis of the soft body of *Magallana gigas* oysters subject to the marine heatwave experiment during six weeks: hardness (a, g); resilience (b, %); cohesion (c, %); springiness (d, %); gumminess (e); chewiness (f). The red line refers to the mean baseline parameter obtained at S0, identical for both treatments. Data is expressed as mean ± SD. The letters a, b, and c represent statistically significant differences between the different sampling points of each treatment, * represents statistically significant differences between the control group and handling group.

4. Discussion

4.1. Oyster Handling Experiment

The main objective of this experiment was to evaluate the effects of a weekly routine of desiccation periods and light tumbling (handling) on the growth and conditioning of *M. gigas,* in comparison to an undisturbed grow-out strategy (control). Results indicated an increase in condition index in the handling treatment, and colour differences, specifically a decrease in luminosity (L*) and an increase in a* parameter towards green colour (green to red axis).

In a three months experiment, Roros (2025) concluded that handling practices, more specifically thumbling and tidal air exposure (desiccation) periods, led to better shaped oysters and higher growth rates. When oysters were grown in submerged and undisturbed conditions, shell growth was more brittle and uneven, and wet meat content was lower. These results were corroborated by Chuku et al. (2025), who found positive correlations between desiccation periods and condition index in a 350-day experiment using the same species. They also found a positive influence in shell quality related to gentle handling techniques, but if done excessively, shell integrity can be hindered. Both these studies used the same species as this experiment, with similar sizes. However, both these studies were performed in the field (on estuarine oyster farms).

In this trial, no statistical differences were found in morphometrics between treatments, although a smaller length and weight were noticed in the handling group, on the other hand, height and width increased. This pattern suggests that the handling group was developing a more cupped shell shape. The decrease in length is related to the initial trimming, during which a large portion of the youngest (and thinnest) shell growth was removed, also explaining the smaller weight increase in weight compared with the control group. The present study only lasted 30 days, due to this substantial difference in duration, only tendencies in growth comparable to the results reported in the other studies (Chuku et al., 2025; Roros, 2025) were observed. If our experiment had continued for a longer period, it was likely that more pronounced differences would be found.

Regarding condition indices, improvements were observed in both treatments for both indices. However, only CI showed significant differences between the handled and control groups. The handling treatment presented a higher CI in relation to the control group (fully submerged and undisturbed). These results are corroborated by Chuku et al. (2025), although they mainly attribute this increase to desiccation periods, they did not find any correlation with thumbling practices. For the AFNOR index, an improvement was observed in both treatments during the trial, although no differences were identified between treatments. However, a mean AFNOR value superior to 9 is already considered a special quality of oyster (Azeredo et al., 2018), and both treatments showed mean values above 20.

In the colour analysis, differences were detected between treatments for L* and a* colour parameters. Luminosity (L*) increased in the control treatment, indicating a lighter flesh tone. In the handling treatment, a less green flesh tone was noticed in the heatwave treatment represented by the higher a* values. In oysters, colour evaluation is usually applied to assess shelf-life degradation in pre-shucked (opened) oysters (Liu et al., 2025), and a clear correlation between the flesh colour of freshly opened oysters and their quality is yet to be established. Several studies reported correlations between genetics and mantle pigmentation (Vu et al., 2020; Xing et al., 2018; Zhu et al., 2023), implying that oyster flesh colour is influenced by a combination of multiple biotic and abiotic factors, such as heritability, health, water conditions, food type and availability.

As for the total colour difference, the values obtained when both treatments were compared they classified as a “very distinct difference”. This difference is clearly visible when the mean values of each treatment are converted into their respective colour (Figure 13). It is important to note that both Colours present a tonality closer to grey than to normal oyster soft body colour, known to range from white to light green (van Houcke et al., 2015). This can be due to the size of the oysters analysed, as the colourimeter may have captured mantle areas with higher pigmentation, leading to the colours observed. When considering statistical analysis, the visual colour difference between treatments further reinforces the results, as a lighter and greener

tonality is consistently observed in the control treatment, both in statistical and visual assessments.

In the texture profile, no statistical differences were detected, but a considerable decrease in mean resilience was noted in the handling treatment. However, the standard deviation is considerably high, making it difficult to obtain a demonstrable difference. Overall, both groups had a decrease in all parameters, but the handling group tended to lower mean values. Given the absence of statistical differences, no firm conclusions can be taken from this analysis. For posterior trials, a longer experiment period and a larger sample size should be considered, allowing a more pronounced impact of treatments on texture. Nevertheless, texture plays a key role in consumer preference and perceived quality, as oysters that offer a slight resistance to the first bite, yet a cohesive meat texture are generally preferred (Lemasson et al., 2017). A deeper understanding of how handling affects texture is therefore critical to ensure higher quality oysters that meet customers' consumer preferences.

In summary, handling practices mainly led to an increase in condition index and to a darker, less green soft body colour in *M. gigas* oysters. The effects on the condition index are corroborated by Chuku et al. study (2025). Whereas a lack of research about the impacts of handling effects on the colour of the oyster's soft body makes it difficult to contextualise the present findings. Several tendencies were noticed in morphometrics and texture, but a longer experiment would likely be required for a statistically significant difference to be noticed.

4.2. Marine heatwave experiment

The simulation on marine heatwave results show the effect of heatwave events in morphometrics, more specifically, a decrease in weight and height growth in the heatwave treatment, and in the condition of *M. gigas* oysters (decreased condition and AFNOR index). Additionally, different colourimetric patterns were observed in the L* and a* colour parameters, alongside distinct colour differences between treatments. The texture profile analysis showed that the exposure to a marine heatwave affects most parameters differently, but a general tendency for lower texture values is observed in the heatwave treatment.

According to several studies where different populations and heatwave profiles (simulated and natural) are analysed, marine heatwave events are identified as a significant stress factor for *M. gigas*, resulting in an immediate physiological disruption and long-term effects on performance and survival (Masanja et al., 2023; Funesto et al., 2023). Elevated temperatures lead to a higher metabolic demand, and thereby the depletion of energy reserves caused by a higher energy requirement, limiting the energy directed to growth and maintenance (Sokolova et al., 2012). Heat stress also promotes oxidative stress through the accumulation of reactive oxygen and lipid peroxidation, at the cellular level, triggering antioxidant defence mechanisms (Xing et al., 2023; Feng et al., 2025). Additionally, thermal stress triggers molecular pathways associated with stress response, including the upregulation of heat shock proteins, with evidence suggesting that effects persist after these heatwave events (Mackenzie et al., 2025; Meistertzheim et al., 2007). Overall, exposure to extreme temperatures can hinder physiological performance, alter metabolic processes, and reduce overall fitness, reducing the physiologic condition of oysters.

In this trial, morphometric differences were found between control and heatwave treatments, more specifically in width and height. The mean width values of the control treatment remained stable throughout the trial, increasing slightly. However, the heatwave treatment presented an irregular pattern, sequentially increasing and decreasing. Concomitantly, mean height progressively increased in the control treatment but slightly decreased in the last sampling.

Since oysters cannot lose shell mass solely due to temperature stress, this odd pattern can be explained by a small sample size and a large width variation within groups. Nevertheless, at the end of the trial, differences were observed between treatments, specifically a higher mean width and height in the control group.

When looking at the mean weight values, even though differences were not found between treatments, both showed a progressive increase in weight over the course of the trial. However, this increase was slightly more noticeable in the control treatment.

Given all that, it is possible to state that marine heatwave events may affect shell growth and may also influence weight growth. Several studies link this decrease in growth with the allocation of energy sources, as a way to combat heat stress (Mackenzie et al., 2025; Sokolova et al., 2012). As energy reserves are channelled to heat stress defence mechanisms, growth is stunted or heavily decreased (Funesto et al., 2023). However, the results found at heatwave treatment mean width are inconclusive, as it is not possible for oysters to lose shell growth unless they are physically damaged. As for the absence of growth in height and slower weight development found in the heatwave treatment is a clear representation of the impact created by marine heatwave events.

When looking at the condition index, both treatments showed a decreasing trend across the trial, both ending up with a mean value considerably lower than the one registered at baseline sampling. When comparing treatments, statistical differences were only found in the sampling performed before the increase in temperature. At a stable temperature within their optimal range, it would be expected for the control treatment to maintain or increase their conditioning. This indicates the possibility of a short acclimation period or the existence of an external stressor hindering their conditioning. Nevertheless, the results obtained for the Heatwave treatment are corroborated by De Marco et al. (2023). In this laboratory study, oysters of the same size were used, but the simulated heatwave profile was longer (30 days) and 2ºC higher (20ºC to 28ºC) than the one used by us (18ºC to 24ºC), with a more sudden increase of 0.5ºC per hour. However, they attributed this decrease in the condition index mainly to the decrease in soft tissue weight, caused by the consumption of the energy reserves. Although the marine heatwave in their studies lasted 30 days, and ours only lasted 10 days, the same tendencies were observed. This indicates that heat stress has a fast first impact on conditioning, which has been found to remain even after the end of the heatwave (Mackenzie et al., 2025), as our findings suggest.

As for the AFNOR index, both treatments increased significantly during the first 15 days of trial (baseline to S1), almost doubling their initial value. After the MHW temperature reached its peak (S2), control treatment remained stable throughout the trial, but heatwave treatment presented a decrease in condition at the end of the trial. When comparing treatments at each sampling point, the same difference is found, whereas the heatwave treatment presents a lower AFNOR index value at the end of the trial. Even considering the decrease found in the heatwave treatment, when grading oysters according to their AFNOR value, they started the trial with a value already superior to 9. This value is the minimum for the criteria needed for their quality to be considered “Special”, and during the rest of the trial, the mean values in both treatments never went below 11. A “Special” grade oyster is categorised as an oyster with a higher meat content, being considerably more desired in the market (Azeredo et al., 2018). Even though both treatments never stopped meeting the requirements for “Special” grade, a progressive decrease in AFNOR index was noted in the heatwave treatment, and if the trial or heat wave were to continue for longer, it was likely that this tendency would continue. As in the condition index, the decrease in AFNOR index can be explained by the loss of soft tissue weight to heat stress response mechanisms (De Marco et al., 2023; Xing et al., 2023), since this Index is also proportional to the soft tissue wet weight.

Regarding the oyster flesh colour, differences were obtained in colour in both treatments. In the control treatment, luminosity (L*) varies throughout the trial. But only at the end of the temperature rise, the control group (S2) presented statistical differences, with the lowest mean value registered, besides the baseline mean value. As for heatwave treatment, after an increase from baseline to S1 (acclimation period at 18ºC), mean Luminosity (L*) values remained stable throughout the trial. When treatments are compared, differences were found at the end of the temperature increase (S2) and at the end of the heatwave period (S3). Whereas the control treatment presented a darker flesh tone (Lower L* values) in S2, and a lighter tone in S3, due to

an increase between samplings. However, it is important to note that during the experiment, the heatwave treatment mean luminosity values never shifted, only the control group did.

When analysing the red to green colour parameters (a*), a more considerable variation was found in both treatments. However, no differences were found in the heatwave treatment treatments a* values, even though variability was observed throughout the experiment. As for the control treatment, surprisingly, the highest mean a* value was observed twice in S1 and S4. Differences were observed when compared to S2 and S3, with the lowest a* values registered. Regardless of differences, besides baseline sampling, all a* values registered were negative. This indicates a green flesh tone over the course of the trial in both treatments. When comparing treatments, differences are found at the end of the temperature decrease (S4), where a greener flesh tone was observed in the heatwave treatment. Even though the highest value registered on this sampling was in the control treatment, its value is closer to 0, which indicates a tonality closer to grey but still green.

As for the b* colour parameters (yellow to blue), differences were found through the course of the trial, between treatments and samplings. Both treatments showed an increase in b* values throughout the trial, even though some fluctuations were observed. Specifically, a decrease in the heatwave treatment at the end of its respective heatwave period. When comparing treatments, a higher mean value was observed in the heatwave treatment at the end of the trial. However, significance was not enough to consider this difference statistically relevant. The only difference found between treatments was at the end of the heatwave period (S3), where a lower b* value was observed in the heatwave treatment. Nevertheless, all values registered at all samplings were on the positive side of the b* axis, indicating a yellow flesh tone in both treatments over the course of the trial.

When looking at the total colour difference, the only sampling point where a higher difference level than “Distinct difference” was found was in S2. In the sampling performed at the end of the temperature rise, a “Very distinct difference” was found between treatments. When looking at the Colour representations, displayed in Figure 17, differences are observable between treatments. However, when comparing treatments L*, a*, and b* values alongside their representative Colour, only an observable difference in Luminosity is distinct to the eye.

Once again, colour analysis has only been applied in oysters to assess post-harvest quality and shelf-life degradation (Liu et al., 2025). In this experiment, it was applied to evaluate the physiological response to heatwave stress. Distinct colourimetric patterns were observed between treatments, specifically in L* and b* values. These differences may be the result of physiological changes, such as changes in mantle pigmentation or tissue structure, related to thermal stress response mechanisms (Jiang et al., 2024). As referred above, previous studies found a correlation between tissue Colourimetry values and changes in structural and biochemical composition (Liu et al., 2025; Li et al., 2022). Although these studies focused on lightly processed oysters (pre-shucked and packed oyster flesh), they support the interpretation that colour variation reflects underlying tissue changes.

To our knowledge, this is one of the first studies applying colour analysis to compare conditioning in *M. gigas*, using soft tissues. The stability presented in the heatwave Luminosity values (L*) contrasts with the variability found in the control treatment, indicating a possible different physiological adaptation to different temperature scenarios. However, the absence of direct biochemical measurements, linked to the colour analysis performed, limits the ability to attribute observed differences to specific physiological mechanisms. Nevertheless, these findings suggest a possible use for soft tissue colour analysis as a tool to detect subtle physiological differences caused by temperature shifts. However, for a more concise validation and expansion on these findings, further studies integrating colour and biochemical data are needed.

In the texture profile, fluctuations were observed in several parameters in both treatments across several texture parameters. Overall, a tendency for lower mean values was observed in the heatwave treatment, more specifically in cohesion, springiness and chewiness. Most of these

differences were not statistically relevant, indicating that temperature has subtle parameter-specific effects on the texture profile.

When looking at the parameters separately, a relatively stable pattern was observed in the hardness values in the control treatment, with no differences found over the course of the trial. At the end of the temperature rise (S2) and Heatwave period (S3), the heatwave treatment had the highest hardness values recorded. At the end of the temperature decrease (S4), the lowest mean values were found in the heatwave treatment. This suggests the occurrence of a transient response to temperature variations that influences hardness during a brief period of time. Regarding resilience, the control treatment presented variations throughout the trial, whereas the heatwave treatment presented no significant differences.

For cohesion and springiness, a relatively stable pattern was observed within treatment through the trial. However, it is important to note that in the heatwave treatment, a progressive decrease in the mean value of these parameters was observed, but the standard deviation was too large for significance to be found. When comparing treatments, in cohesion differences were found at the end of the temperature rise (S2), where the control treatment had higher cohesion. In springiness, differences were found between treatments at the end of the heatwave period (S3) and at the end of the trial (S5). On all occasions, the mean values recorded in the control treatment were higher, suggesting a negative impact on cohesion and springiness parameters related to short-term thermal stress.

In the last two parameters, gumminess and chewiness, a pronounced variation was observed during the trial in both treatments. The peak values within treatments were observed at S2, sampling performed at the end of the temperature rise. A decrease was noticed towards the end of the trial for the two parameters in both treatments, but this pattern led to a more considerable decrease in the heatwave treatment. Significant differences between treatments were only found in gumminess, where the control treatment had a higher mean value, reinforcing the idea that thermal stress may negatively affect texture profiles. Additionally, in the heatwave treatment, the 15-day period was temperature returned to 18ºC (S5), a slight improvement was noted in both parameters, alongside similar patterns. These similarities were expected as gumminess and chewiness are products of the multiplication of almost the same parameters. Gumminess is a product of Hardness multiplied by cohesiveness, and chewiness comes from the multiplication of hardness, cohesiveness and springiness. Alternatively, Chewiness can be obtained by multiplying Gumminess (Hardness x cohesiveness) with Springiness, hence similar patterns and relatability are displayed.

These variations, observed in textural properties, may suggest underlying changes in tissue structure, induced by thermal stress. Even so, texture analysis profile is applied in oysters, mainly to evaluate product quality, more specifically in relation to freshness, processing and storage conditions (Puértolas et al., 2023; Ma et al., 2021). Its application in evaluating physical alterations as a response to heatwave stress remains limited. Although different under testing conditions, prior studies found a link between bivalves' texture parameters and the structural and biochemical characteristics of the tissue (Ma et al., 2021; Lemasson et al., 2017). This suggests that the differences found in this experiment may be indicative of the presence of physiological adjustments that may affect soft tissue texture, instead of a purely physical variation, although biochemical analysis would be needed to confirm this hypothesis.

To our knowledge, this is one of the first studies using texture profile analysis as a tool to compare conditioning treatments' impacts on *M. gigas* soft tissues. A general tendency for reduced texture parameters in the heatwave treatment was observed, suggesting a possible weakening of the soft tissue structure under thermal stress conditions. Regardless of the absence of biochemical or histological analyses to complement the study, these results indicate the possible utility of Texture Analysis Profile as a tool to detect changes in tissues' mechanical properties. Further studies, including histological and biochemical approaches, are required for a stronger validation of these findings.

Overall, simulated marine heatwave conditions affected growth, conditioning, and soft tissue properties of *M. gigas*. Short-term heat exposure reduced growth and condition indices, particularly height and width. AFNOR and condition index values indicated lower conditioning in the heatwave treatment. Soft tissue colourimetry showed minor differences between treatments, with a* and b* values indicating green and yellow tonality in both. Texture analysis revealed fluctuations in both groups, with lower mean values under heatwave conditions, suggesting weakened textural properties under thermal stress. Although most differences were not statistically significant, the trends indicate measurable effects of thermal stress on growth performance and quality.

5. Conclusion

This study aimed to evaluate the effects of handling practices and marine heatwave-stress, during separate trials, on morphometrics, condition and soft tissue colour and texture profile of *M. gigas*. The results demonstrate that both handling practices and marine heatwaves have impacts on oyster performance, but in different ways.

Concerning handling practices, oysters subjected to handling and desiccation periods were associated with improvements in condition index and shell morphology, suggesting a possible enhancement of oyster quality under controlled grow-out conditions. A tendency to an increased morphometric development was also observed, implying that growth is also improved, but a longer experiment period is necessary to verify that. Soft body colour was also affected, whereas these conditions led to an oyster with a less green and darker soft body tonality. Several tendencies were observed among texture parameters, but as in morphometrics, a longer period would be needed to better highlight possible differences. The existing research gap in the way that shifts in oysters' soft tissues, colour and texture parameters translate to quality and conditioning status, limits our ability to further draw conclusions on the effects of handling on these quality indicators. Regardless, these findings support the hypothesis that handling practices have a positive effect on *M. gigas* development and conditioning.

In contrast, a simulated short-term marine heatwave environment had negative impacts on growth and condition, as well as alterations in soft tissue colour and texture properties. An overall tendency to obtain smaller oysters was observed in the marine heatwave treatment, specifically decreased height and weight. Both AFNOR and the Condition index suggested a lower conditioning after a marine heatwave event, but only the AFNOR index presented significant differences. Regarding colourimetry, many variations were observed in both treatments throughout the trial. However, the patterns observed on both treatments are clearly distinct, but both treatments' soft tissues always presented mean colour values within the green and yellow ranges (negative a* values and positive b*values). Texture parameters also fluctuated in both treatments over the course of the trial, but a general tendency for lower mean values was observed in the heatwave treatment, indicating hindered textural properties under thermal stress. As in handling, the research gap in heat stress in colourimetric and textural properties of *M. gigas'* soft tissue limits our capacity to rely on these parameters to support our hypothesis. Even so, they still support the idea that thermal stress can lead to measurable shifts in Colourimetry and texture. Overall, the findings obtained in this study support the initial hypothesis that marine heatwave events have a negative impact on *M. gigas* growth and conditioning.

In summary, the results obtained highlight the potential impact of handling practices and marine heatwaves on *M. gigas* performance and quality, emphasising the risk of climate shifts and the importance of adequate handling strategies.

For future studies, it would be valuable to evaluate different periods of heatwaves, as well as the effects of the event on the opposite side of the spectrum, the Marine cold spell. A more thorough dive into understanding the correlation between heatwave stress and its impacts in colour and texture is also worthwhile, where biochemical and histological changes can also be analysed. This would be critical to fully understand climate change impacts and their risks to *M. gigas* aquaculture productions and their final product quality.

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7. Appendix

Table I - Statistical output of *t*-test analysis of morphometry, indices, colour and texture parameters of both treatments from handling experiment.

		Variation factor	T- test		
			T-value	df	*p*-value
Morphometry	Weight	Treatment (control *vs* handling)	1,116	87	0,267
	Length		-0,301	87	0,764
	Width		-1,040	87	0,301
	Height		0,145	87	0,885
Indices	Condition		-2,302	27	0,029
	AFNOR		0,250	27	0,804
Colour	L*		2,185	20,594	0,041
	a*		-2,698	28	0,012
	b*		0,319	28	0,752
Texture	Hardness		-0,071	25	0,944
	Resilience		-0,567	25	0,576
	Cohesion		0, 634	17,347	0,535
	Springiness		0,607	14,828	0,553
	Gumminess		0,390	25	0,700
	Chewiness		0,898	25	0,189

Table II - Statistical output of *t*-test analysis comparing morphometry, indices, colour and texture parameters between control and heatwave group in the S1 sampling point of the marine heatwave experiment.

		Variation factor	T- test		
			T-value	df	*p*-value
Morphometry	Weight	Treatment (control *vs* heatwave)	0,686	219	0,494
	Length		0,337	219	0,368
	Width		2,832	219	0,005
	Height		-0,293	219	0,77
Indices	Condition		-2,448	19,252	0,024
	AFNOR		-0,751	27	0,459
Colour	L*		1,394	44,805	0,17
	a*		0,495	0,311	0,622
	b*		-1,253	0,108	0,215
Texture	Hardness		0,309	58	0,758
	Resilience		-0,532	58	0,597
	Cohesion		1,025	58	0,31
	Springiness		-0,247	58	0,806
	Gumminess		0,997	58	0,323
	Chewiness		0,79	58	0,433

Table III - Statistical output of *t*-test analysis comparing morphometry, indices, colour and texture parameters between control and heatwave group in the S2 sampling point of the marine heatwave experiment.

		Variation factor	T- test		
			T-value	df	*p*-value
Morphometry	Weight	Treatment (control *vs* heatwave)	0,887	189	0,188
	Length		0,431	189	0,667
	Width		0,738	189	0,461
	Height		0,472	189	0,638
Indices	Condition		0,549	28	0,587
	AFNOR		1,512	28	0,142
Colour	L*		-2,893	58	0,005
	a*		-1,738	53,671	0,088
	b*		-1,641	58	0,038
Texture	Hardness		-1,051	44,178	0,299
	Resilience		1,691	58	0,096
	Cohesion		2,102	58	0,042
	Springiness		0,949	58	0,347
	Gumminess		-0,056	52,534	0,956
	Chewiness		0,168	58	0,867

Table IV - Statistical output of *t*-test analysis comparing morphometry, indices, colour and texture parameters between control and heatwave group in the S3 sampling point of the marine heatwave experiment.

		Variation factor	T- test		
			T-value	df	*p*-value
Morphometry	Weight	Treatment (control *vs* heatwave)	1,185	159	0,238
	Length		0,918	159	0,36
	Width		1,013	159	0,313
	Height		1,249	159	0,213
Indices	Condition		0,079	28	0,937
	AFNOR		1,764	28	0,089
Colour	L*		2,119	58	0,038
	a*		1,002	43,674	0,322
	b*		2,893	40,699	0,006
Texture	Hardness		-0,874	58	0,386
	Resilience		0,239	58	0,812
	Cohesion		1,395	52,85	0,169
	Springiness		2,595	58	0,012
	Gumminess		-0,38	46,243	0,706
	Chewiness		0,107	58	0,916

Table V- Statistical output of *t*-test analysis comparing morphometry, indices, colour and texture parameters between control and heatwave group in the S4 sampling point of the marine heatwave experiment.

		Variation factor	T- test		
			T-value	df	*p*-value
Morphometry	Weight	Treatment (control *vs* heatwave)	1,035	129	0,303
	Length		0,029	129	0,977
	Width		-1,25	129	0,213
	Height		1,158	129	0,249
Indices	Condition		0,681	28	0,501
	AFNOR		0,511	28	0,614
Colour	L*		1,744	54,135	0,087
	a*		2,584	58	0,012
	b*		0,694	58	0,491
Texture	Hardness		0,664	58	0,51
	Resilience		-1,776	58	0,081
	Cohesion		1,465	58	0,148
	Springiness		-0,718	58	0,476
	Gumminess		2,206	58	0,031
	Chewiness		1,682	58	0,098

Table VI - Statistical output of *t*-test analysis comparing morphometry, indices, colour and texture parameters between control and heatwave group in the S5 sampling point of the marine heatwave experiment.

		Variation factor	T- test		
			T-value	df	*p*-value
Morphometry	Weight	Treatment (control *vs* heatwave)	1,655	99	0,101
	Length		1,202	99	0,232
	Width		0,059	99	0,953
	Height		2,418	99	0,017
Indices	Condition		0,997	13,006	0,168
	AFNOR		7,935	27	<0,001
Colour	L*		1,724	58	0,09
	a*		-1,091	58	0,28
	b*		-0,051	52,179	0,959
Texture	Hardness		-0,184	58	0,885
	Resilience		1,111	45,089	0,272
	Cohesion		1,827	58	0,073
	Springiness		2,668	58	0,01
	Gumminess		0,808	58	0,422
	Chewiness		1,053	58	0,297

Table VII - Statistical output of ANOVA analysis of morphometry, indices, colour and texture parameters of control treatment from marine heatwave experiment comparing sampling points.

		Variation factor	Anova			
			F-value	df	p value	Post-hoc
Morphometry	Weight	Sampling point (S1 *vs* S2 *vs* S3 *vs* S4 *vs* S5)	7,394	4	<0,001	S5 ≠ S1 and S2
	Length		0,344	4	0,886	
	Width		1,034	4	0,367	
	Height		2,532	4	0,028	S1 ≠ S5
Indices	Condition		4,215	4	0,004	S2 ≠ S4 and S5
	AFNOR		4,962(H)	4	0,705	
Colour	L*		15,923(H)	4	0,004	S2 ≠ S1, S3, S4 and S5
	a*		5,635	4	<0,001	S2 ≠ S1 and S4; S3 ≠ S4
	b*		9,787	4	<0,001	S5 ≠ S1, S2, S3 and S4
Texture	Hardness		1,864	4	0,12	
	Resilience		3,237	4	0,014	S4 ≠ S2 and S5
	Cohesion		1,392	4	0,24	
	Springiness		2,881(H)	4	0,12	
	Gumminess		2,688	4	0,034	S1 and S2 ≠ S5; S2 ≠ S4
	Chewiness		1,878	4	0,117	

Table VIII - Statistical output of A-nova analysis of morphometry, indices, colour and texture parameters of heatwave treatment from 2º trial (control *vs* heatwave), comparing sampling points.

		Variation factor	Anova			
			F-value	df	p value	Post-hoc
Morphometry	Weight	Sampling point (S1 *vs* S2 *vs* S3 *vs* S4 *vs* S5)	2,847	4	0,015	
	Length		0,19	4	0,966	
	Width		4,45	4	0,001	S1 ≠ S2, S4 and S5
	Height		0,424	4	0,832	
Indices	Condition		4,505(H)	4	0,001	S1 ≠ S3, S4 and S5; S2 ≠ S4 and S5
	AFNOR		5,722	4	<0,001	S5≠ S1, S3 and S4
Colour	L*		3,094	4	0,609	
	a*		6,171	4	0,073	
	b*		5,979	4	<0,001	S2 ≠ S5; S3 ≠ S1, S2, S4, S5; S4 ≠ S5
Texture	Hardness		4,856(H)	4	0,015	S1, S2, and S3 ≠ S4
	Resilience		0,104	4	0,981	
	Cohesion		4,38(H)	4	0,059	
	Springiness		4,467(H)	4	0,205	
	Gumminess		3,489(H)	4	0,001	S4 ≠ S1, S2, and S3; S5 ≠ S1 and S2
	Chewiness		3,351	4	0,012	S2 ≠ S4 and S5