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Physiological Effects of Hooking in Longline Fisheries in Algarve and the Welfare Implications for Fish

Faculdade de Ciências e Tecnologia

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Declaration of authorship

I hereby declare to be the author of this work, which is original and unpublished. Authors and papers consulted are duly cited in the text and are listed in the included references.

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Muito muito obrigado. Não o teria conseguido sem ti.

Beijinhos x

Abstract

This thesis investigates the physiological responses and welfare implications of teleost fish subjected to artisanal longline fisheries in the Algarve region. The study focuses on understanding how different post-capture treatments and varying soak times impact fish vitality and stress markers, including glucose, lactate, cortisol, and osmolality. Both on-vessel fieldwork and in-house experiments were conducted to assess the physiological stress responses of Sparus aurata and other sparid species such as *Diplodus sargus, Diplodus vulgaris, and Pagellus erythrinus*. The results revealed no significant species-specific differences in vitality decline, with all species showing a sharp decrease in vitality overtime. Changes in physiological parameters showed similar pattern for the three species, with increases in lactate and osmolality over time, reaching the highest values at 120 min after capture, while a general decline in glucose concentrations was observed. Cortisol was already elevated at capture and remained high throughout, increasing only in *D. sargus*. The in-house experiments demonstrated that neither post-capture treatment (Dry, Ice, Ice Slurry) nor soak duration (0, 30, 90 minutes) significantly influenced vitality loss, reinforcing that time was the primary driver. Physiological stress indicators showed significant increases in lactate and osmolality over time, with a sharp glucose spike under ice treatment and stable but variable cortisol levels between treatments. This study contributes to the growing body of research on fish welfare in wild-capture fisheries by applying a function-based welfare approach. The findings provide valuable insights into the physiological responses of fish post-capture and offer recommendations for improving fish handling practices in artisanal longlining, with the ultimate goal of minimising stress and enhancing fish welfare. The study underscores the importance of minimising exposure time post-capture to reduce physiological stress in the artisanal longline fishery of the Algarve.

Keywords: Fish Welfare, Physiology, Longline, Artisanal Fishery, Vitality, Physiological stress

Resumo

Esta tese explora as respostas fisiológicas ao stress e as implicações para o bem-estar dos peixes capturados na pesca artesanal com palangre, na região do Algarve. A preocupação com o bem-estar dos peixes tem impulsionado a investigação sobre a forma como os métodos de captura e as práticas de manuseamento pós-captura, incluindo o abate, as respostas ao stress nos peixes e a qualidade do mesmo. Este estudo, uma das primeiras abordagens a este tema em pesca comercial, visa contribuir para colmatar lacunas no conhecimento, fornecendo informações sobre as reações fisiológicas de várias espécies de esparídeos, incluindo o sargo (*Diplodus sargus*), a safia (*Diplodus vulgaris*), a bica (*Pagellus erythrinus*) e a dourada (*Sparus aurata*), à captura e ao manuseamento durante a pesca com palangre. O objetivo principal é avaliar os indicadores de stress, como a glicose, o lactato, o cortisol e a osmolalidade, quer em relação à captura com anzol e duração de permanência no mesmo, , quer à perda de vitalidade em diferentes condições de manipulação e acondicionamento pós-captura: caixa seca, gelo e gelo em água. As atividades contemplaram trabalho de campo a bordo e experiências controladas em laboratório. Os peixes (*D. vulgaris, D. sargus e P. erythrinus)* foram capturados e sujeitos a tratamentos pós-captura que refletiram as práticas típicas da pesca comercial num barco de pesca costeira artesanal com palangre. A bordo foi realizada uma avaliação da vitalidade após a remoção do anzol, na chegada do peixe ao convés, e depois ao fim de 60 e de 120 de exposição ao ar. Foram ainda recolhidas amostras de sangue de peixes nestes intervalos de tempo, utilizadas para dosear indicadores fisiológicos de stress. Em ambiente controlado, num tanque de 10000 litros em circuito aberto, utilizamos douradas que foram pescadas com anzol, no qual foram deixadas por 30 e 90 minutos de forma a simular o tempo potencial de duração numa atividade de palangre na pesca comercial. Após este tempo foram amostradas, a vitalidade registada, e colocadas em caixas secas sem qualquer meio de arrefecimento, em caixas com gelo ou em contentores com uma mistura 1:1 de gelo e água salgada. Fizeram-se avaliações da vitalidade a intervalos regulares, e recolhidas amostras de sangue para quantificar as respostas fisiológicas (medições de glicose, lactato, cortisol e osmolalidade) nas condições pós-captura. Estes indicadores de stress fisiológico estão bem documentados em peixes, sendo que os níveis de glicose e de lactato aumentam tipicamente em resposta às necessidades de energia e ao metabolismo anaeróbico, enquanto o cortisol reflete a resposta endócrina ao stress e as alterações da osmolalidade indicam perturbações iónicas e osmóticas.

Os resultados dos ensaios no mar não revelaram diferenças significativas na vitalidade e nas respostas fisiológicas ao stress entre as três espécies estudadas. Imediatamente após a captura,

todas as espécies exibiram uma vitalidade elevada, sem diferenças significativas nas pontuações iniciais. Aos 60 minutos após a captura, observou-se um claro declínio da vitalidade. No caso da bica, *P. erythrinus*, todos os indivíduos deixaram de reagir até ao intervalo de uma hora após a captura, o que representa uma queda rápida da vitalidade. Esta redução foi aparentemente mais lenta nas espécies do género *Diplodus*, mas sem diferenças estatisticamente significativas. As alterações dos parâmetros fisiológicos apresentaram um padrão semelhante para as três espécies, com aumentos do lactato e da osmolalidade ao longo do tempo, atingindo os valores mais elevados aos 120 minutos após a captura, enquanto se observou um declínio geral das concentrações de glicose. O cortisol, elevado à captura, manteve-se elevado durante todo o período, aumentando apenas em *D. sargus*.

As experiências laboratoriais visavam explorar melhor as respostas fisiológicas ao stress em condições controladas. Os resultados demonstraram que nem o tipo de tratamento pós-captura (caixa seca, gelo, gelo e água) nem a duração da imersão (0, 30, 90 minutos) influenciaram significativamente a perda de vitalidade, destacando o tempo de emersão como principal fator determinante da diminuição da vitalidade. A glicose mostrou um aumento acentuado sob o tratamento com gelo, enquanto os níveis de lactato e osmolalidade aumentaram ao longo do tempo. O cortisol manteve-se relativamente estável, sem diferenças significativas entre os peixes com 0 ou 30 minutos de anzol. No entanto, os níveis de cortisol foram mais elevados nos peixes grupo de 90 minutos no anzol, sobretudo nos colocados em condições secas ou na mistura de gelo e água. Este grupo foi também o único em que o cortisol diminuiu com o tempo. É provável que devido ao longo tempo de captura e luta no anzol, o cortisol já tivesse atingido o seu pico e estivesse numa trajetória descendente. Estes resultados sugerem que o acondicionamento com água e gelo induz a resposta de stress endócrino mais significativa, seguido da caixa seca, enquanto o gelo terá um efeito mais moderado sobre os níveis de cortisol.

As medições de osmolalidade também mostraram diferenças entre os tratamentos. Os peixes em água e gelo apresentaram os níveis mais elevados de osmolalidade, seguidos dos peixes em seco, refletindo uma perturbação do equilíbrio iónico e desidratação. Em contraste, os peixes armazenados em caixas de gelo apresentaram níveis de osmolalidade mais estáveis, provavelmente porque não existem trocas osmóticas com o meio e é evitada a perda de água para o ar.

Globalmente, este estudo fornece novos conhecimentos sobre as respostas fisiológicas e de vitalidade de várias espécies de esparídeos à captura e ao manuseamento pós-captura na pesca artesanal com palangre. Os resultados sublinham a importância das práticas de manuseamento

pós-captura na modulação da resposta ao stress e dos resultados em termos de bem-estar dos peixes. Especificamente, o acondicionamento em água e gelo e caixa seca parecem induzir maior stress metabólico, endócrino e osmótico do que apenas o gelo, particularmente em tempos de imersão mais longos. O gelo também parece reduzir a vitalidade mais rapidamente. Estes resultados têm implicações importantes para a gestão das pescas, uma vez que sugerem que as modificações das práticas de manuseamento pós-captura poderiam melhorar significativamente o bem-estar dos peixes na pesca artesanal com palangre, com impactos éticos e de qualidade do produto.

Palavras chave: Bem-estar em Peixes, Vitalidade, Fisiologia do stress, Pesca com Palangre, Pesca Artesanal,

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Chapter 1 Introduction

1.1 Global and Regional Importance of Fisheries and Longlining

1.1.1 Importance of Fisheries Worldwide and their Challenges

Globally, fisheries are a cornerstone of food security, with fish constituting a major dietary component for more than a third of the world's population (FAO, 2024). Over the past few decades, the importance of fish as a dietary staple has grown significantly, with per capita consumption increasing from around 9 kg per year in the 1960s to approximately 20 kg per year in 2017 (Bondad-Reantaso, 2020). Beyond their nutritional value, fisheries also represent a vital component of the global economy, generating employment for an estimated 33.6 million (FAO, 2024), from small-scale artisanal fishers to large commercial enterprises.

This industry, however, faces growing challenges that endanger its future viability. Uncontrolled anthropogenic stressors, such as overfishing and climate change, have led to the depletion of key fish stocks and disrupted marine ecosystems (Turner et al., 2024; Sumaila & Tai, 2020). Habitat degradation, driven by pollution and destructive fishing practices, further accelerates the decline in biodiversity, making it increasingly difficult for traditional fishing practices to sustain their historical yields. These issues not only threaten the sustainability of fish stocks but also the livelihoods and food security of the coastal communities that depend on them (Srinivasan et al., 2010).

1.1.2 Economic and Cultural Significance of Fisheries in Portugal

In Portugal, fishing is not merely an economic activity but a deeply ingrained tradition with strong historical connotations, integral to the country's culture and society (Pita et al., 2015). The Algarve, the southernmost region of mainland Portugal, exemplifies this connection, where fishing has long been vital to both the economy and local identity. Traditional, artisanal, and labour-intensive methods like longlining are still widely practised, with the Algarve boasting the highest number of multi-gear registered fishers in the country (Pita & Gaspar, 2020), reflecting the region's deep-rooted bond with the sea. The fishing sector is crucial in supporting rural coastal communities, offering employment and income where opportunities are otherwise limited (Pita et al., 2010). Additionally, fish is a vital component of the traditional Portuguese diet, with the Portuguese being the largest consumers of fishery products per capita

in the EU, consuming 56.8 kg per person annually—more than double the EU average (Pita & Gaspar, 2020). Despite accounting for just 4% of total EU-28 landings by quantity, the significance of fisheries to Portugal's cultural heritage and economic sustenance is undeniable, particularly in regions like the Algarve.

1.2 Longlining

1.2.1 Overview

Longlining is a fishing technique that involves the use of a long, horizontal mainline to which numerous hooks are attached via branch lines at regular intervals. These hooks are baited and the longline is then deployed in open water, where it remains untended for a specified period of time (He et al., 2021). The scale of a longline operation can vary significantly; in coastal fisheries, the mainline may extend only a few hundred metres, while in large-scale pelagic fisheries, it can exceed 80km in length.

The basic components of longline gear include the mainline, branch lines (also known as snoods or gangions), hooks, and bait (Figure 1-1). Hooks and branch lines are attached to the mainline using either traditional knots or mechanical crimps and clamps, which may also incorporate swivels to reduce line twisting. Depending on the operation, longlines can be hauled in either by hand or with the assistance of powered reels or drums. The baiting of hooks can be performed manually or with the help of automated machinery.

Longlining is a versatile and widely used method in both coastal and pelagic fisheries, making it a critical component of the fishing industry. Contrary to other fishing methods, the gear used in these fisheries occupies much less space on board and is in general less complex, both in terms of its components and in operation. This reduces and simplifies the infrastructure needed on vessels and implies also less costs in acquiring or substituting gear. Smaller boats can be used and/or more room can be allocated to fish storage, and the gear can be even used from vessels usually committed to other fisheries. As a result, this is one of the most common fishery techniques in artisanal and local fisheries.

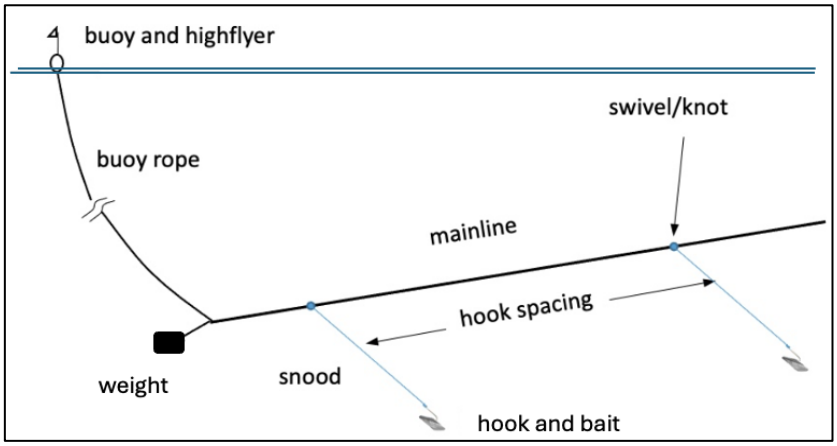

Figure 1-1 Basic components of a set longline used to catch demersal fish. Figure adapted from (He et al., 2021).

1.2.2 Longlining in Portugal and the Algarve

Longlining is a prominent fishing method in Portugal, making up 6.47% of fish tonnage landed in 2018, in comparison the global figure is a considerably lower 2.17% (Pauly et al., 2020). Whilst this makes up a relatively small percentage of catch by weight; bottom trawl and purse seine contributing 35.33% and 24.08% respectively, this catch method is still vital to the fishing exploits of the nation due to targeting higher value catch.

These high-value catches are particularly significant to the smaller, artisanal longline fishers of the Algarve. These fishers typically target demersal species such as those in the Sparidae family, often setting and retrieving their lines by hand during single day or night trips in vessels ranging from 5.5 to 7 metres in length (Pita & Gaspar, 2020). Upon landing, their catch is sold directly to local markets, supporting both their livelihoods and the region's economy.

1.2.3 Challenges associated with longlining

While an effective and widely used method in fisheries, longlining presents several challenges that affect both the sustainability of fish stocks and the welfare of marine species. One of the primary issues is bycatch—the unintentional capture of non-target species, including endangered species such as sea turtles, seabirds, and sharks (Bull, 2007; Gjertsen et al., 2010). Bycatch not only threatens biodiversity, but also complicates management efforts to maintain healthy fish populations (Gilman et al., 2019; Hall et al., 2000). In addition, studies have shown that blood chemistry stress indicators in fish, such as those from yellowfin tuna and other species, are adversely affected by longer soak times. The stress response is linked to both the duration of struggle during capture and the time spent on the hook before retrieval the prolonged soaking of lines increases the likelihood of injuries, which can affect the quality of the catch and the welfare of the fish.

1.3 Welfare

1.3.1 General

Welfare, in its broadest sense, refers to the overall state of well-being of an individual, encompassing both physical and mental health. When applied to animals, the concept becomes inherently complex and multifaceted, as it spans across different species, environments, and human perspectives. In this context, welfare is shaped by the specific conditions in which animals live and the human activities that affect them. Importantly, the definition of "good welfare" often varies based on one's role or interest—whether that be a fisherman, policy maker, activist, or researcher—leading to different interpretations of what constitutes appropriate treatment of animals and their environments (Saraiva et al., 2022).

One of the more foundational definitions that can be found frequently in literature comes from Broom (1986), who characterises animal welfare as "the state of an individual in relation to its attempts to cope with its environment," suggesting that welfare is dependent on the animal's ability to manage and adapt to the conditions it faces. This definition remains central to discussions about welfare because it acknowledges the dynamic relationship between an animal and its surroundings. In more contemporary research, the focus has shifted toward a more nuanced understanding of welfare as a balance between three key factors: the animal's emotional state, its physical health, and its ability to express natural behaviours, allowing it to live in a manner that aligns with its innate needs (Weary & Robbins, 2019).

Studies in the improvement of animal welfare and ethical implications have been gaining traction for decades (Koknaroglu & Akunal, 2013). In recent years, there has been a growing emphasis on the idea that animal welfare is not just about avoiding negative states but also about promoting positive experiences. This shift has led to a focus on the cumulative balance of pleasant and unpleasant experiences that an animal encounters throughout its life (Reimert et al., 2023). This evolving perspective highlights welfare as a dynamic and ongoing process, where the quality of an animal's life is judged by the prevalence of positive states, rather than merely the absence of harm or suffering. Thus, the study of animal welfare has expanded into an increasingly comprehensive field that seeks to ensure animals not only survive but thrive in their environments.

1.3.2 Fish welfare

Where animal welfare science has been expanding over the past few decades, studies into fish welfare, particularly capture fisheries, has notably lagged behind (Waley et al., 2021). The root of this stems from multiple different reasons; prior to 2002, it was a commonly held belief that fish were incapable of perceiving pain, this being due to nociceptors, receptors that preferentially detect potentially painful stimuli, had not yet being identified in fish (Rose, 2002). This has since been disproven, with nociceptors being identified in teleost fish (Sneddon, 2002, 2003), and further empirical studies have shown that fish respond aversively to painful stimuli, supporting the view that they can experience negative welfare states (Huntingford et al., 2006).

Another factor contributing to the slow uptake of fish welfare science is human apathy. The idea of caring for the welfare of an often uncharismatic animal that we rarely interact with, one that does not even share the air we breathe, and is destined for slaughter, has until recently garnered little interest from the general public (Bennett et al., 2015; Lundberg et al., 2019).

However, interest in fish welfare has grown, and the term "fish welfare" now encompasses a wide range of views and opinions (Browman et al., 2019). Diggles et al., (2011) describes three different approaches that can be used to define what constitutes "good" welfare for wild-capture fish. The first is the "feelings-based approach", which focuses primarily on pain and suffering, and equates the absence of these states with good welfare. In the context of wild-capture fish, this approach lacks a solid foundation. After all, pain and suffering are inherent aspects of a wild fish's life, such as predation and conspecific aggression (Braithwaite & Huntingford, 2004), making this approach less applicable when assessing welfare during capture.

The second approach is the "nature-based approach," which defines good welfare as allowing an animal to express its inherent biological nature (Huntingford et al., 2006). Like the feelings-based approach, this is less relevant in wild capture fisheries.

The third approach is the "function-based approach," which holds that good welfare is reflected in an animal's health and the proper functioning of its biological systems. This aligns closely with Broom's (1986) definition of animal welfare more broadly. This "function-based approach" heralds from factual science (Arlinghaus et al., 2007, 2009), and can be based and measured with behavioural, physiological, neurological, pathological and cellular quantifiable data.

Physiological markers, such as glucose, lactate, cortisol and changes in osmolality can be used to quantify stress in fish. As stress can pose significant challenges to the welfare of fish (Sneddon et al., 2016), stress might be able to be used as a quantifiable proxy measure of welfare in fish.

1.3.3 Stress and indicators of physiological stress

The stress response in teleost fish strikes many similarities to that of the terrestrial vertebrates (Bonga, 1997). It is characterised by physiological changes in that arrive in a set hierarchy (K. B. Davis, 2006). The stress response is initiated and controlled by two systems leading to the production of corticosteroids (mainly cortisol in fish) and catecholamines (adrenaline – also called epinephrine - and noradrenaline – or norepinephrine). These factors regulate the secondary stress response that alter the mobilisation and allocation of resources, such as energetic substrates and oxygen to vital areas of the body (Schreck et al., 2016).

Briefly, a stressor is first perceived by the peripheric and central nervous system through sensorial mechanisms and receptors (e.g. vision, tactile, temperature or pain receptors, etc) and the information is relayed to the hypothalamus. Sympathetic innervation stimulates the chromafin tissue that releases adrenaline and noradrenaline, which results in mobilisation of glucose and increase of metabolic rates, the first stage of a “fight or flight” response. In parallel the hypothalamus produces a corticotropin release factor (CRF) that travels to the pituitary where it induces the release of adrenocorticotropic hormone (ACTH), which then circulates through the blood to reach the interrenal tissue, the fish equivalent to the adrenal gland, where it stimulates the production and release of cortisol (Figure 1-2). Cortisol, also known as the stress hormone, is released in the blood stream and will have generalised actions, mediating several responses. Elevated cortisol levels over prolonged periods can weaken the fish’s immune response and growth, compromising health and welfare (Barton & Iwama, 1991).

Although the cortisol response is slower that of catecholamines, cortisol levels rise considerably rapid after exposure to stressors, usually within a few minutes and can take hours to return to normal (Fanouraki et al., 2011). The cascade of tissues involved is termed the hypothalamic-pituitary-interrenal (HPI) axis and its functioning, regulated by strong feedback mechanisms, serves multiple functions (Best et al., 2023).

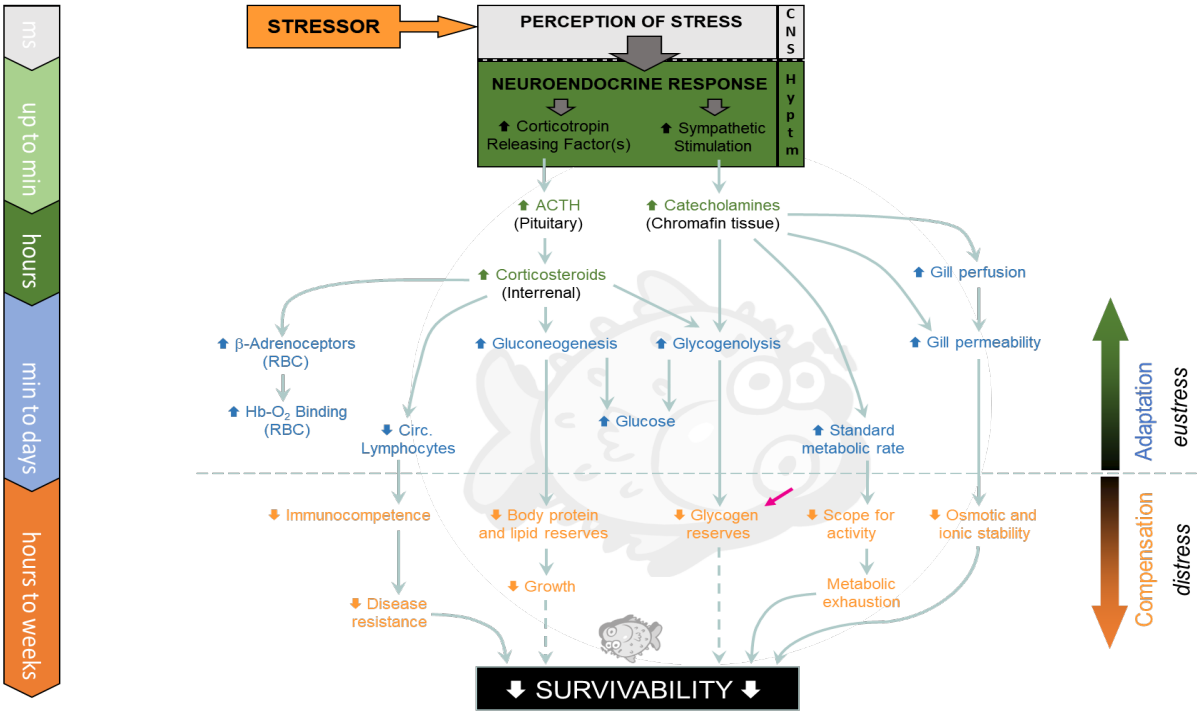

Figure 1-2 Cascade of action in response to the perception of stress, mediated by the hypothalamus-pituitary-interrenal axis and the catecholamines (Adapted from Moyle & Cech, 1996)

Usually, cortisol release and circulating levels are proportionate to the stressor that induce it, which makes it a good indicator of stress. One of its main actions is the breakdown of energy reserves and thus the increase of circulating glucose, which values start increasing shortly after the cortisol rise. Thus, measuring glucose is also a reliable indicator of a stress response, although its levels may be changed by other factors, such as feeding or nutritional status. Lactate is a by-product of anaerobic respiration and a good indicator of excessive exercise or increased metabolism, extending beyond aerobic thresholds. Lactate can be converted in glucose through a process of gluconeogenesis, which is also stimulated by cortisol.

The typical sequence of accumulation and clearance of this substances in blood following the response to a stressor are shown in Figure 1-2, knowledge about the typical sequence, duration and amplitude of these metabolites is helpful to evaluate the intensity of the stress response, and these profiles can be very species specific and eventually stress-specific.

In addition to influencing energy metabolism, other actions of cortisol impact the osmoregulatory ability and immunocompetence. Thus, physiological indicators related to these functions are sometimes used to evaluate the status of a fish after a potential stress event. In specific, osmolality and ion composition may show important alterations due to changes in membrane permeability or modified activity io ion pumps.

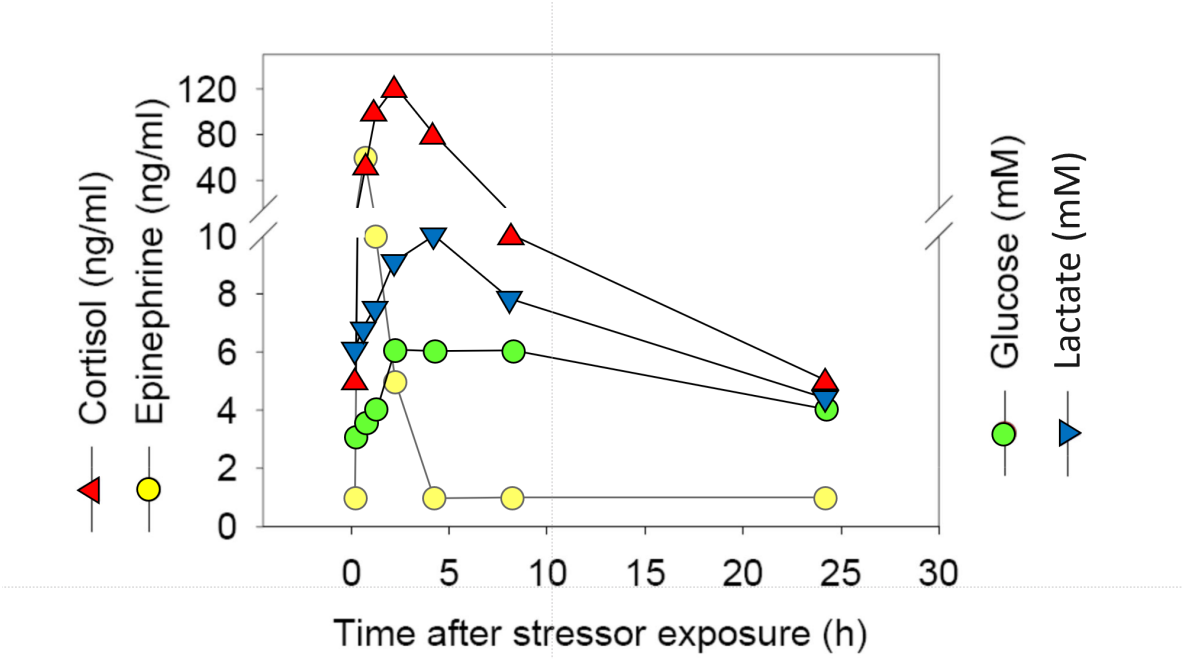

Figure 1-3 Generalised chronological accumulation and clearance of endocrine factors and energy metabolism following a stressful event (modified from Iwama et al., 2004)

1.4 Research gaps

Fish welfare has received increased attention in the recent years, namely in the intensive aquaculture industry due to its equivalency, both in legislation and public perception, to other animal production systems, such as poultry, pigs and cows. However, the introduction of this concept to commercial wild-capture fisheries is challenging and problematic. In general it is not a case of increasing welfare to humanely raise an animal, providing the concepts described above or the 5-freedoms approach (freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury and disease; freedom to express normal behaviour; freedom from fear and distress) (FAWC, 2009) – the objective is to catch and kill the fish, and the question is then how to catch and slaughter a fish, usually in the middle of hundreds or thousands of other fish, depending on the gear used and species targeted, inflicting the least suffering possible.

There is little information on the physiology of many of the species caught in the wild, and only in a very limited number of these will be possible to obtain basal, non-stressed levels for these indicators, thus, the reliability of the interpretations made with the numbers measured is reduced.

1.5 Thesis Focus and Objectives

1.5.1 Aim of the Study

The primary aim of this thesis is to investigate the physiological effects of hooking in artisanal longline fisheries in the Algarve region. Specifically, the research focuses on understanding the impact of different post-capture treatments and soak times on fish welfare. The study integrates both on-vessel data collection and in-house experiments to assess stress responses through vitality assessments and key physiological markers. The findings aim to contribute to a deeper understanding of fish welfare in commercial fisheries and provide insights into the best practices for improving fish handling procedures to minimise stress.

1.5.2 Research Questions:

- What is the impact of hooking on fish welfare and what are the contributing variables?
- Does the duration of soaking influence vitality and how both relate to the stress response. and the progression of physiological indicators during storage?
- Do different post-capture handling conditions (dry box, ice, ice slurry) affect the vitality and physiological stress responses of gilthead bream (*Sparus aurata*)?
- Can the methods be improved or made more humane by modifying storage, namely with cold storage on different ice conditions?
- How do different species stress responses differ to the same capture and post-capture handling conditions?

Chapter 2 Materials and Methods

2.1 On-vessel fieldwork

2.1.1 Study Area

The area of study was waters off the coast of Algarve (Figure 2-1). Algarve is situated in the southernmost part of Portugal, coastally boarded entirely by the Atlantic Ocean. The southern coast runs from Cabo de São Vicente (37°1′ N - 8°59′ W) in the west, to the Rio Guardiana (37º11' N - 7º25' W) and the Spanish border to the east (Alexandre et al., 2022). The distance is approximately 155km, and characterised by a variety of sandy beaches, rocky cliffs, sea caves, and lagoons. The continental shelf in this region is very narrow, ranging from just a few kilometres to 45km (Lobo et al., 2014). The relatively stable Atlantic Ocean primarily influences water in this region, however fluctuation occurs due to seasonal coastal upwelling events and the influx of warm, highly saline waters that enter through the Strait of Gibraltar due to the current known as the Mediterranean Outflow Water (MOW) (Salles et al., 2010).

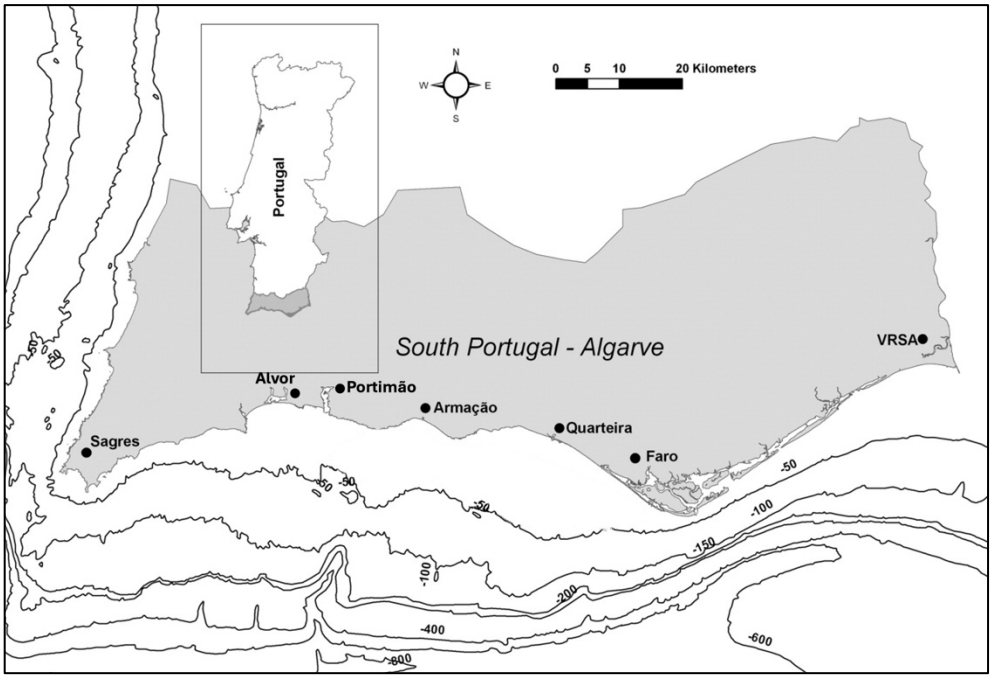

Figure 2-1. Algarve coastline. Adapted from (Monteiro et al., 2010)

The sampling trips aboard the FV Conquistador were carried out within the continental shelf between the fishing villages of Alvor and Carvoeiro (Figure 2-2).

2.1.2 Materials

Table 2-1. Vessel and fishing gear specification aboard FV Conquistador

1. Vessel Specifications	
Length	6 m
Width	NA
Height	NA
Gross Tonnage	NA
Primary Engine (used during travelling)	100 HP, Four stroke
Secondary Engine (used during fishing)	15 HP, Four stroke
2. Gear Specifications	
Gear Type	Set- longline
Mainline length	4.25 Km (approx.)
Mainline diameter	1 mm
Mainline build material	Monofilament nylon
Snood length	1.5m
Snood diameter	0.5mm
Snood build material	Monofilament nylon
Distance between two snoods	2.5 m (approx. 400/Km of mainline)
Hooks/Snood	1
Hook type	No. 13
Hook material	Metal
Hook shape	J- shaped
Hook barb	Present
Hook Measurements: -	(F. Mehanna et al., 2021)
Total length(mm)	23.6
Front (mm)	9.9
Gape (mm)	8.7
Neck (mm)	10.3
Bait type(s)	Frozen: *Sipunculus nudus* *Solen marginatus* *Sepia officinalis*

2.1.3 Methods

Beginning in mid-February 2024 and running intermittently through to the summer months, eight opportunistic research trips took place aboard the Portuguese fishing vessel Conquistador (vessel and gear specifications shown in table 2 -1). The fishing voyages sailed from the port of Alvor in the early hours of the morning, between 0230 and 0300, with only two exceptions sailing at approximately 1730. The fishing vessel steamed out east heading to one of two locations, either remaining coastal or setting off deeper near the edge of the continental shelf (Figure 2-2). This sailing takes between 60 – 90 minutes.

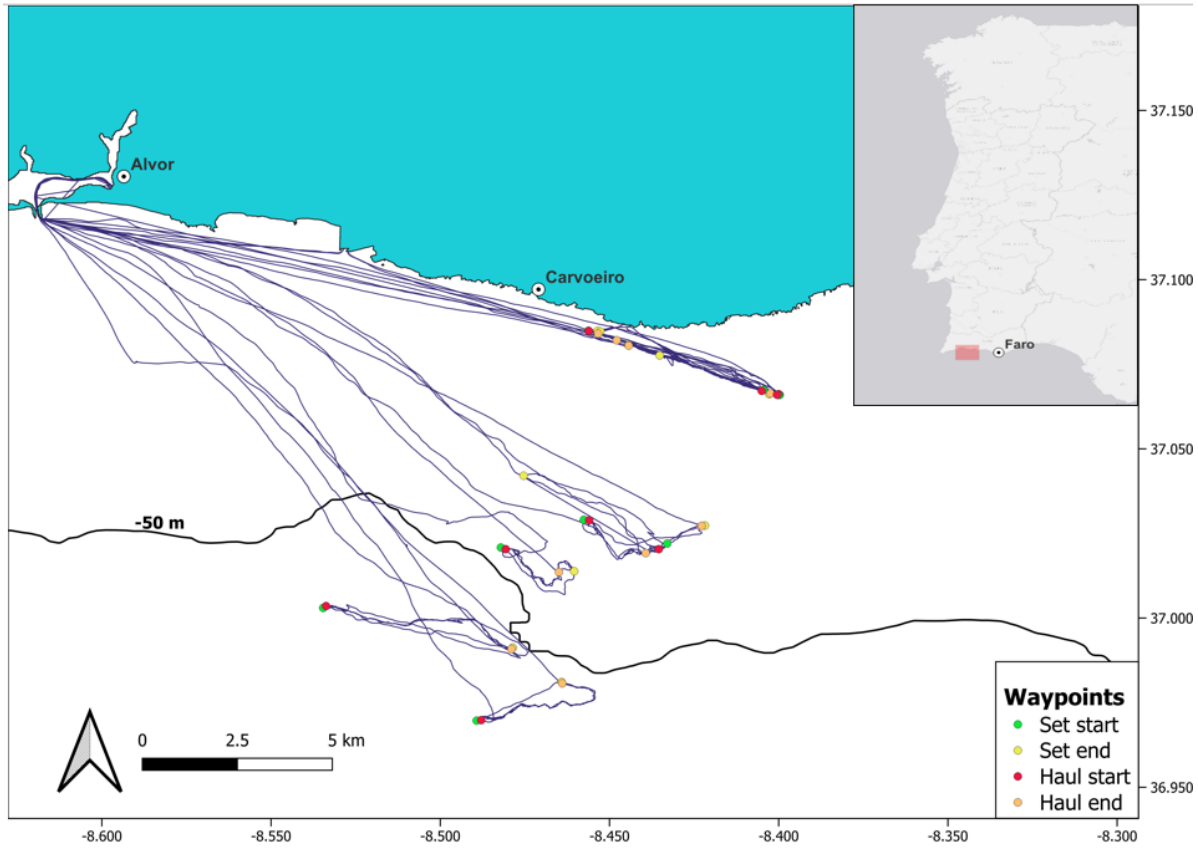

Figure 2-2. Map with sailing locations and waypoints of fishing trips aboard FV Conquistador.

Using GPS waypoints and upon reaching the skipper's desired fishing ground the traditional and, uniquely Algarvian, square longline boxes baited with a variety of frozen bait (Table 2-1. Vessel and fishing gear specification aboard FV Conquistador), of which there are approximately 1700 hooks (Figure 2-3) were set. The setting of the longline typically took 80 minutes, with the depth of the line being set at 8 fathoms and between 21-27 fathoms for the coastal and offshore trips respectively. At the termination of the line setting, the boat turned back on itself (Figure 2-2), slowly returning to the location where the line was set around 45 minutes post the end of the setting. The fisher then proceeded to manually haul the line, removing hooked fish by a sharp pull of the line and then tossing the fish into a sparsely iced box. The line being set near the bottom, the gear targeted demersal fish species and caught an abundant number of sparids. Namely: *Diplodus vulgaris* (Two-banded seabream); *Pagellus acarne* (Axillary seabream); *Pagellus erythrinus* (Common pandora); *Sparus aurata* (Gilt-head seabream); *Diplodus cervinus* (Zebra seabream); *Diplodus sargus* (White seabream). Throughout the course of the fishing trips, vitality and blood physiology were sampled on *D. sargus, D. vulgaris,* and *P. erythrinus*.

Figure 2-3. Left - longline fishing gear being set. Right - baited hooks. Photo credits (Magda Frade/Vighnesh Samel/Ana Marçalo)

2.1.3.1 Visual inspection and Vitality assessment

When the target fish species were landed several initial observations were made. Namely, any wounds or barometric trauma, and signs of bleeding were categorised binomially, bleeding (b) and non-bleeding (nb). The location of the hooking site had five categories, mouth (m), gut (g), the eye (e), hook inside (HI) and hook retained (HR).

Following initial visual inspection, the specimens would be placed in a dry tray of 10 of each targeted species to measure the loss of vitality with time, categorised by the vitality scale in Table 2-2. Vitality was assessed on an hourly basis until vitality stage 1 was reached. At this time, the specimen would be weighed, the length measured and then returned to the rest of the fisher's catch. Therefore, data is shown as T1, which indicate fish at arrival on deck, T2, fish stored on trays on deck 60 min after arrival and T3, fish store on deck for 120 after arrival.

Table 2-2 The vitality scale used for conducting vitality assessments

Vitality Scale	Description	Activity
4	Highly Active	Flopping moving or other body movements
3	Less Active (Vital)	Reaction to touch, regular opercular movement, spasms.
2	Lethargic	Random opercular movement, eye-roll/Vestibulo-Oculo reflex (VOR).
1	Not responsive	No movement or reaction

2.1.3.2 Physiological sampling and analysis

2.1.3.3 Blood sampling

Collection of blood was performed in addition to visual observations and vitality assessments to establish possible correlations between the behavioural indicators and the physiological status of the fish. On selected fishing trips, where the sea conditions would allow, blood samples were taken from species DV, DS and PE, following the same sampling chronology used for visual inspection – at arrival on board (T1), 60 minutes later (T2) and 120 minutes later (T3). In the case of blood collection each fish was only sampled once and vitality recorded at that point, to avoid the influence of repeated handling and blood drawing on blood physiology and fish behaviour/vitality. Approximately 1 ml of blood was drawn from the caudal vein using heparinised (1000 units/ml lithium heparin, Sigma-Aldrich®) 1 ml syringes (Terumo®) and 23G needles (0.6X32 mm, Agani™, Terumo®). The blood was immediately transferred to tubes containing 10 µl of heparin, agitated, and stored on ice until the end of the collection period. Upon returning to the laboratory, the blood samples were centrifuged at 9,500 rpm for 5 minutes at 4°C (Heraeus™ Pico™ 17 Microcentrifuge), and the plasma was stored henceforth at -80°C for later analysis. Blood collection and fish handling were conducted under the FELASA type-C license issued by the DGAV (Government of Portugal). All procedures adhered to Portuguese legislation and European Union guidelines (DL 113/2013, 2010/63/EU).

The blood sampled from the fish caught was analysed for 4 different parameters that can be used as indicators of physiological stress.

2.1.3.4 Glucose

Plasma glucose concentration was measured using a commercial colourimetric kit (Spinreact™ Glucose-TR, Kaplan A. et al., 1984) following the manufacturer's protocol. In brief, the internal standard and plasma samples were mixed in duplicate with the working reagent, which contained the enzymes glucose oxidase (GOD), peroxidase (POD), and phenol-aminophenazone in Tris-buffer (pH 7.4), in a 96-well plate. Glucose oxidase catalyses the oxidation of glucose to gluconic acid, producing hydrogen peroxide as a byproduct. This hydrogen peroxide is then detected by phenol-aminophenazone in the presence of peroxidase, forming a coloured quinone product. The absorbance of this product was measured at 505 nm using a Multi-mode Microplate Reader (Multiskan® GO, Thermo Scientific) with SkanIt™ software. A standard curve was generated by measuring the absorbance of glucose standards ranging from 0 mmol/L to 20 mmol/L, and the glucose concentration in the samples was calculated by fitting the absorbance values to the standard curve equation.

2.1.3.5 Cortisol

To analyse plasma cortisol levels, we utilised the TECAN Cortisol ELISA Kit (Tecan Group Ltd.) following the manufacturer's instructions. Plasma samples and standards were diluted accordingly and dispensed into a 96-well microplate pre-coated with cortisol-specific antibodies. Following the addition of 200 µL of Enzyme Conjugate to each well, the plate was covered and mixed for 10 seconds, then incubated for 60 minutes at room temperature (18-25°C). Post-incubation, the plate was washed three times with 300 µL of diluted Wash Buffer and the excess solution was removed by tapping the plate on a paper towel. Subsequently, 100 µL of TMB Substrate Solution was added to each well, and the plate was incubated for 15 minutes. The reaction was halted with 100 µL of TMB Stop Solution, and the colour change from blue to yellow was observed.

The optical density was measured at 450 nm (with a reference wavelength of 600-650 nm) using a Multi-mode Microplate Reader (Multiskan® GO, Thermo Scientific) with SkanIt™ software. Cortisol concentrations in the samples were calculated by comparing the absorbance values to a standard curve generated from known cortisol standards.

2.1.3.6 Osmolality

Blood plasma osmolality was measured using a vapour pressure osmometer (Vapro Wescor 5520, South Logan, Utah, USA). The process is as thus; firstly, the osmometer is calibrated using a 290 mOsm/Kg standard solution (ELItech Group® Optimole™ Osmolality Standard), following this, 10µL of sample is pipetted onto a sampling disc, inserted into the machine and a reading given after 90 seconds. This process is repeated with each sample, recalibrating every 10th sample.

2.1.3.7 Lactate

A method like that used for glucose was employed to measure lactate concentration in the plasma samples. Lactate levels were determined using a commercial colourimetric kit (Spinreact™ Lactate, Kaplan A et al., 1984), following the manufacturer's instructions. In short, the internal standard and samples were mixed with the working reagent, which consisted of lactate oxidase (LOD), peroxidase (POD), 4-aminophenazone (4-AP), and chlorophenol in PIPES buffer (pH 7.5). The reactions were carried out in duplicate in a 96-well plate. LOD catalyses the oxidation of lactate to pyruvate and hydrogen peroxide, which then reacts with POD, 4-AP, and chlorophenol to form a red quinone compound. The absorbance of the quinone product was measured at 505 nm using the Multi-mode Microplate Reader Multiskan® GO (Thermo Scientific) and analysed with SkanIt™ software. A standard curve was generated by measuring the absorbance of known lactate concentrations (ranging from 0 mmol/L to 15 mmol/L). Lactate concentrations in the samples were calculated by fitting their absorbance values to the standard curve equation.

2.2 In-house experimentation

2.2.1 Rationale

The goal of the in-house experiments, was to replicate the artisanal longline fisheries in a controlled manner, being able to test individual variables and see the impacts this has on fish stress and welfare. Although not directly tackled in this thesis, this allowed to refine methods and to apply technologies to collect additional data such as heartbeat rate and strength applied on hook by the fish, and at the same time, to record swimming and escape/struggling behaviour of hooked and non-hooked fish.

2.2.2 Experimental animals and housing conditions

In-house experimentation took place at Centro do Ramalhete between May and August 2024. Here, aquaculture-raised Sparus aurata (gilthead bream) were housed in a circular 10,000-litre tank with an approximate radius of 2.3 m for a week prior to the start of the experiment for acclimation. The water parameters had an average dissolved oxygen concentration of 6.06 ± 0.62 ppm, a temperature of 23.78 ± 1.53 °C, and a salinity of 38.02 ± 0.74 ppt. The tank was kept outside and therefore followed the natural photoperiod of the Algarve region during this time of year, which ranges from 10:14 (dark:light) in May to 9:15 in August; however, it was covered to prevent direct sunlight from reaching the water. Water was continuously filtered using a recirculating system with mechanical and biological filters.

The acclimation period included the introduction of bait to be used during the experiment (a mixture of mussel, clam, crab, and razor clam) and daily interaction with handlers and the equipment used during the experiment. This included opening the tank lid and testing water parameters. The fish, which had a mean length of 32.90 ± 3.96 cm, a mean weight of 608.14 ± 234.57 g, and a mean Fulton's condition factor of 1.64 ± 0.20 (a measure of the fish's health and body condition), were fasted for 48 hours prior to the beginning of the experiment to increase the likelihood of biting and being hooked.

2.2.3 Materials

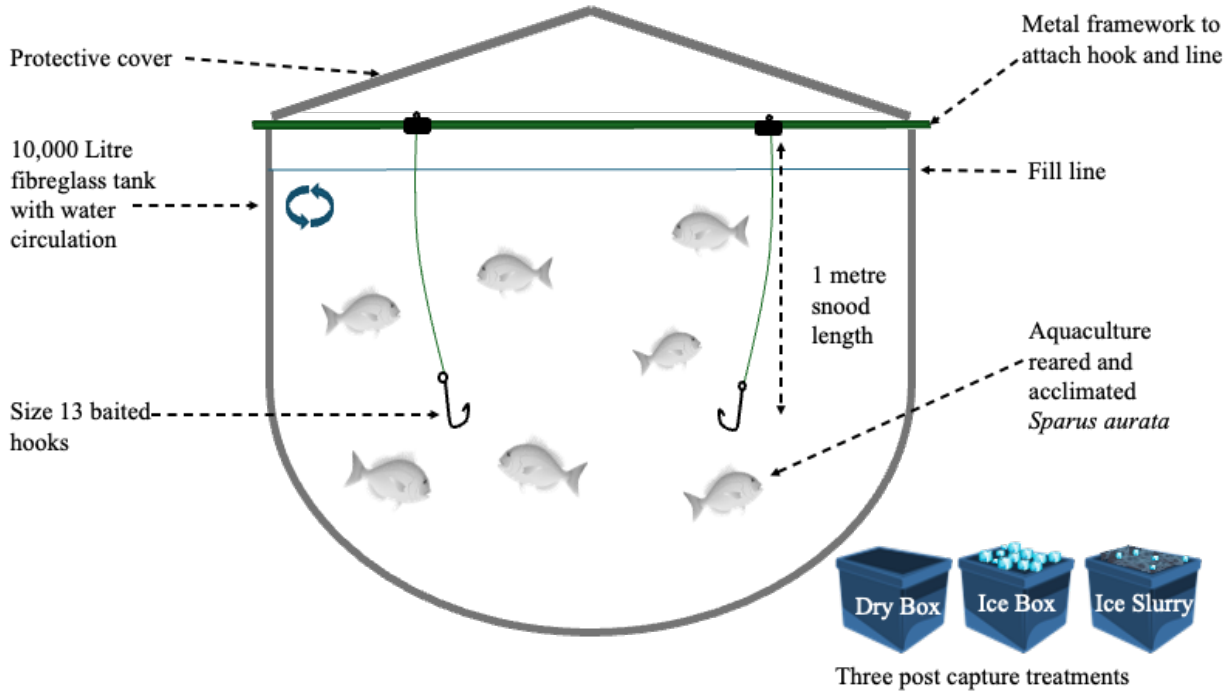

Figure 2-4. Conceptual illustration of the in-house experiment. Aquaculture-reared *Sparus aurat*a were housed in a 10,000-litre fibreglass tank with water circulation. Hooks baited with local bait were suspended 1 metre into the tank. Post-capture treatments included (i) a dry box, (ii) an ice box, and (iii) an ice slurry (50:50 ice and seawater mixture).

2.2.4 Water parameters

Four water parameters were measured during the fish acclimation stage and on experiment days. Dissolved oxygen percentage, oxygen PPM (parts per million), and temperature were recorded using the same Dissovled Oxygen Meter, the VWR OX4110H. This model was changed on 18th June, halfway through the experiment period, due to a malfunction and was replaced by YSI PRO 20 for the remainder of the experiments. The fourth parameter measured was salinity (‰), which was assessed using the VWR EC300 salinity meter. Although not directly related to in this thesis, checking the water parameters allowed for handlers to monitor the water quality and health of fish, whilst the first were acclimated to the handlers.

2.2.5 Experiment day

On the day of the experiment, the tank was set up as displayed in Figure 2-4. Conceptual illustration of the in-house experiment. Aquaculture-reared *Sparus aurat*a were housed in a 10,000-litre fibreglass tank with water circulation. Hooks baited with local bait were suspended 1 metre into the tank. Post-capture treatments included (i) a dry box, (ii) an ice box, and (iii) an ice slurry (50:50 ice and seawater mixture).. Cameras rolling and time noted; the hooks were baited and dropped into the tank simultaneously. While viewing through the tank window, the hooks were observed until one of three things occurred: the fish removed the bait from the hook without getting hooked, in which case the hook was removed without lifting the tank lid, rebaited, and readded to the tank; the hook was left in the tank for more than 10 minutes without any interest from the fish, at which point the hook would be removed, rebaited with an alternative bait without lifting the tank lid, and readded to the tank; or a successful hooking occurred, meaning a fish had taken the bait and was hooked on the line.

Fish hooked on the line were left to soak for a predetermined time, either 30 or 90 minutes. At the end of this period, the specimen was removed from the water with a net, and both the vitality assessment and blood physiology were conducted using the same methodology as in the vessel fieldwork (Table 2-2 and section 2.1.3.3 respectively). The fish was immediately placed in one of three demise boxes: ice, ice slurry (approximately 50:50 ice to salt water), or a dry box with no ice. Vitality was assessed, and blood samples were taken every 15 minutes for 1 hour. The experimental process was organised and executed according to the timeline summarised in Table 2-3, detailing each step and corresponding time intervals.

Table 2-3. Chronology of experiment design.

Time Interval	Activity/Process	Notes
Start of day	Preparation Phase	- Arrival of researchers. - Tank inspection for water quality. - Equipment setup.
Experiment start	Initiating Hooking Process	- Baiting and hooking begin. - Monitoring of fish behaviour. - Timer starts.
T0 (End of Soak)	Post-Capture Start	- Fish removed from hook at 30 or 90 minutes. - Initial blood sample drawn.
	Vitality Assessment	- Fish rated on the vitality scale (1 to 4).
	Post-Capture Treatment Assigned	- Fish placed into: - Dry box (no water or ice). - Ice box (fish in ice). - Ice slurry (fish in 50:50 ice-water).
T1 (15 minutes post)	Blood Sampling & Vitality Recheck	- Second blood sample drawn. - Vitality reassessed.
T2 (30 minutes post)	Blood Sampling & Vitality Recheck	- Third blood sample drawn. - Vitality reassessed.
T3 (60 minutes post)	Final Blood Sampling & Vitality Recheck	- Fourth and final blood sample drawn. - Final vitality score.
	Experiment Conclusion	- Data compiled for blood analysis (glucose, lactate, cortisol, osmolality). - Clean-up and prep for next experiment day.
End of day	Data Compilation & Blood Storage	- Data reviewed and finalised for all samples. - Blood samples stored for analysis.

2.3 Statistical analysis

The data obtained were analysed statistically using RStudio (RStudio Team, 2025). Normality of the data was assessed using the Shapiro-Wilk test, while Levene's test was applied to evaluate the homogeneity of variances. Given that the data met the assumptions of normality and homogeneity, a one-way analysis of variance (ANOVA) was conducted to compare the means of each species across the different time points. When ANOVA indicated significant differences, Tukey's Honest Significant Difference (HSD) post-hoc test was applied to determine specific pairwise differences between groups. For non-normally distributed data, a Kruskal-Wallis test was used as a non-parametric alternative to ANOVA, followed by Dunn's post-hoc test with Bonferroni correction to account for multiple comparisons.

An alpha level of 0.05 was set for all analyses, with differences considered statistically significant at $p < 0.05$. Significant differences between groups are indicated by differing letters above bars or data points, or by asterisks where appropriate, highlighting comparisons with the control group.

All data are presented as mean ± standard error of the mean (s.e.m.) to ensure clarity and transparency in reporting variability. Data visualisation was performed using both Microsoft Excel and ggplot2 package (Yandell & Broman, 2024) within RStudio, enabling consistent and accurate representation of statistical findings through a combination of advanced graphing techniques and straightforward data presentation.

For the in-house experiments, generalised linear models (GLMs) were used to assess the influence of treatment, soak duration, and time point on vitality and physiological variables. Vitality scores were analysed using ordinal logistic regression to account for their ordinal nature. A linear mixed model (LMM) was also applied to account for repeated measures, with time, treatment, and soak duration included as fixed effects and fish ID as a random effect. All GLM and LMM analyses were performed in RStudio (RStudio Team, 2025), using the lme4 package for mixed effects modelling and the ordinal package for ordinal logistic regression. These approaches enabled appropriate analysis of both the data structure and the experimental design.

Chapter 3 Results

3.1 On vessel Results

This section presents the results of the on-vessel data collection, focusing on fish vitality and physiological parameters (glucose, cortisol, lactate, osmolality) across different species and time intervals: T1 (arrival on board), T2 (1 hour post capture), T3 (2 hours post capture). The data reflects the physiological stress responses of different fish species following their capture in longline fisheries.

3.1.1 Effect of Time on Vitality

Vitality scores declined significantly over time (Figure 3-1), with the most rapid reduction occurring between T1 (arrival on board) and T2 (1 hour post-capture). At T1, fish exhibited high vitality scores (mean: 3.33 ± 0.11), but by T2, vitality had declined substantially (mean: 1.89 ± 0.14). By T3 (2 hours post-capture), nearly all fish were unresponsive, with a mean vitality score of 1.08 ± 0.05.

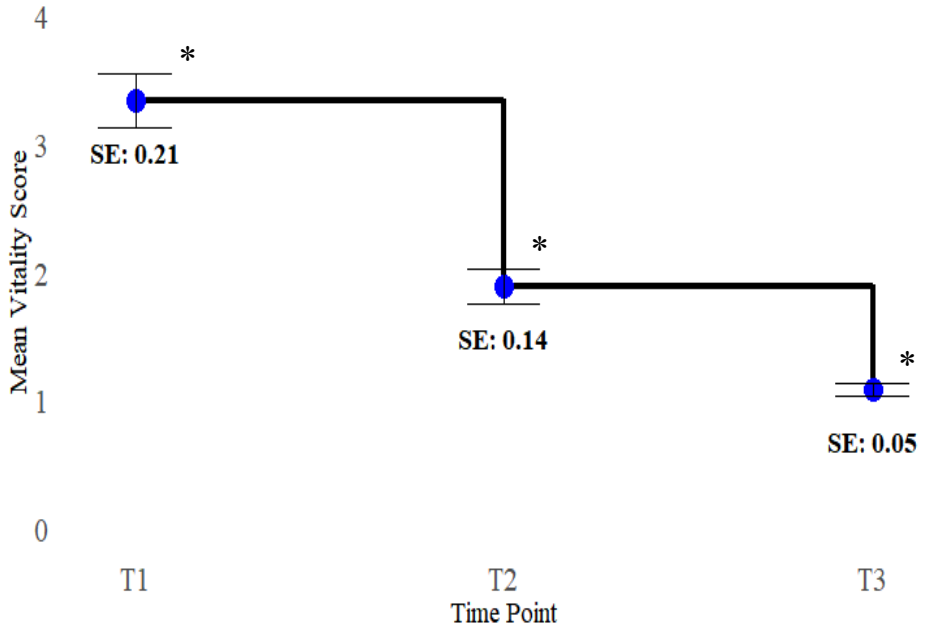

Figure 3-1 Step plot illustrating vitality decline over time of all species. Mean vitality scores (± SE) for T1 (arrival on board), n = 120, T2 (1 hour post-capture), n = 120, and T3 (2 hours post-capture), n = 120. Standard error values are displayed below each point. An asterisk denotes values that are significantly different to both other times points.

A Kruskal-Wallis test confirmed a significant effect of time on vitality ($\chi^2 = 222.66$, df = 2, $p < 0.001$). Post-hoc Dunn's tests revealed significant differences between all time points (T1 vs. T2, T1 vs. T3, T2 vs. T3, all $p < 0.001$). This consistent decline highlights the dominant effect of time post-capture on vitality loss, irrespective of species differences (Figure 3-1).

3.1.2 Vitality Differences Across Species

Given the significant impact of time on vitality, further analysis was conducted to determine whether species exhibited different rates of decline. Mean vitality scores for *Diplodus sargus* (DS, n = 23), *Diplodus vulgaris* (DV, n = 57), and *Pagellus erythrinus* (PE, n = 40) were compared across all time points. At T1, mean vitality scores were similar across species (DS: 3.21 ± 0.21, DV: 3.30 ± 0.12, PE: 3.47 ± 0.13), indicating no immediate species-specific response upon capture. By T3, all species had declined to similar near-zero vitality levels (DV: 1.11 ± 0.04, DS: 1.20 ± 0.10, PE: 1.00 ± 0.00), demonstrating a uniform deterioration in vitality over time.

A Kruskal-Wallis test comparing species across all time points found no significant effect of species on vitality decline ($\chi^2 = 2.71$, df = 2, $p = 0.258$). These results suggest that species identity did not significantly influence the rate of vitality loss, reinforcing the conclusion that time post-capture is the primary driver of vitality decline (Figure 3-2).

3.1.3 Vitality Comparisons Within Each Time Point

To explore whether species exhibited different responses at specific time points, separate Kruskal-Wallis tests were conducted for T1, T2, and T3.

- T1 (Arrival on board): Vitality scores were high across all species, with a median of 4. No significant difference was detected ($\chi^2 = 1.56$, df = 2, $p = 0.458$).
- T2 (1 hour post-capture): A marked decline in vitality was observed, with *P. erythrinus* showing the sharpest drop. However, no significant difference was found between species ($\chi^2 = 2.57$, df = 2, $p = 0.276$).
- T3 (2 hours post-capture): By this stage, all *P. erythrinus* individuals were unresponsive, and species vitality scores converged. A Kruskal-Wallis test showed a weak trend toward significance ($\chi^2 = 4.72$, df = 2, $p = 0.094$), but post-hoc Dunn's tests failed to identify significant species differences after Bonferroni correction.

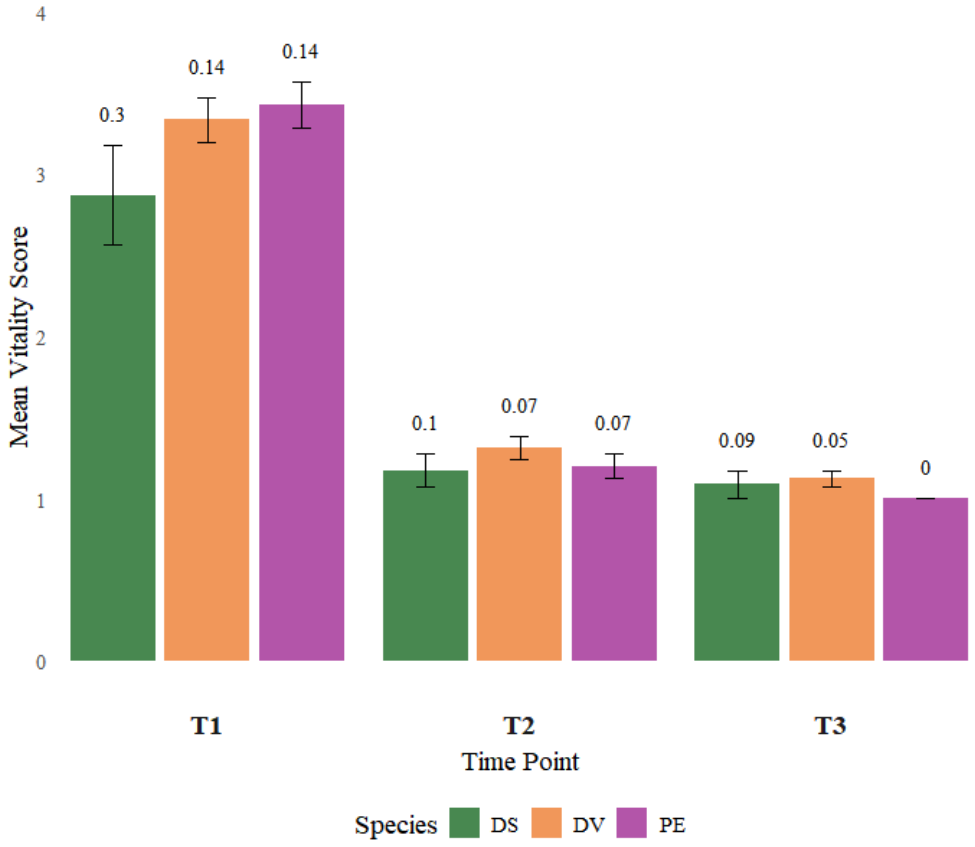

Figure 3-2 Mean vitality scores (± SE) of three fish species across different time points (T1, T2, and T3). Bars represent the mean vitality score for ***Diplodus sargus*** **(n=23),** ***Diplodus vulgaris*** **(n=57),** and ***Pagellus erythrinus*** **(n=40),** with error bars indicating the standard error (SE). Vitality scores were assessed at three time points: T1 (arrival on board), T2 (1 hour post-capture), and T3 (2 hours post-capture). The SE values are displayed above the error bars for clarity. X-axis facet labels indicate the time points, while species colours are consistent throughout. Lack of significant difference is shown by the absence of asterisks.

3.1.4 Summary of Vitality Results

These findings demonstrate that time had a significant effect on vitality, while species did not significantly influence the rate of decline. The sharp decline in vitality across all species sampled suggests that post-capture conditions drive vitality loss, rather than species-specific resilience.

3.1.5 Vitality and physiology

Physiological markers—glucose, cortisol, lactate, and osmolality—were measured for *D. sargus, D. vulgaris,* and *P. erythrinus* at the same time intervals as the vitality assessments (T1, T2, and T3). These parameters were analysed in conjunction with vitality scores to assess metabolic and stress responses following longline capture. The results provide insight into physiological changes over time, which are presented in the following sections.

3.1.5.1 Diplodus sargus

Statistical analysis confirmed significant changes in physiological parameters over time, reflecting an escalating stress response in *D. sargus* following capture. The largest physiological shifts were observed between T1 and T2, aligning with the most pronounced drop in vitality (Figure 3-3).

Glucose

As shown in Figure 3-3, glucose levels exhibited a downward trend over time, declining from 3.72 ± 0.34 mmol/L at T1 to 3.10 ± 1.13 mmol/L at T2, and further to 2.20 ± 0.67 mmol/L at T3. However, a Kruskal-Wallis test did not detect a statistically significant difference across time points ($\chi^2 = 4.95$, $p = 0.084$), suggesting that energy depletion was gradual rather than occurring in a specific interval.

Cortisol

Cortisol levels increased over time, from 189.1 ± 26.36 ng/mL at T1 to 275.8 ± 40.45 ng/mL at T2, and peaking at 285.1 ± 88.44 ng/mL at T3 (Figure 3-3). However, the Kruskal-Wallis test found no significant effect of time on cortisol levels ($\chi^2 = 1.81$, $p = 0.405$), suggesting that while cortisol increased, this change was not statistically pronounced.

Lactate

Lactate levels increased significantly over time, from 4.9 ± 0.64 mmol/L at T1 to 10.8 ± 1.38 mmol/L at T2, and reaching 11.1 ± 1.73 mmol/L at T3 (Figure 3-3). A Kruskal-Wallis test identified a highly significant effect of time on lactate levels ($\chi^2 = 14.86$, $p = 0.0006$). Dunn's post-hoc test with Bonferroni correction indicated that lactate was significantly higher at T2 compared to T1 ($Z = -3.31$, $p = 0.003$) and at T3 compared to T1 ($Z = -2.99$, $p = 0.008$), while no significant difference was observed between T2 and T3 ($Z = -0.05$, $p = 1.000$).

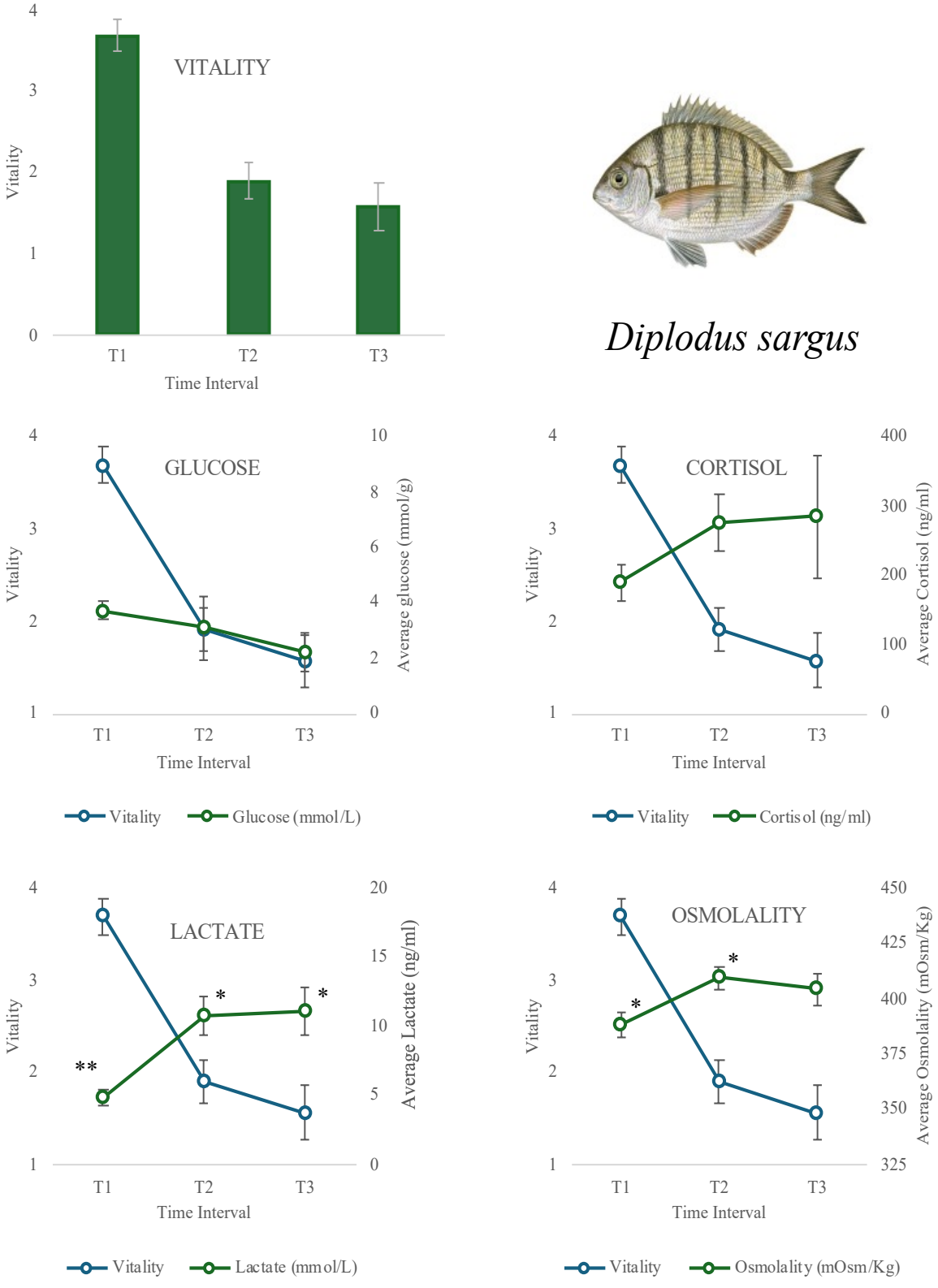

Figure 3-3 Changes in vitality and physiological parameters across different time intervals for *D. sargus*. The bar graph (top left) shows the decline in vitality index over time. **Glucose** (mmol/L, middle left), **Cortisol** (µg/mL, middle right), **Lactate** (mmol/L, bottom left), and **Osmolality** (mOsm/Kg, bottom right) are plotted against time intervals (T1 (n = 16), T2 (n = 10), T3 (n = 7), along with the corresponding vitality index. Error bars represent standard error of the mean (SEM) for each measurement. The image of the fish (*Diplodus sargus*) is shown for species identification (Image credit: Scandinavian Fishing Year Book). * = significant difference from one other time point; ** = significant difference from two.

Osmolality

Osmolality significantly increased over time ($F(2,30) = 4.1$, $p = 0.0267$), rising from 387.87 ± 5.56 mOsm/kg at T1 to 409.60 ± 5.18 mOsm/kg at T2, before slightly decreasing to 404.57 ± 7.25 mOsm/kg at T3 (Figure 3-3). Tukey's post-hoc test confirmed that osmolality was significantly higher at T2 compared to T1 ($p = 0.0297$), while no significant differences were found between T3 and the other time points (T1-T3: $p = 0.1784$; T2-T3: $p = 0.8586$).

The physiological data presented in Figure 3-3 demonstrate a progressive stress response in *D. sargus* following capture. The most significant physiological shifts occurred between T1 and T2, coinciding with a steep decline in vitality. Lactate and osmolality exhibited statistically significant increases, while cortisol and glucose did not show significant variation over time. The stabilisation of certain parameters between T2 and T3 suggests that the fish had reached a physiological threshold, beyond which metabolic and osmotic stress no longer escalated.

3.1.5.2 Diplodus vulgaris

Physiological parameters in *D. vulgaris* changed significantly over time, reflecting increasing physiological stress following capture. The most pronounced changes were observed between T1 and T3, with shifts in glucose, lactate, and osmolality corresponding to declining vitality (Figure 3-4).

Glucose

Glucose levels showed an initial increase from 3.69 ± 0.38 mmol/L at T1 to 4.82 ± 0.59 mmol/L at T2, before declining to 2.53 ± 0.50 mmol/L at T3. A Kruskal-Wallis test confirmed a significant effect of time ($\chi^2(2) = 9.48$, $p = 0.009$). Post-hoc analysis identified a significant difference between T2 and T3 ($p = 0.006$, Bonferroni adjusted), suggesting glucose depletion over time.

Cortisol

Cortisol levels followed a fluctuating pattern, increasing from 147.6 ± 35.49 ng/mL at T1 to 160.0 ± 27.95 ng/mL at T2, before decreasing to 121.0 ± 26.14 ng/mL at T3. A Kruskal-Wallis test found no significant effect of time ($\chi^2(2) = 1.21$, $p = 0.545$), indicating that while levels varied, the changes were not statistically pronounced.

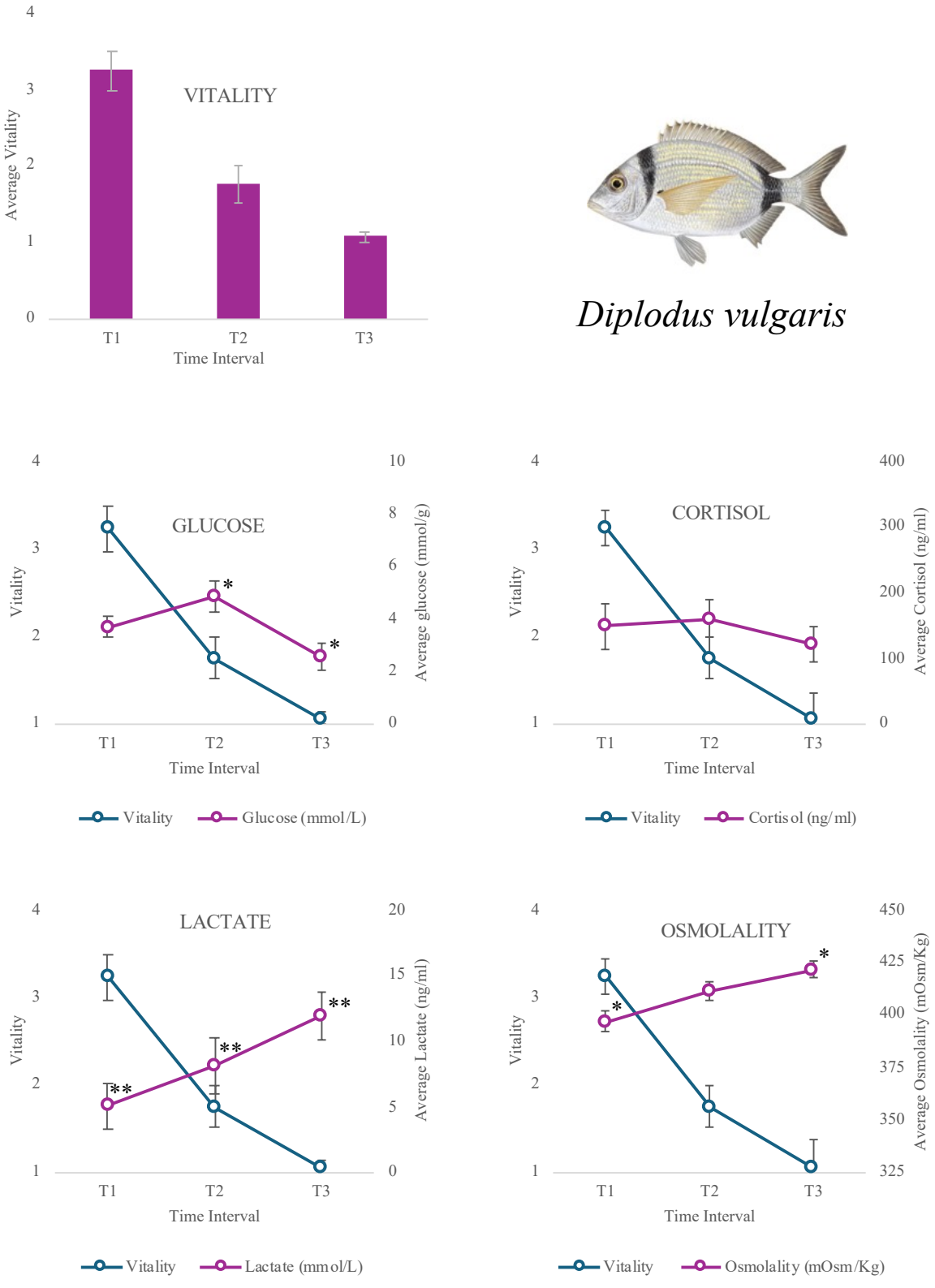

Figure 3-4 Changes in vitality and physiological parameters across different time intervals for *D. vulgaris*. The bar graph (top left) shows the decline in vitality index over time. **Glucose** (mmol/L, middle left), **Cortisol** (µg/mL, middle right), **Lactate** (mmol/L, bottom left), and **Osmolality** (mOsm/Kg, bottom right) are plotted against time intervals (T1 (n = 22), T2 (n = 16), T3 (n = 16)), along with the corresponding vitality index. Error bars represent standard error of the mean (SEM) for each measurement. Image of the fish (*Diplodus vulgaris*) is shown for species identification (Image: Scandinavian Fishing Year Book). * = significant difference from one other time point; ** = significant difference from two.

Lactate

Lactate levels increased steadily across all time points, rising from 5.01 ± 0.60 mmol/L at T1 to 8.00 ± 0.68 mmol/L at T2 and reaching 11.91 ± 0.94 mmol/L at T3. A one-way ANOVA confirmed a significant effect of time ($F(2,51) = 23.22$, $p < 0.001$). Tukey's post-hoc test revealed significant differences between all time points (T1-T2: $p = 0.012$, T1-T3: $p < 0.001$, T2-T3: $p = 0.002$), indicating progressive reliance on anaerobic metabolism.

Osmolality

Osmolality increased over time, from 396.62 ± 4.90 mOsm/kg at T1 to 411.00 ± 4.53 mOsm/kg at T2, peaking at 421.00 ± 3.80 mOsm/kg at T3. A Kruskal-Wallis test confirmed a significant effect of time ($\chi^2(2) = 13.17$, $p = 0.001$), with Dunn's post-hoc test identifying a significant difference between T1 and T3 ($p = 0.001$, Bonferroni adjusted).

Physiological changes in *D. vulgaris* followed a time-dependent pattern, with significant increases in lactate and osmolality, indicating escalating metabolic and osmotic stress. Glucose levels initially increased before declining, while cortisol levels fluctuated without statistical significance. These results align with a progressive physiological response to capture and exposure to air.

3.1.5.3 Pagellus erythrinus

Statistical analysis for *Pagellus erythrinus* indicated no significant changes in physiological parameters across time points, likely due to the small sample size. However, as shown in Figure 3-5, trends in physiological markers suggest ongoing metabolic stress and physiological deterioration following capture.

Glucose

Levels of glucose exhibited a downward trend over time, declining from 5.92 ± 1.09 mmol/L at T1 to 4.04 ± 1.49 mmol/L at T2, and further to 3.96 ± 1.60 mmol/L at T3. A one-way ANOVA did not detect a statistically significant difference across time points (*$F(2,10) = 0.70$, $p = 0.519$*), indicating that while energy depletion occurred, the pattern was not strongly time-dependent.

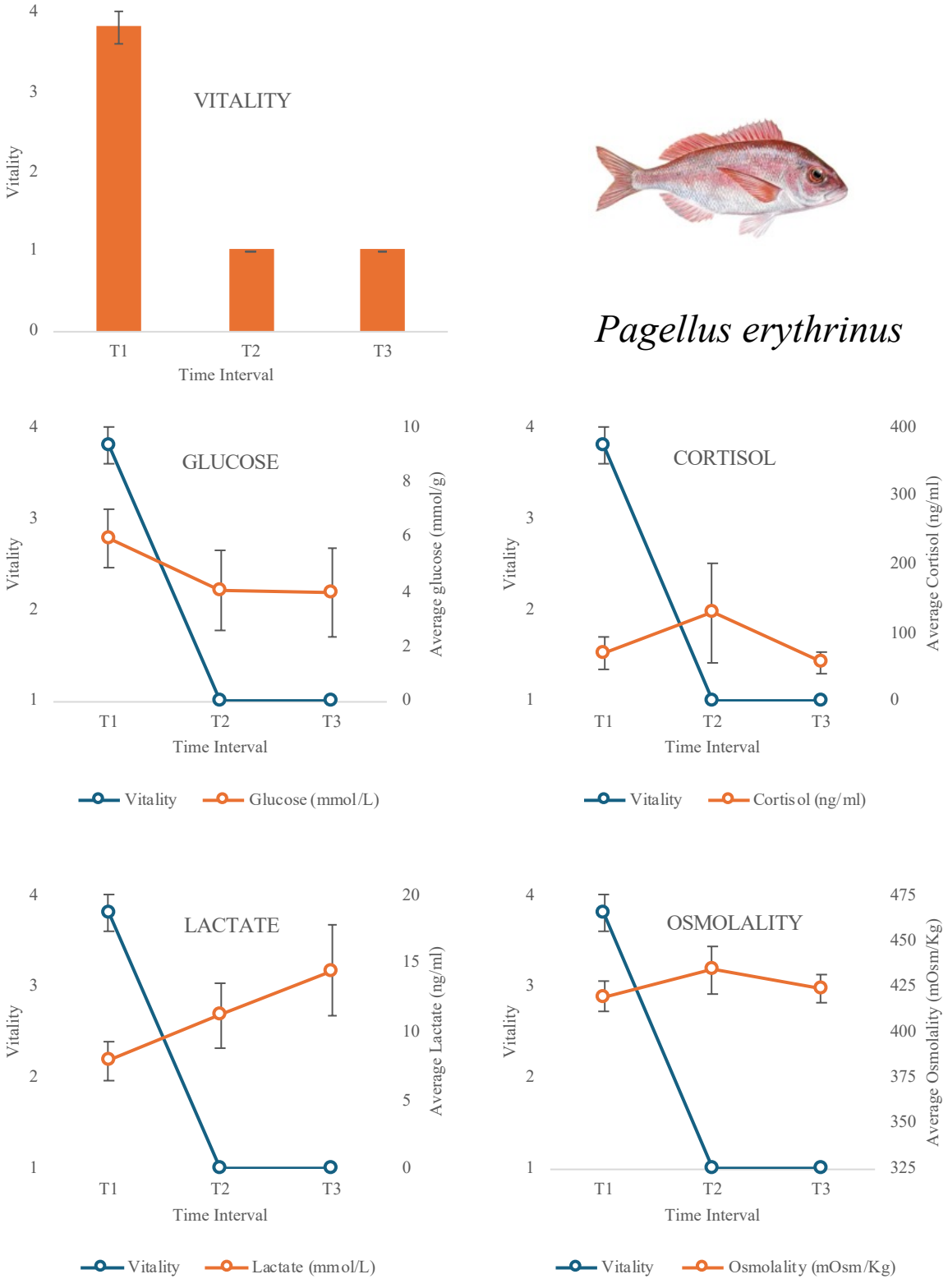

Figure 3-5 Changes in vitality and physiological parameters across different time intervals for *P. erythrinus*. N = 40. The bar graph (top left) shows the decline in vitality index over time. **Glucose** (mmol/L, middle left), **Cortisol** (µg/mL, middle right), **Lactate** (mmol/L, bottom left), and **Osmolality** (mOsm/Kg, bottom right) are plotted against time intervals (T1 (n = 5), T2 (n = 4), T3 (n = 4)), along with the corresponding vitality index. Error bars represent standard error of the mean (SEM) for each measurement. The image of the fish is shown for species identification (Image credit: Scandinavian Fishing Year Book). * = significant difference from one other time point; ** = significant difference from two.

Cortisol

Cortisol levels displayed high variability, fluctuating from 70.05 ± 25.07 ng/mL at T1 to 128.63 ± 71.32 ng/mL at T2, before decreasing to 56.30 ± 15.53 ng/mL at T3. A Kruskal-Wallis test revealed no significant differences across time points ($\chi^2(2) = 0.30$, $p = 0.860$), though the variability suggests individual differences in stress response. The decline at T3 may indicate physiological exhaustion, where cortisol production was no longer sustained.

Lactate

Lactate levels showed a notable increase over time, rising from 7.85 ± 1.36 mmol/L at T1 to 11.17 ± 2.43 mmol/L at T2, and peaking at 14.42 ± 3.31 mmol/L at T3. Although ANOVA did not detect a significant effect of time ($F(2,10) = 1.94$, $p = 0.194$), the effect size ($\eta^2 = 0.28$) suggested a moderate increase in anaerobic metabolism as oxygen deprivation progressed.

Osmolality

Values in osmolality followed a similar increasing trend, with initial levels at 419.20 ± 7.89 mOsm/kg at T1, rising to 433.75 ± 13.29 mOsm/kg at T2, before slightly decreasing to 423.75 ± 7.75 mOsm/kg at T3. ANOVA confirmed no statistically significant effect ($F(2,10) = 0.59$, $p = 0.573$), but the persistently elevated values indicate prolonged osmotic stress.

Overall, *P. erythrinus* exhibited physiological changes consistent with stress, similar to that of *D. sargus* and *D. vulgaris*. All individuals of this species reached a vitality score of 1 (non-responsive) by T2, but statistical analyses did not confirm a significantly different trajectory of physiological decline compared to the other species. The limited sample size likely reduced statistical power, and future studies with larger datasets may provide a clearer understanding of the physiological responses in *P. erythrinus* following capture.

3.1.5.4 Summary

Physiological responses to capture varied among *D. sargus*, *D. vulgaris*, and *P. erythrinus*, with lactate and osmolality increasing over time, while glucose and cortisol showed no clear trends. *P. erythrinus* reached a vitality score of 1 earlier than the other species, though physiological markers did not indicate a significantly different response. The absence of statistical significance across most parameters suggests that physiological changes followed a similar pattern among species.

3.2 In-house experiment

The in-house experiments were designed to assess both the vitality and physiological responses of gilthead bream subjected to different post-capture treatments and soak times post hooking. Fish were exposed to three conditions following capture: a dry box, an ice box, and an ice slurry. These treatments were examined under both 30-minute and 90-minute soak times, with an additional control group for comparison. Unlike the sea experiments, vitality and physiological parameters were recorded at four time points: T0 (immediately after removal from the tank), T1 (15 minutes after T0), T2 (30 minutes after T0), and T3 (45 minutes after T0).

Vitality, measured on a scale from 1 (unresponsive) to 4 (highly active), the same index used in the sea trials and shown in Table 2-2, allowed for an assessment of the immediate behavioural responses and stress experienced by the fish following capture. The decline in vitality over time across the different treatments provides an indication of how rapidly stress factors impacted the fish. The accompanying physiological measurements – including glucose, osmolality, lactate, and cortisol – were used to further quantify the metabolic and stress responses to these treatments. By integrating these vitality and physiological data, the experiments aimed to offer a comprehensive overview of the post-capture stress dynamics in gilthead bream across different handling scenarios.

3.2.1 Vitality

Vitality scores across all treatments exhibited a progressive decline from T0 to T3, with all groups experiencing a substantial reduction in vitality over time (Figure 3-6). The mean vitality scores were initially high, with most fish scoring 4 at T0, but values progressively decreased at subsequent time points.

Effect of Treatment (Dry, Ice & Slurry) on Vitality

A Kruskal-Wallis test was conducted to determine whether vitality differed significantly between storage conditions (Dry, Ice, and Slurry). The results indicated no statistically significant difference between treatments ($\chi^2 = 0.216$, $df = 2$, $p = 0.898$), suggesting that storage condition had no meaningful effect on vitality loss.

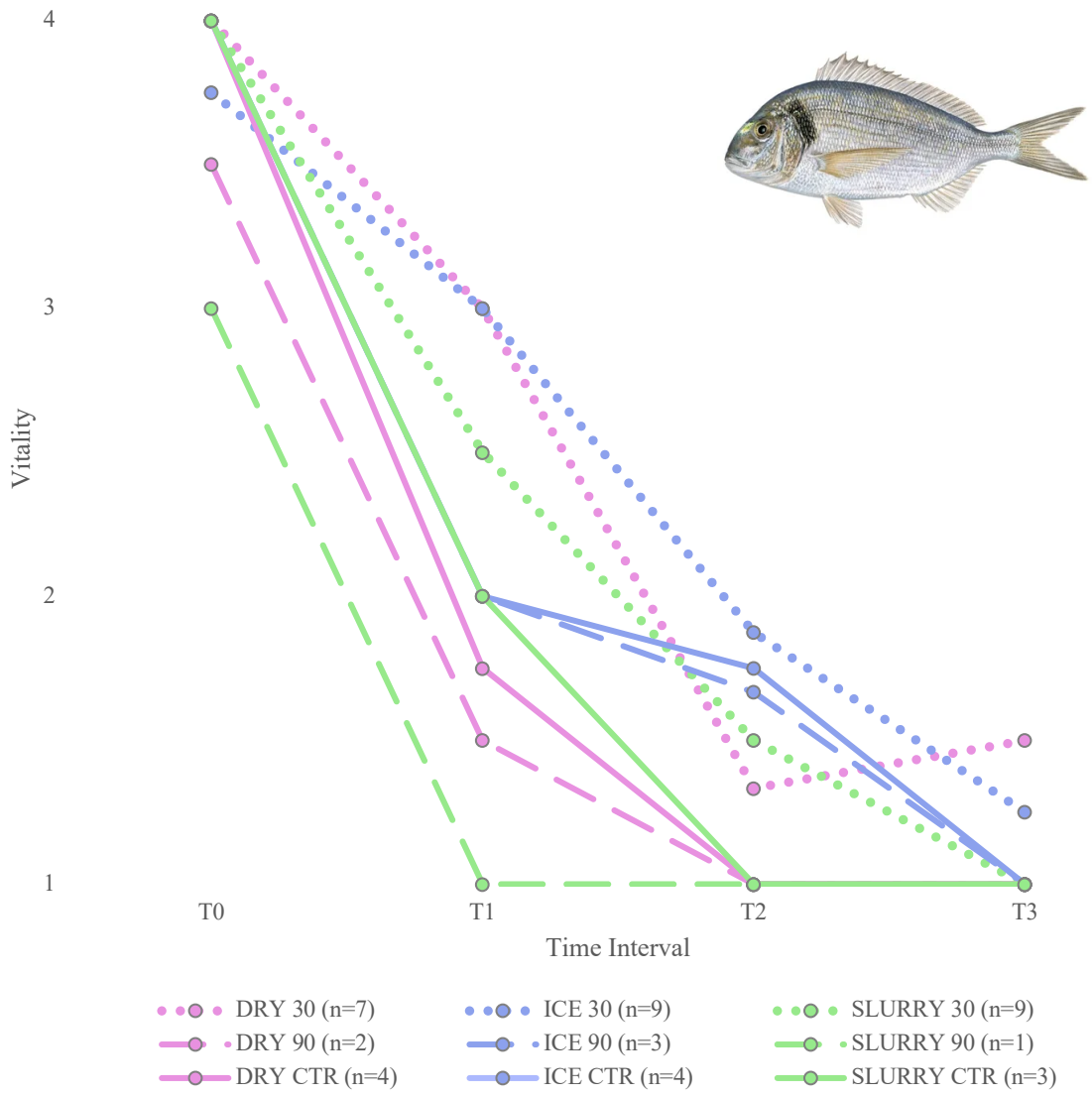

Figure 3-6 Changes in fish vitality over time under different post capture conditions and soak times. The change in vitality across four time intervals (T0, T1, T2, and T3). Pink lines indicate dry conditions, blue lines indicate ice treatments, and green lines indicate ice slurry treatments. Solid lines represent a 90-minute soak time, dashed lines represent the control group, and dotted lines correspond to the 30-minute soak time. Sample size (n) for each group are shown in the legend. Vitality declined significantly over time across all groups (LMM, $p < 0.001$). The image of the fish (*Sparus aurata*) is shown for species identification purposed. Image credit: Scandinavian Fishing Year Book)

Effect of Soak Time (0, 30 & 90) on Vitality

Similarly, a Kruskal-Wallis test was performed to examine the influence of soak time on vitality. The test yielded no significant differences between 0, 30, and 90-minute soak times ($\chi^2 = 3.429$, $df = 2$, $p = 0.18$), indicating that soak duration did not significantly impact vitality scores over time.

Vitality at T0 across soak times

To determine whether soak time influenced initial vitality, a Kruskal-Wallis test was performed using only T0 data. The results showed no significant difference in vitality scores between soak times at T0 ($\chi^2 = 3.997$, df = 2, $p = 0.135$). This indicates that initial vitality is similar across soak durations, reinforcing the finding that soak time does not significantly influence vitality at the moment of capture.

Generalised Linear Mixed Model (LMM) Analysis

A Linear Mixed Model (LMM) was employed to account for repeated measures and assess potential interactions between Time, Soak Time, and Treatment. The model confirmed a significant time effect, with vitality decreasing at each time point:

- T1: $\beta = -2.00$, $p < 0.001$
- T2: $\beta = -3.00$, $p < 0.001$
- T3: $\beta = -3.00$, $p < 0.001$

However, neither soak time nor treatment significantly influenced vitality, and there were no strong interaction effects between time, soak, and treatment.

Ordinal Logistic Regression

Given that vitality scores are ordinal (ranging from 1 to 4), an Ordinal Logistic Regression model was also applied. The results further supported the significant effect of time, with vitality scores declining as time progressed. However, soak time and treatment did not show any significant effects, confirming the findings from both the Kruskal-Wallis and LMM analyses.

Summary of findings

Overall, the results indicate that vitality declines significantly over time, regardless of storage condition or soak time. Neither the treatment type (Dry, Ice, Slurry) nor the soak duration (0, 30, 90 min) significantly affected vitality scores. These findings suggest that post-capture handling conditions had minimal influence on the rate of vitality decline, implying that factors such as air exposure, stress, or individual variation may play a more dominant role in determining fish vitality post-hooking.

3.2.2 Physiology

Physiological responses to post-capture stress were assessed by measuring glucose, lactate, osmolality, and cortisol over time (T0 (at capture), T1(15 minutes post), T2 (30 minutes post), T3 (45 minutes post)) across different soak times (0, 30, 90 min) and post-capture treatments (Dry, Ice, Slurry). Figure 3-7 provides an overview of these trends, illustrating how physiological stress markers varied in response to soak duration and post-capture conditions. Solid lines represent physiological parameters, while dashed lines indicate corresponding vitality scores. Statistical analyses were conducted to determine whether treatment and time effects were significant, with Kruskal-Wallis tests assessing overall differences between groups, followed by pairwise Wilcoxon post-hoc comparisons.

Glucose Response

Glucose levels varied significantly between treatments and over time (Kruskal-Wallis, $\chi2 = 6.408$, df = 2, $p = 0.0406$). Post-hoc Wilcoxon tests identified a significant increase in glucose in the Ice treatment between T0 and T1 ($W = 39$, $p = 0.005$), while no significant changes were detected in Dry ($W = 78$, $p = 0.758$) or Slurry ($W = 71$, $p = 0.227$) treatments.

Soak time had a significant effect on glucose levels, with fish in the 90-minute soak group displaying significantly higher glucose concentrations compared to both the 0-minute soak ($p = 0.041$) and 30-minute soak ($p = 0.049$). These trends are shown in Figure 3-7(Row 1), where Ice-treated fish exhibit a sharp initial glucose spike, followed by stabilisation across all treatments.

Osmolality Response

Osmolality levels did not change significantly over time in any treatment (Kruskal-Wallis, $\chi2 = 8.301$, df = 2, $p = 0.016$). However, post-hoc Wilcoxon tests identified a significant difference between Dry and Ice treatments ($W = 55$, $p = 0.037$), while no significant differences were observed between Dry and Slurry ($W = 76$, $p = 1.000$) or Ice and Slurry ($W = 102$, $p = 0.052$).

Soak time had a strong effect on osmolality, with significant differences across all soak durations (0 min vs. 30 min, $p = 0.014$; 0 min vs. 90 min, $p = 0.044$; 30 min vs. 90 min, $p = 0.001$). These trends are shown in Figure 3-7 (Row 2), where osmolality progressively increases with longer soak durations.

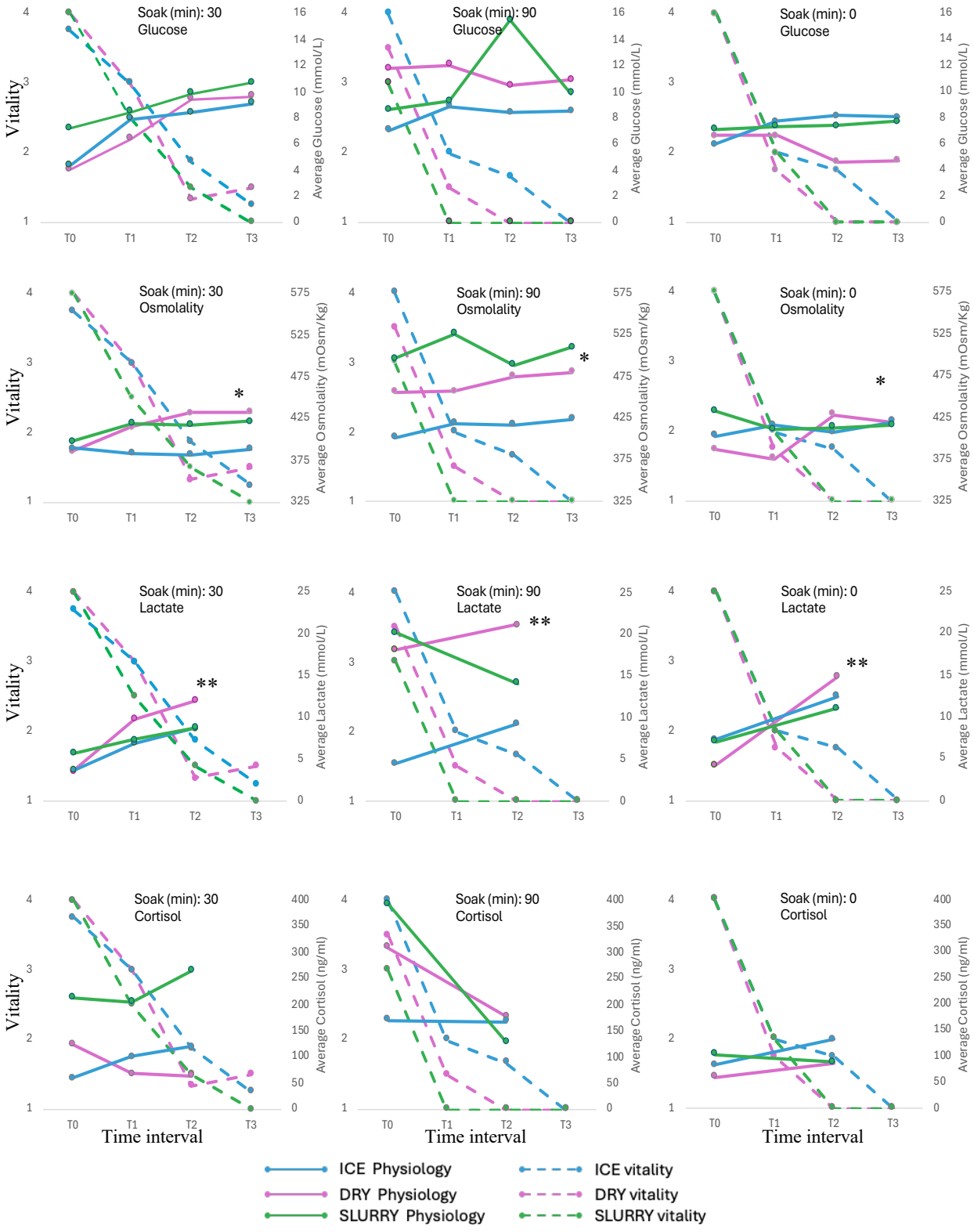

Figure 3-7 Changes in fish vitality and physiological markers (glucose, osmolality, lactate, and cortisol) over time across different soak times, T0, T1, T2 & T3 and post-capture treatments. Each column represents a different experimental condition: **column 1** shows results for a 30-minute soak, **column 2** shows results for a 90-minute soak, and **column 3** represents the control group. The rows correspond to different physiological parameters: **row 1** shows glucose levels and corresponding vitality, **row 2** shows osmolality, **row 3** shows lactate, and **row 4** shows cortisol. Dashed lines represent vitality index, and solid lines represent the physiological parameters. Sample size (n) is denoted in Figure 3-6. * = significant difference from one other treatment; ** = significant difference from two. The colour of the lines dictates post-capture treatments: where pink is **DRY**, blue is **ICE**, and green is **SLURRY**.

Lactate Response

Lactate levels did not differ significantly between treatments overall (Kruskal-Wallis, $\chi2 = 2.231$, df = 2, p = 0.328). However, a significant increase in lactate was observed from T0 to T2 in the Dry and Ice treatments (Dry: W = 16, p = 0.002; Ice: W = 14, p = 0.001), while the increase in the Slurry treatment was not significant (W = 26.5, p = 0.082).

Soak time had a moderate effect on lactate accumulation, with lactate significantly increasing between the 30-minute and 90-minute soak groups (p = 0.026), but no significant difference between the 0-minute and 90-minute soak groups (p = 0.503). These trends are illustrated in Figure 3-7 (Row 3), where all treatments show an upward trajectory in lactate levels, indicating progressive lactate accumulation post-capture

Cortisol Response

Cortisol levels did not change significantly over time in any treatment (Kruskal-Wallis, $\chi2 = 6.134$**,** df = 2, p = 0.046). However, post-hoc Wilcoxon tests revealed a significant difference between Dry and Slurry treatments (W = 28**,** p = 0.025), while no significant differences were detected between Dry and Ice (W = 29**,** p = 0.398) or Ice and Slurry (W = 45, p = 1.000). These trends are illustrated in Figure 3-7 (Row 4), where cortisol levels remain relatively stable over time but differ between Dry and Slurry treatments.

Summary

Glucose levels varied significantly between treatments, with a sharp increase in the Ice treatment at T1 before stabilising, while Dry and Slurry treatments showed no significant changes. Soak time influenced glucose accumulation, with significantly higher levels observed in the 90-minute soak group compared to both the 0-minute and 30-minute soak groups. Osmolality remained stable over time but differed between Dry and Ice treatments, with soak time also having a significant effect, showing a progressive increase across all durations. Lactate levels increased significantly from T0 to T2 in all treatments, with Dry and Ice showing statistically significant increases, while Slurry exhibited a non-significant upward trend. Lactate was also significantly higher in the 90-minute soak group compared to the 30-minute soak group. Cortisol remained stable over time, though post-hoc analysis identified differences between Dry and Slurry treatments. Soak time also influenced cortisol, with significantly higher concentrations in both the 30-minute and 90-minute soak groups compared to the 0-minute soak. These results indicate that glucose and lactate were the most responsive physiological markers to post-capture stress, particularly in Ice and Dry treatments and under longer soak durations, while osmoregulatory and hormonal responses remained more stable.

Chapter 4 Discussion

It is unquestionable that wild-capture fisheries negatively affect the welfare of the fish being caught (Breen et al., 2020), from capture, to handling, to death, welfare is compromised at nearly every stage of their interaction with humans (Waley et al., 2021). Previous research on fish welfare has predominantly centred around fish reared in aquaculture (Barreto et al., 2022), which is understandable as welfare concerns in aquaculture persist throughout the fish's life, rather than beginning as the fish's life is about to end. While the feelings-based and nature-based approaches (Diggles et al., 2011) have their place in the broader discussion of fish welfare, the functional approach was most applicable for this study. The primary aim of this thesis was to evaluate the physiological responses of fish to capture by artisanal longlining in the Algarve. A secondary aim was to investigate the post-capture stress response and vitality loss, with the goal of providing recommendations to improve welfare practices in the fishery.

4.1 Vitality analysis

4.1.1 Capture fishery

During the trials at sea the average vitality of all fish sampled was 3.33 ± 0.12 SE at T1 (time of capture). Meaning that most of the fish caught were vital, showing reaction to touch, regular opercular movement and spasms, with 66.3% of all fish hauled onboard still at vitality 4 (Figure 3-1). Within an hour, the average vitality of all fish had dropped dramatically to 1.89 ± 0.14. At the 2-hour mark, nearly all individuals were unresponsive. Statistical analysis demonstrated a strong effect of time on vitality loss, with post-hoc comparisons revealing significant differences between all intervals ($p < 0.001$). These findings align with existing literature, where prolonged air exposure has been shown to be a major contributor to physiological collapse and mortality in captured fish (Breen et al., 2020; K. B. Davis, 2006; M. W. Davis et al., 2001).

While previous studies have shown that species-specific metabolic traits may influence resilience to capture stress (Barton & Iwama, 1991; Fanouraki et al., 2011; Martínez-Porchas & Martínez-Córdova, 2009), the present study found no significant differences in vitality decline between species. At capture, *Diplodus sargus, Diplodus vulgaris*, and *Pagellus erythrinus* displayed comparable vitality scores, and by T3, all species had converged to near-total loss of responsiveness. Indicating that post-capture conditions, rather than species-specific resilience, were the dominant factors influencing vitality loss.

It is important to note, however, that resilience to stressors is not a sign of higher welfare. Humborstad et al., (2009) found a negative correlation between fish vitality and stress exposure, suggesting that fish that remain active for longer endure prolonged physiological strain. From a welfare perspective, this is problematic, as exposure duration is a critical determinant of stress severity (Huntingford et al., 2006). In this context, improving welfare in longline fisheries does not necessarily mean selecting for fish that resist stress better but rather reducing exposure time to stressors wherever possible.

In the context of artisanal longline fishing in the Algarve, asphyxiation due to air exposure is likely the primary cause of death for captured fish. Air exposure is well-documented as one of the most physiologically demanding aspects of capture, leading to hypoxia, increased anaerobic metabolism, and osmoregulatory imbalance (Figure 4-1) (Cook et al., 2015). During fieldwork excursions, additional probable causes of mortality were observed, including predation by cephalopods while fish remained on the line and injuries from hooks being swallowed and forcefully removed. These findings highlight that while asphyxiation is a dominant factor, a proportion of mortality can also be attributed to physical injuries and predation, which are context-specific to longline fisheries.

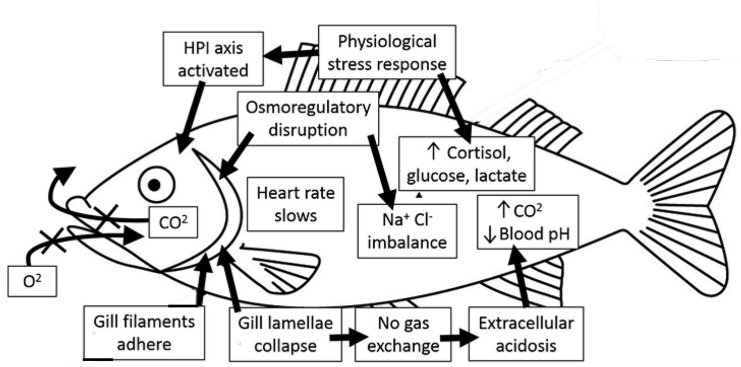

Figure 4-1 Physiological responses of air exposure occurring when fish are removed from water. Adapted from Cook et al., 2015

Another notable observation during the fieldwork was the occurrence of muscle stiffness, or rigor mortis, in a subset of captured fish. Rigor mortis is a well-documented post-mortem physiological process where muscle stiffness arises due to the depletion of adenosine triphosphate (ATP) and the subsequent permanent cross-bridging of actin and myosin filaments in muscle fibres (Martinsdóttir et al., 2009). The onset and progression of rigor mortis can be influenced by stress levels prior to death, with higher stress accelerating the process. Stressful

conditions, such as prolonged air exposure, hypoxia, and physical exhaustion, trigger anaerobic metabolism, leading to rapid ATP depletion and, consequently, faster onset of rigor mortis.

In the context of wild-capture fisheries, the presence of rigor mortis shortly after death is a strong indicator of fish exhaustion and poor welfare, as it reflects high metabolic strain and intense physiological stress experienced by the fish before death. This aligns with findings from Daskalova, 2019) who demonstrated that pre-slaughter stress not only influences muscle biochemistry but also impacts the quality of fish flesh by accelerating rigor mortis onset.

4.1.2 In-house

Current humane slaughter methods in fisheries emphasise rapid and humane euthanasia to minimise stress and suffering. The World Organisation for Animal Health (WOAH) recommends methods such as percussive stunning, electrical stunning, and spiking (ikejime), which aim to render fish unconscious before death occurs (WOAH, 2019). However, in artisanal longlining fisheries such as those in the Algarve, these methods are not feasible due to infrastructure and resource limitations. To address this welfare gap, in-house experiments were conducted to evaluate practical post-capture methods that could bring fish to a vitality score of 1 in the shortest time possible, thereby minimising stress exposure. Additionally, the effect of soak time—the duration fish remain hooked on the line—was assessed to investigate whether prolonged capture duration influenced exhaustion and stress responses, potentially affecting vitality outcomes.

These post-capture treatments were varied between ice box, ice slurry, and dry container conditions, alongside different soak times of 0 (control), 30, and 90 minutes. Slaughter by ice slurry or air exposure is a commonly used method in farmed *S. aurata*, particularly in commercial aquaculture settings (Robb & Kestin, 2002). However, the welfare implications of these practices have been widely questioned, with Van De Vis et al., (2003) describing them as inhumane. The results presented in Figure 3-6 indicate that while vitality scores declined significantly over time across all treatments, there was no statistically significant difference between dry, ice, and ice slurry conditions ($p = 0.898$) or soak times ($p = 0.18$). This suggests that none of the tested methods provided a clear welfare benefit, highlighting a potential welfare gap in artisanal longline practices where humane slaughter techniques are not available.

More recent research by Van Pelt et al., (2024) further criticises traditional methods such as live chilling in ice or ice slurry for European seabass and seabream, stating that such practices prolong consciousness, thereby causing significant stress. Their study found that fish

could remain conscious for up to 40 minutes, a time frame consistent with our study, where some fish had yet to reach vitality stage 1 by T3 (45 minutes post-capture) (Figure 3-6). This comparison underscores the limitations of passive methods in achieving rapid unconsciousness.

The duration of soak time—the length of time fish remains hooked on the line before being retrieved on board—is a crucial factor influencing welfare outcomes in longline fisheries. Prolonged soak times have been associated with increased stress, higher mortality rates, and reduced vitality as fish experience extended periods of hypoxia, physical exhaustion, and predation risk (Veldhuizen et al., 2018). In this study, soak times of 0, 30, and 90 minutes were tested alongside different post-capture treatments, yet no statistically significant differences in vitality scores were observed ($p = 0.18$).

This finding contrasts with previous studies, which generally indicate that extended soak times are more detrimental to fish welfare (T. Ellis et al., 2012; Kerstetter & Graves, 2008; Waley et al., 2021), with maximum soak duration often used as a predictor of post-discard mortality (Broadhurst et al., 2009). A potential explanation for this discrepancy lies in the relatively short soak times used in this experiment. In small demersal longline operations, soak times can be as short as 2–4 hours, allowing livelier fish to be returned to the sea alive and bycatch survival rates to remain high (J. R. Ellis et al., 2008). Additionally, smaller fish species are more susceptible to capture mortality (Diaz & Serafy, 2005) and are more likely to be preyed upon by scavengers following discarding (J. R. Ellis et al., 2008). These factors may contribute to the observed outcomes, suggesting that both soak time and fish size are important considerations in welfare assessments for artisanal longline fisheries.

The absence of a significant soak time effect in this study may also indicate that once fish are removed from the water, their vitality is primarily influenced by air exposure and post-capture handling practices, rather than by the initial duration on the line. Alternatively, stress accumulated during the soak period may have been sufficient to compromise vitality at T0, potentially masking differences between soak times at later stages. However, when comparing vitality as T0, no significant difference was seen between soak times ($p = 0.135$). These findings align with Robb & Kestin, (2002) who observed that in capture fisheries, handling stress post-retrieval often overrides pre-existing stress from soak times, emphasising the importance of rapid and humane slaughter methods to improve welfare outcomes. In artisanal longline fisheries, where humane slaughter methods are often not feasible, reducing soak times may still provide welfare benefits, but only if paired with improved on-board handling practices that limit air exposure and accelerate the transition to vitality 1.

Overall, these findings highlight that while soak time alone may not dictate welfare outcomes, the integration of rapid handling practices and minimisation of air exposure are critical to improving welfare in artisanal longline fisheries

Vitality has been shown to be a good indicator of fish welfare, particularly under challenging sea conditions where blood cannot be drawn (Anders et al., 2023). But when able, analysing blood allows for the evaluation of physiological stress markers, another facet of the functional welfare approach.

4.2 Physiological analysis

The physiological markers observed in this study offer crucial insights into the stress response of Sparids during capture, post-capture handling, and slaughter. These biomarkers: glucose, lactate, cortisol and osmolality, are widely recognised indicators of physiological stress in fish (K. B. Davis, 2006), and their fluctuations provide a window into how different treatments and soak times influence the welfare of the fish.

Glucose

Glucose, a critical energy source for all living things, typically rises in response to stress. This is due to the catecholamine hormones adrenaline and noradrenaline being released during ‘stressful’ situations (Reid et al., 1998). These hormones stimulates liver glycogenolysis, breaking down stored glycogen into glucose (Baruffaldi & Puviani, 1983; K. B. Davis, 2006).

The in-house experiments revealed significant differences in glucose levels between treatments, with the ice treatment showing a sharp increase in glucose concentrations from T0 to T1 ($p = 0.0005$). In contrast, neither the dry ($p = 0.758$) nor the ice slurry ($p = 0.227$) treatments exhibited significant changes in glucose levels over time. Fish subjected to the 90-minute soak displayed higher glucose concentrations compared to the 0-minute (control) and 30-minute soak groups ($p = 0.041$ and $p = 0.049$, respectively). These findings suggest that prolonged soak times, and post-capture ice conditions, contribute to elevated glucose levels, likely due to increased metabolic demand and stress responses triggered by cold exposure. This result going against the findings of Barton & Iwama, (1991), who found the cooling effect to have reduced metabolic stress by slowing down physiological processes.

To assess whether these glucose responses were consistent under real-world conditions, glucose levels were also measured during the at-sea trials, providing insights into how artisanal longline capture influences metabolic stress in different sparid fish species.

At sea, glucose levels exhibited a gradual decline over time across all species tested (*D, sargus, D, vulgaris, P, erythrinus*). Glucose concentrations in *D. sargus* decreased from 3.72 ± 0.34 mmol/L at T1 to 2.20 ± 0.67 mmol/L at T3, with no statistically significant differences across time points ($\chi^2 = 4.95$, $p = 0.084$). In *D. vulgaris*, glucose levels initially increased to 4.82 ± 0.59 mmol/L at T2 before dropping to 2.53 ± 0.50 mmol/L at T3 ($\chi^2(2) = 9.48$, $p = 0.0087$), indicating a metabolic response to capture stress followed by glucose depletion. *P. erythrinus* showed a similar declining trend, with glucose levels falling from 5.92 ± 1.09 mmol/L at T1 to 3.96 ± 1.60 mmol/L at T3, though without statistical significance ($p = 0.519$).

Notably, blood samples during the sea trials were collected at hourly intervals, unlike the quarter-hourly sampling in the in-house experiments, which may have contributed to the observed glucose patterns. The steady decline in glucose concentrations may also reflect the high mortality observed during the sea trials, where nearly all fish were unresponsive (vitality score 1) two hours post-capture, and 58% of individuals reached this state within just one hour (Figure 3-1). This declining trend might indicate that metabolic processes were severely compromised or halted altogether. Additionally, this could be explained by the fact that the blood concentration of glucose depends on the level of glycogen reserves in the tissue, which varies between species and between individuals (Larsson, 1973; Nakano & Tomlinson, 1967). This variability in glycogen reserves might also contribute to the species-specific differences observed in glucose responses, with *D. vulgaris* showing an initial increase while other species exhibited a more consistent decline.

Lactate

Lactate, a byproduct of anaerobic metabolism, and is another key marker of stress (Raposo de Magalhães et al., 2020). Its accumulation indicates a shift towards anaerobic energy production due to oxygen limitation or heightened activity, often seen during the capture process. The in-house experiments demonstrated pronounced treatment-dependent variations in lactate accumulation (Figure 3-7), underscoring the critical role of anaerobic metabolism in mediating physiological stress responses. Fish subjected to dry and ice conditions exhibited a robust and sustained increase in lactate concentrations, with the dry treatment eliciting the most substantial elevation ($p = 0.002$ for dry; $p = 0.001$ for ice). This heightened lactate response suggests an intensified reliance on anaerobic glycolysis, potentially driven by prolonged air exposure (dry treatment) and the physiological shock associated with rapid cooling (ice treatment). Air exposure is known to be a severe stressor as it makes the gill filaments collapse and adhere to each other causing the fish to asphyxiate (Broadhurst et al., 2008).

In the at-sea trials, lactate responses varied across species and time points, reflecting the complex interplay between capture-related stressors and species-specific metabolic capacity. All tested species exhibited an upward trend in lactate concentrations, consistent with increased anaerobic metabolism in response to capture and air exposure. However, statistical analyses did not identify significant differences in lactate accumulation between species ($p = 0.328$), suggesting a generally uniform metabolic response to the capture stressors.

Temperature plays a critical role in modulating anaerobic respiration in fish, as it influences physiological and biochemical processes such as enzyme activity, substrate availability for energy production, and the efficiency of metabolic end-product clearance (Kieffer, 2000). This may partly explain the differences observed between the at-sea and in-house trials, as the aquaculture-raised fish in the controlled in-house experiments were likely acclimated to stable temperatures, enhancing their anaerobic capacity under ice and dry treatments, whereas wild-captured fish at sea, were exposed to variable and potentially less optimal temperatures.

Cortisol

Cortisol is a primary indicator of stress in fish and serves as a key marker of the endocrine response to stress in fish (K. B. Davis, 2006). The initial phase of cortisol production involves neuroendocrine activation, marked by sympathetic nervous system stimulation and the release of adrenaline (epinephrine), alongside hypothalamic signalling to the pituitary gland, which triggers cortisol secretion (Barton, 2002; Donaldson, 1981).

Cortisol levels remained relatively stable over time across all treatments, indicating that acute stress responses may have plateaued or that chronic stressors did not significantly escalate hormonal output. However, post-hoc analysis identified specific differences between dry and slurry treatments, with cortisol concentrations notably higher in the dry condition ($p < 0.05$), suggesting that prolonged air exposure may induce a more pronounced stress response compared to the buffered conditions of the slurry treatment. Additionally, soak time played a critical role in modulating cortisol levels, with significantly higher concentrations observed in both the 30-minute and 90-minute soak groups compared to the 0-minute control ($p < 0.05$). This trend indicates that extended pre-treatment exposure increases stress burden, potentially due to prolonged physical struggle or metabolic demands.

In the at-sea trials, cortisol responses exhibited species-specific variability and temporal fluctuations, reflecting the complex interplay between capture-related stressors and physiological resilience. *D sargus, D. vulgaris, and P. erythrinus* demonstrated generally stable

cortisol levels over time, with no statistically significant differences identified between species ($\chi^2 = 0.30$, $p = 0.860$). However, the variability in cortisol concentrations, particularly the transient increase at T2 followed by a decline at T3, may indicate an acute stress response that reached a physiological threshold, leading to either hormonal exhaustion or adaptive downregulation of the hypothalamic-pituitary-interrenal (HPI) axis (Kieffer, 2000). Cortisol does not peak until about 1–2 h (Gamperl et al., 1994), which may explain the stable cortisol levels observed during the at-sea trials. The lack of a pronounced cortisol peak suggests that sampling times may not have fully captured the hormonal response window, or that fish had already reached a physiological threshold, leading to a subdued endocrine reaction.

Osmolality

The final marker of physiological disturbance used in this study is osmolality, the measure of the concentration of solutes in body fluids (Kammerer et al., 2010). Elevated osmolality indicates disruptions in ionic balance, often exacerbated by prolonged exposure to stressful conditions (Barton et al., 2005). The in-house experiments revealed treatment-dependent differences in osmolality, with the dry and ice treatments inducing significant increases over time ($p = 0.0267$), while the ice slurry treatment maintained more stable osmolality levels. Fish in the dry condition exhibited the highest osmolality values, rising from 387.87 ± 5.56 mOsm/kg at T1 to 404.57 ± 7.25 mOsm/kg at T3, suggesting that prolonged air exposure disrupts osmotic regulation, likely due to dehydration and impaired gill function (Figure 4-1) (Broadhurst et al., 2008).

In the at-sea trials, osmolality responses varied across species and time points, demonstrating a general upward trend in osmolality levels following capture. While statistical analyses did not identify significant differences between species ($p = 0.573$), all species exhibited increased osmolality over time, reflecting the physiological stress associated with air exposure and handling. The at-sea results align with the in-house findings, particularly in demonstrating that prolonged exposure to air and suboptimal handling can exacerbate osmotic imbalances.

These findings adhere with literature demonstrating that extreme stressors, such as air exposure and rapid temperature changes, can compromise osmoregulatory capacity, leading to ion imbalances and altered cellular homeostasis (Bonga, 1997). The absence of significant species-specific differences suggests that the capture and handling conditions imposed similar osmotic stress across the sparid species tested, highlighting the generalised impact of such stressors on osmoregulation.

4.3 Implications of Findings

This study provides valuable insights into the physiological responses of sparid fish to capture and handling practices in artisanal longline fisheries, highlighting key areas where welfare improvements can be made. By examining post-capture treatments, soak times, and at-sea practices, this research offers practical recommendations to enhance fish welfare during and after capture.

Post-Capture Treatments

The findings demonstrate that ice slurry conditions provide a more stable physiological environment for fish compared to dry and ice treatments. Ice slurry minimised metabolic stress markers such as glucose and lactate and helped maintain osmotic balance, suggesting a buffering effect against severe stress. To improve welfare outcomes, fisheries should prioritise ice slurry storage over dry or ice-only conditions, particularly when humane slaughter methods are not feasible. Since hastening the transition to non-responsiveness is critical to improving welfare, ice slurry treatments offer an advantage by reducing the time fish remain under 'stressed' conditions. The cool, semi-aquatic brackish environment not only moderates physiological stress markers but also hastens the transition to non-responsiveness, thereby minimising the duration of conscious suffering.

Soak Time Considerations

Extended soak times were associated with higher stress markers, including elevated cortisol and glucose levels. Although no statistically significant differences were found between the tested soak times (0, 30, 90 minutes), the trend towards increased stress with longer soak durations underscores the importance of minimising soak times wherever possible. Shorter soak times may help reduce the cumulative stress burden on fish, improving vitality scores and ensuring a more humane process before death.

At-Sea Practices

To enhance welfare, artisanal longline fisheries should adopt measures to minimise air exposure, improve handling efficiency, and reduce the duration between capture and storage. The findings do not suggest prolonging vitality loss to enhance welfare.

Chapter 5 Conclusion

This study explored the physiological responses of sparid fish to capture and handling practices in artisanal longline fisheries, providing critical insights into welfare management. The research demonstrated that ice slurry treatments offer the most effective method for minimising stress and achieving a vitality score of 1 as rapidly as possible, thereby reducing conscious suffering. Shorter soak times and improved at-sea handling practices also contribute to enhanced welfare outcomes.

While this study provided valuable data, it also encountered limitations. The lack of humane slaughter methods within the experimental design meant that welfare improvements could only focus on minimising stress before death, not on preventing suffering entirely. Additionally, the variability in environmental conditions during at-sea trials may have influenced the physiological responses observed, highlighting the challenge of comparing controlled and natural settings directly.

Ultimately, these findings contribute to a growing body of research aimed at improving welfare practices in wild-capture fisheries. By advocating for better handling techniques and emphasising the need to balance vitality loss with physiological stress. Improving fish welfare in this fishery would require killing the fish swiftly, with minimal handling and stressors between hooking and slaughter. This must be done while maintaining realistic expectations of what a single fisher on a small boat can achieve, given the practical limitations of manually hauling a longline, as is the reality in South Portugal's artisanal longline fleet.

Future research should aim to refine handling techniques further, explore species-specific responses to post-capture treatments, and assess the effectiveness of alternative welfare strategies in real-world fisheries operations. By continuing to develop evidence-based practices, fisheries can better balance ethical considerations with operational demands, promoting more humane and sustainable fishing practices.

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