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Declaração de autoria

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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Abstract

Inhibitory control is a key component of executive function, influencing adaptability and decision-making in animals. Consequently, this study aims to investigate whether environmental enrichment (EE) enhances inhibitory control in juvenile gilthead seabream (*Sparus aurata*) using the cylinder task. As aquaculture rapidly expands to meet global food demands, the welfare of farmed fish species, such as seabream, has become a critical concern. EE, which involves introducing elements that stimulate fish physiologically and psychologically, has shown significant benefits in various species, including enhanced learning, memory, and stress resilience. This study used 40 juvenile seabreams housed in tanks with and without enrichment to test their cognitive abilities through a series of progressive learning phases. These phases culminate in the Cylinder Task, where fish are challenged to obtain food from a transparent cylinder using the two open ends, evaluating the inhibition of the urge to approach the reward through the transparent walls of the cylinder. All the individuals were previously screened for personality traits (i.e. either more proactive or more reactive). In this study, juvenile gilthead seabream reared in enriched environments exhibited higher behavioral engagement and faster learning during training, with significant improvements in task performance over trials. However, no significant differences in inhibitory control were observed between enriched and non-enriched groups during testing. Instead, larger fish and those with proactive traits, characterized by rapid decision-making and high exploration, performed tasks faster and more accurately. These findings suggest that while EE enhances motivation and learning, inhibitory control may be primarily driven by intrinsic factors like body size and personality rather than environmental complexity, and that adapting EE to individual traits could optimize welfare and efficiency in aquaculture.

Key-words: Aquaculture, Welfare, Cognition, Learning, Intrinsic factors

Resumo português

O aumento contínuo da população tem gerado uma crescente procura por alimentos, especialmente por fontes de proteína animal. Neste contexto, a aquacultura tem assumido um papel fundamental, destacando-se como o setor alimentar com um crescimento mais acelerado a nível global nas últimas décadas. Esta expansão deve-se, em grande parte, à estagnação das pescas extrativas e consequentemente à necessidade de garantir o fornecimento sustentável de recursos marinhos, essenciais sobretudo em países em desenvolvimento. Desde os anos 80, a aquacultura tem crescido de forma exponencial, passando de cerca de 10 milhões de toneladas em 1987 para aproximadamente 156 milhões de toneladas em 2018. Uma vez que o consumo global nesse ano aumentou duas vezes a taxa de crescimento populacional, mais de metade desta produção resultou exclusivamente da aquacultura. No entanto, com esta intensificação têm surgido uma série de desafios, sendo o bem-estar dos animais uma das principais preocupações.

É hoje amplamente reconhecido pela comunidade científica que os peixes são seres sencientes, capazes de experienciar dor, medo, stress e diferentes estados emocionais, tanto negativos como positivos. Esta constatação tem vindo a reformular o conceito de bem-estar animal que atualmente não se limita apenas à ausência de sofrimento físico, mas inclui também a presença de experiências positivas e o respeito pelas necessidades comportamentais, fisiológicas e cognitivas de cada espécie. O bem-estar animal é assim entendido como o resultado do equilíbrio entre emoções positivas e negativas experienciadas ao longo da vida do indivíduo. Apesar desta evolução conceptual, grande parte das práticas em aquacultura continua a priorizar apenas o crescimento, a conversão alimentar e outros parâmetros produtivos, negligenciando as necessidades comportamentais e psicológicas dos animais. As condições frequentemente observadas na aquacultura industrial introduzem um conjunto de estímulos para os quais os peixes raramente estão preparados para lidar, tais como restrições, elevadas densidades populacionais/agregações não naturais, manipulações frequentes e falta de estímulos ambientais. Este tipo de stress tem impactos fisiológicos e comportamentais significativos, podendo comprometer o crescimento, a resposta imunitária e até funções cognitivas destes animais. A exposição constante a estímulos inadequados ao contexto evolutivo da espécie pode levar a alterações comportamentais, como agressividade excessiva, letargia ou comportamentos repetitivos, além de afetar negativamente a aprendizagem e a plasticidade cerebral. A mitigação do stress e a promoção de ambientes mais naturais e estimulantes são por isso prioridades para melhorar o bem-estar dos peixes criados em sistemas de aquacultura.

Uma das estratégias mais eficazes e promissoras para enfrentar estes desafios é o enriquecimento ambiental, um fornecimento de estímulos ambientais – como elementos físicos, sensoriais, sociais, cognitivos ou estruturais - que aumentam a complexidade do ambiente e permitem aos peixes expressar comportamentos naturais. O enriquecimento pode incluir estruturas tridimensionais que simulam esconderijos ou barreiras visuais (enriquecimento físico), variações de substrato (enriquecimento sensorial), objetos manipuláveis (enriquecimento ocupacional), modificações no regime alimentar (enriquecimento alimentar) ou estímulos sensoriais específicos para a espécie em questão (enriquecimento social). A implementação de técnicas de enriquecimento tem demonstrado benefícios consistentes em várias espécies, como a melhoria do desempenho cognitivo, maior flexibilidade comportamental, redução de comportamentos agressivos e aumento da resiliência ao stress. No caso da dourada, por exemplo, foi observado que o enriquecimento ambiental pode promover comportamentos exploratórios e melhorar capacidades associadas à aprendizagem espacial.

O estudo da cognição em peixes tem vindo a ganhar destaque devido ao aumento de investigação em bem-estar animal e este termo refere-se a um conjunto de processos mentais como perceção, memória, atenção, tomada de decisão e resolução de problemas. Durante muito tempo, os peixes foram considerados animais de comportamento puramente instintivo e limitado, mas estudos recentes revelam que é um tema mais complexo. Hoje sabemos que muitas espécies de peixes possuem capacidades cognitivas sofisticadas, que incluem memória a longo prazo, aprendizagem social, reconhecimento de congéneres e até comportamentos cooperativos e estas descobertas desafiam ideias preconcebidas e demonstram que o cérebro dos peixes, embora estruturalmente diferente do de mamíferos e aves, é funcionalmente capaz de processar informação complexa e adaptar o comportamento ao contexto. As capacidades cognitivas dos peixes são influenciadas por diversos fatores, incluindo a complexidade ambiental, a presença de congéneres, a exposição ao stress, a história de desenvolvimento e até traços individuais como personalidade ou tamanho corporal. A investigação tem mostrado que peixes criados em ambientes enriquecidos apresentam maior neurogénese, melhor desempenho em tarefas de aprendizagem e memória, e respostas comportamentais mais adaptativas quando comparados com peixes mantidos em ambientes não enriquecidos. Paralelamente, estudos indicam que o stress crónico pode comprometer estas funções afetando negativamente a capacidade de adaptação, aprendizagem e resolução de problemas. Para avaliar os níveis cognitivos dos animais, podem ser analisadas várias capacidades cognitivas, tais como o controlo inibitório. Esta função executiva permite ao indivíduo suprimir respostas impulsivas ou automáticas em vez de uma ação mais deliberada. O controlo inibitório é essencial na resolução de problemas, na tomada de decisões e na regulação do comportamento social e em humanos está associado à inteligência, sucesso académico e controlo emocional e em animais a sua presença indica flexibilidade comportamental e capacidade de adaptação. Uma das formas mais comuns de avaliar o controlo inibitório em diferentes espécies é com a tarefa do cilindro. Nesta tarefa, o animal deve resistir ao impulso de aceder diretamente a uma recompensa visível colocada no interior de um cilindro transparente, sendo necessário contornar a estrutura para aceder ao alimento. Esta tarefa já foi aplicada com sucesso em primatas, aves e, mais recentemente em peixes, demonstrando que o controlo inibitório não depende apenas do tamanho do cérebro, mas também de fatores ecológicos e comportamentais.

A dourada (*Sparus aurata*) é uma espécie marinha de grande importância económica no Mediterrâneo e Atlântico Oriental, amplamente utilizada em aquacultura e a sua robustez, tolerância ambiental e comportamento social tornam-na uma espécie adequada para estudos de comportamento e cognição. No entanto, apesar da sua relevância comercial, são ainda escassas as investigações que exploram as suas capacidades cognitivas mais complexas, como as funções executivas. Até agora, os estudos têm-se centrado sobretudo em aspetos relacionados com a personalidade, resposta ao stress e aprendizagem espacial, havendo pouca informação sobre o impacto do enriquecimento ambiental no controlo inibitório desta espécie.

O presente estudo teve como principal objetivo avaliar os efeitos do enriquecimento ambiental no controlo inibitório de juvenis de dourada, utilizando a tarefa do cilindro como ferramenta de avaliação cognitiva. Foram utilizados 40 indivíduos, criados em tanques com e sem enriquecimento ambiental, submetidos a várias fases de treino progressivo até à execução da tarefa final. Os resultados revelaram que os peixes criados em ambientes enriquecidos exibiram maior envolvimento comportamental e aprendizagem mais rápida durante o treino, com melhorias significativas no desempenho ao longo das tentativas, contudo, no teste de controlo inibitório, não foram observadas diferenças significativas entre os grupos. Em contrapartida, peixes maiores e com traços de personalidade proativos, caracterizados por tomada de decisão rápida e alta exploração, executaram a tarefa com maior rapidez e precisão.

Estes resultados sugerem que, embora o enriquecimento ambiental potencie a motivação e a aprendizagem, o controlo inibitório é principalmente influenciado por fatores intrínsecos, como tamanho corporal e personalidade e ajustar o EE às características individuais pode otimizar o bem-estar e a eficiência em aquacultura.

Palavras-chave: Aquacultura, Bem-estar, Cognição, Aprendizagem, Fatores intrínsecos

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Abbreviations list

BL - Body length

BW - Body weight

CCMAR - Algarve Marine Sciences Center

EB - Exploratory behavior

EE - Environmental Enrichment

Eff - Effort

F - Fail

FS - Post-fail

LEOA - Experimental Laboratory for Aquatic Organisms

LR - Learning rate

MT - Million tonnes

NE - Not Enriched

NI - No Interaction

S - Success

T - Tonnes

1. Introduction

As the human population grows, the demand for food is rising, requiring intensive protein production and increasing the dependence on farmed animals (Saraiva et al., 2022). To meet the demand for seafood, which is vital for nutrition and food security, especially in developing countries, aquaculture has expanded rapidly (Belton et al., 2018; Cojocaru et al., 2022; Thilsted et al., 2016). For several decades, aquaculture has been the fastest-growing food sector globally and this growth is essential because capture fisheries have leveled off, and higher production is needed to maintain or improve the per capita fish consumption as the population increases (Figure 1.1) (Bjørndal et al., 2024; FAO, 2024).

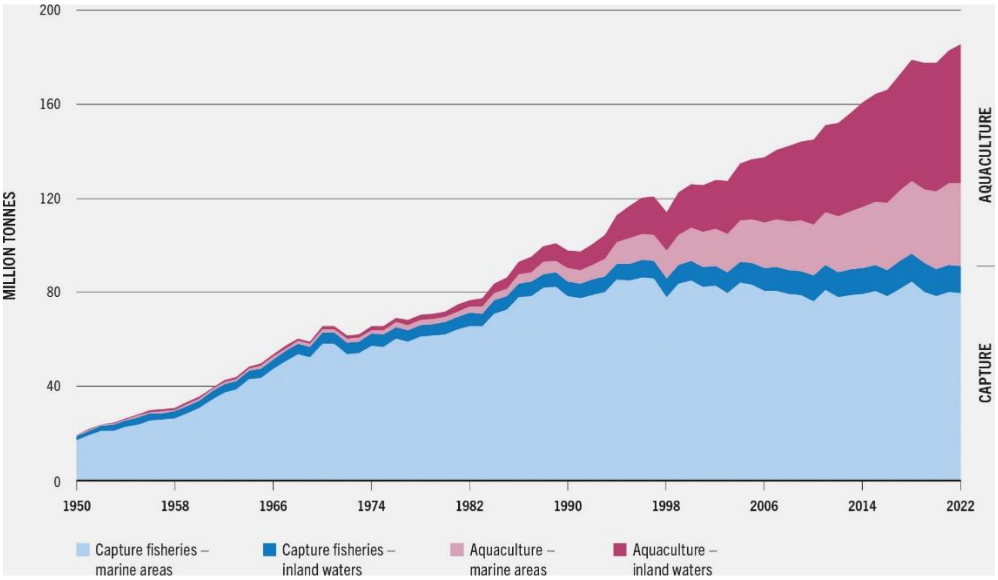

Figure 1.1: Evolution of aquaculture and fisheries production between 1950 and 2022 expressed in live weight equivalent (FAO, 2024).

Aquaculture, i.e the farming of aquatic animals, plants and algae, has grown substantially since the 1980s. In 1987, aquaculture production was about 10 million tonnes (Mt). By 2018, global consumption increased at twice the rate of the population growth at accounted for approximately 156 Mt of fish and shellfish, with 52% of this supply coming from aquaculture (Barreto et al., 2022; FAO, 2020; Naylor et al., 2021). However, intensifying aquaculture practices presents several challenges, including significant concerns about fish welfare. In addition to biodiversity loss, environmental damage, pollution from waste and uneaten food, antibiotic use and poor sustainability, welfare abuses have also emerged as critical problems (Brown & Dorey, 2019; Franks et al., 2021; Saraiva et al., 2019). Scientific evidence indicates that fish can experience pain, fear, stress, and distress, underscoring the need to improve their welfare (Sneddon & Brown, 2020). These animals are capable of forming relationships within and between species, possess complex cognitive abilities such as learning and memory, assess risks and benefits to make strategic behavioural decisions, and exhibit positive and negative affective states, demonstrating consciousness and confirming their status as sentient beings deserving better welfare (Saraiva et al., 2022; Sneddon & Brown, 2020).

The concept of animal welfare encompasses the physical and emotional well-being of animals and has traditionally been defined as the individual's state as it copes with the environment (Broom, 1991). More recently, the definition has evolved to emphasise not only the absence of suffering but also the presence of positive experiences, such as having some control over their environment (agency), using natural skills effectively (competence), and being able to pursue meaningful goals like exploring, nesting, or socialising (goal-directed behaviour) (Hansen, 2023; Rault et al., 2025). Today, animal welfare is also understood as the overall balance of emotions an animal experiences throughout its life - focusing on whether positive feelings, like comfort or pleasure, generally outweigh negative ones, such as fear or frustration (Rault et al., 2025). Initially, research on animal welfare focused on livestock and laboratory animals but it has recently extended to fish, other vertebrates, and invertebrates (Huntingford et al., 2006; Kristiansen & Bracke, 2020). Ensuring the welfare of fish intended for human use is critical for several reasons, including ethical standards, economic implications, and consumer demands. Proper welfare conditions for fish in zoos, aquariums, and scientific research are essential to maintain their natural behaviour and obtain reliable research results. Maintaining appropriate welfare conditions in aquaculture leads to better growth rates, lower disease incidence, and higher-quality products (Dara et al., 2023). Stress can significantly impact these factors, making it crucial to avoid unnecessary suffering during capture, rearing, and slaughter (Dara et al., 2023). Despite increasing regulations especially at European level (Candela et al., 2020), some aquaculture practices still cause avoidable stress during processes like handling, pre-slaughter, and slaughter, highlighting the need for more research and rigorous application of welfare standards (Arechavala-Lopez et al., 2022; Dara et al., 2023). There is a significant knowledge gap regarding the welfare of many farmed aquatic species compared to terrestrial farm animals and the existing knowledge primarily focuses on production traits rather than welfare (Saraiva et al., 2019). Research has often pushed the physiological limits of many fish species regarding growth, fertility, and size, resulting in highly artificial conditions (Huntingford, 2004). Under farming conditions, environments are frequently designed based on economic and ergonomic requirements, often neglecting welfare considerations (Arechavala-Lopez et al., 2022). However, the artificial conditions of captivity, particularly in industrial aquaculture, introduce a range of stimuli for which fish are seldom equipped to handle, such as restrictions, unnatural aggregations, barren environments, frequent handling, and other artificial stressors that differ significantly from the conditions under which these species evolved (Ashley, 2007; Conte, 2004; Maia et al., 2024). These stressors can significantly compromise fish welfare, leading to physiological and behavioural disturbances, and highlighting the urgent need to address and mitigate stress in aquaculture practices (Conte, 2004). A growing body of scientific evidence indicates that fish are capable of experiencing a variety of affective states (Brown & Dorey, 2019; Lambert et al., 2022), and each species has evolved within a specific ecological context, developing adaptations, behaviours, and coping mechanisms suited to their natural environments - traits that are often not considered in farming conditions (Helfman et al., 2009; Kristiansen et al., 2020).

According to recent studies in the European Union, consumers are becoming increasingly concerned about the conditions under which fish are farmed (Brydges & Braithwaite, 2009). Beyond that, the perception that better welfare conditions also help product quality and profitability has led to the research and application of environmental enrichment (EE) techniques, providing opportunities for fish to experience positive aspects in their environment (Arechavala-Lopez et al., 2022). Environmental enrichment can be understood as the provision of environmental stimuli—such as motor, sensory, cognitive, social, or structural elements—that allow farmed fish to express natural behaviours and meet their physiological, behavioural, and psychological needs, encompassing the three major components that define animal welfare in terms of proper biological functioning: the functional, natural, and feeling-based (Arechavala-Lopez et al., 2022; Huntingford & Kadri, 2014). The science of EE is constantly improving, reflecting the growing diversity of enrichment types and the wide range of species-specific responses observed across aquaculture systems (Zhang et al., 2022). The existing knowledge on the effects of EE on fish welfare was recently reviewed (Arechavala-Lopez et al., 2022), evaluating possible benefits for the industry and providing guidelines for fish farmers, researchers, and other stakeholders. Five EE categories were proposed: physical, sensorial, occupational, social, and dietary. Physical enrichment involves adding physical complexity such as structures, objects, or any structural modification to increase the heterogeneity of the rearing environment (Näslund & Johnsson, 2016). This category includes structures that provide shelter or add complexity and substrates suitable for bottom-dwelling species at specific life stages (Arechavala-Lopez et al., 2022). Sensorial enrichment requires a good understanding of the biological needs and sensory world of the target species since fish have substantial differences in their sensory systems compared to terrestrial animals (Arechavala-Lopez et al., 2022; Saraiva et al., 2018; Sneddon, 2018). Methods for sensorial enrichment include visual, auditory, chemical (olfactory, taste), hydromechanical, and electrical stimuli (Arechavala-Lopez et al., 2022). Occupational EE aims to introduce diverse challenges into the rearing environment to prevent monotony and boredom. This category can include psychological devices that provide challenges or control over the environment and enrichment that encourages physical exercise (Bloomsmith et al., 1991). Social enrichment involves the presence of other individuals and their interactions and the space available for these interactions or for avoiding others. It is crucial to understand whether the species is solitary or tends to form shoals at different life stages and if they cohabit with other species in the wild (Arechavala-Lopez et al., 2022). Dietary enrichment refers to food type or feeding strategies (distribution, quantity, periodicity) that affect foraging behaviour or food intake but does not include diet composition, which is considered "internal" or "nutritional" enrichment. Feeding strategies are important in dietary enrichment, as regimens, schedules, and procedures can significantly affect fish welfare (Arechavala-Lopez et al., 2022). Recent studies have demonstrated that EE can significantly benefit fish, enhancing behavioural flexibility, improving spatial learning and memory, and reducing aggression and anxiety-like behaviours (Barcellos et al., 2018; Braithwaite & Salvanes, 2005; Salvanes et al., 2007; Zhang et al., 2021).

There are several examples of the benefits of environmental enrichment in specific species. In rainbow trout (*Oncorhynchus mykiss*), EE promotes faster recovery of opercular beat rates and reduces the coefficients of variation in plasma cortisol following a stressor (Brydges & Braithwaite, 2009). In zebrafish (*Danio rerio*), EE attenuates the effects of chronic unpredictable stress, measured in trunk cortisol and brain reactive oxygen species (Marcon et al., 2018). In juvenile black rockfish (*Sebastes schlegelii*), EE reduces basal stress levels, measured in visceral cortisol levels and opercular beat rate (Zhang et al., 2021). Similar effects are found in Atlantic salmon (*Salmo salar*), where EE reduces plasma cortisol, improves behaviour, and reduces fin deterioration (Näslund et al., 2013). In juvenile gilthead seabream (*Sparus aurata*), EE improves welfare by reducing aggression and interactions with the net pen, reducing fin erosion, and enhancing exploratory behaviour, spatial orientation, learning abilities, and physiological brain functions which are skills that are usually neglected on farms, even though they are key to positive welfare (Arechavala-Lopez et al., 2019, 2020; Fife-Cook & Franks, 2019).

One of the most recent and rapidly developing areas in fish welfare research is cognition. The field of animal cognition investigates the mental processes that help animals perceive, learn, and make decisions (Ebbesson & Braithwaite, 2012). Fish have been shown to exhibit cognitive flexibility, inhibited control, and individual variation in problem-solving abilities - capacities that are increasingly recognized as important for welfare (Arechavala-Lopez et al., 2022; Kristiansen et al., 2020; Vila-Pouca et al., 2025). For instance, studies on rainbow trout reared in enriched environments demonstrate improved learning and behavioural adaptability, which underline the need to align farming conditions with the cognitive capacities of different species to promote better well-being (Kristiansen et al., 2020; Vila-Pouca et al., 2025). Cognition includes perception, attention, memory formation, and executive functions like learning and problem-solving (Drigas & Karyotaki, 2019). Historically, the study of animal cognition has focused on birds and mammals, particularly non-human primates, due to a misconception that learning played little role in the behaviour of reptiles and fishes (Font et al., 2023). For much of scientific history, fish were viewed as automatons with behaviour controlled by unlearned predispositions. Ethologists described their behaviour as fixed action patterns triggered by environmental cues. However, fish are ancient vertebrates that have diversified immensely over 500 million years, occupying nearly every aquatic niche (Brown et al., 2011). The redundant view of a linear progression of behavioural and neural sophistication from fish to mammals has been exposed by Darwin's theory of evolution, which shows no progressive hierarchy in cognitive complexity (Brown et al., 2011). Recent research has revealed that fish exhibit sophisticated behaviours and that learning is crucial in their behavioural development, have impressive long-term memories, comparable to other vertebrates, and their neural architecture shares similarities with mammals, providing comparable processing power (Broglio et al., 2003; Brown, 2001; Warburton, 2003), and their cognitive abilities in many fields are similar to those of non-human primates (Bshary et al., 2002; Laland & Hoppitt, 2003; Odling-Smee et al., 2011). Fish have evolved complex cultural traditions and strategies of manipulation, deception, and reconciliation (Brown & Laland, 2003; Bshary et al., 2002), they recognize individuals, monitor social prestige, and cooperate in foraging, navigation, reproduction, and predator avoidance (Fitzpatrick et al., 2006; Griffiths, 2003; Huntingford et al., 1994; Johnstone & Bshary, 2004; McGregor, 1993). Early comprehensive reviews, such as those by Kieffer and Colgan in the 1990s, highlighted the role of learning in fish behaviour (Kieffer & Colgan, 1992). Subsequent reviews and studies have expanded our understanding of fish cognition, emphasising the need for further investigation into their behavioural flexibility and cognitive abilities (Brown, 2003).

Cognitive abilities in fish are widely believed to be most dramatically influenced by environmental conditions early in development. However, recent evidence suggests that fish brains remain plastic across all life stages (Ebbesson & Braithwaite, 2012; Näslund et al., 2012). Both the social and physical conditions that a fish experiences are known to improve neural development and cognitive abilities however chronic exposure to social stress has been shown to decrease neural proliferation (Johansen et al., 2012; Sørensen et al., 2013). While the consequences of long-term social stress on cognitive performance in fishes have not been well studied, most studies suggest that social stress impairs learning (Laudien et al., 1986; Olla & Davis, 1989; Sørensen et al., 2013). In the cichlid *Cichlasoma paranaense*, isolation decreased performance on an associative learning task (Brandão et al., 2015). Juvenile guppies (*Poecilia reticulata*) reared under stressful, crowded conditions were worse at learning from experienced conspecifics how to navigate a labyrinth compared to conspecifics raised at lower densities (Chapman et al., 2008). Cognitive abilities can also be influenced by the conditions experienced early in development or even the conditions experienced by parents (Eriksen et al., 2011; Moore et al., 2019). While other studies have directly linked maternal investment to offspring cognitive abilities, there is evidence in salmonids that cognitively demanding tasks like foraging (Leblanc et al., 2011) and schooling (Tierney et al., 2009) are affected by maternal conditions and egg investment. In other animals, such as honeybees *Apis mellifera*, increased maternal investment in workers improved their performance in a later associative learning experiment (Scheiner, 2012) and in goats, exposure to certain plant species before foraging increased their efficiency in locating these plants, with this learned preference also transmitted to their offspring (Arviv et al., 2016; Zobel & Nawroth, 2020).

In general, the assessment of cognitive abilities in laboratory-raised fishes may be biased by the quality of care received by parents and the conditions experienced during rearing (Jonsson & Jonsson, 2014; Sørensen et al., 2013). The degree of environmental enrichment is considered the primary mechanism for differences in cognitive capacity between captive and wild fishes (Näslund et al., 2012). Generally, increased complexity results in enhanced brain growth (Kihslinger & Nevitt, 2006) and faster rates of neural proliferation (Dunlap et al., 2011; Salvanes et al., 2013; Von Krogh et al., 2010). For instance, adult zebrafish isolated in structurally enriched environments showed increased telencephalic cell proliferation after only one week (Von Krogh et al., 2010). These neuroanatomical differences have been linked to improvements in several aspects of cognition, including foraging on novel prey (Brown &

Laland, 2003), hiding from predators (Salvanes & Braithwaite, 2005), and spatial learning (Salvanes et al., 2013).

To assess the cognitive levels of animals, various cognitive skills can be analysed, such as inhibitory control. Inhibitory control is a core executive function that enables animals to manage their attention and behaviour, overriding internal predispositions or resisting external lures (Chudasama, 2011; Diamond, 2013; Gilbert & Burgess, 2008). This function is crucial for blocking impulsive behaviours and is linked to performance in various cognitive tasks (Band & van Boxtel, 1999; Shamosh et al., 2008). Inhibitory control is associated with adult intelligence and predicts children's academic achievement and cognitive competence (Band & van Boxtel, 1999; Duckworth et al., 2012; Duckworth & Seligman, 2005; Mischel et al., 1989). In animals, it predicts problem-solving abilities and other cognitive skills (Boogert et al., 2011; Kralik et al., 2002). While traditionally considered a feature of humans (Shettleworth, 2009) and large-brained vertebrates (Genty & Roeder, 2006), inhibitory control is also observed in species with smaller brains (Kabadayi et al., 2016), such as certain birds and fish (Brown et al., 2011; Bshary et al., 2002). The detour paradigm, also known as the Cylinder Task, is a classic method in comparative cognition used to investigate inhibitory control (MacLean et al., 2014). In this task, animals are presented with a transparent cylinder containing a visible reward, and the subject must inhibit the urge to reach it through the transparent barrier and instead detour to one of the open ends to successfully retrieve food (Kabadayi et al., 2016). The cylinder task has been applied across a wide range of species, including mammals, birds, and fish, to explore how different ecological and evolutionary pressures shape self-regulatory behaviour (MacLean et al., 2014). Feeding ecology significantly shapes inhibitory control abilities (Harvey et al., 1980; Marshall & Wrangham, 2007; Tomasello & Call, 1997). For example, gummivorous marmosets (*Callithrix jacchus*) and insectivorous tamarins (*Saguinus Oedipus*) show different inhibitory control strategies that align with their feeding behaviours (Nadler, 2017). In primates, inhibitory control is linked to social complexity and dietary breadth, suggesting that these factors influence the development of cognitive abilities (Nadler, 2017). Comparative analyses on self-control in 36 mammalian and bird species showed that self-control is common and strongly co-varies with absolute brain size (MacLean et al., 2014). In primates, self-control is positively correlated with dietary breadth but not with group size (MacLean et al., 2014). However, another study found that inhibitory skills in primates were related to social organization rather than diet, indicating that social complexity is linked to inhibitory skills (Amici et al., 2008). Feeding ecology has long been assumed to shape cognitive capabilities in primates (Harvey et al., 1980; Marshall & Wrangham, 2007; Milton, 1981; Tomasello & Call, 1997). For example, marmosets waited longer for a larger reward rather than accepting immediate, smaller recompense (Stevens et al., 2005). Conversely, tamarins travelled further for greater rewards in a spatial discounting task (an experimental method used to assess impulsivity and decision-making in animals), fitting their foraging behaviour (Stevens et al., 2005). Similarly, chimpanzees (*Pan troglodytes*), which generally rely on small, distributed food patches and fluctuating fruit resources, showed stronger inhibitory skills in a delayed gratification task than bonobos (*Pan pansicus*), which live in environments with more stable food sources, suggesting that different feeding ecologies influence foraging effort and cognitive abilities (Rosati et al., 2007). Comparable patterns have also been observed in fish species such as guppies (*Poecilia reticulata*). This species demonstrates adaptive changes in foraging decisions and social learning depending on factors such as food availability and predation risk, highlighting how environmental variability influences cognitive flexibility. Recent research shows that wild guppies increase social learning under high predation risk, while domesticated populations adjust learning strategies based on current environmental pressures, supporting the idea that feeding ecology shapes inhibitory control and related cognitive skills (Guigueno et al., 2025; Laland & Reader, 1999).

Gilthead seabream (*Sparus aurata*), is a species within the *Sparus* genus, belonging to the ray-finned fishes (Actinopterygii) and the order Perciformes (Jobling, 2011). It has a silvery grey body with a distinctive black spot at the lateral line and a golden band between its eyes (Jobling, 2011; Mhalhel et al., 2023). This fish inhabits sandy seabeds, seagrass beds, and surf zones, typically at depths of around 30 meters but can go as deep as 150 meters. It is eurythermal and euryhaline, tolerating a wide range of temperatures and salinities, and is found in estuaries and coastal waters (Mhalhel et al., 2023; Verhaegen et al., 2007). As an opportunistic feeder, its diet includes bivalves, gastropods, polychaetes, and echinoderms (Pita et al., 2002; Wassef & Eisawy, 1985). It is distributed in the Black and Mediterranean seas, and the Eastern Atlantic Ocean, and has recently been found in the colder waters of the Celtic Sea and the English Channel (Mhalhel et al., 2023). This species is really valuable in aquaculture, particularly in the Mediterranean Sea (Mhalhel et al., 2023). Intensive production began in the early 1980s using marine cages and land-based recirculating aquaculture systems (Mhalhel et al., 2023). Production increased from 1,800 tonnes (T) in the late 1980s to 45,000 T in 1997, and reached 258,754 T in 2020, making it the 33rd most-reared fish species in the world (FAO, 2020; Mhalhel et al., 2023). The leading producers are Turkey, Greece, Egypt, Tunisia, Spain, and

Italy, with Turkey and Greece being the largest exporters, and Italy the largest importer (FAO, 2020; Mhalhel et al., 2023). In 2018, seabream production in transitional systems under semi-intensive or extensive regimes in Portugal was approximately 308 T, corresponding to 60.3% of total fish production in these environments (Rocha et al., 2022). Until 2010, it was the most extensively cultured marine fish species in Portugal, after which turbot production reached its peak (Ramalho & Dinis, 2011). Since then, gilthead seabream has remained the second most cultured fish species nationally and are the leading species in coastal aquaculture (Rocha et al., 2022b). The rapid growth of seabream farming is due to the species' robustness, plasticity, and reliable juvenile supply from hatcheries (Mhalhel et al., 2023). Although research on the behaviour and cognitive abilities of gilthead seabream has increased in recent years, the current body of knowledge remains limited. Existing studies have explored aspects such as spatial learning, personality traits, and the influence of environmental enrichment on behavioural plasticity (Arechavala-Lopez et al., 2020; Castanheira et al., 2013; Millot et al., 2009). However, fundamental cognitive processes, particularly executive functions such as inhibitory control, are still poorly understood in this species, underscoring the need for further empirical investigation.

The present study aims to evaluate the cognitive abilities of gilthead seabream using the Cylinder Task. As described above, this task has been widely used in mammals and birds but its application in fish remains limited, with successful results reported in only a few species, such as guppies (Lucon-Xiccato et al., 2017), Nile tilapia (Brandão et al., 2019) and zebrafish (Santacà et al., 2019). Despite its importance for aquaculture, Gilthead seabream has not yet been studied in this context. As it is commonly reared in captivity, investigating its cognitive capacities is particularly relevant for informing husbandry practices that address behavioural needs and enhance their welfare. Additionally, this study examines the impact of environmental enrichment on cognitive performance in this species, thereby contributing to broader discussions in the fields of cognition, behaviour, and welfare.

2. Materials and Methods

2.1. Ethics statement

All animal care and experimental procedures were conducted in compliance with 2023DGV/000066293 issued by Direção Geral de Alimentação e Veterinária, Ministério da Agricultura, Florestas e Desenvolvimento Rural, Portugal, in compliance with the European

(Directive 2010/63/EU) and Portuguese (Decreto-Lei no. 113/2013 de 7 de Agosto) legislation for the use of laboratory animals.

2.2. ManyFishes project

This experiment was conducted under the project ManyFishes (https://themanyfishes.github.io/), a multi-laboratory collaboration dedicated to assessing the cognitive abilities of various fish species through behavioural tests. The aim is to create a freely accessible database to address some limitations in cognitive studies and compare the results across species. Combining the ManyFishes project with the need to increase animal welfare in aquaculture, this study was organized by the Fish Ethology and Welfare group of CCMAR.

2.3. Animals and housing

This study was performed in the Experimental Laboratory for Aquatic Organisms (LEOA), a bioterium for maintaining and cultivating aquatic organisms to support teaching and research located at the University of Algarve, campus of Gambelas (Figure 2.1).

Figure 2.1- Bioterium at the Experimental Laboratory for Aquatic Organisms (LEOA) located at the University of Algarve. The laboratory is equipped with multiple tank systems for maintaining and culturing fish under controlled conditions, supporting research in animal welfare and behavior.

For this experiment, 40 juveniles of gilthead seabream (*Sparus aurata*) with a mean body weight (BW) of 24.4 ± 2.03g and a mean body length (BL) of 10.2 ± 0.37 cm were housed at day 4th of September 2024 in four 200 L housing tanks (85 x 47 x 50 cm), each divided into two halves by vertical perforated opaque partitions, allowing the water to pass but not the individuals. Two housing tanks had environmental enrichment (EE) on each side, consisting of three plastic plants, and the other two did not (NE - Figure 2.2). Each half of the tank contained one group of five fish that were maintained at an average water temperature of 19.3 ºC, oxygen at 9.7 mg/L, pH of 7.6 ± 0.05, salinity of 36.0 PSU, luminosity of 955.5 lux, and with a 12:12 photoperiod cycle starting at 8 o'clock and ending at 20 o'clock. All the fish were tagged with an 8-mm mini transponder (Trovan Ltd., UK) on day 6th of September to ensure that the animals had enough recovery time until the beginning of the experimental procedures, which took place at the beginning of November. The tagging procedure was done to guarantee the identification of each individual across the trials. Prior to the start of the experimental training, one individual of a EE group died. To minimise potential behavioural changes within the group, the individual was not replaced, resulting in a final sample size of 39 individuals (N=5 per group, except for one NE that had 4 individuals).

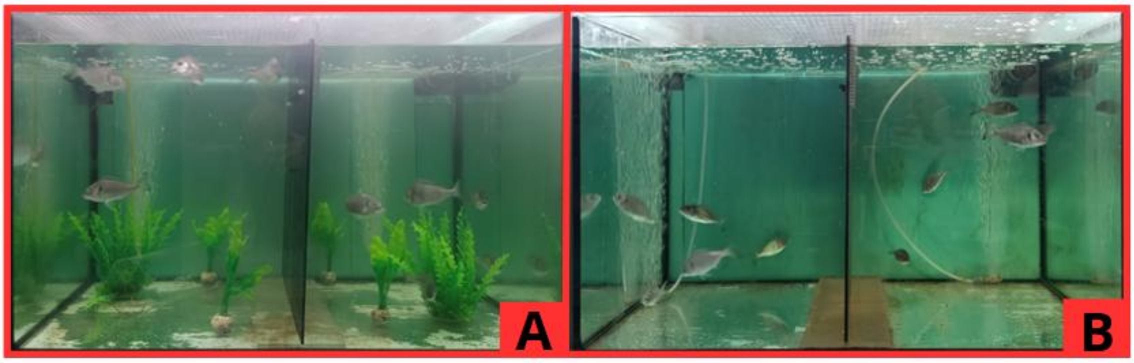

Figure 2.2- Pictures of housing tanks. A- Housing tank with environmental enrichment, which consists of three plastic plants; B- Housing tank without environmental enrichment. All the tanks had an aerator and five juveniles of gilthead seabream (*Sparus aurata*) on each side of the tank, which is separated by a perforated opaque partition.

2.4. Experimental set-up

To perform this experiment, a 320L (200 x 40 x 40 cm) tank was utilized and divided in three parts with removable opaque and transparent partitions. The first compartment was named the Waiting compartment (100 x 40 x 40 cm), and it was utilized to keep the individuals of the group that were waiting to be trained/tested, avoiding netting stress before the activity, and giving more time to the individuals to acclimate and habituate to the new environment (Figure 2.3-A). Next to the Waiting compartment and separated from it by an opaque partition (40 x 37.5 cm), there was another compartment called the Holding compartment (20 x 40 x 40 cm), where the individual who was being trained or tested waited between trials and started all the activities (Figure 2.3-B). The last compartment was the Experimental compartment (80 x 40 x 40 cm), and it was where the training/testing was performed (Figure 2.3-C). The separation between the Holding and Experimental compartments consisted of two partitions, each measuring 40 × 37.5 cm. Opaque partitions were always used prevent the fish from seeing the other side. A second transparent partition between B and C was added against to it and this transparent partition allowed the subject to view the experimental setting for a few seconds before the test began, ensuring that all subjects had equal exposure to the testing arena prior to the start of the trial. Minor modifications could occur according to the phase of the study, such as the addition of some elements to the Experimental compartment, like the cylinder, the plate, and the "set" plate + cylinder.

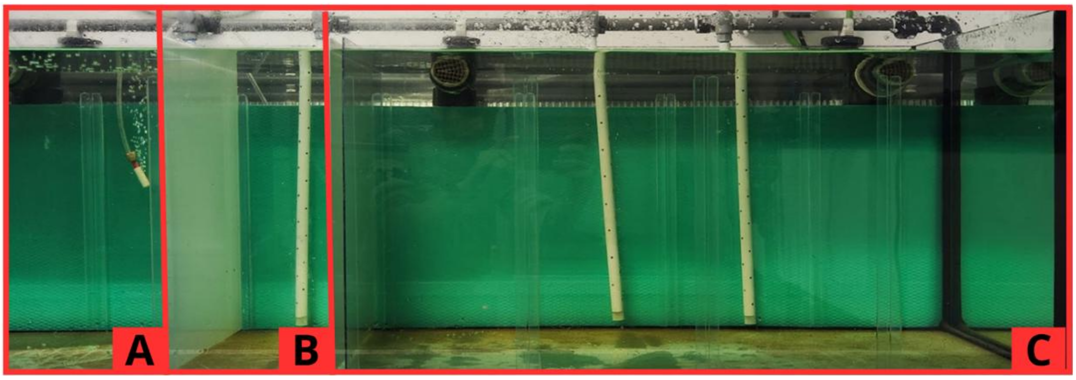

Figure 2.3- Experimental tank divided with opaque and/or transparent partitions in three compartments. A- Waiting compartment: Compartment where the untested fish wait in a group for their turn to be trained or tested; B- Holding compartment: Compartment where the individual fish being trained or tested waits between trials; C- Experimental compartment: Compartment where the fish perform the training or test.

2.5. Experimental design

As required by the cylinder task, this experiment is divided in two parts:

Training -the individuals are trained throughout three learning phases, being exposed to each component separately which involves an increasing level of difficulty according to the phase (Phase 3 being the hardest one since is the last one before the real test)

and

Testing -individuals perform the final test a certain number of times (trials), and the data are collected.

Due to the number of individuals and the specificity of the protocol for executing the Cylinder Task, the groups were tested one at a time following the scheme shown in Figure 2.4.

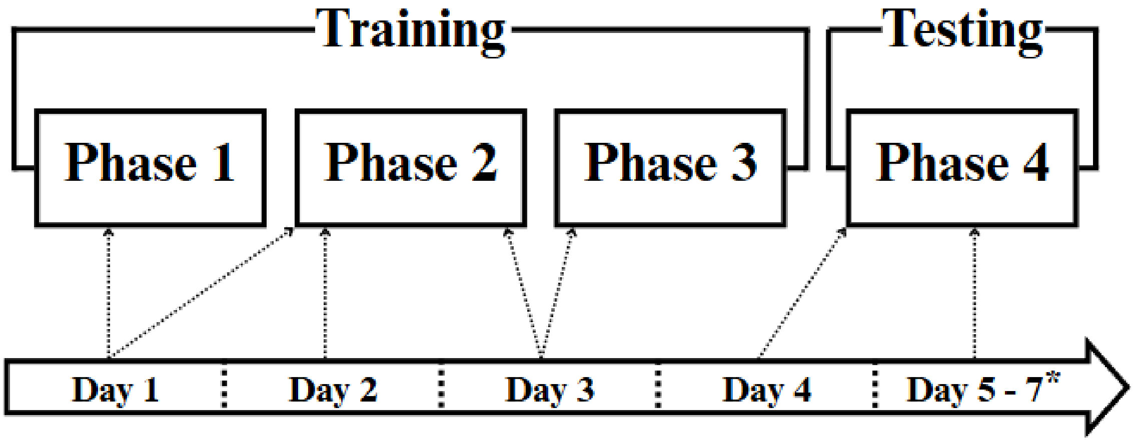

Figure 2.4: Schematic representation of the experimental design during 5 consecutive days. The experiment is divided into two parts: Training (includes Phases 1 to 3 and is conducted from day 1 until day 3) and Testing (includes Phase 4 and is done on days 4 and 5). Each phase is inside a box with dotted arrows indicating the specific days on which each phase was done. The horizontal arrow at the bottom illustrates the temporal sequence of the study. The asterisk on day 5 indicates that, while the testing phase (Phase 4) was typically completed over two days (days 4 and 5), half of the groups required an extended testing period due to a higher number of trials, resulting in Phase 4 being conducted over four days instead of two (days 4, 5, 6 and 7).

2.5.1. Phase 1 – Plate training

The Plate Training (Figure 2.5) is the first and simplest phase, where the goal is to help the individual associate a square white plate (10 x 10 cm), marked with a green dot in the centre to enhance the food's salience, with the reward (a small, one-bite piece of shrimp). To achieve this, the individual begins each trial in the holding compartment. After an acclimation time of ten minutes and once the setup is ready, the opaque partition is removed, followed by the transparent partition, allowing the fish to access the plate with the reward (located 40 cm from the partition). In each trial, the individual had 5 minutes to obtain the reward (success), and if this did not happen, the plate was removed, and the trial was considered a failure. To complete this training phase and progress to the next, the animal must succeed in five out of six consecutive trials.

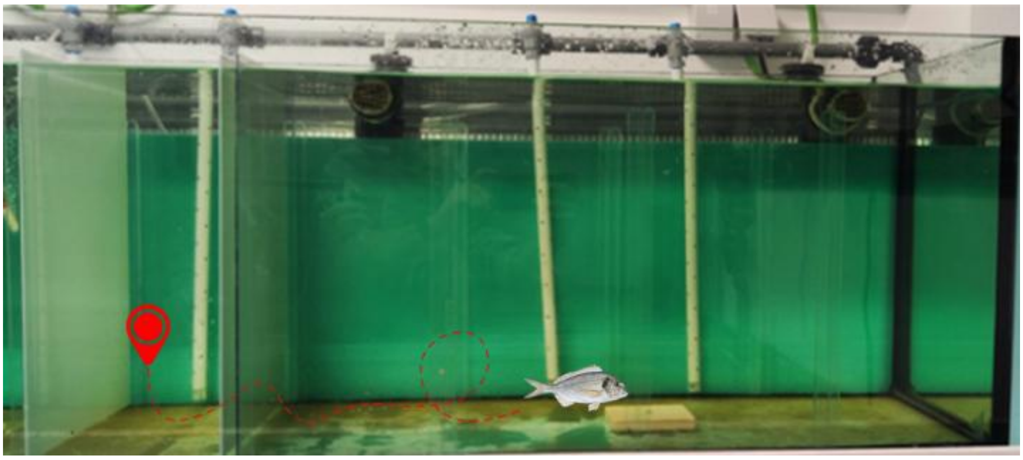

Figure 2.5: Phase 1 – Plate Training setup. Illustration of the experimental tank during the Plate Training phase. The fish starts in the holding compartment (left, marked by the red pin), and the reward is placed on the white plate with a green dot (near the fish image). The dashed line is a visual representation of the fish's movements toward the reward.

2.5.2. Phase 2 – Cylinder familiarization

The objective of the Cylinder familiarization phase, as the name suggests, is to allow individuals to habituate and freely explore the transparent cylinder (22 cm in length and 10 cm in diameter) for 48 hours (equivalent to two waking and sleeping cycles). Unlike the other phases, this one was conducted in the housing tanks, as gilthead seabream is a species typically maintained in groups. This facilitated the process for the animals, as the objective of this phase was to allow them to become comfortable with the presence of the transparent cylinder and to interact with it. Performing this phase in the housing tanks prevents the stress associated with transferring the fish between tanks and avoids the need for habituation to the new tank, and consequently provides more time for the fish to calmly explore the novel object. Data about this phase was not collected since this was just a step to facilitate the process for the animals.

2.5.3. Phase 3 – Forced cylinder

The forced cylinder phase is the last stage before the test phase, and its objective is to ensure that the fish experiences both entry into and exit from the cylinder at least once. The procedure is similar to that of Phase 1: the individual began in the holding compartment, and the opaque partition was removed. However, instead of the transparent partition, a new transparent partition with a hole and an attached cylinder was used to separate the holding and the experimental compartments, requiring the fish to pass through the cylinder to access the reward. The food reward, as in Phase 1, consisted of the square white plate with a green dot and a piece of shrimp, which was placed 18 cm away from the cylinder (Figure 2.6). Each trial lasted five minutes, and if the fish did not pass through the cylinder within that time, the opaque partition was replaced, and the trial was repeated after a ten-minute break. To complete this phase and proceed to the final test, the fish had to pass through the cylinder once, regardless of whether it consumed the reward.

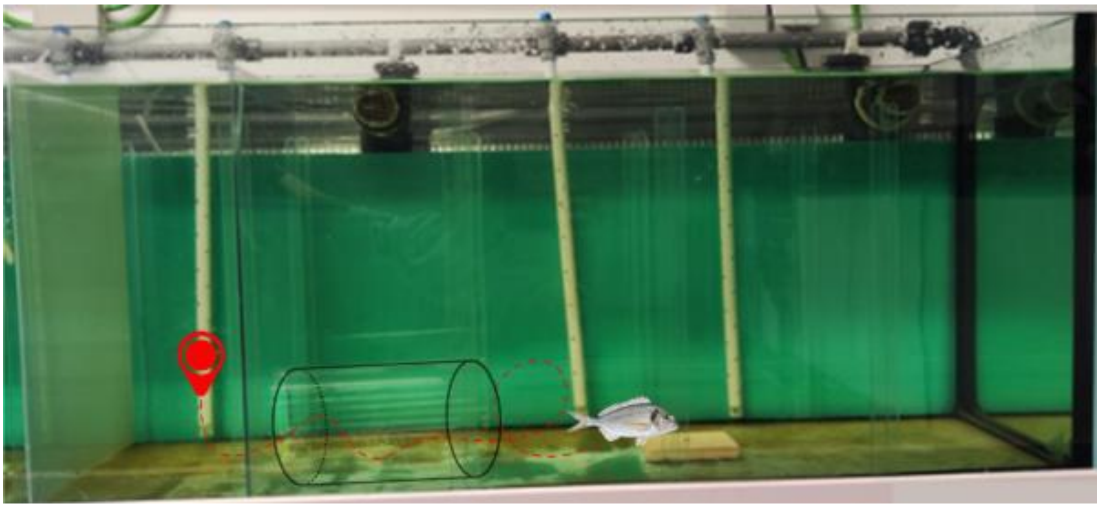

Figure 2.6: Phase 3 – Forced Cylinder setup. Illustration of the experimental tank during the Forced Cylinder phase. The fish begins in the holding compartment (left, marked by the red pin) and must pass through the transparent cylinder (attached to the transparent partition) to reach the white plate with the food reward (near the fish image). The dashed line is a visual representation of the fish's movements toward the reward.

2.5.4. Phase 4 – Inhibitory Control test

The Inhibitory Control Test is the central focus of the study, where the presence of this specific cognitive mechanism in fish was evaluated. The objective was for the individual to detour the cylinder, enter from one of the open sides, and reach the reward without touching the cylinder itself. The setup of the test combines the square white plate with the dot attached to the cylinder. This apparatus was placed at the centre of the experimental compartment, parallel to the rear wall of the tank, with the reward positioned above the green dot inside the cylinder (Figure 2.7). Since this was the final test, and to enhance the fish's attention to the task, each individual previously received a "warm-up" plate trial, following the same procedure as in Phase 1. The testing process was similar to the training phases: the individual began the test in the holding compartment; the opaque partition was removed, followed by the transparent partition, marking the start of the trial. In this final phase, two EE and two NE groups (N=20 fish) underwent a minimum of 10 trials and a maximum of 20 trials, while the other half (two EE and two NE groups, N=19 fish) had between 20 and 30 trials to either succeed or fail the task, giving more opportunities to the progression of the individuals and to evaluate if more trials influences the ratio of success or learning rate. A ten-minute intertrial interval was used to allow the fish to calm down and reduce test-related stress. As in previous phases, each trial lasted 5 minutes. However, in this phase, there were three possible outcomes: Success (S) –the individual detours the cylinder and obtains the reward without contact–, Fail (F) –the individual touches the cylinder–, and No Interaction (NI) –the individual did not interact with the cylinder (in which case the trial is repeated until the maximum number of trials).

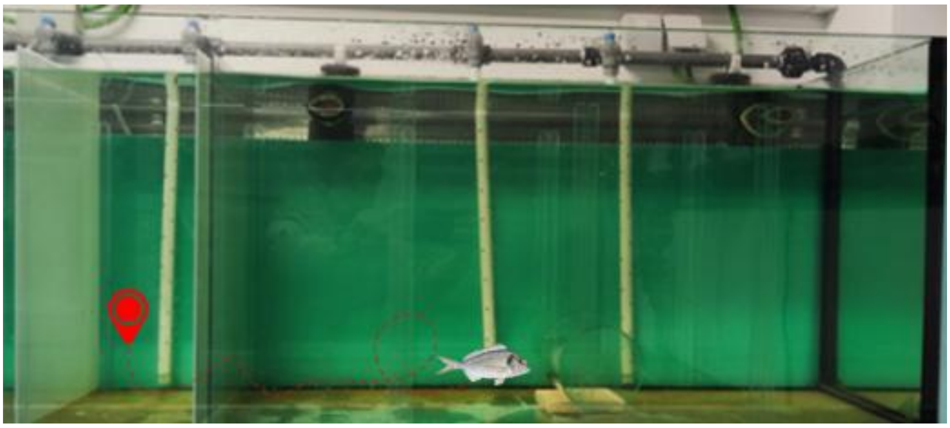

Figure 2.7: Phase 4 – Inhibitory Control Test setup. Illustration of the experimental tank during the final test phase, where the fish must detour the transparent cylinder to access the reward placed inside it (near the fish image) without touching the apparatus. The dashed line is a visual representation of the fish's movements toward the reward.

2.6. Statistical analysis

All statistical analyses were conducted using R (version 4.3.2; R Core Team, 2023). Data were first assessed for normality and homogeneity of variances through visual inspection—using Q-Q plots and residual plots—and formal statistical tests, including the Shapiro-Wilk test and Levene's test, as implemented in the car package (Fox & Weisberg, 2019). When the assumptions of parametric testing were violated, often due to non-normal distributions, appropriate non-parametric methods were applied, including the Wilcoxon rank-sum test, the Kruskal-Wallis test, and Dunn's post hoc test, conducted using tools from the rcompanion (Mangiafico, 2025), FSA (Ogle et al., 2025), and DescTools (Signorell, 2025) packages.

Statistical significance was defined as α = 0.05, with adjustments for multiple comparisons applied where necessary, using procedures such as the Bonferroni or Holm corrections. To account for repeated measures, individual fish were identified by unique subject IDs and analyzed using linear or generalised linear mixed-effects models, fitted with the lme4 package (Bates et al., 2015). Model adequacy and residual behaviour were examined using simulation-based diagnostics provided by DHARMa (Hartig, 2024). When model interpretation required group comparisons, estimated marginal means and pairwise contrasts were computed using emmeans (Lenth, 2024).

Throughout the workflow, data preparation and transformation were performed using dplyr (Wickham et al., 2023) and the tidyverse (Wickham et al., 2019). Visualizations—used both for diagnostic checks and presentation of results—were created using ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2023), and extended with ggdist (Kay, 2024) and ggbeeswarm (Clarke et al., 2023) to clearly and effectively display distributions, uncertainty, and individual-level data.

In addition, AI-assisted tools (ChatGPT by OpenAI) were utilized to support the data analysis process by helping determine the appropriate statistical tests for various situations, providing clarification on methodological choices, and assisting in the development of R code. All outputs were critically reviewed, validated, and adapted by the author.

2.6.1. Data and data processing

As shown in Table 2.8, thirteen variables were collected and used to perform the statistical analysis. The initial morphometric measurements were collected on 23 October, several weeks before the start of the experimental period, since the procedure required to obtain them would have influenced and created an additional stress to the fish that could affect the performance of the fish. The final morphometric measurements were obtained a few days before each group completed the full experimental protocol (training and testing). Due to this, the nearest values to the real measurements of each individual at the time of training and testing were the final measurements (final body length and final body weight). Among all the behavioural variables, the “FS” (Post-fail) outcome was designed to identify cases in which a fish initially failed the cylinder task by making contact with the cylinder but subsequently succeeded within the 5-minute trial. For example, a fish that touched the cylinder after 2 seconds was recorded as having failed at that point; however, if it persisted and successfully completed the task within the remaining 4 minutes and 58 seconds, this was noted as a “Post-fail”.

Table 2.8.: Experimental variables and data collection overview

Variable	Description	Testing	Training	Observations
Treatment	EE vs NE	X	X	

<table>
<tr><td>Group</td><td>G1 – G8</td><td>X</td><td>X</td><td colspan="2"></td></tr>
<tr><td>ID</td><td>Tag code for individual identification</td><td>X</td><td>X</td><td colspan="2"></td></tr>
<tr><td>sBW</td><td>Starting body weight</td><td>X</td><td>X</td><td colspan="2" rowspan="4">Morphometric measurements were used to calculate the mean and median of the weight and length when needed.</td></tr>
<tr><td>fBW</td><td>Final body weight</td><td>X</td><td>X</td></tr>
<tr><td>sL</td><td>Starting length</td><td>X</td><td>X</td></tr>
<tr><td>fL</td><td>Final length</td><td>X</td><td>X</td></tr>
<tr><td>Trial</td><td>Trial number</td><td>X</td><td>X</td><td colspan="2">Different depending on the Phase and Group.</td></tr>
<tr><td>Outcome</td><td>Success (S), Failure (F), Post-fail (FS) or Timeout (NI)</td><td>X</td><td>X</td><td colspan="2">The FS outcome is exclusively used in the testing part.</td></tr>
<tr><td>Time</td><td>Time to S or F</td><td>X</td><td>X</td><td>Training: Every F outcome is a fail per time out.</td><td>Testing: If Time to F = 300 means NI (no interaction), but if Time to F < 300 is a real F.</td></tr>
<tr><td>Post-fail</td><td>Time to S after F</td><td>X</td><td></td><td colspan="2">Post-fail exists when, in a testing trial, a fish fails with Time < 300 and keeps trying until it succeeds.</td></tr>
<tr><td>Food bites</td><td>Number of attempts to reach the food</td><td>X</td><td></td><td colspan="2"></td></tr>
</table>

According to the intended analysis, these variables were processed, including converting categorical variables to factors and removing incomplete or irrelevant entries. In addition, some of these variables were used to create new ones, such as:

- LR – Learning rate was computed for each individual by fitting a linear regression model estimating the change in response time across trials, with the slope of the regression line representing the LR, such that more negative slopes indicated faster learning. According to individual slopes five behavioural types were created: Fast Learners (LR ≤ -40) showed steep performance improvement, Moderate Learners (-40 < LR ≤ -15) demonstrated gradual improvement, Slow Learners (-15 < LR < 0) showed minimal improvement, Failures (LR = 0) exhibited no learning, and Explorers (LR > 0) performed worse over time, giving more attention to exploration over time instead of the reward. These thresholds were chosen based on noticeable gaps in the LR distribution, helping to distinguish meaningful differences in learning performance and behavioural strategy (Figure 2.9).

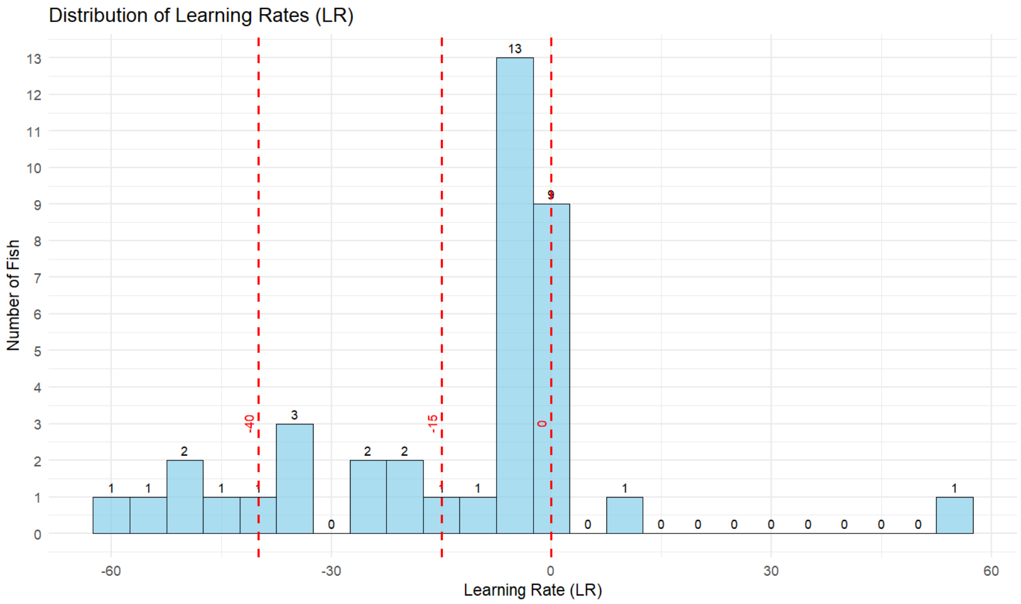

Figure 2.9: Histogram of the distribution of fish learning rate (LR), where it was calculated as the slope of response time across trials. Individuals were categorized into behavioural types based on LR thresholds — Fast Learners (≤ –40), Moderate Learners (–40 < LR ≤ –15), Slow Learners (–15 < LR < 0), Failures (LR = 0), and Explorers (LR > 0).

- Latency – This variable was scored on a 4-point scale, ranging from 5 to 2, based on the response time for each individual. The scoring system rewarded faster responses with higher scores, reflecting greater responsiveness. Specifically, trials with response times ≤ 54seconds received a maximum score of 5; times between 55 and 108 seconds were scored as 4; responses between 109 and 299 seconds were scored as 3; and failures (maximum time reached at 300 seconds) were assigned the lowest score of 2. This ordinal scale allowed the comparison of latency across individuals while accounting for performance quality. Groups are not equally divided because the observed response times ranged from a minimum of 4 seconds to a maximum of 156 seconds (excluding failures), and the score thresholds were defined to reflect meaningful differences in performance rather than to produce equally sized groups.

- EB – Exploratory behaviour was scored using a 4-point ordinal scale, ranging from 5 to 2, based on the time taken during a specific training phase (Phase 3). Faster exploratory responses were assigned higher scores to reflect greater engagement or curiosity. Individuals who reached the maximum time limit (300 seconds) received a score of 2, indicating no exploration. Response times ≤ 98 seconds were scored as 5, those between 99–194 seconds as 4, and times between 195–299 seconds as 3. This scoring system provided a standardised measure of exploratory behaviour across subjects. The groups are not equally divided due to the observed response times ranged from a minimum of 5 seconds to a maximum of 292 seconds (excluding failures), and the score intervals were set to reflect meaningful differences in exploration rather than to produce equally sized groups.

- Eff – Effort was quantified as the number of food bites per unit time, calculated as (food bites/time(s)) × 100. This normalised measure reflects the intensity of effort relative to available time. To allow for standardised comparison across individuals, the effort values were divided into tertiles (three equally sized groups) based on the distribution of the data. Subjects in the lowest tertile (least effort) received a score of 3, those in the middle tertile received a score of 4, and those in the highest tertile (greatest effort) were assigned a score of 5. This ordinal variable served as a proxy for individual variation in motivational or energetic investment.
- Efficiency – This variable was categorised based on the average response time for each subject. Trials where the response time was equal to the maximum allowed time (300 seconds) were considered failures. Otherwise, efficiency was classified into three levels: fast (< 100 s), moderate (100–199 s), and slow (200–299 s).

2.6.2. Training

2.6.2.1. Skill acquisition across trials and treatment

To evaluate whether time to complete Phase 1 varied significantly across trials and between treatments (EE or NE), a repeated-measures analysis of variance (ANOVA) was performed using the ezANOVA function. The within-subject factor was trial (T1 to T5), the between-subject factor was treatment, and the dependent variable was the time. To further explore the effects inside each treatment, separate models were also conducted for individuals in EE and NE treatments. Sphericity for the within-subject effects and interactions was assessed using Mauchly’s test, and when this assumption was violated, Greenhouse-Geisser and Huynh-Feldt corrections were automatically applied. Bonferroni-adjusted pairwise comparisons were used for post hoc analysis to identify specific differences between trials.

2.6.2.2. Differences in task success and efficiency

To further examine individual variation in phase 1, the data were aggregated per individual by summarising performance across the trials. For each subject, descriptive statistics were calculated, including the median of the time and the interquartile range (IQR). Based on the individual's median task time, fish were classified into four efficiency levels:

Fast (<100 seconds), Moderate (100–199.9 seconds), Slow (200–299.9 seconds), and Fail (300 seconds). Before performing categorical tests, contingency tables were generated to explore the distribution of the outcome across treatment and groups. Due to non-normal distributions and the presence of ties (i.e. two individuals with the same time to succeed), non-parametric tests were employed: Wilcoxon rank-sum tests were used to compare the time between treatments, and Kruskal–Wallis tests assessed differences in time among groups. Additionally, Chi-square and Fisher's exact tests were applied to examine the relationship between treatments and both outcome and efficiency. Finally, Spearman's rank correlation analyses evaluated associations between time and both outcome and efficiency.

2.6.2.3. Environmental and social influences on training outcome

To assess the effects of treatment, outcome, and group on individual task performance across phase 1, linear mixed-effects models (LMMs) were used, accounting for repeated measures, and for each model, fish ID was included as a random effect to control for individual variability. Separate models were run to examine the interaction between treatment and both outcome and group, and the main effect of treatment alone. Estimated marginal means (EMMs) were computed for each model, followed by pairwise comparisons to explore significant interactions.

2.6.2.4. Assessing individual differences in learning performance

Individual differences in learning performance were assessed by aggregating data by ID. Descriptive statistics summarised performance differences across efficiency levels, treatments, and groups. Due to the non-normality of the data, Kruskal-Wallis tests were used to evaluate differences in average time and both outcome and efficiency, and between treatment and both time and outcome. When significant effects were found, Dunn's post-hoc tests with Bonferroni correction were conducted for pairwise comparisons. Individual rankings were created, one ranking based on time and the other based on the comparison to the "worst" individual's time. Finally, boxplots illustrated differences in task completion time across efficiency categories, highlighting how mean task time varies between efficiency categories.

2.6.3. Testing

To understand whether the treatment, number of trials, and their interaction influence test performance, the dataset was first processed to calculate the total number and percentage of all the outcomes (F, S, FS, and timeout), and the total number of trials for each individual. Plots and t-tests were performed between tanks, considering all outcomes to determine if there were any significant differences. After that, the FS results were used as F (counting as the test finished in the first touched on the cylinder) to perform the rest of the analysis. Linear models were fitted for each outcome percentage with tank, total number of trials, and their interaction as predictors, and to account for the proportional nature of the data, beta regression models were subsequently applied. Predicted probabilities of each outcome across the observed range of trials were generated for visualization.

2.6.3.1. Comparison of time to succeed and morphometric variables across Blocks

To analyse performance across experimental groups, the dataset was filtered to include only trials with successful outcomes, and the individuals were split into two blocks: Block 1 (groups 1–4) and Block 2 (groups 5–8), because the second half of the fish were tested 1 month after the first half. This timing difference was taken into consideration, given that neural development and growth could have differed between blocks at the time of testing. Shapiro–Wilk tests indicated that the distribution of performance times was not normal within groups, and due to that, a Wilcoxon rank-sum test was applied to compare performance between Block1 and Block2. After that, to evaluate whether variation in body size or weight could account for differences in performance, starting body weight (sBW), final body weight (fBW), starting length (sL), and final length (fL) were also compared between blocks. Shapiro–Wilk tests indicated that these variables did not significantly depart from normality, so independent-samples *t*-tests were used to assess block differences.

2.6.3.2. Treatment and trial effects on binary success outcome

To analyse treatment and trial effects on a binary success outcome, the data were filtered to include just the first ten trials, excluding those trials that were failures per NI. Exploratory visualizations were generated using ggplot2 to assess distributions and relationships between variables, including bar plots for outcome frequency by treatment, line plots for success rates across trials, and boxplots for time to complete the test. Statistical modelling was conducted using generalised linear models (GLMs) with a binomial family to evaluate the effect of treatment and trial on the binary success outcome. Models included main effects and interaction terms, with success coded as a binary variable (1 = S, 0 = F and F/S). To further assess these effects while accounting for repeated measures within individuals, generalised linear mixed-effects models (GLMMs) were fitted with trial (continuous) and treatment (categorical) as fixed effects and fish ID as a random intercept. Additional models included random slopes for trial to test individual variation in learning trajectories, and interaction terms to evaluate whether treatment influenced changes in success over time.

2.6.3.3. Feeding effort across individuals and treatments

The dataset was initially filtered to exclude individuals with missing bite counts, since this analysis was based on that variable. The effort was defined as the number of bites divided by Post-fail time, representing bites per second. The individuals were categorised into three efficiency groups based on their outcomes: Efficient (success), Moderate (failure and success), and Inefficient (failure). Due to the effort data not meeting parametric assumptions, non-parametric tests were used to assess differences across groups, such as Kruskal-Wallis rank-sum tests, evaluating whether effort varied significantly by treatment, group, trial, and efficiency category. When significant differences were detected, Dunn's post-hoc tests with Bonferroni correction were applied for pairwise comparisons to control Type I error inflation. Spearman's rank correlation coefficients were computed to explore associations between effort and other continuous variables, with significance determined by the p-values.

2.6.4. Personality

To perform this analysis, behavioural measurements from both the training and test phases were used: from the training phase, the learning rate, latency, and exploratory behaviour, and from the test phase, effort. The training variables were standardized and entered into a principal component analysis (PCA), and the resulting component scores were used for clustering. Individuals were then assigned to behavioural clusters using model-based clustering. Differences in effort between clusters were evaluated with a one-way ANOVA, followed by Tukey's post-hoc tests for pairwise comparisons.

3. Results

3.1. Training

3.1.1. Skill acquisition across trials and treatment

A repeated measures ANOVA showed that fish performance changed significantly over the phase 1 ($N = 39$), with a small-to-moderate effect size (ANOVA, $F(4, 152) = 9.28$, $p < 0.01$, ges = 0.048) . Fish were significantly slower to complete the task during Trial 1 compared to Trials 3, 4, and 5 (all ps $\leq$ .013), suggesting a pattern of learning or habituation with repeated exposure. When considering treatment groups (EE, $N = 19$; NE, $N = 20$), there was no significant main effect of treatment (ANOVA, $F(1, 37) = 0.12$, $p = 0.732$, ges = 0.002), but the interaction between trial and treatment was marginally significant after correction for sphericity violation (Greenhouse-Geisser corrected ANOVA, $F(4, 148) = 2.69$, $p = 0.033$). Within the EE groups ($N = 19$), a similar pattern emerged, with a significant main effect of trial found (ANOVA, $F(4, 72) = 8.30$, $p < 0.001$, ges = 0.115). Post hoc comparisons indicated that fish in this group took significantly longer time in Trial 1 than in Trials 3, 4, and 5 (all ps = .013), demonstrating clear improvement in task efficiency. In contrast, the NE group ($N = 20$) did not show a significant change across trials (ANOVA, $F(4, 76) = 2.05$, $p = .096$, ges = 0.013).Mauchly's test indicated a violation of sphericity, and the Greenhouse-Geisser correction confirmed the absence of a significant effect (corrected ANOVA, $p = 0.133$), suggesting that fish in this group did not benefit from repeated exposure in the same way as the EE individuals.

3.1.2. Differences in task success and efficiency

Individual task performance during phase 1 ($N = 39$) exhibited substantial variation. The median completion time was 51.38 seconds (SD = 99.48), with a mean time slightly higher at 68.79 seconds (SD = 94.89). This disparity, along with a strong positive skew in the distribution, reflects a majority of fast performers alongside a small subset of fish who consistently failed to complete the task within the 300-second time limit (see Figure 3.1 for distribution).

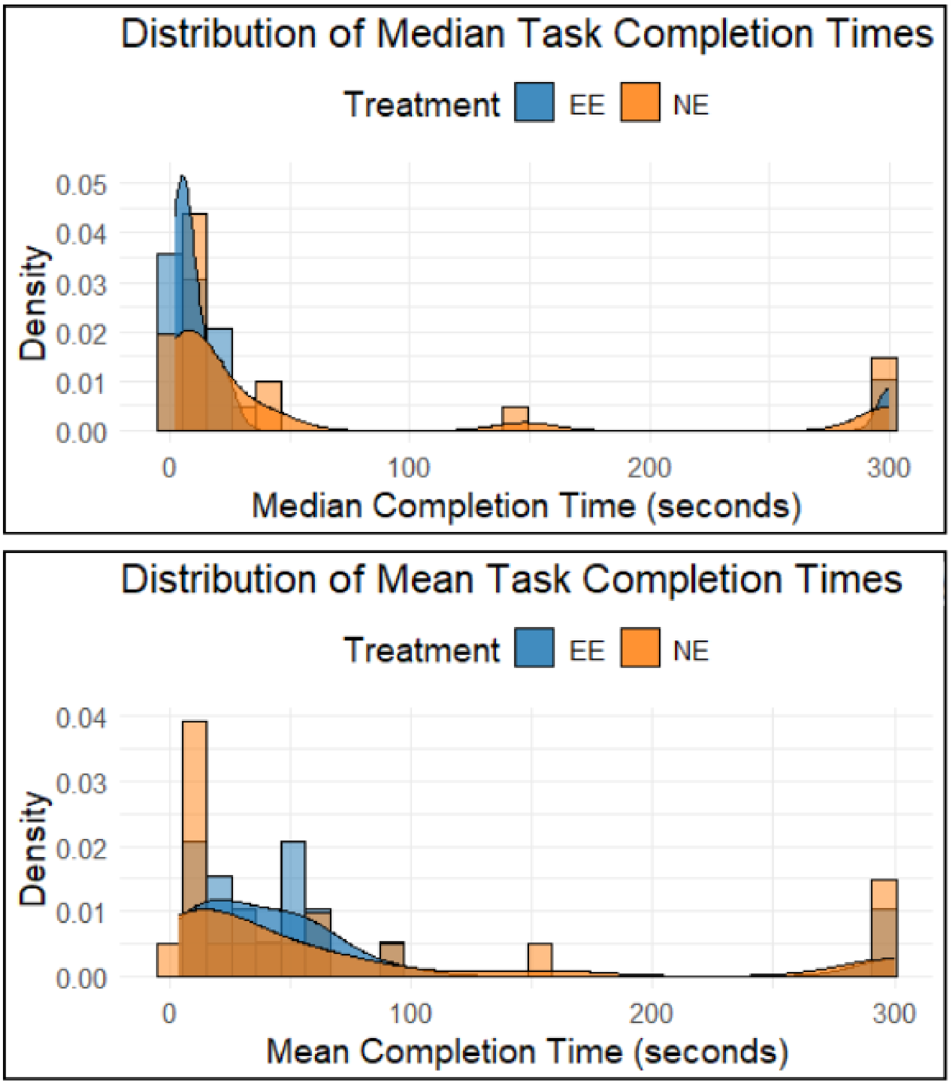

Figure 3.1: Distribution of task completion times during training for fish from enriched (EE) and non-enriched (NE) environments. The top panel shows histograms and density curves of median completion times, while the bottom panel displays the same for mean completion times. Both distributions exhibit a strong positive skew, reflecting a majority of fast performers alongside a smaller group of individuals who failed to complete the task within the 300-second limit. These graphs illustrate the substantial variability in individual task performance across both measures.

Spearman's rank correlation analyses revealed a significant positive association between duration and outcome ($\rho = 0.58$, $p < 0.00011$). These results indicate that longer completion times were strongly correlated with an increased likelihood of failure, supporting the validity of the ordinal efficiency classification that categorised individuals as fast, moderate, slow performers, or failures.

Despite this variation in individual performance, comparisons between treatments revealed no statistically significant differences. Neither median (Wilcoxon rank-sum test, $W = 146$ and $p = 0.22$) nor mean completion times (Wilcoxon rank-sum test, $W = 206$ and $p = 0.66$) differed significantly between enriched and non-enriched environments. Similarly, Kruskal-Wallis tests across housing groups showed no significant differences in median ($\chi^2 = 10.57$, df = 7, p = 0.16) or mean task times ($\chi^2 = 10.95$, df = 7, p = 0.14). Categorical analyses using Chi-square and Fisher's Exact Tests found no significant association between treatment and either task outcome or efficiency category (both p = 1).

3.1.3. Environmental and social influences on training outcome

To assess the influence of treatment and outcome on phase 1 (N = 39) performance, a linear mixed-effects model (LMM: time ~ outcome * treatment + trial + (1|ID)) was applied. The interaction between outcome and treatment was not statistically significant (ANOVA: F(1,85) = 0.004, p = 0.963), indicating that the effect of outcome did not differ significantly between enriched (N = 19) and non-enriched (N = 20) tanks. In a separate model (LMM: time ~ group * treatment + trial + (1|ID)) assessing group differences, no significant main effects or interactions were detected (ANOVA: all F < 2.1, all p-values > 0.10), suggesting that group assignment (G1–G8) did not influence task completion time. Similarly, the model evaluating enrichment treatment alone (LMM: time ~ treatment + trial + (1|ID)) showed no significant difference in estimated task times between enriched and non-enriched conditions (ANOVA: F(1,90) = 0.11, p = 0.7323).

3.1.4. Assessing individual differences in learning performance

To assess whether efficiency categories precisely reflected variation in task performance, we compared mean times across the classified groups (N = 39). The Kruskal-Wallis test revealed a significant difference among efficiency levels ($\chi^2 = 14.939$, df = 2, p = 0.00057), confirming that this classification captured meaningful distinctions in behaviour. Individuals in the "fast" category completed the task in an average of 31.13 seconds, and those in the "moderate" category took approximately 155.60 seconds. In contrast, no significant differences were found in mean task time ($\chi^2 = 0.203$, df = 1, p = 0.653) or task outcome ($\chi^2 = 0.17$, df = 1, p = 0.680) between treatments, suggesting that the presence or absence of enrichment did not influence performance.

3.2. Testing

To provide a clearer overview of performance in final test, the real numbers and percentages of all possible outcomes (F, S, FS, and timeout) were presented for each treatment as we can see in Figure 3.2 Statistical analyses revealed notable differences in behavioural outcomes between tanks like in the F outcome that was significantly more frequent in the EE tank, both in terms of average percentage (p = 0.0007) and count (real numbers, p = 0.010). The FS outcome showed a marginal trend toward significance, with p = 0.057 for percentages and p = 0.094 for total counts, suggesting a possible behavioural divergence between tanks. In contrast, success and timeout did not differ significantly between tanks, with p-values respectively p = 0.54 for average percentage and p = 0.23 for counts and p = 0.36 for average percentage and p = 0.69 for real numbers.

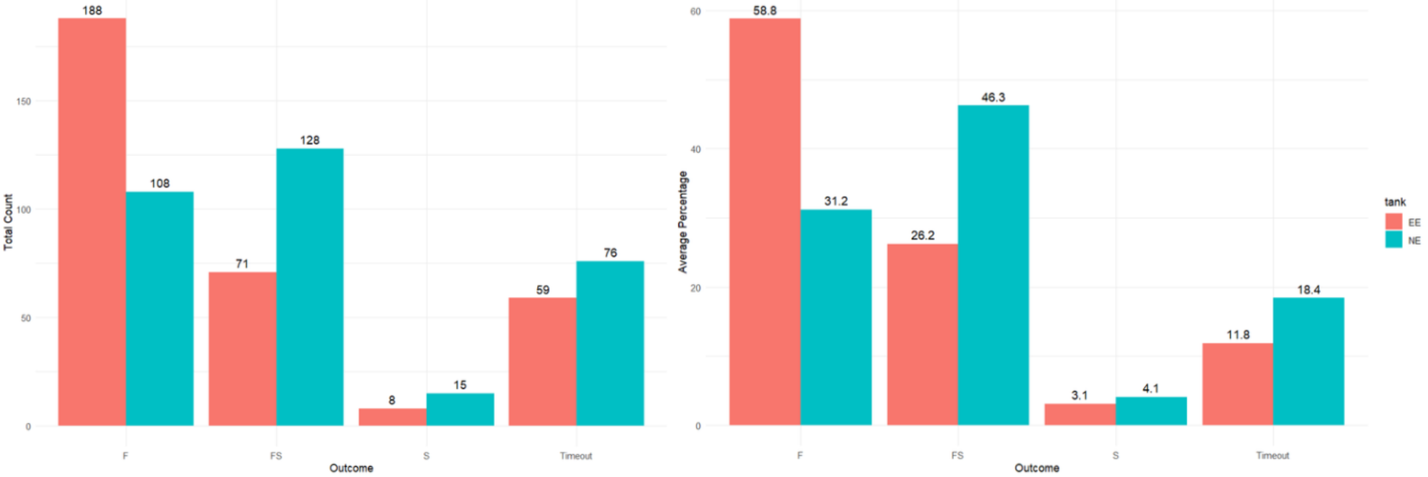

Figure 3.2: Comparison of outcomes (F, FS, S timeout/NI) between EE and NE tanks using both total counts and average percentages. The left panel shows the total number of trials resulting in each outcome where the EE tank had notably more F outcomes (188 vs. 108), while FS was more frequent in the NE tank (128 vs. 71). Successes and timeouts were relatively low in both tanks. The right panel displays the average percentage of each outcome per fish, showing similar trends. Statistical analysis revealed a significant difference in F outcomes (p = 0.010 for counts; p = 0.0007 for percentages), and a marginal trend for FS (p = 0.094 for counts; p = 0.057 for percentages). No significant differences were found for S or Timeout (p = 0.54 for counts; p = 0.23 for percentages and p = 0.36 for counts; p = 0.69 for percentages respectively).

To proceed with the analysis, the data without the post-fail outcome was used (using the FS as F), and the total number of trials per individual ranged from 10 to 30. The mean percentage of successes was low (mean ± SD: 3.6% ± 4.3%), while failures were predominant (mean ± SD: 81.2% ± 19.4%) and timeouts occurred at an intermediate frequency (mean ± SD: 15.2% ± 19.0%). Linear models examining the percentage of successes revealed no significant effects of tank (NE: p = 0.197), total trials (p = 0.220), or their interaction (p = 0.105). In contrast, the percentage of failures decreased significantly with total trials (p = 0.006), while tank (p = 0.808) and the interaction (p = 0.382) were not significant. The percentage of timeouts increased significantly with total trials (p = 0.002), whereas tank (p = 0.909) and the interaction (p = 0.666) did not reach significance (Figure

3.3). Because outcome proportions are bounded between 0 and 1, beta regression models were also applied, which largely confirmed the linear model results while providing additional resolution. Success probability showed highly significant effects of tank (NE: p < 2 × 10^-16), total trials (p < 2 × 10^-16), and their interaction (p < 2 × 10^-16), indicating that fish in NE generally had lower predicted success than those in EE, with the difference becoming more pronounced as the number of trials increased. Failure probability decreased with total trials (p = 0.019), but tank (p = 0.713) and the interaction (p = 0.831) were not significant, and timeout probability increased with total trials (p = 0.047) while tank (p = 0.381) and the interaction (p = 0.651) remained non-significant.

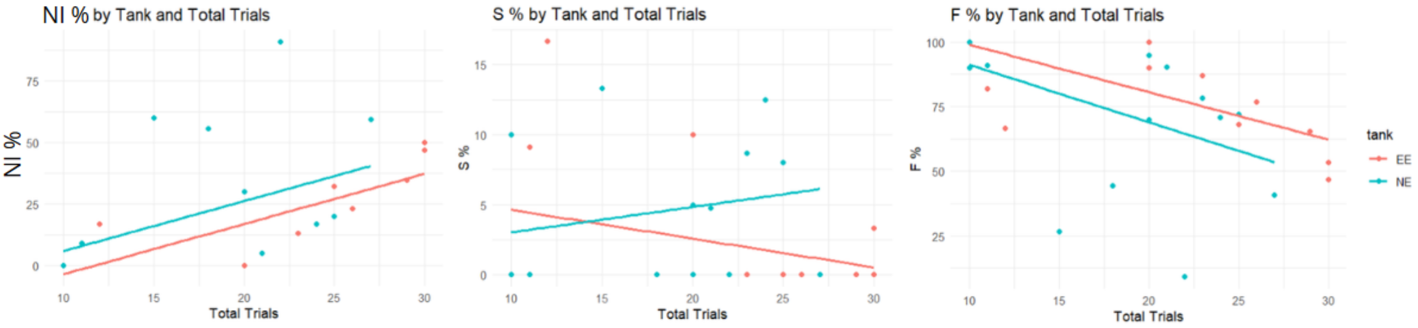

Figure 3.3: Relationship between total trials and outcome proportions (NA, S, and F) across tanks. Each panel shows the percentage of no interaction (NA on the left), success (S in the middle), and failure (F on the right) as a function of the total number of trials, separated by treatment group (EE in red and NE in blue). Points represent individual responses colored by tanks, and regression lines indicate the trend for each treatment.

3.2.1. Comparisons of time to succeed and morphometric variables across Blocks

This analysis showed a significant effect of block (Set of 4 groups, Block1 – G1 to G4 – Block2 – G5 to G8) on performance time, with fish in Block2 (N = 19) taking less time to succeed than those in Block1 (N = 20; Wilcoxon rank-sum test: W = 89, p = 0.012). The second half of the fish was tested 1 month later than the first half, which could have influenced performance due to differences in neural growth and development at the time of testing (Figure 3.4).

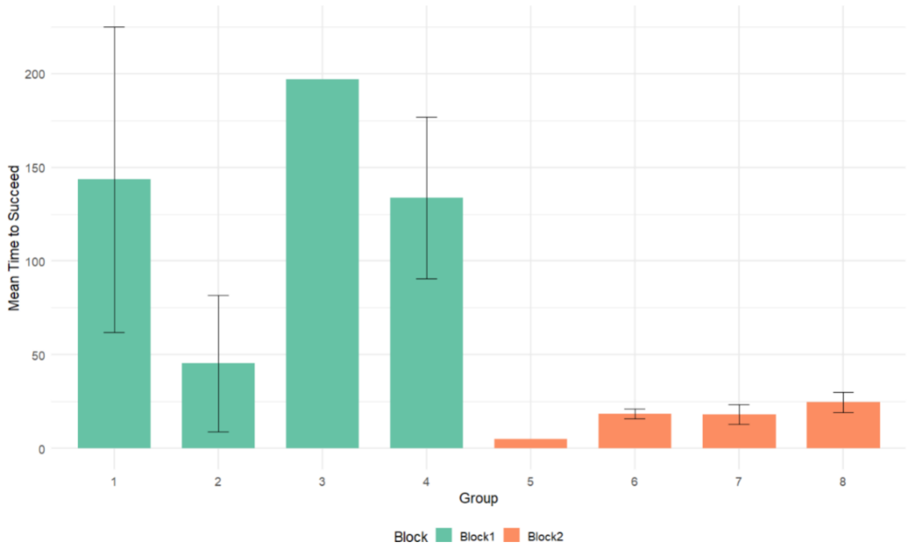

Figure 3.4: Mean time to succeed across groups. Bars represent the mean time to complete the task for each group (groups 1–8), with error bars indicating the standard error of the mean. Groups 1–4 (Block 1) and groups 5–8 (Block 2) were tested 1 month apart, which may have contributed to differences in performance times. Fish in Block 2 generally took less time to succeed compared to Block 1, reflecting potential effects of growth and neural development.

Consequently, the starting body weight (sBW) and starting length (sL) did not differ significantly between blocks (sBW: Block1 meaN = 14.03 g, Block2 = 13.39 g, $p = 0.588$; sL: Block1 = 8.20 cm, Block2 = 7.92 cm, $p = 0.349$), indicating comparable initial size. In contrast, final body weight (fBW) and final length (fL) were significantly higher in Block2 compared to Block1 (fBW: Block1 = 27.12 g, Block2 = 42.91 g, $p < 0.001$; fL: Block1 = 11.14 cm, Block2 = 13.06 cm, $p = 0.00024$), reflecting differences in growth at the time of testing (Figure 3.5).

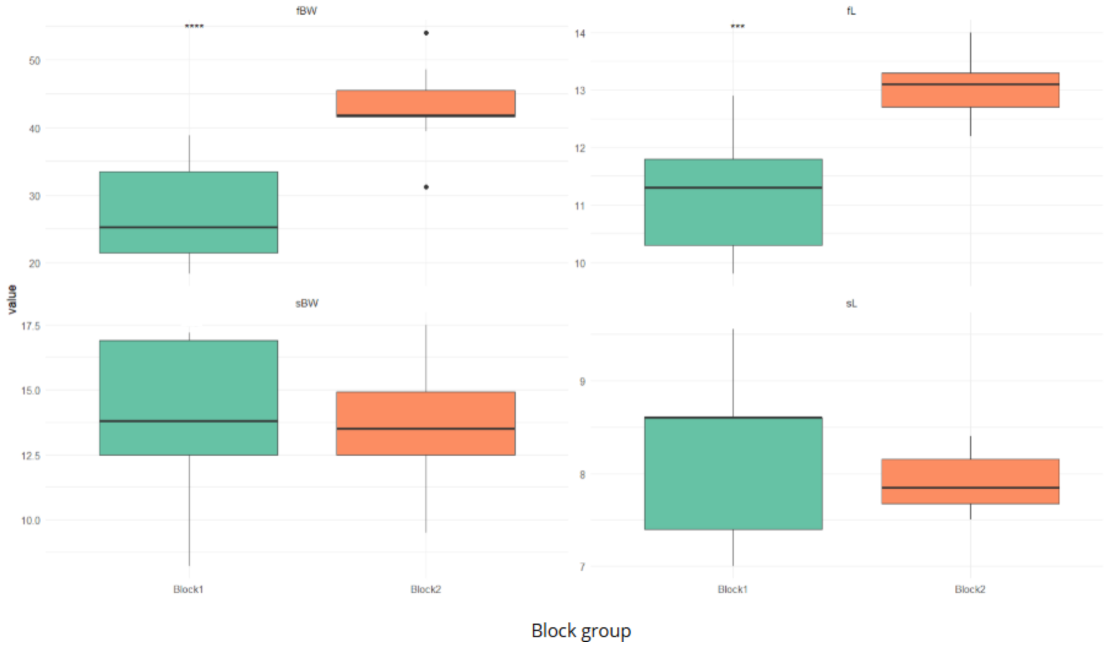

Figure 3.5: Comparison of variable values across Block groups. Box plots display the distribution of four variables (sBW, fL, sBW, and sL) for Block 1 (groups 1–4) and Block 2 (groups 5–8). Each plot illustrates the median, interquartile range, and outliers.

3.2.2. Treatment and trial effects on binary success outcome

This analysis showed no significant effect of EE (N = 19) on response accuracy, so fish exposed to the EE did not perform significantly better or worse than those in the NE treatment (N = 20; $\beta = -0.21$, SE = 0.57, $z = -0.376$, $p = 0.707$). The estimated odds ratio was 0.80, with a 95% confidence interval, indicating no reliable difference in the probability of successful responses between the two treatments. Additionally, the effect of trial progression was examined using polynomial terms; however, none of these terms were statistically significant ($p > 0.70$), suggesting that accuracy remained consistent across trials and did not follow a systematic pattern over time.

3.2.3. Feeding effort across individuals and treatments

A Kruskal-Wallis test revealed a significant effect of enrichment (N = 19) on individual effort levels ($\chi^2 = 15.30$, df = 1, $p < 0.001$). Specifically, individuals housed in EE exhibited significantly greater effort (mediaN = 42.5, interquartile range [IQR] = 36.0–48.0) compared to those in the NE (N = 20) condition (mediaN = 29.0, IQR = 24.0–34.5). These results indicate that increased environmental complexity enhances constant behavioural engagement (Figure 3.6).

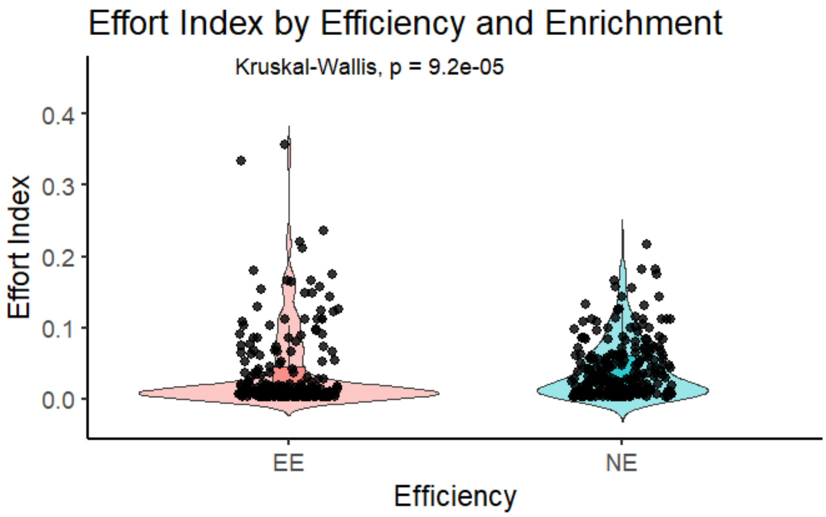

Figure 3.6: Feeding effort index across environmental conditions. Violin plot illustrating individual effort indices under EE and NE conditions. Individuals in EE exhibited significantly higher effort levels (mediaN = 42.5, IQR = 36.0–48.0) compared to NE (mediaN = 29.0, IQR = 24.0–34.5), as confirmed by a Kruskal-Wallis test (χ^2 = 15.30, df = 1, $p < 0.001$). These findings support the hypothesis that environmental enrichment enhances sustained behavioural engagement.

Further analysis of effort across the eight experimental groups revealed that individuals in Group 5 (G5, N = 4) exhibited the highest levels of effort (mediaN = 51.0, IQR = 47.5–55.0). Effort scores in this group were significantly greater than those observed in Groups 2 (NE, N = 5), 3 (EE, N = 5), 4 (NE, N = 5), and 6 (EE, N = 5) (all $p < 0.001$, with Bonferroni correction). However, as Group 5 included only four individuals, this finding should be interpreted with caution, as the observed differences may reflect individual-level variability rather than a consistent group-level effect.

3.3. Personality

The analysis of behavioural patterns during the training phase revealed four distinct clusters of individuals (Figure 3.7), each reflecting unique combinations of learning rate, latency, and exploratory behaviour.

Cluster 1 (N = 7) included individuals with slightly negative learning slopes (mean LR = –2.93, SD = 1.37), short initial latencies (meaN = 10.1 s, SD = 5.19), and moderate exploratory activity (mean EB = 19.4 s, SD = 10.2), representing moderate learners with quick responses and low-to-moderate activity. Cluster 2 (N = 19) consisted of individuals with more negative learning slopes (mean LR = –15.4, SD = 15.8), intermediate latencies (meaN = 38.5 s, SD = 36.8), and higher exploratory activity (mean EB = 146.0 s, SD = 107.0), reflecting intermediate learners with moderate responsiveness and higher activity. Cluster 3 (N = 6) was characterized by individuals with positive learning slopes (mean LR

= 9.12, SD = 22.3), long latencies (meaN = 265.0 s, SD = 85.3), and high exploratory activity (mean EB = 190.0 s, SD = 82.7), representing slow learners with delayed responses and high exploratory tendencies. Finally, Cluster 4 (N = 3) combined very high negative learning slopes (mean LR = –54.0, SD = 5.07) with moderate latencies (meaN = 60.7 s, SD = 5.03) and very high exploratory activity (mean EB = 297.0 s, SD = 5.77), corresponding to rapid learners with high exploratory activity.

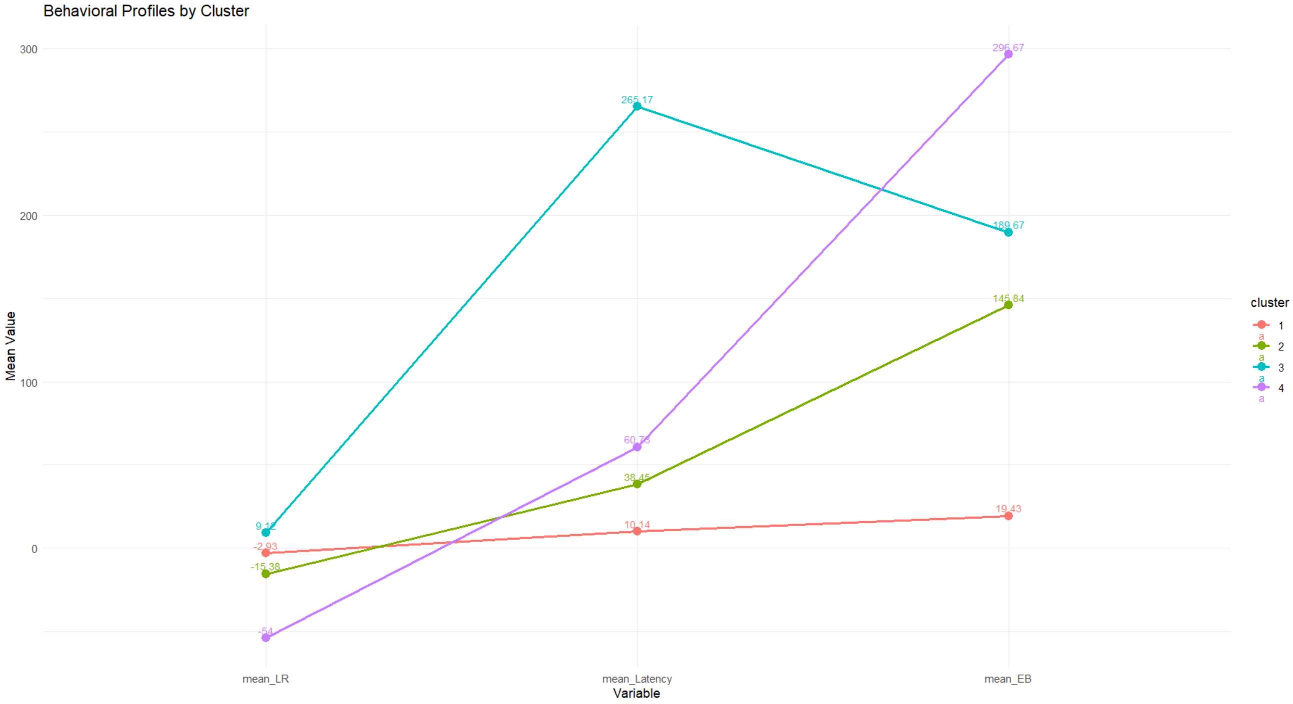

Figure 3.7: Behavioural profiles of individuals across the training phase, grouped by PCA-based clusters. Each line represents the mean values of three behavioural variables: learning rate (LR), latency, and exploratory behaviour (EB) for a given cluster. Clusters capture distinct combinations of behavioural tendencies: Cluster 1 — moderate learners with quick responses and low-to-moderate activity; Cluster 2 — intermediate learners with moderate responsiveness and higher activity; Cluster 3 — slow learners with delayed responses and high exploratory tendencies; Cluster 4 — rapid learners with high exploratory activity. Values above the points indicate the mean for each variable.

Comparisons of effort during the experimental phase revealed clear differences between these clusters. A one-way ANOVA indicated a significant effect of cluster on effort (N = 35, F(3,31) = 3.32, p = 0.032), with post-hoc Tukey tests showing that individuals in Cluster 3 (mean effort = 0.80%, SD = 0.58) invested significantly less effort than those in Cluster 1 (mean effort = 7.70%, SD = 5.80; p = 0.028). No other pairwise differences reached significance. Together, these findings illustrate that the behavioural phenotypes defined during the training phase—learning rate, latency, and exploratory behaviour—capture meaningful variation among individuals, and that these phenotypic differences are reflected in subsequent effort during the experimental phase.

4. Discussion

This study investigated the effects of environmental enrichment on inhibitory control in juvenile gilthead seabream (*Sparus aurata*) using the cylinder task, a widely used paradigm for assessing executive functions. The results suggest that EE promotes greater behavioural engagement and improves learning during training, but it does not significantly enhance inhibitory control during testing. Instead, individual factors such as body size and personality appear to play a more significant role in task performance.

During the training phase, fish housed in EE demonstrated significant improvements in task performance over repeated trials. Specifically, individuals in the EE treatment were slower in Trial 1 but significantly faster in Trials 3 to 5, indicating that EE supported learning. This finding is consistent with research showing that enriched environments enhance cognitive abilities in fish by encouraging exploration and neural development (Näslund & Johnsson, 2016; Salvanes et al., 2013). In contrast, fish in non-enriched (NE) tanks showed no significant improvement across trials, suggesting that a lack of environmental stimulation may limit learning or motivation. However, during the testing phase, EE did not lead to better inhibitory control; both EE and NE fish performed similarly in terms of task accuracy and response times, indicating no significant treatment effect. This aligns with studies suggesting that inhibitory control may be less influenced by short-term environmental changes and more dependent on inherent cognitive traits (Lucon-Xiccato et al., 2017). Notably, successful trials were completed faster than failures, a pattern seen in both treatment groups, suggesting that efficiency reflects behavioural differences rather than random variation.

Body size played a critical role in task performance, with larger fish consistently outperforming smaller ones. Fish in Block 2, tested a month later than Block 1, had significantly higher final body weight and final length, and they completed the task faster. This suggests that larger body size, likely associated with greater developmental maturity, enhances task efficiency. Larger fish may have better-developed sensory and motor systems, allowing for quicker responses, or they may exhibit greater confidence when approaching novel tasks, reducing hesitation (Bensky et al., 2017). Although initial body size did not differ significantly between blocks, we may have taken into account that the final body measurements were the most accurate values at the time of training/testing, and that these final measurements showed significant differences, indicating that size can influence individual performance. This size advantage may reflect physiological advantages, such as improved neural development or metabolic capacity, which enable larger fish to navigate cognitive challenges more effectively (Brydges et al., 2011). These findings highlight body size as a key factor in cognitive performance, with implications for understanding how physical development influences behaviour in fish.

Another clear effect of environmental enrichment was observed in feeding effort (percentage of bites per time). Fish in EE tanks exhibited significantly higher effort levels, with median effort scores notably higher than those in NE tanks. This suggests that EE fosters greater motivation and engagement, consistent with studies showing that environmental complexity encourages goal-directed behaviour and reduces neophobia in fish (Marchetti & Nevitt, 2003; Salvanes & Braithwaite, 2005). However, the exceptionally high effort in Group 5 should be interpreted cautiously due to its small sample size (N = 4), which may reflect individual variation rather than a consistent treatment effect.

Among these internal traits, personality emerged as a major factor shaping individual differences in performance. Cluster analysis identified four distinct behavioural profiles based on learning rate, latency, and exploratory behaviour. Cluster 1 fish were quick and efficient, Cluster 2 showed moderate traits, Cluster 3 included slow, cautious fish, and Cluster 4 comprised fast learners with high exploration. These profiles align with research on behavioural syndromes in fish, where bold and shy traits shape cognitive outcomes (Sih & Del Giudice, 2012) but given the complexity of the data, it is difficult to adjust it to this simpler type of classification. For example, Cluster 4's proactive, exploratory fish likely excelled due to rapid decision-making, while Cluster 3's cautious approach led to lower effort but greater persistence. Personality thus accounted for substantial variation in task performance, often more than environmental treatment.

These findings have significant implications for animal welfare in captive settings, particularly for species like gilthead seabream. Environmental enrichment (EE) enhances behavioural engagement and learning efficiency, which can reduce stress and boredom in fish, fostering a more naturalistic environment that aligns with welfare principles outlined by the Five Domains Model (Mellor et al., 2020). By promoting exploratory behaviour and motivation, as evidenced by higher feeding effort in EE fish, such practices could mitigate the negative effects of barren housing, such as abnormal behaviours or reduced cognitive function, improving overall well-being. However, the lack of impact on inhibitory control underscores that EE should be tailored to individual traits like personality and body size to maximize benefits, ensuring that welfare interventions account for variability among fish to avoid unintended disparities. In aquaculture practices, incorporating EE could enhance production outcomes by improving fish adaptability and resilience. Studies show that enriched rearing environments lead to better growth rates, disease resistance, and post-release survival in farmed fish (Näslund & Johnsson, 2016; Salvanes et al., 2013), potentially leading to higher sustainability in seabream farming. These findings support the use of cost-effective environmental enrichment in aquaculture to enhance welfare and efficiency, but additional research is required to apply these benefits on a commercial scale.

5. Conclusion

This study demonstrates that environmental enrichment (EE) enhances learning and behavioural engagement in juvenile gilthead seabream (Sparus aurata) during the cylinder task, but it does not significantly improve inhibitory control. Fish in enriched environments showed faster learning rates and higher feeding effort, indicating that EE fosters motivation and exploration. However, body size and personality emerged as stronger predictors of task performance, with larger fish and proactive individuals exhibiting greater efficiency. These findings highlight the complex interplay between environmental factors and intrinsic traits in shaping cognitive outcomes. In aquaculture, implementing cost-effective EE could improve fish welfare by reducing stress and promoting naturalistic behaviours, potentially enhancing growth, resilience, and sustainability. Nevertheless, the limited impact of EE on inhibitory control suggests that welfare interventions should be tailored to individual characteristics, such as size and behavioural profiles, to maximize benefits. Future research should explore the effects of prolonged enrichment, varying developmental stages, and scalable EE strategies in commercial aquaculture to further optimize welfare and production outcomes for species like gilthead seabream.

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