

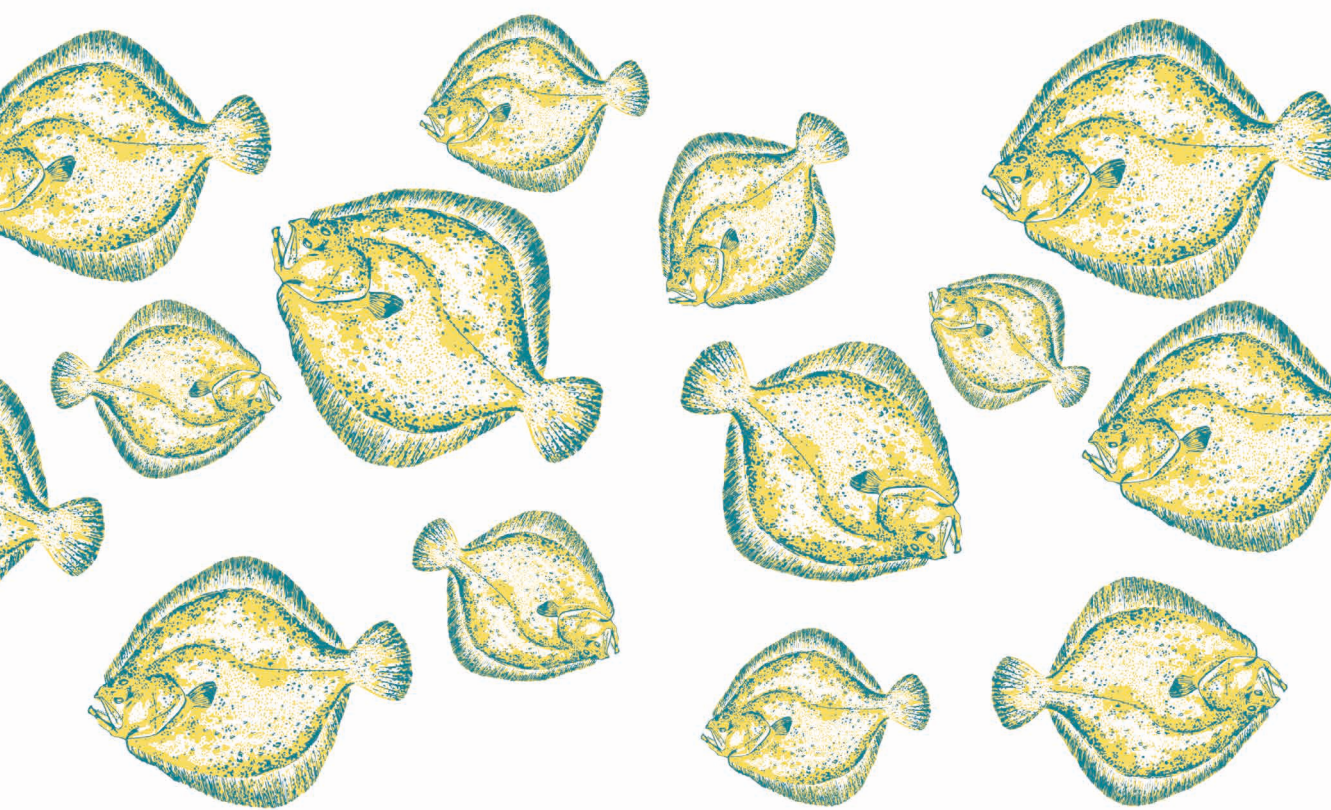
A GUIDE ON

FISH WELFARE

IN

SPANISH AQUACULTURE

(Vol. 5): Welfare of turbot



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SCOPE OF THE GUIDE

Given the importance of aquaculture fish welfare for producers, consumers, and the authorities responsible for ensuring compliance with the best available practices, APROMAR, as the representative body of the aquaculture fish industry in Spain, internally proposed the development of a series of volumes dedicated to this relevant subject. For this reason, based on the relationships previously established with stakeholders across the sector's value chain, we invited companies, public administration bodies, scientists, and professionals from organizations dedicated to animal welfare to work together. The proposal put forward was to develop a set of guides compiling the best available practices to ensure the proper observance of fish welfare in Spanish aquaculture. This guide (volume 5), on the welfare of turbot, published in 2025, has been preceded by

2022- Vol. 1. Guide on fish welfare in Spanish aquaculture.

2023- Vol. 2. Guide on fish welfare in Spanish aquaculture. Welfare of European seabass.

2023- Vol. 3. Guide on fish welfare in Spanish aquaculture. Welfare of gilthead seabream.

2024- Vol. 4. Guide on fish welfare in Spanish aquaculture. Welfare of rainbow trout.

These guides may be downloaded in Spanish and English from APROMAR's website (<https://apromar.es/guia-bienestar/>).

The first volume established common concepts, whereas the subsequent ones identified a set of welfare indicators, the critical welfare points within the production system, and a series of species-specific aquaculture best practices.

In addition to including proposals related to training and communication, the guides address the main challenges and opportunities currently present in this field. The entities responsible for their development agree that they should be understood as dynamic and continuously evolving documents, subject to regular review and updating as new scientific knowledge, regulatory advances, and technological developments emerge. The aim is to ensure that the recommendations and practices contained therein remain at the forefront of aquaculture fish welfare.

This document is primarily intended for aquaculture companies in Spain and for all individuals involved in the sector, both those who work directly with fish and those responsible for organizational and management functions. It will also be of value to public administrations, policymakers, the scientific, technological, and educational communities, as well as to society as a whole.

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EXECUTIVE SUMMARY

Turbot (*Scophthalmus maximus*) production in Spain represents one of the most consolidated and specialized branches of marine aquaculture in Europe. It is concentrated mainly in Galicia, where environmental conditions (temperature, water quality, and the availability of coastal space) are highly favorable for its cultivation. Spain is the leading producer within the European Union and one of the global leaders, with a stable and upward-trending production of approximately 8,000–10,000 tons per year in recent years. Turbot is a high-value-added product that supplies both domestic consumption and exports to European food service and retail markets.

The business sector comprises a few large companies that account for most of the production and have actively contributed to the preparation of this guide. This structure gives the industry a distinctly industrialized profile, characterized by significant investment in technological innovation, biosecurity, and genetic improvement. In addition, there is close collaboration with research centers and universities to advance knowledge on turbot welfare, as well as on other key areas such as nutrition and environmental sustainability. The business sector also works closely through APROMAR in engaging with public authorities and participating in European forums, promoting the product and defending shared interests.

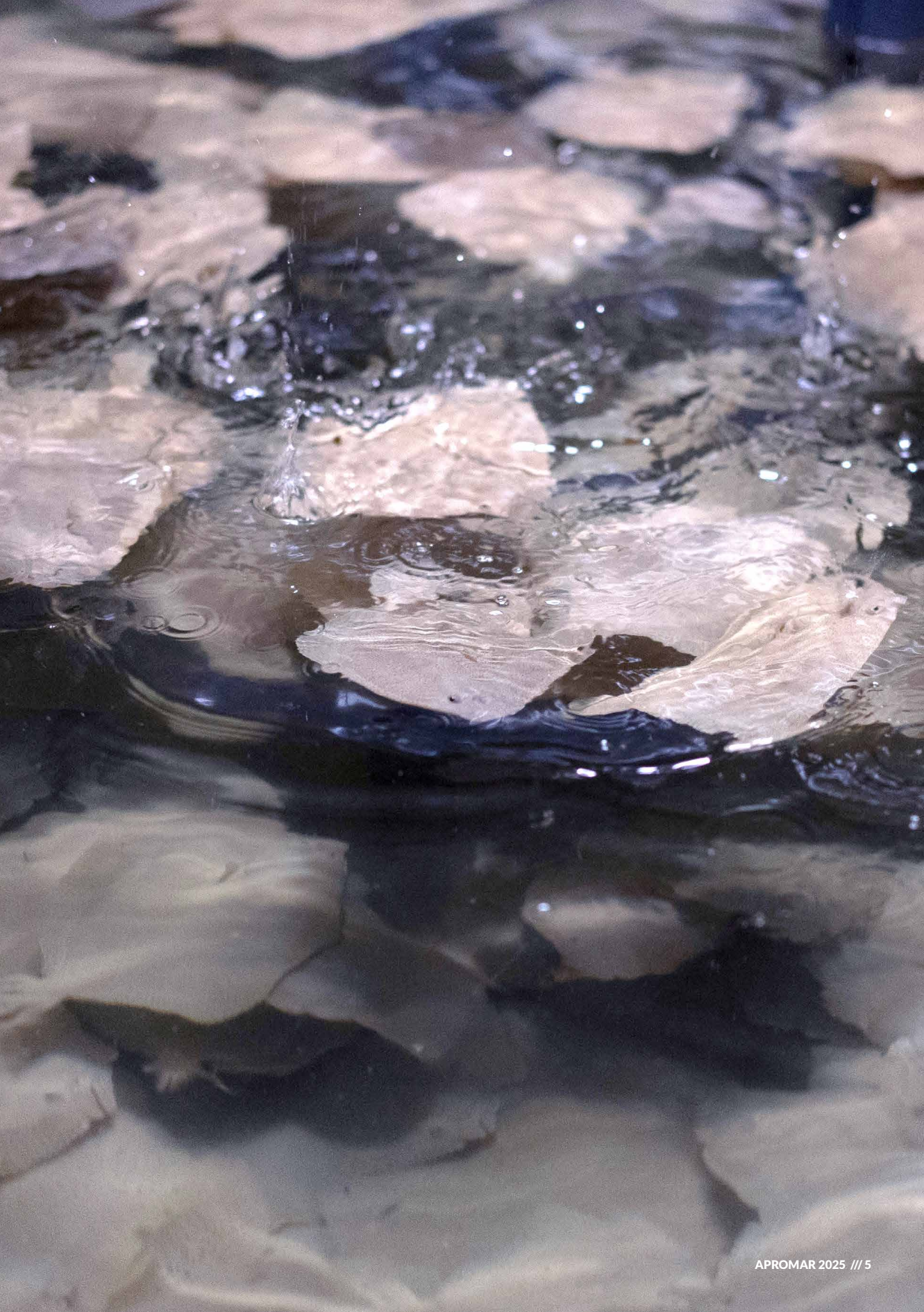
Turbot welfare within the aquaculture facilities of these companies is an absolute priority. As is widely recognized, proper fish production depends on ensuring fish welfare, meaning that optimal growth and development of aquaculture fish are only possible when welfare parameters are adequately met. Aquaculture pro-

ducers are responsible for maintaining animal welfare, both as part of their commitment to sustainable production and because it is directly linked to the profitability of any enterprise.

For the first time, a single document brings together the most recent scientific knowledge on turbot welfare, presenting a series of operational indicators for its assessment. These indicators are specific, quantifiable, and adaptable to the different life stages and production systems used. The guide also identifies the critical welfare points during the stages of reproduction, incubation, fry rearing, pre-fattening, and fattening. It addresses practices related to common on-farm procedures, including feeding, environmental conditions and confinement, handling and maintenance, reproduction, animal health, transport, stunning, and slaughter.

The training of personnel and the involvement of company management are key factors in ensuring fish welfare under controlled conditions. This document acknowledges their importance and is conceived as a tool for both training and outreach.

Funded through national and European resources, this welfare guide is the result of a collaborative effort involving turbot-producing companies, APROMAR staff, scientists from several Spanish universities, animal welfare organizations and public administration (see page 2). The guide includes the following: a) the biological characteristics of turbot, b) operational welfare indicators of various types, c) critical points in different rearing systems and developmental stages, d) best practices for turbot aquaculture, e) staff training and communication, and f) the main challenges facing the sector.





1. INTRODUCTION

The European turbot is a flatfish belonging to the family *Scophthalmidae*. This species is still frequently referred to in the literature as *Psetta maxima*, but the updated and currently accepted scientific name is *Scophthalmus maximus*¹. The turbot is widely distributed along the European Atlantic coast (northeastern and eastern Atlantic Ocean). It is also found along the shores of Morocco, the Black Sea, and the Mediterranean Sea, although it is less common in the latter.

It is a flat, oval, and asymmetrical fish with a disk-shaped body and small, prominent eyes located on the left side. Initially, turbot larvae are symmetrical, but starting around day 15 of development, the right eye begins to migrate to the left side, completing the process in about 30 days. The ocular side is pigmented and mimetic, with brownish and greenish tones covered by numerous light and dark spots of various sizes that imitate the color of the seabed where it lives². The blind (right) side is whitish. The skin lacks scales but is covered with irregularly distributed bony tubercles. The dorsal and anal fins extend widely from the head to almost the tail, contributing to swimming and bottom movement. Although there is no sexual dimorphism in skin pigmentation, females are generally larger due to their faster growth. Turbot can reach a length of up to one meter.

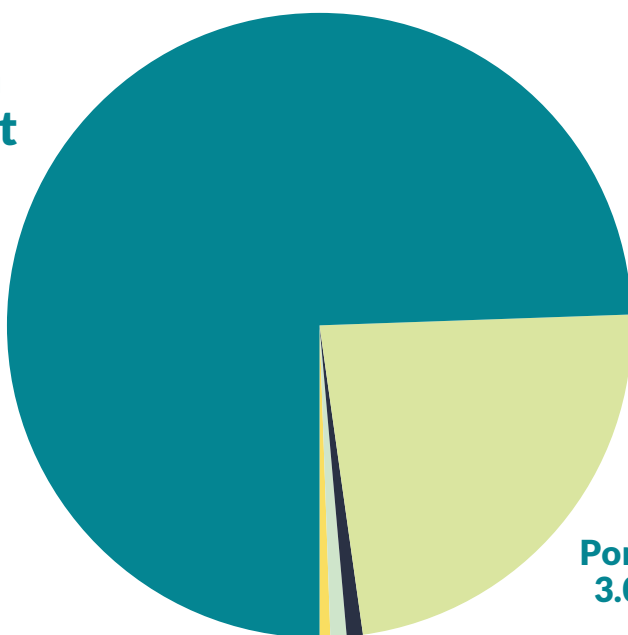
In the wild, the turbot is a marine species inhabiting cold-to-temperate waters, living mainly over sandy, rocky, or mixed bottoms at depths of up to 150 meters³. It can occasionally inhabit brackish waters. The turbot is carnivorous: juveniles feed on mollusks and crustaceans, while adults primarily feed on fish and cephalopods. During its larval phase, it lives in the water column, floating, but once metamorphosis is complete, it settles on the bottom and becomes a benthic species². During the juvenile stage, the turbot is found in coastal areas, gradually moving toward deeper waters as it grows.

The turbot is a dioecious species whose reproduction occurs once a year in spring: between February and April in the Mediterranean and from May to July in the Atlantic. Female turbot reach sexual maturity at around 40 cm in length (2 years), while males are capable of fertilization at about 30 cm (3 years). For spawning, turbot gather in selected spawning grounds located at depths of around 30 meters. The female swims while simultaneously releasing eggs, followed by one or several males that release sperm to fertilize the eggs suspended in the water. Females spawn several sequential batches every 2–4 days, during which they release approximately three million transparent eggs of about 1 mm in diameter. Fertilized eggs are pelagic, and incubation usually lasts about one week (depending on temperature), after which the larvae hatch.

Turbot aquaculture began in the 1970s in Scotland (United Kingdom), with the full production cycle being closed during the 1980s. It was subsequently introduced in France and Spain⁴. Initially, due to the scarcity of juveniles, the number of facilities in Spain was rather limited. Increased knowledge of the species, technological development, and the reorganization of the sector have led to growth both in production and in the number of countries cultivating turbot. Today, Spain is the world's largest producer of turbot, which is also farmed in Denmark, Germany, Iceland, Ireland, Italy, France, Norway, the United Kingdom, and Portugal, and was formerly farmed in the Netherlands. The turbot has also been introduced into other regions (notably Chile in the late 1980s) and more recently into China⁴. Worldwide, total aquaculture production of turbot in 2023 was 75,140 tons, with Spain being the leading producer in Europe (9,600 t), followed by Portugal (3,000 t), Italy (100 t), France (100 t), and the Netherlands (50 t). At present, all turbot farming in Spain takes place in Galicia (100% of national production, 12.8% of total global production)⁵.



Spain
9.600 t



Portugal
3.000 t

The
Netherlands
50 t

France
100 t

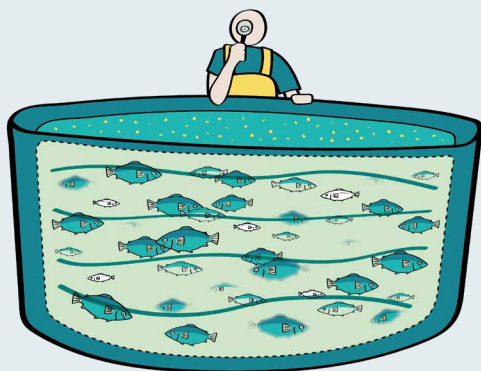
Italy
100 t

Figure 1. Distribution of turbot production in Europe's main producing countries (percentage of total European volume produced in 2023) ⁵.

2. WELFARE INDICATORS FOR TURBOT

Aquaculture rearing systems present specific challenges for fish welfare due to the differing biological needs, capacities, and responses to stress or pain among species, individuals, and life stages. Welfare indicators must therefore be species-specific, allowing for a reliable, objective, and recurrent assessment of welfare status in relation to the species, size, and production systems involved. These indicators serve as tools not only for evaluating the procedures implemented within the company but also for adopting the necessary measures to promote fish welfare. However, depending on the characteristics of the rearing system and other circumstances, some indicators may be more applicable and relevant than others. The selection of welfare indicators must take into account the species' lifestyle, morphology, and behavior. Based on experience with turbot aquaculture systems and the existing literature, the main welfare indicators for this species are described below:

2.1. Physical or External Indicators



These are known as operational indicators based on the fish's outward appearance. They provide information on body condition or external appearance at the population level. The external indicators for turbot, independently of relevance, include:

Condition Factor

The condition factor (K) is a widely accepted indicator for assessing the nutritional status of fish ⁶. It is calculated using the following formula: $K = (\text{body weight (g)} / \text{length (cm)}^3) \times 100$. A K value equal to or close to 1 is considered the threshold for a healthy fish in good condition ⁷. Since the condition factor varies with species, life stage, and season, it is difficult to define exact values that indicate a reduction in overall welfare across the life cycle. Although commonly used in other species, this methodology is not typically applied to turbot, mainly due to fish morphology. It relies instead on the correlation between body weight and age. Nevertheless, an extremely high condition factor in farmed turbot may indicate over-feeding or limited space ⁸. In contrast, very low K values may suggest a loss of body mass caused by inadequate feeding or chronic stress ⁹⁻¹⁰, both of which negatively affect turbot welfare.

Population Dispersion

It is important to monitor size dispersion within each population using methods such as distribution analysis or the variation coefficient. In turbot, differences in size within the same population or rearing unit may result directly from social interactions among individuals, including hierarchy formation or competition for food, in

addition to possible genetic differences ¹¹⁻¹². Social hierarchies or interactions in turbot can be influenced by several factors such as temperature, lighting, feeding regime, and stocking density, among others ^{11,13-16}. These findings highlight the importance of good husbandry practices to maintain the most homogeneous and stable size distribution possible, thereby ensuring fish welfare and optimizing growth performance.

Skin Condition

The skin is considered the largest organ of fish, capable of reflecting changes associated with different physiological or environmental conditions occurring during development ⁷. This organ performs several essential functions, including physical and chemical protection and the regulation of ion and water exchange. Fish are constantly exposed to their surroundings, which makes the skin and mucus their first line of defense. The condition of the turbot's epidermis is thus a key indicator of its health and welfare, as it can significantly influence its general condition or indicate the presence of harmful stressors in the environment (chemical, physical, or biological) ¹⁸. Stressful situations (e.g., high densities, poor water quality) can alter the skin's structure and immune capacity, thereby increasing susceptibility to infections ¹⁹⁻²¹. In addition, some procedures carried out during production that involve physical contact with the fish (such as handling and harvesting) can cause alterations or lesions in the skin, increasing the risk of infection. Assessing skin health requires examining the integrity of the mucous layer and identifying any loss of epidermal tissue that may affect deeper layers. In turbot, this also includes the inspection of possible damage to the characteristic bony tubercles of its skin. It is therefore of great importance to identify active lesions—such as inflamed areas, ulcers, hemorrhages, or superficial wounds—as they can be rapidly colonized by microorganisms present in the environment.

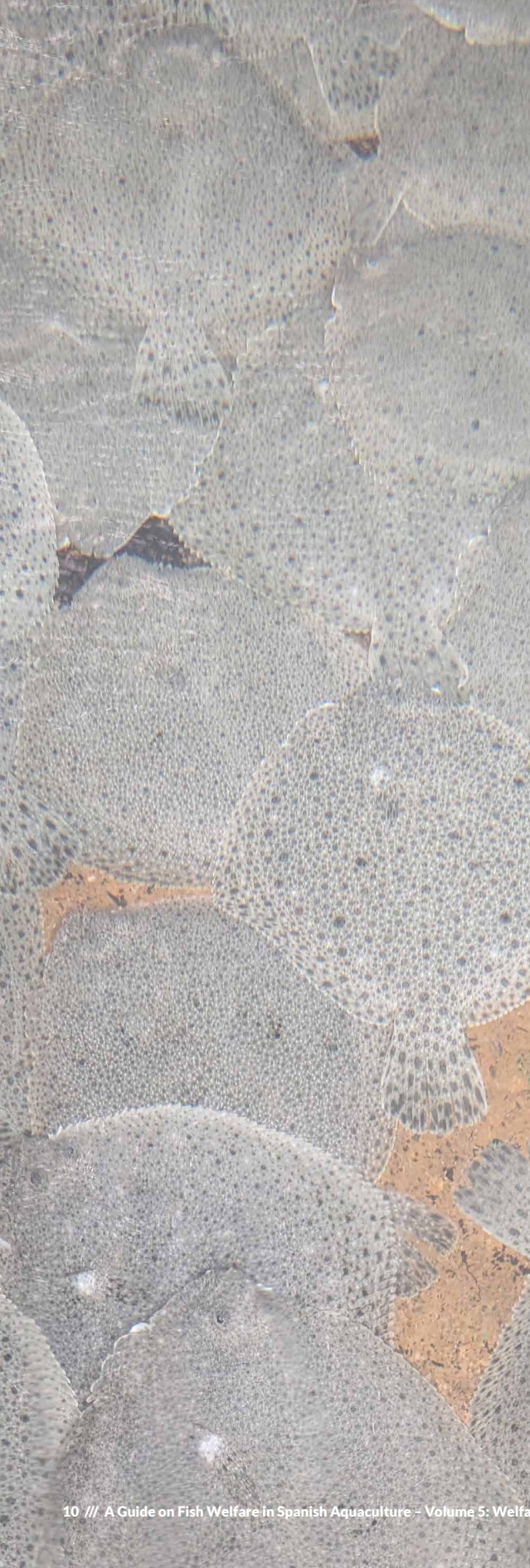
Skin Coloration

Variations in skin color or pigmentation are often good indicators of rearing conditions or physio-

logical alterations in fish. In turbot, these variations may be influenced by lighting, substrate characteristics, dietary changes, or disease ²²⁻²⁶. The different skin colorations observed in turbot are mainly due to variations in the number and distribution of chromatophores ²⁷. Moreover, diseases caused by bacteria or viruses may lead to darkening of the pigmented (ocular) side and the fins ²². Occasionally—especially during the metamorphic phase of larvae—abnormal pigmentation or color aberrations can occur, also influenced by light conditions, substrate, or diet ²⁴. Among the most common pigmentation abnormalities in turbot are albinism on the ocular side and darkening or ambicoloration of the eyeless side ²⁸⁻²⁹. Although these abnormal pigmentations have not been associated with welfare problems in turbot ²⁹, they may nonetheless result in a reduced market value.

Physical Malformations

Turbot may exhibit body malformations, and individuals with irregular body shapes or cranial, body, and—more severely—fin deformities are occasionally observed ²⁹⁻³¹. Cranial anomalies include jaw deformities, underdeveloped operculum, and incomplete migration of the right eye. Body malformations are among the most frequent in turbot, primarily involving anomalies in the vertebral column (e.g., vertebral fusion, scoliosis, lordosis, and kyphosis). Both spinal and cranial deformities significantly affect the external morphology and growth of turbot. Similarly, deformities in the caudal, dorsal, and anal fins are more common than in the pectoral and pelvic fins. Severe deformities of the caudal fin are generally associated with major spinal deformities and produce noticeable external changes. Occasionally, alterations in the swim bladder have also been described, with consequences for buoyancy, development, and larval survival ³². Most of these malformations occur during the larval or early stages and are primarily due to genetic predisposition, as well as adverse feeding or rearing conditions, especially during metamorphosis. When body malformations are severe and easily visible, they can impair swimming and feeding, reduce growth, and even lead to high mortality rates ²⁹⁻³⁰. Individuals with less



severe physical malformations may reach advanced stages of the production cycle if not detected during grading in earlier phases, resulting in low swimming and feeding efficiency, slow growth, and higher stress levels, all of which negatively affect their welfare and potentially that of the group.

Fin Condition

The condition of the fins is a critical parameter to consider because fins contain pain receptors (nociceptors) and therefore serve as an indirect indicator of turbot health and welfare. Fin damage can result from a variety of causes, most notably abrasion against the tank or its components, the presence of pathogens, poor water quality, nutritional deficiencies, or improper handling³³⁻³⁴. Fin injuries typically begin as erosion, tearing, or thickening, which make the tissue more susceptible to infection. In more severe cases, they may progress to hemorrhages, which may indicate the possible presence of pathogens^{20,22,35}.

Gill Condition

Beneath the opercula are the gills, which should display a bright red color and a moist appearance, without mucous coatings, clumping of the gill filaments, whitish discoloration, or unpleasant odors. Such conditions may indicate health and welfare problems that increase the fish's vulnerability. Additionally, a visual examination of the gills can evince water quality issues, as water represents the main risk factor due to the high exposure of this organ to the environment. Gill inspection can also reveal signs of possible bacterial or parasitic infections³⁶⁻⁴⁰.

Eye Condition

Due to the protruding and unprotected nature of turbot's eyes, improper handling can cause mechanical damage and ocular desiccation (cloudy or opaque eyes), which may lead to the development of ulcers. Another aspect to consider is inflammation of the tissues and fluid accumulation behind the eyes, which can cause eye protrusion (exophthalmia), indicating poor water quality conditions as well as various pathological disorders⁴¹⁻⁴⁷.

Mortality

The occurrence of disease outbreaks or environmental and/or management-related problems in turbot farming can lead to increased mortality^{2,48}. The mortality rate refers to the percentage of fish that die within a given period. It is a direct, population-level indicator widely used in welfare assessment. Although retrospective in nature, it is associated with multiple factors—including infectious, viral, and parasitic diseases; environmental conditions; genetics; husbandry; and nutrition. Recording daily mortality at any stage provides valuable information, serving as an indicator of serious anomalies within the population or rearing system. Mortality rates and causes vary depending on the phase of the production cycle, so maintaining daily records of mortality and morbidity is essential for detecting welfare issues.

2.2. Behavioral Indicators

Observing and understanding turbot behavior is crucial for improving welfare and reducing stress responses to daily farming practices, which in turn enhances farm performance. Behavioral alterations, particularly in activity or feeding, have often been used to identify possible variations related to welfare. However, these behaviors must be established and analyzed according to each developmental stage and situation; therefore, the overall set of behavioral data is essential for assessing fish welfare⁴⁹. The following operational indicators provide information on turbot behavioral patterns at both the individual and group level, forming part of the direct, animal-based welfare indicators.

Activity and Distribution

Aspects related to fish swimming behavior, such as activity or speed, provide data that facilitate the understanding of how fish adapt to their environment. Turbot's swimming activity varies according to the developmental stage and is mainly associated with light and feeding periods⁵⁰.

In the pre-metamorphic stage, turbot larvae alternate between periods of high activity, associated with feeding, and phases of lower activity,

during which the larvae may remain almost motionless, moving primarily with the water current. Occasionally and quite suddenly, they exhibit short bursts of swimming with random and complex trajectories, after which they rest and then resume swimming⁵¹⁻⁵². In tanks, larvae show an irregular and variable three-dimensional distribution, making use of all the available space⁵¹. If turbot larvae move vigorously or cluster in a confined area of the tank outside feeding periods, it may be an indicator of food scarcity, health issues, or changes in environmental conditions (lighting, temperature, salinity)⁵³⁻⁵⁶.

During metamorphosis, turbot whose right eye is in the process of migration tend to swim more actively than in the previous phase, moving within the water column, near the tank bottom, or even touching the surface. After metamorphosis, turbot settle on the bottom, adopting a benthic lifestyle and thereby greatly reducing their swimming activity. In the wild, turbot display an innate burrowing behavior⁵⁷⁻⁵⁸; however, in farming conditions where no substrate is available, they tend to seek physical contact with other individuals, often lying partially over one another. This behavior is possibly linked to the social hierarchies observed in captive turbot, where individuals pile upon each other, with dominant fish usually remaining at the bottom of the group⁵⁹. Therefore, considering their gregarious behavior in captivity, turbot kept under good environmental and welfare conditions tend to exhibit a broad but aggregated distribution in specific areas of the tank⁶⁰.

Appetite and Feeding Behavior

Turbot are carnivorous, predatory fish that use both chemical and visual cues to detect and capture prey⁶¹⁻⁶², and it is precisely during feeding that they display the highest levels of activity⁶³. Feeding behavior in fish depends on appetite (the urge to feed themselves), sensory ability to locate food, capacity to seize, manipulate, and ingest it, and on physiological factors that determine how food is digested and assimilated¹².

Appetite and feed intake in turbot are influenced by environmental factors and tank conditions, feeding strategies, and the fish's physiological state (stress, health, etc.), as well as by

the palatability of the feed ^{50,52,56,64}. During the earliest stages, turbot fry feed throughout the water column, showing greater activity near the surface ^{52,65}, whereas larger turbot exhibit more vertical movements, swimming vigorously from the bottom to the surface.

Excessive or agitated swimming activity may indicate inadequate feeding. Conversely, a lack of appetite or feeding response may signal unsuitable environmental conditions (e.g., poor water quality, inappropriate lighting), health or stress problems, or uncontrolled overfeeding, all of which reflect serious welfare issues ^{20,56,66}.

Abnormal Movements

Abnormal movements are generally associated with health and welfare issues. In turbot, individuals or populations affected by disease may display erratic, inverted, or spiral swimming, typically accompanied by reduced feed intake and respiratory distress ^{20,22,67,68}. Another abnormal behavior observed in this species is thigmotaxis, characterized by a tendency to remain at the periphery of the tank in continuous contact with the walls. This behavior is often linked to inadequate lighting or wall coloration and can severely compromise the health and survival of the fish ⁶⁴. In addition, stereotypies—repetitive, invariant behaviors without an apparent function—have been documented in various animal species and are generally associated with environmental deprivation or lack of stimulation under farming conditions.

Aggressiveness

Aggressive behavior can occasionally be observed in turbot, although it is not very common. An increase in aggressiveness among individuals within the same population may result from food scarcity, leading to heightened competition or hierarchical dominance, particularly during the early developmental stages ^{11,12,69}. Such aggressiveness usually disappears when appropriate feeding conditions are provided ¹⁴. While direct methods for monitoring behavior are currently available (e.g., direct observation, video recording, etc.), an indirect way to evaluate aggressive behavior is through the observation of scars or marks on the skin (see external indicator: *Skin Condition*).

Ventilatory Frequency and “Gasping for Air”

Respiratory rate is an indicator of oxygen consumption in fish, including turbot, and is considered one of the stress responses to environmental changes ^{70,71}. In general, a high respiratory rate indicates low respiratory efficiency and/or an increased oxygen demand, which in turbot may be caused by poor water quality (low oxygen concentration, presence of contaminants), unsuitable environmental conditions (high temperature), stress situations, or health problems ^{20,70–73}. Therefore, observing the back-and-forth motion of the opercula can serve as a useful tool for visually assessing the respiratory status of turbot and evaluating their welfare. Likewise, the presence of individ-

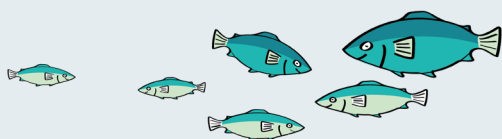




uals “gasping for air” (i.e., repeatedly opening and closing their mouths) is another clear sign of welfare problems in turbot, usually associated with elevated respiratory frequencies.

Vestibulo-Ocular Reflex (VOR)

In fish, when assessing loss or recovery of consciousness, several progressive symptoms or visual indicators can be resorted to, such as the reappearance of opercular movement, fin movement, response to tactile stimuli, recovery of balance, and the vestibulo-ocular reflex (VOR)^{74,75}. Although not considered a behavioral indicator, the VOR is an externally observable neurological reflex manifested as compensatory eye movement when the fish attempts to move its eyes horizontally while conscious⁷⁶, and it is directly related to brain function. Its reappearance is one of the first clear signs of recovery of consciousness following stunning or exposure to sedatives or anesthetics. The VOR, therefore, may serve as an efficient and easy-to-use indicator for assessing possible loss and/or recovery of sensibility in turbot during operational anesthesia and stunning procedures⁷⁵. Caution is recommended when using the VOR as an indicator of loss of consciousness, however, since the only completely reliable method to confirm instantaneous and effective stunning is through direct measurement of the loss of brain activity using electroencephalograms (EEGs)^{77,78}.



2.3. Environmental Indicators

These parameters are used to obtain essential information about the environment in which the fish are kept—specifically, water conditions and external factors that may influence their welfare or be affected by alterations in it. The following are the main environmental indicators relevant to turbot:

Water Temperature

Temperature is a very important factor due to the poikilothermic nature of fish (their body temperature depends on the surrounding environment) and its close relationship with other environmental parameters. The optimal temperature range for turbot is between 14 °C and 19 °C, while their tolerance limits extend from 12 °C and 20 °C^{13,70,79–82}, which coincides with the temperature range of the species’ farming areas. Within this range, the optimal temperatures vary slightly depending on the developmental stage. Any alteration in environmental (and therefore body) temperature has a direct impact on metabolic function and, consequently, on welfare—especially when temperature changes occur abruptly (e.g., during handling, transfer, or transportation). Furthermore, specimens exposed to very high temperatures (above 20 °C) or very low temperatures (below 5–8 °C) may experience physiological stress conditions that can become critical for survival^{21,83,84}.



Salinity

Salinity refers to the concentration of salts or dissolved ions in the water. In Spain, turbot production is generally carried out in seawater, with a salinity of around 35–38 psu; therefore, salinity is not considered a particularly useful welfare indicator within the Spanish sector. Nevertheless, due to its biological characteristics, the species can be reared in less saline waters, adapting to salinities as low as 8–10 psu^{82,85,86}, depending also on temperature¹³.

Dissolved Oxygen

Dissolved oxygen (DO), expressed in mg/L, is a key parameter in aquaculture, since poikilothermic organisms have oxygen requirements that depend on their metabolic rate, which in turn varies with environmental temperature. There is also an inverse relationship between oxygen solubility and water temperature: the higher the

water temperature, the lower the solubility of oxygen, and therefore the lower its concentration or availability. For this reason, DO is one of the most critical factors for turbot and can pose a serious risk when fish are reared at high densities without supplemental oxygen⁸². Nevertheless, turbot possess certain adaptive physiological traits (e.g., hemoglobin polymorphism, cutaneous respiration) that make them highly tolerant to low DO levels^{72,81,87}. This tolerance varies according to the production stage, as juvenile and adult turbot are more resistant to low oxygen concentrations (hypoxia) than larvae. In general terms, the minimum recommended DO concentration for turbot is around 6 mg/L; below 3 mg/L, they stop feeding, and mortality risk increases at concentrations near 1 mg/L^{82,88}. Conversely, there is no biological risk associated with oxygen supersaturation. It has been demonstrated that, in this species, the persistence of low DO values (chronic hypoxia) is more detrimental than short-term decreases (acute exposure).

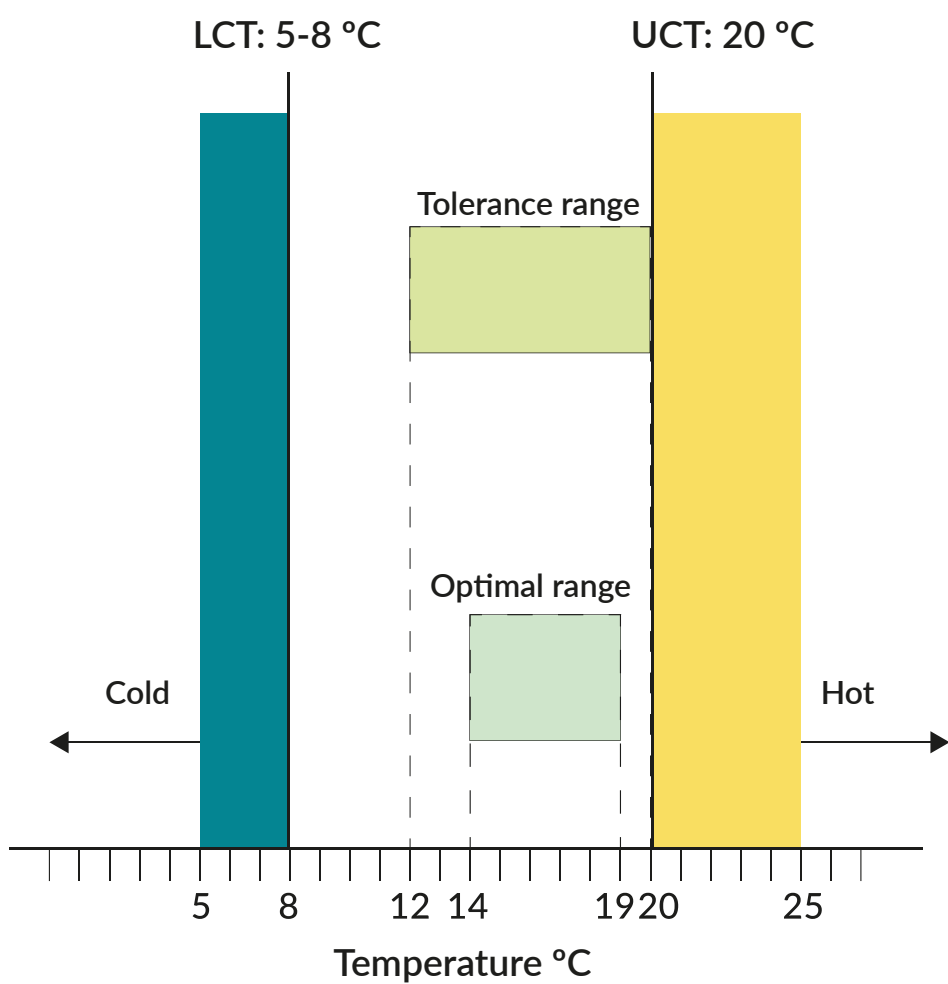


Figure 2. Figure of the thermo-neutral zone of turbot, with the optimal temperature range, tolerance thresholds, and critical temperatures: lower critical temperature (LCT), upper critical temperature (UCT).



Carbon Dioxide

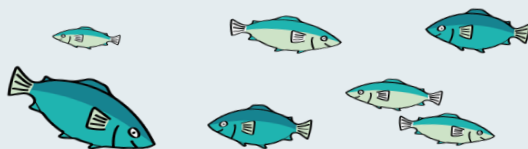
Carbon dioxide (CO_2) is highly soluble in water and exhibits significant toxicity. When dissolved, it forms carbonic acid, which decreases the pH of the water (increasing acidity). Furthermore, high CO_2 concentrations in the water increase blood CO_2 levels, leading to hypercapnia, respiratory acidosis, and a reduced affinity of hemoglobin for oxygen (Bohr effect), as well as a diminished oxygen transport capacity of hemoglobin (Root effect). These physiological changes result in increased respiratory rate, lethargy, and digestive and metabolic disorders, which can ultimately cause death⁸⁹⁻⁹². In less severe cases, growth can also be affected. Dissolved CO_2 concentrations above 9–13 mg/L may negatively impact turbot growth, metabolism, and welfare^{90,92}.

pH

pH is a measure of the acidity or alkalinity of water and depends strongly on temperature and the concentration of CO_2 in the medium. It has been shown that pH is an important factor influencing mortality rates in turbot reared at high densities without water exchange, owing to the strong effect of water acidity on ammonia toxicity and dissolved oxygen (DO) levels, as well as on the affinity and/or capacity of hemoglobin to transport oxygen. In general, turbot display a wide tolerance range for pH, but values below 5.5 or above 8.5 should be avoided^{94,95}.

Ammonia and Nitrogen Compounds

The accumulation of these compounds in tanks results from fish excretions and decomposing organic matter, possibly due to overfeeding. Ammonia can exist in ionized form (NH_4^+) or non-ionized form (NH_3), the latter being highly toxic to fish⁹⁶. Both forms are present in water, but their ratio depends on water pH and temperature. Nitrosomonas bacteria break down ammonia into nitrites (NO_2^-), which are highly toxic to fish. These are subsequently converted by *Nitrobacter* bacteria into nitrates (NO_3^-), which are far less toxic and generally do not cause problems in aquaculture operations. Open systems benefit from constant water renewal, while recirculating aquaculture systems (RAS) use biofilters containing *Nitrobacter* bacteria to reduce nitrite concentrations. Nitrite can enter the bloodstream through the gills, forming methemoglobin in red blood cells, which prevents oxygen uptake. Turbot are more “ureotelic” than many other teleosts, excreting approximately 20–26% of their total nitrogen in the form of urea⁹⁷. For turbot, the recommended maximum concentrations are approximately 0.1 mg/L of ammonia, 0.1 mg/L of nitrites, and 125 mg/L of nitrates^{92,93,95,96,98,99}, although their toxicity will depend on water acidity, temperature, and rearing conditions.



Turbidity and Suspended Solids

In turbot farming, it is very important to use clean water. Turbidity refers to water clarity and the amount of solid material suspended in the water. Both parameters are closely related, as turbidity increases with suspended solids. In turbot, the presence of suspended solids reduces water quality and oxygen concentration, impairs vision and respiration, decreases feeding activity, and increases stress levels ^{66,100–102}.

Water Flow and Velocity

Other parameters related to water quality include flow rate and velocity, which are closely related. Water flow and current velocity can influence turbot welfare both positively and negatively: an optimal velocity ensures good water renewal, promotes more homogeneous distribution, and stimulates moderate physical activity (especially in early life stages). However, if water velocity is too low, renewal is reduced, leading to the accumulation of organic matter (excretions, uneaten feed), suspended solids, and toxic compounds. Conversely, if the velocity is excessively high, feeding behavior and the natural movement of the fish may be adversely affected ^{90,92,103,104}.

Lighting

When discussing lighting, we refer mainly to the photoperiod, light intensity, and spectral characteristics of the light. Controlling and regulating lighting is highly important during the reproduction and larval development of turbot, as these processes are carried out in indoor tanks. It is imperative that the biological rhythms be maintained during turbot rearing, as doing so has a positive impact on reproduction, metabolism, development, and disease resistance, while also reducing stress ^{56,105,106}. Conversely, disrupting biological rhythms can act as a stress factor for turbot, activating the stress response axis and leading to reduced feed utilization, which in turn diminishes growth and gonadal maturation ^{15,56,107–109}. Additionally, the color or light spectrum (wavelength), as well as the color of the

tanks, can specifically affect the body development of turbot embryos, larvae, and juveniles ^{64,110–113}. Turbot grow-out is usually carried out outdoors under natural photoperiod conditions, where tarps or canopies are used to regulate light intensity and prevent sunlight from damaging the skin and eyes of the fish ². When grow-out takes place indoors, artificial lighting is required, but the species' circadian rhythms must always be maintained.

Stocking Density

This parameter refers to the density of fish being reared within a given enclosure or volume, measured as biomass per volume of water (kg/m^3) or individuals per liter of water (ind/L) in the case of turbot during pre-metamorphic stages (fry), and as biomass per surface area (kg/m^2) or individuals per surface area (ind/m^2) in later stages (juveniles and adults), due to the benthic behavior of the latter. References to turbot density may also be expressed in terms of the percentage of surface area covered by individuals. Stocking density directly affects fish welfare but varies depending on the production stage, water quality and conditions (flow rate, dissolved oxygen, temperature, etc.), feeding management, and the size and characteristics of the production system.

Turbot is a species capable of tolerating relatively high stocking densities, owing to its benthic habits, low activity, and tolerance to low oxygen concentrations ⁸². A wide variety of density practices and recommendations exist depending on the production system ². However, when turbot stocking density is high, water quality, behavior, feed conversion efficiency, size distribution, and even skin coloration and integrity are affected—resulting in increased stress levels as well as greater susceptibility to pathogens and disease ^{14,114–119}. In any case, turbot welfare must always be ensured through the maintenance of appropriate stocking densities and the use of other operational welfare indicators ¹²⁰.



2.4. Laboratory Indicators

Laboratory indicators provide an objective and quantifiable means of assessing turbot welfare, offering valuable information about the fish's internal condition beyond what can be detected through external observation. These parameters make it possible to identify physiological imbalances and early signs of stress, even before evident morphological or behavioral changes appear. In aquaculture, their application provides producers with a complementary, practical, and reliable tool for analyzing animal welfare at specific times and under defined conditions, thereby contributing to more precise and controlled farm management. The most relevant laboratory indicators are described below:

Hormonal and Metabolic Indicators

Environmental conditions—both biotic (density, competition, social interactions, etc.) and abiotic (handling, temperature, salinity, dissolved oxygen, water quality, etc.)—significantly influence the biology of turbot². When these conditions exceed the fish's physiological and behavioral capacity to maintain homeostasis, an integrated stress response known as the general adaptation syndrome is triggered, aiming to restore internal balance. If these emergency mechanisms prove insufficient and/or exposure to the stressor is prolonged, the stress response enters a chronic phase characterized by resource depletion, during which the animal's welfare becomes severely compromised¹²¹.

Intense disturbances activate a series of physiological mechanisms mediated by the neuroendocrine stress response, which in its initial phase involves the primary stress response or rapid release of hormones into the bloodstream, mainly cortisol and catecholamine, whose concentration levels are quantifiable and can therefore serve as welfare indicators. As in other teleost species, cortisol has been used as a marker of the stress response in turbot. It can be detected not only in plasma but also in other media such as skin mucus, feces, and even tank water, particularly under prolonged stress conditions. In adult turbot, plasma cortisol concentrations typically range between 1 and 10 ng/mL, but under acute stress, they can increase 10–20-fold. Multiple methods exist for quantification, each with advantages and limitations depending on the experimental context. These include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which differ in sensitivity, specificity, cost, and practical applicability. Although plasma determination is considered the standard due to its precision, it requires blood sampling and specialized facilities¹²².

Although regulated by a complex negative feedback loop, cortisol release into the bloodstream has been widely recognized as an important indicator of stress in fish. This mechanism causes cortisol levels to decline over time when the stress stimulus is persistent. Since chronic stress compromises fish physiology, blood cortisol measurement should be complemented with other indicators. Thus, understanding the degree of invasiveness,



reliability, and variability of this method is key to selecting the most appropriate analytical strategy and ensuring interpretable results without compromising animal welfare.

Catecholamine (adrenaline and noradrenaline) are released from the chromaffin tissue of the kidney into the bloodstream immediately after the perception of a stressor. However, their short duration, low levels, and the technical difficulties involved in measuring them in turbot, limit their usefulness as welfare indicators beyond the initial stress response¹²³.

The increase in stress hormone levels triggers a secondary response characterized by alterations in hematological and metabolic parameters that compromise animal health and welfare. Among these are the levels of respiratory gases (oxygen and CO₂) in the blood, which generally range around 60 ± 25 mm Hg; the pH (7.2–7.5); and certain ions such as HCO₃⁻ (8–12 mM), all of which can serve as good indicators of welfare, especially in situations where the fish has limited access to oxygen and/or experiences blood acidosis caused by intense motor activity or other stressors^{123,124}. Oxygen constraint also results in increased hemoglobin concentration, which in turbot shows baseline values in the range of 50–70 g/L, as well as hematocrit levels ranging from 15–20%. Under hypoxia-induced stress, these may rise to approximately 70–80 g/L and 20–25%, respectively.

Among metabolic indicators, the concentration of plasma glucose, with a basal range of 1.5–2.5 mM, may increase 2–3-fold under stressful con-

ditions, a response not always observed¹²⁵. Likewise, plasma lactate concentration—normally between 0.5 and 1.5 mM—can also double or triple in certain stress situations, particularly those involving oxygen deficiency and activation of anaerobic metabolism¹²³. In cases of malnutrition or starvation, parameters such as albumin (16–17 g/L) or total blood protein (42–45 g/L) may also serve as indicative markers of welfare impairment¹¹⁸.

From a technical standpoint, these parameters can be quantified in plasma, blood serum, or mucus using specific commercial kits (such as those for glucose, lactate, or hemoglobin), following sample collection and subsequent centrifugation. Although these analyses are normally performed in the laboratory, many parameters can also be measured *in situ* on the farm using portable devices. Nevertheless, as noted previously, the interpretation of these data requires caution due to factors such as individual variability, circadian rhythms, and the environmental conditions under which the samples are obtained¹⁰⁶.

Molecular Indicators

The quantification of specific gene expression using molecular techniques holds great potential for identifying welfare alterations. Among the most relevant genetic markers are those associated with the physiological stress response, such as corticotropin-releasing factor (CRF), glucocorticoid receptors (GR), and the expression of chaperone proteins (HSP70, HSP90)¹²⁶. Likewise, the overexpression of markers linked to immune

response—such as tumor necrosis factor (TNF- α), interleukins (IL-1 β and IL-8), and peroxisome proliferator-activated receptor alpha (PPAR α -1)—is of great interest for evaluating welfare impairment associated with the presence of pathogens or parasites^{127,128}.

Among molecular markers studied in turbot, those related to redox status are also noteworthy. Stressful situations can trigger the generation of reactive oxygen species (ROS), which compromise cellular integrity if not counteracted by endogenous antioxidant systems^{8,129}. In this context, the production of antioxidant enzymes such as superoxide dismutase (*sod*), catalase (*cat*), glutathione peroxidase (*gpx*), and glutathione S-transferase (*gst*), as well as the expression of their corresponding genes, is of great interest, as these reflect the fish's ability to neutralize free radicals and maintain cellular homeostasis^{8,73,115,118,129,130}. Proper regulation of these enzymes and genes not only contributes to metabolic balance but also protects muscle tissue from oxidative damage, a factor directly related to welfare and to the preservation of structural characteristics^{8,129}.

Most welfare indicators used in fish are derived from studies of situations in which welfare is compromised, while quantifiable factors associated with the experience of positive welfare states are very few. In this regard, certain parameters related to neuronal development in fish have been identified and linked to cognitive stimulation, such as the expression of genes encoding brain-derived neurotrophic factor (BDNF) and neurogenic differentiation factor 1 (NDF1), both of which are associated with neuronal proliferation. Similarly, some monoaminergic neurotransmitters in the brain, such as serotonin and dopamine, known to play a critical role in modulating the stress response in fish^{131,132}, also mediate emotional states like arousal, anxiety, and fear, serving as physiological indicators of emotional welfare. However, the quantification and interpretation of these indicators are complex due to the lack of standardized reference values, as they depend on factors such as the fish's developmental stage, environmental context, and individual variability among spec-

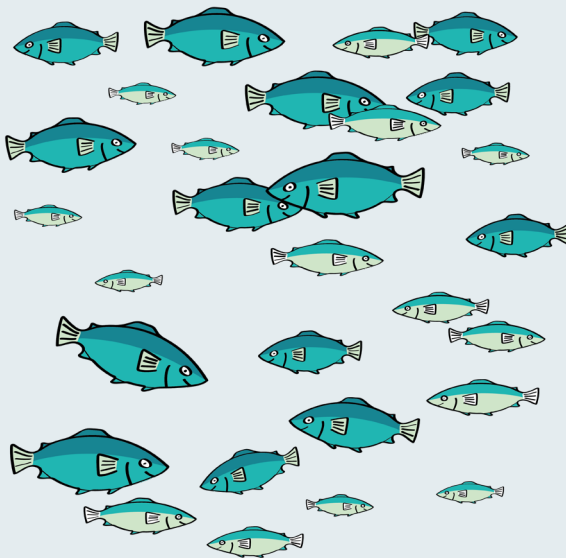
imens. For this reason, their assessment should be carried out by specialized personnel.

Immunological Indicators

Immunological indicators make it possible to assess health status and the incidence of infections. The innate immune response can be measured through the bacteriolytic activity of mucus or plasma, lysozyme concentration, complement activity, and the phagocytic capacity of leukocytes¹³³. These responses are rapidly activated in the presence of infectious agents or various types of environmental stress.

The adaptive immune response in turbot is primarily evaluated by quantifying immunoglobulins (Ig) in plasma—particularly IgM, which is the main immunoglobulin in teleost fish. IgM levels increase significantly following vaccination or infection, reflecting the activation of the adaptive response and the production of specific antibodies against the corresponding antigen or pathogen¹³⁴. Total leukocytes in whole blood can also serve as a general indicator of immune status, especially under conditions of infection or inflammation, complementing hematological and biochemical analyses.

Finally, it should be noted that no single physiological welfare indicator is definitive on its own. Any parameter under study must always be interpreted holistically, in comparison with other welfare indicators, and ideally by trained personnel.





3. CRITICAL WELFARE POINTS IN REARING SYSTEMS AND DEVELOPMENTAL PHASES

The most common practice for turbot farming in Spain is intensive monoculture, carried out in land-based facilities supplied with open-flow seawater. The following sections describe

the different rearing procedures and systems used for turbot according to their developmental phases, identifying the critical welfare points in each case:

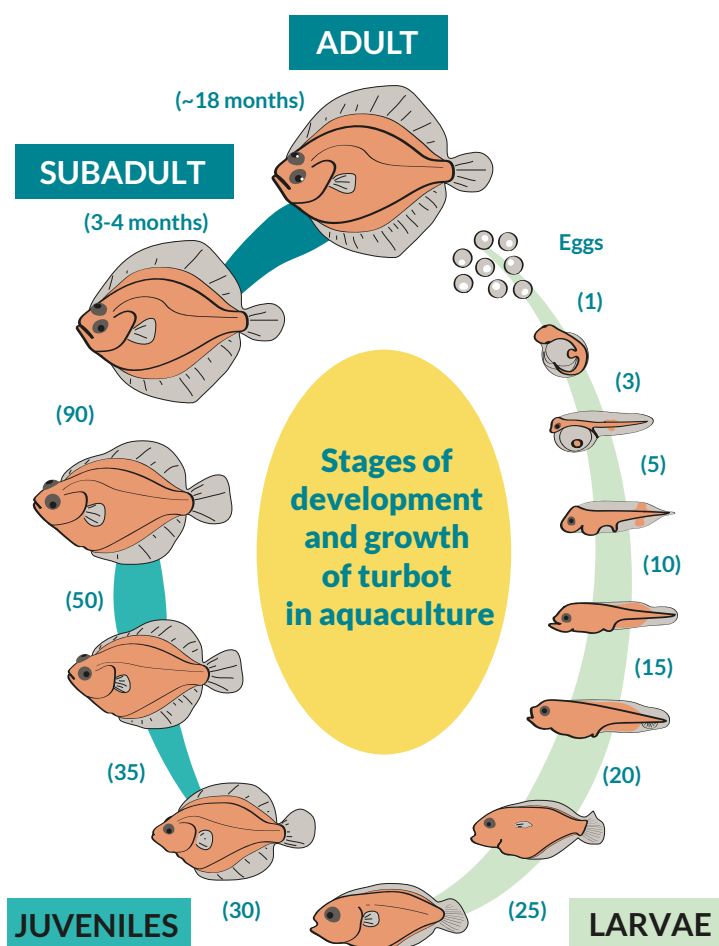


Figure 3. Stages of development and growth of turbot in aquaculture. Days into brackets.

3.1. Broodstock and Egg-incubation

Breeding centers produce eggs from broodstock kept under highly controlled conditions. Broodstock management requires strict biosecurity measures; therefore, it is essential to keep these individuals separated from other production stages. Broodstock typically comprises fish between 2 and 7 years old, weighing 6 to 8 kg, and selected from productive company stocks. It is important to note that both the quality and quantity of gametes decrease with age, so broodstock are routinely renewed. They are kept in spawning tanks, generally made of concrete or fiberglass, of various shapes and sizes, at densities ranging from 10 to 20 kg/m², with an approximate 1:1 ratio of males to females. Gametogenesis lasts around three months and occurs under specific photoperiod (16 hours of light)

and temperature (13–14 °C). conditions. These parameters are carefully controlled in hatcheries to enable spawning throughout the year. Hormonal induction is not a common practice, although it may occasionally be applied to synchronize spawning among broodstock.

Broodstock are periodically and individually examined manually to assess their health, gonadal development, and the quality of future spawn, as well as to collect eggs or sperm for fertilization. Feeding is carried out once daily to apparent satiation using broodstock-specific diets. Feed intake tends to decrease as the spawning period approaches, during which only males and immature females are fed ². Occasionally, and under veterinary prescription, broodstock may be vaccinated by injection to protect them against potential risks present in facility conditions. Transfers or movements of broodstock between tanks are also carried out individually,

Table 1: Aspectos más relevantes para el bienestar de los rodaballos durante la fase de reproducción

Aspectos más relevantes para el bienestar de los rodaballos durante la fase de reproducción		
Ambiente y Confinamiento	Diseño y dimensiones	Uso del espacio, distribución
	Iluminación y Temperatura	Influyen en reproducción
	Calidad del agua	Salud y bienestar general
	Caudal del agua	Renovación, oxigenación
	Densidades	Uso del espacio, Interacciones sociales
Manejo y Mantenimiento	Revisiones gonadales	Práctica pautada con manipulación y exposición al aire
	Inducción hormonal	Procedimiento excepcional por inyección
	Limpieza de tanques	Operaciones rutinarias inducen estrés
Alimentación	Estrategia de alimentación	Cubrir necesidades fisiológicas y comportamentales
Salud animal	Revisiones de salud	Observaciones rutinarias con manipulación y exposición al aire
	Vacunación, Tratamientos	Procedimiento pautado, por inyección y manipulación
Transporte	Traslados	Con redes o lonas, a nivel individual. Distancias muy cortas
Punto final	Sacrificio	Vejez, mala calidad de puesta. Aturdimiento por percusión, sacrificio por inmersión en agua-hielo

using nets or stretchers, and generally last only a few seconds. At the end of their productive cycle, broodstock are stunned by percussion and subsequently slaughtered by bleeding in ice water or by thermal shock through immersion in ice water.

After fertilization, the eggs are kept in cylindro-conical tanks at 13–14 °C, at a density not exceeding 5,000 eggs per liter of water. They hatch after approximately five days of incubation, producing larvae about 3 mm long with closed mouths that rely on their yolk sacs for nutrition during the endotrophic feeding period ².

Biosecurity in turbot farms is of vital importance at both the broodstock stage and in later phases, as it is essential to prevent the introduction and spread of infectious diseases within the facilities. Consequently, companies maintain a Biosecurity Plan, consisting of a risk analysis and a set of implemented measures and protocols aimed at ensuring the health, sustainability, and profitability of aquaculture production. These include, among others, management of the water supply, selection of healthy animals, control of staff and visitor access, disinfection and sanitation of equipment and facilities, quarantine of new animals, and safe disposal of diseased fish.

3.2 Hatchery (Larval and Fry Stages)

Larval culture begins with larvae measuring approximately 3 mm in length and weighing 0.1–0.2 mg, and continues until weaning is complete—that is, when feeding becomes entirely based on inert feed and turbot are considered fry ². During the first three days after hatching, the larvae feed on their yolk reserves. From that point on, exogenous feeding begins with rotifers. The start of feeding is a critical point in terms of biosecurity, as fry have an underdeveloped immune system. Around day 12, newly hatched *Artemia* are introduced and maintained until days 15–17 post-hatching. Subsequently, *Artemia metanauplii* are added, combined with formulated feed from days 20–25 until weaning, which occurs around day 30. Larval culture is carried out in circular, square (with rounded

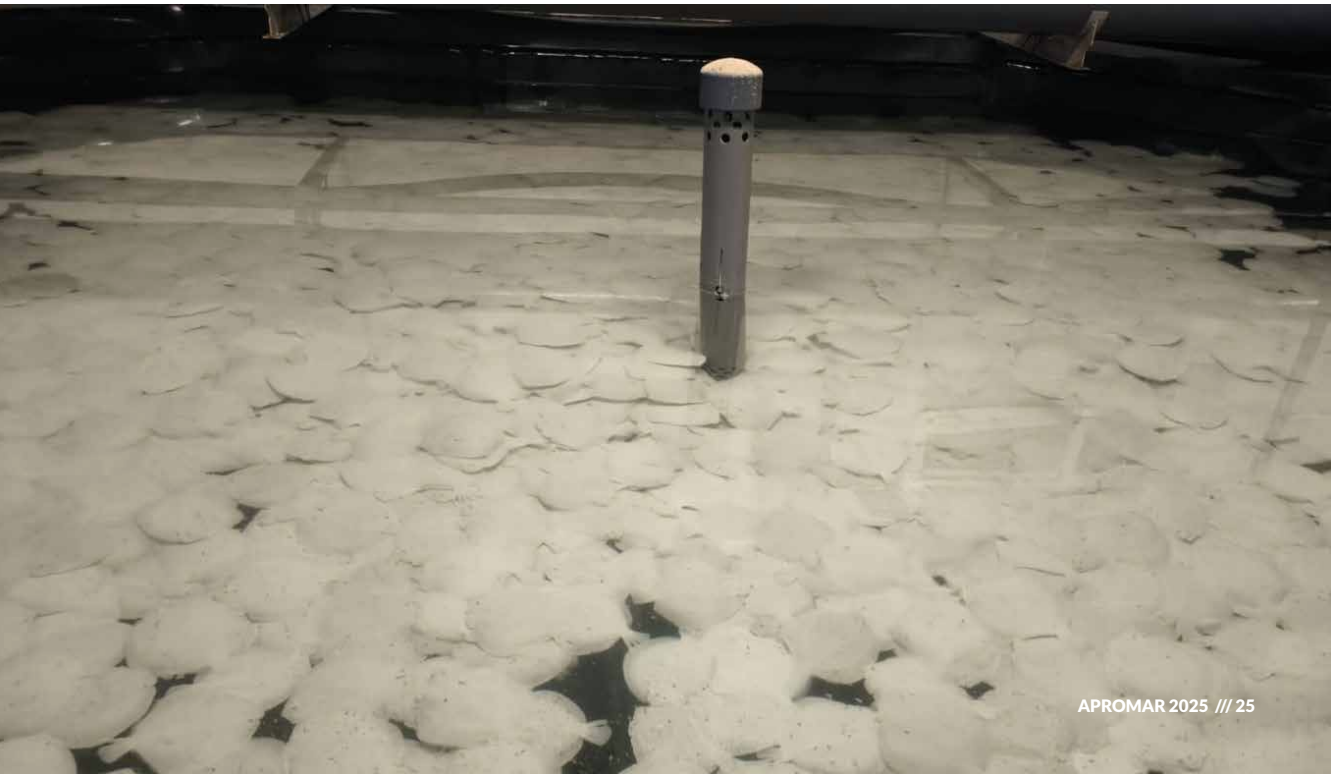
corners), or cone-bottom tanks made of fiberglass or concrete, with volumes ranging from 5 to 20 m³ and water columns from 1.5 to over 2 meters deep. During this larval phase, final densities of 2–3 fish per liter are reached. Survival rates during larval culture are typically above 50%.

From days 30–35 onward, the larvae begin feeding exclusively on formulated feed, marking the start of the fry phase, which continues until approximately day 90 of life. During this larval and fry-rearing stage, metamorphosis takes place, characterized by the migration of the right eye from the ventral or blind side to the dorsal side of the turbot, accompanied by an increasing tendency to remain on the bottom of the tank. Turbot fry tanks may also vary in shape and size, with approximate volumes between 2 and 5 m³ and depths of less than one meter. At the end of the fry phase, densities of 4.5–15 kg/m² of tank surface area can be reached, and survival rates are generally very high—around 90% ².

During the fry stage, size grading is extremely important due to the variation in size among individuals. This process is carried out manually or using specialized equipment (such as roller graders), along with the manual removal of deformed individuals. When any handling process or air exposure is prolonged, anesthesia (for example, tricaine or benzocaine) is used to reduce stress in turbot during manipulation. During this phase, and before transfer to pre-fattening and fattening facilities, fry are vaccinated up to two doses by immersion against the main diseases affecting them, such as vibriosis and flexibacteriosis. Turbot fry are deprived of feed the day before vaccination and subjected to fasting for 24–72 hours prior to transport or other potentially stressful handling. Fry are removed from the rearing tanks using pumps or nets and transported by road in monitored and temperature-controlled tanks, at densities that may reach 100 kg/m². Transport duration can vary—from very short transfers within the same hatchery facility to as long as 72 hours—depending on the final destination, with transport conditions adjusted accordingly.

Table 2: Most relevant aspects of turbot welfare during the breeding phase

Most relevant aspects of turbot welfare during the breeding phase		
Environment and confinement	Design and dimensions	Use of space, distribution
	Lighting and temperature	Influence on reproduction
	Water quality	Overall health and welfare
	Water flow rate	Renovation, oxygenation
	Stocking densities	Use of space, social interaction
Handling and maintenance	Gonadal checkups	Scheduled procedure with handling and air exposure
	Hormonal induction	Exceptional procedure via injection
	Tank cleaning	Routine operations induce stress
Feeding	Feeding strategy	Satisfaction of physiological and behavioral needs
Animal health	Health checkups	Routine observations with manipulation and exposure to air
	Vaccination, treatments	Scheduled procedure, injection and handling
Transporte	Transfer	With individual landing nets or tarps. Very short distances
Punto final	Slaughter	Old age, poor egg quality – stunning by percussión, culling by immersion in ice-water



3.3. Pre-fattening and Fattening

The pre-fattening phase begins with juvenile turbot weighing 2–10 g and ends when they reach 60–100 g, while the fattening phase extends from 60–100 g up to commercial size. Most turbot are reared to a market weight of 1.5–2 kg, although they may be sold smaller (300–500 g) or larger (3–4 kg), depending on market demand. The pre-fattening period generally lasts about 3–4 months, while the fattening one takes approximately 18 months, depending on the target market size. In total, the lifespan of a turbot in captivity is around 1.5–2 years. Pre-fattening and fattening tanks for turbot vary widely in shape and size, and are generally made of concrete or fiberglass, circular or square with rounded corners, with depths of up to one meter and surface areas of approximately 10–30 m² for pre-fattening and 40–140 m² for fattening. These are typically open-flow systems located outdoors—either under roofed structures or in open-air facilities covered with individual or grouped canopies—to prevent damage from excessive solar radiation. At the end of the pre-fattening phase, maximum densities of up to 50 kg/m² can be reached, although average densities are around 20 kg/m². During the fattening phase, densities can reach 60–70 kg/m², with average values near 40 kg/m².

During the pre-fattening and fattening periods, sampling and size grading are common and important practices to obtain batches that are as homogeneous as possible. These procedures can be carried out manually or using specialized equipment (such as roller graders). Tubs filled with water are typically used for transfers between tanks following each procedure. The fish are removed from the tanks with hand nets or conveyor belts and placed into these tubs. At the beginning of the pre-fattening phase, vaccination is typically performed by intraperitoneal injection, always based on a risk analysis established in the facility's health plan. The most common vaccines are against vibriosis and/or flexibacteriosis. To avoid digestion during handling, turbot are not fed the day before vaccination, as this process involves high oxygen consumption and energy expenditure, which makes the fish more defenseless.

A fasting period is also applied before slaughter, typically lasting 24–72 hours. During slaughter, turbot are gathered with nets and transferred using hand nets or conveyor belts into tubs for transport to the slaughter area, or they may be slaughtered near the rearing tanks. The main slaughter method involves immersion in ice water. When bleeding by gill cutting is performed, fish are first mechanically stunned by percussion and then immersed in ice water. Stunning by electronarcosis and other alternative methods are currently being investigated and evaluated by the industry.



Table 3: Most relevant aspects of turbot welfare during the pre/fattening/fattening phase

Most relevant aspects of turbot welfare during the pre/fattening/fattening phase		
Environment and Confinement	Design and dimensions	Use of space, distribution
	Temperature and oxygen	Essential control of outdoor grow-out
	Water flow rate	Oxygenation, renewal
	Water quality	Overall health and welfare
	Stock densities	Overall health and welfare
Handling and Maintenance	Tank cleaning	Routine operations (may induce stress)
	Removal of dead fish	Routine operations (may induce stress)
	Classification/ separation	Manual or grading machine
Feeding	Feeding strategy	Key
	Fasting	Before transfer, handling, and slaughter
Animal Health	Checkups and sampling	Includes handling and/or slaughter
	Vaccinations	Handling and intraperitoneal injections, mainly
Transport	Relocation/Transfer	Pumping, conveyor belts or nets
Final Phase	Pre-slaughter	Concentration, fasting, and handling
	Slaughter	Various methods, occasional prior stunning (percussion)



4. WELFARE AND BEST PRACTICES IN TURBOT REARING

For the proper evaluation and monitoring of welfare and rearing practices in turbot, it is recommended to use **essential operational indicators** (external, behavioral, and environmental) across the different procedures and production phases (see Appendix). It is also advisable to apply additional indicators to achieve a more precise and comprehensive assessment, as these provide greater representativeness—provided that their use is feasible and does not interfere with routine activities that could compromise turbot welfare. Furthermore, it is recommend-

ed to keep a **record of values from monitored indicators** to objectively evaluate the welfare of turbot throughout the various procedures and critical points of their life cycle. This enables the design and implementation of effective management and operational measures.

Design, Dimensions, and Environmental Conditions of Facilities

The design and dimensions of the enclosures must be appropriate to the species' biology, allowing turbot to satisfy their physiological and behavioral needs, while ensuring optimal conditions, water quality, and sufficient space to move freely throughout the facility. In aquaculture operations such as hatcheries or grow-out units, the design of tanks should be oriented toward maximizing turbot welfare. To this end, it is essential to use materials that prevent injuries or abrasions, such as concrete, fiberglass, or other suitable materials for aquaculture. Tanks should be circular or have rounded corners, which allows fish to maintain normal behavior and make efficient use of available space. Likewise, the color and lighting of the facilities should be suitable and not harm the animals' health (e.g., ocular damage caused by excessive brightness) or disrupt their biorhythms. In outdoor rearing systems exposed to natural light, awnings or covers should be used to protect rearing units from excessive solar radiation and adverse weather conditions. Furthermore, ambient noise and vibration levels should be reduced to the lowest possible level to prevent stress.

As for stocking density, it is recommended not to exceed 15 kg/m² in hatchery units (fry and

4.1. Environment and Confinement



*Selected essential operational indicators to be recorded and monitored for the assessment of turbot welfare in relation to **environment and confinement** throughout all production stages.*



juveniles), 50 kg/m² during the pre-fattening phase, and 70 kg/m² during the fattening phase. Broodstock should also be kept in tanks with a capacity greater than 20 m² and at densities below 15 kg/m². Before these limits are reached, close monitoring of welfare indicators should be intensified to ensure good animal condition. During spawning, tank size may be reduced (without increasing density) to facilitate access to the fish and the performance of routine operations such as cleaning, egg collection, and monitoring, all of which help improve welfare during reproduction. Environmental conditions should replicate natural spawning conditions through temperature control (13–14°C) and lighting (a 16-hour light, 8-hour dark photoperiod), maintaining natural biorhythms and spawning duration. By alternating different broodstock groups, spawning can be achieved year-round using separate populations. Hormonal injections should be used only in exceptional cases, giving preference to natural spawning. In any case, essential operational welfare indicators must always be used to evaluate and ensure that fish are reared under good rearing conditions.

Monitoring of Water Quality and Flow Parameters

The quantity and quality of water are key factors in ensuring turbot health and welfare. To this end, several essential and non-essential water-related parameters must be regularly monitored and recorded (see Appendix). Frequency monitoring should satisfy each parameter's importance and its relevance to fish welfare. Regular monitoring enables the detection and

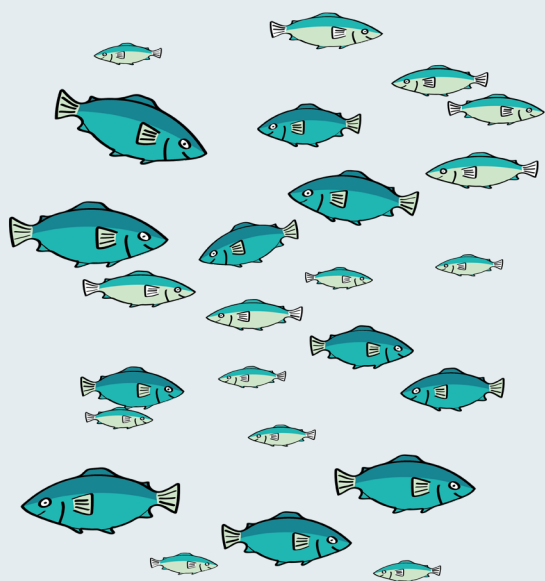
assessment of potential adverse environmental changes affecting the population, whether caused by aquaculture operations themselves, other anthropogenic impacts, or meteorological events. Continuous monitoring, therefore, provides a much more accurate picture of the conditions in which the fish live, allowing timely intervention to prevent or mitigate impacts on their welfare.

In turbot farming, monitoring must be carried out systematically, as frequently as possible, and at several locations and depths within each enclosure or production unit, depending on facility design and prior risk assessment. This ensures that the parameters measured accurately reflect the conditions experienced by the fish across the largest possible water volume. Besides, water flow should be studied and adjusted for each production phase to facilitate dispersion, ensure adequate water renewal and aeration, and minimize abrupt changes in water quality parameters. When necessary, pure oxygen should be injected into the water flow to increase dissolved oxygen levels.

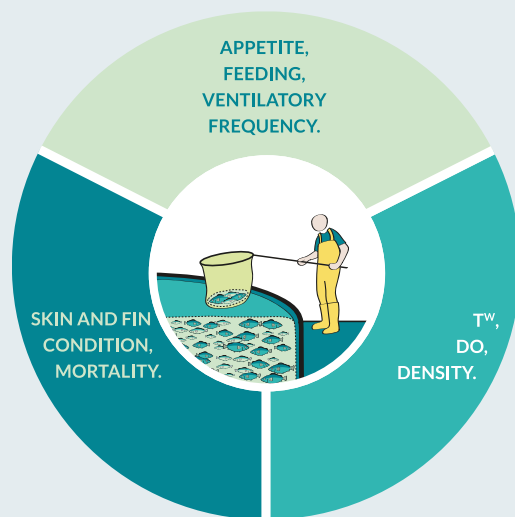
Each facility must have an action plan in place for situations in which any parameter deviates from optimal ranges. Water quality values may fluctuate, and if they reach levels harmful to turbot (especially during hatchery stages), the producer must provide the means to minimize animal suffering. For this reason, each facility and species should have a Fish Welfare Plan (see Section 4.d, Animal Health), as well as a standard operating procedure specifying action thresholds for each parameter and the measures to be taken in the event of deviations.

Management Plan for Interaction with Local Fauna and Predators

Turbot production systems located outdoors are often situated in areas far from urban centers, where some level of interaction with local wildlife and ecosystems might occur. The presence of predators (such as piscivorous birds or small mammals) could cause stress and mortality in turbot, leading to economic losses. However, due to the design and operation of modern rearing facilities, interactions with local fauna and predators are virtually nonexistent. In addition, production companies conduct environmental impact studies and implement monitoring plans to ensure that their activities remain sustainable and respectful of the surrounding natural environment. The typical predators present in the area are described in each company's environmental assessment procedures, and potential interactions with local fauna—especially with possible predators—are addressed in compliance with current regulations ([Law 33/2015](#)). Guidelines are also in place to prevent the attraction of local wildlife to the farms as much as possible (for example, avoiding the feeding of wild animals) and to restrict access to the fish by surrounding predators (for example, the use of nets to prevent piscivorous birds from entering rearing enclosures or barriers to block small terrestrial mammals). Additionally, these guidelines stipulate the methods to be applied for safely removing predators from the facilities should they gain access.



4.2. Handling and Maintenance



Selected essential operational indicators to be recorded and monitored for the assessment of turbot welfare in relation to handling and maintenance throughout all production stages.

Handling of Turbot

During turbot farming, handling procedures are necessary for farm operations, such as size grading and sorting, among others. These procedures can be potentially stressful, and if performed incorrectly, the fish may suffer abrasions, wounds, or mucus loss because of crowding and netting. Such handling can also trigger a physiological stress response^{125,135}, increasing the risk of infection, osmoregulatory problems, and even locomotor impairment. For this reason, turbot handling must be carried out carefully and limited to what is strictly necessary, ensuring that fish are in good health before any procedure is performed. Routine handling operations such as health inspections, growth monitoring, or size grading should be conducted properly by trained personnel using specialized equipment, with due attention to animal welfare and biological needs. It is advisable to use nets or other devices (such as water-filled tubs, shower tables, or submerged conveyor belts) when handling

larger individuals (including broodstock) as well as pump-equipped flow-through water systems that allow the movement of smaller fish, thereby minimizing direct contact and stress. Sudden changes in temperature during handling or transfer must be avoided, and adequate oxygen levels must be maintained in the water. Supplemental oxygenation systems should therefore be used to ensure sufficient saturation levels. All materials and equipment used for handling (nets, hoses, buckets, pumps, etc.) must be properly designed and maintained to prevent injury and kept in perfect working condition; regular inspections are recommended to ensure optimal performance. In cases where direct manual handling is required, operators must have received appropriate training and apply good practices (e.g., wearing gloves to prevent skin damage and infections, using nets for transfer, properly supporting the fish with both hands) to ensure the highest welfare standards for turbot ¹³⁶.

Limiting Air Exposure

Handling is often associated with fish being exposed to air, which is considered a welfare risk factor for turbot ¹³⁶. The inability to breathe while out of water, combined with the full body weight unsupported by buoyancy, induces stress and consequently reduces welfare. This stress can increase disease susceptibility and even diminish reproductive capacity and quality. Therefore, air exposure should be limited and ideally avoided altogether ¹³⁶. When avoidance is not possible, exposure time must be minimized as much as possible to safeguard fish welfare. Fish should be kept moist, either by maintaining a thin film of water over the body or by using wet surfaces or equipment providing continuous water flow. The fish's full body weight should be supported throughout handling to prevent injury. If prolonged exposure is unavoidable (e.g., during an extended reproductive maturity check or a detailed health examination and treatment), turbot should be sedated or anesthetized, their bodies kept moist, and recovery carefully monitored after handling. Nonetheless, recent studies ¹³⁷ have shown that turbot do not exhibit physiological stress responses during air exposures lasting up to one minute, suggesting that this species can tolerate short-term air exposure without significant welfare compromise.

Grading Procedure

Size grading is used to reduce the variation in body size among individuals within a batch, thereby minimizing competition for feed and ensuring similar access to food for all fish. However, this procedure is stressful and can negatively affect turbot welfare; therefore, the frequency of grading should be limited to the minimum necessary. It should also be considered that repeated exposure to adverse stimuli (such as handling, manipulation, and air exposure) can have cumulative negative effects on the fish over time. An effective feeding strategy can help reduce size variability and, consequently, the need for grading. When grading is required, specialized equipment and methods should be employed to minimize stress (e.g., conveyor belts and shower tables that maintain water contact for larger fish, grading boxes, sweep nets, or pumping systems for smaller fish), whenever feasible. During size grading or sorting for deformities, the time fish spend undergoing these procedures should be kept as short as possible and all personnel involved must be properly trained and qualified. Post-grading monitoring of turbot recovery is also recommended to ensure animal welfare following the procedure.

Crowding Procedures

Crowding can be a stressful process and should be preceded by risk assessment and coordinated management between production and welfare personnel. If performed incorrectly, it can cause abrasions and skin injuries that may lead to serious infections. However, it is not considered a high-risk factor for turbot welfare ¹³⁶. Even so, crowding events should be kept to a minimum, such as for vaccination, grading, or harvesting, using low stocking densities, minimizing both duration and frequency, and employing nets of appropriate size and in good condition. The procedure must always be carried out by trained and experienced personnel. Water quality parameters may be affected during crowding; so, at a minimum, oxygen levels should be monitored, along with fish behavior (e.g., signs of surface gasping) and external condition (e.g., skin and gill health). These observations help ensure that crowding intensity remains within acceptable limits and that fish do not experience excessive



stress. If signs of severe stress appear, the procedure should be immediately halted.

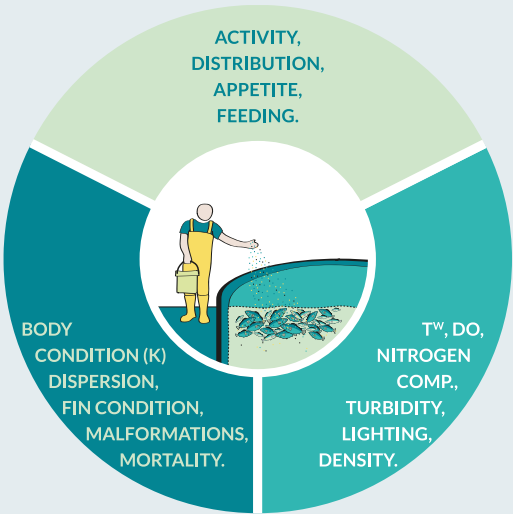
Cleaning of Rearing Units and Systems

In turbot farming carried out in tanks, cleaning and disinfection procedures are an integral part of the Biosecurity Plan, aiming at removing organic material to eliminate or inactivate pathogenic agents. The procedure will depend on its purpose (prevention, control, or eradication of disease) and on the production phase, while ensuring that the cleaning methods employed do not compromise the welfare of the fish. It is recommended to use siphons or vacuum systems during the larval and post-larval stages to clean the tank bottoms and walls while the fish remain inside. For deeper disinfection procedures, fish should be transferred to another tank so that the original unit can be drained and thoroughly cleaned using disinfectants, detergents, or other legally authorized products.

Collection of Mortalities and Moribund Fish

Dead and moribund fish can serve as sources of disease transmission within rearing systems and may also contribute to deterioration of water quality. Additionally, it is important to record mortalities for effective farm management, as unusually high mortality rates can signal underlying health or welfare issues that require investigation. Ideally, daily observation and monitoring of dead and moribund individuals should be performed by authorized personnel, and any mortality levels above normal should be analyzed to determine the cause and managed accordingly. The removal of dead fish should be carried out regularly, according to operational conditions, to maintain proper hygiene. In cases where moribund fish are observed, they should be promptly removed from the water and humanely euthanized (e.g., through anesthetic overdose). Samples should be collected for subsequent analysis and diagnosis, in accordance with the company's Health Plan.

4.3. Feeding



Selected essential operational indicators to be recorded and monitored for the assessment of turbot welfare in relation to feeding behavior throughout all production stages.

Appropriate Feeding Strategy

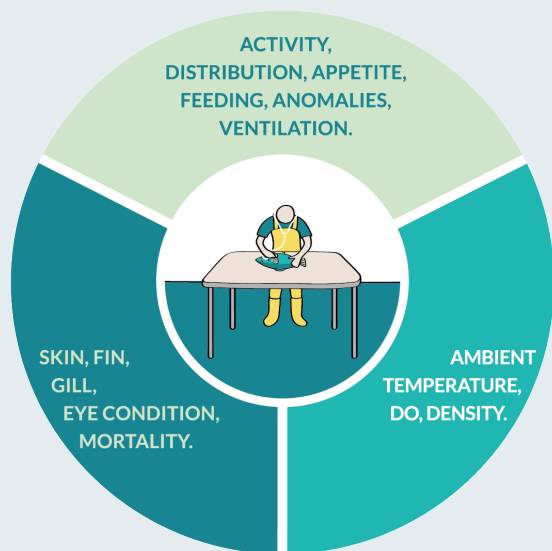
A well-designed feeding strategy plays a crucial role in maintaining the welfare, health, and production efficiency of turbot rearing, as feeding regimes, schedules, and feed characteristics have a significant impact on overall performance and fish condition. The number of daily feedings depends on the stage of the production cycle, but appetite and feed intake throughout the day, season, and prevailing environmental conditions (e.g., water temperature) must always be considered. Additionally, the feeding strategy should ensure that all turbot have equitable access to feed, minimizing competition between individuals.

Management of Fasting Periods

As a management practice, fasting serves several purposes: improving operational efficiency, optimizing flesh quality, and supporting animal welfare. It is also an essential practice for ensuring food safety. By emptying the intestine before stressful procedures, fasting reduces the fish's metabolic rate and oxygen demand, improving tolerance to acute stress. Moreover, it minimizes the risk of carcass contamination during evisceration, thereby enhancing product hygiene and food safety. Fasting effectiveness, however, depends largely on its duration, as prolonged food deprivation can compromise both welfare and production performance. Extended fasting increases turbot sensitivity to stress. Therefore, fasting periods should only be applied when strictly necessary and always in accordance with the farm's Veterinary Health Plan (at least when performed for the first time), ensuring both fish welfare and food safety. During handling, fasting duration should be minimized and must not exceed 20 °C/day or 48 hours, whichever occurs first; i.e., it should be adjusted according to season and water temperature. Fasting before slaughter may be extended up to a maximum of 50 °C/day or 72 hours, whichever comes first. Similarly, during transport, fasting should not exceed 75 °C/day or five days. Repeated fasting periods should be avoided to allow the fish to recover; thus, consecutive fasting events must not be carried out. If fasting needs to be extended, it should only be done for welfare reasons and under strict veterinary supervision. In all cases, a broad set of indicators should be used to monitor and assess welfare status, ensuring the timely application of corrective management measures.



4.4. Animal Health



Selected essential operational indicators to be recorded and monitored for the assessment of turbot welfare in relation to animal health throughout all production stages.

Animal Welfare Plan

Each facility is recommended to have a specific Animal Welfare Plan, adapted to the size of the farm and aligned with the minimum requirements outlined in Annex II of [Royal Decree 348/2000](#). These are summarized as follows: a) a description of the structural and environmental conditions of the facility; b) an assessment of risk factors for animal welfare, including natural disasters (such as floods, earthquakes, tsunamis, strong waves, currents, the presence of predators, or fires), according to the characteristics of the site; and c) an action plan detailing the measures to be implemented to address the identified risks. In addition, the plan must be designed and periodically reviewed by a veterinarian and/or the professional responsible for animal welfare. The review frequency should be specified in the plan itself, with a suggested minimum of one review every two years. The plan should include: a) the name of the person responsible for the welfare and health of the fish; b) critical procedures that could affect fish health and welfare; c) common or

potential diseases, including their symptoms, diagnostic methods, and treatments; d) operational procedures to ensure fish health and welfare in response to the issues that may arise; and e) a detailed protocol for welfare assessment based on specific operational indicators.

Vaccination

Vaccination has been proven to be an effective tool for reducing the incidence of disease and improving fish health and welfare. Therefore, vaccination is recommended as a preventive measure. Vaccine use is regulated by [Royal Decree 666/2023](#), which governs distribution, prescription, dispensing, and application. For turbot, the most common vaccines correspond to two diseases prevalent in the main farming regions: Flexibacteriosis, caused by *Tenacibaculum maritimum*, and Vibriosis, caused by bacteria of the *Vibrio* genus. However, vaccination can be a stressful process since it involves handling, crowding, air exposure, and the injection itself, which may cause side effects. The procedure should therefore be carried out in a manner that minimizes stress at every step. Post-vaccination monitoring should be conducted to detect any adverse reactions or impacts on turbot welfare.

Antibiotic Treatments

Antibiotics are an essential tool for combating bacterial diseases, both in humans and in animal farming systems such as aquaculture. Their use is regulated under ([Royal Decree 666/2023](#)), which explicitly prohibits prophylactic use. Antibiotics must only be administered under veterinary prescription and supervision. Their application should be strictly limited to diagnosed cases and never for preventive purposes, as misuse can lead to the development of bacterial resistance, thereby reducing their effectiveness and leaving fewer options to treat disease. The use of antibiotics classified as critically important for human health should only occur when strictly necessary and as a last resort. Each farm's Health and Welfare Plan must designate the person responsible for deciding on treatments following a confirmed diagnosis and clearly state the justification for their use. When required by a diagnosis, and only if no alternative options exist, antibiotics may be used to safeguard fish welfare, but never as a substitute for good farm-

ing and husbandry practices. Every aquaculture company should have a plan to reduce antibiotic use, defining specific quantitative and time-bound targets. All antibiotic treatments must be recorded and reported, including the reason for use, to enable evaluation of progress toward reduction goals.

Non-Antibiotic or Antimicrobial Treatments

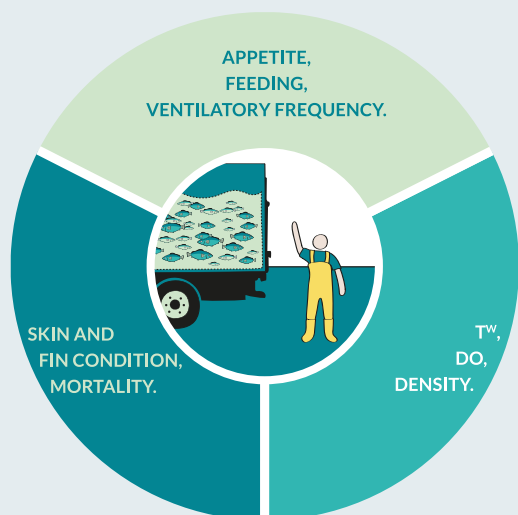
Each company must maintain a Comprehensive Health Plan, overseen by a licensed veterinarian and implemented by the aquaculture producer in accordance with current regulations. Preventive approaches, such as the use of natural immunostimulants, probiotics, and prebiotics, should be prioritized over reactive treatments to minimize the need for therapeutic intervention. The use of antimicrobials and any other treatment is governed by [Royal Decree 666/2023](#), which regulates the distribution, prescription, dispensation, and use of veterinary medications. As with antibiotics and vaccines, these treatments must never replace sound husbandry and management practices.

Recording and Definition of Mortality by Cause

Although fish death rates are retrospective indicators of farm events, they remain valuable for detecting diseases or underlying issues that may compromise welfare. Therefore, they should be recorded regularly and, whenever possible, the causes investigated. In production practice, these data are usually reported as total or mean values for die-off fish batches, as well as morbidity and mortality rates by production phase and time period, expressed in both absolute (kg) and relative (%) terms. It is also advisable to include losses resulting from selective culling or discards in the overall figures. Occasionally, a sharp increase in deaths may occur, indicating an acute event that reflects a critical health or welfare problem on the farm. Tracking the frequency of such occurrences provides complementary information to improve management practices. All acute cases should be documented along with the identified or suspected cause.



4.5. Transport



Selected essential operational indicators to be recorded and monitored for the assessment of turbot welfare in relation to transport throughout all production stages.

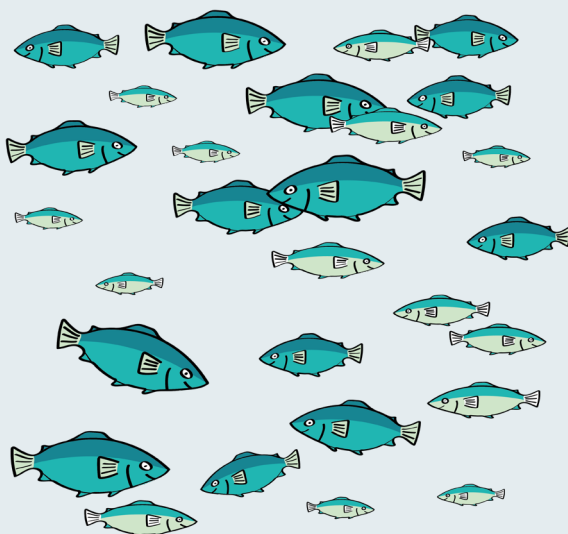
Short- and Long-Distance Transport

The transfer of individuals between units or facilities is a critical stage for welfare. To avoid additional stress, only fish in good health should be transported, except in emergencies, which require veterinary approval. Sedatives may be used before or after transport to facilitate handling, although this practice is not common. During transport, an additional oxygen supply must be provided either through aeration or injection of pure oxygen to maintain oxygen saturation levels between 150% and 200%. Transport and holding containers must: a) be thermally insulated; b) be filled with water of the highest possible quality, ideally sourced from the same location as the fish; c) lack sharp corners or have rounded edges to prevent skin abrasions and mechanical injury; and d) be sufficiently large to allow adequate fish movement and ensure comfort during transport.

After transportation, a water renewal protocol must be established to gradually equalize the temperature between the transport water and

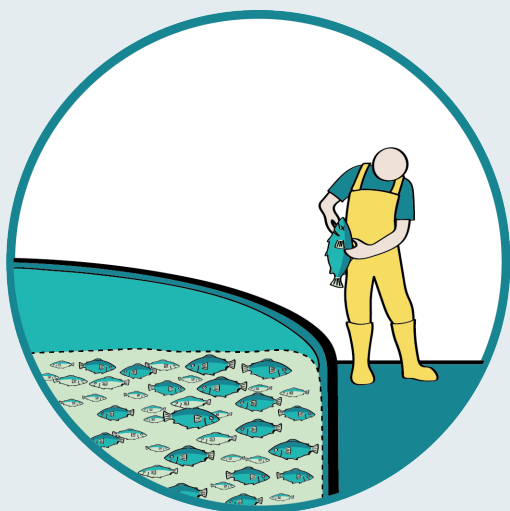
that at the destination, thereby reducing potential negative impacts on welfare. Regarding feeding, turbot should undergo a fasting period of 14 to 48 hours beforehand to reduce excretion rates. For broodstock, stocking density should be kept low, varying inversely with journey duration and water temperature, while maintaining oxygen saturation levels around 100%. The handling of broodstock must be carried out swiftly and carefully, ensuring that travel time is minimized and that water quality conditions are maintained throughout. Whenever possible, temperature and dissolved oxygen levels should be continuously monitored during transit, along with fish behavior and other welfare indicators.

Animal welfare legislation governing transport is currently defined by [Council Regulation \(EC\) N° 1/2005](#) of 22 December 2004 on the protection of animals during transport and related operations, and by [Royal Decree 990/2022](#) of 29 November, concerning animal health and welfare standards during transport. These regulations establish a series of requirements, including authorization of the transporter and transport vehicle, identification of the operator, origin, and destination, including departure and arrival times, availability of a contingency plan (for both the transporter and the transport organizer), recording of mortality rates and water changes made during the journey, and documentation of any intermediate facilities or stops. All transport vehicles must be authorized for the movement of live fish within Spanish territory and for journeys exceeding 8 hours within the European Union.





4.6. Slaughter Procedures



Slaughter represents the final stage of the animals' life cycle. Article 3.1 of [Council Regulation \(EC\) n° 1099/2009](#) of 24 September 2009, on the protection of animals (including fish) at the time of killing, establishes that “during the slaughtering or related operations, animals shall be spared any avoidable pain, distress, or suffering.”

Management of Discards and Moribund Fish (Not for Human Consumption)

Throughout the production cycle, there are several stages in which turbot are sorted and culled from the rearing process, either for welfare or production reasons. Additionally, sick or moribund fish may be found in the facilities and must be removed from the water. All such fish must be humanely euthanized, ensuring that they do not experience unnecessary suffering. The use of anesthetic agents followed by euthanasia by overdose is recommended, since these individuals are not intended for human consumption.

Stunning and Slaughter (for Human Consumption)

The slaughter of fish for human consumption involves a series of sequential operations culminating in death. These stages include fasting, crowding and harvesting, stunning, and killing ¹³⁶. Each

of these steps is highly significant for the welfare of farmed turbot and must be performed properly and in coordination, considering additional key factors such as equipment design, a commitment to updating methods, and personnel training.

As previously noted, it is important to limit the frequency and duration of crowding events as well as fasting periods, since the latter can help fish better tolerate stress and improve product quality, while also serving as a food safety requirement. However, the legal requirements on stunning and slaughter methods set out in ([Council Regulation \(EC\) 1099/2009](#)) do not apply to fish in aquaculture.

Spanish aquaculture companies currently employ various stunning and/or slaughter methods, with direct immersion in ice-water being the most common. However, both the World Organization for Animal Health (WOAH), issued in 2008 and last updated in 2012, and the Council of Europe's 2005 Recommendation concerning the welfare of farmed fish, do not recognize immersion in ice-water as a stunning method, but rather as a killing method. Furthermore, the European Food Safety Authority (EFSA) ¹³⁶ has stated that direct immersion in ice-water is not recommended for the slaughter of turbot in aquaculture, as it does not constitute a humane stunning technique. Therefore, it is recommended that fish reaching market size and harvested for consumption be effectively stunned before killing to ensure their welfare.

In this regard, it is important to ensure that the protocols applied at the final stage of the production cycle involve a stunning method followed by a killing method, so that the animals die while still in an unconscious state. Stunning should induce immediate loss of consciousness and should last until death occurs, thereby preventing the fish from experiencing pain or suffering during slaughter. Some Spanish companies use mechanical stunning methods, such as percussion in turbot, particularly in cases where bleeding is performed, killing the fish by gill cutting followed by immersion in ice-water. Other alternatives, such as stunning by electro-narcosis, are currently being explored within the sector and remain experimental for turbot. Despite scientific progress, questions remain regarding the effectiveness and welfare implications of these methods⁷⁸. The Spanish aquaculture sector

is therefore actively working to develop humane stunning and slaughter methods that are both effective and commercially viable. These efforts take into account additional considerations such as worker safety, technical feasibility in aquatic environments, potential environmental impacts, and effects on product quality and food safety for fish destined for human consumption.

Verification of Proper Stunning and Slaughter

To ensure the effectiveness of both stunning and slaughter, it is essential to verify that each has been performed correctly and efficiently. In the case of the former, verification involves confirming that the fish have indeed reached a state of unconsciousness, which requires species-specific studies and validation. In turbot, several reflexive and behavioral responses can be observed and quantified⁷⁵. These include the vestibulo-ocular reflex (VOR), the response to puncture, the ability to regain posture, respiration

through opercular movement, and the reaction to electrical stimuli. These have been described as operational indicators of loss of consciousness in turbot⁷⁵, although they must be interpreted with caution⁷⁷⁻⁷⁸. True confirmation of unconsciousness, however, can only be precisely assessed through the analysis of brain activity, using highly specialized methodologies such as electroencephalography (EEG)^{78,140}. While the routine application of EEG in turbot farms is not feasible, it should be employed to validate different stunning methods and, more broadly, the entire slaughter process, helping to determine the techniques most suitable for the species. Moreover, EEG analysis can be useful for confirming the validity of sensory and behavioral responses as indicators of consciousness, thereby facilitating their use to ensure that routine protocols are applied correctly and that loss of consciousness is maintained until death occurs. In general, before any processing, it must be verified that the fish show no vital signs, ensuring that the slaughter protocol has been humanely conducted.



5. TRAINING AND COMMUNICATION



5.1. Internal and Institutional Training

Each company should implement measures to train its personnel in fish welfare, using manuals, internal courses, external training programs, and lectures delivered by in-house or outsourced specialists. The level of training may vary depending on the position held, and records of all training activities must be maintained. It is essential to convey and reinforce fish welfare concepts among personnel who work directly with the animals, as well as among those responsible for decision-making that may affect their welfare. Studies have shown that training staff in animal welfare strengthens their bond with the animals under their care, improves their attention to observable welfare indicators by helping them understand their causes, and ultimately enhances the quality of animal husbandry. Training should be provided at the start of employment and periodically renewed (recommended every two years) to reinforce key concepts and bring staff up to date on new scientific and technical advances in this continuously evolving

field. In addition to job-specific content, training programs should generally include: i) the concept of welfare and sentience in fish and other aquatic animals; ii) best practices related to welfare management; iii) welfare indicators, both general and species-specific; iv) common issues, such as diseases or injuries; and v) examples of good and poor practices.

Training in aquaculture should be promoted both internally within companies and at the institutional level. Collaboration with public and academic institutions is encouraged to integrate animal welfare education into official certification or qualification programs required for aquaculture activities and fish handling, such as the Nautical-Fishing Professional Identity Card. Furthermore, animal welfare training should be incorporated into various educational levels (vocational programs, undergraduate, and master's degrees), as doing so will help build an industry that is both aware of and responsive to the challenges and opportunities associated with this essential concept.

5.2. Communication and Outreach

It is important that the entire supply chain, beginning with producers, actively communicates and disseminates information on the practices being implemented in the farming of animals destined for consumption, as well as the specific measures being taken to enhance them. In this regard, APROMAR publishes its Sustainability Report on a biennial basis (www.apromar.es), and it is recommended that each producer contribute to and take responsibility for communicating their own practices and policies.

The production of fish, as with any other animal species, should be conducted in the most transparent manner possible, not only to prevent crit-

icism, but also to inform and engage the public in the production process, thereby enabling consumers to make informed choices about the products they purchase, should they wish to do so. In doing so, the aquaculture industry can

position itself alongside other sectors of animal protein production, demonstrating to the public the commitment and sense of responsibility that fish farmers already have toward the aquatic animals under their care.



6. CHALLENGES AND OPPORTUNITIES



enhancing welfare in captive fish ¹⁴¹. Since turbot remain in farms for long periods throughout their production cycle, the implementation of environmental enrichment, whether structural, occupational, or sensorial, may help meet their behavioral and physiological needs when appropriately adapted to the production phase and system. Despite extensive scientific knowledge regarding the positive effects of enrichment strategies in many fish species, studies in turbot remain scarce, representing a greater challenge for industrial-scale application. The sector, therefore, has an opportunity to collaborate with researchers in the investigation and validation of enrichment methods specifically adapted to production systems, ensuring positive welfare outcomes without negatively affecting animal health or productivity.

Precompetitive Collaboration for the Improvement of Welfare

The European and Spanish aquaculture industries are advancing and evolving through innovation in technologies and processes, including those related to animal welfare. However, there remains a significant challenge in improving farming practices to ensure and enhance fish welfare. Achieving this goal requires collaboration among public administrations, producer associations, research institutions, and society at large to develop relevant knowledge and technologies to support welfare improvement. The immediate outcome of these collaborative efforts will be an overall enhancement in fish welfare, which will also serve as a differentiating factor and increase the international competitiveness of the sector.

Development and Application of Environmental Enrichment

The aquaculture sector faces the challenge of evolving and adopting new methods that improve the living conditions of turbot. Strategies such as environmental enrichment are considered valuable tools for diminishing stress responses and

Light is extremely important in turbot farming and can serve as an enrichment strategy. In indoor tanks, the use of natural light spectra or short wavelengths (e.g., blue light) should be encouraged to improve larval development ^{64,110-113} and maintain circadian rhythms, thereby promoting multiple positive effects on turbot welfare ^{56,105-106}. Likewise, excessively dark or bright tank colors should be avoided ^{25,64}. For outdoor rearing systems, using tarpaulins or awnings above the tanks is essential to regulate light intensity and prevent skin and eye damage caused by solar radiation ².

Several studies suggest that the use of different substrates, such as sand or gravel, may serve as environmental enrichment for flatfish species, promoting their benthic behavior and interaction with the bottom (e.g., burying, hiding, resting), while also improving health by reducing skin lesions and infections (see references in 141). However, structures, substrates, and enrichment objects can accumulate organic matter (from feed or feces), complicating cleaning and disinfection and potentially compromising fish health and welfare, as well as increasing operating costs ¹⁴¹. In turbot, the use of

rigid structures serving as shelters in broodstock tanks has been shown to improve egg production and viability ¹⁴², while the inclusion of diagonal structures can stimulate exploratory behavior ¹⁴³. Nevertheless, inadequate enrichment design or placement can cause behavioral disturbances, physiological stress, or physical injuries, increasing the risk of infection, stress, or mortality.

Furthermore, enrichment may sometimes elicit negative responses in certain fish, such as fatigue, neophobia, territoriality, or aggression. All these factors must be carefully considered when planning enrichment strategies, and operational welfare indicators (see Annex) should be used to monitor and evaluate the welfare effects and identify any negative impacts on production outcomes.

Welfare Monitoring, Follow-up, and Assessment Tools

Developing detailed and effective protocols will be a priority in the coming years to ensure the accurate evaluation and management of animal welfare in aquaculture farms operating within the European Union. Welfare assessment tools must rely on operational indicators that allow for the objective quantification of welfare over time and under varying conditions. Technological innovation and the application of tools tailored to specific rearing conditions or species should enable precise and rapid welfare evaluations, while also supporting the development of innovative management strategies. For instance, the integration of automated sensors for physicochemical and microbiological water monitoring, observation and surveillance cameras, and artificial intelligence systems will facilitate continuous monitoring of welfare indicators within automated operational frameworks. Such technologies must be practical for use in commercial facilities, enable early detection of animal welfare risks, and support informed decision-making. The adoption of continuous monitoring systems, together with technological and computational advances, will open new opportunities for innovation in welfare surveillance and assessment. Therefore, through collaboration with the public sector and research institutions, the aquaculture industry faces the challenge of promoting these technological advances to ensure their effective implementation in the near future.

Development and Application of New Health Treatments

At present, there are no specific health treatments for all diseases or pathogens that occur in turbot farming facilities. As a result, producers often rely on broad-spectrum or non-specific treatments, which are not always effective. Research and development of pathogen-specific treatments, particularly vaccines, would strengthen the preventive approach and improve the effectiveness of farm health plans. Such advancements would enable more efficient control of certain diseases, reduce reliance on antibiotics, and enhance the health and welfare of farmed turbot. They would also reduce the risk of bacterial resistance associated with antibiotic use, thereby promoting more sustainable and responsible aquaculture practices.

Implementation of Humane Slaughter Techniques

Scientific research has demonstrated that fish are sentient beings capable of experiencing fear, pain, and suffering throughout their lives, including during slaughter. This understanding raises questions about the humaneness and effectiveness of certain slaughter methods currently used in turbot production for consumption.

Slaughter techniques influence not only the welfare of the fish but also the quality, shelf life, and organoleptic properties of the final product. The sector therefore faces the challenge of proactively developing and adopting more humane and innovative slaughter techniques, adapting existing technologies to the Spanish context through collaborative efforts. This represents an opportunity to improve fish welfare, enhance product quality, and gain access to markets and certification schemes that require or reward humane slaughter practices.

Genetic Selection and Research

Turbot farming is relatively recent compared with other aquaculture species produced in Spain and Europe, and certain production situations may still compromise fish welfare and reduce performance. Although turbot has reached a high level of domestication, comparable to other species of greater production and distribution ¹⁴⁴, aquaculture producers face the

challenge of developing genetic selection programs that continue to improve the plasticity and resilience of the species, enabling adaptation to new environments and pathogens while promoting welfare. It is essential, therefore, that any research into the genetic improvement of turbot be accompanied by welfare studies to ensure that selective breeding practices do not compromise their welfare.

Welfare Certification in Turbot Aquaculture

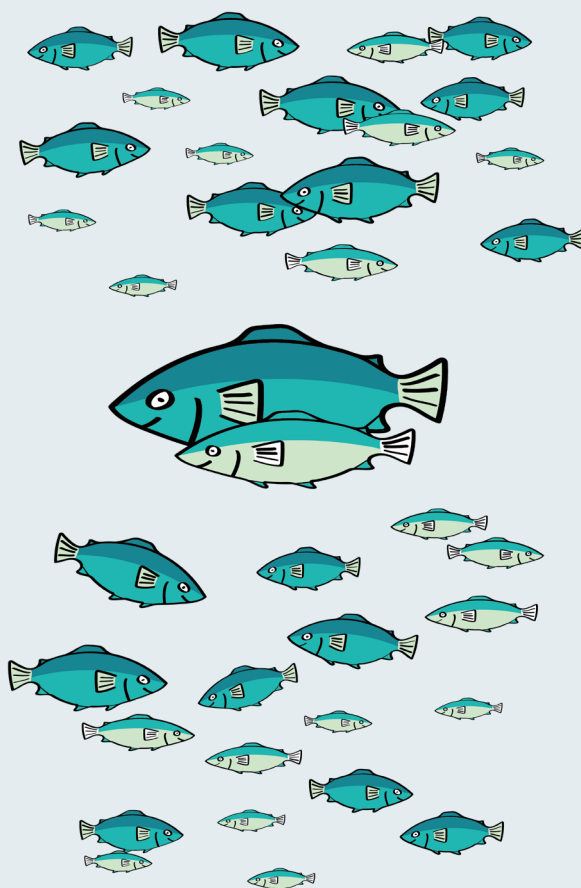
One of the main challenges for the sector is effectively communicating its practices to the various members of the supply chain and to the end consumer — not only to raise awareness, but also to promote recognition of the standards under which fish are farmed. Certification schemes are among the most widely used and sought-after tools to evaluate, verify, and ensure compliance with the standards that are established or required. Most current aquaculture certifications, however, have yet to include welfare parameters that can guarantee these practices throughout the production and distribution chain. Because certification standards are often complex and not widely understood, they do not always facilitate effective communication with consumers. While certifications cannot replace transparent corporate communication, they are useful for establishing and improving welfare practices and for enhancing communication within the supply chain. This presents several opportunities for development, such as requesting the inclusion of welfare parameters for turbot within existing certification schemes or creating welfare standards within already recognized proprietary quality labels.

Climate Change Implications for Turbot Welfare

Climate change and its impact on aquaculture are an established reality, significantly transforming the sector, particularly in systems dependent on natural environmental conditions. Protecting fish welfare requires anticipating these changes and taking coordinated action before conditions in tanks become unsuitable or economically unsustainable. Ensuring welfare under these evolving conditions depends on sufficient understanding of species biology to adequately meet their environmental and physiological needs.

In Spain, increasing average temperatures and the growing frequency of heatwaves may affect water temperature, oxygen concentrations, and algal bloom, among other parameters, both in turbot farms and in water sources supplying aquaculture facilities. These changes may pose risks to fish welfare, making efficient water quality monitoring and robust management plans essential for timely response. Climate change will also affect bacterial communities in the water, including potential pathogens, and may lead to the emergence of new contaminants, all of which have direct implications for fish health and welfare.

Measures to mitigate the effects of climate change may include improvements in farm design and location, selection of resistant fish strains or species, development of enhanced fish monitoring and weather forecasting systems, and improved control and management of water quality parameters. Although these measures will pose challenges, the alignment of economic and production objectives with the goals of animal welfare protection provides reason for optimism¹⁴⁵.





7. SELECTED BIBLIOGRAPHY

1. Bailly, N. & Chanet, B. *Scophthalmus rafinesque*, 1810: the valid generic name for the turbot, *S. maximus* (Linnaeus, 1758) [Pleuronectiformes: Scophthalmidae]/*Scophthalmus Rafinesque*, 1810: le nom de genre valide du turbot, *S. maximus* (Linnaeus, 1758) (Pleuronectiformes: Scophthalmidae). **Cybium Int. J. Ichthyol.** **34**, 257–262 (2010).
2. Rodríguez, J. L. Cultivo del Rodaballo (*Scophthalmus maximus*). 4–43 (2011).
3. Vladislav Shlyakhov (Laboratory of Aquatic Biological Resources, A.-B. S. B. of V. et al. IUCN Red List of Threatened Species: *Scophthalmus maximus*. **IUCN Red List Threat. Species** <https://www.iucnredlist.org/en> (2020).
4. FAO - Psetta maxima. https://www.fao.org/fishery/docs/CDrom/aquaculture/I1129m/file/es/es_turbot.htm.
5. APROMAR. La acuicultura en España. (2024).
6. Nash, R. D., Valencia, A. H. & Geffen, A. J. The origin of Fulton's condition factor— setting the record straight richard. **Fisheries** **31**, 236–238 (2006).
7. Lloret, J., Shulman, G. & Love, R. M. **Condition and Health Indicators of Exploited Marine Fishes**. (John Wiley & Sons, 2013).
8. Križanac, S. et al. Comparative Study of Physiological Changes in Turbot *Scophthalmus maximus* in Different Living Conditions. **Appl. Sci.** **12**, 4201 (2022).
9. Blanquet, I. & Oliva-Teles, A. Effect of feed restriction on the growth performance of turbot (*Scophthalmus maximus* L.) juveniles under commercial rearing conditions. **Aquac. Res.** **41**, 1255–1260 (2010).
10. Kerambrun, E., Henry, F., Rabhi, K. & Amara, R. Effects of chemical stress and food limitation on the energy reserves and growth of turbot, *Scophthalmus maximus*. **Environ. Sci. Pollut. Res.** **21**, 13488–13495 (2014).
11. Sunde, L. M., Imsland, A. K., Folkvord, A. & Stefansson, S. O. Effects of size grading on growth and survival of juvenile turbot at two temperatures. **Aquac. Int.** **6**, 19–32 (1998).
12. Irwin, S., O'Halloran, J. & FitzGerald, R. D. The relationship between individual consumption and growth in juvenile turbot, *Scophthalmus maximus*. **Aquaculture** **204**, 65–74 (2002).
13. Imsland, A. K., Sunde, L. M., Folkvord, A. & Stefansson, S. O. The interaction of temperature and fish size on growth of juvenile turbot. **J. Fish Biol.** **49**, 926–940 (1996).
14. Irwin, S., O'Halloran, J. & FitzGerald, R. D. Stocking density, growth and growth variation in juvenile turbot, *Scophthalmus maximus* (Rafinesque). **Aquaculture** **178**, 77–88 (1999).
15. Stefánsson, M. Ö., FitzGerald, R. D. & Cross, T. F. Growth, feed utilization and growth heterogeneity in juvenile turbot *Scophthalmus maximus* (Rafinesque) under different photoperiod regimes. **Aquac. Res.** **33**, 177–187 (2002).
16. Gomez-Raya, L. et al. The relationship between feed efficiency, growth and group dominance dynamics in turbot (*Scophthalmus maximus*). **Span. J. Agric. Res.** **16**, e0604–e0604 (2018).
17. Landeira-Dabarca, A., Abreu, C. S. R., Álvarez, M. & Molist, P. Changes in marine turbot (*Scophthalmus maximus*) epidermis and skin mucus composition during development from bilateral larvae to juvenile flat fish. **J. Fish Biol.** **99**, 2018–2029 (2021).
18. Novoa, B., Nun˜ez, S., Fernández-Puentes, C., Figueras, A. J. & Toranzo, A. E. Epizootic study in a turbot farm: bacteriology, virology, parasitology and histology. **Aquaculture** **107**, 253–258 (1992).
19. Jia, R. et al. Stress and immune responses in skin of turbot (*Scophthalmus maximus*) under different stocking densities. **Fish Shellfish Immunol.** **55**, 131–139 (2016).
20. Devesa, S., Barja, J. L. & Toranzo, A. E. Ulcerative skin and fin lesions in reared turbot, *Scophthalmus maximus* (L.). **J. Fish Dis.** **12**, 323–333 (1989).
21. Huang, Z.-H., Ma, A.-J. & Wang, X.-A. The immune response of turbot, *Scophthalmus maximus* (L.), skin to high water temperature. **J. Fish Dis.** **34**, 619–627 (2011).
22. Bloch, B. & Larsen, J. L. An iridovirus-like agent associated with systemic infection in cultured turbot *Scophthalmus maximus* fry in Denmark. **Iridovirus- Agent Assoc. Syst. Infect. Cult. Turbot Scophthalmus Maximus Fry Den.** **15**, 235–240 (1993).

23. Estevez, A. & Kanazawa, A. Effect of (n-3) PUFA and vitamin A Artemia enrichment on pigmentation success of turbot, *Scophthalmus maximus* (L.). **Aquac. Nutr.** **1**, 159–168 (1995).
 24. Bolker, J. A. & Hill, C. R. Pigmentation development in hatchery-reared flatfishes. **J. Fish Biol.** **56**, 1029–1052 (2000).
 25. Li, X. et al. Colour preferences of juvenile turbot (*Scophthalmus maximus*). **Physiol. Behav.** **156**, 64–70 (2016).
 26. Wu, L. et al. Comparative transcriptome analysis reveals growth and molecular pathway of body color regulation in turbot (*Scophthalmus maximus*) exposed to different light spectrum. **Comp. Biochem. Physiol. Part D Genomics Proteomics** **49**, 101165 (2024).
 27. Faílde, L. D., Bermúdez, R., Vigliano, F., Coscelli, G. A. & Quiroga, M. I. Morphological, immunohistochemical and ultrastructural characterization of the skin of turbot (*Psetta maxima* L.). **Tissue Cell** **46**, 334–342 (2014).
 28. Venizelos, A. & Benetti, D. D. Pigment abnormalities in flatfish. **Aquaculture** **176**, 181–188 (1999).
 29. Lv, X. et al. Osteological ontogeny and allometric growth in larval and juvenile turbot (*Scophthalmus maximus*). **Aquaculture** **498**, 351–363 (2019).
 30. Tong, X. H. et al. Skeletal development and abnormalities of the vertebral column and of the fins in hatchery-reared turbot *Scophthalmus maximus*. **J. Fish Biol.** **80**, 486–502 (2012).
 31. Aydin, İ. et al. Skeletal development and malformations in the early life stage of diploid and triploid turbot (*Scophthalmus maximus*). **Aquaculture** **550**, 737886 (2022).
 32. Padros, F. & Crespo, S. Swimbladder pathology during larval development of turbot (*Scophthalmus maximus* L.). **ICES Mar Sci Symp** **201**, 159–162 (1995).
 33. Latremouille, D. N. Fin Erosion in Aquaculture and Natural Environments. **Rev. Fish. Sci.** **11**, 315–335 (2003).
 34. Ellis, T. et al. Fin Erosion in Farmed Fish. in **Fish Welfare** 121–149 (John Wiley & Sons, Ltd, 2008).
- doi:10.1002/9780470697610.ch9.
35. Lei, Q., Yin-Geng, W., Zheng, Z. & Shao-Li, Y. The First Report on Fin Rot Disease of Cultured Turbot *Scophthalmus maximus* in China. **J. Aquat. Anim. Health** **18**, 83–89 (2006).
 36. Dyková, I., Figueras, A., Novoa, B. & Casal, J. F. *Paramoeba* sp., an agent of amoebic gill disease of turbot *Scophthalmus maximus*. **Dis. Aquat. Organ.** **33**, 137–141 (1998).
 37. Leiro et al. An amoeba associated with gill disease in turbot, *Scophthalmus maximus* (L.). **J. Fish Dis.** **21**, 281–288 (1998).
 38. Villamil, L., Figueras, A., Toranzo, A. E., Planas, M. & Novoa, B. Isolation of a highly pathogenic *Vibrio pelagius* strain associated with mass mortalities of turbot, *Scophthalmus maximus* (L.), larvae. **J. Fish Dis.** **26**, 293–303 (2003).
 39. Coscelli, G. A. et al. Acute *Aeromonas salmonicida* infection in turbot (*Scophthalmus maximus* L.). Histopathological and immunohistochemical studies. **Aquaculture** **430**, 79–85 (2014).
 40. Qi, T. et al. Acute low-dose phosphate disrupts glycerophospholipid metabolism and induces stress in juvenile turbot (*Scophthalmus maximus*). **Sci. Total Environ.** **861**, 160430 (2023).
 41. Lamas, J., Noya, M., Figueras, A. & Toranzo, A. E. Pathology associated with a viral erythrocytic infection in turbot, *Scophthalmus maximus* (L.). **J. Fish Dis.** **18**, 425–433 (1995).
 42. Branson, E., Riaza, A. & Alvarez-Pellitero, P. Myxosporean infection causing intestinal disease in farmed turbot, *Scophthalmus maximus* (L.), (Teleostei: Scophthalmidae). **J. Fish Dis.** **22**, 395–399 (1999).
 43. Iglesias, R. et al. *Philasterides dicentrarchi* (Ciliophora, Scuticociliatida) as the causative agent of scuticociliatosis in farmed turbot *Scophthalmus maximus* in Galicia (NW Spain). **Dis. Aquat. Organ.** **46**, 47–55 (2001).
 44. Bermúdez, R. et al. Effects of symmetric triazinone (toltrazuril) on developmental stages of *Enteromyxum scophthalmi* parasitizing turbot (*Scophthalmus maximus* L.): A light and electron microscopic study. **Aquaculture** **254**, 65–71 (2006).
 45. Padrós, F., Zarza, C., Dopazo, L., Cuadrado, M. & Crespo, S. Pathology of *Edwardsiella tarda* infection in turbot, *Scophthalmus maximus* (L.). **J. Fish Dis.** **29**, 87–94 (2006).
 46. Pereiro, P., Figueras, A. & Novoa, B. Turbot (*Scophthalmus maximus*) vs. VHSV (Viral Hemorrhagic Septicemia Virus): A Review. **Front. Physiol.** **7**, (2016).
 47. Feng, H. et al. Ocular bacterial signatures of exophthalmic disease in farmed turbot (*Scophthalmus maximus*). **Aquac. Res.** **51**, 2303–2313 (2020).
 48. Fernández-Pato, C. Aspectos biológicos y tecnológicos del cultivo del rodaballo *Scophthalmus maximus* L., 1758. (Ministerio de Agricultura, Pesca y Alimentación (España), 1998).

49. Martins, C. I. M. *et al.* Behavioural indicators of welfare in farmed fish. **Fish Physiol. Biochem.** **38**, 17–41 (2012).
50. Skiftesvik, A. B. Changes in Behaviour at Onset of Exogenous Feeding in Marine Fish Larvae. **Can. J. Fish. Aquat. Sci.** **49**, 1570–1572 (1992).
51. Kasumyan, A. O., Ryg, M. & Døving, K. B. Effect of amino acids on the swimming activity of newly hatched turbot larvae (*Scophthalmus maximus*). **Mar. Biol.** **131**, 189–194 (1998).
52. Bruno, E., Mahjoub, M.-S., Hansen, B. W., Munk, P. & Støttrup, J. G. Feeding behavior and capture success of turbot *Psetta maxima* larvae during the transition from upright to tilted swimming position. **Aquat. Living Resour.** **30**, 35 (2017).
53. Huse, I. & Skiftesvik, A. B. Qualitative and quantitative behaviour studies in starving and feeding turbot (*Scophthalmus maximus* L.) larvae. <https://agris.fao.org/search/en/providers/125318/records/6748e0258834da021e380c82> (1985).
54. Skiftesvik, A. B. & Bergh, Ø. Changes in Behaviour of Atlantic Halibut (*Hippoglossus hippoglossus*) and Turbot (*Scophthalmus maximus*) Yolk-Sac Larvae Induced by Bacterial Infections. **Can. J. Fish. Aquat. Sci.** **50**, 2552–2557 (1993).
55. Imsland, A. K., Folkvord, A. & Stefansson, S. O. Growth, oxygen consumption and activity of juvenile turbot (*scophthalmus maximus* L.) reared under different temperatures and photoperiods. **Neth. J. Sea Res.** **34**, 149–159 (1995).
56. Ceinos, R. M. *et al.* Differential circadian and light-driven rhythmicity of clock gene expression and behaviour in the turbot, *Scophthalmus maximus*. **PLOS ONE** **14**, e0219153 (2019).
57. Iglesias-Estévez, J. & Rodríguez-Ojea, G. Fitness of hatchery-reared turbot, *Scophthalmus maximus* L., for survival in the sea: first year results on feeding, growth and distribution. <https://digital.csic.es/handle/10261/315342> (1994).
58. Kristensen, L. D., Sparrevohn, C. R., Christensen, J. T. & Støttrup, J. Cryptic behaviour of juvenile turbot *Psetta maxima* L. and European flounder *Platichthys flesus* L. **Open J. Mar. Sci.** **4**, 185–193 (2014).
59. Imsland, A. K., Nilsen, T. & Folkvord, A. Stochastic simulation of size variation in turbot: possible causes analysed with an individual-based model. **J. Fish Biol.** **53**, 237–258 (1998).
60. Martínez-Villalba, A. Desarrollo de un certificado de bienestar animal en el rodaballo (*Psetta maxima*) en la etapa de engorde. **Tesis Master En Segur. Aliment. Fac. Vet. Univ. CEU Cardenal Herrera 50 Pp** (2021).
61. Holmes, R. A. & Gibson, R. N. A comparison of predatory behaviour in flatfish. **Anim. Behav.** **31**, 1244–1255 (1983).
62. Knutsen, J. A. Feeding behaviour of North Sea turbot (*Scophthalmus maximus*) and Dover sole (*Solea solea*) larvae elicited by chemical stimuli. **Mar. Biol.** **113**, 543–548 (1992).
63. Champalbert, G. & Le Direach-Boursier, L. Influence of light and feeding conditions on swimming activity rhythms of larval and juvenile turbot: *Scophthalmus maximus* L.: An experimental study. **J. Sea Res.** **40**, 333–345 (1998).
64. Sierra-Flores, R. *et al.* Effects of light spectrum and tank background colour on Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) larvae performances. **Aquaculture** **450**, 6–13 (2016).
65. Huse, I. Feeding at different illumination levels in larvae of three marine teleost species: cod, *Gadus morhua* L., plaice, *Pleuronectes platessa* L., and turbot, *Scophthalmus maximus* (L.). **Aquac. Res.** **25**, 687–695 (1994).
66. Mallekh, R., Lagardère, J. P., Bégout Anras, M. L. & Lafaye, J. Y. Variability in appetite of turbot, *Scophthalmus maximus* under intensive rearing conditions: the role of environmental factors. **Aquaculture** **165**, 123–138 (1998).
67. Johansen, R. *et al.* Characterization of nodavirus and viral encephalopathy and retinopathy in farmed turbot, *Scophthalmus maximus* (L.). **J. Fish Dis.** **27**, 591–601 (2004).
68. Montes, A., Figueras, A. & Novoa, B. Nodavirus encephalopathy in turbot (*Scophthalmus maximus*): Inflammation, nitric oxide production and effect of anti-inflammatory compounds. **Fish Shellfish Immunol.** **28**, 281–288 (2010).
69. Sæther, B.-S. & Jobling, M. The effects of ration level on feed intake and growth, and compensatory growth after restricted feeding, in turbot *Scophthalmus maximus* L. **Aquac. Res.** **30**, 647–653 (1999).
70. Waller, U. Factors influencing routine oxygen consumption in turbot, *Scophthalmus maximus*. **J. Appl. Ichthyol.** **8**, 62–71 (1992).
71. Wu, Z. *et al.* Effects of waterborne Fe(II) on juvenile turbot *Scophthalmus maximus*: analysis of respiratory rate, hematology and gill histology. **Chin. J. Oceanol. Limnol.** **30**, 193–199 (2012).
72. Maxime, V., Pichavant, K., Boeuf, G. & Nonnotte, G. Effects of hypoxia on respiratory physiology of turbot, *Scophthalmus maximus*. **Fish Physiol. Biochem.** **22**, 51–59 (2000).
73. Jia, Y. *et al.* Physiological response of juvenile turbot (*Scophthalmus maximus* L.) during hyperthermal stress. **Aquaculture** **529**, 735645 (2020).

74. Kestin, S. C., Robb, D. H. & Van De Vis, J. W. Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. **Vet. Rec.** **150**, 302–307 (2002).
75. Morzel, M., Sohier, D. & Van De Vis, H. Evaluation of slaughtering methods for turbot with respect to animal welfare and flesh quality. **J. Sci. Food Agric.** **83**, 19–28 (2003).
76. Davis, M. W. Fish stress and mortality can be predicted using reflex impairment. **Fish Fish.** **11**, 1–11 (2010).
77. Lambooi, B. et al. Evaluation of Electrical Stunning of Atlantic Cod (*Gadus morhua*) and Turbot (*Psetta maxima*) in Seawater. **J. Aquat. Food Prod. Technol.** **22**, 371–379 (2013).
78. Saraiva, J. L., Brijs, J., Cabrera-Álvarez, M. J., Arechavala-Lopez, P. & Gräns, A. Blueprint for research to detect loss of consciousness and/or sensibility of fish at slaughter. (2025).
79. Iglesias, J., Olmedo, M., Otero, J. J., Peleterio, J. B. & Solórzano, M. R. Growth, under laboratory conditions, of turbot, *Scophthalmus maximus*, from the Ría de Vigo (north-west Spain). **Mar. Biol.** **96**, 11–17 (1987).
80. Burel, C. et al. Potential of plant-protein sources as fish meal substitutes in diets for turbot (*Psetta maxima*): growth, nutrient utilisation and thyroid status. **Aquaculture** **188**, 363–382 (2000).
81. Boeuf, G., Boujard, D. & Ruyet, J. P.-L. Control of the somatic growth in turbot. **J. Fish Biol.** **55**, 128–147 (1999).
82. Ruyet, J. P.-L. Turbot (*Scophthalmus maximus*) Grow-out in Europe: Practices, Results, and Prospects. **Turk. J. Fish. Aquat. Sci.** **2**, 29–39 (2002).
83. Van Ham, E. H. et al. Environment affects stress in exercised turbot. **Comp. Biochem. Physiol. A. Mol. Integr. Physiol.** **136**, 525–538 (2003).
84. Ji, L. et al. Low temperature stress on the hematological parameters and HSP gene expression in the turbot *Scophthalmus maximus*. **Chin. J. Oceanol. Limnol.** **34**, 430–440 (2016).
85. Gaumet, F., Boeuf, G., Truchot, J.-P. & Nonnotte, G. Effects of environmental water salinity on blood acid-base status in juvenile turbot (*Scophthalmus maximus* L.). **Comp. Biochem. Physiol. A Physiol.** **109**, 985–994 (1994).
86. Gaumet, F., Boeuf, G., Severe, A., Le Roux, A. & Mayer-Gostan, N. Effects of salinity on the ionic balance and growth of juvenile turbot. **J. Fish Biol.** **47**, 865–876 (1995).
87. Imsland, A. K., Brix, O., Nævdal, G. & Samuelsen, E. N. Hemoglobin Genotypes in Turbot (*Scophthalmus maximus* Rafinesque), Their Oxygen Affinity Properties and Relation With Growth. **Comp. Biochem. Physiol. A Physiol.** **116**, 157–165 (1997).
88. Pichavant, K. et al. Effects of hypoxia on growth and metabolism of juvenile turbot. **Aquaculture** **188**, 103–114 (2000).
89. Hermann, Bernd. T., Wuertz, S., Vanselow, K. H., Schulz, C. & Stiller, K. T. Divergent gene expression in the gills of juvenile turbot (*Psetta maxima*) exposed to chronic severe hypercapnia indicates dose-dependent increase in intracellular oxidative stress and hypoxia. **Aquat. Toxicol.** **206**, 72–80 (2019).
90. Schram, E. et al. Impact of increased flow rate on specific growth rate of juvenile turbot (*Scophthalmus maximus*, Rafinesque 1810). **Aquaculture** **292**, 46–52 (2009).
91. Stiller, K. T. et al. The effect of carbon dioxide on growth and metabolism in juvenile turbot *Scophthalmus maximus* L. **Aquaculture** **444**, 143–150 (2015).
92. Sun, G., Li, M., Wang, J. & Liu, Y. Effects of flow rate on growth performance and welfare of juvenile turbot (*Scophthalmus maximus* L.) in recirculating aquaculture systems. **Aquac. Res.** **47**, 1341–1352 (2016).
93. Grøttum, J. A., Staurnes, M. & Sigholt, T. Effect of oxygenation, aeration and pH control on water quality and survival of turbot, *Scophthalmus maximus* (L.), kept at high densities during transport. **Aquac. Res.** **28**, 159–164 (1997).
94. Shuang-Yao, W. et al. Effects of seawater pH on survival, growth, energy budget and oxidative stress parameters of juvenile turbot *Scophthalmus maximus*. **Iran. J. Fish. Sci.** **17**, 675–689 (2018).
95. Mota, V. C. et al. The effect of low pH on physiology, stress status and growth performance of turbot (*Psetta maxima* L.) cultured in recirculating aquaculture systems. **Aquac. Res.** **49**, 3456–3467 (2018).
96. Ruyet, J. P.-L., Galland, R., Le Roux, A. & Chartois, H. Chronic ammonia toxicity in juvenile turbot (*Scophthalmus maximus*). **Aquaculture** **154**, 155–171 (1997).
97. Dosdat, A. et al. Nitrogenous excretion in juvenile turbot, *Scophthalmus maximus* (L.), under controlled conditions. **Aquac. Res.** **26**, 639–650 (1995).
98. Alderson, R. The effect of ammonia on the growth of juvenile Dover sole, *Solea solea* (L.) and turbot, *Scophthalmus maximus* (L.). **Aquaculture** **17**, 291–309 (1979).

99. van Bussel, C. G. J., Schroeder, J. P., Wuertz, S. & Schulz, C. The chronic effect of nitrate on production performance and health status of juvenile turbot (*Psetta maxima*). **Aquaculture** **326–329**, 163–167 (2012).
100. Holmes, R. A. & Gibson, R. N. Visual cues determining prey selection by the turbot, *Scophthalmus maximus* L. **J. Fish Biol.** **29**, 49–58 (1986).
101. Borges, M.-T., Morais, A. & Castro, P. M. L. Performance of outdoor seawater treatment systems for recirculation in an intensive turbot (*Scophthalmus maximus*) farm. **Aquac. Int.** **11**, 557–570 (2003).
102. Kerambrun, E. et al. Growth and condition indices of juvenile turbot, *Scophthalmus maximus*, exposed to contaminated sediments: Effects of metallic and organic compounds. **Aquat. Toxicol.** **108**, 130–140 (2012).
103. Almansa, C., Reig, L. & Oca, J. Use of laser scanning to evaluate turbot (*Scophthalmus maximus*) distribution in raceways with different water velocities. **Aquac. Eng.** **51**, 7–14 (2012).
104. Li, X. et al. Effect of flow velocity on the growth, stress and immune responses of turbot (*Scophthalmus maximus*) in recirculating aquaculture systems. **Fish Shellfish Immunol.** **86**, 1169–1176 (2019).
105. Polat, H., Öztürk, R., Terzi, Y., Aydin, I. & Kucuk, E. Effect of photoperiod manipulation on spawning time and performance of turbot (*Scophthalmus maximus*). **Aquac. Stud.** **21**, (2021).
106. Petereit, J., Lannig, G., Baßmann, B., Bock, C. & Buck, B. H. Circadian rhythm in turbot (*Scophthalmus maximus*): daily variation of blood metabolites in recirculating aquaculture systems. **Metabolomics** **20**, 23 (2024).
107. Imsland, A. K., Dragsnes, M. & Stefansson, S. O. Exposure to continuous light inhibits maturation in turbot (*Scophthalmus maximus*). **Aquaculture** **219**, 911–919 (2003).
108. Imsland, A. K. & Jonassen, T. M. Growth and age at first maturity in turbot and halibut reared under different photoperiods. **Aquac. Int.** **11**, 463–475 (2003).
109. Imsland, A. K. et al. Long-term effect of photoperiod manipulation on growth, maturation and flesh quality in turbot. **Aquaculture** **416–417**, 152–160 (2013).
110. Wu, L. et al. Effects of different light spectra on embryo development and the performance of newly hatched turbot (*Scophthalmus maximus*) larvae. **Fish Shellfish Immunol.** **90**, 328–337 (2019).
111. Wu, L. et al. Growth, stress and non-specific immune responses of turbot (*Scophthalmus maximus*) larvae exposed to different light spectra. **Aquaculture** **520**, 734950 (2020).
112. Wu, L. et al. Influence of light spectra on the performance of juvenile turbot (*Scophthalmus maximus*). **Aquaculture** **533**, 736191 (2021).
113. Han, M. M. et al. The plasticity of vision and body development of turbot *Scophthalmus maximus* larvae Under different light spectra. **Aquac. Res.** **51**, 3347–3357 (2020).
114. Xian, L., Ying, L. & Blancheton, J.-P. Effect of stocking density on performances of juvenile turbot (*Scophthalmus maximus*) in recirculating aquaculture systems. **Chin. J. Oceanol. Limnol.** **31**, 514–522 (2013).
115. Liu, B., Jia, R., Han, C., Huang, B. & Lei, J.-L. Effects of stocking density on antioxidant status, metabolism and immune response in juvenile turbot (*Scophthalmus maximus*). **Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.** **190**, 1–8 (2016).
116. Aijun, M. et al. Turbot *Scophthalmus maximus*: stocking density on growth, pigmentation and feed conversion. **Chin. J. Oceanol. Limnol.** **24**, 307–312 (2006).
117. Jia, R. et al. Stress and immune responses in skin of turbot (*Scophthalmus maximus*) under different stocking densities. **Fish Shellfish Immunol.** **55**, 131–139 (2016).
118. Liu, B., Fei, F., Li, X., Wang, X. & Huang, B. Effects of stocking density on stress response, innate immune parameters, and welfare of turbot (*Scophthalmus maximus*). **Aquac. Int.** **27**, 1599–1612 (2019).
119. Liu, B. et al. Stocking density effects on growth and stress response of juvenile turbot (*Scophthalmus maximus*) reared in land-based recirculating aquaculture system. **Acta Oceanol. Sin.** **36**, 31–38 (2017).
120. Saraiva, J. L., Rachinas-Lopes, P. & Arechavala-Lopez, P. Finding the “golden stocking density”: A balance between fish welfare and farmers’ perspectives. **Front. Vet. Sci.** **9**, 930221 (2022).
121. Wendelaar Bonga, S. E. The stress response in fish. **Physiol. Rev.** **77**, 591–625 (1997).
122. Lemos, L. S., Angarica, L. M., Hauser-Davis, R. A. & Quinete, N. Cortisol as a Stress Indicator in Fish: Sampling Methods, Analytical Techniques, and Organic Pollutant Exposure Assessments. **Int. J. Environ. Res. Public Health** **20**, 6237 (2023).
123. Pichavant, K. et al. Effects of hypoxia and subsequent recovery on turbot *Scophthalmus maximus*: hormonal changes and anaerobic metabolism. **Mar. Ecol. Prog. Ser.** **225**, 275–285 (2002).

124. Guo, T. *et al.* Accumulated CO₂ affects growth, acid-base regulation and ion balance of turbot (*Scophthalmus maximus*) in a recirculating aquaculture system. **Aquaculture** **578**, 740070 (2024).
125. Waring, C. P., Stagg, R. M. & Poxton, M. G. Physiological responses to handling in the turbot. **J. Fish Biol.** **48**, 161–173 (1996).
126. Li, J. *et al.* Phenethylamine Is a Potential Density Stress Pheromone in Turbot (*Scophthalmus maximus*). **Fishes** **8**, 506 (2023).
127. Fajardo, C. *et al.* Early Molecular Immune Responses of Turbot (*Scophthalmus maximus* L.) Following Infection with *Aeromonas salmonicida* subsp. *salmonicida*. **Int. J. Mol. Sci.** **24**, 12944 (2023).
128. Ronza, P. *et al.* Integrating Genomic and Morphological Approaches in Fish Pathology Research: The Case of Turbot (*Scophthalmus maximus*) Enteromyxosis. **Front. Genet.** **10**, (2019).
129. Kerambrun, E., Sanchez, W., Henry, F. & Amara, R. Are biochemical biomarker responses related to physiological performance of juvenile sea bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*) caged in a polluted harbour? **Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.** **154**, 187–195 (2011).
130. Lu, Y. *et al.* Insight into the heat resistance of fish via blood: Effects of heat stress on metabolism, oxidative stress and antioxidant response of olive flounder *Paralichthys olivaceus* and turbot *Scophthalmus maximus*. **Fish Shellfish Immunol.** **58**, 125–135 (2016).
131. Gesto, M., López-Patiño, M. A., Hernández, J., Soengas, J. L. & Míguez, J. M. The response of brain serotonergic and dopaminergic systems to an acute stressor in rainbow trout: a time course study. **J. Exp. Biol.** **216**, 4435–4442 (2013).
132. Gesto, M., López-Patiño, M. A., Hernández, J., Soengas, J. L. & Míguez, J. M. Gradation of the Stress Response in Rainbow Trout Exposed to Stressors of Different Severity: The Role of Brain Serotonergic and Dopaminergic Systems. **J. Neuroendocrinol.** **27**, 131–141 (2015).
133. Zhao, L., Sun, J. & Sun, L. The g-type lysozyme of *Scophthalmus maximus* has a broad substrate spectrum and is involved in the immune response against bacterial infection. **Fish Shellfish Immunol.** **30**, 630–637 (2011).
134. Fontenla Iglesias, F. Vacunación en rodaballo, *Scophthalmus maximus* (L.): análisis de la respuesta inmunitaria y desarrollo de nuevos adyuvantes. (Universidade de Santiago de Compostela, 2019).
135. Waring, C. P., Poxton, M. G. & Stagg, R. M. The physiological response of the turbot to multiple net confinements. **Aquac. Int.** **5**, 1–12 (1997).
136. European Food Safety Authority. Species-specific welfare aspects of the main systems of stunning and killing of farmed turbot - - 2009 - EFSA Journal - Wiley Online Library. (2009).
137. Míguez, J. M. *et al.* Efficient handling of turbot under industrial production conditions: how long air exposure triggers the stress response? **Poster Commun. Book Abstr. Aquac. Eur. Congr. AE2025** (2025).
138. Ashley, P. J. Fish welfare: Current issues in aquaculture. **Appl. Anim. Behav. Sci.** **104**, 199–235 (2007).
139. Villalba, A. M. *et al.* Seasonal comparison of uniform pre-slaughter fasting practices on stress response in rainbow trout (*Oncorhynchus mykiss*). **Aquaculture** **596**, 741750 (2025).
140. Ramírez-Rodríguez, C. M. *et al.* A new perspective on electrical stunning and live-chilling in the industrial slaughter of turbot: insights from electroencephalography. (2025).
141. Arechavala-Lopez, P., Cabrera-Álvarez, M. J., Maia, C. M. & Saraiva, J. L. Environmental enrichment in fish aquaculture: A review of fundamental and practical aspects. **Rev. Aquac.** **14**, 704–728 (2022).
142. Teixeira, D. S. R. Efeito do enriquecimento ambiental no sucesso reprodutivo e bem-estar animal nos reprodutores de pregado ((*Scophthalmus maximus* *Scophthalmus maximus* L.). <https://repositorio-aberto.up.pt/handle/10216/164739> (2024).
143. Fernández, B. & Brians, M. J. Cultivo de peixes planos en tanques de moluscos aplicando técnicas de enriquecimento ambiental. **Foro Rec Mar Ac Rías Gal** **26**, (2025).
144. Teletchea, F. & Fontaine, P. Levels of domestication in fish: implications for the sustainable future of aquaculture. **Fish Fish.** **15**, 181–195 (2012).
145. Huntingford, F. A., Kadri, S. & Saraiva, J. L. Welfare of cage cultured fish under climate change. in **Climate Change on Diseases and Disorders of Finfish in Cage Culture, 3rd edition** (CABI Publishing, 2023).

8. APPENDIX

Table 5: ESSENTIAL indicators of well-being and **RECOMMENDED** for turbot, taking into account the different procedures and phases of production.
 *Note: This selection of indicators was carried out using the **DELPHI** method, with the participation of producers, scientists, and members of animal protection associations, all experts in turbot production and/or welfare.

TURBOT		BREEDERS							HATCHERIES						GROW-OUT				
Categories	Indicators	Environment and confinement	Handling and maintenance	Feeding	Animal health	Transport	Reproduction	Slaughter	Environment and confinement	Handling and maintenance	Feeding	Animal health	Transport	Slaughter	Environment and confinement	Handling and maintenance	Feeding	Animal health	Stunning and Slaughter
Physico-somatic or external	Condition factor (K)																		
	Population dispersion																		
	Skin condition																		
	Skin coloration																		
	Malformations																		
	Fin condition																		
	Gil condition																		
	Eye condition																		
	Mortality																		
Behavioral	Activity and distribution																		
	Appetite and feeding behavior																		
	Abnormal movements																		
	Aggressiveness																		
	Ventilatory frequency																		
	VOR																		
Environmental	Temperature																		
	Salinity																		
	DO																		
	CO ₂																		
	pH																		
	Nitrogen compounds																		
	Turbidity/Suspended solids																		
	Water Flow																		
	Lighting																		
	Stocking density																		

