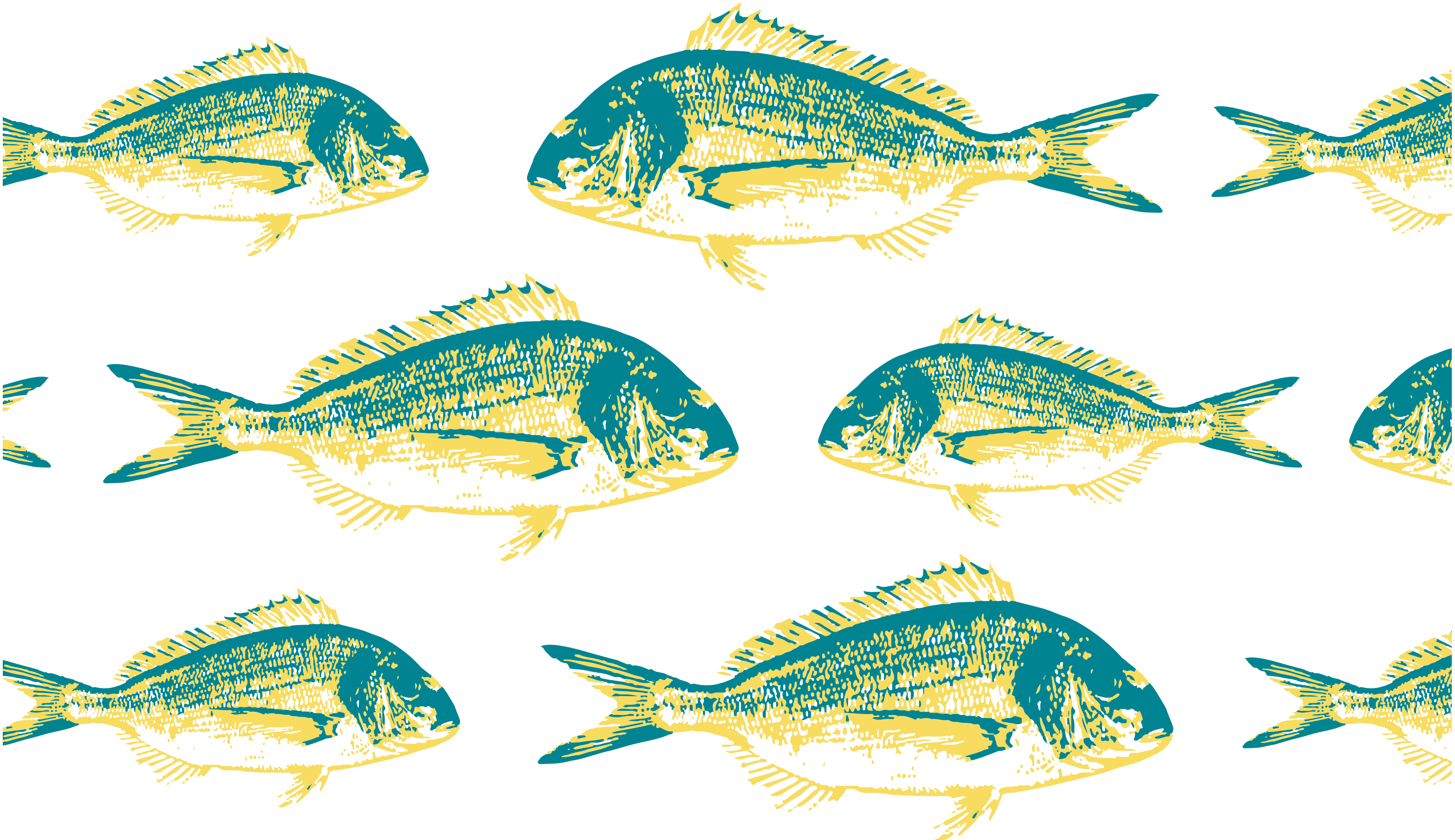
A GUIDE ON FISH WELFARE IN SPANISH AQUACULTURE

(Vol. 3): Welfare of gilthead sea bream



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SCOPE OF THE GUIDE

In 2022, **APROMAR** published an initial guide on fish welfare providing information on aquaculture activity in Spain regarding fish welfare. The guide dealt with fundamental issues and generalities, establishing concepts and common principles, as well as developing the first fish welfare guidelines in concurrence with organizations beyond the sector. The guide came into being from an **APROMAR** initiative, and its text resulted from a collaborative and participatory exercise between **APROMAR** member companies together with several animal welfare and protection associations and organizations (Animal Welfare Observatory, Compassion in World Farming International, and FishEthoGroup association), Spanish universities (*Universidad Autónoma de Barcelona*, *Universidad Politécnica de Madrid*, *Universidad de Cádiz*, and *Universidad Complutense de Madrid*), public research organizations (Mediterranean Institute of Advanced Studies-CSIC) and the Ministry of Agriculture, Fisheries, and Food. That document had been conceived as the first volume of a collection that would be specifically devoted to each of the fish species in Spanish aquaculture.

This is the second of the guides by species, published in the wake of another dedicated to the welfare of sea bass (*Dicentrarchus labrax*). The present one focuses on gilthead sea bream (*Sparus aurata*). It identifies a series of welfare indicators, critical points for welfare in production systems, and a wealth of good practices in aquaculture. In addition, training and communication proposals are offered, together with an analysis of the challenges and opportunities in this area. The different organizations that developed the contents of this guide have agreed that it should necessarily be considered a living document in which new advances in scientific knowledge and technological developments will lead, in the coming years, to inevitable revisions and updates to ensure the best provision of recommendations and practices at the forefront of farmed fish welfare.

The guide is especially aimed at aquaculture companies in Spain and all people working in the sector, whether in direct contact with fish or in work organization or management tasks. It will also be of interest to public administrations and legislators, as well as to the scientific, technological, and educational communities, and society in general.

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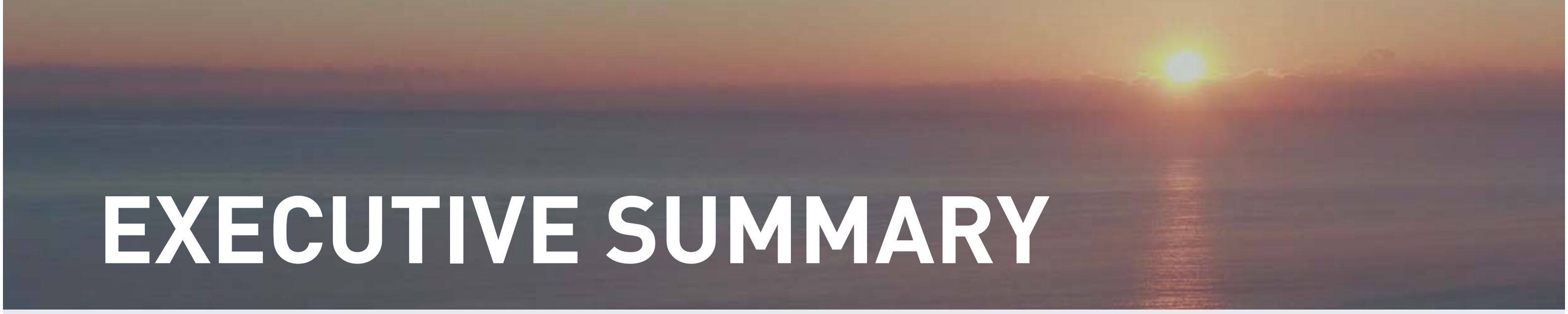


EXECUTIVE SUMMARY

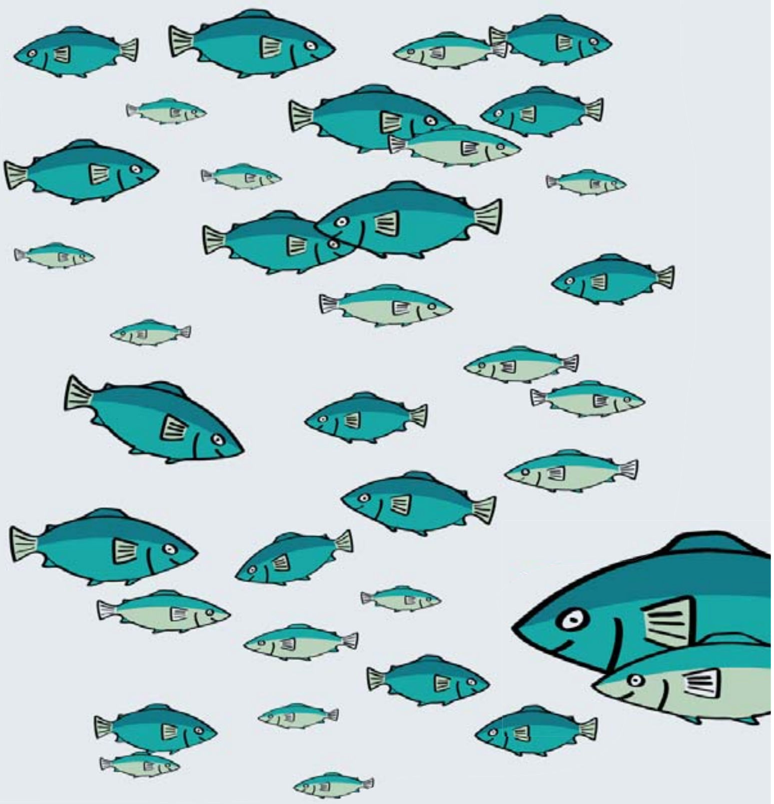
The Guide on Fish Welfare in Spanish Aquaculture - Concepts and Generalities (Vol. 1), was launched in 2022, marking the start of a collection aimed at compiling best practices in animal welfare, especially those related to fish raised in Spanish farms. After a second volume on the welfare of seabass, the **third volume** of this series focuses on gilthead sea bream (*Sparus aurata*), a species native to the Atlantic Ocean and the Mediterranean Sea and one of the most widely farmed in Spain.

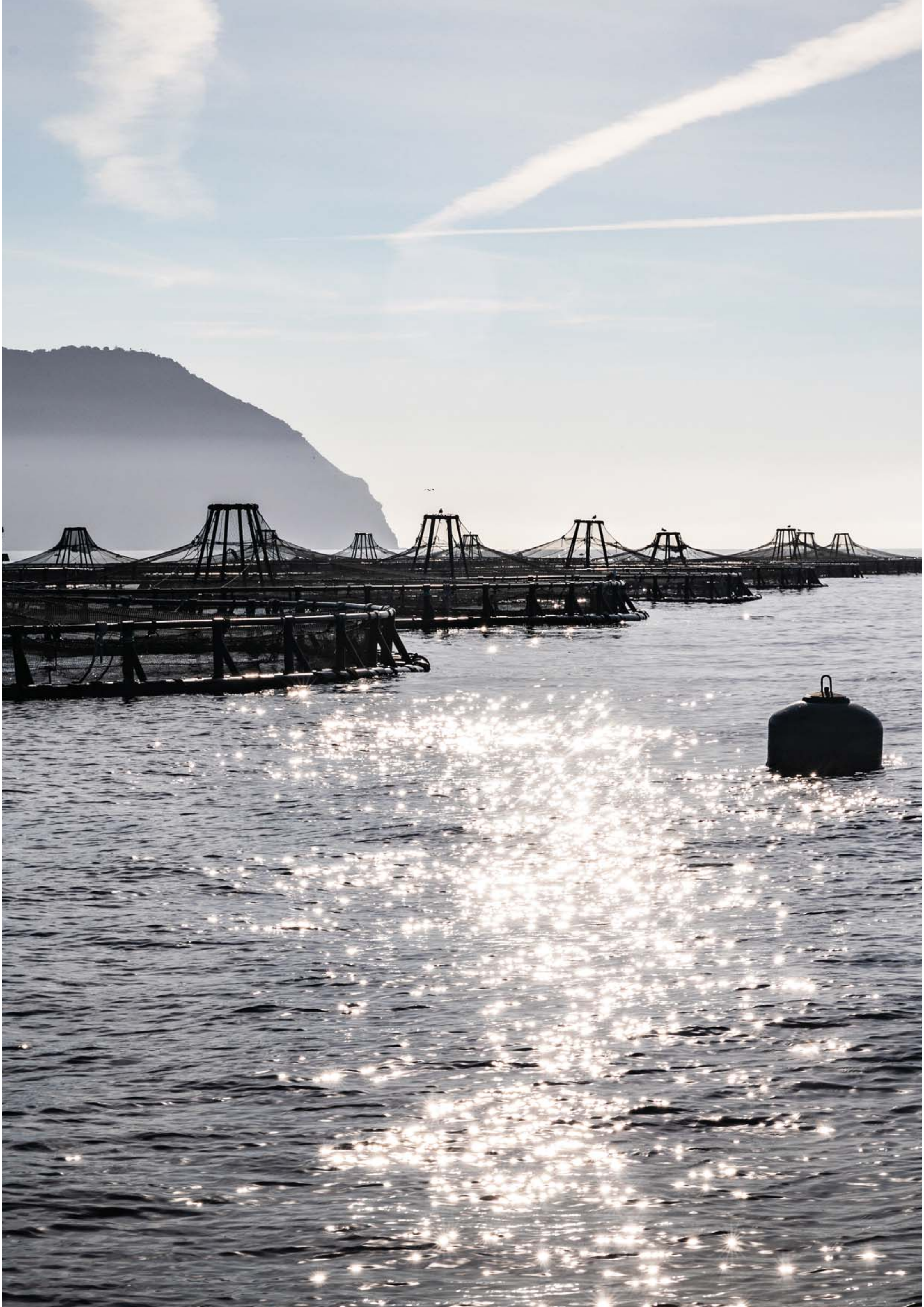
Sea bream production reached 8,932 tons in 2022, representing about 15.2% of Spanish fish production, with a market value of 43.7 million euros, consolidating Spain as the world's fifth largest producer of sea bream. [1] Funded with national and European resources, the Guide on welfare of sea bream is the result of collaboration between several key players in the Spanish aquaculture sector, including MAPA, APROMAR, scientific experts in animal welfare, production companies, and animal protection organizations (NGOs).

This guide addresses areas ranging from biological characteristics of sea bream to operational welfare indicators, critical points in different farming systems, developmental stages, good farming practices, personnel training, communication, and new challenges. This is the first time a single document has synthesized the latest scientific knowledge on sea bream welfare, providing specific, quantifiable, and adaptable indicators for different life cycle stages and production systems. The guide also highlights the intrinsic relationship between animal welfare and good practices in aquaculture, offering tools to evaluate how effective their implementation is in areas such as feeding, environment and confinement; stunning and slaughter; handling and management; reproduction, animal health, and transport.

The guide acknowledges the importance of continuous personnel and management training in production companies, as a skilled workforce positively impacts the welfare of captive fish. It is presented as an educational and informative tool, highlighting the need to update training and include specific sections addressing welfare concepts, assessment methods, and issues related to captive fish production.

Finally, it highlights that the sustainable and responsible development of Spanish aquaculture faces several challenges, one of the most significant being to ensure the welfare of the fish raised in this industry. The last chapter of the guide lists essential factors, such as the application of humane slaughter techniques, consideration of climate change, intersectoral collaboration, research, science, and technology, as well as the need for a binding legal framework that adequately protects the welfare of aquatic animals.





1. INTRODUCTION

The gilthead sea bream (*Sparus aurata*), belongs to the Sparidae family. It is an emblematic fish in the Spanish and Mediterranean gastronomy, from the British Isles to Cape Verde and throughout the Mediterranean Sea. It is a coastal species, encountered in varied bottoms, mainly sandy or rocky and seagrass meadows. They tend to be solitary, although they occasionally swim in small schools, the youngest individuals being observed in shallow coastal waters, reaching up to 30 m depth while the adults inhabit deeper waters, around 50 m.

Its common name comes from the characteristic golden stripe between its two eyes. The fish has a smooth, dark spot at the level of the operculum, and a high, oval body, compressed on both sides, its dorsal line presenting a marked convexity. It measures up to 70 centimeters long and has a silvery coloration, with a green, gray, or bluish back. Although exceptional, some adults have been observed to reach a length of up to 1 m and a weight between 5 and 7 kg. It has a single elongated, dorsal fin with 11 spiny rays in the anterior part and 13 or 14 soft rays. The pectoral fins are long and pointed. The caudal fin, covered by a black band, separates into two portions by a slight depression. The anal fin has three spiny and 13 or 14 soft rays. The head is large and rounded and the lips are thick. The first row of teeth comprises pointed structures attached to strong jaws, followed by multiple molar teeth.

Sea bream is essentially carnivorous. Their strong teeth allow them to feed on bivalve mollusks (clams, oysters, mussels, etc.), gastropods and crustaceans, which they crush before eating. They can also feed on small fish, cephalopods, and occasionally even algae and marine phanerogams. Sea bream can withstand large variations in salinity (euryhaline species), tolerating and preferring brackish water, so it is common to find them in coastal lagoons, estuaries, or river mouths and streams. They are sensitive, however, to sudden changes in water temperature, so in winter, they move into deeper waters to reproduce. It is a protandrous hermaphroditic species; that is, they are males at birth until they reach approximately two years of age, and from three years old, they become mature females. Some individuals act as males their entire lives. Besides age, size also has a bearing on it, although they usually go hand in hand. From 600 g onward, males typically change sex to females.

Sea bream is one of the most appreciated and studied species in aquaculture, and today, one of the species with the highest production throughout the Mediterranean (mainly Türkiye, Greece, Egypt, Tunisia, Spain, Italy, Cyprus, and Croatia). The total aquaculture production of sea bream in Europe and the rest of the Mediterranean Arc for 2022 was 320,630 tons or 1.8% higher than the previous year. [1] The production of farmed sea bream in Spain reached 8,932 tons in 2022, with the Valencian Community leading it with 5,620 t (63% of the total), followed by the Murcia Region with 1,327 t, (15% of the total), Andalusia (815 t, 9%), the Canary Islands (790 t, 9%), and Catalonia, with 380 t (4% of the total). [1]

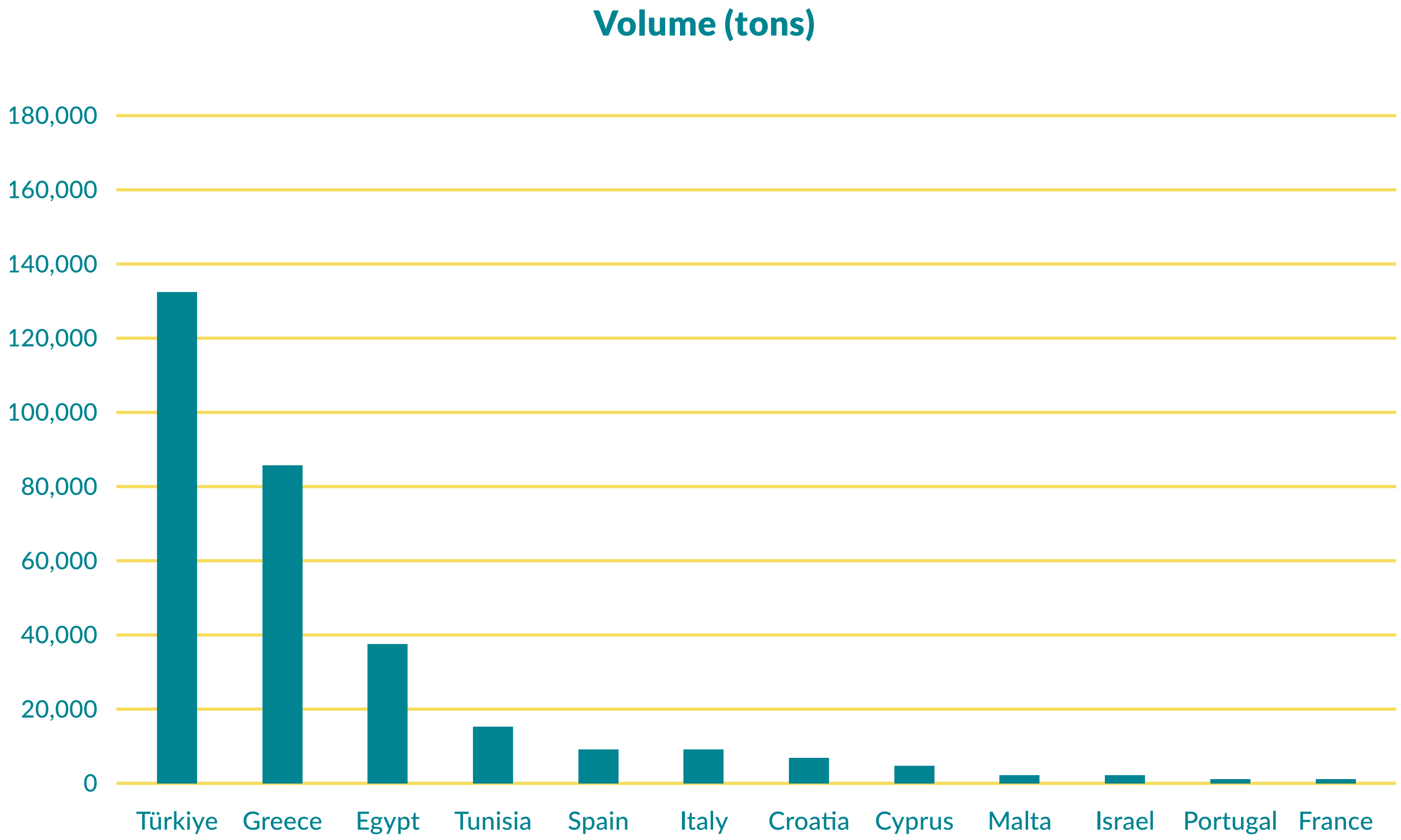
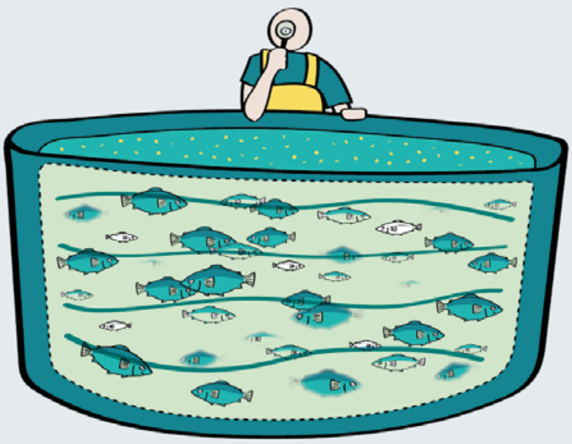


Figure 1. Distribution of sea bream production in different countries per volume (tons) (source: APROMAR 2023[1])

2. WELFARE INDICATORS FOR GILTHEAD SEA BREAM

Aquaculture farming systems present specific challenges for fish welfare due to their different biological needs and capacities to cope with pain or stress, which may vary between individuals and species throughout the life cycle. Welfare indicators, therefore, need to be specific, allowing reliable and objective assessment of the welfare status of the species, its size, and its production systems. They are tools that allow for both the evaluation of the procedures carried out in the company and the adoption of the necessary measures to promote welfare of fishes. However, depending on the characteristics of the farming system and other circumstances, some indicators are more applicable and relevant than others. Possible welfare indicators for sea bream are described below:



2.1. External or physical-somatic indicators

Known as direct indicators based on the appearance of the animal, they provide information on the body condition or external appearance of the fish. The most used in sea bream are:

Condition factor (*K*)

It is an index that relates the length to the weight of the fish, $K = (\text{body weight (g)}/\text{length (cm)}^3) \cdot 100$. It allows evaluation of the body's condition and development, reflecting its nutritional status and welfare. A very high K value means the fish is overfed, with excess abdominal fat and lipid content. Conversely, if the value is low, it will indicate that the animal may be thin, thus experiencing severe malnutrition. It is worth mentioning that the values of this index may be influenced by genetic factors, seasonality, and its respective water temperature, since sea bream may come from a growth phase (end of summer) or from lethargy (end of winter), as well as by the feeding strategy (amount of feed supplied or ingested), life stage of the fish (age), and the production system. In general terms, K values between 1 and 1.5 are interpreted as optimal for the growth and welfare of gilthead sea bream. [2–4]

Population dispersion

It is important to monitor the size dispersion in each stock, either by analyzing its distribution or the coefficient of variation (CV) [5]. Among the possible causes of a high dispersion in a batch of sea bream, we can find: a) genetics, which governs the differential growth of each individual in the population; b) the production strategy applied through previous classifications or continuous breeding; and c) a correct feeding, insofar as it can reduce aggressiveness and competition among fish, especially in the early stages of development. [5–7]

Skin condition

The skin is the outermost covering or envelope of the body and is therefore the part most exposed to the environment, in continuous contact with microorganisms and physicochemical changes in the water. In fish, the skin is well adapted against injury and pathogens, also serving for respiration, excretion, and osmoregulation. It can be affected by nutritional deficits or water quality, as well as by various procedures during production, especially those involving physical contact with the animal (e.g. handling, harvesting, etc.). To determine the skin condition, one must check the state and integrity of the scales, mucosity, and whether there is a loss of epidermal tissue compromising deeper layers: dermal/subdermal/muscular. Additionally, it is important to determine whether there are any active lesions such as ulcers, hemorrhages, or superficial wounds, [8,9] as they can be rapidly affected by bacteria found in the marine environment.

Skin coloration

Changes in sea bream skin pigmentation may be due to infection or disease, diet composition, or social or environmental stress conditions. [10–15] Under stress, hormones cause a reorganization of the chromatophores of the skin, so that the coloration can be affected and exhibit important changes, serving as an effective and quick indicator of the state of the animal at that precise moment. Alterations in the welfare of gilthead sea bream would be reflected with changes in their natural coloration, with darker or duller patterns. For example, stressful situations or excitements induce a darkening of the skin on sea bream. [16]

Physical malformations

In sea bream, it can be observed cases of malformations of the opercula, jaws, or dorsal spine, among others (see [17–30]). Although the etiology of the malformations is not entirely clear in some cases, the following have been identified as possible causes: genetic predisposition, nutritional deficiencies, water quality, current velocity, egg incubation temperature, infectious diseases, sources of stress in early stages, etc. Generally, they start to be observed in the fry and juvenile stages and may remain until adulthood in case of improper purification in previous stages.

Fins condition

Assessing fin condition can be determined by whether they are eroded, thickened, malformed, split, or hemorrhagic, as these are direct causes of rearing conditions, e.g. excessively high densities, strong currents, recent improper handling, disease, or increased aggressiveness. Thus, the loss of integrity in their epidermal barrier may constitute the gateway for various pathogens present in the water, thus triggering a systemic disease in the fish. The condition of a fish's fins can indicate the presence of viruses and pathogens [31–37] and reflect the overall well-being of the fish.

Gills condition

The gills have several functions related to respiration, osmoregulation, ion exchange, and immune defense, and are susceptible to alterations. For example, a variation in water quality parameters will influence the health and condition of the gills. This will affect oxygen uptake, especially in conditions where metabolic demand increases, such as during exercise and osmoregulation, which is important for excretion and homeostasis. In case of pathogens in the water, the gills will come into direct contact with them. Diagnosis can be made by direct observation since, in some circumstances, parasites, lesions, or abnormal coloration patterns can be distinguished in gilthead sea bream. [38–41]

Eyes condition

In aquaculture, the most frequent pathology in the eyeball corresponds to mechanical trauma derived from handling, [42] followed by exophthalmia, cataract, rupture, and hemorrhage. [43] Some ocular alterations may be due to nutritional deficiencies, diseases, or inadequate lighting. [44] Visually impaired fish tend to have decreased foraging and defense abilities, usually ending up with total vision loss. In the case of sea bream, we can find individuals with eye lesions, cataracts, exophthalmia, blindness, or viral or bacterial infections. [45–47]

Mortality

The percentage of mortality indicates the number of fish dead as a function of the total population in a short period (e.g. one day). In the case of sea bream, it is possible to observe mortality episodes that follow different patterns, from hyperacute to chronic. It is necessary to investigate and categorize the possible causes, which can be due to diseases, predators, storms, or other adverse environmental conditions. [36,48–50] Cumulative mortality, over a given time interval, is a retrospective indicator. It can help observe trends that negatively impact the health and welfare of fish and that, if not corrected in time, will continue to recur. Daily mortality should be recorded as a function of the total population and production stage. [8] As in the case of sea bass, [51] a rate higher than 2% per month in the fattening stage in sea bream may constitute a warning criterion and require a thorough evaluation of the possible causes and remedies to be implemented, which can be increased in earlier stages such as hatchery or nursery.


2.2. Behavioral indicators

Observing and understanding fish behavior is crucial to improving welfare, reducing stress response to daily husbandry practices, and optimizing farm performance. Behavioral alterations, specifically swimming, have been used to highlight possible welfare-related variations due to stress or disease. However, this must be established and analyzed based on the species and situations under study. [52] The following operational indicators provide information on the behavioral pattern at the individual and group level, forming part of the direct animal-based indicators. They are detailed below:

Swimming activity and distribution

In general terms, distribution and swimming speed indicate how fish react to the surrounding environment. Observing them as a group can help guide the diagnosis of animal welfare. [53] The way sea bream use space, shift speed and direction (accelerations), in tandem with group structure and cohesion, provides useful information about their social and environmental relationships and state of health. [54–57] Different environmental gradients may occur during rearing, such as changes in temperatures, currents, or light conditions. [58,59] This can make fish seek out more favorable areas and move away from others, affecting their distribution. In addition, acute stress events trigger avoidance behaviors, which can be observed and related to their distribution on the bottom of cages and tanks. [60] Predators in the environment also affect swimming activity and distribution. Sea bream with good environmental and welfare conditions are expected to observe a wide and uniform distribution of individuals, making extensive use of space and maximizing the distance between them, with synchronous or asynchronous swimming and normal average speed for the species.

Surface activity

In most cases, surface activity is related to the animals' interest in food (e.g. frenetic activity), promoted by appetite or competition for the food resource (*see indicators of feeding and anticipation*), so a decrease in such activity prior to feeding may indicate some affection to their welfare. Conversely, an increase may be related to critical situations for fish welfare, when not related to feeding. For example, in situations of hypoxia or poor water quality, "gasping" may be observed where fishes burst the surface with their mouths, and individuals in poor health may show lethargic behavior, loss of balance, or reduced maneuverability, sometimes with fins sticking out of the water. [53,61]

Abnormal movements

Abnormal behaviors called stereotypies are defined as "repetitive and invariable behavioral patterns having no obvious purpose or function," [62] which in many animal species has led to construe this occurrence as a response to a lack of stimuli and/or loss of interest experienced by animals in captivity. In the case of sea bream, stereotyped behaviors can be observed, as well as ono-desirable behaviors such as erratic movements, lethargy or immobilization, thigmotaxis (a tendency to stay on the periphery or to move along the walls or nets), scototaxis (preference for shaded areas), or apathy [8,54,63–65]. If welfare conditions are optimal, no abnormal behaviors should be observed.

Aggressiveness

Behaviors such as attacks and bites are considered aggressive or agonistic behaviors of fish [53] in response to inadequate captive conditions or welfare deficiencies. These behaviors may be exhibited in varying proportions and affect other fish. Generally, these agonistic or aggressive behaviors in sea bream are the result of genetic selection or social interactions (hierarchies), competing for resources (or lack thereof) such as food or space. [14,34,34,54,66–68] In addition, they can be enhanced if there are differences in size or shape between individuals of the same group, a fact that is usually more evident in the early stages of development. It should be noted that an indirect way of assessing these behaviors can be by direct observation of darker skin coloration patterns, or by counting wounds, scars or signs of bites on the skin (*see external indicators: skin coloration and condition.*).

Appetite and feeding behavior

The amount of food consumed when the fish has the opportunity to do so is a behavior influenced by various factors such as energy reserves and gastric emptying, seasonal adaptations and water temperature, health status, or stress level. [69,70] Likewise, the frequency of feeding, the amount of food, or its physical and organoleptic characteristics (e.g. composition and palatability) influence the availability and appetite of sea bream and thus its feeding behavior. [34,69,71–74] Qualitatively, the appetite of sea bream can be measured by direct observation when the food is offered, [8] i.e., based on the demand for food according to the quantities estimated for its optimal development. In this case, it is convenient to combine it with other indicators, such as the condition factor (K) or surface activity.

Anticipatory behavior

When animals are subjected to a programmed feeding schedule, they generally synchronize their locomotor and feeding behavior with feeding times, showing anticipatory activity several minutes or hours before feeding time. [75] On numerous occasions, the smell of feed, the presence of humans, or the sound of the engines from feeding boats may sufficiently trigger this learning behavior. Therefore, this behavior is a good indicator of both welfare and feeding strategy. [75] An emotional state of restlessness, coloration changes, agitation, and frenetic activity, with forays to the water surface, can be observed in sea bream. [76–79]

Ventilatory frequency

It is defined as the number of times the operculum opens and closes in one minute. Alterations such as increased frequency caused by greater oxygen demand may be related to low oxygen saturation, increased metabolism, decreased water quality, physical exercise, functional gill damage, or stress conditions. [80,81] A direct way to account for this is by counting the opercular movement of the fish, widely used to monitor the effects of sedatives and anesthetics in fish. [82]

Vestibulo-ocular reflex

In living beings, the assessment of consciousness is typically conducted through a series of involuntary movements. For instance, in fish, the corneal reflex has been linked to brain activity in vertebrates. [83] Studies have established that the vestibulo-ocular reflex (VOR), which involves a horizontal displacement of the eyes when an individual moves along their longitudinal axis, can serve as a similar indicator. This reflex is one of the simplest movements to monitor, and its absence indicates loss of consciousness, as seen when fish are anesthetized or stunned. [84,85] However, before reaching VOR loss, other indicators of progressing unconsciousness may manifest, including impaired balance, lack of response to tactile stimulation, and alterations or cessation of respiratory patterns (opercular movement).

2.3. Environmental indicators

These parameters, related to water and external factors, are used to obtain information about the environment where the fish are found, which can influence their welfare. They are detailed below:

Water temperature

Fish are poikilotherms, their metabolism being directly influenced by water temperature and its fluctuations. Any temperature change will directly impact their metabolic function and consequently, their welfare, especially if it occurs abruptly. Therefore, it is necessary to consider and monitor the optimal range in which the fish will be free from physiological alterations affecting their growth and welfare. [43] Sea bream is not a species particularly sensitive to low temperatures. Its lower temperature limit is around 5 °C, [86] and its tolerance range is between 11 °C and 28 °C, being able to tolerate up to 30 °C. [87–91] However, this tolerance may vary depending on the stage of development of the fish, and the seasonality, geographical area, type, and location of the rearing facilities. For example, the optimum temperature for breeders is between 14 and 20 °C. [92] For the hatching of eggs and the development of gilthead sea bream larvae, the temperature ranges between 14 and 26 °C, the optimum being around 19 - 20 °C. [92–95] The optimal temperature for growth is between 25 and 30 °C. [95,96] Besides, sudden temperature changes should be avoided, keeping them below 5 °C/day. [97]

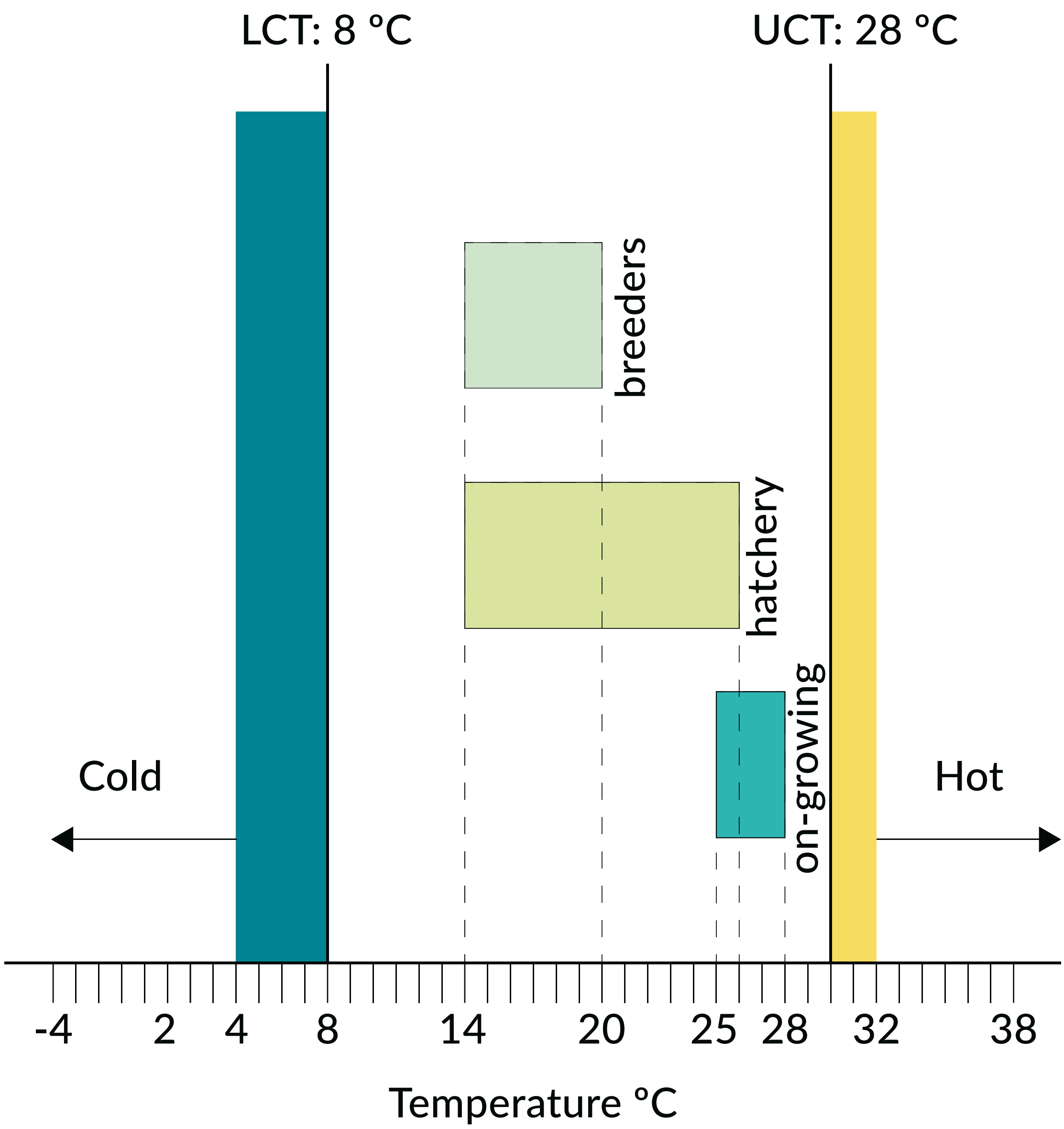
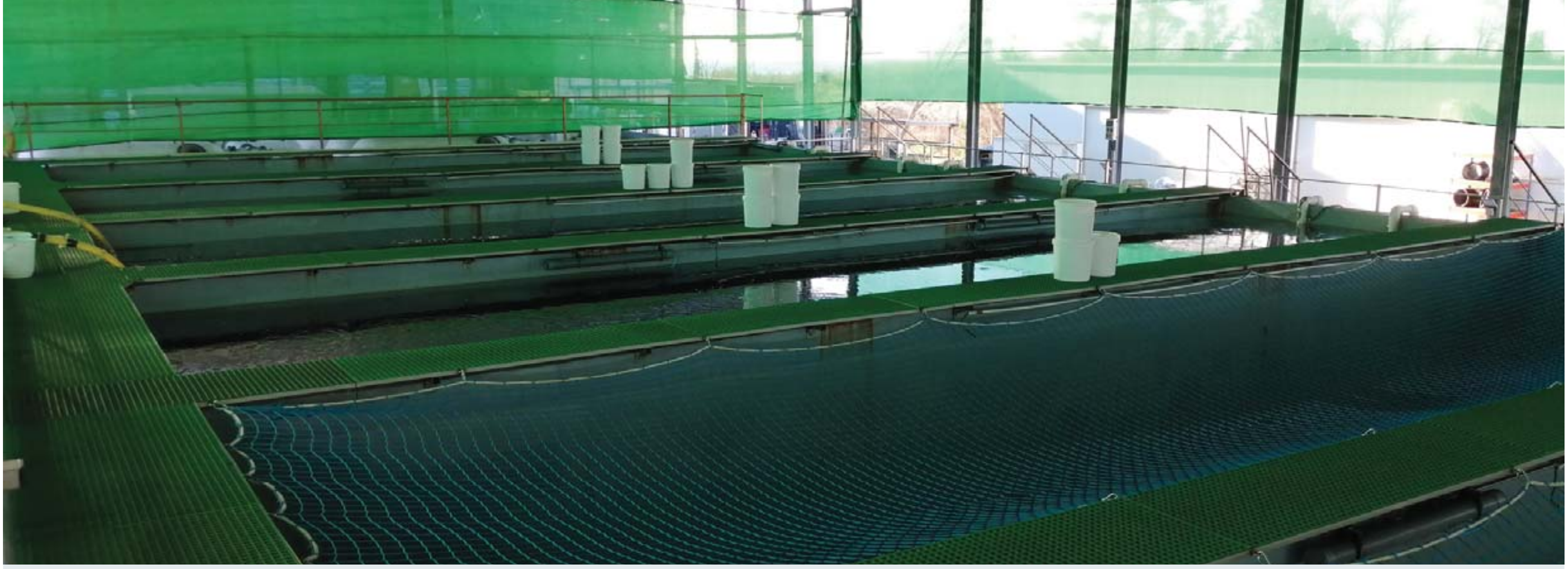


Figure 2. Graph of the thermo-neutral zone of the gilthead sea bream, with the optimal range in different developmental stages, the lower critical temperature (LCT), and the upper critical temperature (UCT).



Salinity

Salinity indicates the amount of salt or ions dissolved in the water. Sea bream is a euryhaline species, i.e., it tolerates a wide range of salinity in the water, being able to cope with extreme environmental salt variation and adapt to environments of different salinity. [98] It can inhabit from very little brackish water (>3‰) to seawater (38-40‰), and high salinity in extreme cases (<60‰). [99,100] Generally speaking, its optimum range is around that of seawater, but in the early stages of development it may be wider than that of adults, as fry adapt more easily to these changes (especially to brackish water) [101,102] However, the physiological adaptations of sea bream to different salinities depend on temperature, so both indicators should be evaluated together. [103]

Dissolved Oxygen

Dissolved oxygen (DO) levels in water serve as a critical indicator of water quality and fish welfare. The concentration of dissolved oxygen can be affected by various factors and stressors. For instance, situations that trigger heightened stress responses, such as high population densities typical of intensive rearing or routine handling, can elevate oxygen consumption, thereby reducing its concentration in each area or timeframe. Furthermore, as temperature rises, so does the demand for oxygen, underscoring the importance of monitoring both parameters concurrently. [104,105] Particularly noteworthy are cases involving land-based production ponds, where, due to the presence of algae or phytoplankton, nighttime oxygen consumption may reach harmful levels. In the case of sea bream, the optimal DO concentration should ideally approach saturation or, at the very least, remain above 70%, [106] never dipping below 40%. [97] It is pertinent to mention that sea bream appears to exhibit lower susceptibility to low oxygen conditions as compared to sea bass. [106]

Carbon dioxide (CO_2)

Carbon dioxide (CO_2) dissolved in water is a by-product of animal and plant respiration, as well as the combination of hydrocarbons. In aquaculture, CO_2 from fish respiration, especially under high-density rearing or recirculation (RAS) conditions, lowers water pH, favoring the toxicity of other metals or alterations in the microbiology of the water. There is a direct correlation between the concentration of CO_2 in water and in blood, which can lead to a situation of hypercapnia that reduces the hemoglobin capacity to transport oxygen in sea bream. Its concentration in water is highly dependent on pH, salinity, and temperature of the environment. Throughout the production process, there are several procedures and situations where oxygen consumption by the fish can become greater, increasing the concentration of CO_2. Acute and chronic exposure above 25 mm Hg PCO2 (> 30 mg/l CO_2) is stressful and can negatively affect the growth and welfare of sea bream. [107]

pH

It is a measure of acidity or alkalinity of water, highly dependent on temperature and CO_2 concentration in the environment. In general, sea bream has a wide range of tolerance to pH, espe-

cially in larval stages (pH: approx. 5-9) [108], but it is recommended not to exceed the pH thresholds of 6.5 (lower) and 8.5 (upper). [97]

Total ammoniacal nitrogen

Ammonia (NH_3) results from protein catabolism, and in combination with water, forms the ammonium ion (NH4+). With respect to total ammoniacal nitrogen (TAN), it represents the sum of both ions. Ammonia is neurotoxic and also affects the osmoregulation and functionality of the gills, which can cause problems with respiration, metabolic regulation, feeding, and growth in gilthead sea bream.12, [109–112] For example, TAN levels of 5.93 mgL-1 at 10 ppt, 11.72 mgL-1 at 20 ppt, and 19.38 mgL-1 at 30 ppt have been determined to be lethal. [113] According to EFSA (2008) [97], a UIA-N concentration of 0.26 mg L-1 can be considered a safe long-term limit for juvenile sea bream in seawater. TAN is not a welfare problem for sea bream in offshore growing net-pens, since it is generally diluted to non-limiting levels by currents. [97]

Nitrites and nitrates

Both ions have the potential to alter respiration in fish. For example, nitrites (NO_2-) can bind to hemoglobin transforming it into methemoglobin, decreasing its ability to transport oxygen. [114,115] However, nitrite toxicity will be mediated by the interaction of factors such as fish size and species, duration of exposure, and water quality. [116] Nitrate (NO_3-), is the final product of the nitrification chain, resulting from the oxidation of nitrite. Although it presents adverse effects similar to those of nitrite, [117] its impact on fish toxicity and welfare is usually lower. [118] However, good water recirculation and oxygenation avoids high concentrations of nitrate and nitrite in the medium, to which sea bream is quite resistant, also depending on the salinity (the lower the salinity, the higher the toxicity). At normal salinities of marine water, levels below 30 mg/L of nitrites and 150 mg/L of nitrates do not present toxicity problems.

Turbidity and Total Suspended Solids

Turbidity refers to the degree of water transparency influenced by the presence of diluted compounds and suspended particles, whereas total suspended solids (TSS) encompass both organic and inorganic materials with a diameter exceeding 1 micrometer in a given volume. [119] The properties of suspended materials are significant as they can elevate biological oxygen demand and, in the case of small abrasive particles, inflict damage to gill tissue, compromising oxygen transfer and rendering fish more susceptible to secondary infections by microorganisms. [43] Furthermore, elevated levels of suspended solids in water volumes hinder animal observation and, consequently, diagnosis. [120] These parameters are critical for assessing water quality in sea bream farms, particularly in closed circulation systems, where optimal conditions mandate low turbidity with low TSS levels (preferably zero or <10-20 mg/L). [121,122]

Water current velocity

It is usually measured in terms of body length (BL) and expressed in body length per second (BL/s).

Current velocity can positively or negatively influence the fish. For example, an optimal speed will allow good water renewal and physical exercise. Conversely, if the current is extremely strong, floating structures and net-pens may be altered and individuals may exhibit signs of fatigue and difficulty in maintaining their position, to the point of exhaustion. Excessive current may even induce deformation of the structure in the case of floating net-pens. [123] However, if the velocity is low, the water exchange will not be sufficient, thus reducing the quality and impacting the welfare of the fish. [124] Sea breams have been reported to maintain speeds of up to 5 BL s-1 (Ucrit), their optimum velocity (Uopt) being between 2.5 and 4.5 BL/s, although it depends on their stage of development. [55,125–128]

Lighting

The quality of lighting can be determined based on two main parameters: light intensity and photoperiod. Light intensity refers to the amount of light emitted by a specific source towards a particular direction. Photoperiod refers to the duration of light/dark hours to which fish are exposed and influences their activity and circadian biological rhythms. Different measures are often used, but the most used is Lux (lx), understood as the amount of light illuminating an area of 1 m². If high intensity is used, light has the capacity to cause direct damage to the fish's retina. [129] Conversely, low intensity light can reduce or inhibit their activity. Therefore, lighting is vital for inducing spawning, reproduction, and growth. However, it is not possible to regulate the photoperiod during the rearing phase carried out in ponds or off-shore facilities, although sometimes its intensity can be regulated through shading. The natural day:night phases of the natural species' distribution area must always be respected to avoid affecting their chronobiology. [130] Under ideal conditions for sea bream, the natural photoperiod should be maintained during fattening, with relevant seasonal variations and a light intensity between 150 and 500 lux (500-1,000 lux in larvae). [92]

Population density

Known as the fish biomass per unit volume of water (kg/m³). the volume occupied by fish varies depending on the stage and duration of the production process, rearing system, water temperature, size of the individual, and species. Problems associated with density typically arise from lack of space, leading to increased physical contact between fish, as well as factors such as water quality and difficulty in accessing food. It is recommended to use other operational indicators to assess the impact of density on fish welfare. [132] Under intensive sea bream farming conditions, densities at the end of each cycle are typically around 15-20 kg/m³ in on-growing sea-cages, or close to 10 kg/m³ in broodstock. It is important to reinforce evaluations of these operational indicators if these density thresholds are exceeded. The calculation of density is not always precise, and production data indicates that density limits may be exceeded in the final stages of intensive cycles. In such cases, it is essential to ensure sea bream welfare by utilizing additional indicators if these limits are surpassed.

2.4. Laboratory indicators

Stress hormones and metabolic indicators

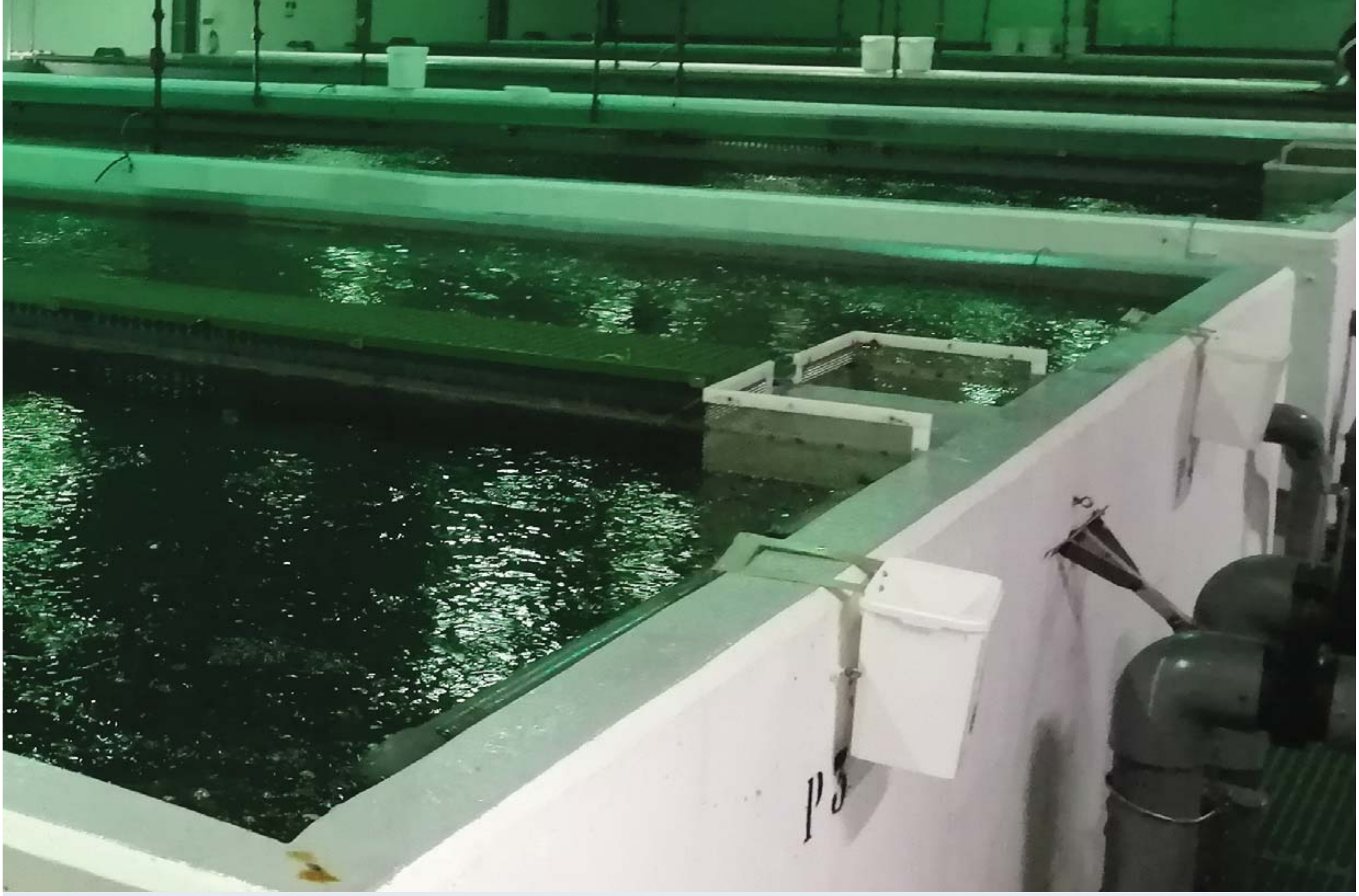
Cortisol is one of the hormones released after any stressful situation (physical, environmental, social). It multiplies its concentration in physiological fluids by a factor of 5 to 100, being a good indicator of acute stress and lack of well-being, although not so much of chronic stress, since its regulation reduces this increase substantially. In terms of experimentation, the measurement of cortisol in plasma is carried out by means of ELISA or Enzyme-Linked Immunosorbent Assay kits, which requires a specific laboratory in addition to blood collection. Cortisol is produced in the cephalic kidney of the fish and transferred to the blood, so it is found in plasma and other structures such as skin mucus and urine. It can even be deposited in the scales of the fish in case of chronic stress, to finally be excreted to the external water. Sea bream presents its optimum levels below 10ng/mL, acute stress increases these levels up to 80-100 ng/mL, and moderate chronic stress ranges between 20-40 ng/mL.

In aquaculture research, the cortisol concentration in water is most interesting since it is a non-invasive method. Its feasibility and correlation with blood cortisol have been demonstrated in recirculating aquaculture systems (RAS), and in some cases in on-growing cages (at the center, with medium-high densities), with values lower than plasma cortisol. Other stress hormones such as adrenaline, corticotropin-releasing hormone (CRH), and adrenocorticotropic hormone (ACTH) can also be indicators, although the difficulty to measure (CRH, ACTH) and, especially, speed of secretion (adrenaline) prevent their use in practice.

Other physiological indicators relevant to this field, pertaining to stress and monitorable, are those that include metabolic analysis, especially blood glucose and lactate levels, which typically rise in plasma following stress. These analyses require a small blood sample, followed by centrifugation, and examination using specialized commercial kits. Plasma samples can be stored frozen for future reference. Additionally, hematological analyses such as hematocrit, red blood cell count, and hemoglobin concentration, provide insight into oxygen uptake and transport capacity from the gills to the tissues. They also require a fresh blood sample and immediate processing with commercial kits for hemoglobin determination, centrifugation for hematocrit assessment, and microscopic examination for red blood cell count.

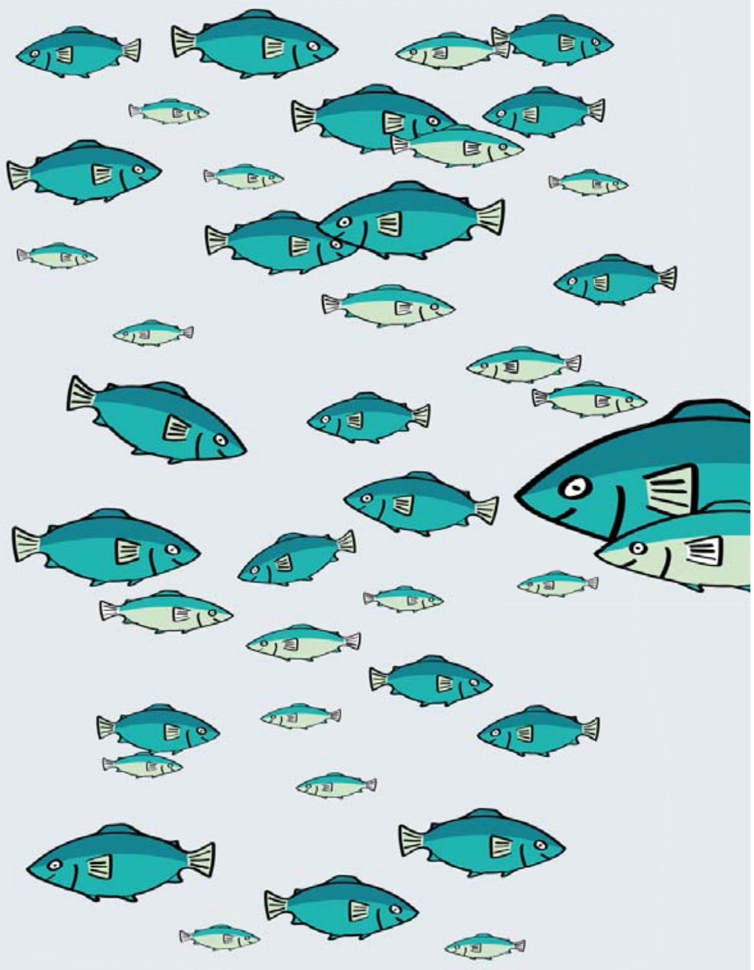
Immunological and morphopathological

In case of suspicion of a pathology, it is advisable to obtain blood or tissue samples from the fish. These samples can help determine the presence of pathogens and should be processed in a specialized laboratory. In research settings, immune indicators can also provide insights into the degree of incidence. The innate immune response allows for the determination of both the levels



of bacteriolytic activity of plasma or skin mucus and the activity of specific defense proteins such as lysozyme or complement, as well as the capacity for phagocytosis. Meanwhile, the adaptive immune response can be assessed by determining plasma immunoglobulin (Ig) levels, which increase in case of infection. However, it is important to note that these levels may vary depending on factors such as age and species. Additionally, the white blood cell or leukocyte count in plasma may increase in case of infection and can be combined with hematological and immunological determinations in complete blood count.

In the field of production, all these indicators translate into useful tools in situations such as lack of visualization of operational and productive indicators or within the context of research and development (R&D) such as monitoring vaccination or response to different feed formulations, among others. In addition, it is necessary to establish baseline values for all these parameters depending on the species, life stage, and rearing environment.



3. CRITICAL WELFARE POINTS IN FARMING SYSTEMS AND FISH DEVELOPMENT STAGES

The different sea bream farming systems and their development stages are described below, identifying the critical points in terms of welfare:

3.1. Breeders and egg-incubation

Breeding centers produce eggs from breeders under very controlled conditions. The natural spawning season for gilthead sea bream extends from November to February and even April, depending on the area. However, at fish-farms, eggs from broodstock are obtained throughout the year, varying the photoperiod and temperature. In addition, they are periodically checked to know the state of gonadal development and the quality of future eggs.

Broodstock are reared in spawning tanks, generally made of cement or fiberglass, of different shapes and sizes, at densities of about 10 kg/m^3, at an optimum temperature of 16-18 °C. Puberty in gilthead sea bream varies with sex. Males are sexually mature in the second year of life (size: 20-30 cm, weight: 150-300 g), while females reach maturity at the end of the third year of life (size: 30-40 cm, weight: more than 600 g). In breeding conditions, efforts are made to keep fish between 2 and 5 years old, ensuring the introduction of between 25 and 30% of 2-year-old individuals every year. This is due to their protandrous hermaphroditism, behaving first as males and, around the third year in 80% of the population, transitioning to females.

The transfer of sea bream to the breeding facilities is carried out individually and carefully, using nets, tarpaulins, or stretchers. A light anesthesia is applied to them to reduce stress during transport, facilitating the maneuver.

Egg collection is carried out in a natural way, channeling the surface outflow of water in the broodstock tank. The collected eggs are placed in containers with water where the viable ones are separated from the nonviable ones, according to their buoyancy. The selected viable eggs are then placed in incubation tanks. The incubation temperature is around 19-20 °C and lasts about two days (or 40-45 °C/day).

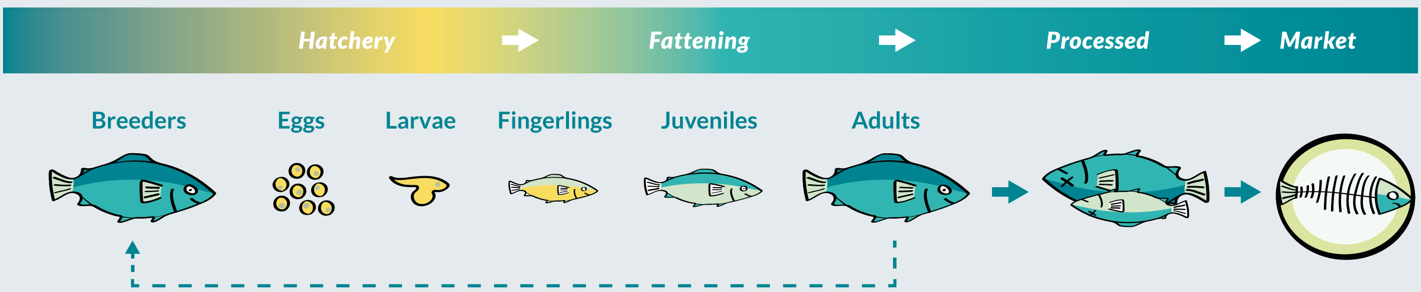


Table 1: Most relevant aspects of gilthead sea bream welfare during the breeding phase

Most relevant aspects of gilthead sea bream welfare during the breeding phase		
Environment and confinement	**Design and dimensions**	Use of space, distribution
	Lighting and temperature	Influences reproduction
	Water quality	General health and welfare
	Stocking Densities	Use of space, social interaction
	Sex ratio	Social interaction, reproduction
Handling and maintenance	**Gonadal examinations**	Scheduled procedures with manipulation
	Hormonal induction	Specific procedure with manipulation, severe
	Tank cleaning	Routine operations induce stress
Feeding	**Feeding strategy**	Meeting physiological and behavioral needs
Animal health	**Health checkups**	Routine observation with specific manipulation
	Treatments	Scheduled procedure
Transport	**Relocation**	With hand-nets or tarps on an individual basis
Final phase	**Slaughter**	Old age or poor egg quality

3.2. Hatcheries and nurseries

The newly hatched sea bream larvae are about 3 mm long and weigh between 0.1 and 0.15 mg. They are blind, symmetrical, and not very active, swimming passively on the water surface. The yolk reserves are completely reabsorbed after the first week and the inflation of the bladder takes place when the larva is about 10-15 mm long, acquiring the shape of an elliptical vesicle. Metamorphosis takes place between 45-60 days of life (10-15 mm in length and 40-60 mg in weight), giving rise to a fry with characteristics like those of the adult.

Exogenous feeding starts around the 4th-5th day of life. Feeding is usually started with rotifers, enriched with microalgae or commercial products (specific concentrates of trace elements, attractants, vitamins, and specific fatty acids, etc.). From day 18 of life, gradual feeding with enriched Artemia metanauplii and nauplii is started until day 55-60 of life, at which time the larvae are already weaned and begin to feed exclusively on dry feed.

Larval rearing ponds are typically large (5,000-20,000 liters) and are equipped with surface cleaners and bottom aeration, allowing for adequate inflation of the swim bladder and preventing potential malformations. Transfer of the larval culture to the weaning tank is done manually (with a dip net and in buckets or pails) or by using tubes connecting both tanks and pumped water.

When the fry reach a weight of 0.1-0.2 grams (at about 70 or 80 days of life), sorting is usually performed, where fish with malformations or without a swim bladder are discarded. Dry feed is used for feeding, and the number of feedings is high, with the possibility of using automatic feeders. At this stage, it is important to classify the fish by size, which is done by letting them pass through variable gauge classifiers (grading boxes). When they reach 100-120 days of life, their average weight is about 0.5-2 g, and they are transferred to the nurseries. The transfer of gilthead sea bream from weaning to pre-fattening and nursery is done by using pipes connecting ponds to which water is pumped (i.e. Pin-Pin pump). The oxygen needs of the fry in the pre-fattening phase of sea bream are high, so oxygen is usually injected at the water inlet. This also results in better fish health, improved feed utilization, and growth. Feeding consists of dry feed with a minimum frequency of 4-6 per day, frequently resorting to the use of automatic feeders under constant supervision of operators.

During this phase it is advisable to classify individuals according to size, generally using sorting machines. In this way, better control of the welfare indicators is achieved, which is essential at this stage. Depending on the malformation rates observed through quality checks, depuration processes can be also carried out, removing those individuals with clear external malformations. This procedure is usually performed manually by specialized personnel on designed tables for sorting (smooth light tables). For transfer or sorting, the gilthead sea bream is usually lightly anesthetized.

During pre-fattening, sea bream juveniles are vaccinated before being sent to the on-growing facility. There may be one or two vaccination processes, by immersion or by peritoneal injection, with polyvalent vaccines. In addition, routine health and condition inspections are carried out during the entire hatchery/nursery phase, and various treatments (prophylactic or metaphylactic baths) may be applied under veterinary decision, prescription, and supervision by the company's integrated health plan. The product, dosage, and duration of these treatments are always adapted to the circumstances and the animals.

The transfer of juveniles at the end of the pre-fattening phase to the open-sea farms can be carried out by land or sea transport. Depending on the location of the origin and destination facilities, the duration varies according to the distance to be covered and the methods used. Before loading for transport, the sea breams are subjected to a fasting period (24-48 h, depending on temperature, method, and transport time). Subsequently, they can be lightly sedated to reduce stress during loading, and the water is renewed for transport. The juveniles are pumped from their pond of origin through flexible pipes to the transport tanks. During transport, control and management of water density and quality is essential

Land transport is mainly carried out with special trucks for transporting live fish, equipped with several individual tanks (approx. 2 m^3) and an oxygen and air injection system. Transport by sea is carried out by well-boats, vessels equipped with large tanks (over 50 m^3), with oxygen supply and water renewal system directly from the sea. Conveyance by well-boat allows for continuous water renewal during transport and for unloading of fish directly into the hatchery. Unloading from the truck is usually done by gravity through flexible pipes; and from the boat, with pumps after the fish have been grouped in the tank.

Table 2: Most relevant aspects for gilthead sea bream welfare during the hatchery/nursery phase

Most relevant aspects for gilthead sea bream welfare during the hatchery/nursery phase		
Environment and confinement	**Design and dimensions**	Use of space, distribution
	Lighting and temperature	Intensity, photoperiod, and temperature, phases
	Aeration	Oxygenation, homogenization, (mainly for larvae)
	Water quality	Health and general well-being
	Stocking densities	Use of the space, social interactions
Handling and maintenance	**Manipulation**	Stress easily. Sedatives
	Tank cleaning	Routine operations induce stress.
	Classification/sorting	Manual sizing
	Deformities	Extensive handling. Sedatives
Feeding	**Feeding strategy**	Very voracious, reducing cannibalism, different phases
	Fasting	Prior to transport or handling
Animal health	**Biosecurity**	Extreme hygiene, high infection risk
	Health checkups	Includes handling and/or sacrifice
	Treatments	Prophylactic or metaphylactic baths prescribed and supervised by a veterinarian
	Vaccination	Baths or injections
Transport	**Relocation**	With buckets or tubes (larvae), nets or tubes (fingerlings)
	Long distances	Transfers to on-growing facilities
Final phase	**Slaughter**	Culled or sacrificed by overdosing

3.3. On-growing land-based and off-shore

In Spain, the on-growing phase of sea bream is mainly carried out in floating open-sea net-pens and, to a lesser extent, in land-based facilities. Each fish reaches commercial size between 13-16 months (varying up to 18 months in cold areas and 12 months in the Canary Islands), with a weight of between 350-400 g. Sea bream tolerates temperatures between 12 °C and 30 °C, although the optimum growth temperature is between 23 °C and 25 °C.

Marine farms consist of floating net-pen units in the sea, far from the coastline and exposed to adverse marine weather conditions (temperatures, currents, waves). They consist mostly of circular structures made of high-density polyethylene, with a double floating crown and a top railing. They have a net depth of 10-20 m (pouch-like) and a diameter between 16 and 38 m (up to 30 m in the pouch). The sea bream density is usually no more than 20 kg/m^3.

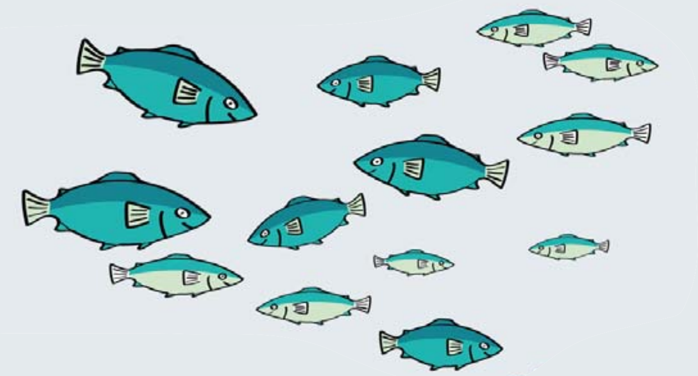
Most operations carried out in marine facilities (net changes, harvesting, maintenance, etc.) are complex, require high specialization, and are performed from farm service boats. The feed consists exclusively of nutritionally balanced dry feed to cover physiological needs. The feeding systems employed on the farms vary, ranging from manual to automatic feeders, often involving specialized crews. The number of feedings depends mainly on the method used, water temperature, and size of the sea bream. Before harvesting, farmers use small-sized pens where fish are transferred, or a purse seine inside the net-pen or pond, forming a smaller shoal to group only the targeted individuals. Subsequently, they are extracted with brailing devices (nets) to slaughter them, a process that is generally carried out by immersing the sea breams in a mixture of water with approximately 50% ice, in isothermal tanks located on the fishing vessel, at a temperature of no more than 1 °C.

Table 3: Most relevant aspects of gilthead sea bream welfare during the on-growing phase

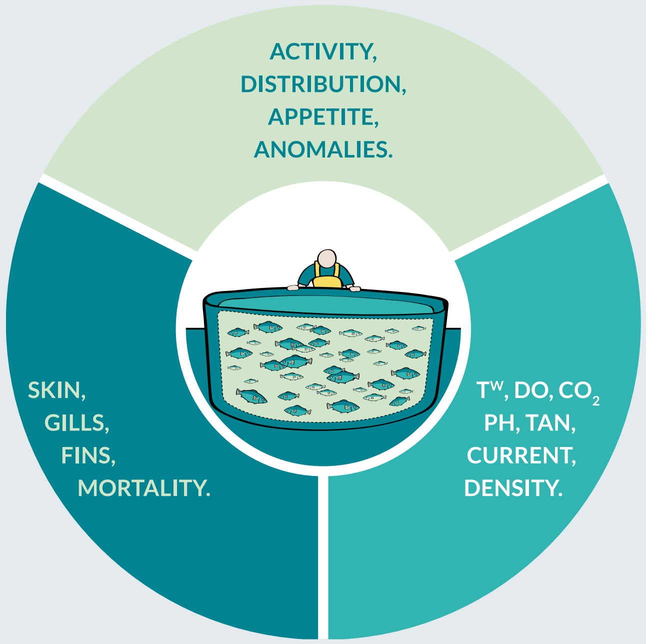
Most relevant aspects of gilthead sea bream welfare during the on-growing phase		
Environment and confinement	**Design and dimensions**	Use of the space, distribution (only intensive)
	Temperature and oxygen	Lack of oxygen (essential in land-based ponds)
	Water exchange	Oxygenation, condition of nets (on-growing sea-cages)
	Water quality	General health and well-being
	Stocking densities	General health and well-being
	Climatology	High relevance (in both systems)
	Predators	Several present (in both systems)
	Boats (noise)	Unavoidable (essential in on-growing sea-cages)
Handling and maintenance	**Net cleaning**	Routine operations by scuba divers (on-growing sea-cages)
	Culling	Routine operations (may induce stress)
	Classification/sorting	With nets, extensive handling
	Net change	Specific operation (on-growing sea-cages)
Feeding	**Feeding strategy**	Essential
	Fasting	Prior to transport or handling
Animal health	**Health checkups**	Includes handling and/or sacrifice
	Treatments	Health control
Transport	**Relocation**	Unusual (nets, pumps, or connecting rearing units)
	Long distances	Transfer to on-growing facilities
End of cycle	**Pre-slaughter**	Concentration, fasting
	Slaughter	Stunning before slaughter

4. WELFARE AND GOOD PRACTICES IN SEA BREAM FARMING

For an adequate evaluation and monitoring of sea bream welfare and farming practices, it is suggested to use essential operational indicators (external, behavioral, and environmental) during the various procedures and production phases (see *Appendix*). Likewise, the application of other indicators for a more precise and complete evaluation is also advised, as long as it is feasible, without impairing the adequate development of routine activities that may compromise the welfare of the sea bream. For detailed information on each indicator, please refer to *section 2* (*Welfare indicators for gilthead sea bream*). In addition, it is recommended to keep a record of the values of the monitored indicators to enable an objective assessment of the welfare of gilthead sea bream at the different critical points. This will facilitate the formulation and implementation of appropriate management actions and measures.



4.1. Environment and confinement



Suggested essential operational indicators to be recorded and monitored to assess the welfare of gilthead sea bream regarding environment and confinement.

Design and size of the facilities

The design and dimensions of the enclosure must be adequately adapted to the specific needs of the species, guaranteeing the satisfaction of their physiological and behavioral needs, and ensuring the best conditions, quality, and sufficient space to allow free movement. In addition, they should avoid harmful materials or structures that may cause wounds or other physical damage. Likewise, the color and lighting of the enclosure should be appropriate, and environmental noise must be minimized to avoid inducing stress and disturbing the biorhythm of the fish. It is important to prevent potential health issues such as eye damage from excessive illumination.

Broodstock should, therefore, be housed in circular concrete or fiberglass tanks with rounded corners, each having a capacity surpassing 20 m^3 and densities of less than a 10 Kg/m^3 to ensure well-being. Additional indicators should be monitored in case established limits are exceeded. During spawning, the tank may be reduced to 5 m^3 and 1 m deep (without increasing density) to facilitate access and routine operations such as cleaning, egg collection, and monitoring. Furthermore, the natural environmental conditions of reproduction should be simulated, maintaining the length of the spawning period, and alternating between the different stocks to achieve clutches with different populations all year round. This way, hormonal injections will be circumvented, thus promoting natural spawning.

In hatcheries, gilthead sea bream should be kept in cement or fiberglass tanks, circular or with rounded corners, an optimum volume between 10-20 m^3, a minimum depth of 1-1.5 m, and optimum densities allowing them to develop normal behavior. An efficient use of space should also be sought to safeguard a minimum distance between individuals. Basic operational welfare indicators must also be used to evaluate and ensure a good welfare status under the densities used. In addition, the tanks should be equipped with surface cleaners and bottom aeration devices to ensure an adequate distribution of larvae in the water column, facilitate swim bladder inflation, and prevent possible future malformations.

In land-based pre-fattening and fattening facilities (semi-intensive rearing), dimensions of enclosures and rearing systems must have sufficient volume and space to meet fish biological needs. Pre-fattening is carried out in either open, flow-through elongated raceways systems or round, close circuit ponds, depending on the design and capacity of the facilities. In the case of on-growing open sea-cages, the net-pens must have a diameter between 12-50 m, a minimum depth of about 10 m, and densities that can reach 15-20 kg/m^3. The nets can be made from a varied supply of materials, with a mesh size suitable for the size of the sea bream being reared, kept in good condition to allow good water exchange, and avoid unwanted escapes or entanglements.

Monitoring water quality and flow parameters

Water quality is essential to ensure the health and welfare of fish. To this end, there are several essential and non-essential water-related parameters that should be monitored and recorded on a regular basis (see *Appendix*). This allows the control and evaluation of possible adverse environmental changes in the population, caused by the aquaculture practice itself, other anthropogenic impacts, or meteorological events. Therefore, regular monitoring will provide a much more accurate picture of the conditions in which the fish live and will allow timely reaction to avoid or mitigate the impact on the fish.

In land-based rearing systems (tanks or ponds), monitoring should be carried out systematically, as frequently as possible and in various zones and depths of each enclosure or production unit, based on a prior risk assessment. This ensures the determination of the parameter experienced by the fish in as large volume as possible. The frequency will depend on the parameter and, to some extent, on the systems available. In addition, it is necessary to carry out a study of the water flow suitable for the different production phases, which facilitates dispersion, ensures a good renewal of the water volume, guarantees aeration, and reduces abrupt changes in the quality parameters.

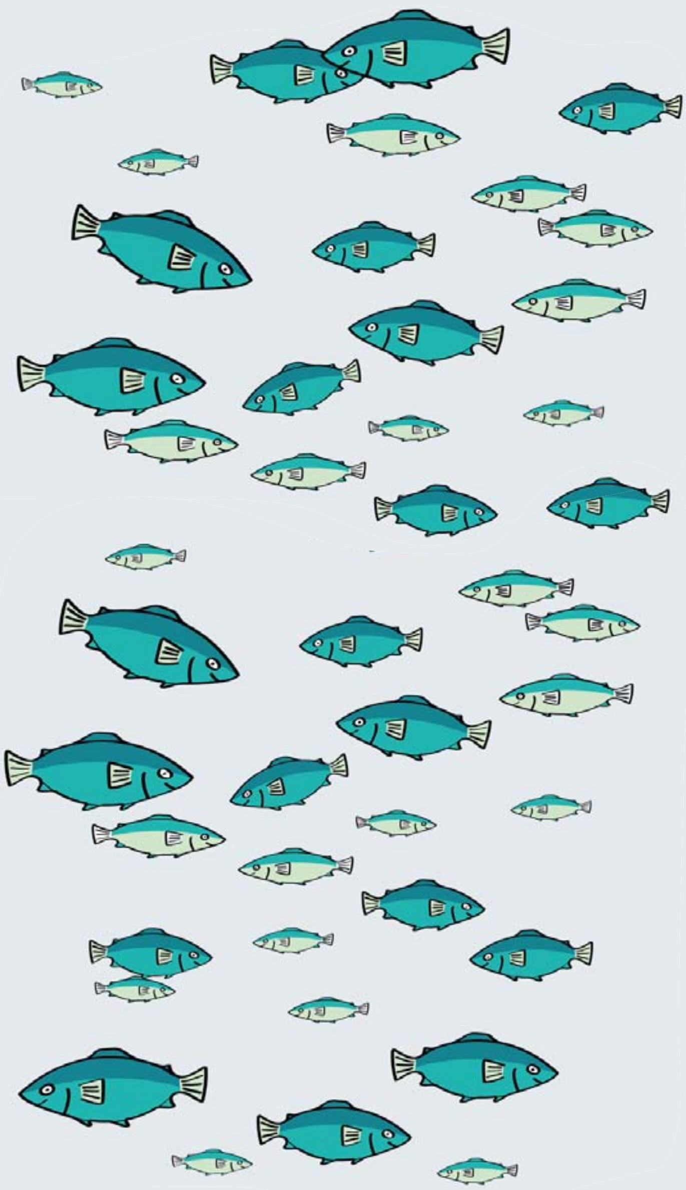
In the case of on-growing sea-cages, monitoring should also be carried out systematically, as frequently as possible, and in various zones and depths of each enclosure or production unit. However, the location of the facility must be considered since it depends on the local weather conditions and currents, which must therefore be monitored. Furthermore, optimal maintenance of the net-pens must be performed to allow for a good exchange of water with the exterior.

The Animal Welfare Plan of each facility should include action plans in cases where any parameter deviates from optimal ranges. Occasionally the values of water quality parameters may fluctuate and reach levels detrimental to farmed gilthead sea bream, possibly influenced by environmental conditions. However, as the fish are still under the farmer's responsibility, means should be provided so reduce or avoid fish suffering. Therefore, it is recommended that the Animal Welfare Plan for each facility and species, or the standard procedures, contemplate a course of action that breaks down the reaction thresholds for each parameter and countermeasures in case of an unfortunate incident.

Management plan for local fauna and predators interactions

Outdoor gilthead sea bream production systems are usually located in coastal areas (ponds, estuaries) or in the open sea (floating net-pens), where they interact with local fauna and local ecosystems. The presence of wild predators (birds, fish, marine mammals) can cause stress in sea bream, as well as a series of damages to the facilities, helping bring about the spread of diseases and economic losses. For this reason, the production companies have environmental impact studies and monitoring plans in place to ensure a sustainable activity that is respectful of the surrounding nature.

In environmental assessment procedures conducted by companies, regarding their facilities, interactions with local fauna and potential predators are addressed in accordance with applicable regulations. The initial environmental assessment process includes descriptions of the typical predators in the area where the facility is located. Guidelines are in place to minimize the attraction of local fauna to the farms such as refraining from feeding wildlife, and procedures are implemented to prevent access to fish by surrounding predators. For instance, nets may be used to deter fish-eating birds from entering farming enclosures. Additionally, protocols are established for removing predators from the facilities in case such incidents occur.



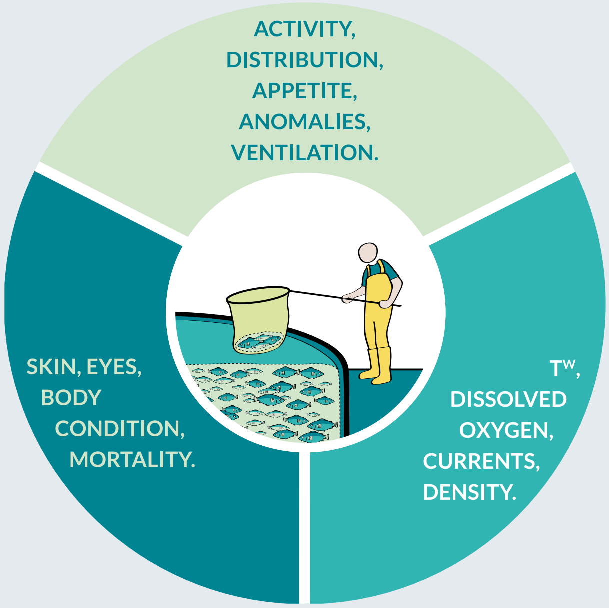
4.2. Handling and maintenance

Handling of gilthead sea bream

Handling of sea bream must be cautious, limited to what is strictly necessary, and focused on individuals in good health condition. Routine handling procedures such as health inspections, growth and size classification, should be conducted carefully by qualified personnel and specialized machinery to minimize stress and attend to their well-being and biological needs. When circumstances permit, using sedatives (e.g. for broodstock) or pumps for handling and moving fish (e.g. in hatcheries and land-based facilities) is recommended. Additionally, sudden temperature changes during handling or transfers should be minimized to prevent a long-lasting source of stress of moderate severity. When handling an individual out of the water, the entire body weight should be supported. Supplemental oxygen supply should be ensured by additional diffusers in the water to maintain oxygen saturation levels. Materials and equipment used for handling (such as nets, tubes, buckets, pumps, etc.) should be designed to prevent physical damage and maintained in perfect condition through routine inspections. Operators should be properly trained and wear gloves during direct handling to prevent skin damage and possible infections.

Avoiding or limiting exposure to air

Handling sea breams out of the water causes a high stress response, decreasing their well-being, making them more susceptible to diseases, and potentially reducing their reproductive capacity and quality. For this reason, exposure to air must be minimized, with a maximum handling time of 15 seconds if necessary, ensuring the fish remain moist. If longer handling times are unavoidable, such as during sexual maturity checks or parasite counts, sedation or anesthesia should be used to minimize stress, keeping the body moist, the eyes covered, and observing their recovery after handling.



Suggested essential operational indicators to be recorded and monitored to assess the welfare of gilthead sea bream regarding handling and maintenance.

Classification procedure

Size grading limits size variation within the same batch and can help reduce aggression and improve access to food. It is, however, a stressful process for fish and should be minimized. Repeated exposure to handling or adverse stimuli can have a cumulative negative impact on fish welfare over time. A good feeding strategy can help reduce size variability and therefore, grading. Passive methods such as grading boxes (fry), sweep nets or pumps, and specialized machinery with no direct handling (juveniles) should be used whenever possible to reduce stress.

Sea breams are sometimes subjected to a classification process when they reach 0.2-0.5 g, where those with absent or malformed swim bladders are discarded. Sedation or anesthesia is recommended for capturing and classifying them during this process, as well as the use of a hypersaline medium ranging between 50 ppt and 70 ppt (for larger sea breams) to reduce osmotic shock, and also observe their subsequent recovery after the process. During sorting or depuration due to deformities (when they reach 2-5 g), sea breams must be anesthetized, and manual sorting must be carried out by trained personnel under appropriate conditions.

Management of concentration maneuvers

Concentration maneuvers, which involve gathering fish into dense groups, are highly impactful processes requiring prior risk assessment and coordination between production and welfare managers. It causes a high stress response in gilthead sea bream, which can last for days before they recover. It significantly impoverishes well-being and gives rise to chafing and skin wounds that can lead to serious infections. Repetitions of concentrations maneuvers during sampling and harvesting should be limited, to the minimum necessary, concentrating low densities of fish, reducing their duration and frequency, and using nets of adequate size and in good condition. Furthermore, water parameters are affected significantly and can even damage the gills and affect the health of the fish. Therefore, water parameters must be monitored during concentrations, at least oxygen levels, and the behavior of the fish should be observed to ensure that the concentration intensity is not excessive and that the fish are not suffering excessive stress, in which case the process should be stopped.

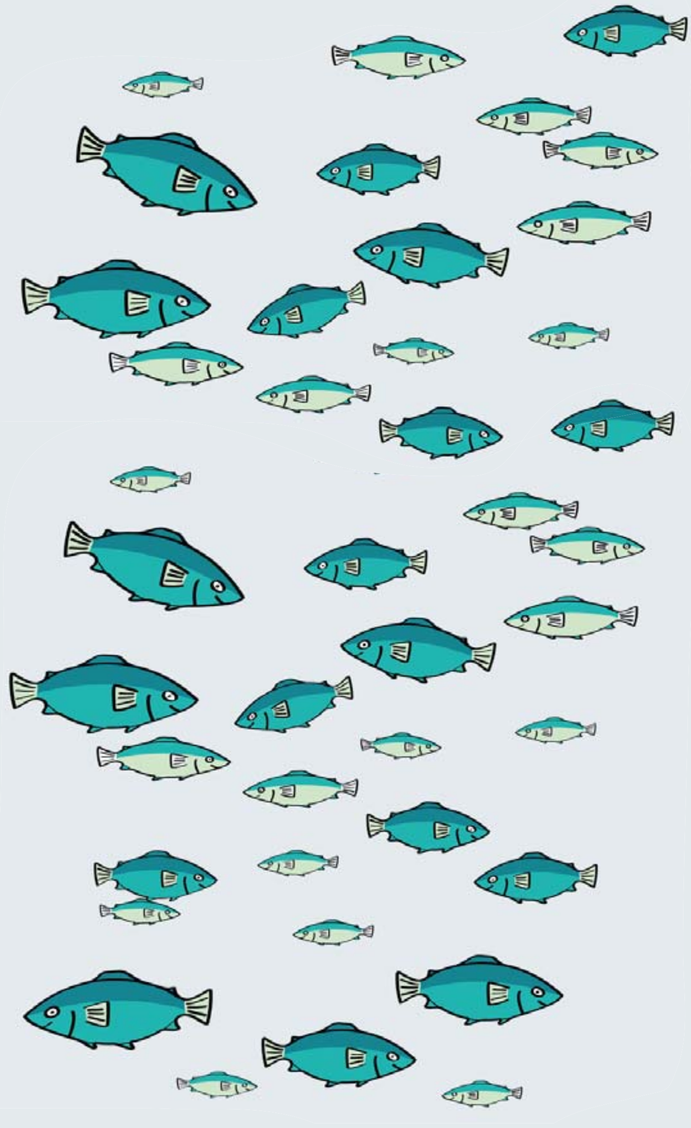
Cleaning the hatchery enclosure

In sea bream production in tanks, cleaning and disinfection is part of the Biosecurity Plan. The objective is to eliminate organic material to destroy or inactivate pathogenic agents. The procedure will depend on the purpose (prevention, control, or eradication of diseases), applying in all cases the appropriate methodology to ensure the cleaning and elimination of infectious agents from the tanks without harming the welfare of the sea bream during the process. It is recommended to use siphons or vacuum cleaners to wash the bottom and walls of the tanks when fish are present. In the case of deeper disinfection, with hypochlorite, detergent, or other legally authorized products, fish should be moved to another tank.

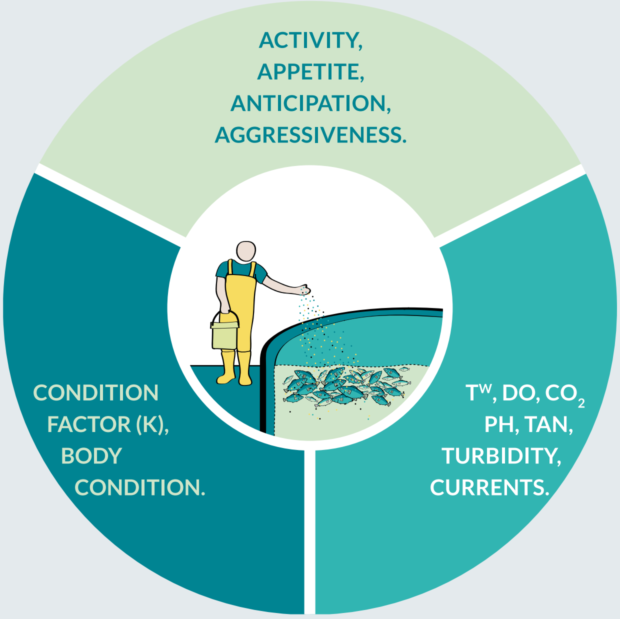
In the case of open-sea cages, net cleaning should be ensured through routine cleaning *in situ* or changes, depending on the installation and conditions, with the option of using periodic treatments with anti-fouling products. The net must be changed if it is in poor condition or when it is necessary to change the mesh size.

Culling and moribund fish management

Mortality and the presence of moribund fish can be a source of disease spread within enclosures. In addition, it is important to count dead fish for farmers management, raising the alarm if the mortality is too high, as well as appropriately analyze the cause of death. Ideally, observation and monitoring of dead and moribund individuals should be done daily by an authorized person in charge, who should analyze above-normal mortalities to ascertain causes and proceed accordingly. Dead and moribund sea bream must be removed from the water to guarantee hygiene. The latter must be humanely sacrificed (see section 4.6) so that they do not suffer unnecessarily.



4.3. Feeding



Suggested essential operational indicators to be recorded and monitored to assess the welfare of gilthead sea bream regarding feeding.

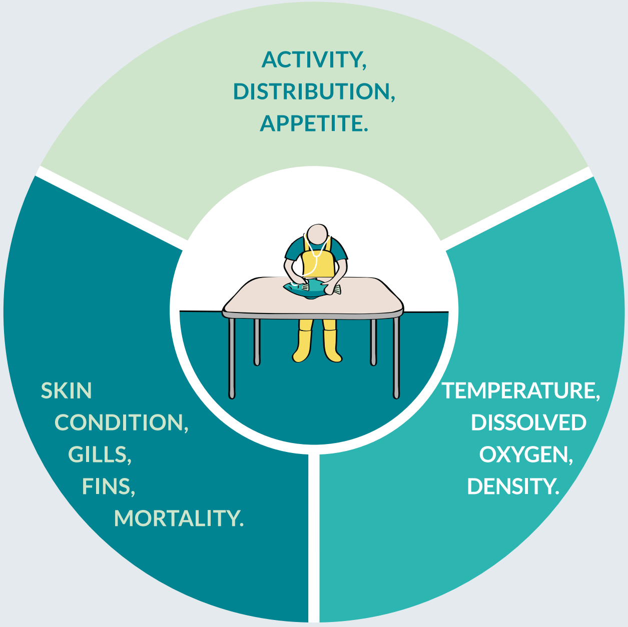
Adequate feeding strategy

A good feeding strategy can help maintain welfare, health, and efficiency in fattening, as feeding regimes, schedules, and feed characteristics have a major impact. The number of feedings will depend on the rearing cycle, but the appetite and intake of the fish throughout the day should always be considered. Sea bream may be quickly satisfied during one feeding and digest promptly (especially at high temperatures), so they may need more than one daily feeding for 5-6 days per week. In a land-based production system, there is the option of using automatic feeders, as they are well adapted to self-demand. Feed for broodstocks is usually based on dry feed supplemented with fresh fish, squid, or mussels during the spawning season.

Management of fasting periods

Food deprivation increases the sensitivity of sea bream and induces stress, which may trigger aggression. However, fasting may be necessary for welfare, as well as a requirement for food safety and product improvement. Minimizing its metabolism and emptying its digestive tract early will temper its stress response. During handling, fasting should be reduced without exceeding 48 hours or 50 °C/day, whichever comes first; the duration will depend on the season and water temperature. Furthermore, to avoid repeated food deprivation over time, in transit, or while harvested, fasting can be extended up to a limit of seven days. In this case, it should only be for the sake of fish welfare and under strict veterinary control or, in the case of sea-cages, due to occupational hazards (storms that prevent navigation and feeding in the hatcheries). In either case, the use of a wide range of indicators to monitor and evaluate their welfare status is recommended to allow the application of management measures in time.

4.4. Animal health



Suggested essential operational indicators to be recorded and monitored to assess the welfare of gilthead sea bream regarding animal health.

Animal Welfare Plan

By March 2027, each facility must have a specific Animal Welfare Plan, which must include the minimum considerations of Appendix II of Royal Decree 159/2023. These are summarized in: a) a description of the structural and environmental conditions of the farm, b) an assessment of risk factors for animal welfare, including the risk of natural disasters (such as floods, earthquakes, tsunamis, strong waves, currents, presence of predators or fires) according to the characteristics of the place where the farm is located, and c) an action plan with measures to be adopted against the identified risks. In addition, this plan must be designed and regularly reviewed by a veterinarian or professional responsible for animal welfare. The periodicity of the review should be defined in the plan itself, suggesting a review at least every two years. The plan should include: a) a person responsible for fish welfare and health; b) critical procedures that may affect fish health and welfare; c) frequent or susceptible diseases, as well as their symptoms, diagnosis, and treatment; d) action procedures to ensure fish health and welfare according to the cases that arise; e) a detailed welfare assessment protocol based on specific operational indicators.

Vaccination

Vaccines are proven to be an efficient tool to limit disease incidence and thus ensure better fish health and welfare. Even so, the process can be stressful because it involves gathering individuals, time out of the water, handling, and injections. Vaccination should not be a reason to relax practices during rearing and should never mask bad practices. The process should be carried out minimizing the stress caused by all its steps throughout the whole course of action, i.e., both its effectiveness and the possible effects on sea bream after vaccination should be monitored.

Non-antibiotic or antimicrobial treatments

Integrated Health Plans must be kept by each company, which according to current regulations, must retain a responsible veterinarian, or be implemented by the fish farmer. Antimicrobial treatments and practices are governed by RD 666/2023, which regulates the distribution, prescription, dispensation, and use of veterinary medications.

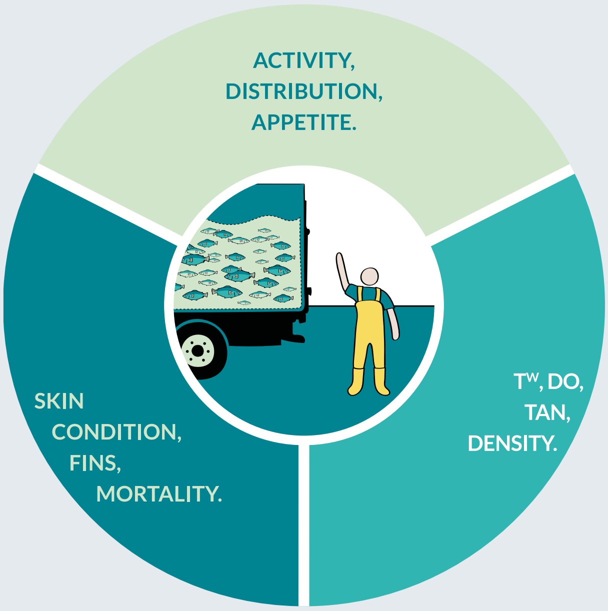
Recording and definition of mortality by cause

Although mortality is a retrospective indicator of what has happened on the farm, it is still valuable, linked to the detection of underlying diseases or problems that may affect fish welfare. Therefore, it should be recorded periodically and, where possible, its origin investigated. The Health and Welfare Plan should break down the mortalities to be included in the mortality rate with their respective investigation plans.

In practice, it is more common to speak of averages when talking about mortality in a company that may have many die-off fish batches. For its calculation, it is recommended: i) to use the mortality of each die-off batch for the last two years, moving in a rotational way; ii) that the mean must be accompanied by measures of dispersion; and iii) to use the median and mode to better report the mortality experienced.

Occasionally, mortality can increase rapidly and is referred to as acute mortality, indicating a critical health or welfare problem at the fish farm. An acute mortality event in a flock is defined as a mortality of 1% or more in a week. Accounting for the number of occurrences of these events provides supplemental information on the most appropriate management of the animals for their health and welfare. Acute mortality events should be recorded along with the cause that brought them about.

4.5. Transport



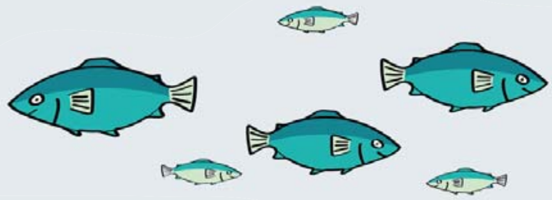
Suggested essential operational indicators to be recorded and monitored to assess the welfare of gilthead sea bream regarding transport.

Short or long-distance transport

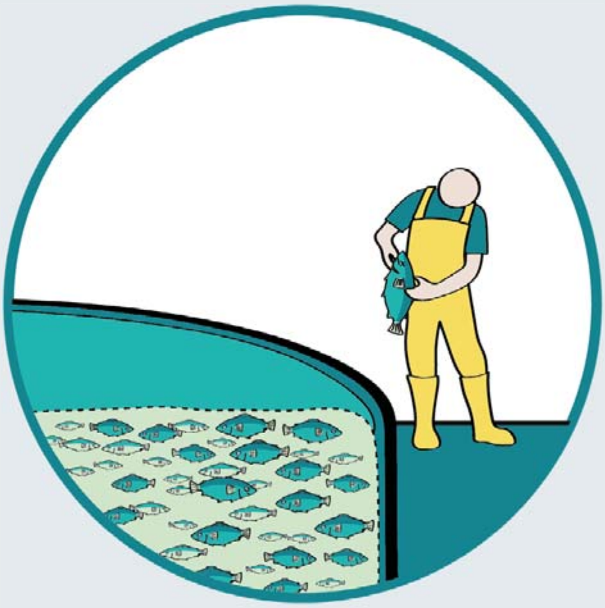
Moving individuals from one unit or facility to another is a critical stage for welfare. To avoid additional stress, storage and transport containers may only transport individuals in good health and welfare, except in emergencies, in which case, veterinary approval must be obtained. Sedatives may be used for pre- and post-transport handling, allowing 1.5 hours of water renewal before transport. In addition, a spare supply of oxygen should be provided, either by aeration or injection of pure oxygen, to maintain a saturation level between 150-200%. Due to their practicality and low installation cost, oxygen cylinders are preferred for short-distance transportation, whereas liquid oxygen containers are the preferred choice for long-distance due to their greater autonomy. In addition, storage and transport containers should: a) be thermally insulated, b) be filled with (filtered) seawater from the place where the fish were obtained, c) have no corners or be provided with rounded corners to avoid skin abrasions and mechanical shocks, and d) be large enough to allow the fish a good degree of movement. After transport, a water renewal schedule should be carried out to balance the temperature between the transport water and the destination water, thus reducing negative effects on welfare. Regarding feeding, sea breams should fast between 24-48 hours before transport to reduce their excretion rate, as long as their cannibalistic behavior is kept controlled.

About transport of broodstocks, density should be kept low and inversely proportional to transportation time and water temperature, with oxygen saturation around 100%. Speed and care are recommended in the handling of sea bream broodstock, so they should be transported to the destination in the shortest time possible while maintaining water quality conditions. Depending on the size of the fish, the density in short transports with favorable climatic conditions should not exceed 30 kg/m^3, whereas in long transports the density should be limited to 10-15 kg/m^3. To transport sea bream alevine, it is recommended not to exceed densities of 50-60 kg/m^3. Furthermore, temperature and dissolved oxygen saturation levels should always be controlled during transport (safe values ranging from 85-120%), monitoring fish behavior and other indicators whenever possible.

The legislation on animal welfare during transport and related operations is currently contained in Council Regulation (CE) No 1/2005 of 22 December 2004 and in Royal Decree 990/2022, of 29 November, which establish a series of requirements such as the authorization of the carrier and the means of transport, accreditation of the owner, origin, date and time of departure, place, date and time of destination, having a contingency plan, mortality records, water changes made during the journey, and farms visited by the vehicle. Transport vehicles must be authorized for the movement of fish on long journeys (more than 8 hours) in the European Union and for short trips in Spanish territory.



4.6. Stunning and slaughtering procedure



Slaughter is the final phase of the fish life cycle, which bears a great significance on their welfare. According to the scientific opinion of the European Food Safety Authority (EFSA) on the general approach to welfare and the concept of sentience in fish, fish can experience pain. [97] Article 3.1 of Regulation (EC) No 1099/2009 on the protection of animals at the time of death states that no avoidable pain, distress, or suffering shall be caused to animals during slaughter or related operations.

Management of discards and moribund fish (non-consumption)

Throughout the production cycle, there are several points where gilthead sea bream not continuing with the rearing process are classified and discarded (swim bladder removal, deformities, etc.). In addition, we can find sick or dying fish in the facilities, which must be removed from the water. All these fish should be killed humanely, so that they do not suffer unnecessarily. The use of anesthetics and killing individuals by overdose is recommended.

Stunning and slaughtering (consumption)

The slaughter of fish for consumption consists of a series of concatenated activities that conclude with the death of the fish. These phases are fasting, concentration, water extraction, stunning, and killing. They are all very relevant from the welfare point of view and should be carried out in an adequate and coordinated manner, in addition to other important issues such as equipment design, commitment to update methods, and personnel training.

The importance of limiting the frequency and duration of concentrations, as well as that of fasting periods, has already been explained, as the latter can help the fish in their response to stress, a requirement for food safety and product improvement. Taking into account that the process of slaughtering sea bream does not take place in a specific slaughterhouse on land but in the production facilities themselves, the occupational safety of farm personnel, the material feasibility of the processes in the aquatic environment (especially in open sea aquaculture), the impact on the environment, and the repercussions on the quality and safety of the final product destined for human consumption must also be taken into consideration.

Currently, there is a European legislative framework on slaughter that the sector complies with, and in which the use of immersion in ice slurry is accepted as a method to slaughter sea bream (for consumption) in aquaculture. However, advances in science raise questions about this methodology, to the point that it is not considered humane (EFSA [97]; OMSA). In that sense, fish that reach commercial size and are harvested for consumption should be effectively stunned before slaughter. [97] Stunning must cause an immediate loss of consciousness or insensibility which should last until death, preventing the fish from experiencing pain or suffering during slaughter. Sea bream must first be retrieved from the ponds or sea-cages with large brails or water pumps, so it is necessary to concentrate them without causing them harm.

At present, stunning is recommended for gilthead sea bream. However, it has yet to be implemented in real farming conditions in Spain. With “wet” electrical stunning, the fish are kept in water-filled pipes and the electrical current is transmitted through the liquid. In contrast, in “dry or semi-wet” electrical stunning, the fish are removed from the water and placed on a conveyor belt that acts as a terminal. The other terminal is formed by curtain-like plates that touch the belt as it advances, closing the circuit and allowing electricity through. However, it is important to adjust the electrical parameters to achieve an effective and long-lasting stunning until the death of the fish, without compromising the welfare of the animal, the quality of the final product, or the safety of the workers.

The Spanish industry considers highly important to stay at the forefront of developments in animal welfare and is actively and proactively involved (and investing) in exploring and incorporating available technologies and innovation processes that permit optimizing slaughter processes, ahead of future changes in legislation. In these developments, fish welfare, farmed fish quality, and workers’ safety must always be considered.

Verification of proper stunning and slaughtering

To ensure the effectiveness of stunning and slaughter, it should be verified that both have been carried out correctly and effectively. In the case of stunning, it must be assured that the fish involved have reached the state of unconsciousness. However, such a state can only be assessed by analyzing brain activity, which is rather inoperative. Therefore, it is recommended to use the cessation of vestibulo-ocular reflex (VOR) and opercular movement. If the fish show signs of consciousness, they should be stunned by a secondary stunning method as quickly as possible. In addition, signs of consciousness should be continuously monitored to ensure that consciousness is not regained before death occurs. Before any processing, fish should be monitored to ensure that they have been properly killed and show no vital signs.

5. TRAINING AND COMMUNICATION

5.1. Internal and institutional training

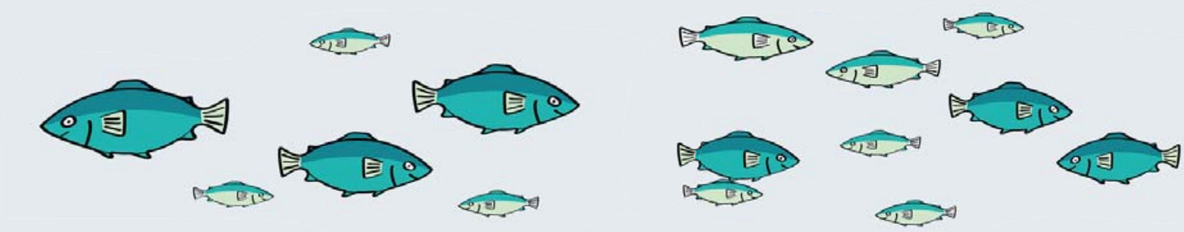
Each company should implement measures to train its personnel in fish welfare through manuals, internal courses, external training, and presentations by internal or external specialists. Such training can be advanced depending on the job positions held, and there should be attestation and records of such training.

It is important to transmit and consolidate the concept of fish welfare to the personnel working directly with the fish and to those making decisions that will affect their well-being. It has been proven that training personnel in animal welfare improves their bond with the animals under their care, hones their attention to indicators that are directly discernable by better understanding the reasons behind them, and, finally, enhances their care for the animals.

To this end, training by companies should take place at the beginning of the contractual relationship and be repeated from time to time (every two years is recommended) to consolidate concepts and update personnel on new developments in a field that is in continuous expansion. The training, in addition to job-specific information, should contain general information:

- The concept of welfare and sentience in fish and other aquatic animals.
- Good practices related to welfare.
- Welfare indicators. Both general and those specific to the species.
- Common problems: diseases, wounds, etc.
- Examples of good and bad practices.

Training within aquaculture should be carried out both internally, at the companies, and implemented institutionally. An understanding should be sought with the institutions to integrate welfare training in official courses leading to certifications or qualifications for aquaculture activity and work with fish, such as the nautical-fishing professional identity card. In addition, there are training courses at various educational levels (vocational training, degrees, masters) in which incorporating welfare training would help achieve an industry that is aware of the difficulties and possibilities related to this concept.

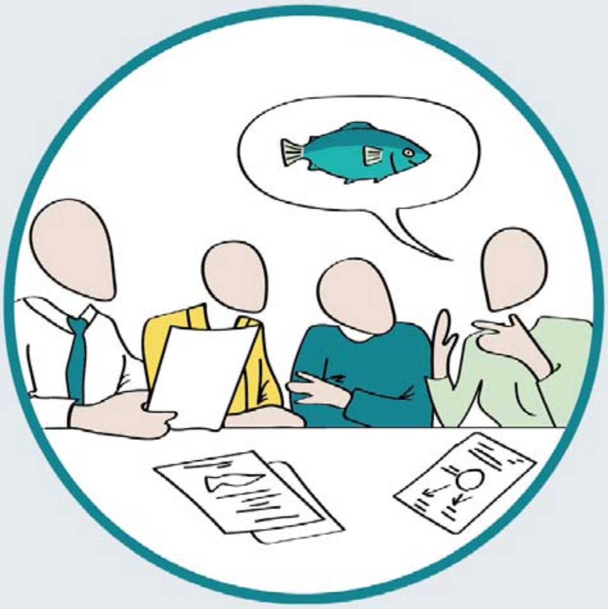


5.2. Communication and outreach

It is important that the supply chain, starting with the producers, disclose and make known what practices are being carried out to raise animals for consumption, and how they are trying to improve them concretely. To this end, **APROMAR** publishes a biannual issue of its Sustainability Report (www.apromar.es).

The production of fish, or any other animal, should be carried out in the most transparent way possible, not only to avoid criticism but also to inform and integrate the public in the production process and allow them to choose their products with all the information available, if they so wish. In this way, the industry can compare itself with other animal protein production industries, showing the public the responsibility that aquaculture farmers already feel for the animals in their care.

6. CHALLENGES AND OPPORTUNITIES



Precompetitive collaboration for welfare improvement

European and Spanish aquaculture are advancing and evolving, innovating their technologies and processes. However, their production is stagnant. Regarding welfare, there is a clear bias towards certain species and in a segmented manner among companies. This is particularly the case of Spanish aquaculture. There is a challenge to continue working on welfare practices for the whole sector, promoting collaboration between different fronts: public administrations, production associations, public research organizations, and society, to develop the relevant knowledge and technology to improve these endeavors. This will result in the improvement of both animal welfare and competitiveness at the international level.

Application of humane slaughter techniques

Advances in science have shown that fish are sentient beings and experience fear, suffering, and pain during the last moments of their lives, generating uncertainty about the efficacy of the slaughter methods currently applied to fish for consumption. Slaughtering techniques affect not only the welfare of the fish but also the product's final quality, shelf life, and organoleptic qualities. Currently, the sector is willing to face the challenge of developing more innovative and humane methods, to adapt existing technology to the Spanish context, through collaborative work. Thus, it is also an opportunity to improve the welfare of these aquatic animals, improve the product, and access markets requiring humane slaughter.

Development and application of environmental enrichment

Strategies such as environmental enrichment are considered a good tool to reduce the stress response and improve the welfare of fish in captivity. [133] Given that sea bream resides on farms for extended periods during the production cycle, environmental enrichment (whether structural, occupational, or sensory) tailored to meet their behavioral, physiological, or psychological needs. Adjusted to the specific type and stage of production, environmental enrichment is a valuable strategy to reduce stress responses and enhance welfare. [54,56,134] Although there is ample scientific knowledge on the positive effects of this strategy on fish welfare, there is no commercial-scale test on sea bream today. This is an opportunity for the sector to research and develop tools to improve the living conditions of sea bream in all their rearing stages. With this in mind, it would be possible to incorporate structures or apply water currents that provide refuge areas and stimulate sea bream's maneuverability and swimming activity. However, some structures or objects may accumulate particles of organic matter (from food and feces), making cleaning and disinfection difficult, and compromising the health and general welfare of the fish. If design of the structures is inadequate it can cause physical disturbance or damage, increasing the risk of infection, stress, or death. Another aspect to consider is that enrichment may cause negative stimuli in some fish, such as fatigue, neophobia, territoriality, or aggressiveness. All these factors should be considered when planning the strategy to be implemented, and operational indicators (see *Appendix*) should be used to monitor and evaluate the possible effects of enrichment on fish welfare and the counterproductive effects on other production factors.

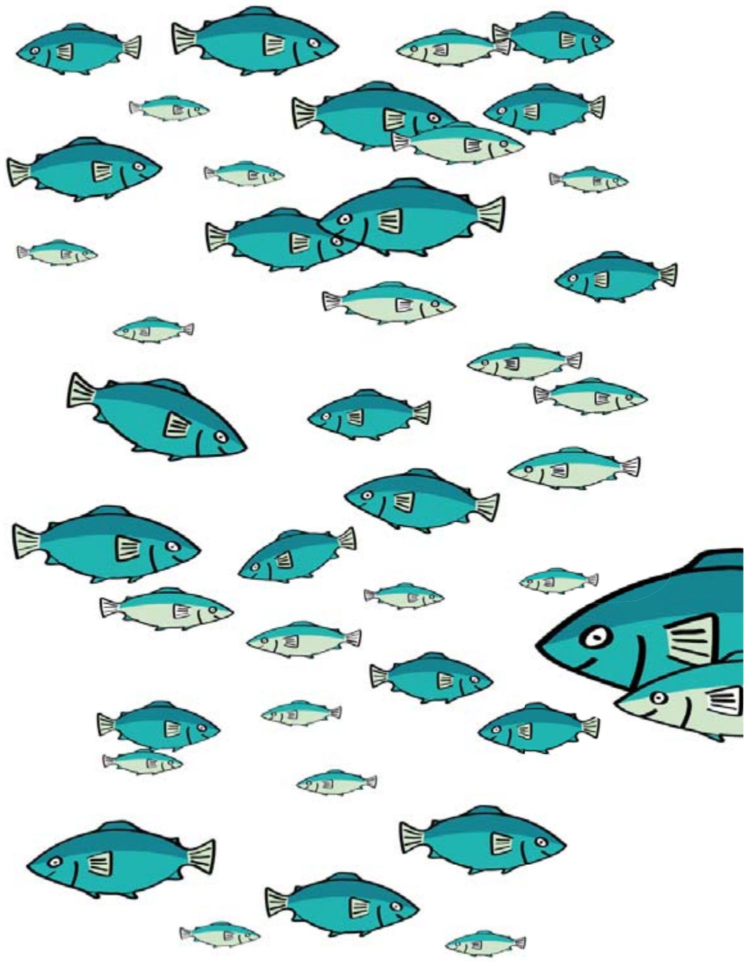
Research, science, and technology

Increasing the current knowledge about the welfare parameters of the species most produced in southern Europe, as is the case of gilthead sea bream, is a priority, to allow a correct evaluation and determination of its welfare. The technological development and application of advanced tools adapted to their farming conditions would enable precise and rapid monitoring of fish welfare and a much more effective management response. For example, the application of artificial intelligence allows the monitoring of animal welfare parameters with high precision and in a more automated way, facilitating early detection of problems and, therefore, better decisions. Genetic selection that promotes adaptability and sturdiness in gilthead sea bream should be a way forward in business research.

Implications of climate change on fish welfare

Climate change and its impacts on aquaculture are a fact of life, profoundly altering industry, especially in open systems exposed to weather and climate, such as pond and sea-cage farming. Increasing our knowledge on the biology of fish reared at different stages and their diversity and the science of welfare will help us cope with the inevitable consequences of climate change. Protecting the welfare of fish makes imperative that climate changes anticipate, and coordinated action must be taken before it becomes impossible to ensure that fish raised in ponds or sea-cages have the environmental conditions necessary for good welfare status. The welfare imperative here is to ensure that enough is known about the biology of the species and strains in question to enable their welfare needs being met, before intensive farming of such fish is initiated, and lessons are learned through trial and error.

In addition to the expected steady increases in temperature, fish cultured in open systems will also experience an increasing number of acute events such as storms, algal blooms, and heat waves, with adverse effects on their welfare. Little can be done to protect them against such events except using available computational tools and appropriate management strategies. Measures to mitigate the impact of climate change could include, for example, improvements in farming units design, making structures stronger and more submersible, preference for resilient fish strains (and species), relocation of fish farms, and improved fish monitoring and weather forecasting systems. These responses are challenging, but the fact that economic and production goals are moving in the same direction as welfare protection demands is encouraging. [135]



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8. APPENDIX

Table 4: **ESSENTIAL** (E) and **RECOMMENDED** (R) welfare indicators for gilthead sea bream according to different procedures and phases of production.
*Note: this selection of indicators has been prepared based on the **DELPHI** method, relying on aquaculture producers, scientists, and members of animal protection associations, all experts on gilthead sea bream production and welfare.

		BREEDERS							HATCHERIES						ON-GROWING				
Category	Indicators	Environment and confinement	Handling and management	Feeding	Animal health	Transport	Reproduction	Sacrifice or end point	Environment and confinement	Handling and management	Feeding	Animal health	Transport	Sacrifice or end point	Environment and confinement	Handling and management	Feeding	Animal health	Stunning and slaughtering
Physical or external	Condition factor (k factor)			E					R	E	E				R		E	R	
	Malformation						E		E			E			R				
	Skin coloration	E	E	R	R		R		E	E	E		R		R	R		E	
	Skin condition	E	E	R	E	E	E		E	E	E	E	E		R	R	R	E	R
	Fin condition	E	E	R	R	E	R		E	E	E	E	E		E	E	R	E	
	Gill condition	E			E				E			R			E			R	R
	Eye condition				R				R	E	E	R	E			R			
	Mortality	R	E		E	E	E		E	E	E	E	E		R	E	R	E	R
Behavioral	Level of activity	E	E	R	E	E	E		E	E	E	E	E		E	E	E	R	E
	Surface activity			R							R						E		
	Distribution	E	E	R	E	E	E		E	R		R	R					R	
	Aggressiveness			R							E						E		
	Anomalies	E			R		R		E			R	R						
	Appetite	R	E	E	E	E	E		E	E	E	E	E		E		E	R	
	Food anticipatory activity										E						E		
	Ventilatory frequency	E							E	E		R	R	E	R	R			
	VOR							E						E					E
ENVIRONMENTAL	Temperature	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
	Salinity	R	R	R	R	R	R		R	R	R			E					
	Dissolved oxygen	E	E	E	E	E	E		E	E	E	E	E	E	E	E	E	E	E
	Carbon dioxide	E				R	R		E	R	E								
	pH	E				R	R		E	R	E								
	TAN	E		E		R	R		R	R	E	R	E						
	Nitrites/Nitrates	R		R		R			R	R									
	Turbidity/TSS	R		R	R	R			R	R	E	R	R		R				
	Current flow speed	R		R	R	R			E	R	E				R				
	Lighting	R				R			R	R			E						
	Stocking density	E	R			R			E	E	R	E	R		R	R	R	R	R