Improving the welfare of European sea bass and gilthead sea bream

COMPASSION in world farming Food Business

Foreword

European sea bass and gilthead sea bream are sentient beings and must be provided with a good quality of life in a farmed environment. This document summarises research relevant to the rearing phase of sea bass and sea bream, as a basis for our recommendations to improve fish welfare through the provision of good housing, good feeding, good health and opportunities to express appropriate behaviour. This is in line with the adapted Five Freedoms model of Welfare Quality.

Background

Gilthead sea bream and European sea bass are fish commonly found in the Mediterranean and along the North-eastern Atlantic coast. They will migrate to warm waters, with optimal temperatures for both species is roughly 18-26°C (EFSA) 2008), but have a wide temperature and salinity tolerance. They are both carnivorous – sea bass mainly feeds on other fish, and sea bream will eat mainly worms and molluscs.

European sea bass (*Dicentrarchus labrax)* and gilthead sea bream (*Sparus aurata*) are economically important aquaculture species and represent greater than 95% of all marine fish species farmed in the Mediterranean (Lembo, Carbonara, Scolamacchia, Spedicato, & McKinley, 2007). In 2013, Turkey accounted for nearly half (46%) of world production of sea bass, followed by Greece (24%), Spain (10%) and Italy (5%) (EUMOFA, 2017). The same four countries accounted for 75% world production of sea bream: Greece (36%), Turkey (23%), Spain (12%) and Italy (3.4%) (EUMOFA, 2017). Based on figures from the Food and Agriculture Organization of the United Nations, worldwide production in 2015 equated to 417-556 million individual sea bream and 325-406 million sea bass (Mood & Brooke, 2015). In 2016, production of sea bream and sea bass was estimated to be 185,980 tonnes (FAO Fisheries and Aquaculture Department, n.d.-b) and 191,003 (FAO Fisheries and Aquaculture Department, n.d.-a), respectively (European Commission, 2017). Yet, during the 'Seize the day for fish welfare' event hosted by the European Parliament in 2018, officials stressed that this sector of aquaculture currently fails to meet the standards that the World Animal Health Organisation (OIE) has set for all EU Member State signatures ("Round table: Seizing the day for Fish Welfare," n.d.).

GOOD ENVIRONMENT

Although they are very different species, aquaculture production of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) is carried out using similar systems. In the Mediterranean, 82-85% of sea bass and sea bream farms use sea cages followed by land based intensive tanks or raceways (10%) and semi-intensive production in earth ponds (8%) (Muir & Basurco 2000; Jawad, 2012; Jobling, 2010). Production in Greece, Turkey, and northern Spain predominantly use intensive on-growing floating sea cage systems in lagoons, sheltered bays or semi-exposed and offshore conditions, whereas France, Italy and southern Spain mainly use land-based systems (B. & J., 2000; Jawad, 2012).

Historically, sea cages were placed in well-protected, largely enclosed coastal sites which resulted in oxygen deficits and cage fouling. This led to the development of flexible and durable plastic pens suitable for conditions 1-3 km offshore at depths of 18-45 metres where currents are stronger allowing for improved water quality (EFSA 2008). Usually, gilthead sea bream (5 g) reach typical commercial size (350-400 g) in less than two years while European seabass take up to three years to reach commercial size (400-500 g) (EFSA, 2008).

Current manipulation

In terms of natural swimming behaviour, both species make substantial annual migrations between coastal and offshore waters during the winter months. Therefore, an appropriate current may be required for improved sea bass and sea bream welfare (Jobling, 2010). For instance, Ibarz *et al.*, (2011) found that sea bream which were held in a state of sustained swimming had increased body weight, growth rate, and improved nutrient-use efficiency, compared with fish with voluntary swimming activity. Also, Ferreira (2012) found that rearing sea bream at current speeds in the range of 0.3 to 0.5m/s was optimal in terms of FCR, O_2 consumption and fillet quality. However, it is important to note that optimal current speeds differ among individuals (Marras, Claireaux, McKenzie, Nelson, & Nilsson, 2010). Forced swimming of juvenile sea bass and sea bream has been correlated with spine deformities (Chatain, 1994). Therefore, further research is required to better understand the relationship between appropriate current and welfare with respect to individual differences and life stages.

Enrichment

Sea bream are benthic species that are usually found near the sea bed in rocky and seagrass meadows or on sandy grounds (FAO Fisheries and Aquaculture Department, n.d.-b). Therefore, increased structural complexity (e.g. gravel, sand, pebbles, and plants) of fish rearing environment may improve the cognitive abilities and welfare of fish. A series of experiments demonstrated that environmental enrichment in the form of coloured substrate (particularly blue) may be beneficial for both fish welfare and production. Gilthead sea bream showed enhanced growth, suppressed aggression, and an altered size distribution in tanks with blue substrate, compared with those held in barren tanks (Batzina, Dalla, Papadopoulou-Daifoti, & Karakatsouli, 2014). Furthermore, providing a blue or red-brown substrate reduces brain serotonergic and dopaminergic activity, suggesting lower stress experienced by these fish, when compared to fish held in tanks with barren or green substrates (Batzina, Dalla, Papadopoulou-Daifoti, *et al.*, 2014; Batzina, Dalla, Tsopelakos, Papadopoulou-Daifoti, & Karakatsouli, 2014). Batzina & Karakatsouli (2012) also reported that providing a blue substrate resulted in sea bream with a higher condition factor (a common measurement of general condition of the fish, calculated by dividing the fish's weight by its cubed length ($K=W/L^3$)), reduced aggression, and better fillet quality (in addition to improved final mass, specific growth rate, mass gain and food conversion ratio) than those with barren, green or red-brown substrates. While this form of enrichment may only be possible in land-based or closed containment systems, providing an ecologically appropriate substrate appears to provide some environmental stress relief in sea bream with additional performance benefits.

Noise stressors

The acoustics of the farm environment may induce chronic stress. Sound travels roughly 4.5 times faster in 25°C saltwater than air at 20°C[1]. Anthropogenic noise pollution has increased rapidly over the recent decades and "*offshore aquaculture noise, and in particular the sea soundscape, adversely influences the oxidative status and the immune function of gilthead sea bream determining a mild stress condition that could affect the sea bream welfare*" (Filiciotto *et al.*, 2017, pp. 1895). Offshore aquaculture noise includes typical noise from a sea cage: "sea background and boat noises" and "cage machinery". In fact, 25 years ago the National Research Council (1994, as cited in Celi *et al.*, 2016) reported that vessel traffic noise emissions accounted for 90% of the acoustic energy that

---

[1] http://hyperphysics.phy-astr.gsu.edu/hbase/Tables/Soundv.html

humans emit into the sea. Sea bream exposed to only 10 days of acoustic stress (recordings of motorboats, including recreational boats, hydrofoil, fishing boats and ferry boats) had significantly increased values of ACTH, cortisol, glucose, lactate, haematocrit, Hsp70, cholesterol, triglycerides and osmolality. While it is difficult to control offshore aquaculture noise, precautions should be taken to prevent acoustical stressors.

Stocking density

The concept of a minimum rearing space per animal is more complex for fish than for terrestrial species, as fish utilise a three dimensional medium (Conte, 2004; Ellis *et al.*, 2002). Additionally, stocking density is not uniform at any point in time; it will increase as fish grow or decrease following grading and therefore, it is hard to measure precisely in the farm environment.

Land based systems are much more expensive to build and maintain leading to producers stocking fish at much higher densities (60-90 kg/m^3) than sea cages to make them economical. This can have detrimental effects to fish welfare. In sea cages, stocking densities for seabass and seabream range from 5-20 kg/m^3 for fish weighing 2.5-150 g and 10-20 kg/m^3 for larger fish (>150 g), depending on site and cage characteristics (Appendix 1) (EFSA 2008; Jobling, 2010). While the majority of sea bass and sea bream production is done in sea cages, research is conducted almost exclusively in land-based tank systems, as experimental conditions are easier to control and manage. Therefore, care must be taken when applying results found under experimental conditions to applied settings. Also, stocking density alone cannot be used as a good indicator to predict welfare as it involves consideration of many interrelated parameters, such as physical space and the physiological need for water to provide oxygen and dilute and remove waste products, and food availability (Håstein, Scarfe, & Lund, 2005). This issue is further complicated as it is difficult to compare studies where the researchers have used different parameters for their experiments; for example, different temperatures and feeding regimes, and also creating different thresholds for what they consider to be 'low' and 'high' stocking densities (see Appendix 1). In addition, stocking density effects may also be life-stage specific, because while many fish species, including young European sea bass, are gregarious and form schools, adult European sea bass and gilthead sea bream are less gregarious, living solitarily or in small schools (EFSA 2008). Consequently, selecting inappropriate stocking densities may have a greater effect on European sea bass and gilthead sea bream than other, more gregarious, farmed species.

GILTHEAD SEA BREAM

Key results from scientific publications on the effect of different sea bream stocking densities (please see Appendix 1 for further information and examples of industry practice):

- Montero *et al* (1999) reported that stocking densities of 40 kg/m^3 showed a four-fold increase in cortisol levels when compared with stocking densities of 10 kg/m^3.They also showed that high stocking densities in juvenile gilthead sea bream also produce a chronic stress situation, reflected by high cortisol levels, immunosuppression and altered metabolism.

- Sangiao-Alvarellos *et al* (2005) showed that cortisol levels rises to a five-fold increase when stocking densities of 70 kg/m^3 are compared to 4 kg/m^3.

- Sánchez-Muros *et al* (2017) found no differences in cortisol levels in fish stocked at 20 kg/m^3 when compared to fish stocked at 5 kg/m^3. However, fish stocked at the higher density did display a significant reduction in growth rate.

- Batzina *et al* (2014) found that at higher (9.7 to 29.9 kg/m^3) compared to lower (4.9 to 14.7 kg/m^3) stocking densities, juvenile sea bream showed less aggressive behaviour and fish sizes were more evenly distributed, suggesting a more favourable social environment.

- Canario *et al* (1998) tested juveniles and found that growth rate was higher in the lowest density (groups were stocked at 0.35, 1.3 and 3.2 kg/m^3). The authors noted differences in social behaviour – in the high density group the fish shoaled more, were less aggressive, and occupied the whole water column. In the low density group there were more aggressive interactions, fish tried to defend a territory near the bottom of the tank and shoaled less. The better growth rate in the low group may have been because they seemed to swim less but further research is needed.

- In sea cages, Papoutsoglou, Costello, Stamou, & Tziha (1996) suggested that the poor water quality found (in terms of high ammonia and low oxygen levels) would be improved if stocking densities were lower than 16 kg/m^3.

EUROPEAN SEA BASS

Scientific publications and main results on the effect of different values of stocking densities in sea bass (please see Appendix 1 for further information and examples of industry practice):

- European sea bass showed higher stress levels at high densities (100 compared with 80 and <10 kg/m^3), as indicated by expression of stress related genes (Gornati *et al.*, 2004).

- Di Marco *et al* (2008) found higher levels of non-esterified fatty acids (NEFA, a stressor indicator) in sea bass stocked at 45 kg/m^3 than at 30 kg/m^3 and 15 kg/m^3. Also, fish stocked at the higher density were more sensitive to crowding.

- Santos *et al* (2010) showed that feed intake and growth decreased at high stocking densities (50.5 kg/m^3 and 75.4 kg/m^3) compared with lower densities (8.1 kg/m^3; 25.2 kg/m^3) without controlling for water quality. They also observed that feed intake reduction was compensated by a decrease in swim speed. These effects are interpreted as the combined effect of crowding and deteriorated water quality.

- In sea cages, Papoutsoglou *et al* (1996) suggested that the poor water quality (in terms of high ammonia and low oxygen levels) would be improved if stocking densities were lower than 16 kg/m^3.

There are no comprehensive studies that compare a useful and wide range of stocking densities for sea bass and bream to fully evaluate the effect of density in sea cages. Also, very few studies have researched stocking densities under commercial sea cage or earthen pond farming conditions; almost all scientific evidence refers to juvenile fish reared in tanks. However, there appears to be a trend for higher stocking densities being associated with poorer welfare (e.g. higher stress hormone levels and lower growth rates) even when water quality is controlled. For sea bream at least, there is also some evidence that at the other end, much lower densities can also be detrimental as social stress can increase when behaviour turns to territory defence rather than group shoaling. Stocking densities of between 10 and 20 kg/m^3 are typical in commercial sea cages (see Appendix 1).

Based on the scientific information available, we recommend that sea bream are reared at as low a density as possible while ensuring fish behaviour is consistent with shoaling. We therefore recommend stocking at the lower end of the range practised (i.e. 10-15 kg/m^3). This allow fish to disperse to more favourable areas when water conditions are sub-optimal to gain access to feed, find a preferred temperature or dissolved oxygen level and prevent forcing fish into unfavourable and stressful conditions. Fish behaviour should be monitored and welfare outcome measures used (i.e. check for evidence of fin erosion or biting) to ensure that the social environment does not become a stressor.

Ideally, at each site, environmental factors as well as behaviour should be regularly monitored. Poor welfare can occur at any given stocking density and stocking densities should be reviewed after every production cycle. There is lack of scientific evidence of the effect of stocking densities in sea bass and sea bream in sea cages, therefore more research is needed to give more specific recommendations.

Water Quality

Water quality has a fundamental role in the health and welfare of farmed sea bream and sea bass. Indeed, one of the principal concerns about high stocking density is that it can lead to a deterioration in water quality. Oxygen, temperature, salinity and turbidity are all important parameters. Water circulation also plays a vital role in disposing of waste products and allowing oxygenated water to circulate. Some of these factors can be controlled by farm management practices while others are related to the environmental characteristics of the site and should be assessed prior to starting farming.

Gilthead sea bream and European sea bass are both eurythermal and euryhaline, meaning that they are able to function well at a range of temperatures and water salinities (FAO Fisheries and Aquaculture Department, n.d.-a, n.d.-b). However rapid and elevated changes of temperature and salinity close to limits are more likely to lead to poor welfare. Moreover, temperatures lower than 15°C may lead to a pathological condition known as 'winter disease' or 'winter syndrome' (Ibarz *et al.*, 2010) – see disease section. Optimal temperature parameters for both species are showed in Appendix 2.

It is also important that saturated oxygen levels are closely monitored and adjusted according to stocking densities, especially at high stocking densities, where oxygen uptake increases in swimming fish (Carbonara, Scolamacchia, Spedicato, Zupa, Mckinley, *et al.*, 2015). Hypoxia (oxygen deficient water of less than 7.4mg O_2/L) is a stressful condition and has been demonstrated to reduce growth rate and feeding activity (Pichavant *et al.*, 2001). Low levels of dissolved oxygen could also result in gill lesions and high haematocrit levels (Araújo-Luna *et al.,* 2018; Carbonara, Scolamacchia, Spedicato, Zupa, McKinley, *et al.*, 2015; Di Marco *et al.*, 2017). In sea cages, dissolved oxygen could be a welfare issue at high temperatures and EFSA (2008) recommends that oxygen should be above 40% saturation and monitored daily. Sea cage design, position and current conditions can be limiting factors and should be considered in order to limit risk of hypoxia. In order to maintain the welfare of sea bass and sea bream, for all stages the oxygen saturation should be kept as close as 100% of saturation as practicable. Normally a saturation between 70 and 110 is acceptable and oxygen concentration should be preferably kept over 5 mg l^{-1}. The minimum saturation should never drop below 40% for more than a few hours for adults and below 70% for larvae. Feeding should be reduced or stopped if a decrease in dissolved oxygen, which could harm the fish is to be expected.

In the wild, both European sea bass and gilthead sea bream react behaviourally to seasonal temperature fluctuations by migrating into deeper waters in order to move away from changes in temperature (Claireaux, Couturier, & Groison, 2006; Ibarz *et al.*, 2010). Although they do experience temperature fluctuations in their natural environment, drastic, abrupt changes in temperature induce stress responses especially where fish are kept in confined conditions such as sea cages or ponds and are unable to move away from sub-optimal conditions. A temperature range between of 18-24°C appears to be optimal for both species in terms of feed efficiency, activity and metabolism with absolute lower and upper limits of 14 (15°C for gilthead sea bream) to 29°C (EFSA, 2008). Temperatures lower than 15°C greatly increase the risk for winter disease. To mitigate the likelihood of winter disease, husbandry management leading up to and during winter includes focusing on fish health, not growth, reducing feed rations as well as providing winter feed diets (more energy per kg of feed as well as different essential amino acids and minerals).

Therefore, CIWF recommends that neither species is exposed to rapid changes in temperature, salinity and oxygen levels are carefully monitored and adjusted to account for biomass to prevent hypoxia. CO_2, pH, and ammonia are generally regulated by ambient waters streams and are not welfare issues in sea cages (EFSA, 2008).

GOOD FEEDING

Feeding

Feeding may be done by hand, by computer-controlled automatic feeders at regular intervals (2-3 times a day) or using on-demand feeding systems. Regular feeding is important to reduce the risk of cannibalism, particularly in sea bream (M. Jobling, 2010). Andrew, Noble, Kadri, Jewell, & Huntingford (2002) found that on-demand feeding systems reduced competition between fish during feeding in both sea bass and sea bream and further hypothesized this would lead to improved growth and production efficiency. However, poor adaptation to feed distribution in sea cages has been observed (EFSA, 2008). As previously mentioned, following feed ration recommendations for sea bream is especially important leading up to the winter months when feeding naturally reduces as fish adjust their metabolism rate to compensate for falling water temperatures fall (Ibarz *et al.*, 2010). Reduced feeding is essential to prevent winter disease (see Good Health section).

Compassion recommends that food for sea bream and sea bass must be of optimal quality for fish, especially in winter periods for sea bream to prevent winter disease. The feeding method used must minimise competition and hence aggression and ensure that all the fish have access to feed.

Fishmeal

Commercial feeds for both species typically consist of high-energy, dry pellets containing 43–50% protein, around 12-25% fat and 20% carbohydrates (Grigorakis, Alexis, Taylor, & Hole, 2002; M. Jobling, 2010; Ökte, 2002). Feed for gilthead sea bream contains 10-15% less fat content than that for European sea bass as sea bream weighing 45 g or more are able to use lipids for energy and spare protein exclusively for growth (Ökte, 2002).

Special attention should be paid to the quality of commercially manufactured diets for sea bass and sea bream as the formulation is variable and may be nutrient deficient (EFSA 2008). The risk of dietary insufficiencies is less of an issue in sea cage systems as commercial feed is complemented by natural feed (live prey from the sea) (EFSA, 2008); however, decreased growth, survival rates and decreased welfare can occur due to nutrient deficiencies. For example, vitamins C and E have been found to be are particularly important for immune health. Gilthead sea bream fed diets supplemented with vitamins C and E were better able to cope with the stress inflicted on the immune system due to high stocking densities (40 kg/m^3 vs 20 kg/m^3) as shown by an increase serum lysozyme activity – an indicator of immune activity (Montero, Marrero, *et al.*, 1999). Furthermore, deficiencies in vitamin C and vitamin D can cause anorexia, scale loss, internal and external haemorrhage, depigmentation, poor wound healing (Tort *et al.*, 2004), and spinal deformities (Andrades, Becerra, & Fernández-Llebrez, 1996).

Another consideration of feed formulation is where the nutrients are sourced. Sea bream and sea bass are carnivorous species and their feed contains a proportion of animal protein and oil sourced from wild-caught fish. The use of wild-caught fish for reduction to fishmeal and fish oil (FMFO, by so called 'reduction fisheries') which is then added to farmed fish represents food wastage as a majority of these fish are, in fact, human edible and energy is inevitably lost during the process. The welfare of the fish caught by reduction fisheries is very poor during capture, landing and killing; there is no humane slaughter practised. Therefore, the FMFO industry has substantial negative welfare consequences and should be addressed.

In an attempt to address FMFO issue, and due to their low cost, plant protein and lipid sources have been used over marine-derived ingredients (Brill, Horodysky, Place, Larkin, & Reimschuessel, 2019; Ganga *et al.*, 2011; Montero *et al.*, 2010; Piccinno *et al.*, 2013; Torrecillas *et al.*, 2019). In fact, in order to meet Naturland's[2] organic standards, feeds from animal origin should be in limited amount and alternatives are preferred (see Table 1). However, their use is related with several side-effects on performance, health, or disease resistance. For example, solely plant-based sources lack essential nutrients such as the amino acid taurine which it had negative effects on fish ability to see colours (Brill *et al.*, 2019); replacing fish derived oil with vegetable oils in sea bream fish feed also effected plasma cortisol levels in response to stress (linseed oil increased basal plasma cortisol levels, while soya bean oil delayed the cortisol response) (Ganga *et al.*, 2011) and lowered resistance to pathogens (Montero *et al.*, 2010); thus, affecting fish welfare negatively.

[2] https://www.naturland.de/images/01_naturland/_en/Standards/Naturland-Standards_Aquaculture.pdf

Therefore, further research is needed to identify the mechanisms of alternative ingredients to guarantee that the contents satisfy fish physiological requirements.

Compassion recommends that the amount of fishmeal and fish oil (FMFO) in sea bass and sea bream feed be reduced as much as possible, while still providing for the nutrition needs of the farmed fish. This can be done by replacing some of the FMFO with other ingredients that can meet nutritional requirements, e.g. fish trimmings (or waste from other agricultural processes where suitable, e.g. poultry), algal oils, etc.

Table 1. Label regulations: Feeds

Naturland, 2018	**Species**	**Feed**
	Supplementary regulations for the culture of Perciformes (perch-like), Carangiformes (jack-like) and Gadiformes (cod-like) fish species in marine net cages	**8.1** For certain culture systems an upper limit for the application quantity feed/area can be determined (ref. B. Supplementary regulations for specific farming systems and animal species). **8.2** Type, quantity and composition of feed must take into account the natural feeding methods of the concerned animal species. The activity level and the condition of the animals mainly give indications in this respect (e.g. corpulence factor, fat tissue). **8.3** All the feed stuff of vegetable origin must be produced in accordance with Naturland standards[11]. Additionally, feed from animal origin in limited amount and defined quality (s. 8.5.) is permitted. Supplements and additives in animal feed are dealt with in Naturland's processing standards, under the heading "Feed". **8.4** Feed from genetically altered organisms or their products is not permitted. **8.5** If feed ingredients of animal origin (particularly fishmeal/oil) have to be used for the culture of carnivorous[12] species with higher protein requirements, the following basic principles shall be respected: • The animal components in feed shall, where acceptable for nutritional physiological reasons, be replaced by vegetable products. Where feed is used which is not produced in the course of the farm's aquatic food chains, the proportion of animal components in the feed shall be lower than 100%. Provisional maximum values are set in Part B. II. (Supplementary Regulations for specific farming systems and animal species) • Feed shall not be obtained from conventionally reared terrestrial or aquatic animals. • In order to work towards a responsible utilisation of wild fish stocks, special standard requirements are set on the origin of fishmeal/oil • Fishmeal made from a certain species must not be used as feed for the same species.

		8.6 Feeding of natural pigments (e.g. in the form of Phaffia yeast or microorganisms)[13] is permitted.
Friend of the Sea[3]	Generic	Using animal feed certified by Friend of the Sea, when available on the market for the species bred is recommended. Alternatively, using trimmings from processing of edible products is recommended. Using animal feed produced by IFFO certified plants such as Responsible Sourcing / Responsible Production is required. GMO and growth hormones are not allowed.

Fasting

Food deprivation increases the sensitivity to the stress induced by high stocking densities of both European sea bass (Lupatsch, Santos, Schrama, & Verreth, 2010) and gilthead sea bream (Sangiao-Alvarellos *et al.*, 2005). However, prior to certain management practices such as grading, transport, disease treatment and slaughter, standard practice is to withdraw feed and fast fish. While fasting prior to slaughter is done in order to give the gut time to empty (more hygienic processing), and lower perivisceral fat (Grigorakis & Alexis, 2005) and firm the flesh (Beveridge, 1996) as per consumer preference, it is also done for welfare reasons. Emptying the gut reduces physiological stress by reducing metabolism, oxygen demand and waste production (Ashley, 2007a). Reduction in waste production, in turn, reduces the bacteria load in fish (López-Luna, Vásquez, Torrent, & Villarroel, 2013). Longer periods of starvation prior to slaughter may also affect production quality, for example, a recent study found that starving sea bream for more than 24 hours accelerated post mortem deterioration (increased pH, colour loss, higher bacterial counts, increased Quality index) when compared to 48 and 72 hours (Alvarez *et al.,* 2008). Flos *et al.* (2002), Huidobro *et al.* (2001), and Huidobro and Tejada (2004) all mention periods of fasting lasting 24 and 48 h in order to empty the gastrointestinal tracts in gilthead sea bream. In 2007, a questionnaire sent to fish farmers (Ferreira Pinto *et al.*, 2007) observed that 1 day was considered to be the minimum fasting period, with 8 days being the maximum. However, reasons for extending this period beyond 48 h included variations in the market price for the fish and the time needed to empty the cage or pond. While it is generally accepted that fish are tolerant to fasting (Navarro & Gutiérrez, 1995), prolonged feed deprivation may lead to aggression, cannibalism and affect welfare (Smith & Reay, 1991); therefore, the length of time fish are fasted should be limited.

Compassion recommends that fasting periods should be no longer than is required for the gut to empty and to reduce oxygen requirements. More research into the precise time required in degree days for this is needed but based on industry practice fasting for 24-48 hours, according to temperature, to enable gut evacuation in this time period appears to be practical. Therefore, sea bream and sea bass should not be fasted for longer than 48 hours at any one time for welfare reasons and fish should never be fasted for presumed flesh quality benefits. In hotter periods, this time should not exceed 24 hours. Procedures should be in place to ensure that this maximum time is adhered to for every fish in the pen. For example, where multiple harvests/days are required to slaughter all fish in a pen, the fish should be segregated so that fasting times can be adhered to. Records of the dates and duration of fasting should be kept.

[3]https://friendofthesea.org/wp-content/uploads/FOS_Aquaculture_Marine_rev2_03112014_en.pdf

GOOD HEALTH

Disease

Infectious diseases pose a risk to any intensive cultivation setting where large numbers of animals are housed in close proximity (Poppe, Barnes, & Midtlyng, 2002). Disease may cause animal welfare problems as well as significant stock and economic losses. There are several diseases that exist in sea bream and sea bass aquaculture. However, one of the major concerns is the condition referred to as "winter disease" in sea bream since it may cause high mortalities during the coldest months and acute mortality episodes when the temperature rises (Sarusic, 1999; Tort, Rotllant, & Rovira, 1998). This syndrome is mainly related to decreases in water temperatures to levels much lower than those recommended for optimal welfare conditions. The exposure to low temperatures affects the fish immune system producing a suppression of T-cell mitogenic or antibody responses (Bly, Buttke, Meydrech, & Clem, 1986; Clem *et al.*, 1984; Miller & Clem, 1984). Also, winter syndrome increases plasma cortisol, decreases the complement and lysozyme activities, and reduces circulating lymphocytes (Tort *et al.*, 1998). Thus, it decreases the ability of fish to resist attacks by opportunistic bacteria, viruses and parasites. Wild gilthead sea bream migrate to greater depths (warmer waters) when surface temperatures start to decline (Davis, 1988). However, the problem of low temperature may be critical in intensive cultured gilthead sea bream because the fish are unable to move to warmer waters. Symptoms include 'multi-organ dysfunction showing reduced sensitivity to stimuli, erratic swimming, pale and friable liver, necrotic muscles, atrophy of the exocrine pancreas, and distended digestive tract' (Ibarz *et al.*, 2010). Proper management (focusing on fish health during winter months instead of growth), feed management (reducing feed rations as temperatures drop), as well as using feed formulated to mitigate the effects of thermal metabolic stress (see below) have been promising. Winter feed diets high in lipids, marine-derived ingredients, vitamins (especially C and E) and minerals have had positive effects increasing growth, reducing the metabolic effects of thermal stress as well as improving immunity (L. Bavčević, S. Petrović, M. Crnica, & E. Corazzin, 2006; Schrama *et al.*, 2017; Tort et al., 2004). For more information on diseases of gilthead seabream and European seabass, please see Appendices 3 and 4.

Vaccines

Vaccines have proven effective against many of the bacterial pathogens of farmed fish but should be used in conjunction with good management practices. Both injectable and short-acting immersion vaccines have been used successfully, however therapeutic treatments themselves may be stressful to fish, depending on the delivery method. Many of the therapeutic agents, vaccines or protective immunostimulants, can be delivered in the feed without the need for handling and manipulation, which is better for welfare. For example, feeding fish glucan in low doses several weeks prior to a stressor shows potential for reducing the immunosuppressive effects of stress (Meena *et al.*, 2013) but care must be taken that this does not mask poor production methods, and all preventative treatment strategies need full welfare assessments. However, vaccine treatments that involve taking fish out of water, crowding or any other handling procedure will cause stress to fish.

Use of antimicrobials

Antimicrobials are commonly used both as a preventative measure as well as to combat disease outbreaks (Romero, Gloria, & Navarrete, 2012). One study showed that antimicrobials were present in sea bream muscle samples longer than previously thought and suggested that allergic reactions or resistance to antimicrobials may develop with high fish consumption over time (Rosa *et al.*, 2018). Antimicrobial resistance has become a serious problem and concerns have arisen regarding the impact antimicrobial resistance on the effectiveness of medical treatments. Therefore, the practice should be phased out. Furthermore, the Responsible Use of Medicines in Agriculture Alliance (RUMA) has formulated guidelines for the responsible use of antimicrobials in fish production[4] which should be strictly followed. For example, initiating treatment only under direct veterinary approval, following treatment protocols strictly and completing the entire course of treatment using correct dosages.

The development of natural immunostimulants (pre- and pro-biotics) seems promising (Cordero, Morcillo, Cuesta, Brinchmann, & Esteban, 2016); for a review see Carbone & Faggio (2016); however, addressing the highly intensive systems as the underlying cause of irresponsible antibiotic use is also vital.

Compassion recommends that all disease treatments should be recorded in the veterinary health and welfare plan and only when prescribed by a vet. Guidelines produced by RUMA regarding the Responsible Use of Antimicrobials in Fish Production[1] and the Responsible Use of Vaccines and Vaccination in Fish Production[1] should be followed. Disease risk should be assessed on a site-by-site basis and prevention via vaccination should be prioritised. The veterinary health and welfare plan should outline planned husbandry procedures, risk assessments, disease monitoring and details of all treatments carried out. The continued development of cost-effective authorised vaccines should be supported by producers' organisations and the veterinary profession. High levels of antibiotic use in farming systems is indicative of health and welfare problems at a systemic level and should be immediately addressed.

ABILITY TO EXPRESS NATURAL BEHAVIOURS

Many standard management practices are likely to cause stress for European sea bass and gilthead sea bream, including crowding, grading and handling.

Crowding is a stressful procedure that may cause lesions, as fish are unable to avoid each other and can be a prime cause of poor welfare (Southgate & Wall, 2001). The main problem is often a lack of sufficient oxygen as well as elevated levels of ammonia as at high stocking densities increase oxygen uptake fish (Carbonara, Scolamacchia, Spedicato, Zupa, Mckinley, *et al.*, 2015) and the increase in fish excrement leads to gill damage (Araújo-Luna *et al.*, 2018). Gilthead sea bream experienced significant rapid increases in blood cortisol and glucose following short-term crowding and it took 2-3 days for their immune system to recover. Similarly, European sea bass stocked at 45 kg/m^3 for six weeks then crowded at 100 kg/m^3 for only 15 minutes took 24-48 hours to recover (Ortuño, Esteban, & Meseguer, 2001). Folkedal *et al* (2018) investigated the effect of classic Pavlovian conditioning on sea bream exposed to an aversive stimulus finding conditioned fish

[4]https://www.ruma.org.uk/responsible-use-of-antimicrobials-in-fish-production/

switched from a fright reaction to anticipating a reward. As fish may be crowded up to 15 times during harvesting, classic Pavlovian conditioning to the repeated procedure may potentially make an otherwise stressful experience rewarding.

Grading is a managing practice carried out to prevent large variation in size of individual fish. However, grading is a high stress situation that leads to aggression, cannibalism, and further growth differentiation (EFSA 2008) and may cause physical injury, such as fin damage (Person-Le Ruyet & Le Bayon, 2009). Lordosis – an abnormal V-shaped curvature of the spine – can also be caused by trauma during handling (Chatain, 1994) as well as an increase in plasma cortisol, α-MSH, glucose, lactate, osmolality and plasma Na, Cl, and Mg (Arends, Mancera, Muñoz, Wendelaar Bonga, & Flik, 1999). European sea bass and gilthead sea bream are graded up to three times. To minimise the negative effects on growth, fish quality and production, grading is typically done when fish are 25-40 g and, again, around 100 g (EFSA 2008).

Many farm management activities involve **handling** of fish. Handling is stressful and often entails removal from the water, therefore, it should only be carried out when absolutely necessary. Care must be taken at all stages to avoid abrasions and removal of scales and the fish's protective mucus coat, which serves as a physical and chemical barrier to infection as well as being important in osmoregulation and locomotion (Ashley, 2007). Once out of water the fish should be kept moist, handled using wet hands and for a maximum time of 15 sec, unless anaesthetised (RSPCA guidelines).It is important to note that in EFSA's report on the animal welfare aspects of husbandry systems for farmed European sea bass and gilthead sea bream, says "handling (not according to best practice) was judged to occur in nearly all farms, affecting the entire population for approximately 30 days with moderate severity" (EFSA, 2008).

Fish coping styles

While investigating the effects of rearing gilthead sea bream at different stocking densities (up to 20 kg/m^3) as well as handling stress applied to the fish raised at 10 kg/m^3 on various physiological behavioural response parameters, Sánchez-Muros *et al.*(2017) found no physiological and few behavioural effects of treatment. However, they stated that the variation between individual responses in the behavioural tests (open field, neophobia, and object presentation repeat) was 'remarkable'. Two types of personality have been described in animals: proactive – individuals with active coping style or bold, aggressive personalities; and reactive – passive coping, shy, or non-aggressive individuals. In fish, personality has been linked to growth performance and feed conversion, metabolism, cortisol responsiveness, and learning.

Indeed, Millot *et al.* (2009b) found wild European sea bass were initially bolder but decreased their risk-taking behaviour over time, whereas farmed-fish were consistent in their risk-taking behaviour. Risk-taking behaviour (boldness) was positively correlated to competitive ability (bolder fish were quicker in gaining access to food). High metabolic demands have been linked to high risk-taking in foraging, as a higher metabolic demand of individuals means that they must be more successful in increasing feed intake (Careau *et al.*, 2008). This correlation between an increase in metabolism (as demonstrated by an increase in activity and oxygen consumption) and risk-taking behaviour has been demonstrated in gilthead sea bream (Herrera *et al.*, 2014). However, the increased energetic demand does not seem to have negative effect on growth. In fact, Millot *et al.* (2009a) found that selecting for increased growth in sea bass seemed to concurrently select for bolder personality. Furthermore, selecting for growth had a greater effect on personality than selecting for the behavioural trait (boldness) itself. They concluded that bolder fish are better adapted to the artificial environments of commercial production.

Nonetheless, aggression is a trait of a bold, proactive personality. Therefore, selecting for fast growing, bold fish may simultaneously select for increased aggression which can impair fish welfare. Aggression has, ironically, been linked to lower feed intake and growth dispersion, chronic stress, and disease vulnerability (see (Martins *et al.*, 2012)). Castanheira *et al.*, found both risk-taking (2013a) and aggression (2013b) to be consistent behaviours in gilthead sea bream, the latter being linked to lower cortisol values, and 'likely to be distinctive traits of [active] coping styles' (Table 2).

Table 2. Fish coping styles

(Table adapted from Castanheira *et al.*, 2017)

Source	**Species**	**Tests**	**Details**
(Castanheira *et al.*, 2013b)	Gilthead sea bream	Restraining/ aggression	Fish with lower cortisol levels (proactive) when exposed to stress are more aggressive.
(Castanheira *et al.,* 2013a)	Gilthead sea bream	Feed intake recovery, novel object, restraining, risk taking	Behavioural differences are consistent over time and predictable based on other behaviours. Possibility to predict behaviour in groups from individual personality traits.
(Herrera *et al.*, 2014)	Gilthead sea bream	Risk-taking, hypoxia	Risk-taking was positively correlated to movement and metabolism.
(Ferrari *et al.*, 2014)	European sea bass	Feed intake recovery, exploration, restraining, risk-taking, hypoxia	Behavioural differences were not consistent over time or across context in individual-based tests. In contrast, strong individual consistency was observed for all variables measured in group-based tests. Hypoxia-avoiders has lower cortisol rate and high activity and were higher risk takers: the three characteristics of proactive coping style.
(Millot *et al.*, 2009a)	European sea bass	Exploration + swimming activities after stimulation	Whatever the level of domestication and selection presented the same flight response and stimulus exposure induced a significant decrease in exploratory behaviour and swimming activity. Only one generation of captivity could be sufficient to obtain fish presenting the same coping style characteristics (bolder) than fish reared for at least two generations.
(Millot *et al.*, 2009b)	European sea bass	Risk taking	Wild fish were generally bolder than selected fish during two-first days of test but showed a decrease in risk-taking behaviour during a third-day test. Selected fish showed a constant

			increase in their risk-taking behaviour over time.

Compassion recommends that all the fish-handling practices should be planned and prepared in advance in order to have the appropriate equipment available. It should be done carefully in order to decrease the natural fish escaping reactions, which can lead to over-excited swimming behaviour which leads to injuries, exhaustion, stress and ultimately to poor welfare. Grading, crowding and handling should be done with care, by trained professionals and only when needed. Using classical Pavlovian conditioning for rewarding aversive stimuli may be used to reduce stress in situations where adverse experiences cannot be avoided. Welfare outcomes should be measured and recorded for sea bass and sea bream and include parameters such as swimming behaviour, feeding behaviour, skin and fin damage and skeletal deformities. Further work to develop more behavioural indicators of positive welfare for sea bass and sea bream are required.

APPENDIX 1. Stocking densities in scientific literature for gilthead sea bream and European sea bass

Source	Species	Density (extremes)	Detail
Requirements of certification schemes			
Naturland 2018[5]	Perciformes (perch-like), Carangiformes (jack-like) and Gadiformes (cod-like) fish species in marine net cages	Shall not exceed 10 kg/m^3	In no case shall the animals display any injuries (e.g. of the fins) indicating too high stocking densities.
Soil Association 2017[6]	Sea bass and sea bream	Open water containment systems: 15 kg/m^3; in earth ponds of tidal areas and coastal lagoons: 4 kg/m^3	According to the Organic aquaculture regulation Nº 889/2008→amended 710/2009).
Examples in commercial practice			
Common practices in Turkey (Ökte, 2002)	Sea bream	2-5g: 100-150 kg/m^3 20-50g: 70-100 kg/m^3 >50g: 12-15 kg/m^3	These figures can change, according to many variables, like temperature and the dissolved oxygen concentration at the cage site.
Common practices in Turkey (Mente *et al.*, 2012)	Sea bream	Low SD: 4 kg/m^3 High SD: 15 kg/m^3	Common practice for conventional sea cages: SD of 15-20 kg/m^3.
FAO, 2005[7]	Sea bream	Extensive: 0.0025kg/m^3 Semi-intensive: 1 kg/m^3 Intensive: 15-45 kg/m^3	Massive oxygen injection is needed to ensure fish survival in intensive systems.
EFSA, 2008	Sea bream	2.5-150g: 5-10 kg/m^3 >150g: 10-20 kg/m^3	"Commercial experience."
(Jobling, 2010)	Sea bream	SD usually lower in sea cages:10-15 kg/m^3 Higher in land-based tanks and raceways 15—50 kg/m^3	

[5]https://www.naturland.de/images/UK/Naturland/Naturland_Standards/Standards_Producers/Naturland-Standards_Aquaculture.pdf
[6]https://www.soilassociation.org/media/15726/soil-association-aquaculture-standards-v1-3-may-2017.pdf
[7]http://www.fao.org/fishery/culturedspecies/Sparus_aurata/en

Research comparing different stocking densities			
(Araújo-Luna, Ribeiro, Bergheim, & Pousão-Ferreira, 2018)	Sea bream	Low SD: 5 kg/m^3 High SD: 20 kg/m^3	No effect of density.
(Batzina, Dalla, Papadopoulou-Daifoti, *et al.*, 2014)	Sea bream	Lower SD: 4.9 kg/m^3 (start) to 14.7 kg/m^3 (end of experiment) Higher SD: 9.7 kg/m^3 (start) to 29.9 kg/m^3 (end of experiment)	Lower aggression and less size variation in the higher density group.
(Montero, Izquierdo, *et al.*, 1999)	Sea bream	Low SD: 10.04 kg/m^3 High SD: 40.8 kg/m^3	High SD resulted in 4-fold increase in cortisol.
(Sánchez-Muros *et al.*, 2017)	Sea bream	Low SD: 5 kg/m^3 High SD: 20 kg/m^3	Reduced growth at high SD; No differences in total antioxidant capacity (TEAC) and lipid peroxidation (MDA) in the liver or the adrenocorticotropic hormone (ACTH), cortisol, alanine aminotransferase (AAT) and glucose in plasma over time; Physiological parameters did not reveal important differences among treatments (MD, HD, LD, H).
(Sangiao-Alvarellos *et al.*, 2005)	Sea bream	"Normal" SD: 4 kg/m^3 High SD: 70 kg/m^3	High SD: 5-fold increase in cortisol; 20% increase in plasma glucose; 60% decrease in liver glycogen; 20% increase in gluconeogenic potential in the liver; 100% increase in liver glucose phosphorylating capacity; 30% decrease in capacity for phosphorylating glucose of gills; 80% increase in the capacity of phosphorylating glucose of kidneys; 2.5-fold increase in brain ATP levels; Food deprivation increased the sensitivity of gilthead sea bream to the stress induced by HSD.
(Valente *et al.*, 2011)	Sea bream	Extensive: 0.03 kg/m^3	

		Semi-intensive: 0.5-4.5 kg/m³ Intensive: 10–70 kg/m³	
(Di Marco *et al.*, 2017)	Sea bream and sea bass	15 kg/m³	
(Papoutsoglou *et al.*, 1996)	Sea bream and sea bass	16 kg/m³	
(Roncarati, Melotti, Dees, Mordenti, & Angellotti, 2006)	Sea bream and sea bass	Low SD: 0.2 kg/m³ High SD: 40 kg/m³	A density of 20 kg/m³ is considered acceptable for raising healthy on-growing seabass and seabream juveniles.
FAO, 2005[8]	Sea bass	Stocking densities 20-35 kg/m³	In intensive (land-based) systems.
EFSA, 2008	Sea bass	150g: 5-10 kg/m³ >150g: 10-20 kg/m³	"Commercial experience."
(M. Jobling, 2010)	Sea bass	SD usually lower in sea cages: 20 kg/m³ Higher in land-based tanks and raceways 40-50 kg/m³	
(Abou Zied, 2010)	Sea bass	Low SD: 1.0 kg/m³ High SD: 2.0 kg/m³	1.5 kg/m³ stated to be optimal.
(Carbonara, Scolamacchia, Spedicato, Zupa, Mckinley, *et al.*, 2015)	Sea bass	Low SD: 10 kg/m³ High SD: 50 kg/m³	Muscle activity of high SD was two times higher than low SD.
(Roque d'Orbcastel et al., 2010)	Sea bass	Low SD: 10 kg/m³ High SD: 100 kg/m³	No differences in cortisol values nor susceptibility to nodavirus; No differences in growth rate between high and low SD but fish at 20 and 40 kg/m³ had higher growth rates
Di Marco *et al.*, 2007 as cited in EFSA, 2008	Sea bass	Low SD: 10 kg/m³ High SD: 100 kg/m³	Early mortality at high SD; Even distribution and "quiet" low SD; Polarized displacement and high swimming speeds at high SD; -10% feed intake and -14% growth rate at high SD.

[8] http://www.fao.org/fishery/culturedspecies/Dicentrarchus_labrax/en

			No differences in the feed conversion ratio and cortisol levels, susceptibility to nodavirus or other stress indicators (Na+, K+, glucose, pH, haematocrit).
(Di Marco *et al.*, 2008)	Sea bass	Low SD: 15 kg/m^3 Medium SD: 30 kg/m^3 High SD: 45 kg/m^3	Stress test induced higher levels of cortisol and NEFA and lower levels of glucose high SD fish.
(Person-Le Ruyet & Le Bayon, 2009)	Sea bass	Experimental conditions: Low SD: 20 kg/m^3 High SD: 120 kg/m^3 Farm conditions: Lower SD in sea cage: <25 kg/m^3 Higher SD in recirculating aquaculture system (RAS): 60 kg/m^3	100–250 g fish: Fin damage 10X higher in high SD. 350–890 g fish: Lower fin damage at high SD.
(Santos *et al.*, 2010)	Sea bass	Low SD: 8.1 kg/m^3 High SD: 75.4 kg/m^3	Increased density levels reduced feed intake and growth; Extreme low and extreme high SD resulted in increased feed conversion ratio compared to medium SD; Increased density reduced swimming speeds; No differences in control: blood cortisol, glucose and lactate parameters; however, a stress test induced higher levels of plasma cortisol in high SD fish.

APPENDIX 2. Water quality parameters in literature for gilthead sea bream and European sea bass

	Species	Water Temperature	Water Quality	Detail
Naturland, 2018[9]	Perciformes (perch-like), Carangiformes (jack-like) and Gadiformes (cod-like) fish species in marine net cages			"The water quality (e.g. temperature, pH, salinity, oxygen, ammonium and nitrate concentrations) must conform to the natural requirements of the species in question."
Friend of the Sea[10]	Generic		Ammonium (NH_4) ≤1 mg/L Nitrate (NO_3 -) ≤15 mg/L Nitrite (NO_2) ≤1 mg/L Phosphorus (PO_4) ≤0.2 mg/L Dissolved Oxygen ≥5 mg/L CO_2 <2.0 ppm Cd ≤0.05 mg/kg Pb ≤0.03 mg/kg Zn ≤0.02 mg/kg Cu ≤0.01 mg/kg Trophic Index value <6	The water quality parameters and the sediment parameters under the sea cages shall comply with the provisions of the existing FAO regulations. Water quality parameters shall be monitored at least once every six months. The distance between the lower part of the cage and the bottom shall be at least 15m.
(Araújo-Luna *et al.*, 2018)	General		Gill damage when unionized ammonia > 0.05 mg/L	
(Blancheton, 2000)	Sea bass	22-24ºC	pH 6.5-8.3 O_2 >90 CO_2< 40 Ammonium (NH_4) ≤2 mg/L Nitrate (NO_3^-) ≤100 mg/L	

[9]https://www.naturland.de/images/UK/Naturland/Naturland_Standards/Standards_Producers/Naturland-Standards_Aquaculture.pdf
[10]https://friendofthesea.org/wp-content/uploads/FOS_Aquaculture_Marine_rev2_03112014_en.pdf

			Nitrite (NO_2) ≤2 mg/L	
(Claireaux & Lagardère, 1999)	Sea bass	2-32°C		In autumn the falling of sea temperature below 10 °C is associated with their migration into deeper, warmer water.
EFSA, 2008	Sea bass	10-20°C for eggs, larvae 8-28°C for larger fish	O_2 should be 40% above saturation pH: 6.5-8.5; pH below 5 and above 9 impair growth and welfare. CO_2: Lethal concentration in juvenile seabass (LC 50 at 96 h, at 15°C) is close to 112.1 m CO_2/L (50.4 mm Hg). Ammonia: 0.26-mg/L UIA-N	Minimum and maximum survival water temperatures are 2-32°C. Ammonia in seawater is not a welfare issue in on-growing cage systems because it is diluted generally at non-limiting levels by the ambient water streams.
EFSA, 2008	Sea bream	12-22°C for eggs, larvae 8-30°C for larger fish	O_2 should be 40% above saturation pH: 6.5-8.5; pH below 5 and above 9 impair growth and welfare	Minimum and maximum survival water temperatures are 5-34°C. Sea bream are sensitive to cold temperatures. Acute temperature decreases (from 15°C to 9°C) have been shown to be significant thermal stressors. When cold-induced fasting is prolonged, it significantly affects metabolism and physiology of sea bream, and it has been associated with the onset of winter disease.

FAO, 2005[11]	Sea bream	18-26 °C		
FAO, 2005[12]	Sea bass	13-18 °C		A recirculation system, to control water temperature (between 13-18°C) is used during autumn/winter.
(M. Jobling, 2010)	Sea bream	"Optimal" 18-26°C		Not possible to control temperature and other water quality parameters in cages.
(Ökte, 2002)	Sea bream	Up to 30ºC		Do not tolerate cooler waters unlike sea bass. Daily feed ration of sea bream can double with a temperature increase from 12-22ºC. O_2 5 mg/L is the minimum required by fish during growout. Sea bream is more sensitive to low oxygen than sea bass. Oxygen is also very important to feed conversion: low oxygen results in high FCR.
(Roque d'Orbcastel *et al.*, 2010)	Sea bass		Dissolved Oxygen ≥5 mg/L Ammonia Nitrogen (NH4; TAN) 0.5-2 mg Nitrite-Nitrogen (NO_2-N) 0.5–2 mg Dissolved Oxygen 6-7 mg/L CO_2 <40 mg/L	

[11]http://www.fao.org/fishery/culturedspecies/Sparus_aurata/en
[12]http://www.fao.org/fishery/culturedspecies/Dicentrarchus_labrax/en

(Person-Le Ruyet & Le Bayon, 2009)	Sea bass	13-16°C 25°C		Fin damage 5x higher at 25°C than 13-16°C Fin damage 35% lower at 53% O_2 saturation than at 105%).

APPENDIX 3. **Gilthead sea bream – diseases and control measures (FAO, 2005)**

DISEASE	AGENT	TYPE	SYNDROME	MEASURES	
Pasteurellosis (Pseudotuberculosis)	*Photobacterium damselae* subsp. *Piscicida*	Bacterium	Anorexia; focal necrosis of the gills	Vaccination of broodstock and juveniles; use of immunostimulant and vitamin treatments; good hygiene and disinfection of water supply; antibiotics	Pasteurellosis is a significant disease affecting sea bass and sea bream. When there is an outbreak, the disease is usually controlled by the use of approved antibiotics given with the feed. Pasteurellosis prevention can be effectively achieved by the use of a correct vaccination protocol using commercial vaccines, although the efficacy of these vaccines needs to be improved.
Vibriosis	*Photobacterium damselae* subsp. *damselae*	Bacterium	Dark skin; lethargy; distended abdomen; haemorrhages	Avoid use of feed with very high lipid levels; antibiotics	Vibriosis prevention can be effectively achieved by the use of a correct vaccination protocol using commercial vaccines but it can still be a serious problem in hatcheries as protection can only be achieved after vaccination at 2-3 grams. Careful and efficient sanitary controls on farms including prophylactic measures such as a vaccination with rapid diagnostic and treatment programmes should be recommended as the main ways to control this disease.
	Vibrio alginolyticus	Bacterium	Haemorrhages; dark skin; skin lesions	Good hygiene; antibiotics	
	Vibrio anguillarum	Bacterium	Lethargy; anorexia; head down position	Good hygiene and disinfection of water supply; antibiotics	
Lymphocystis	*Iridoviridae*	Virus	Whitish pseudotumour	Reduce feeding rate; reduce biomass; avoid additional stress on diseased fish;	Lymphocystis is a benign disease that spontaneously disappears if rearing conditions are correct. Good husbandry conditions during the infection should be

				low pathogenicity - no treatment	implemented for a quick and total recovery and stressful and rough manipulation should be avoided.
Aquareovirus	Aquareovirus	Virus	None	Low pathogenicity - no treatment	
Distended Gut Syndrome (DGS)	Virus-like particle	Virus	Distended abdomen; disoriented spinning motion; immobility with the head down	Effective UV treatment of incoming water during first larval stages	
Parasitic Enteritis	*Myxidium leei*	Endoparasite	Lethargy; distended abdomen; hyperpigmentation	Avoid stressing fish; no treatment	
Gill fluke infections	*Diplectanum aequans*; *D. laubieri*	Monogenean trematode	Skin cloudiness; focal reddening with excess mucus production; epithelial hyperplasia; gill haemorrhages	Correct prophylaxis; good husbandry condition	Preventive treatments using formalin or hydrogen peroxide are useful but cannot always be carried out. Therefore, routine net and tank cleaning operations, in addition to other preventive measures, are the most reliable means to control the level of the parasites and keep the disease at a low level.
Winter Disease Syndrome	*Pseudomonas anguilliseptica* (multifactorial)	Bacterium	'Belly up' syndrome, with or without the presence of haemorrhaging	Effective disinfection and dry-out period for land-based fattened units; adapt feeding regime prepare fish for winter period; antibiotic treatment ineffective *in vivo*	Correct management before the cold season (avoid feeding when temperatures are low and reduce stressful management) minimises the risk of the disease. Correct nutritional and husbandry measures before the cold period to prepare the fish to achieve an adequate metabolic status should be encouraged.

APPENDIX 4. European sea bass – diseases and control measures (FAO, 2005)

DISEASE	AGENT	TYPE	SYNDROME	MEASURES	
Viral encephalo-retinopathy	Nodavirus	Virus	Nervous symptoms	Good prophylaxis; good husbandry conditions	Broodstock testing for Nodavirus carriers, disinfection of the incoming water and strict hygiene of the facility and husbandry practices can be effective measures to guarantee the quality of fry and juveniles supplied to ongrowing units. No commercial vaccines are available and there is no treatment. As the management of the mortality is a critical issue in the control of the disease, the procedures for the removal of dead or moribund fish should be improved.
Vibriosis	*Vibrio anguillarum*; *Vibrio ordali*; *Vibrio* spp	Bacteria	Anorexia; darkening; skin ulcers; abdominal distension; splenomegaly; visceral petechiation; necrotic enteritis	Fry vaccination; antibiotic treatment	Vibriosis prevention can be effectively achieved by the use of a correct vaccination protocol using commercial vaccines but it can still be a serious problem in hatcheries as protection can only be achieved after vaccination at 2-3 grams. Careful and efficient sanitary controls on farms including prophylactic measures such as a vaccination with rapid diagnostic and treatment programmes should be recommended as the main ways to control this disease.
Photobacteriosis or Pseudotuberculosis	*Photobacterium damsela* subsp. *Pasteurella*	Bacterium	Anorexia; darkening; splenomegaly; miliary lesions of spleen or spleen granulomatosis (chronic form)	Antibiotic treatment	
Pasteurellosis (Pseudotuberculosis)	*Photobacterium damselae* subsp. *Piscicida*	Bacterium	Anorexia; focal necrosis of the gills	Vaccination of broodstock and juveniles; use of immunostimulant and vitamin treatments; good hygiene and disinfection of	Pasteurellosis is a significant disease affecting seabass and sea bream. When there is an outbreak, the disease is usually controlled by the use of approved antibiotics given with the feed. Pasteurellosis prevention can be effectively achieved by the use of a correct vaccination protocol using commercial vaccines, although the

				water supply; antibiotics	efficacy of these vaccines needs to be improved.
Mycobacteriosis	*Mycobacterium marinum*	Bacterium	Emaciation; poor growth; hypertrophic kidney and spleen with granulomas	Good prophylaxis	
Epitheliocystis	*Chlamydia*-like	Bacterium	Miliary nodules on skin or gills	Good prophylaxis	
Amyloodiniasis	*Amyloodinium occelatum*	Dinoflagellate	Skin darkening; skin dusty appearance (velvet disease)	Freshwater treatment	
Cryptocaryoniasis	*Cryptocaryon irritans*	Ciliates	Skin lesions; white spot or multifocal white patches (marine white spot disease)	Freshwater treatment	
Scuticociliatosis; other ciliatosis	*Philasterides dicentrarchi*; *Uronema* sp.; *Tetrahynema* sp.	Ciliates	Skin and gill lesions; depigmentation; ulcerations; skin area haemorrhages	Freshwater treatment	
Myxosporidiosis	*Shaerospora dicentrarchi*; *S. testicularis*; *Ceratomyxa labraci*	Myxosporidia	Reduced production; reduced growth rate; low mortality	No treatment	
Microsporidiosis	*Glugea* sp.	Microsporidia	Reduced production; low mortality	No treatment	
Gill fluke infections	*Diplectanum aequans*; *D. laubieri*	Monogenean trematode	Skin cloudiness; focal reddening with excess mucus production; epithelial hyperplasia; gill haemorrhages	Correct prophylaxis; good husbandry condition	Preventive treatments using formalin or hydrogen peroxide are useful but cannot always be carried out. Therefore, routine net and tank cleaning operations, in addition to other preventive measures, are the most reliable means to control the level of the parasites and keep the disease at a low level.
Anisakis infection	*Anisakis* spp.	Nematoda	Larvae in coelomatic cavity	Correct prophylaxis	
Isopodiasis	*Ceratothoa oestroides*; *Nerocilla*	Crustacea (isopods)	Growth retardation; gills and skin	Correct prophylaxis	

	orbiguyi; *Anilocra physoides*		tissue necrosis; adults and larvae on fish		

REFERENCES

Abou Zied, R. (2010). *EFFECT OF STOCKING DENSITY ON GROWTH PERFORMANCE AND FEED UTILIZATION OF SEA BASS (Dicentrarchus labrax) IN CAGES SUSPENDED ON NATURAL POND* (Vol. 24).

Andrades, J. A., Becerra, J., & Fernández-Llebrez, P. (1996). Skeletal deformities in larval, juvenile and adult stages of cultured gilthead sea bream (Sparus aurata L.). *Aquaculture*, *141*(1–2), 1–11. https://doi.org/10.1016/0044-8486(95)01226-5

Andrew, J. E., Noble, C., Kadri, S., Jewell, H., & Huntingford, F. A. (2002). The effect of demand feeding on swimming speed and feeding responses in Atlantic salmon Salmo salar L., gilthead sea bream Sparus aurata L. and European sea bass Dicentrarchus labrax L. in sea cages. *Aquaculture Research*, *33*(7), 501–507. https://doi.org/10.1046/j.1365-2109.2002.00740.x

Animal welfare aspects of husbandry systems for farmed European seabass and gilthead seabream - Scientific Opinion of the Panel. (2008). *EFSA Journal*, *6*(11), 844. https://doi.org/10.2903/j.efsa.2008.844

Araújo-Luna, R., Ribeiro, L., Bergheim, A., & Pousão-Ferreira, P. (2018). The impact of different rearing condition on gilthead seabream welfare: Dissolved oxygen levels and stocking densities. *Aquaculture Research*, *49*(12), 3845–3855. https://doi.org/10.1111/are.13851

Arends, R. J., Mancera, J. M., Muñoz, J. L., Wendelaar Bonga, S. E., & Flik, G. (1999). The stress response of the gilthead sea bream (Sparus aurata L.) to air exposure and confinement. *The Journal of Endocrinology*, *163*(1), 149–157. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10495417

Ashley, P. J. (2007a). Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science*, *104*(3–4), 199–235. https://doi.org/10.1016/j.applanim.2006.09.001

Ashley, P. J. (2007b). Fish welfare: Current issues in aquaculture. *Applied Animal Behaviour Science*, *104*(3–4), 199–235. https://doi.org/10.1016/J.APPLANIM.2006.09.001

Batzina, A., Dalla, C., Papadopoulou-Daifoti, Z., & Karakatsouli, N. (2014). Effects of environmental enrichment on growth, aggressive behaviour and brain monoamines of gilthead seabream Sparus aurata reared under different social conditions. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *169*, 25–32. https://doi.org/10.1016/J.CBPA.2013.12.001

Batzina, A., Dalla, C., Tsopelakos, A., Papadopoulou-Daifoti, Z., & Karakatsouli, N. (2014). Environmental enrichment induces changes in brain monoamine levels in gilthead seabream Sparus aurata. *Physiology and Behavior*, *130*, 85–90. https://doi.org/10.1016/j.physbeh.2014.03.023

Batzina, A., & Karakatsouli, N. (2012). The presence of substrate as a means of environmental enrichment in intensively reared gilthead seabream Sparus aurata: Growth and behavioral effects. *Aquaculture*, *370–371*, 54–60. https://doi.org/10.1016/J.AQUACULTURE.2012.10.005

Beveridge, M. C. M. (1996). *Cage Aquaculture* (2nd ed.). Oxford, U.K.

Blancheton, J. P. (2000). Developments in recirculation systems for Mediterranean fish species. *Aquacultural Engineering*, *22*(1–2), 17–31. https://doi.org/10.1016/S0144-8609(00)00030-3

Bly, J. E., Buttke, T. M., Meydrech, E. F., & Clem, L. W. (1986). The effects of in vivo acclimation temperature on the fatty acid composition of channel catfish (Ictalurus punctatus) peripheral blood cells. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *83*(4), 791–795. https://doi.org/10.1016/0305-0491(86)90148-3

Brill, R. W., Horodysky, A. Z., Place, A. R., Larkin, M. E. M., & Reimschuessel, R. (2019). Effects of dietary taurine level on visual function in European sea bass (Dicentrarchus labrax). *PLOS ONE*, *14*(6), e0214347. Retrieved from https://doi.org/10.1371/journal.pone.0214347

Canario, A. V. M., Condeça, J., Power, D. M., & Ingleton, P. M. (1998). The effect of stocking density on growth in the gilthead sea-bream, *Sparus aurata* (L.). *Aquaculture Research*, *29*(3), 177–181. https://doi.org/10.1046/j.1365-2109.1998.00954.x

Carbonara, P., Scolamacchia, M., Spedicato, M. T., Zupa, W., Mckinley, R. S., & Lembo, G. (2015). Muscle activity as a key indicator of welfare in farmed European sea bass (Dicentrarchus labrax L. 1758). *Aquaculture Research*, *46*(9), 2133–2146. https://doi.org/10.1111/are.12369

Carbonara, P., Scolamacchia, M., Spedicato, M. T., Zupa, W., McKinley, R. S., & Lembo, G. (2015). Muscle activity as a key indicator of welfare in farmed European sea bass (*Dicentrarchus labrax* L. 1758). *Aquaculture Research*, *46*(9), 2133–2146. https://doi.org/10.1111/are.12369

Carbone, D., & Faggio, C. (2016). Importance of prebiotics in aquaculture as immunostimulants. Effects on immune system of Sparus aurata and Dicentrarchus labrax. *Fish & Shellfish Immunology*, *54*, 172–178. https://doi.org/10.1016/J.FSI.2016.04.011

Celi, M., Filiciotto, F., Maricchiolo, G., Genovese, L., Quinci, E. M., Maccarrone, V., … Buscaino, G. (2016). Vessel noise pollution as a human threat to fish: assessment of the stress response in gilthead sea bream (Sparus aurata, Linnaeus 1758). *Fish Physiology and Biochemistry*, *42*(2), 631–641. https://doi.org/10.1007/s10695-015-0165-3

Chatain, B. (1994). Abnormal swimbladder development and lordosis in sea bass (Dicentrarchus labrax) and sea bream (Sparus auratus). *Aquaculture*, *119*(4), 371–379. https://doi.org/10.1016/0044-8486(94)90301-8

Claireaux, G., Couturier, C., & Groison, A.-L. (2006). Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (Dicentrarchus labrax). *Journal of Experimental Biology*, *209*(17), 3420–3428. https://doi.org/10.1242/jeb.02346

Claireaux, G., & Lagardère, J.-P. (1999). Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. *Journal of Sea Research*, *42*(2), 157–168. https://doi.org/10.1016/S1385-1101(99)00019-2

Clem, L. W., Faulmann, E., Miller, N. W., Ellsaesser, C., Lobb, C. J., & Cuchens, M. A. (1984). Temperature-mediated processes in teleost immunity: Differential effects of invitro and invivo temperatures on mitogenic responses of channel catfish lymphocytes. *Developmental & Comparative Immunology*, *8*(2), 313–322. https://doi.org/10.1016/0145-305X(84)90038-7

Conte, F. . (2004). Stress and the welfare of cultured fish. *Applied Animal Behaviour Science*, *86*(3–4), 205–223. https://doi.org/10.1016/j.applanim.2004.02.003

Cordero, H., Morcillo, P., Cuesta, A., Brinchmann, M. F., & Esteban, M. A. (2016). Differential proteome profile of skin mucus of gilthead seabream (Sparus aurata) after probiotic intake and/or overcrowding stress. *Journal of Proteomics*, *132*, 41–50. https://doi.org/10.1016/J.JPROT.2015.11.017

Davis, P. S. (1988). Two occurrences of the gilthead, Sparus aurata Linnaeus 1758, on the coast of Northumberland, England. *Journal of Fish Biology*, *33*(6), 951–951. https://doi.org/10.1111/j.1095-8649.1988.tb05545.x

Di Marco, P., Petochi, T., Marino, G., Priori, A., Finoia, M. G., Tomassetti, P., … Poli, B. M. (2017). Insights into organic farming of European sea bass Dicentrarchus labrax and gilthead sea bream Sparus aurata through the assessment of environmental impact, growth performance, fish welfare and product quality. *Aquaculture*, *471*, 92–105. https://doi.org/10.1016/J.AQUACULTURE.2017.01.012

Di Marco, P., Priori, A., Finoia, M. G., Massari, A., Mandich, A., & Marino, G. (2008). Physiological responses of European sea bass Dicentrarchus labrax to different stocking densities and acute stress challenge. *Aquaculture*, *275*(1–4), 319–328. https://doi.org/10.1016/j.aquaculture.2007.12.012

Ellis, T., North, B., Scott, A. P., Bromage, N. R., Porter, M., & Gadd, D. (2002). The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology*, *61*(3), 493–531. https://doi.org/10.1006/jfbi.2002.2057

EUMOFA. (2017). The EU Fish Market.

FAO Fisheries and Aquaculture Department. (n.d.-a). Cultured Aquatic Species Information Programme - Dicentrarchus labrax (Linnaeus, 1758). Retrieved June 21, 2019, from http://www.fao.org/fishery/culturedspecies/Dicentrarchus_labrax/en#tcNA00C5

FAO Fisheries and Aquaculture Department. (n.d.-b). Cultured Aquatic Species Information Programme - Sparus aurata (Linnaeus, 1758). Retrieved June 21, 2019, from http://www.fao.org/fishery/culturedspecies/Sparus_aurata/en

Ferreira, J. G., Saurel, C., & Ferreira, J. M. (2012). Cultivation of gilthead bream in monoculture and integrated multi-trophic aquaculture. Analysis of production and environmental effects by means of the FARM model. *Aquaculture*, *358–359*, 23–34. https://doi.org/10.1016/J.AQUACULTURE.2012.06.015

Ferreira Pinto, J., Nunes, M.L., Cardoso, C., 2007. Feeding interruption and quality of cultured gilthead sea bream. Food Chem. 100, 1504–1510.

Filiciotto, F., Cecchini, S., Buscaino, G., Maccarrone, V., Piccione, G., & Fazio, F. (2017). Impact of aquatic acoustic noise on oxidative status and some immune parameters in gilthead sea bream Sparus aurata (Linnaeus, 1758) juveniles. *Aquaculture Research*, *48*(4), 1895–1903. https://doi.org/10.1111/are.13027

Flos, R., Reig, L., Oca, J., Ginovart, M., 2002. Influence of marketing and different landbased systems on gilthead sea bream (Sparus aurata) quality. Aquac. Int. 10, 189–206.

Folkedal, O., Fernö, A., Nederlof, M. A. J., Fosseidengen, J. E., Cerqueira, M., Olsen, R. E., & Nilsson, J. (2018). Habituation and conditioning in gilthead sea bream (*Sparus aurata*): Effects of aversive stimuli, reward and social hierarchies. *Aquaculture Research*, *49*(1), 335–340. https://doi.org/10.1111/are.13463

Ganga, R., Montero, D., Bell, J. G., Atalah, E., Ganuza, E., Vega-Orellana, O., … Izquierdo, M. (2011). Stress response in sea bream (Sparus aurata) held under crowded conditions and fed diets containing linseed and/or soybean oil. *Aquaculture*, *311*(1–4), 215–223. https://doi.org/10.1016/J.AQUACULTURE.2010.11.050

Gornati, R., Papis, E., Rimoldi, S., Terova, G., Saroglia, M., & Bernardini, G. (2004). Rearing density influences the expression of stress-related genes in sea bass (Dicentrarchus labrax, L.). *Gene*, *341*(1–2), 111–118. https://doi.org/10.1016/j.gene.2004.06.020

GRIGORAKIS, K., & ALEXIS, M. N. (2005). Effects of fasting on the meat quality and fat deposition of commercial-size farmed gilthead sea bream (Sparus aurata, L.) fed different dietary regimes. *Aquaculture Nutrition*, *11*(5), 341–344. https://doi.org/10.1111/j.1365-2095.2005.00351.x

Grigorakis, K., Alexis, M. N., Taylor, K. D. A., & Hole, M. (2002). Comparison of wild and cultured gilthead sea bream (Sparus aurata); composition, appearance and seasonal variations. *International Journal of Food Science and Technology*, *37*(5), 477–484. https://doi.org/10.1046/j.1365-2621.2002.00604.x

Håstein, T., Scarfe, A. D., & Lund, V. L. (2005). Science-based assessment of welfare: aquatic animals. *Revue Scientifique et Technique (International Office of Epizootics)*, *24*(2), 529–547. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16358506

Huidobro, A., Tejada, M., 2004. Gilthead seabream (Sparus aurata): suitability for freezing and commercial alternatives. J. Sci. Food Agric. 84, 1405–1413.

Huidobro, A., Pastor, A., López-Caballero, M.E., Tejada, M., 2001. Washing effect on the quality index method (QIM) developed for raw gilthead sea bream, Sparus aurata. Eur. Food Res. Technol. 212, 408–412

Ibarz, A., Felip, O., Fernández-Borràs, J., Martín-Pérez, M., Blasco, J., & Torrella, J. R. (2011). Sustained swimming improves muscle growth and cellularity in gilthead sea bream. *Journal of Comparative Physiology B*, *181*(2), 209–217. https://doi.org/10.1007/s00360-010-0516-4

Ibarz, A., Padrós, F., Gallardo, M. Á., Fernández-Borràs, J., Blasco, J., & Tort, L. (2010). Low-temperature challenges to gilthead sea bream culture: review of cold-induced alterations and 'Winter Syndrome.' *Reviews in Fish Biology and Fisheries*, *20*(4), 539–556. https://doi.org/10.1007/s11160-010-9159-5

Jawad, L. A. (2012). SPARIDAE: BIOLOGY AND AQUACULTURE OF GILTHEAD SEA BREAM AND OTHER SPECIES - Edited by M. A. Pavlidis and C. C. Mylonas. *Journal of Fish Biology*, *81*(6), 2100–2101. https://doi.org/10.1111/j.1095-8649.2012.03468.x

L. Bavčević, S. Petrović, M. Crnica, & E. Corazzin. (2006). *EFFECTS OF FEEDING*

STRATEGY ON GROWTH OF SEA BREAM (SPARUS AURATA L.) DURING WINTER–SPRING AND POSSIBLE IMPLICATIONS FOR »WINTER DISEASE« SYNDROME. Croatian Journal of Fisheries : Ribarstvo (Vol. 64). [Ribozajednica]. Retrieved from https://hrcak.srce.hr/4666

Lembo, G., Carbonara, P., Scolamacchia, M., Spedicato, M. T., & McKinley, R. S. (2007). Use of muscle activity indices as a relative measure of well-being in cultured sea bass Dicentrarchus labrax (Linnaeus, 1758). In *Developments in Fish Telemetry* (pp. 271–280). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-1-4020-6237-7_25

López-Luna, J., Vásquez, L., Torrent, F., & Villarroel, M. (2013). Short-term fasting and welfare prior to slaughter in rainbow trout, Oncorhynchus mykiss. *Aquaculture*, *400–401*, 142–147. https://doi.org/10.1016/j.aquaculture.2013.03.009

Lupatsch, I., Santos, G. A., Schrama, J. W., & Verreth, J. A. J. (2010). Effect of stocking density and feeding level on energy expenditure and stress responsiveness in European sea bass Dicentrarchus labrax. *Aquaculture*, *298*(3–4), 245–250. https://doi.org/10.1016/j.aquaculture.2009.11.007

M. Jobling, S. P. (2010). Finfish aquaculture diversification. Wallingford, UK; Cambridge, MA: CABI. Retrieved from https://doi.org/10.1079/9781845934941.0000

Marras, S., Claireaux, G., McKenzie, D. J., Nelson, J. A., & Nilsson, S. (2010). Individual variation and repeatability in aerobic and anaerobic swimming performance of European sea bass, Dicentrarchus labrax. *The Journal of Experimental Biology*, *213*(1), 26–32. https://doi.org/10.1242/jeb.032136

Meena, D. K., Das, P., Kumar, S., Mandal, S. C., Prusty, A. K., Singh, S. K., … Mukherjee, S. C. (2013). Beta-glucan: An ideal immunostimulant in aquaculture (a review). *Fish Physiology and Biochemistry*, *39*(3), 431–457. https://doi.org/10.1007/s10695-012-9710-5

Mente, E., Stratakos, A., Boziaris, I., Kormas, K., Karalazos, V., Karapanagiotidis, I., … LEONDIADIS, L. (2012). *The effect of organic and conventional production methods on sea bream growth, health and body composition: A field experiment. Scientia Marina* (Vol. 76). https://doi.org/10.3989/scimar.03411.07C

Miller, N. W., & Clem, L. W. (1984). Temperature-mediated processes in teleost immunity: differential effects of temperature on catfish in vitro antibody responses to thymus-dependent and thymus-independent antigens. *Journal of Immunology (Baltimore, Md. : 1950)*, *133*(5), 2356–2359. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6384365

Montero, D., Izquierdo, M. S., Tort, L., Robaina, L., & Vergara, J. M. (1999). High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, Sparus aurata, juveniles. *Fish Physiology and Biochemistry*, *20*(1), 53–60. https://doi.org/10.1023/A:1007719928905

Montero, D., Marrero, M., Izquierdo, M. ., Robaina, L., Vergara, J. ., & Tort, L. (1999). Effect of vitamin E and C dietary supplementation on some immune parameters of gilthead seabream (Sparus aurata) juveniles subjected to crowding stress. *Aquaculture*, *171*(3–4), 269–278. https://doi.org/10.1016/S0044-8486(98)00387-1

Montero, D., Mathlouthi, F., Tort, L., Afonso, J. M., Torrecillas, S., Fernández-Vaquero, A., … Izquierdo, M. S. (2010). Replacement of dietary fish oil by vegetable oils affects humoral immunity and expression of pro-inflammatory cytokines genes in gilthead sea bream Sparus aurata. *Fish & Shellfish Immunology*, *29*(6), 1073–1081. https://doi.org/10.1016/J.FSI.2010.08.024

Mood, A., & Brooke, P. (2015). Fishcount. Retrieved from http://fishcount.org.uk/

Muir J. (ed.), Basurco B. (ed.). Mediterranean offshore mariculture. Zaragoza : CIHEAM, 2000. 215 p. (Options Méditerranéennes : Série B. Etudes et Recherches; n. 30). Advanced Course of the CIHEAM Network on Technology of Aquaculture in the Mediterranean on 'Mediterranean Offshore Mariculture', 1997/10/20-24, Zaragoza (Spain).

National Research Council. (1994). *Low-Frequency Sound and Marine Mammals: current knowledge and research needs*. National Academies Press (US). https://doi.org/10.17226/4557

Navarro, I., & Gutiérrez, J. (1995). Fasting and starvation. *Biochemistry and Molecular Biology of Fishes*, *4*, 393–434. https://doi.org/10.1016/S1873-0140(06)80020-2

Ökte, E. (2002). Grow-out of sea Bream Sparus aurata in Turkey, particularly in land-based farm with recirculation system in Canakkale: better use of water, nutrients and space. *Turkish Journal of Fisheries and Aquatic Sciences*, *2*, 83–87.

Ortuño, J., Esteban, M. A., & Meseguer, J. (2001). Effects of short-term crowding stress on the gilthead seabream (Sparus aurata L.) innate immune response. *Fish & Shellfish Immunology*, *11*(2), 187–197. https://doi.org/10.1006/FSIM.2000.0304

Papoutsoglou, S., Costello, M. J., Stamou, E., & Tziha, G. (1996). Environmental conditions at sea-cages, and ectoparasites on farmed European sea-bass, Dicentrarchus labrax (L.), and gilt-head sea-bream, Sparus aurata L., at two farms in Greece. *Aquaculture Research*, *27*(1), 25–34. https://doi.org/10.1111/j.1365-2109.1996.tb00963.x

Person-Le Ruyet, J., & Le Bayon, N. (2009). Effects of temperature, stocking density and farming conditions on fin damage in European sea bass (*Dicentrarchus labrax*). *Aquatic Living Resources*, *22*(3), 349–362. https://doi.org/10.1051/alr/2009047

Piccinno, M., Schiavone, R., Zilli, L., Sicuro, B., Storelli, C., & Vilella, S. (2013). *Sea Cucumber Meal as Alternative Protein Source to Fishmeal in Gilthead Sea Bream (Sparus aurata) Nutrition: Effects on Growth and Welfare* . *Turkish Journal of Fisheries and Aquatic Sciences* (Vol. 13). DOI:10.4194/1303-2712-v13_2_12

Pichavant, K., Person-Le-Ruyet, J., Bayon, N. Le, Severe, A., Roux, A. Le, & Boeuf, G. (2001). Comparative effects of long-term hypoxia on growth, feeding and oxygen consumption in juvenile turbot and European sea bass. *Journal of Fish Biology*, *59*(4), 875–883. https://doi.org/10.1111/j.1095-8649.2001.tb00158.x

Poppe, T. T., Barnes, A. C., & Midtlyng, P. J. (2002). Welfare and ethics in fish farming. *Bulletin of the European Association of Fish Pathologists*, *22*(2), 148–151.

Romero, J., Gloria, C., & Navarrete, P. (2012). Antibiotics in Aquaculture – Use, Abuse and Alternatives. *Health and Environment in Aquaculture* . https://doi.org/10.5772/28157

Roncarati, A., Melotti, P., Dees, A., Mordenti, O., & Angellotti, L. (2006). Welfare status of cultured seabass (Dicentrarchus labrax L.) and seabream (Sparus aurata L.) assessed by blood parameters and tissue characteristics. *Journal of Applied Ichthyology*, *22*(3), 225–234. https://doi.org/10.1111/j.1439-0426.2006.00741.x

Roque d'Orbcastel, E., Lemarié, G., Breuil, G., Petochi, T., Marino, G., Triplet, S., … Blancheton, J.-P. (2010). Effects of rearing density on sea bass (*Dicentrarchus labrax*

) biological performance, blood parameters and disease resistance in a flow through system. *Aquatic Living Resources*, *23*(1), 109–117. https://doi.org/10.1051/alr/2009056

Rosa, J., Leston, S., Castro, M., Freitas, A., Barbosa, J., Pardal, M. Â., … Ramos, F. (2018). Evaluation of antimicrobials residues in farmed gilthead seabream (Sparus aurata) after administration through medicated feed. *Food Control*, *86*, 110–116. https://doi.org/10.1016/J.FOODCONT.2017.11.005

Round table: Seizing the day for Fish Welfare. (n.d.). Retrieved June 21, 2019, from https://www.animalwelfareintergroup.eu/2018/06/06/round-table-seizing-the-day-for-fish-welfare/

Sánchez-Muros, M. J., Sánchez, B., Barroso, F. G., Toniolo, M., Trenzado, C. E., & Sanz Rus, A. (2017). Effects of rearing conditions on behavioural responses, social kinetics and physiological parameters in gilthead sea bream Sparus aurata. *Applied Animal Behaviour Science*, *197*, 120–128. https://doi.org/10.1016/J.APPLANIM.2017.08.004

Sangiao-Alvarellos, S., Guzmán, J. M., Láiz-Carrión, R., Míguez, J. M., Martín Del Río, M. P., Mancera, J. M., & Soengas, J. L. (2005). Interactive effects of high stocking density and food deprivation on carbohydrate metabolism in several tissues of gilthead sea breamSparus auratus. *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, *303A*(9), 761–775. https://doi.org/10.1002/jez.a.203

Santos, G. A., Schrama, J. W., Mamauag, R. E. P., Rombout, J. H. W. M., & Verreth, J. A. J. (2010). Chronic stress impairs performance, energy metabolism and welfare indicators in European seabass (Dicentrarchus labrax): The combined effects of fish crowding and water quality deterioration. *Aquaculture*, *299*(1–4), 73–80. https://doi.org/10.1016/j.aquaculture.2009.11.018

Sarusic, G. (1999). *Clinical signs of the winter disease phenomenon in sea bream (Sparus aurata, L.). Bulletin- European Association of Fish Pathologists* (Vol. 19).

Schrama, D., Richard, N., Silva, T. S., Figueiredo, F. A., Conceição, L. E. C., Burchmore, R., … Rodrigues, P. M. L. (2017). Enhanced dietary formulation to mitigate winter thermal stress in gilthead sea bream (Sparus aurata): a 2D-DIGE plasma proteome study. *Fish Physiology and Biochemistry*, *43*(2), 603–617. https://doi.org/10.1007/s10695-016-0315-2

Smith, C., & Reay, P. (1991). Cannibalism in teleost fish. *Reviews in Fish Biology and Fisheries*, *1*(1), 41–64. https://doi.org/10.1007/BF00042661

Southgate, P., & Wall, T. (2001). Welfare of farmed fish at slaughter. *BMJ In Practice*, *23*(5), 277. https://doi.org/10.1136/inpract.23.5.277

Thorburn, M. A., Teare, G. F., Martin, S. W., & Moccia, R. D. (2001). Group-level factors associated with chemotherapeutic treatment regimens in land-based trout farms in Ontario, Canada. *Preventive Veterinary Medicine*, *50*(1–2), 165–176. https://doi.org/10.1016/S0167-5877(01)00192-1

Torrecillas, S., Montero, D., Domínguez, D., Robaina, L., Izquierdo, M., Torrecillas, S., … Izquierdo, M. (2019). Skin Mucus Fatty Acid Composition of Gilthead Sea Bream (Sparus Aurata): A Descriptive Study in Fish Fed Low and High Fish Meal Diets. *Fishes*, *4*(1), 15. https://doi.org/10.3390/fishes4010015

Tort, L., Rotllant, J., Liarte, C., Acerete, L., Hernández, A., Ceulemans, S., … Padros, F. (2004). Effects of temperature decrease on feeding rates, immune indicators and histopathological changes of gilthead sea bream Sparus aurata fed with an experimental diet. *Aquaculture*, *229*(1–4), 55–65. https://doi.org/10.1016/S0044-8486(03)00403-4

Tort, L., Rotllant, J., & Rovira, L. (1998). Immunological suppression in gilthead sea bream Sparus aurata of the North-West Mediterranean at low temperatures. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *120*(1), 175–179. https://doi.org/10.1016/S1095-6433(98)10027-2

Valente, L. M. P., Cornet, J., Donnay-Moreno, C., Gouygou, J. P., Bergé, J. P., Bacelar, M., … Cardinal, M. (2011). Quality differences of gilthead sea bream from distinct production systems in Southern Europe: Intensive, integrated, semi-intensive or extensive systems. *Food Control*, *22*(5), 708–717. https://doi.org/10.1016/J.FOODCONT.2010.11.001

Vazzana, M., Cammarata, M., Cooper, E. ., & Parrinello, N. (2002). Confinement stress in sea bass (Dicentrarchus labrax) depresses peritoneal leukocyte cytotoxicity. *Aquaculture*, *210*(1–4), 231–243. https://doi.org/10.1016/S0044-8486(01)00818-3