

Chapter 3

Stress and welfare in sparid fishes

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Abstract: As in other vertebrates, fish experience stressful situations after exposure to both abiotic and biotic noxious stimuli. The current procedures used in aquacultured fish may also induce stress responses that involve neuroendocrine activation and a number of physiological, metabolic, molecular, and genetic changes. The causes for these changes are due to stress messengers and physiological responses designed to overcome the altered situation. Thus, in this chapter, the physiology of the stress response, including stress perception, the nature of the response, the molecular and neuroendocrine activation, the metabolic and energetic arrangements and the regulation of the response are presented with particular reference to sparid fishes. Furthermore, aspects related to the concept of fish welfare will be also discussed in the present chapter.

Key words: *Acanthopagrus*; gilthead sea bream; *Pagrus*; red sea bream; silver sea bream; sparids; *Sparus*; stress; welfare

3.1 Introduction

It is not easy to define the term stress or welfare in fish, as these words or concepts have a subjective component related to human experience and emotion. Stress in fish has been defined in different ways. For instance, stress is defined as a situation that places an animal out of the normal physiological scope, imposing an excessive energetic or physiologic load to the animal, or resulting in an imbalanced situation that the animal has to overcome after a challenge. All these definitions emphasize both the existence of a relevant

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challenge for the fish, the risk of being unable to contend with this situation with the current physiological resources and the onset of a number of responses at different physiological levels to circumvent these adverse challenges.

Stress-related literature emphasizes the consequences of physiological imbalance and the risk for homeostasis after a stressor challenge. Following this approach, the stressor would cause an imbalance and endogenous physiological mechanisms would operate in order to counteract and reverse the consequences and recover a state of equilibrium. Therefore, the central idea is the imbalance of homeostasis. Recently, another related concept, allostasis has emerged, of which the consequences of the stressor would be integrated into the overall response in terms of an additional load to the normal function. The animals have therefore to recruit additional energy resources to cope with the stressor, and so the magnitude or direction of the stress response would depend on the allostatic load. Within this concept, physiological imbalance would depend on the costs or the load that the stressor imposes. Therefore, this concept can include a wide range of stimuli, from moderate environmental changes, for example, temperature variations, that may not even be considered as stressors, though involving a load increase, to severe stressors with high costs and high overload (Figure 3.1).

Nevertheless, it becomes clear that the experience of a stress situation may not necessarily lead to negative consequences and, in fact, can result in an adaptive process. Thus, fish may respond more efficiently when a stressor recurs a second time after a previous encounter. This adaptive process takes place as the fish may learn from past experiences both in terms of behavior and physiological responses and, therefore, resulting in fish having improved performance when faced with further stress situations. This significant adaptation involves better fitness.

On the contrary, the failure of this process, that is, when fish cannot adapt to the stress situation, leads to maladaptation that involves low performance, physiological imbalance, and eventually death if conditions persist. This maladaptation is more common under two conditions: chronic stress and presence of combined stressors. Chronic stressors, such as crowding or contamination, involve a direct effect of the stressor causing a specific injury, and/or a switch in the metabolic or energetic pathways to compensate for these effects. This normally involves higher energy expenditure, increased use of energetic reserves, and activation of selected alarm or sentinel type of processes, such as stimulation of signaling among immune and endocrine systems, or cell protection processes, for example, heat-shock or acute phase proteins. The persistence of the stressor and the continuation of these responses lead to exhaustion of resources, followed by malfunction of key regulatory systems, such as the osmoregulation or immune competence. While chronic stress is more related to the slow

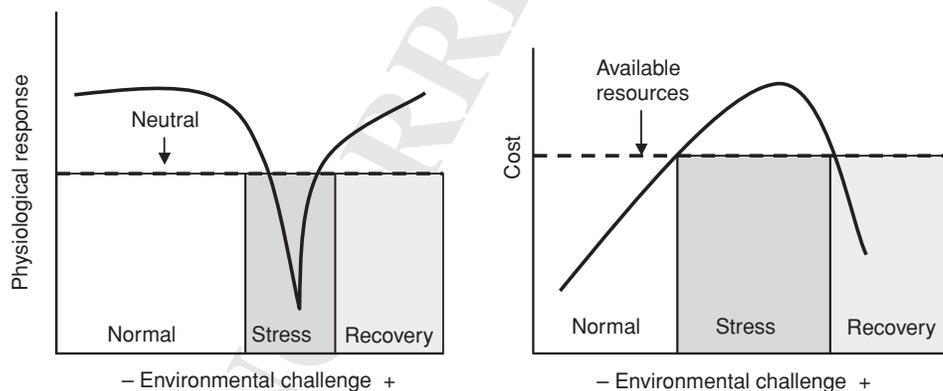


Figure 3.1 Homeostasis (left) and allostasis (right).

and progressive exhaustion of resources because of the persistence of the stressor, the case of combined stressors is more related to a reiterative activation of alarm response mechanisms without chances for adaptation, as new stressors have not been encountered in the previous stress experience.

3.2 Fish stress and fish welfare

Besides fish stress, the perception of fish welfare gained recognition over the last years as another step in the consideration of the treatment of fish. Welfare and stress have important implications, although in the past stress was a concept that was often used in the literature of fish biology, whereas welfare an ethical issue of public concern and debate. One could assume that the welfare status should mean a lack of stress and vice versa; however, this is not correct since both terms are not completely opposite. Stress situations have an adaptive value, and there is no justification for the assumption that the expression of stress responses always compromise health or welfare (Korte *et al.* 2007). The reaction developed by the animals to overcome the effects of the stressor may be incorporated as a learning experience and be utilized to negotiate another stress episode in the future. On the other hand, it is true that when the stress situation cannot be resolved, fish will enter into a situation where welfare is compromised.

The concept of welfare is open to much debate because it is difficult to be defined in precise, scientific terms, and because it always involves anthropocentric, normative judgments about the meaning of quality of life. One of the key questions on welfare that has deserved special attention during the last years is whether fish are a sentient animal, that is, whether fish have the ability to feel or perceive subjectively and/or whether fish can feel pain. No definitive answer exists for this question (Rose 2002; Chandroo *et al.* 2004; Braithwaite & Boulcott 2008), but an increasing amount of evidence has been published supporting the idea that fish can feel pain through neural processes that are situated in different neural structures than the respective sites in mammals (Sneddon 2003).

In the last decade, the applied concept of welfare in fish has been based on the five freedoms originally defined for farmed animals (Farm Animal Welfare Council 2005). These include: (a) freedom from hunger and thirst, by ready access to fresh water and a diet to maintain full health and vigor; (b) freedom from discomfort, by providing an appropriate environment including shelter and a comfortable resting area; (c) freedom from pain, injury, or disease, by prevention or rapid diagnosis and treatment; (d) freedom to express normal behavior, by providing sufficient space, proper facilities and company of the animal's own kind; and (e) freedom from fear and distress, by ensuring conditions and treatment, which avoid mental suffering.

Thus, by adapting the freedoms principle fish welfare can be defined by satisfying the following three conditions (Huntingford & Kadri 2008): (a) the fish can adapt to its environment and be in good health, with all biological systems functioning properly; (b) the fish is able to express its own behaviors and the behavioral needs; (c) the fish is free of negative experiences, such as pain, hunger, and has access to positive experiences, such as social companionship, in the case of social species.

Regarding aquaculture, the need for identifying parameters that could be applied on site has become a necessity. Welfare promotes survival and overall fish quality and has become an increasing commercial issue, as most of the retailers and the distributors have started using labels for good practices in fish farms. In terms of measuring welfare, various indicators or indices that are related to the fish itself can be devised, that is, their physical condition, their physiological status and their behavioral condition. Nevertheless, there are also external parameters that are related to environmental (as water quality) and husbandry (as handling, confinement, stocking density, etc.) conditions. Finally, slaughtering and the procedures around antemortem events are key points regarding fish welfare and product quality.

3.3 The physiology of the stress response

3.3.1 Perception

Responses to stressors start when a fish perceives a certain threat. The perception phase involves one or numerous sensors to fire signals to the central nervous system. These endogenous or exogenous signals are ending up in the brain and concentrating in the hypothalamus. From the brain hypothalamic nuclei, a neuroendocrine response will be triggered (Figure 3.2). Acute stressors can rapidly trigger the response mechanisms as the process of perception is immediate. However, some low intensity stressors may not induce such a response in the first place. In these cases, it is the accumulation or the increase in intensity that will complete the perception. In other cases, perception may come from other physiological compartments. Low intensity infections may not be perceived at the beginning of the process, and it is only after some time when infection spreads that the interaction between immune and neuroendocrine systems start to generate a response (Acerete *et al.* personal observations).

3.3.2 The nature of the response

Though stress response and mechanisms are qualitatively the same in fish, the quantitative response can be very different at the level of species or even the individual. It is well known that some species are much more susceptible to the same stressor than others as far as the cortisol response is considered (Barton 2002), and the intensity of the responses driven by this hormone will also be different.

Other than species differences, individual differences can also be of relevance. It has been shown in most vertebrates and also in fish that coping strategies can vary among individuals. On one side the shy-type animals develop a resistance type of response with low aggressiveness, saving energy and avoiding conflict (reactive style of coping). On the other hand, bold-type animals show aggressive behavior, a strong fighting response and an evident display of energy (proactive coping style). Therefore, within the same species and even the same batch, differences can be significant. In fact, these different coping strategies involve different ways of using an individual's physiological resources, and it has been shown that shy coping styles normally involve a higher level of cortisol and the reverse for the bold types. Therefore, the consequences of such divergence in hormonal responses would lead to differences in secondary responses, such as osmoregulation, metabolism, and immune response.

Naturally, genetics play a role in modulating the response to stress. Different strains or families may show variations in the intensity of the stress response (Thorland *et al.* 2007). In addition, it has been shown that

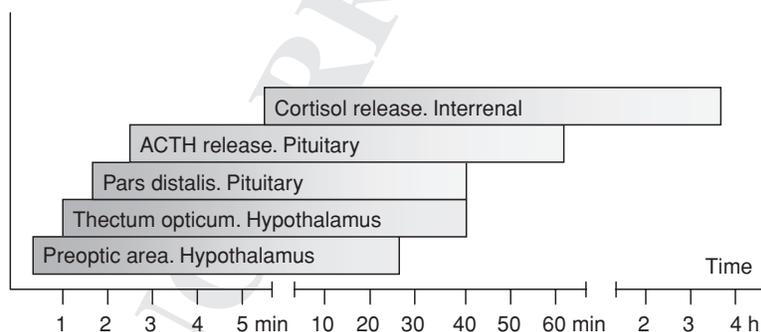


Figure 3.2 Time course of the initial hypothalamic and pituitary changes after stress.

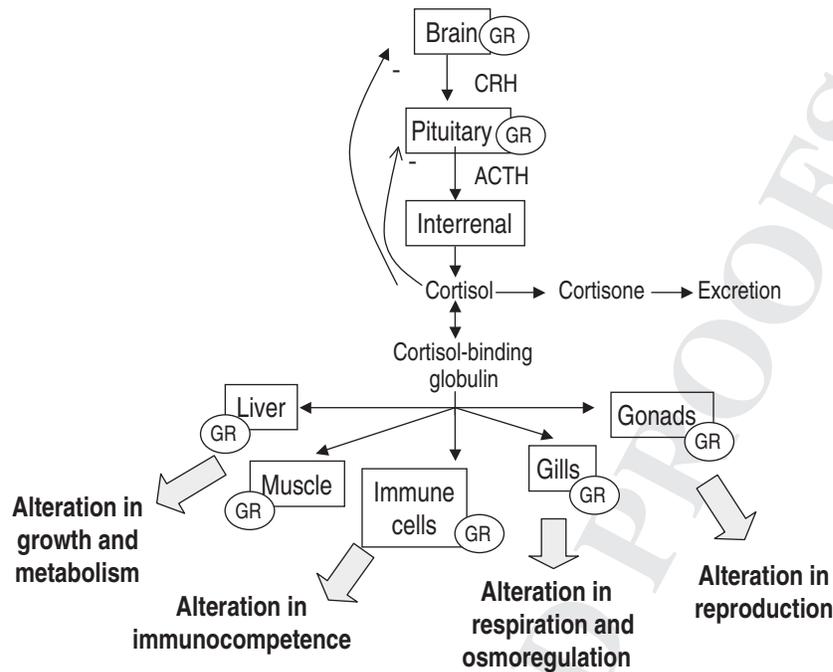


Figure 3.3 Neuroendocrine and metabolic changes during stress episodes.

cultured fish may be selected for their response to a particular stressor, for instance, in terms of the cortisol response (Pottinger & Carrick 1999), or in terms of the lysozyme response (Fevolden *et al.* 1999). Other than inconsistent or mixed responders, family selection for either high or low cortisol responders shows a consistent response in the subsequent generations.

3.3.3 Neuroendocrine activation and stress messengers

Once the perceptive signal reaches the nervous system, a number of neuroendocrine mechanisms are evoked (see Figure 3.3). First, hypothalamic hormones are released, mostly through two axes, the sympathetic-chromaffin axis and the hypothalamic-pituitary axis. In the first axis, neural fibers activate neurons in the chromaffin cells of the head kidney, releasing catecholamines, epinephrine (E), and norepinephrine (NE). In the second axis, hypothalamus releases CRH (corticotropin releasing hormone), an activator of the ACTH (adrenocorticotrophic hormone) synthesized by the corticotrophic cells of the adenohypophysis and released to the blood stream. ACTH then stimulates the production of the corticosteroid hormone, cortisol by the interrenal cells.

As far as sparids are concerned, the neuroendocrine activation and the stress response are similar to that of other fish species studied to date (Rotllant *et al.* 2000a, 2000b). Experiments in sea bream show that acute stressors generate a classical and sudden release of catecholamines followed by a sharp increase in cortisol that remains in blood for 3–4 hours, before returning to basal levels (Kelly & Woo 1999a; Rotllant *et al.* 2000a). Nevertheless, it may be noticed that there are species specific differences in the magnitude of the response. Thus, it has been shown that European sea bass shows a significantly higher level of cortisol in plasma under

resting conditions compared to other fish and that the fold increase after an acute stressor is of a lesser magnitude (Rotllant *et al.* 2003a).

3.3.4 Gene activation

Some stress responses may be very rapid, occurring within seconds or minutes and involve neural communication or hormonal release, whereas others activate processes that may involve genetic activation and therefore take more time. The appearance of genomics as a new technology allows for the opportunity to study these processes. Studies have been published regarding the genomic response to stress, identifying the set of genes involved in the alarm response to stress events in sparids (Sarropoulou *et al.* 2005; Mackenzie *et al.* 2006; Castillo *et al.* 2008). Definitely, more data will appear in the near future regarding such a genomic response.

3.3.5 Metabolic and energetic arrangements

During a physiological response to stress, it can be observed that fish and other vertebrates experience a shift in energetic and metabolic patterns. Thus, post stress, energetic resources are drawn to sustain the costs, that is, increase in cardiovascular and respiratory rhythms, increase in tissue perfusion, increase in blood flow to tissues, increase in energy use, and a higher demand for muscles. Therefore, metabolic tasks, such as growth or high cost processes, may be retarded or delayed as may happen with cell production in the immune system. On the other hand, other metabolic derangements are derived from the specific action of stress hormones (catecholamines and cortisol). As these hormones have receptors in a wide range of tissues, their activation may cause an imbalance or deregulation in specific tissues, causing secondary alterations, as in the case of the cortisol effect in the gills resulting in osmoregulatory imbalance.

3.3.6 Regulation of the first response

Stress hormones have a limited half-life. They are released into the blood stream and bind to tissue receptors, thereby generating secondary changes. The unbound hormones are processed in the liver and the kidney and are later excreted. The limited time activity for these hormone fractions is one way to regulate the extent of their action. Another manner of being regulated is by the presence of hormone receptors in the hypothalamus and the pituitary. An increase in plasma hormones produces a negative feedback on the release mechanism at the neuroendocrine level (Figure 3.3).

3.4 Stress indicators

Several indicators have been used for detecting stress in fish and some can be applied to sparids. At the gene level, few studies have been published at present in sparids, although a number of experiments are being conducted in the last years, so further data is expected in the near future (Sarropoulou *et al.* 2005; Mackenzie *et al.* 2006). Generally, stress induces an important effect on the metabolism and on energy allocation, as a great number of related genes are regulated after exposure of fish to different stressors (Krasnov *et al.* 2005; Mackenzie *et al.* 2006).

At the molecular level, a number of indicators have been measured and several new ones have been proposed from both gene identification studies and microarray analyses. Concerning sparids, Ribas *et al.* (2004) showed that enolase may be a relevant indicator of stress as it is modulated after applying different kinds of stressors, such as crowding and antigen treatment and that it is expressed in many tissues. Similarly, other authors have referred to different genes that are up- or down regulated after stress activation and could also be used as

indicators (Doñate *et al.* 2007; Bermejo-Nogales *et al.* 2008). Among them, heat shock proteins and acute phase proteins have been used as molecular indicators, although not much information is available for the gilthead sea bream *Sparus auratus* (Bermejo-Nogales *et al.* 2008). In contrast, there is much literature on heat shock protein (HSP) induction in response to a variety of stressors, for the silver sea bream *Sparus sarba* and the black sea bream *Mylio macrocephalus*. Apart from marked induction of HSP70 family by acute heat shock (Deane & Woo 2005a, 2005d), sea bream infected with live *Vibrio alginolyticus* had higher hepatic HSP70 levels one day following pathogen administration (Deane *et al.* 2004), potentially caused by damaged cellular proteins or hypoxaemia. Administration of bacterial extracellular products containing amylase, caseinase, gelatinase, lipase, chitinase, and hemolysin activities, resulted in elevation of HSP70 within two days of infection (Deane *et al.* 2004). Exposure of silver sea bream to hypersaline stress (50 psu seawater) led to a marked increase in the transcript abundance of both heat shock cognate 70 (*hsc70*) and *hsp70* (Deane & Woo 2004). In black sea bream, *Mylio macrocephalus*, chronically exposed to either hyposmotic, isoosmotic, seawater or hypersaline conditions, hepatic HSP60, HSP70, and HSP90 levels were lowest in isoosmotically acclimated fish (Deane *et al.* 2002), and in silver sea bream exposed to the same salinity regime, *hsp70* transcript abundance was also lowest in isoosmotic adapted fish (Deane & Woo 2004). In *Sparus sarba* exposed to nitrite stress, there were marked increases in the amount of HSP70 family in gills, kidney and liver and HSP90 levels in kidneys, and liver (Deane & Woo 2007). The stress of heavy metals or organochlorine exposure was followed by marked induction of *hsp70* and *hsc70* gene expression in black sea bream (*Mylio macrocephalus*) fibroblasts (Deane & Woo 2006a). HSP70 was also induced in blood cells of silver sea bream, *Sparus sarba*, following exposure to cadmium, lead and chromium (Fulladosa *et al.* 2006).

Concerning circulating hormones, most studies have dealt with cortisol as a reliable and sensitive indicator of stress response. In sparids, resting levels of cortisol in plasma are between 1 and 10 ng mL⁻¹ to rise at about 90–100 ng mL⁻¹ in acutely stressed fish (Table 3.1). A recent study on silver sea bream, *Sparus sarba*, described the importance of cortisol and HSP70 as major elements in the fish stress response (Deane *et al.* 2006). In this species, cortisol induced HSP70 synthesis together with concomitant protection against apoptosis in the fibroblasts, while similar doses of cortisol reduced HSP70 and caused apoptosis in the macrophages (Deane

Table 3.1 Cortisol response to acute stressors in sparid fishes

Acute stressor	Cortisol (ng mL ⁻¹)	Time (hours)	Species	Author
Handling	160	1	<i>S. aurata</i>	Rotllant <i>et al.</i> (2001a)
Handling	54	2	<i>P. pagrus</i>	Rotllant <i>et al.</i> (1997)
Handling	109	1	<i>S. aurata</i>	Tort <i>et al.</i> (2001)
Physical disturbance	90	0.5	<i>S. aurata</i>	Ortuño <i>et al.</i> (2002)
Hormone treatment	140	4	<i>S. aurata</i>	Teles <i>et al.</i> (2005)
Air exposure	144	0.1	<i>S. aurata</i>	Tintos <i>et al.</i> (2006)
High salinity	160	1	<i>S. aurata</i>	Sangiao-Alvarellos <i>et al.</i> (2005)
Low salinity	200	1	<i>S. aurata</i>	Sangiao-Alvarellos <i>et al.</i> (2005)
Air exposure	70	1	<i>S. aurata</i>	Barton <i>et al.</i> (2005)
Lps treatment (immune challenge)	150	2	<i>S. aurata</i>	Acerete <i>et al.</i> (2008)
Air exposure	130	1	<i>S. aurata</i>	Barton <i>et al.</i> (2005)
Background & netting	220	2	<i>P. pagrus</i>	Van Der Salm <i>et al.</i> (2006)
Air exposure	120	0.5	<i>S. aurata</i>	Van Anholt <i>et al.</i> (2004)
Air exposure & confinement	489	0.5	<i>S. aurata</i>	Arends <i>et al.</i> (1999)
Air exposure	202	1	<i>S. aurata</i>	Arends <i>et al.</i> (1999)
Mean values	162	1.2		

et al. 2006). Other hormones have been measured in plasma after stress, but to a lesser extent. Thus, in the sea bream, *Sparus aurata* stress causes a clear reduction in growth hormone (GH) levels both after acute and chronic stress (Rotllant *et al.* 2000a, 2000b, 2001b). When the silver sea bream, *Sparus sarba*, is exposed to cadmium the pituitary GH levels are markedly suppressed (Woo & Man 2009). The importance of GH acting as a reliable indicator of stress in fish has been extensively reviewed (Deane & Woo 2009). From the available information, it is clear that GH levels in fish can be extensively modulated in response to a variety of stressors, including salinity, temperature, pollutants, disease, handling, confinement, crowding, and nutritional stress (Deane & Woo 2009). In sparids, GH has a modulatory effect on HSPs as GH attenuates HSP70 expression in macrophages (Deane *et al.* 2007) and in the liver (Deane & Woo 2010) of the silver sea bream. Exposure of silver sea bream blood cells to chemical stressors (camptothecin) resulted in apoptosis, which could be protected by GH (Deane & Woo 2005d). Melanocyte stimulating hormone (MSH) is also affected by stressors, and it is apparently more sensitive to chronic stressors (Rotllant *et al.* 2000b). Epinephrine has also been measured in sparids, showing significant changes after acute handling, chronic crowding, temperature change, and anesthesia (Fanouraki *et al.* 2007).

Metabolic changes have been measured through different indicators in plasma and tissues and the energetic and metabolic pathways in muscle and liver (Mommsen *et al.* 1999). Thus, stress induces significant changes in the plasma glucose levels, free fatty acids or plasma lactate, although the later is more sensitive to acute and physical stressors (Acerete *et al.* 2004).

There was a twofold elevation of serum glucose and lactate levels in the silver sea bream, *Sparus sarba*, exposed to crowding stress within a tank without seawater re-circulation (Woo & Kelly 1995). Similar hyperglycemia and hyperlactemia, accompanied by hepatic glycogenolysis occurred in black sea bream, *Mylio macrocephalus*, exposed to hypoxic stress (Woo & Wu 1984). The stress of starvation in red sea bream, *Chrysophrys major*, led to a marked decline in plasma-free fatty acid and liver glycogen levels, but plasma glucose and lactate concentrations were unaffected (Woo & Murat 1981). Starved red sea bream, *Chrysophrys major*, exposed to cold stress (13°C) appeared to rely on heightened liver transamination as evidenced by marked stimulation of hepatic transaminase activities (Woo & Fung 1981). High temperature stress resulted in upregulation of phosphoenolpyruvate carboxykinase expression and plasma glucose in the black sea bream *Acanthopagrus schlegeli* (Choi *et al.* 2007). Besides metabolic changes, chronic stress generally leads to growth suppression in fish, which could be a reflection on the suppressive effect of cortisol on hepatic insulin growth factor (IGF-I) expression in silver sea bream (Leung *et al.* 2008).

Osmoregulatory parameters, such as plasma or serum osmolality and ion concentrations appear to be sensitive indicators of measuring stress in fish. Both environmental changes in water quality but also in hormone release may induce changes in gills and kidneys, thus impairing osmoregulation and causing changes in plasma osmolality and ion levels. For example, hypoxic stress induced marked elevations in serum Na⁺, K⁺, and Ca²⁺ levels in black sea bream, *Mylio macrocephalus*, indicating significant events of osmoregulatory derangements (Woo & Wu 1984). A recent study on exposing silver sea bream, *Sparus sarba*, to nitrite stress reported changes in other indices of osmoregulatory dysfunction such as elevation in gill sodium pump activity and on decline in kidney aquaporin water channel abundance (Deane & Woo 2007). Osmoregulatory dysfunction is also evident upon exposure of *Sparus (=Rhabdosargus) sarba* to the toxic alga *Chattonella marina* (Xu *et al.* 2008) and bacterial pathogen *Vibrio alginolyticus* (Deane & Woo 2005c), as evidenced by changes in plasma osmolality, Na⁺ and Cl⁻ concentrations, and gill Na⁺-K⁺-ATPase activity.

3.5 Responses to stressors in Sparids

Although most of the array of responses to stressors is similar in most fishes, sparids show a clear response to acute stressors. Few studies focus on the use of catecholamines for assessing acute or chronic stress responses (Fanouraki *et al.* 2007), while there is an extensive bibliography on plasma cortisol concentrations. Acute

Table 3.2 Cortisol response to chronic stressors in sparid fishes

Chronic stressor	Cortisol (ng mL ⁻¹)	Time (days)	Species	Author
Crowding	28	14	<i>S. aurata</i>	Sangiao-Alvarellos <i>et al.</i> (2005)
Crowding + food deprivation	41	14	<i>S. aurata</i>	Sangiao-Alvarellos <i>et al.</i> (2005)
Crowding	10	7	<i>S. aurata</i>	Rotllant <i>et al.</i> (2001a)
Crowding	25	2	<i>S. aurata</i>	Ortuño <i>et al.</i> (2002)
Multiple	20	3	<i>S. aurata</i>	Ortuño <i>et al.</i> (2002)
Ca deficiency	40	56	<i>S. aurata</i>	Abbink <i>et al.</i> (2004)
Chemicals	40	1	<i>S. aurata</i>	Teles <i>et al.</i> (2005)
Cd exposure	42	4	<i>S. sarba</i>	Man (2008)
High salinity	9	8	<i>S. aurata</i>	Laiz-Carrion <i>et al.</i> (2005)
High salinity	10	14	<i>S. aurata</i>	Sangiao-Alvarellos <i>et al.</i> (2005)
Low salinity	15	5	<i>S. sarba</i>	Kelly and Woo (1999b)
Low salinity	22	21	<i>S. sarba</i>	Kelly and Woo (1999b)
Low salinity	21	3	<i>A. schlegeli</i>	Chang <i>et al.</i> (2007)
Bacterial infection	135	10	<i>S. sarba</i>	Deane <i>et al.</i> (2001)
Crowding	23	14	<i>S. aurata</i>	Barton <i>et al.</i> (2005)
Crowding	16	14	<i>S. aurata</i>	Montero <i>et al.</i> (1999b)
Crowding	13	21	<i>S. aurata</i>	Bermejo-Nogales <i>et al.</i> (2008)
Crowding	13		<i>S. aurata</i>	Fanouraki <i>et al.</i> (2007)
Crowding	73	11	<i>S. aurata</i>	Arendts <i>et al.</i> (1999)
Crowding	112	60	<i>S. sarba</i>	Woo and Kelly (1995)
Mean values	35	14		

stressors may induce a marked increase in plasma cortisol compared to unstressed resting levels. Table 3.1 shows the mean levels of cortisol on sparids subjected to acute stressors, reaching levels as high as 200 ng mL⁻¹ (Sangiao-Alvarellos *et al.* 2005) for *Sparus aurata* and 220 ng mL⁻¹ for *Pagrus pagrus* (Van Der Salm *et al.* 2004). As a representative measure, the mean value of plasma cortisol after acute stress according to 15 different studies is around 133 ng mL⁻¹ at one hour after the stressor was applied. Taking into account that resting values for plasma cortisol are around 1–10 ng mL⁻¹, acute stressors induce a 100-fold increase of cortisol in plasma (Table 3.1).

The magnitude of the cortisol response to chronic stressors varies due to the nature and intensity of the stress stimuli, the differences in the sampling design and the analytical method used for cortisol determination. Nevertheless, chronic stressors generally induce a lesser increase in plasma cortisol concentrations than do acute stressors, that is, around 33 ng mL⁻¹ after a period of about 2 weeks, following chronic stress encounter (Table 3.2).

Sparids and the gilthead sea bream, in particular, may show relevant physiological alterations when subjected to environmental stressors. However, the gilthead sea bream is particularly sensitive to low temperatures and may be affected by the winter syndrome, a multifactorial syndrome driven by a decrease in temperature below 13°C (Tort *et al.* 1998a, 2004). Under these circumstances, fish cease feeding, show signs of decreased activity and changes in a number of physiological, metabolic, and immune functions. Therefore, it may be assumed that temperatures lower than 13°C may be stressful for *Sparus aurata*, but also that these temperatures coincide with other potential stressors or opportunistic pathogens that may induce the syndrome, sometimes causing mortality. However, for several Asiatic sea bream species, it appears that the opposite situation prevails; high temperatures appear to be stressful for fish. This was demonstrated by high cortisol levels in warm acclimated

black sea bream, *Acanthopagrus schlegeli* (Choi *et al.* 2007). It may be pertinent to note that the Asiatic sea bream species, such as *Sparus sarba* (silver sea bream) and *Chrysophrys major* (red sea bream) do not exhibit the winter syndrome. In fact, cold temperature acclimation (12°C) may actually benefit growth in these species (Woo 1990), as evidenced by marked stimulation of GH (Deane & Woo 2006b), hepatic IGF-I mRNA (Deane & Woo 2005a), and glucose-6-phosphate dehydrogenase expression (Deane & Woo 2005b). In this regard, the gilthead sea bream appears to behave differently from the Asiatic sea bream species since acclimation to warm temperatures generally led to reduced stress and higher plasma GH levels (Mingarro *et al.* 2002).

Temperature alone may not be the sole cause for the winter syndrome but one of the inducers; firstly, only some fish species exhibit the syndrome, whereas the majority of fish do not. Secondly, other stressors may be eventually present in fish farms. In addition, experiments performed under laboratory conditions using the same temperature range and feeding regimes were not able to simulate the syndrome in the gilthead sea bream (Gallardo *et al.* 2003; Ibarz *et al.* 2007).

Compared with other groups, sparids appear not to be particularly sensitive to ordinary physical and husbandry stressors present in intensive rearing systems, as suggested by various indicators. Cortisol concentrations after acute stress may increase up to 140–200 ng mL⁻¹ as shown before, indicating a high reactivity to stressors, but values return to basal levels in about 4 hours and other less severe stressors generally induce a lower increase in plasma cortisol concentration. In terms of behavior, sparids seem to tolerate husbandry stressors better than other cultured species. In the silver sea bream, *Sparus sarba*, resting cortisol level (Woo and Kelly 1995; Deane & Woo 2005a) is very similar to those reported for the gilthead sea bream, and cold temperature stress has virtually no effect on serum cortisol level (Deane & Woo 2005a). Sea bream under disease stress (vibriosis), exhibited no elevation in serum cortisol until reaching the final moribund stage (10 days) when serum cortisol was markedly elevated by ~15-fold (Deane *et al.* 2001). For instance, compared to European sea bass, the response to the human presence is of lower avoidance reaction; similarly, fish farmers may perform grading exercises in the gilthead sea bream with minor disturbance and losses. One of the reasons for such a difference may be either genetic or because of a faster process of domestication, although the culture of both species started almost simultaneously. Nevertheless, some works have also described significant physiological differences that may explain such a different result. Thus, the European sea bass shows a higher basal level of cortisol in the head kidney where it is produced and released, and the fold-difference between basal and stressed levels are lower than sea bream (Rotllant *et al.* 2003b). It has been suggested that a basal cortisol secretion would be produced in resting unstressed European sea bass whereas no secretion would be produced in the sea bream, explaining in part the higher fold-secretion observed in the sea bream.

3.6 Aquaculture and fish welfare

Despite the fact that the concept of welfare has many facets and that there is no universally accepted method of assessing farmed fish welfare (Turnbull *et al.* 2008), there is no doubt that welfare in relation to farmed fish is of increased interest for scientists, fish farmers, consumers, stakeholders, and governmental bodies. When considering farmed fish, application of generalized welfare concepts requires more consideration due to species and ontogenetic differences, to the greater impact of environmental factors on fish biology and to the plasticity of responses of fish to changes in the abiotic and biotic environment. In addition, major factors affecting farmed fish welfare are related to the production system and production cycle, to husbandry and management practices, to behavioral interactions, to genetic selection and to the impact of disease and disease control measures.

Most sparid farming operations are vertically integrated, that is, they carry out all stages of the production cycle, from egg production to harvesting. During the ordinary farming procedures, fish are exposed to disturbances that may impair their welfare. Potential stressors can be found in all stages of the production cycle and include cleaning routines, grading, handling and manipulation, crowding and confinement, sexing of broodstock, feed

distribution mode, transportation between units, prophylactic measures, and use of chemicals; the presence of predators, boats and divers are also factors of disturbance for ongrowing fish kept in sea cages (EFSA 2008). On the other hand, as pointed out by Korte *et al.* (2007), ad libitum food availability together with an impoverished environment may cause stereotypic and compulsive behaviors in farmed animals.

3.6.1 *The influence of abiotic factors on the welfare of farmed sparids*

Sparidae inhabit tropical and temperate littoral or inshore waters (sometimes brackish waters) and, therefore, are in mostly eurythermal and euryhaline species, tolerating a wide range of temperatures and salinity. However, rapid and substantial changes of temperature close to the thermal limits are more likely to lead to poor welfare, and early life-stages have a more limited temperature tolerance. Finally, prolonged cold-induced fasting in sea cages affects the metabolism and physiology, in certain species like the gilthead sea bream, *Sparus aurata*, leading to the onset of the winter disease; available data suggest that 12°C could be a threshold temperature for the metabolic activity of the gilthead sea bream (Ibarz *et al.* 2003). Data on optimum salinity levels at the different developmental stages are lacking for most of the farmed Sparidae. Tandler *et al.* (1995) reported that the growth rate and survival in the gilthead sea bream larvae were higher at salinity 25 than at 40. In another study on the juvenile gilthead sea bream (20 g mean body weight), brackish water (salinity 12) acclimated fish showed a better growth with respect to seawater (38) or low salinity water (6) acclimated fish (Laiz-Carrión *et al.* 2005). Juvenile black bream, *Acanthopagrus butcheri*, are able to survive and grow at salinities, ranging from freshwater (0) to 24; however, growth was greater at salinity 24 in association with the highest food intake and most efficient food conversion ratio (Partridge & Jenkins 2002).

Sparids are also tolerant species capable of coping with large ranges of dissolved oxygen concentrations. However, it should be noted that relative oxygen consumption increases with temperature, activity, food intake, and stress level, while it decreases with increasing body size. In addition, the concentration of oxygen availability in fish varies across different production systems; for instance, in cages dissolved oxygen may be a limiting factor at high temperatures (EFSA 2008). Finally, different developmental stages have different oxygen requirements. Low dissolved oxygen conditions (10.3–16.6%) during somatogenesis caused centrum defects, which are the major vertebral deformity in cultured red sea bream, *Pagrus major* (Hattori *et al.* 2004). Cerezo Valverde *et al.* (2006) suggested, for the common dentex (*Dentex dentex*) with a body weight of 117–745 g and reared at water temperatures from 13.9 to 28.1°C, an optimal dissolved oxygen saturation of above 70–75%, suboptimal values of between 70 and 35%, and anything below 35% being unsafe. Suitable oxygen levels for *D. dentex* seem to be similar with respect to other sparids of interest in aquaculture.

The prime source of carbon dioxide (CO₂) within aquaculture systems is through fish respiration. It can become an important welfare issue only in intensified production systems, when oxygen injection is introduced, or when well or spring water is used. CO₂ concentration depends on pH, temperature, water salinity, on the respiration of the fish, and other organisms, and in the case of recirculated systems on the concentration of the H⁺ produced by the biological filter (Kaiser & Wheaton 1983). In sea cages, water pH variations are so minor to be considered as a welfare issue. However, in flow-through land-based farms and in recirculating systems, a careful monitoring of pH and CO₂ concentrations should be performed daily (EFSA 2008). Blancheton (2000) suggested for juveniles and adult sea bream that CO₂ concentrations should not exceed 40 mg L⁻¹ in recirculated and flow-through systems with supplement oxygenation.

Water quality is another important parameter for fish welfare. Among water quality criteria, ammonia (both unionized and ionized form of total ammonia nitrogen) is one of the most significant limiting factors for growth and survival. In farming conditions, the major source of the ammonia present in the water is through fish metabolism, while a minor source is through the decomposition of uneaten food (MacIntyre *et al.* 2008). Ammonia in seawater is not a welfare issue in net pen sea cage systems because it is diluted generally at nonlimiting levels by the ambient water streams (EFSA 2008). According to Dosdat *et al.* (2003), a concentration

of 0.26 mg L^{-1} UIA-N (unionized ammonia) can be considered as a safe long-term limit for seawater fish. Water renewal is also a critical factor, as a reduced specific water flow often results in the accumulation of excretion products from fish, in the accumulation of suspended solids and in low pH values that may impair growth performance and health status. In cage culture, proper site selection, antifouling practices and appropriate changing of nets are recommended to ensure sufficient water exchange (EFSA 2008). Despite the fact that there is limited information about optimum flow rate requirements in tank systems, farmers are using their own experience to avoid negative impacts for poor water quality on production. High speed water currents may also become a welfare issue since it may cause, in the early production stages, deformities and/or damage to the fins.

3.6.2 *The influence of biotic factors on the welfare of farmed sparids*

Stocking density and its effect on growth, performance, physiology, and behavior has been well studied in aquaculture species. High stocking density has been shown to produce, in several cultured sparids, chronic stress, poor feed utilization, poor growth, alterations in metabolism, physiology, behavior, and mortality (Rotllant *et al.* 1997; Montero *et al.* 1999b; Sangiao-Alvarellos *et al.* 2003). However, recent studies have shown that the effect of stocking density on intensively reared fish is mediated through the available space for the needs of fish, water quality, and social interactions (Ellis *et al.* 2002; Fanouraki *et al.* 2007; Turnbull *et al.* 2008). Unfortunately, there is a limited number of studies with stocking density manipulation under controlled water quality and social interactions. A recent study on the red porgy, *Pagrus pagrus*, showed that when group and tank sizes were reduced, even at a low stocking density (5 kg m^{-3}), the maintenance cost is higher than the one at high stocking density (25 kg m^{-3}) (Fanouraki *et al.* 2007). In another study on simulated transport of red porgy's fry, stocking density (10, 20, 30, 40 kg m^{-3}) did not affect unionized ammonia and ammonium water concentrations or mortality in groups exposed to a water renewal rate of 100%, while increasing values of estimated parameters with increasing stocking density was observed in groups with no water renewal (Pavlidis *et al.* 2003).

Aggressiveness and competition for food and/or space can negatively affect survival, growth and welfare of farmed fish. The impact of these parameters on welfare has been mainly studied in salmonids and little data is available for sparids. In gilthead sea bream juvenile (1.3–14.4 g) competition for access to food was induced and growth was negatively correlated with increased stocking density (0.35, 1.3, and 3.2 kg m^{-3}), but these effects did not seem to be related to intraspecific interactions as assessed by changes in size variability (Canario *et al.* 1998). Similarly, in other studies it was shown that dominant gilthead sea breams bit at food particles more often than subordinate members and also had the highest relative specific growth rate (Goldan *et al.* 2002). Subordinate fish showed lower feed intake and utilization and reduced growth, with a lower immunological potential (Montero *et al.* 2009). Feeding regime also affects competition for food in on-growing gilthead sea bream (Andrew *et al.* 2004). Agonistic behavior (biting of the tail, cannibalism) is the main cause of high mortalities in early production stages in certain sparids like the common dentex, *Dentex dentex* (Koumoundouros *et al.* 2004). From a practical point of view, the development of competition and aggression can be prevented or diminished by simultaneously adjusting stocking density, ensuring proper feed quality, access, and distribution.

3.6.3 *The influence of husbandry and management on farmed sparids welfare*

Fish at all stages of the production cycle of intensive rearing are exposed to several disturbances, like grading, crowding, confinement, transport between units, cleaning activities, use of anesthetics, and prophylactic agents that may cause physical injury, stress and impair fish health, quality, and welfare (Tort *et al.* 2002, 2003). However, when fish are handled by skilled personnel with special care and established protocols, and only for essential

husbandry and veterinary purposes, their welfare may be improved in the long run. For example, vaccination prevents the use of medicines and enhances fish health; grading prevents the development of aggressive behavior and cannibalism resulting in a better feed utilization and better fish performance.

Other stressors different from physical disturbance may be related to current husbandry procedures, such as feeding or nutrition components. Thus, high plasma cortisol concentrations and other hematological alterations, as well as immunosuppressive status were observed in gilthead sea bream fed diets deficient in fatty acids (Montero *et al.* 2001b; Montero *et al.* 2004). These high cortisol values were similar to those found in fishes reared under high density (Montero *et al.* 2001b). Similarly, supplementation of a low vitamin E diet results in a stress profile consisted of high levels of cortisol, immune suppression (Montero *et al.* 1998), an increase in erythrocyte fragility, a reduction in stress tolerance and overall survival rate (Montero *et al.* 2001a).

The method for fish stunning or slaughtering also has relevant influences on physiological variables and, as a consequence, on the meat quality. Handling and crowding prior to slaughter and immersion in ice slurry, which is the only method used for harvesting sparids, evoke a certain degree of stress resulting in a physiological response (Tort *et al.* 2002; Braithwaite & Boulcott 2008). Thus, electrically stunned sea bream show lower ATP depletion than fish immersed in ice slurry (Giuffrida *et al.* 2007). However, such methods are not feasible for commercial harvesting of marine sparids, and there is a need to develop alternative slaughtering methods that will improve fish welfare and increase fish quality.

3.7 Prospects on welfare research in aquaculture

The increasing protein demand for human consumption is not being currently afforded by fisheries and aquaculture will have to fulfill such a demand. A number of technologies in different areas of the production chain are being developed in order to increase the production. Another important component is also the sustainability of such technologies. The challenge is, therefore, to increase the production without affecting wild biological resources, natural stocks, or local environments.

One area of welfare research is behavioral adaptation. Although limited experiments have been performed on sparids, fish can adapt to particular husbandry conditions if adequate procedures are followed and enough time is provided. It is known that fish kept confined in low numbers may develop a tense social behavior, including aggressive responses to the other fish, while in high numbers fish behave in schools, and it is empirically known that fewer social problems are encountered. In sea bream, it has also been demonstrated that fish kept in low numbers display active social hierarchies, generating dominant fish and subordinate fish, resulting in inefficient culture conditions in growth and health.

A second aspect of welfare research is breeding selection. Years ago, scientists and aquaculturists tried to perform selection procedures in order to obtain better performances on growth, disease resistance, and stress resistance. These procedures were based on family selection and in terms of stress resistance. Selection was based on the intensity of plasma cortisol response after handling and confinement stress (Pottinger and Carrick 1999).

Attempts to select for stress response have also been performed in gilthead sea bream although the work has only followed for a limited number of generations. In gilthead sea bream, generally 10–15% of fish exhibit consistently a high (HR) or low (LR) cortisol response, and the response is conserved over the next generation (Tort *et al.* 2001). Selection data in gilthead sea bream showed that HR fish displayed lower performance in reproductive success and growth and, therefore, low responders were more appropriate for selection (Montero *et al.* unpublished results). Another selection procedure for stress responsiveness is genetic manipulation although few attempts have been carried out in fish and particularly in sparids.

A third area of improvement is the recirculating technology or Recirculating Aquaculture Systems (RAS). With RAS technology (see also Chapter 6), the use of water is drastically reduced as most of the water is

recirculated and, at the same time, the system is carefully controlled in terms of quality (water chemistry, oxygen, temperature, and nitrogenous wastes). With such a system, water of higher quality is available with the important consequence that fish can be stocked at higher densities, thus increasing fish production. It has been shown that species, such as European seabass and perch can be stocked at densities of up to 100 kg m^{-3} without significant mortalities or health problems and still sustain standard growth rates. (Gornati *et al.* 2004). This technology also applies for sparids. An experimental marine fish farm based on RAS technology has been proposed for the intensive culture of silver sea bream (Woo *et al.* 1988; Woo 1997); an RAS based on a system of biodiscs has been developed for intensive culture of silver sea bream, *Sparus sarba*, and serum cortisol levels in fish reared in recirculating water is 25-fold lower than those reared without recirculation and fivefold lower than sea-pen cultured fish (Woo & Kelly 1995). This demonstrates the ability of the RAS to reduce stress in cultured fish. Whether it can be currently applied to most species will involve that the current criteria regarding crowding densities as “deleterious” should be revised.

Finally, another important area of work for welfare is the use of nutritional components and the role of specific nutrients in decreasing the effects of stressors or counteracting their effects, an area extensively developed for sparid culture. It has been found that sea bream deficient in essential fatty acids show a decrease in muscle lipids and particularly in eicosapentanoic acid, but also an increase in plasma cortisol and a decrease in nonspecific immune defenses, such as complement (Montero *et al.* 1998, 2001a). Similarly, a stressed status with higher levels of plasma cortisol is observed in fish-fed diets deficient in vitamin C or vitamin E, together with other alterations, such as increased erythrocyte fragility and altered hematological profile (Montero *et al.* 1999a, 1999b, 2001b). When fish that are fed these deficient diets are subjected to crowding stress, commonly occurring in aquaculture, cortisol levels increase while the disease resistance and immunocompetence indicators decrease (Montero *et al.* 1999a, 2001b). On the other hand, supplementation of vitamins C and E, and particularly vitamin E, may counteract the effects of crowding stress, thereby reducing immunosuppressive effects of high density (Montero *et al.* 1999b). Moreover, supplementation of sea bream larvae with arachidonic acid may induce different effects depending on the stressor, that is, down-regulation of cortisol after air exposure, but up-regulation after salinity exposure. Although most of the mechanisms behind these changes are not known, an *in vitro* study with gilthead sea bream juveniles demonstrated that highly unsaturated fatty acids induce an increase in cortisol production in the interrenal cells of the head kidney, particularly arachidonic acid and eicosapentanoic acid (Ganga *et al.* 2006), underlying the importance of the components in the corticosteroid response. In addition, over the last years, there has been a great interest in obtaining sustainable feed for fish including sparids, by replacing fish oil with vegetable oils. Although, this may induce changes in growth, stress resistance and immune resistance in sea bream when replaced at levels higher than 60% (Montero *et al.* 2007). Higher plasma GH levels were observed in gilthead sea bream-fed high lipid diets, possibly reflecting the consequence of a compensatory protein sparing response (Company *et al.* 1999).

All approaches have improved in the last years by the advancements in genomic technologies. A number of European and American projects resulting from genome studies in fish have been looking at the responsive genes for stress susceptibility and stress response. This has produced a large quantity of genomic data and a number of tools, in particular microarrays, with the intent of discovering a set of genes responsible for such a response. Up to now the results are not conclusive, mainly because the phenomenon of stress is a complex one, involving most physiological systems and compartments. Therefore, the results of the genomic expression in microarrays involve a large amount of affected genes, most related to metabolism, as the stressors produce a shift in the metabolic and energetic pathways to allowing energy for coping with effect of such stressors. Nevertheless, some trends appear to be emerging from these works. In the brain, there is an increase in genes encoding for metalloproteins, energy driving processes and catabolism, and a decrease in immune defense genes. In the liver, there is a decrease of heat shock proteins, upregulation of immune-related genes, an increase of genes involved in energetics and reprogramming liver machinery and the responsive genes to corticosteroids (Krasnov *et al.* 2005; Sarropoulou *et al.* 2005). In the near future, it is expected that genomics can provide useful information

to manage tools either in fish selection for reduced stress, assessment of stressor effects or assessment of effects of immunostimulants.

3.8 Conclusions

The physiology of stress in fish, including sparids, has been well characterized. Neuroendocrine, metabolic, energetic, and immune changes have been determined after exposure to biotic, abiotic, and husbandry stressors. However, wide areas of research still need to be investigated, such as brain physiology, the ability of fish to adapt to recurrent stressors and the mechanisms underlying the neuro-immuno-endocrine connections. In addition, there has been an increase in concern for fish welfare that will definitely be extended to reared sparids as was done with salmonid fish. The incorporation of fish care, stress avoidance, and welfare issues will become an essential part of the commercial management in sparid farms, and, as a consequence, a retrieval or reduction in the intensity of stressors and a level of welfare will be achieved.

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