SCIENTIFIC OPINION

Animal welfare aspects of husbandry systems for farmed European seabass and gilthead seabream[1]

Scientific Opinion of the Panel on Animal Health and Welfare

(Question N° EFSA-Q-2006-149)

Adopted on 22nd October 2008

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PANEL MEMBERS*

The Scientific Panel for Animal Health and Welfare (AHAW) of the European Food Safety Authority adopted the current Scientific Opinion on 22 October 2008. The Members of the AHAW Scientific Panel were:

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* A minority opinion was expressed from Prof. Donald Broom based on the view that the accepted Report and adopted Opinion are incomplete and that in order to answer the mandate from the European Commission, the introductory chapters on the welfare, biological functioning and farming of fish should be included (Annex II).

SUMMARY

Following a request from the European Commission, the AHAW Panel was asked to deliver a Scientific Opinion on the animal welfare aspects of husbandry systems for farmed seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*). The Scientific Opinion was adopted on 22 October 2008.

From the available data, factors affecting farmed seabass and seabream welfare were identified which led to conclusions in the Scientific Opinion. These factors are grouped as: abiotic and biotic factors and behavioural interactions, food and feeding, husbandry and management, genetic selection and the impact of disease and disease control measures. A risk assessment was carried out to obtain a ranking of risk and compare the production systems.

Sea basss and sea bream are eurythermal and euryhaline species, tolerating wide range of temperature and salinity variations. Rapid and elevated changes of temperature close to the thermal limits are more likely to lead to poor welfare. Seabass and seabream are tolerant species capable of coping with large ranges of dissolved O_2 concentrations through physiological adjustments. In cages, however dissolved O_2 is a limiting factor at high temperatures. O_2 saturation in outlet water should be monitored daily and should be maintained above 40% saturation. Further studies are recommended on the combined effects of high O_2 and CO_2 levels on different stages. Seabass and seabream can be considered tolerant to pH variations. There is an increased risk of poor welfare at pH values below 6.0 and above 8.5. The daily monitoring of the water pH in recirculated (RC) and ongrowing flow-through (FT) systems are recommended. More studies are needed to evaluate the combined effects of low pH and elevated CO_2 concentrations. Super saturation is a rare but serious cause of loss in farmed fish, with serious welfare implications when it occurs. CO_2 concentration depends on pH, temperature and salinity of the water as well as the respiration of the fish and other organisms. Its management is complex in RC systems and can become a welfare issue. Studies on the CO_2 tolerance and possible welfare implications are recommended. High stocking densities and insufficient water flow may result in build up of ammonia in the water. Sub-lethal concentrations of ammonia can damage the gills and also impair immune function leading to increased susceptibility to infectious disease but further research is necessary to determine potential welfare effects of long term exposure to low levels of unionized ammonia nitrogen. Photoperiod is an important factor conditioning larvae growth and development and also the induction of spawning. The welfare consequences of artificial photoperiod, if any, are not fully understood. Ammonia and other metabolites may cause poor welfare where inadequate water flows occur. There is, however, very limited information about flow rate requirements in tank systems

Stocking density can affect welfare because of its consequences on fish social interactions and water quality. Stocking density per se (biomass/volume) cannot be used as a good indicator to predict welfare. Intra-specific aggression (including cannibalism) in post larvae can be problematic and avoidance by maintaining grading and adequate stocking densities and water flow is necessary. At the post larval stage, husbandry parameters, such as adequate stocking density, water flow and feed access should be maintained to avoid intra-specific aggression. While predation damage and the presence and predation activities are undoubtedly a significant welfare issue for seabass and seabream in certain systems, there is no systematic data available on the scale of the problem. Clear guidance based on scientific evidence on the issue of predator control should be developed and provided to the fish farming industry.

Larval first feeding is a very sensitive stage where both high quality and abundant live feeds has to be provided to the fish in order to obviate a welfare compromise. Post larvae and

ongrowing stages are less sensitive to feeding strategy providing that even access to feed is allowed to all fish in order to avoid aggression. At the larval stage, inadequate size and quantities of live feed in the diet can cause empty gut, metabolic stress, impaired growth and at worse fasting leading to death. Fish are exposed to various husbandry stressors during all stages of the life cycle in intensive culture conditions that can lead to injury, stress, increased disease susceptibility and impaired performance. Proper equipment, handling and anaesthetic protocols are important to minimize stress and physical damage associated with handling procedures. Assessment of seabream and seabass sex and sexual maturation by urogenital catheterization biopsy is an invasive stressful procedure that may threaten fish health and reproductive performance. Viral Nervous Necrosis is an important disease for seabass production with major implications in fish behaviour and welfare as no commercial vaccines are available and no treatment is possible. Monogenan parasites, winter syndrome, vibriosis and pasteurellosis are common problems in most farms and can become a significant welfare problem if not effectively controlled. Availability of veterinary medical products for seabream and seabass is very limited. Vaccines have made a significant contribution to controlling serious infectious diseases; however further research is recommended.

The risk assessment outcomes showed that in the majority of farms monitoring of health and production management is carried out to a high standard, with the possible exception of handling procedures. Hazards when they occur are generally quickly detected and corrected. Poorly formulated feed and poor storage, which may cause low level chronic effect and may go undetected was highly scored hazard for a number of life stages and across production systems (except extensive). The failure of fish to adapt to feed distribution modes and feed storage conditions during the summer months are important hazards in some stages and are open to improved management. The lack of availability of authorised anaesthetics for use in broodstock was an important hazard. Disturbance to the fish due to routine management is inevitable to a degree but management practices should be implemented that minimise the effect on fish.. There were no significant differences between larve, juveniles and ongrowers in flowthrough tanks compared with recirculation systems. The main hazards were associated with management (e.g.handling, disturbance, poor tank hygiene), feed and disease. In extensive systems predation and water temperature were important hazards for ongrowers and juveniles. The impact of infectious and non-infectious diseases in flowthrough, recirculated and also in extensive systems is an important hazard.

Key words: seabass, seabream, welfare, risk assessment, fish-farming, abiotic factors, biotic factors, feeding, husbandry, disease.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Council Directive 98/58/EC concerning the protection of animals kept for farming purposes lays down minimum standards for the protection of animals bred or kept for farming purposes, including fish.

In recent years growing scientific evidence has accumulated on the sentience of fish and the Council of Europe has in 2005 issued a recommendation on the welfare of farmed fish[2]. Upon requests from the Commission, EFSA has already issued scientific opinions which consider the transport[3] and stunning-killing[4] of farmed fish.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of this and in order to receive an overview of the latest scientific developments in this area the Commission requests EFSA to issue a scientific opinion on the animal welfare aspects of husbandry systems for farmed fish. Where relevant, animal health and food safety aspects[5] should also be taken into account. This scientific opinion should consider the main fish species farmed in the EU, including Atlantic salmon, Gilthead seabream, Seabass, Rainbow trout, carp and European eel and aspects of husbandry systems such as water quality, stocking density, feeding, environmental structure and social behaviour.

Due to the great diversity of species it was proposed that separate scientific opinions on species or sets of similar species would be more adequate and effective. It was agreed to subdivide the initial mandate into 5 different questions:
Question 1. In relation to Atlantic salmon
Question 2. In relation to trout species
Question 3. In relation to carp species.
Question 4. In relation to seabass and gilthead seabream
Question 5. In relation to European eel

This Scientific Opinion refers only to Question 4 as referenced above

ACKNOWLEDGEMENTS

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2 Recommendation concerning farmed fish adopted by the Standing Committee of the European Convention for the protection of animals kept for farming purposes on 5 December 2005

3 Opinion adopted by the AHAW Panel related to the welfare of animals during transport -30 March 2004

4 Opinion of the AHAW Panel related to welfare aspects of the main systems of stunning and killing the main commercial species of animals- 15 June 2004

5 Food Safety aspects are addressed by a Scientific Opinion of the BIOHAZ Panel (Food Safety aspects of Animal welfare aspects of husbandry systems for farmed fish, Question N° EFSA-Q-2008-297).

CONCLUSIONS AND RECOMMENDATIONS

1. OUTCOMES FROM THE DATA PRESENTED IN THE SCIENTIFIC REPORT

1.1. Abiotic Factors

1.1.1. Temperature

Conclusions

- Seabass and seabream are eurythermal fish. Minimum and maximum survival water temperatures are 2-32 °C and 5-34 °C for seabass and seabream respectively. Early life-stages, eggs, larvae and juveniles have more limited temperature tolerance during ontogenesis.
- Temperature tolerance is highly dependent on acclimation. Rapid and substantial changes of temperature close to the thermal limits are likely to lead to poor welfare.
- Seabream 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 seabream, and it has been associated with the onset of winter disease.

Recommendations

- For seabass, the temperature ranges 10-20 °C for eggs, larvae and 8-28 °C for larger fish should be recommended in terms of acceptable welfare.
- For seabream, the temperature ranges 12-22 °C for eggs, larvae and 8-30 °C for larger fish should be recommended in terms of acceptable welfare.
- During incubation and early development stabilized water temperature should be maintained. During the ongrowing phase, any rapid changes of temperature should only occur within the recommended thermal ranges. Temperature changes exceeding 5 °C /day should be avoided

1.1.2. Oxygen

Conclusions

- The available dissolved oxygen (mg l^{-1}) in water depends on temperature, salinity, partial pressure of ambient oxygen, stocking density and water renewal.
- Seabass and seabream are very tolerant species capable of coping with large ranges of dissolved oxygen concentrations through physiological adjustments. The relative oxygen consumption (mg O_2 kg^{-1} fish h^{-1}) in both species increases with temperature, activity, feed consumption and stress level, while it decreases with increasing body size.
- The concentration of oxygen available to fish varies across different production systems. In cages, dissolved oxygen is a limiting factor at high summer temperatures. Such problems do not normally arise in flow-through or recirculated systems except in

the event of mechanical breakdown. At 40% oxygen saturation feed intake and growth are impaired in seabass and seabream.

- The combination of high oxygen (230-250% O_2 saturation) and high carbon dioxide (50-60 mg $CO_2\ l^{-1}$) increases mortality in seabass after bacterial challenge.

Recommendations

- The oxygen saturation in outlet water should be monitored daily and, as a guideline, should be maintained above 40% saturation. Siting, cage design, cage orientation, bio-fouling control and stocking density should be optimised to avoid chronic hypoxic conditions in cages

Recommendations for future research

- Further studies are needed on the combined effects of high O_2 and CO_2 levels on different life stages.

1.1.3. pH

Conclusions

- Seabass and seabream are able to maintain balanced acid/base concentrations and constant internal pH even when pH in ambient waters varies widely. Both species can therefore be considered as tolerant to pH variations.
- In cages, seawater pH variations are too small to become a welfare issue.
- There is an increased risk of poor welfare at pH values below 6.5 and above 8.5 for both seabass and seabream and mortality can occur when fish are exposed abruptly to a pH below 4.5 and above 9.4, but this is unlikely to happen in normal practice.
- In flow-through land based farms, pH level depends mainly on CO_2 concentrations due to fish respiration. In case of low water renewal rate combined to high levels of oxygen supplementation in inlet waters, the CO_2 concentrations per se in ambiant water may have detrimental effects on fish before the altering pH levels.
- In recirculated systems, pH depends on CO2 concentrations and also on the level of H+ produced by the biological filter. When CO2 is removed by the use of packed columns, the pH may nevertheless reach low values (<6) and there is limited information on the welfare implications of such circumstances.

Recommendations

- Water pH should be monitored closely in recirculatedg and ongrowing flow-through system using low water renewal and supplementary dissolved oxygen.
- Water pH values in the range 6.5 to 8.5 ensure good welfare in seabass and seabream. pH values below 5 and above 9 impair growth and welfare.

Recommendations for future research

- The effect of pH on early life stages is not well-known and further investigations are required as this is a particularly vulnerable element of the production cycle.
- More studies are necessary to evaluate the combined effects of low pH and elevated CO_2 concentrations

1.1.4. *Carbon Dioxide*

Conclusions

- Carbon dioxide (CO_2) concentration depends on pH, temperature and salinity of the water as well as the respiration of the fish and other organisms in the water its management is complex in recirculating systems and can become a welfare issue.
- CO_2 concentration is not normally a welfare issue in cage systems, but can become one in some circumstances in flow through systems.
- Lethal concentration in juvenile seabass (LC 50 at 96 h, at 15 °C) is close to 112.1 m CO_2 l^{-1} (50.4 mm Hg). Seabass can, however, compensate for blood acidosis at water concentrations of CO_2 around 55 mg l^{-1} and no mortality or reduced growth has been observed under chronic hypercapnic conditions (up to 75 mg l^{-1} for 45 days). There is no available information for seabream.

Recommendations for future research

- Further research is recommended in relation to the CO_2 tolerance of seabream and possible welfare implications.
- Since nephrocalcinosis would appear to be a factor of welfare significance even at low levels of CO_2, practical studies are justified under commercial conditions on seabass and seabream farms to determine threshold CO_2 levels.

1.1.5. *Supersaturation*

Conclusions

- Super saturation is a rare but serious cause of loss in farmed fish, with serious welfare implications when it occurs.

1.1.6. *Ammonia*

Conclusions

- High stocking densities and insufficient water flow may result in build up of ammonia in the water. Ammonia is present in ionised and un-ionised forms. The level of the more toxic form, the un-ionised ammonia is dependent on total ammonia level, pH, temperature and salinity.
- Sub-lethal concentrations of ammonia can damage the gills and also impair immune function leading to increased susceptibility to infectious disease.
- The 0.26-mg l^{-1} UIA-N concentration can be considered as a safe long-term limit in seawater for seabass juveniles.
- Ammonia in seawater is not a welfare issue in on-growing cage systems because it is diluted generally at non-limiting levels by the ambiant water streams.

Recommendations for future research

- Further research is necessary to determine potential welfare effects of long term exposure to low levels of unionized ammonia nitrogen.

1.1.7. Salinity

Conclusions

- Seabass and seabream are euryhaline fish capable of tolerating both high saline waters and freshwater environments. Gradual changes in salinity are not a welfare issue in seabass and seabream farming.
- Despite their tolerance of wide salinity ranges, both species are sensitive to rapid changes in salinity.

Recommendations

- Seabass and seabream should not be subjected to rapid and significant changes of salinity.

1.1.8. Light/ Photoperiod

Conclusions

- Photoperiod is an important factor conditioning larvae growth and development and also the induction of spawning. Photoperiod treatments can be employed to advance or delay the spawning time.
- The welfare consequences of artificial photoperiod, if any, are unknown.

Recommendations

- Photoperiod manipulation to modify the male sex ratio in seabass is becoming a useful husbandry tool. The welfare implications of this are not known and this area should be investigated.

1.1.9. Water renewal / Water exchange rate / Specific water flow

Conclusion

- Ammonia and other metabolites may cause poor welfare where inadequate water flows occur in all seabass and seabream culture systems. There is, however, very limited information about flow rate requirements in tank systems
- In cage culture there is a particular risk of poor welfare in the case of inappropriate site selection and unfavourable managerial practices. The measurement of the water renewal rate in cages is difficult and the measurement of water oxygen concentration is the most accurate and current-induced measure of water quality

Recommendations

- In cage culture proper site selection, antifouling practices and appropriate changing of nets are recommended to ensure sufficient water exchange.

Recommendations for future research

- More research is needed to explore risks of poor welfare at different water renewal rates in seabass and seabream in the different life stages.
- There is a significant requirement for further studies on definition of inter-relationships between water flow, water quality and stocking density in culture systems for both seabass and seabream.

1.2. Biotic factors / Behavioural interactions

1.2.1. Stocking density

Conclusions

- Stocking density per se (biomass/volume) cannot be used as a good indicator to predict welfare. Stocking density can affect welfare because of its consequences on fish social interactions and water quality. In addition, crowding can lead to poor welfare.

Recommendations

- In considering the effects of stocking density on farmed seabass or seabream it is recommended that monitoring of the condition of the fish and the water quality is the appropriate route for preventing poor welfare.
- When short-term high stocking densities are necessary for husbandry manipulations, close attention to water quality, fish health and behaviour should be maintained.

1.2.2. Intraspecific interactions: Aggression and competition

Conclusions

- Intra-specific aggression (including cannibalism) in post larvae can be problematic and its prevention by maintaining a uniform size distribution, adequate stock density and water flow is necessary.
- Feeding regime determines competition for feed amongst gilthead seabream which can influence feeding behaviour and feeding efficiency.

Recommendations

- In pre-ongrowing seabream, the development of competition and aggression can be prevented, principally by simultaneously adjusting stocking density and ensuring proper feed access.
- In seabass post-larvae, husbandry parameters, such as adequate stocking density, water flow and feed access should be maintained to avoid intraspecific aggression.

1.2.3. Predation

Conclusions

- While predation damage and the presence and predation activities are undoubtedly a significant welfare issue for seabass and seabream in certain systems, there is no systematic data available on the scale of the problem.
- The efficacy of the methods developed to prevent or minimise predation are very variable. There is also a lack of any rigorous scientific investigation or clear practical advice for farmers on the methods to be used to control the predation.
- Birds may predate upon seabass and seabream in ponds, lagoons and sea cages but predation by marine mammals is not documented.

Recommendations

- In pond and lagoons systems, predation should always be controlled (by netting or other methods), whenever possible. Control of cormorants is particularly difficult and the welfare of the fish as well as the cormorants should be considered in any control strategy.
- Clear guidance based on scientific evidence on the issue of predator control should be developed and provided to the fish farming industry.
- Data should be collected on the extent of predation in seabass and seabream culture, the control methods used and their efficacy, including their impact on predators.

1.3. Food and Feeding

Conclusions

- Larval first feeding is a very sensitive stage where both high quality and abundant live feeds has to be provided to the fish in order to preserve good welfare.
- Post-larvae and ongrowing stages are less sensitive to feeding strategy providing that even access to feed is allowed to all fish in order to avoid aggression.
- Inadequate feed formulation and quality problems can induce larval deformities and impaired growth.

Recommendations

- Feed quantity, distribution and quality should be sufficient to avoid poor welfare associated with stress and intra specific interactions including competition and cannibalism at larvae, juvenile and pre-ongrowing stages.

1.3.1. Food deprivation and starvation

Conclusions

- At the larval stage, food deprivation can have severe effects on fish. Inadequate size and quantities of live feed in the diet can cause empty gut, metabolic stress, impaired growth and at worse fasting leading to death.
- Ongrowing and brood fish are more tolerant to feed deprivation but nevertheless if prolonged it can lead to inappropriate social interactions affecting welfare.

1.4. Husbandry and Management

Conclusions

- Fish are exposed to various husbandry stressors during all stages of the life cycle in intensive culture conditions that can lead to injury, stress, increased disease susceptibility and impaired performance.
- Proper equipment, handling and anaesthetic protocols are important to minimize stress and physical damage associated with handling procedures.
- Eggs and larvae are fragile in handling and abrupt temperature changes that can lead to mortalities and developmental deformities.
- Out-of-season spontaneous spawning is feasible in both seabass and seabream by the use of photoperiod and temperature manipulation. Hormonal induced spawning is not in practice for seabream and has a limited use in seabass broodfish.
- Broodstock should always be handled by well-trained personnel and under sedation to minimize physical damage and stress. Currently there is only one anaesthetic approved for use in fish that is generally considered less suitable than other unlicensed alternatives
- Grading is an important part of husbandry at the juvenile and pre-ongrowing stages as it prevents the development of aggression and cannibalism, results in better performance and facilitates daily husbandry activities. However, grading may cause physical injury and stress.
- Sort-term handling, crowding and confinement and daily cleaning activities may result in the activation of the classical physiological stress response in fish, however, physiological and behavioural changes are normally reversible and fish recover within 24 h following exposure to the stressor.
- Intense activities like prolonged crowding and transportation between units or between different fish farms may cause physical injury and major physiological and behavioural changes where the animal is unlikely to cope or adapt to the stress being imposed.
- Assessment of seabream and seabass sex and sexual maturation by urogenital catheterization biopsy is an invasive stressfull procedure that may threaten fish health and reproductive performance.

Recommendations

- Fish should be handled solely by skilled personnel and only for essential husbandry and veterinary purposes.Handling should be performed with special care to avoid physical injury and damages and to minimize stress.
- Abrupt changes in water temperatures should be avoided during transport of eggs, larvae and fry between the different units of the hatchery or between different hatcheries.
- There is a need for authorised anaesthetics and prophylactic agents for use in seabass and seabream farming.

Recommendations for future research

- Further research on the use of ultrasonogaphy and other non invasive methods in sex and sexual maturity determination in seabream and sebass is necessary.

1.5. Genetic selection impact on welfare

Recommendations for future research

- Research is necessary on monitoring if genetic selection programs affect traits that will negatively impact welfare.

1.6. Impact of disease on welfare

1.6.1. Viral Nervous Necrosis (VNN) / Viral encephalopathy and retinopathy (VER) / Nodavirus infection

Conclusions

- VNN is an important disease in seabass production. VNN affects the central nervous system and has major implications in fish behaviour and welfare
- 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.

Recommendations

- Official and non-official survey and control programmes should be recommended
- The efficacy of the specific biosecurity measures for this condition should be further improved.
- Development of effective vaccines should be encouraged
- 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.

1.6.2. Monogenean infections

Conclusions

- Monogenan parasites are common and persistent problems in most seabream and seabass farms and can become a significant problem with welfare implications if they are not effectively controlled.
- 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.

Recommendations

- In order to maintain the level of the parasites and the disease at a low level prophylactic controls at farm level should be improved.
- A wider range of antiparasitic drugs against monogenean infections should be developed and made available.

1.6.3. Winter syndrome

Conclusions

- Winter syndrome is a disease that is associated with strong metabolic and immunological disturbances in seabream in some farming practices at low temperatures for long periods.
- Correct management before the cold season (avoid feeding when temperatures are low and reduce stressful management) minimises the risk of the disease.

Recommendations

- The low temperature at which there is increased risk of winter syndrome disease in each area where sea bass are farmed should be determined in relation to the fish strain and farming conditions
- Correct nutritional and husbandry measures before the cold period to prepare the fish to achieve an adequate metabolic status should be encouraged.

1.6.4. Vibriosis

Conclusions

- Vibriosis can be a secondary pathology often associated with unappropriate handling and management procedures in intensive systems.
- Vibriosis are a common group of diseases that affect seabass and seabream but the disease is usually controlled by the use of approved antibiotics given in the feed,
- 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.

Recommendations

- 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.

1.6.5. Pasteurellosis

Conclusions

- Pasteurellosis is a significant disease affecting seabass and seabream. 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.

Recommendations

- 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.
- Research on vaccines with increased efficacy and reduced side effects should be envisaged.

1.6.6. Lymphocystis

Conclusions

- Lymphocystis is a benign disease that spontaneously disappears if rearing conditions are correct

Recommendations

- Good husbandry conditions during the infection should be implemented for a quick and total recovery and stressful and rough manipulation should be avoided.

1.7. Disease Control Measures

Conclusions

- Availability of veterinary medical products approved for seabream and seabass is very limited and this constitutes an important risk for poor welfare caused by disease.

Recomendations

- Measures should be taken to facilitate rapid and beneficial release of efficacious veterinary medicinal products.

1.7.1. Vaccination

Conclusions

- Vaccines have made a significant contribution to controlling serious infectious diseases in seabream and seabass.

Recomendations for future research

- Research for development of vaccines with a long lasting immunity should be carried out.

1.7.2. Biosecurity

Conclusions

- Individual farm biosecurity strategies with mandatory protocols are a major advantage in controlling the spread serious infectious disease.

Recomendations

- Seabass and seabream fish farms should be operated under agreed biosecurity plans subject to audit by a veterinarian.

2. RISK ASSESSMENT

2.1. Risk Assessment Discussion

2.1.1. *Eggs*

In this life stage, in both flow-through and recirculated production systems all hazards occurred with low probability (scoring 1 or 2). Hazards considered at the egg stage were important because of embryonic abnormalities, which may persist through later life stages. It should be taken into consideration that welfare *per se* is not relevant at this life stage.

- Flow-through

In the flow-through system the highest ranked risks were sudden change in temperature and inappropriate transport between units, which would cause significant mortality, followed by low water renewal and a sudden change in dissolved oxygen. The high score for inappropriate transport was mainly attributable to the fact that a high proportion of the population was affected. The sudden change in temperature has the highest severity score.

- Recirculation

Three hazards stood out in the recirculation system: low water renewal, inappropriate transport and sudden change in temperature.

A range of deformities will arise from the listed hazards, some of which may result in mortality after days, others will resolve in time.

2.1.2. *Larvae*

- Flow-through and Recirculation systems

The highest ranked hazards (in ranked order) for larvae in flow-through and recirculation systems were:

1. Inadequate stocking density
2. Inappropriate water velocity
3. Inadequate feed formulation and storage conditions
4. VNN / VER / Nodavirus infection

It should be pointed out that there is a big scoring difference between the four highest scored hazard and the rest of the hazards in the list (~0.010 vs ~0.003, see Tables).

- The inadequate stocking density hazards scored highly because they persist for 40 days and occurred relatively frequently
- Inappropriate water velocity scored highly mainly because it had a high severity score (3) and the effect lasted 21 days.
- Iinadequate feed formulation/storage conditions had a severity scores of 3.

- Disease (VER/VEN/nodavirus) was the fourth highest ranked hazard. These diseases only occur infrequently (probability score = 1) but affect a high proportion of the population with high severity. The outcome is frequently death, thus the mortality score is also high. The period of clinical disease, prior to mortality, is approximately 5 days.

2.1.3. Juveniles

This stage lasts about 50 to 90 days. Fish are growing rapidly and removal of fish generally occurs twice to maintain a consistent stocking density (kg/m^3). Three systems were considered: flow-through, recirculation, and extensive.

- Flow-through and Recirculation systems

- In both systems poor tank hygiene receives a much greater score than any other hazard, attributable to a high severity score, a high proportion of the population affected and long duration (70 days).
- VNN/VER/nodavirus was an important hazard during the larval stage and remains important (second most highly ranked hazard) for juveniles in flow-through tanks and recirculation systems but not in extensive systems.
- Monogenean infections received high scores in tanks and recirculation systems. There is a lower probability of the disease hazards occurring in the recirculation systems, compared with the flow-through tanks. However, in recirculation systems the impact of monogeneans may be more severe. Poor biosecurity will lead to infection that will last 30 days.
- Abdominal adhesions due to vaccination (vaccine side effects) also ranked highly. Other adverse effects of vaccination by bath challenge were considered to be handling and crowding, which were considered separately. Handling did not feature as a highly ranked hazard. However data presented in the Scientific Report clearly indicates that handling is a welfare hazard, especially because the lack of anaesthetic and repeated manipulations of juveniles during this period for different purposes.

- Extensive

In extensive systems, considered hazards were different to the other 2 systems. Overall fewer hazards were identified, (n=4) and the value of the highest ranked hazard (predation) was approximately half the value of the highest ranked hazard in the more intensive sytems.

- Predation was by a very wide margin the most important hazard. Its high score was due to the severity (mainly physical injury) and duration of the effect of the hazard.
- Extreme temperature was another important hazard because whilst the conditions occurred relatively infrequently, if affected a high proportion of the population and affected them for on average 14 days.

These hazards reflect the nature of the systems. There exists an inherently reduced capacity to influence the environmental parameters in extensive compared with other systems.

2.1.4. Ongrowers

Handling issues and inadequate feed formulation / storage were the most important hazards in sea cages, recirculated and flow-through tanks.

Inadequate feed formulation and storage scored highly because it affects a high proportion of the population, has a relatively high probability (3) and severity score (3) but above all because of a high estimated duration (150 days). 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 (2).

In recirculated and flow-through tanks disturbances mechanical failure and. Mechanical failure may cause stress because of the decline in water quality parameters. It is assumed that the fault is corrected relatively promptly and the fish recover relatively quickly (if they have survived). Similarly, adverse water quality parameters in recirculation systems are relatively short-lived since the farmer rectifies the problem or the fish die rapidly. The effect of untrained personnel is always expressed through the other hazards of the list, therefore untrained personnel as a has not been scored. In sea cages, poor adaptation to feed distribution was assessed as a high risks

Clear difference exists between the extensive and the other systems. In extensive systems few hazards were identified for ongrowers compared with other systems. Almost no handling occurs in extensive systems at this stage. Feed formulation is not an important issue in extensive systems as natural feed represents an important complement. The main hazards for ongrowers in extensive systems are disease, and predation. In sea cages, protective nets normally prevent bird predation. Failure in the system leading to predation will be, in general, quickly corrected and so predation does not score highly. In more extensive systems protective nets are not practicable.

In extensive and semi-intensive systems, algal blooms and lack of artificial oxygenation system may cause hypoxia. It did not however achieve a high score because it had a low probability score (1) and duration was estimated to be only 7 days.

The difference between the extensive and other systems reflect the nature of the inherently lower level of disease management and predator control that is attainable under extensive systems. However, these systems are better in terms of reduced disturbance and feed formulation and storage scoring a much lower.

2.1.5. Broodstock

The welfare of broodstock is particularly important since fish might be fertile for a number of years. Two hazards stood out: high stocking density and not using anaesthetics. The manipulation of broodstock without the use of anaesthetics occurs because anaesthetics are not licensed for seabass or bream in the EU. Manipulation without anaesthesia was considered to be a hazard with low severity (1) but affects all broodstock for approximately 63 days during this life stage. Similarly high stocking density received a low severity score (1) but since it lasted for 300 days the final score was high.

The following hazards attained considerably lower scores: inadequate feed formulation and storage conditions, inadequate feed size and improper sexing. Inaccurate sexing of broodstock may lead to an absence of males, and female seabream may retain eggs leading to reproductive dysfunctions and/or mortality.

2.2. Conclusions

- In the majority of farms monitoring of health and production management is carried out to a high standard, with the possible exception of handling procedures. Hazards when they occur are generally quickly detected and corrected.
- Inadequate feed formulation was a highly scored hazard for a number of life stages and across production systems (except extensive). It scores highly because it occurs relatively frequently, and lasts for much of the life stage. This hazard is partly outside of the control of the fish farmers and is an issue that needs to be pursued with the feed manufacturers. The main problem lies with the quality of the protein.
- The failure of fish to adapt to feed distribution modes and risks associated with poor feed storage conditions during the summer months (which results in a decline in feed quality), are important risky hazards in some stages and are open to improved management.
- The lack of availability of authorised anaesthetics for use in broodstock was an important hazard in this life stage.
- Poor handling was highlighted in a number of life stages and could be improved through training and better management.
- The highest ranked hazard for larvae was inappropriate water velocity.
- Disturbance to the fish due to routine management is inevitable to a degree but management practices should be implemented that minimise the effect on fish.
- There were no significant differences between larve, juveniles and ongrowers in flowthrough tanks compared with recirculation systems. The main hazards were associated with management (e.g.handling, disturbance, and poor tank hygiene), feed and disease.
- In extensive systems predation and water temperature were important hazards for ongrowers and juveniles. The impact of infectious and non-infectious diseases in flow through recirculated and also in extensive systems can also be considered as an important hazard.

SCIENTIFIC REPORT OF EFSA

ANIMAL WELFARE ASPECTS OF HUSBANDRY SYSTEMS FOR FARMED EUROPEAN SEABASS AND GILTHEAD SEABREAM [1]

Prepared by Working Group on seabass /seabream welfare

(Question No EFSA-Q-2006-149)

Issued on 22nd October 2008

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SUMMARY

The scientific report on animal welfare aspects of husbandry systems for farmed European sea bass and Gilthead seabream constitutes the background document to the opinion adopted by Panel on Animal Health and Welfare adopted on 22nd October 2008.

The Scientific report presents the life history and an overview of the production systems to the considered species: European seabass (*Dicentrarchus labrax*) and Gilhead seabream (*Sparus aurata*). Although the species are taxonomically very different they are often produced in similar production systems. In Europe diversified systems, extensive, semi intensive and intensive, are used seabass and seabream production.

The report reviews the production factors potentially affecting welfare of farmed seabas and seabream. Those factors were grouped in categories: abiotic, biotic, feed and feeding, husbandry and management, genetic, disease and disease control measurers.

The risk assessment approach was applied to assess the welfare risk of each of the factors identified as hazards for various life stages in various production systems. However the environmental conditions affecting welfare are often inter-related factors and that is not reflected in the applied method. Risk assessment was based on scientific literature, expert opinion and practical experience.

Key words: seabass, seabream, welfare, production systems, production factors, abiotic, biotic, feed, husbandry and management, genetic, disease, disease control measures, risk assessment.

ACKNOWLEDGEMENTS

This scientific / technical report was prepared by the working group on Seabass/seabream Welfare. The European Food Safety Authority wishes to thank the members of the WG for their contribution to the preparation of this report: Ronald Roberts (Chairman), Ed Peeler (Risk Assessor) Marie-Laure Bégout, Gilles Lemarié, Giovanna Marino, Michalis Pavlidis and Francesc Padrós.

The scientific co-ordination for this Scientific Report has been undertaken by the EFSA AHAW Panel Scientific Officers: Oriol Ribo, Ana Afonso and Tomasz Grudnik.

PANEL MEMBERS

This scientific report was peer reviewed by the Members of the Scientific Panel for Animal Health and Welfare (AHAW) of the European Food Safety Authority. The scientific report was used as the basis for a scientific opinion adopted on 22nd October 2008. The members of the AHAW Scientific Panel were:

Bo Algers, Harry J. Blokhuis, Anette Bøtner, Donald M. Broom, Patrizia Costa, Mariano Domingo, Mathias Greiner, Daniel Guemene, Jörg Hartung, Frank Koenen, Christine Müller-Graf, David B. Morton, Albert Osterhaus, Dirk U. Pfeiffer, Mohan Raj, Ron Roberts, Moez Sanaa, Mo Salman, J. Michael Sharp, Philippe Vannier and Martin Wierup.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Council Directive 98/58/EC concerning the protection of animals kept for farming purposes lays down minimum standards for the protection of animals bred or kept for farming purposes, including fish.

In recent years growing scientific evidence has accumulated on the sentience of fish and the Council of Europe has in 2005 issued a recommendation on the welfare of farmed fish[2]. Upon requests from the Commission, EFSA has already issued scientific opinions which consider the transport[3] and stunning-killing[4] of farmed fish.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of this and in order to receive an overview of the latest scientific developments in this area the Commission requests EFSA to issue a scientific opinion on the animal welfare aspects of husbandry systems for farmed fish. Where relevant, animal health and food safety aspects[5] should also be taken into account. This scientific opinion should consider the main fish species farmed in the EU, including Atlantic salmon, Gilthead seabream, Seabass, Rainbow trout, carp and European eel and aspects of husbandry systems such as water quality, stocking density, feeding, environmental structure and social behaviour.

Due to the great diversity of species it was proposed that separate scientific opinions on species or sets of similar species would be more adequate and effective. It was agreed to subdivide the initial mandate into 5 different questions:
Question 1. In relation to Atlantic salmon
Question 2. In relation to trout species
Question 3. In relation to carp species.
Question 4. In relation to seabass and gilthead seabream
Question 5. In relation to European eel

This Scientific Opinion refers only to Question 4 as referenced above

2 Recommendation concerning farmed fish adopted by the Standing Committee of the European Convention for the protection of animals kept for farming purposes on 5 December 2005

3 Opinion adopted by the AHAW Panel related to the welfare of animals during transport -30 March 2004

4 Opinion of the AHAW Panel related to welfare aspects of the main systems of stunning and killing the main commercial species of animals- 15 June 2004

5 Food Safety aspects are addressed by a Scientific Opinion of the BIOHAZ Panel (Food Safety aspects of Animal welfare aspects of husbandry systems for farmed fish, Question N° EFSA-Q-2008-297).

SCOPE AND OBJECTIVES OF THE REPORT

European seabass *Dicentrarchus labrax* and Gilthead seabream *Sparus aurata* production in the various European countries is often done using similar systems, although they are very different species belonging to different taxonomic families, for this reason it was decided to consider both species in the same report. The differences in their life cycle have been explained in Chapter 2.

The various areas where specific welfare risks have been considered to exist have been defined and analysed in relation to the different stages in the life history of the two species and the type of production system when differences occur between the 2 species these were highlighted. Chapter 3 provides evidence concerning major factors affecting the welfare of the 2 farmed species. Wherever possible information used to inform the report is taken from the available scientific literature but recourse has often been necessary to expert opinion or to the experience of producers. Where use of such information was necessary, it is indicated in the text. The scientific information is in general more available to seabass than seabream and extrapolation is not always possible.

A Risk Assessment approach was followed to describe possible welfare risks. The risk assessment methodology is explained in Chapter 5. The information about potential hazards forms the basis for the hazard identification in the assessment of risks associated with particular husbandry conditions for seabream and seabass. This risk assessment had three objectives: i) transparency ii) to allow a ranking of the most important hazards and iii) to attempt a comparison of the different production systems for each life stage.

Although it is recognised that transportation of live seabream and seabass and their slaughter may involve welfare issues, these were excluded from the terms of reference and are not considered.

The objective of the report is to highlight those aspects of current husbandry systems for trout in Europe which may increase the likelihood of negative welfare effects on the fish and to recommend areas where modification of the system as currently used may assist in reducing this risk.

1. Taxonomy

The European seabass (*Dicentrarchus labrax,* Linnaeus, 1758) is a member of the recently revised family of Moronidae, which also includes congener *Dicentrarchus punctatus* (Bloch, 1972). Seabass has an elongated body with a more or less straight dorsal head profile and two clearly differentiated dorsal fins and a rather high tail. They can reach a maximum length of 1 m and 15 kg weight. Their bodies are grey to greenish black along the back (dorsally), grey-silver laterally and white silver along the belly (ventrally). The mouth is moderately protractile and vomerine teeth are present only in the anterior part. This characteristic and the absence of black spots on the back and sides at the adult stage distinguish *D. labrax* from *D. punctatus*. The operculum bone has two flat spines and a range of small forward-directed denticles in the lower part of pre-operculum bone. The first dorsal fin has a typical triangular shape with 8-10 spiny rays; the second dorsal fin is trapezoid in shape with 12- 13 soft rays, the anal fin has 3 spines and 10-12 soft rays, and the caudal fin is slight forked with a large caudal peduncle (Figure 1).

Gilthead seabream (*Sparus aurata*, Linnaeus, 1758) is a member of the Family Sparidae, which includes 24 species in the Mediterranean and 2 migrants from Red Sea established in Eastern Mediterranean. Seabream have an oval, compressed and rather deep body and can reach a maximum length of 70 cm and 5 kg weight. Molar-shape teeth are arranged in 2-4 rows on the jaw and become very big in fish longer than 20 cm. The body is essentially silver-grey coloured, with large red-rimmed black blotch or patch starting behind the operculum and extending forward over the upper side. A golden frontal stripe is present between the eyes. A black longitudinal line crosses the dorsal fin, the other fins are light grey and the tips of the caudal fin are edged in black. The dorsal fin has 11 spines and 13-14 soft rays and the anal fin has 3 spines and 11-12 soft rays. The snout and superorbital areas are scaleless (Figure 2).

Whenever in this report seabass and seabream are mentioned it refers to *Dicentrarchus labrax* (European seabass) and *Sparus aurata* (Gilthead seabream).

Figure 1. **Adult seabass**

Figure 2. **Adult seabream**

2. Life history

2.1. Seabass (*Dicentrarchus labrax*): Life stages

Seabass are common in the Mediterranean Sea, the Black Sea and along the North Eastern Atlantic coasts, from Norway to Senegal. In 1994, it was introduced into Iceland from France for aquaculture. It inhabits coastal waters down to 100 m depth, but it is commonly found in estuarine areas and coastal lagoons which it enters during summer and then returns to the sea during the winter period (Tortonese et al., 1984). Both juveniles and adults often overwinter in Mediterranean coastal lagoons. It’s a eurythermal and euryhaline species, tolerating temperatures between 2 and 32°C and surviving in freshwater and high salinity waters up to 50[6]. Young fish inhabit coastal waters, are gregarious and form schools, also with other fish. Juveniles feed mainly on small crustaceans and small fish. Adult fish are less gregarious, feeding mainly on crabs and shrimps and they change their feeding rhythms (diurnal and nocturnal) on a seasonal basis.

Seabass hunts both individually and in group. The group hunting concerns both adults and juveniles. The seabass of similar size hunt in a coordinated way on pelagic fish schools in the water column, a first individual attacks the schools soon followed by others. Juveniles exhibit gregarious behaviour especially evident during seasonal migrations while adults tend to be

[6] 'Salinity', defined as the ratio of two quantities of the same unit, is a 'dimensionless quality', *i.e.* takes no units. (UNESCO, 1985. The International System of Units (SI) in Oceanography. Report of IAPSO working group on symbols, units and nomenclature in physical oceanography (SUN). IAPSO Publication Scientifique, no. 32, UNESCO technical papers in marine science, no. 45).

more solitary except during the hunting periods (Barnabé, 1980; Bas Peired, 2002). Juveniles coordinated schools are observed for fish of similar sizes. The placement of the groups in the water column varies in accordance with the size of the individuals on it: the smaller individuals swim at lower depth (0 to 2 m), the larger fish at higher depth, and this in order to avoid intra specific cannibalism. Such shoaling disperses at dusk and seabass display little movement during night-time and stay in close contact with the bottom. The gregarious behaviour of seabass juveniles has been observed on the wild and in captivity (Barnabé, 1980). In captivity strong behavioural disturbances (Anthouard, 1987) and reduced growth (Stirling, 1977) have been observed when seabass is kept alone.
Underwater observations have shown that sea bass may occasionally exhibit aggressive and territorial behaviours. Seabass has indeed two types of behaviours when attacked by a larger individual: move away or adopt a defensive position. In that later case, the attacked individual, if it is smaller than the opponent, raises the dorsal fin and rays in order to enlarge its size (Brosowski, 1999). There are however very little evidence of aggressive interactions among fish of the same size.

Broodstock (or Breeders)

Seabass are a gonochoristic species and gonads are undifferentiated during the first year of life. Sexual maturity generally occurs at 3 years in males and 4 years in females in the Mediterranean Sea, and a little later, between 4 and 7 years in the Atlantic. Under farming conditions, puberty is attained at 2 years in males (average body weight of 200 g) and at 3 years in females at 700 g body weight. This reproductive strategy results in better growth performances in females (39 %) during the second year of life (Gorshkov et al., 2004). Male biased populations (70-90 %) are usually produced in cultured conditions, with male fish exhibiting precocious sexual maturation (Zanuy et al., 2001). Sexual precocity is undesirable in fish farming because it is correlated with negative aspects such as reduced growth and feed conversion, and new techniques for production of all female stocks (Pavlidis et al., 2000) and sterile fish (Gorshkov et al., 2002), as well as delaying fish puberty, have been recently developed.

Seabass spawn once per year. In Mediterranean waters, female gonadal maturation starts in September and spawning occurs from December to March in estuaries and in-shore areas where salinity is close to seawater 30 and temperature at 12-14 °C. At Northern latitudes, spawning is delayed to June and it is more restricted in length. Spawning is inhibited at temperature below 9 °C and above 16 °C (Jennings and Pawson, 1991). Optimum thermal range for gametogenesis is between 13-16°C, minimum temperature between 9-10 °C and maximum temperature 18 °C for Mediterranean seabass populations. Gametogenesis is blocked above 21-22 °C (Moretti et al., 1999).

Photopheriod and temperature are key environmental cues to control reproductive cycle in seabass and both are widely used in fish hatcheries. Manipulation of day length and temperature (Bye, 1987; Zanuy et al., 1986) are used to alter spawning time, e.g. increased temperature during winter for delaying spawning, and low temperature (<15 °C) for synchronization of spontaneous spawning. Seabass breed spontaneously in captivity, however, hormone manipulations are often used to synchronize or increase the yield of eggs. Ovarian stimulation by GnRH analogues induces a group synchronous oocyte development and multiple spawnings in females (Mayer et al., 1990; Alvariño, 1992; Forniés et al., 2001).

Seabass is a very fecund species, absolute fecundity increasing with body size (Mayer et. al 1990). Absolute fecundity varies from 293 000 to 358 000 eggs / kg body weight in seabass from Atlantic Sea (Kennedy and Fitzmaurice, 1972) and from 492 000 to 955 000 eggs / kg in Mediterranean seabass (Bou Ain, 1977).

Eggs

Seabass spawn pelagic eggs, small in size and spherical in shape. Egg diameter approx 1.15-1.2 mm, but is larger (1.2-1.5 mm) at northern latitudes (Barnabè, 1980). Hatching starts approximately 72 hours after fertilization, at 14 °C.

In hatcheries, egg quality is a key factor to guarantee good larval performance and high survival rates. Many factors have been indicated as possible determinants of eggs' quality (Kjorsvik et al., 1990; Bromage et al., 1992), among others the nutritional status of broodstock (Bruce et al., 1999) which is mainly affected by the energy-yield (Cerda et al., 1994) and polyunsaturated fatty acid content in the diet (Bell et al. 1997; Navas et al. 2001). Egg size and dry weight, buoyancy, regular shape of vitellus, regular cell divisions, egg lipid droplets of proper size (400-440 μm) and number (1-6), fertilization and hatching rates, and biochemical composition are common criteria used for evaluating egg quality (Devauchelle, Coves, 1988; Carrillo et al., 1995). However, a clear relationship between morphological and biochemical characteristics of the eggs and viability of the larvae has still not been shown.

Fertilised eggs are sensitive to physical shock which may occur during collection, disinfection and incubation, packaging and transportation (Moretti et al., 1999, Escaffre, et al., 2001).

Larvae

At hatching, pre-larvae are around 3.3-4.0 mm long, and lack functional eyes and a mouth. The yolk sac is almost ½ of whole body length; lipid droplets (1-4) fuse during embryonic development and good quality larvae generally show a single lipid droplet (0.40-0.45 mm). Within the following 3-4 days a mouth is developed and opens, eyes and larval body pigmented and pectoral fins are also developed. Swim bladder inflation occurs in two steps: primary inflation starts when larvae are 5.5 mm long and is completed by day 16, when larvae reach 6.5-7.0 mm body length. The swimbladder expands progressively between day 20 and 35 (Chatain et al., 1994). Caudal and anal fins develop by day 20 and dorsal and ventral fins from day 40 (Marino et al., 1993). Scales appear from day 70. The larval rearing period approximately lasts around 80 days, depending on water temperature and larval rearing system. Larval rearing consist of two phases; the first one (*exogenous phase*) starts at a size of around 4.0 mm and is based on feeding on live foods. The second phase (*weaning*) starts around day 40 aiming to wean the fish off live feed and onto commercial microdiets.

Juveniles

At the final stages or at the end of the weaning phase, juveniles are much sturdier than post-larvae (Moretti et al., 1999) and can be moved from larval or weaning tanks to pre-ongrowing facilities. During this period seabass juveniles are fed on artificial diets, usually graded 2-3 times for size and deformities evaluation until they reach a size of 1-2.5 g. Juveniles produced for stocking sea cages are kept in flow-through tanks until they reach approximately 10 g size.

2.2. Seabream (*Sparus aurata*): Life stages

Seabream is common throughout the Mediterranean and less frequent in the south-eastern regions. It is found along the Eastern Atlantic coasts, from Great Britain to Senegal; it is common around the Canary Islands but quite rare in the Black Sea.

Seabream is a euryhaline and eurythermal species, although it is less tolerant to low temperatures (lower lethal limit is 3-4 °C) and salinity fluctuation than seabass. Like seabass, the gilthead seabream spends most of its life in coastal and lagoon waters, moving into deeper waters (around 150 m) during the spawning period, which occurs from October to December in northern latitudes and from December to May in Southern Mediterranean where fish are exposed to higher water temperature (Zohar et al., 1995). In the open sea, it is found on sea grass beds and sandy bottoms between 30 and 150 m. It is a sedentary fish, living solitary or in small schools. After spawning, adults come back to lagoons and the young fry migrate in spring towards estuarine waters in search of food and mild temperature. Growth is faster in fish living in brackish waters, reaching about 15 cm at the end of June and 150-200 g at the end of the first year of life (Ardizzone et al., 1988). Young seabream generally feed on micro-invertebrates and, when larger in size, on shrimps and shellfish, which they find in sediments and which, they easily crush by means of their strong molar teeth.

Broodstock (Breeders)

Seabream is a protandrous hermaphrodite (Zohar, 1978). It develops as a male by the end of the first year (>100 g) and becomes female at the end of the second year when size exceeds 30 cm and 600 g. Sex-reversal takes place after the end of the spawning season (May), when the testicular part of the gonad degenerates and the ovarian part develops. This process is completed by the following September. The fish can then develop into functional females or reabsorb the ovarian tissue and develop testes. During this period sex-reversal is socially controlled (Happe and Zohar, 1988). In hatcheries, broodstock management requires careful attention since the introduction of young fish increases the number of older males that reverse into females. In addition, the presence of older females inhibits sex-reversal in younger males. Also, the quality of eggs is affected by the sex ratio in tanks (Zohar et al., 1995). A balanced sex-ratio used in farms is usually 1:2 (male: female) in groups over 9 breeders, and 1: 1 in smaller groups.

Seabream reproduction is controlled by social, light and thermal cues. Optimum thermal range for gametogenesis is between 15-17 °C, minimum temperature between 13-14 °C and maximum temperature around 20 °C in Mediterranean seabream populations. Gametogenesis is blocked at 21-22 °C (Moretti et al., 1999). Shifting gametogenesis is mainly induced by manipulation of photoperiod (year long season -shifted photoperiod cycles; compressed or extended photoperiod cycles; alterations of constant photoperiod regimes) and secondarily by temperature reduction.

There is only one breeding season per year, but each female can spawn several times during the reproductive period. Females are sequential spawners and they can lay up to 20 000-30 000 eggs per day for a period up to 3-4 months (Zohar et al., 1995). Absolute fecundity is very high: 800 000 eggs per kg body weight (Moretti et al., 1999) or 2-3 million eggs per kg BW per season (Zohar et al., 1995). Prior to spawning, seabream change body colour and typical courtship behaviour is observed, even under captive conditions. Seabream usually spawn spontaneously in captivity and optimum spawning temperature is around 15-19 °C, depending on latitude. Spawning is induced by use of gonadotrophin releasing hormone analogues in some parts of Europe.

Eggs

Eggs of seabream are pelagic, spherical, very small in size (0.94-0.99 mm) and have a single large lipid droplet (230-240 μm). Hatching starts 48 hours after spawning at 16-17 °C Morphological and biochemical parameters used to evaluate egg quality are the same as for seabass.

Larvae

At hatching, seabream larvae are around 3 mm long and the yolk sac is 1/3 of larval length. Eyes pigmented when larvae are 3.8 mm long at 4 days post-hatching. The swimbladder starts to inflate at 4 mm larval length and completed at 15 mm, when the yolk sac is completely resorbed (3-4 dph). Caudal fin develops from day 15, and between day 40 and 45 the dorsal and ventral fins develop and scales appear (Moretti et al., 1999).

Larval metamorphosis starts when larvae are approximately 16-17 mm length by day 40. As in the case of seabass, larvae rearing consist of two phases: the exogenous, based on live feed, and weaning based on special formulated microdiets (for details see section 4.3).

Juveniles

At the final stages or at the end of the weaning phase, juveniles are moved from larval or weaning tanks to the pre-growing sector where they are reared, mainly in flow-through tanks, until they usually reach a size of 1.0-2.5 g. Larger size juveniles (20 g or even in some areas up to 50 g) are used for stocking sea cages and, recently, also for extensive systems (see chapter **Error! Reference source not found.**).

The appearance of marketable size gilthead seabream is one of the most important parameters of product's quality. Farmed fish should have a pigmented operculum, distinct abdominal yellow line, bright yellow arc between the eyes, and golden shine over the dorsal area. Fasting, nutritional factors and stress are factors that can lead to undesirable skin pigmentation alterations in farmed fish (Gomez et al., 2002; Gouveia et al., 2002; Grigorakis and Alexis, 2005).

Table 1. **Farmed life cycle seabass**

Production Stage	Approximate Duration (days)	Temperature (°C)	Weight range (g)
Broodstock:			
Active gametogenesis	120	21-8 (Sept.-March)	800-3,000
Spawing	60	12-15	
Eggs	3	14-17	
Larvae	80	15-19	0.003-0.2
Pre-ongrowing	100	15-22	0.2-2.5 (intensive/extensive)
	140	15-22	>2.5 (cage)
Ongrowing	480	10-25	2.5 -400

Table 2. **Farmed life cycle seabream**

Production Stage	Approximate Duration (days)	Temperature (°C)	Weight range (g)
Broodstock:			
Active gametogenesis	100	23-9 (August-Febr.)	400-1,500
Spawing	150	15-18	
Eggs	2-2.5	16-17	
Larvae	50	16-19	0,001-0.2
Pre-ongrowing	100		0.2-2.5 (intensive)
	140		>2.5 (cage/extensive)
Ongrowing	480	13-25	2,5-400

Figure 3. **Life-cycle and production phases of European seabass, *Dicentrarchus labrax*, reared under intensive farming conditions.**

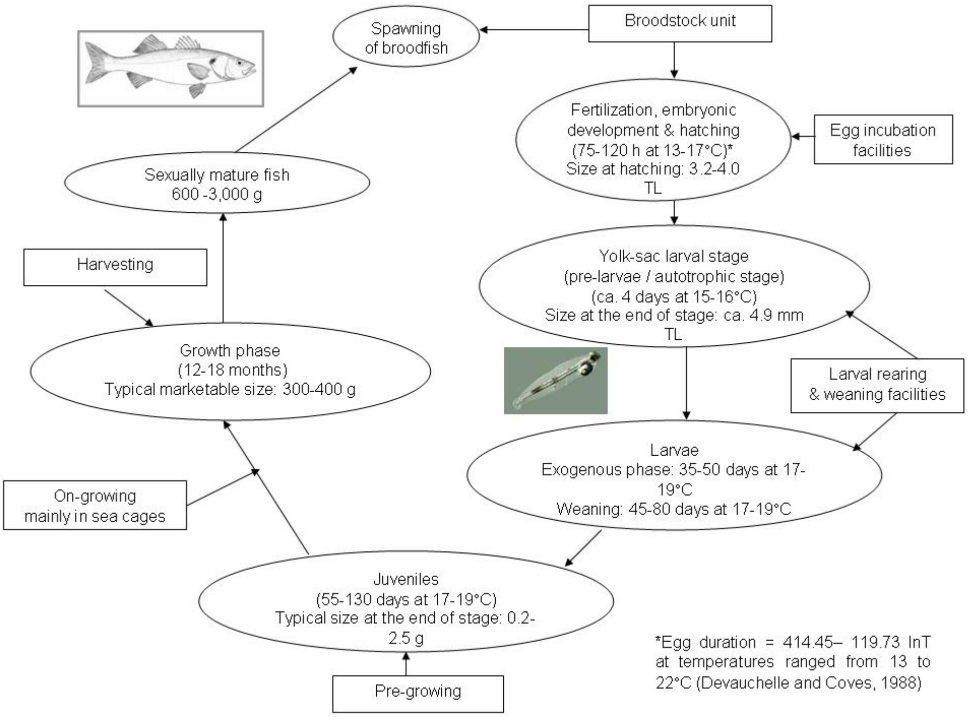
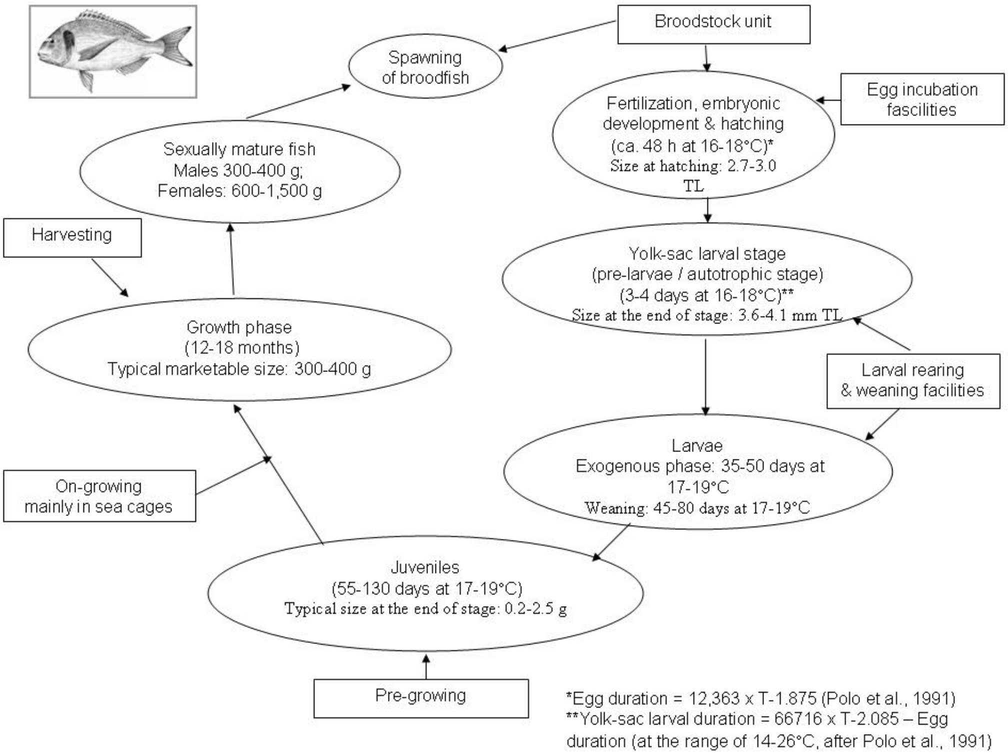


Figure 4. **Life-cycle and production phases of gilthead seabream, *Sparus aurata*, reared under intensive farming conditions.**



3. Overview of production systems in europe

3.1. Main production systems

The diversified character of European coastal aquaculture along with an array of historical and socio-economic factors allow the use of extensive, semi-intensive and intensive systems for most stages of seabass and seabream production (Basurco, 2000; 2004).

Hatchery

Most seabass and seabream fish farming operations are vertically integrated, i.e. they carry out all stages of the production cycle: egg production, larvae rearing, weaning, juvenile production (or pre-ongrowing) and ongrowing. ‘Hatchery’ is the term used for production not only of larvae but for mass production of juveniles, which are the basis for the subsequent ongrowing. Marine fish hatcheries are technologically complex, relatively expensive facilities, and their operation requires skilled personnel (www.fao.org). A hatchery consists of several distinct sections for the various phases in the production cycle. Hatchery facilities include: (1) broodstock units and egg harvesting facilities; (2) live feed (phytoplankton and zooplankton) production units; (3) egg incubation and larval rearing facilities; and (4) nursery/juveniles facilities, each with their specific technological requirements. The typical end product is juveniles 4-5 cm long, weighing 1.0–2.5 g.

Broodstock management

Broodstock management (origin of the stock, selection of broodfish, abiotic and biotic parameters, sex ratio and social interactions, breeder renewal strategy and handling procedures) is a prerequisite for the control of reproduction and gamete production. Egg management is important to avoid any welfare problems at later post-hatching life-stages.

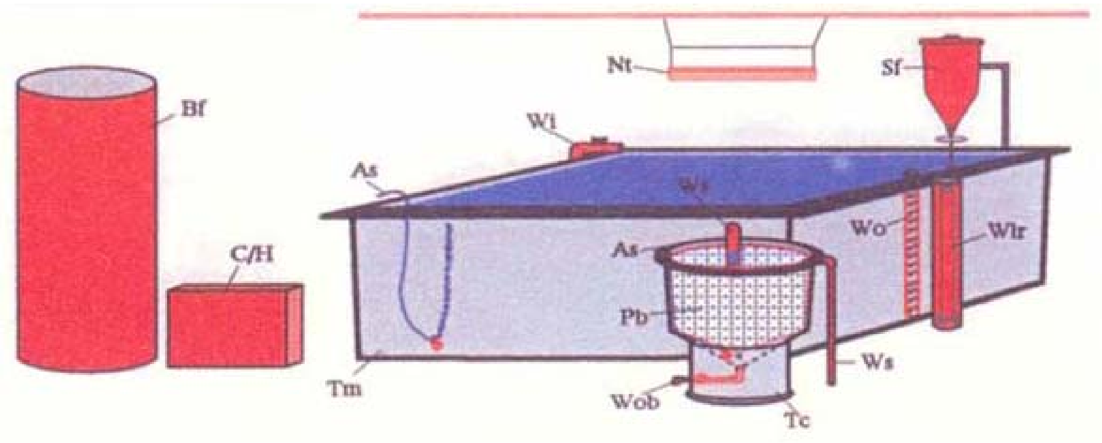
Seabream and seabass broodstock may be wild or farmed. Wild fish are currently preferred by the industry to maintain genetic variability in the broodstock; but they have to be conditioned to captivity for at least six months to recover from stress before being used as broodstock (Moretti et al., 1999). Upon arrival wild fish are selected and stocked in quarantine tanks away from the other rearing units in order to check their health status, for the fish to recover from abrasions and other lesions that occurred during catching, and to eliminate possible external parasites. The use of farmed broodstock gives the opportunity to select fish on the basis of their individual characteristics and performance. In most farms, broodstock are selected by empirical criteria, such as the health status, body size, shape, and behaviour. Specific family genetic selection programmes (e.g. for faster growth rate and disease resistance) have started in some hatcheries, although these are not yet routine.

Seabass and seabream are seasonal spawners, but all-year-round egg production is possible by manipulating photoperiod and temperature. Broodstock are normally reared in sea cages or in outdoor land based tanks until sexual maturation and then transferred to broodstock facilities. Seabream broodstock usually includes five age groups, from age 1 (young fish) to age 5 (old females). Seabream sex reversal takes place at the end of the reproductive period, is socially induced and lasts approximately 4 months. During this period, older females are replaced, annually, with young fish since the quality and the quantity of the eggs decrease with age. The dimension, the sex-ratio, the balance between the number of young males introduced and the number of older females has to be carefully managed. Ovarian biopsy is often performed to determine the sex. Breeders are often anaesthetised and examined by ovarian biopsy to evaluate the sex, the stage of gonad maturation and for hormone treatment with GnRH for spawning synchronization.

Two distinct feeding regimes are usually applied to seabass and seabream broodstocks: a maintenance diet after spawning until the start of gametogenesis, and a boosted diet, enriched in n-3 PUFA and essential micronutrients during gametogenesis and spawning. Maintenance diet includes both dry feed (0.5 % biomass) and fresh feed (1.0 % biomass) using a combination of cuttlefish, sardines, hake/whiting, crustacea and molluscs.

During the breeding period, dry feed is integrated with commercial integrators and natural components, such as squid oil, to provide nutrients essential for ensuring proper gametogenesis and good quality eggs and larvae. Distribution of dry feed during the maintenance period usually takes places 6 times per week, and for fresh feed twice a week and this is increased up to six times per week during vitellogenesis. Feed is manually distributed to reduce waste and to prevent deterioration of water quality in tanks. During spawning, seabass are generally starved for few days whereas seabream, which may spawn for several weeks, are regularly fed.

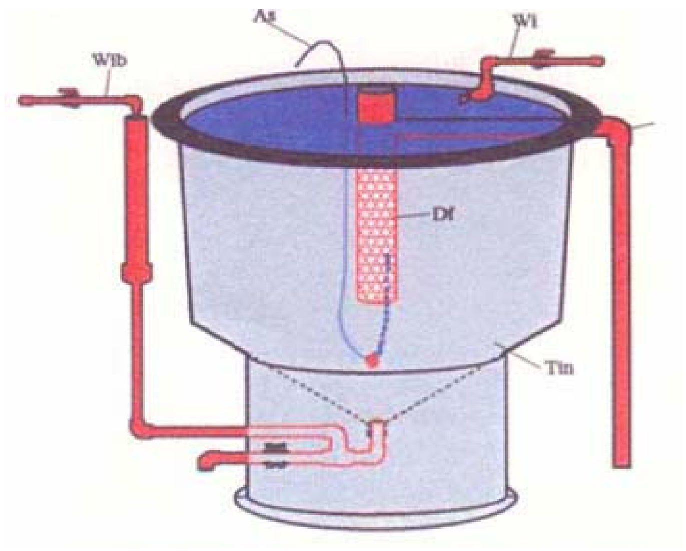
Current industrial practice is for brood fish to be held in land based indoor concrete round or sub-square tanks of 10 m^3 minimum volume at a low stocking density (2-5 kg m^{-3}). Each tank is usually equipped with several accessories including air and oxygen supply, a surface water inlet and an outlet for emptying the tank, light tubes and automated timers for photoperiod control, cooling or heating systems for temperature control, biological filters (in the case of close water recirculation system) and an external regulator for adjusting the tank’s water level (Figure 5).



As: air supply; Bf: biological filter; C/H cooling/heating system; Nt; fluorescent lamps; Pb: plamktonic basket of 500 µm mesh size; Sf: self-feeder; Tc: Egg collector; Wi: water inlet; Wlr: water level regulator; Wo: water outlet; Wob; collector water outlet; Ws: overflow water outlet, Tm: main tank

Figure 5. **Broodstock tank with its accessories and egg collector.**
SOURCE: Divanach et al. 2003

During spawning, the broodstock tank is also equipped with an external egg collector. Classical egg collectors are made of a PVC cylinder with large lateral and bottom openings screened by a plankton basket of 400-500 μm mesh size, an air supply (to keep eggs floating), and a bottom or/and an overflow water outlet to maintain the water level (Figure 6) (Moretti et al., 1999).



As: air supply. Df: drainage filter with 360 μm mesh size. Tín: incubator tank; Wi: surface water inlet; Wib: bottom water inlet; Wo: water outlet.

Figure 6. **Egg incubator**
SOURCE: Divanach et al. 2003

Viable floating eggs are collected through the overflow water outlet of the tank into the collector. After the eggs are removed from the egg collector, they are deposited in a transparent cylindrical conical tube and egg quality is assessed by the degree of buoyancy (Chatain, 1994). Fertilized eggs are normally disinfected and then transferred, daily, either to the egg incubators or directly in the larval rearing tanks or even transported to other hatcheries. Egg incubators are usually plastic or fibre glass tanks of 250-500 l volume with a smooth inner surface and equipped with water from a surface and a bottom inlet, and a central aeration supply placed near the tip of the conical bottom. Water is drained from the egg incubator through a central cylindrical filter of 360 μm mesh size connected to the water outlet (Anonymous, 2002). Egg incubation at a typical cylindro-conical 500 l tank is usually performed at an initial density of 200-400 egg l^{-1}. The incubator is supplied with filtered sea water from both the base and the water surface of the tank at an average range of 35-40% h^{-1} (Divanach, 1985). Water temperature is usually in the range of 12-15 for seabass and 16-19 for seabream, salinity 32-40, oxygen concentration 7.0-8.0 mg l^{-1}; oxygen saturation 80-100% and pH 7.9-8.1 (Moretti, 1999; Anonymous, 2002).

Larviculture

All hatchery larval rearing techniques/methodologies used for seabass and seabream are based on live prey. Based on larval density of the rearing system these techniques can be categorized into intensive, mesocosm or extensive larviculture, while based on the quality of the rearing medium can be divided into clear water, green water or pseudo-green water larvae rearing methods (Divanch, 1985; Divanach and Kentouri, 2000; Papandroulakis et al., 2002).

Intensive techniques

Larval rearing of seabass and seabream is a typical intensive technology involving sophisticated indoor rearing techniques in which productivity is totally dependent on personnel and technology. They are characterised by high larval densities in small tanks under strict controlled hydraulic and environmental conditions. Larviculture technology used in Mediterranean hatcheries differ mainly in relation to larval density (semi-intensive: 30-50 larvae l^{-1}; intensive: 80-100 larvae l^{-1}; hyper-intensive: 150-200 larvae l^{-1}), the volume of tanks, photoperiod and light intensity, and live feed production. The so called “French technique” is usually applied to seabass larvae and is characterised by a dark environment for 5-7 days after hatching and the use of *Artemia nauplii* as a first feed. A second intensive technique takes place in lighted environment 16 hours light then 8 hours dark, when temperature remains below 21 °C, light intensity 1 000-3 000 lux at water surface using micro-algae, rotifers and brine *Artemia* and is utilised for both seabass and seabream (Moretti et al., 1999).

During the first 4-6 weeks of rearing, larvae are kept in indoor fibreglass tanks of 6-10 m^3 volume. Seawater is recirculated through biofilter or pumped in flow-through systems and often heated during winter. Water temperature during incubation and yolk sac resorption is the same as spawning temperature, and it is slowly increased at approximately 0.5 °C per day to reach 18 °C in seabass and up to 20 °C in seabream. Particular attention is given to maintain water temperature constant in larval tanks, avoiding any fluctuation that will exceed 0.5 °C in 24h. Drops in water temperature may occur during night when water renewal is nil or very low. High water temperature during early ontogenesis increases the malformation rate, especially skeletal and splanchno-cranial deformities (Boglione et al., 1989). Salinity is usually the same as at spawning (33-38). Although, both seabream and seabass larvae growth is better at lower salinities (25-30), it is generally maintained unchanged to avoid osmotic stress and additional labour in the hatchery. Water renewal rate depends on the larviculture system, design and size of the tanks, larval biomass and the quality of the rearing medium. In closed re-circulating systems water renewal ranges from 10% h^{-1} at the start to 40% h^{-1} at the end of the rearing; in flow-through systems (intensive or mesocosms) water renewal ranges from 5-10% day $^{-1}$ at the start to 100-150% day $^{-1}$ at the end of the rearing (Papandroulakis et al., 2002). Dissolved oxygen is maintained between 80% and 100% saturation levels. Total ammonia is kept below 0.5 ppm. Fresh micro-algae and zooplankton are added daily to larval tanks so screens of different dimensions are employed to retain particles during the day and larger dimensions at night to allow flushing of effete algae and zooplankton. Water surface is cleaned by means of skimmers to facilitate swim bladder inflation until day 20.

Mesocosm techniques

Mesocosms are indoor or semi-outdoor hatcheries, for mass larviculture production based on two main different philosophies: in the extensive one, the food chain is basically endogenous and complemented with exogenous input when signs of overgrazing are seen (natural bloom or clear water method) or the green water method; in the intensive system, the food is mainly exogenous (clear water or pseudo-green water method) (Divanach and Kentouri, 2000). Larvae are reared at relative low densities (2-8 larvae l^{-1}), in relatively large (< 100 m^3 - usually 30-50 m^3) cylindrical, black glass-fibre, tanks of 1.5-2.5 m depth (Divanach and Kentouri, 2000; Lavens and Sorgelos, 1996). Tanks are filled with filtered sea water of 32-41 salinity and unicellular green algae species are introduced into the initial culture. During the yolk-sac larvae stage temperature is usually maintained at 15-21 °C (yolk-sac absorption in seabream is completed in 3 days at 21 °C, 4 days at 18°C and more than 5 days at less than 17°C), oxygen saturation at 80-100%, pH at 7.9-8.3, and ammonia less than 0.5 mg l^{-1} and nitrite below 0.2 mg l^{-1} (Anonymous, 2002). Water renewal is set at 8% of tank volume per day. The heterotrophic stages are performed in similar abiotic conditions under a 24L:0D photoperiod of low light intensity (200 – 1 000 lux). As soon as the food consumption of the growing larval biomass exceeds the net zooplankton production, new zooplankton, rotifers, *Artemia* or artificial feeds are added. As larvae grow, water exchange rate gradually increases from 10% to 200% of tank volume per day. During the period of swim bladder inflation water surface is cleaned by the use of peripheral skimmers three times per day (Anonymous, 2002). A well-managed semi-extensive mesocosm in a 60 m³ rearing tank may enable a production of up to 25-50 000 seabream or 50-100 000 seabass fry, within 25-40 days. The quality of fry which survive within such a system is often good with less than 5% deformities or non-inflated swim bladders (Lavens and Sorgelos, 1996, Boglione et al., 2001). However, larval production with the mesocosm of an extensive or semi-intensive nature is not a widely used technique. The intensive mesocosm is a recent approach and seems to be a well-performing technique of larviculture with the advantages of both extensive and semi-intensive techniques without their inconveniences (Papandroulakis et al., 2004).

Extensive techniques

Larviculture is performed at low densities (0.1 to 1 larvae l^{-1}) in large outdoor ponds, with minor personnel interference and no control of environmental parameters. Feeding is exclusively based on the endogenous bloom of zooplankton and endogenous primary production. Human operations are restricted to the preparation of the pond, the initialisation of the food chain, the inoculation of eggs or larvae and the harvesting (Anonymous, 2002). Success of production is based on the climatic conditions, intensity and longevity of the food chain. Such extensive techniques are not now, however, widely used in Europe (Saroglia and Ingle, 1992).

Weaning system

Weaning differs from the larviculture system essentially because of ending live feed supply and the setting up of a truly intensive rearing system based on the automatic distribution of dry feed (Moretti et al., 1999). In most hatcheries, weaning take places in rearing units similar to those of intensive larviculture systems, equipped with large round or rectangular indoor tank (10-25 m^3 volume). Water is generally provided by a flow-through water system, filtered (50-100 um) and sterilised by a UV-light system. Where environmental conditions are not favourable, water is partially recirculated, heated and passed through a biofilter, as in the larviculture systems. Tanks are equipped with filters of different mesh sizes (1-3 mm) according to fish size. Water quality and hydrodynamic conditions in weaning tanks are key parameters for keeping good rearing conditions. Water circulation depend on the size, the shape and the depth of rearing tanks and should be adjusted to eliminate debris and faeces, to favourite the distribution of oxygen in tanks, and to provide proper current conditions for swimming activity and feeding under automatic feeders. Computer systems should be used to keep under strict control environmental water parameters in tanks, at least for dissolved oxygen concentration.

During early weaning, co-feeding with *Artemia metanauplii* and dry feed is generally performed to minimize stress and cannibalism and to reduce difference in fish size. Moist food was also used in the past, but today there is a tendency to replace live feed with micro-particulated extruded diets. Recent research has led to the formulation of a compound diet that is well adapted for larvae and can totally replace live prey (Cahu and Zambonino, 2001). New formulations contained highly specific raw materials, high metabolisable energy content, high quality protein with an optimum amino-acid balance, and high HUFA content with high DHA/EPA ratio are available. Significant improvements have also been achieved to maintain feed particles stable in water and in the automatic feeders. Feed distribution is carried out at regular intervals (6-8 times per day) avoiding under- and over-feeding. Under-feeding may trigger cannibalism and over-feeding may increase ammonia concentration and reduce water quality.

Pre-ongrowing system

After 40-50 days, larvae are weaned off live feed and on to commercially formulated fish food of a very fine particle size. When fish are capable of consuming commercial feed, at a size of approximately 0.25 g, they are transferred to the pre-on-growing tanks. The pre-on-growing phase is necessary in order to: (a) avoid deterioration of water quality in the larvae rearing tanks, due to the increased size and waste production of fish; (b) avoid stress of fish during transportation and acclimatisation directly to the on-growing facilities (mainly floating sea cages); and (c) avoid unnecessary grading, cannibalism and the outbreak of disease in the sea cages.

Pre-on-growing take place mainly in land based indoor flow-through tanks of various shape and size (circular fibreglass tanks 20 – 200 m^3 in volume; raceways > 36 m^3; and less common in small sub square fibreglass tanks 1.2-2 m^3). However, as water quality control is becoming more critical, an increasing number of hatcheries are currently using recirculation systems for pre-on-growing purposes. Optimum growth is achieved at water temperatures of 20-22°C but in most of the cases fish are exposed to ambient temperature (15-22 °C). Oxygen concentration is usually kept above 4.5 mg l^{-1}. Initial stocking density is usually 0.25 kg m^{-3} increasing at the end of the phase up to 15 - 30 kg m^{-3} (Kentouri, 1996). Water renewal is 0.5 m^3 h^{-1} kg^{-1} and 1.25 m^3 h^{-1} kg^{-1} for densities of < 1.5 kg m^{-3} and > 15 kg m^{-3} respectively (Kentouri, 1996). Fish reach the traditional ongrowing stocking size of 2.0 to 5.0 g in a period of 55 to 140 days depending mainly on the size of the incoming fish, the water temperature, the design and size of the tanks, and the management of the farm. Nowadays, hatcheries are also keeping to the pre-on-growing facilities juveniles up to the size of 10-20 g or even more (50-60 g), to satisfy the demand by offshore farms to stock larger fish, as they reduce the need for initial grading. Fish are fed commercial dry feeds from automatic feeders, self-feeders or by hand feeding (2 – 4 times per day).

Routine management during the pre-on-growing involves monitoring of environmental parameters and water quality, grading, disease monitoring and vaccination, removal and disposal of dead fish, and sampling for quality control (mainly operculum and skeletal deformities). Grading take place usually once (some farms may perform up to three gradings) to avoid cannibalism and adverse effects on growth.

Ongrowing systems

Ongrowing of seabass and seabream may take place under intensive, semi-intensive or extensive systems. Intensive systems include (a) net cages (b) land-based flow-through systems and (c) land based recirculation systems. According to Basurco (2000) in Mediterranean countries, 82% of farms use cages, followed by land based intensive raceways or tanks (10%) and semi-intensive production in earth ponds (8%).

Net cages

The dominant intensive ongrowing system for seabass and seabream is floating net cages. These may be used in lagoons, sheltered bays or semi-exposed and offshore conditions (Basurco, 2004). Originally, cages used to be placed at well-protected, largely enclosed sites. Issues relating to oxygen deficits and cage fouling during summer months, coupled with the scarcity of suitable sites and issues related with the management of the coastal zone, resulted in the development of off-shore cage technology. Many different types of cages are used in Mediterranean. Over the years, the old wooden or steel framed pens have been gradually abandoned in favour of plastic framed pens, either circular or rectangular, which are flexible and economic and may withstand strong wave action. These allow cages to be anchored further from the shore where sea currents are stronger and the organic matter loading of the water is dispersed. Most farms use cages in a distance of 1-3 km from the shore, at water depths ranging from 18 to 45 m. Farms usually employ cages of different dimensions (smaller for pre-fattening and larger for fattening) with a diameter range of 10 to 30 m (Basurco et al., 2000). Besides sea cages, large rigid floating structures resembling an oil-rig are operating in the Spanish Mediterranean coast (Basurco et al., 2000). Almost all of the existing types of open sea cages employ mechanised systems for feeding and harvesting. Submersible cages with systems for automation and control of feeding and inspection of fish have also been used.

Ongrowing of both species in floating sea cages is the fastening system mainly used in the Mediterranean basin. Juveniles are transferred from the pre-growing tanks into the sea cages usually at a size of 2.5-5.0 g. However, for the seabream it is possible to stock juveniles at a smaller (0.3-2 g) or, recently, at larger size (8.0-10.0 g). Farmers prefer to stock younger fish (2.5- 150 g) at a density of 5 to 10 kg m^{-3} and older fish (> 150 g) at a density of 10-20 kg m^{-3} (commercial experience). Routine management during the intensive ongrowing phase in sea cages involves monitoring of environmental parameters (mainly water temperature and oxygen), grading, sampling of fish, disease monitoring, removal and disposal of dead fish, maintenance of cages and gear, and harvesting (Beveridge, 1996). Grading (0-2 times) may take place at the summer months at a size of 25-40 g and around 100, in order to avoid adverse effects on growth, fish quality and production. Samples of fish may be taken at regular intervals to check fish quality and to determine stocking and feeding policies. Cages, nets and moorings are checked at regular intervals. Inspection of net bags is carried out from a boat or from the cage walkway or using divers. Special care is also taken over mesh size of the nets to secure sufficient water exchange and good hygiene. The frequency of net changing varies from 1 to 5 times for the whole ongrowing period, depending upon site location, design of cage and management. Antifouling cage chemicals are also used. These chemicals are classified as pesticides and must be approved by National authorities as antifouling chemicals (Read and Fernandez, 2003). There are no data available on their effect on fish welfare.

Feed is distributed by automatic feeders, modulated-automatic feeding systems or hand feeding. Common size pre-fattened fish reach first commercial size of 300 to 400 g (representing approximately 30% of sales) at 12 – 18 months and the size of 400 – 600 g (representing approximately 40% of sales) around 24 months (commercial experience), depending mainly on the climate, site and managerial practices of the farm.

Harvesting is performed in sea cages when the weather conditions are safe for the workers. Prior to harvesting, fish are starved in order to firm the flesh and give the gut time to empty (Beveridge, 1996). The length of starvation (usually 24-78 h) depends on the water temperature and feeding rate (www.fao.org). Fish are crowded into a relative small area so that they can be harvested usually with dip-nets or, less often, by vacuum pumps. Seabass and seabream are killed by thermal shock, i.e. chilled in a few minutes from sea temperature to about 2-3 °C by immersion in ice slurry (Smart, 2001).

Land based flow-through systems

Land-based intensive flow-through systems aim to produce high value fish at high stocking densities. Juveniles are transferred from the pre-growing tanks into the outdoor tanks (50-300 m^3 volume) at a size of 2.5-5.0 g. Ongrowing is performed in concrete or earth tanks, raceways or ponds, based on the use of pumped water with oxygen supplementation (Basurco, 2000). Younger fish (2.5g) are stocked at a density ranging from 5 to 10 kg m^{-3} and reached a final stocking density of 20-35 kg m^{-3}. Routine management involves monitoring environmental parameters (water temperature and oxygen), grading, sampling of fish, disease monitoring, removal and disposal of dead fish. Feeds are distributed by automatic feeders and automatic feeding systems are sometimes used for seabream. The commercial size of 300- 400 g is reached after 14–18 month of rearing and represents about 50% of commercial sales. There is a tendency today to extend the rearing period to 24 months in order to reach a larger commercial size (500-600 g). The high energy costs and capital cost of construction have latterly made land based flow-through systems less competitive (De la Pomelie, 1995; Blakstad et al., 1996; Basurco, 2000). In a number of systems particularly in France and Italy these problems have to some extent being mitigated by use of cooling effluent flows from power stations or from geothermal waters at constant temperature.

Land based recirculated systems

Indoor systems using bio-filtration to treat re-circulated and temperature controlled water are also used in some instances for ongrowing. This is less economic than the use of such systems for hatcheries where water volumes are significant lower. Recirculating systems (RS) are not closed systems and need to be renewed with low quantities of clean water (also called refreshment water or replacement water) at a level depending on the total fish biomass, the quantity of ingested food and the design (size and performances of the different units of the treatment line of the water). In most of the cases, the quantity of the refreshment water is between 2 and 10 m^3 per kg of ingested food (Blancheton, 2000).

There are various designs for re-circulating systems to accomplish several processes in water such as pumping, removal of particulate matter (uneaten food and fish-waste), oxygenation, gas removal (CO_2 and N_2), biological filtration to remove ammonia and nitrite wastes, cooling and heating, and buffering of water pH levels. These processes can be achieved by simple or several interconnected units. Pumping is provided by the means of rotary pumps or airlifts. Sand filters and rotating microscreens (80 to 100 µm mesh size) are used to remove particulate wastes with efficiency close to 60% (Blancheton, 2000). Carbon dioxide and gaseous nitrogen removal are achieved by packed columns or other stripping systems Oxygenation is achieved by bubbling (low efficiency) or by injection methods (containers under pressure, U-tube, hydro-injector, packed columns). Biological filtration (ammonia→nitrite→nitrate) is achieved by suitable bacteria developed on various substrates (stones of expanded clay, plastic ribbons or various shapes). Cooling and heating is achieved by the use of plastic or titanium exchangers. Peristaltic pumps supply concentrated soda into the RS to buffer the pH. The adequate use of such systems requires skilled manpower, much more than cage or flow-through systems.

Semi-intensive systems

Semi-intensive farming is based on pond culture. Ponds are modified environments where it is possible to simulate and accelerate natural processes. This is achieved by controlling water flow (development of sufficient canals and making new openings to the open sea), vegetation control, integrating the availability of food and rearing at low densities (0.2 – 2 kg m^{-3}). The production is higher than in the extensive system and amounts to 500-700 kg ha^{-1} yr^{-1}. A few farms in Portugal and Spain use this system for the production of approximately 1,500 tons of seabream but it is not a significant production system in the context of European aquaculture.

Extensive systems

Extensive farming includes (a) coastal lagoon management and (b) *vallicultura*. Around 500 000 hectares of coastal lagoons still survive in the Mediterranean and their exploitation is based on the migratory behaviour of some wild species, including seabass and seabream, which spend their juvenile and growing phase until reaching sexual maturity in such areas. Such extensive farming makes use of both wild immigrant fish and hatchery-reared juveniles. Such culture still remains a traditional activity in several regions and a tool for the conservation of biological resources and ecosystems in sensitive coastal lagoon areas.

Extensive aquaculture is based both on wild fry and hatchery fry that are restocked in spring (March–April). When the fish are preparing to migrate back to the sea they are harvested at the confluence of the lagoon and the open sea by fixed capture devices. Italian 'valliculture' is a peculiar farming activity differing from the management of the rest of the traditional lagoons. All valli systems consist of enclosed wetlands located within the large lagoon systems of the Northern Adriatic. The valli are traditionally extensive with a productivity ranging between 30 kg ha^{-1} yr^{-1} (Valli di Comacchio) and 150 kg ha^{-1} yr^{-1} (Valli Venete). Valliculture supplies about 70% of Italian aquaculture production from coastal lagoons. The traditional management of the "valli da pesca" uses wild and hatchery juveniles. The undersized fish, captured in complex weir systems (lavorieri) in the autumn, are held in deeper ponds (called 'sverno' ponds) for winter recovering and released into the lagoons for another season's growth before harvesting. Water level and salinity are controlled by hydraulic management in embanked portions of the lagoons (www.fao.org). In extensive culture, seabass reach a commercial size of 400-500 g in 30-36 months. Seabream reach 150-200g in 10 months and a market size of 400-500g in 18-20 months. Today, the productivity of extensive systems and valliculture is significantly reduced, mainly because of the marked impact of ichthyophagous birds, such as the cormorant, especially on juvenile stages and the negative impact of human activity on coastal environment (Marino et al., 2004). Extensive culture only contributes 10% of total European seabass and seabream production.

Table 3. **Production systems for seabass and seabream**

Production Stage	Most frequent Production Systems in EEA	Other Production Systems
Broodstock	Tanks flow-through	Tanks recirculated
Eggs and larvae	Tanks flow-through Tanks recirculated	Mesocosms (large volume technique)
Pre-ongrowing	Tanks flow-through	Tanks recirculated
Ongrowing	Cages Tanks flow-through	Tanks recirculated (seabass) Ponds Extensive system (lagoons)

3.2. Production in Europe

Precise data on production by the various systems of production is not available at European level. Regarding ongrowing the biggest production is from Greece mostly from cage systems, tanks flow through and extensive, semi-intensive system are used in some European countries but overall number of sites and production is much lower.

Table 4. **Seabass and Seabream hatchery production systems.**

Hatcheries			No. of sites producing millions of juveniles					
Data for 2007	No of sites in the country	Production of fingerlings (million)	<5	5- 10	10-20	20-30	30-50	>50
Greece	28	464,8	3	7	9	4	3	2
Turkey	18	268,2	3	6	7	1	0	1
Italy*	17	102	10	3	3	1	0	0
France	6	73,8	3	2	1	0	0	0
Spain	7	88,2	2	1	3	1	0	0

Source: Pavlina Pavlidou (Hatcheries Division Manager, Selonda Group), 2007. "Present and Future status of Mediterranean fry production". In What future for Mediterranean Marine Aquaculture?" PROFET Policy Workshop, 22-23 November 2007 – Athens, Greece.
(available at www.profetpolicy.info/index.php?option=com_contentandtask=viewandid=85andItemid=178)
* data 2006 from Italian Association of fish producers and ICRAM

Table 5. **Seabass and Seabream ongrowing production systems**

	No of sites	Production System			
		Sea cages	Tanks flow-through	Extensive and semi-intensive (lagoons/ponds)	Recirculated
Greece	173	99.5%	0%	0.5%	0%
Turkey	**	97%	1%	2%	0%
Italy*	191	28%	38%	34%	na
France	35**	48%	50%	1%	1%
Spain	58 **	90%	0%	10%	0%
Cyprus	7	100%			
Portugal	**	8%	11%	81%	
Malta	4	100%	0%	0%	0%
UK	1	0%	0%	0%	100%

Sources: 1. FEAP, Aquamedia; 2. COST867 Varese Meeting; 3. Federation of Greek Maricultures, 4. CIHEAM, 2000
* data 2006 from Italian Association of fish producers and ICRAM. Only extensive system (not integrated with semintensive and /or hatchery units)
** Expert opinion. Data on production sites is not available.

Table 6. **Seabass and seabream ongrowing production by production system**

	Sea cages	Tanks flow-through	Extensive and semi-intensive (lagoons/ponds)	Recirculated	Data 2005 Total Production (tonnes)
Greece	80100	0	420	0	80520
Turkey					64924
Italy*	8900	7690	1200	0	17790
France	3100	3000	50	50	6200
Spain					29592
Cyprus					2144[1]
Portugal					4000
Malta	1650				1650
UK				1000	1000
total	93750	10690	1670	1050	207820

1Official data (2007), V. Papadopoulos, Department of Fisheries and Marine Research.
* data 2006 from Italian Association of fish producers and ICRAM

4. Welfare of seabream and seabass

4.1. Abiotic Factors

4.1.1. Temperature

Seabass and seabream are classified as eurythermal fish (Stickney, 1994). Seabass is able to tolerate temperature ranging from 2 to 32 °C (Barnabè, 1990) and seabream between 5 and 34 °C. In extensive and semi-intensive coastal systems, water temperature can vary significantly influencing both the duration of production cycles and feeding management. Most seabass and seabream production in Europe is intensively produced in cages (87.5%) or in flow-through tank systems (10%) designed to operate under ambient water temperature conditions (11-25 °C). In such systems variation in water temperature is less extreme and is not normally a welfare issue, as the temperature change is invariably within the normal range for the species. In recirculated aquaculture systems and in flow-through water tanks using warm water from heated effluents (from fossil fuel and nuclear power plants) or geothermal waters temperature variations are usually too small to become a potential risk under normal operation conditions.

Thermal effects, however, interact with other abiotic factors, such as dissolved oxygen, salinity and food supply and greatly depend on age, population and strains. Intra-specific differences in thermal tolerance and growth-temperature relations are observed between fish populations of different geographic origin (Conover et al., 1997). In seabass, the optimum temperature for growth is reported as being 24°C (Tesseyre, 1979; Ravagnan, 1992). Juveniles from the Eastern Mediterranean stop growing at less than 11 °C and have a lethal lower limit of 2 °C (Doimi, 1990). Juveniles from Western Mediterranean demonstrated increased growth rate at temperatures from 13 to 25 °C, with maximum growth at 26 °C, decreasing at 29°C. Maximum specific growth rate was at 26°C and maximum feed intake at 27.5 °C (under oxygen concentration to saturation, Person et al., 2004). In seabass juveniles from Welsh waters, growth did not occur at 7 °C and was high at 18 °C (Russell et al., 1996). Variation in thermal preference and limits between populations may be related to genetic differences and normal ranges of temperature at the geographical origin and may influence biological responses and sensitivity to environmental changes. The origin, therefore, of the seabass strains and their temperature tolerance is important in relation to good welfare on sites where water temperature can reach extreme limits.

Temperature tolerance is also highly dependent on acclimation. Rapid increases in water temperature reduced dissolved oxygen concentration and increased fish metabolic rate, and are potentially harmful to health and welfare. Both seabass and seabream (Requena et al., 1997) acclimated rather quickly to high and medium temperatures, when temperature changes occurred within the tolerated thermal ranges for species. For example, oxygen consumption increased about twice in seabass when temperature increased from 15 to 25 °C (Person et al., 2004), and almost three times when temperature increased from 20 and 30 °C (Dalla Via et al., 1998), without any detrimental effects on growth.

Rapid changes in ambient water temperature are not so frequent during the farming cycle and are essentially associated with final harvesting, transfer from one water body to another, and live hauling. During harvest and fish transfer, water levels are reduced and as a result water temperatures may be modified. Dissolved oxygen may also be significantly decreased as a result of increased fish metabolism during handling and confinement. These effects result in stress leading either to mortality or reduced disease resistance in exposed fish. High temperature may also act as a stressor in farms using shallow ponds and tanks with low water exchange during diurnal temperature in summer.

Acute cold stress is reported as being less frequent due to the high specific heat capacity of water and, therefore, rarely to occur in seabass and seabream farming. The degree of tolerance of both species to low temperature depends on several factors, such as the rate of temperature drop, fish health status, energy storage, turbidity and water current (Ravagnan, 1992). Seabream has a thermal preference of 18 – 26 °C, but it is more sensitive to low temperature than seabass. The lower lethal limit for seabream in culture systems is around 5 °C (Ravagnan, 1978). Fish activity and growth rate below 15 °C are minimal (Tort et al., 1998) and food is refused at 12 °C. In winter time, when cold-induced fasting is prolonged, especially in North Mediterranean Sea (Spain, Italy and Croatia) cold temperature significantly affects metabolism and physiology of seabream. Cold water temperature and fasting decreased plasma proteins (albumin and α1-globulin), calcium and potassium ions and significantly increased serum glucose levels (Sala- Rabanal et al., 2003; Gallardo et al., 2003). In the long term, cold temperature (8 °C) altered lipid and protein metabolism in liver and induced the mobilisation and use of lipids from peri-visceral fat and muscle. Hepatic metabolism was also significantly compromised, as suggested by the significant reduction in protein synthesis, the mobilisation of glycogen and the accumulation of non-polar lipids at low temperature (Ibarz et al., 2005; 2007). Cold water and nutritional deficiencies have often been associated with immunosuppression in seabream. A reduction in complement, lysozyme and agglutination activities (around 50%) and phagocytic activities (15-20%) were reported in seabream cultured in sea cages in Northern Spain during winter (Tort et al., 1998), as well as in seabream in Italy (Galeotti et al., 1998). Fish also become very sensitive to opportunistic pathogens and the “winter disease syndrome” could often occur (see Chapter 4.6.3).

Seabream seem to be more adaptable to low but stable temperatures than to decreasing temperature changes (Tort. et al., 2004). Acute temperature decreases from 18 °C to 9 °C proved to be a significant thermal stressor in seabream (Rotllant et al., 2000), activating the hypothalamic pituitary interrenal (HPI) axis, increasing serum cortisol levels and inducing secondary stress responses at metabolic and osmotic level. Physiological perturbances were not restored after 15 days and fish suffered from chronic cold stress. The lack of compensatory response in oxygen consumption at 8 °C for 20 days is also considered a lack of acclimation to cold temperatures (Ibarz et al., 2003). Cold temperature (<=12 °C) was also reported to affect the immune system of seabass (Scapigliati et al., 1999; Cecchini and Saroglia, 2002; Bagni et al., 2005), mainly reducing complement activity and immunoglobulin content in peripheral blood.

4.1.2. Oxygen

Dissolved oxygen is of primary significance in fish farms and its concentration is influenced by other abiotic (i.e. temperature, salinity, water quality) and husbandry factors (i.e. high stocking density, water renewal) (Willoughby, 1968 and Piper, 1970). Supplemental oxygen is now commonly used in flow-through and recirculated land-based farms (Colt and Orwicz, 1991). Oxygen consumption (mg O_2 kg^{-1} h^{-1}) in fish depends on the developmental phase and size of fish and it is influenced by temperature, salinity, oxygenation levels and feeding rate. Oxygen consumption in seabass reared under routine conditions increases linearly with increasing temperatures, from 140 to 300 (mg kg^{-1} h^{-1}) in the range of 13-29 °C (Person-Le Ruyet et al., 2004). In seabream, oxygen consumption values of 147 to 209 have been recorded between 12-18 °C (Ibarz et al., 2003). Environmental oxygen levels can directly affect activity, feeding behaviour, growth performance, physiology and immune response of seabass and seabream. Seabass seem to cope quite well with low dissolved oxygen concentrations (hypoxia); physiological response (i.e. cortisol, glucose, hematocrit) is similar in fish kept under hypoxic (3-4.5 mg O_2 l^{-1}) and normoxic (7.4 mg l^{-1}) conditions. Long term exposure to oxygen saturation below 80% (at 22 °C) impaired feed intake and growth in seabass (Pichavant 2001), mainly due to a reduced feed intake. Specific growth rate was 24% lower in hypoxic (40% air saturation) than in normoxic (86% air saturation) treatments

(Thetmeyer, 1999) and growth was stopped at 38% oxygen saturation (2.77 mg O_2 l^{-1}, Pichavant, 2001). Under severe hypoxic conditions, seabass decrease oxygen consumption and blood PO_2 is reduced (Gilmour, 1998; Claireaux and Lagardere, 1999). However, blood oxygen carrying capacity does not seem to be affected under chronic hypoxic conditions (seawater PO_2 =40 mmHg for 40 days) and so it is assumed that different adaptive strategies for the control of blood gas transport under chronic low ambient oxygen concentrations are adopted (Pichavant et al., 2003) compared with other species (Gilmour, 1998). In seabass the oxidative status of the plasma does not seem to be significantly influenced by low oxygen levels (3.5 mg O_2 l^{-1}). Hypoxia, however, enhances plasma total antioxidant content (Di Marco et al. 2007a) as observed in other fish species (Martinez-Alvarez et al., 2005). This mechanism is considered as an evolutionary adaptation, having a protective role during oxygen deficient conditions, which allows fish to cope with oxidative stress arising from tissue re-oxygenation (Hermes-Lima and Zenteno-Savin, 2002). The concentration of oxygen available varies across different production systems. In cages, dissolved oxygen becomes a limiting factor when water renewal is low and stocking density and temperature high. Such problems do not normally arise in flow-through or recirculated systems except in the event of mechanical breakdown.

If an increase in environmental dissolved oxygen level occurs (hyperoxia), important effects are observed in fish physiology. Mild hyperoxia (150% oxygen saturation) does not affect blood acid-base regulation, or the immune system or the growth performances of seabass (Scapigliati et al., 1999; Cecchini and Caputo, 2003) while severe hyperoxia (up to 230-250% oxygen saturation) significantly affects seabass physiology. Seabass (200-250 g) exposed for 5 weeks to hyperoxygenated waters (250% saturation) shows physiological disturbance with significant modifications in acid base status (Cecchini and Caputo, 2003), similar to those observed under hypercapnia (Cecchini et al., 2001). Recent studies (Di Marco et al., 2007a) confirm this finding, indicating that hyperoxygenation, up to 250%, also induces some physiological disturbances in the oxidative balance of seabass compared to moderate hyperoxia (150% O_2 saturation). Hyperoxia (up to 250% O_2 saturation) does not cause a chronic cortisol stress response in seabass (Di Marco et al., 2007b).

The effects of environmental dissolved oxygen concentrations on fish physiology also depend on the concentration of other dissolved gases. When seabass are exposed to combined hyperoxia (230-250% O_2 saturation) and hypercapnia (50-60 mg CO_2 l^{-1}), significant changes in ion concentrations (decreased plasma Na^+ and increased plasma K^+) are observed, indicating ionic regulation disturbance (Marino et al., 2007). Similar variations, in both Na^+ and K^+ plasma levels, were described in Atlantic salmon smolts exposed to combined hyperoxia and hypercapnia (Brauner et al., 2000). This may suggest a maladaptive response to acidosis compensation, probably due to an impairment of gill function caused by intense hyperoxygenation. An increased risk of oxidative stress for impairment of antioxidant defences may also occur under hyperoxic and hypercapnic conditions in response to elevated oxidative conditions (Di Marco et al., 2007a), as already reported in other species (Dabrowski et al., 2004). Hyperoxygenation and hypercapnia were also found to increase mortality in seabass after bacterial challenge (WEALTH, 2008).

4.1.3. pH

Sea water pH is generally claimed to be stable and buffered at around 8.1- 8.2. However, pH fluctuation in brackish ponds and low-salinity lagoons may occur in relation to phytoplankton respiration, which removes carbon dioxide under high light intensity and inversely, produces carbon dioxide and increases water pH at night (Wedemeyer, 2000).

In fish farming, carbon dioxide produced by fish respiration can significantly reduce water pH under intensive conditions such as recirculated systems and open-flow systems with low water renewal and oxygen supplementation. In recirculated systems, pH depends on CO_2 concentrations and also on the level of H^+ produced by the biological filter (Kaiser and Wheaton, 1983). When CO_2 is removed by the use of packed columns, the pH may nevertheless reach low values (<6) and there is limited information on the welfare implications of such circumstances.

In cages, pH variations are too small to represent a welfare issue. Data on acute and chronic effects of acidic and basic waters in seabass and seabream are very scarce in on-growers, and non-existent for larvae or juveniles. An unpublished study was carried out on 100g seabass in a research station in the French Mediterranean. The study exposed seabass to 5 different stabilized pH regimes (5.3, 6.2, 7.0, 8.0 and 9.0 approx.) for 42 days, by adding continuously adequate quantities of concentrated acid (Hydrochloric acid) or alkali (sodium hydroxide) in the inlet seawater of the experimental tanks. No significant differences were shown in swimming behaviour, feed intake, growth and feed conversion ratio inside a pH range between 6.0-8.5. Beyond these values, swimming and feeding activities and growth performances were impaired (Lemarié, unpublished). In previous trials, up to 30% mortality was recorded in seabass acutely exposed to pH 4.5 and 9.4.

Reduction in pH affects seabass by influencing ion transportation at gill level and can lead to osmotic imbalance (Cecchini et al., 2001; Grottum and Sigholt, 1996). The recovery of physiological blood pH values is primarily due to adjustments in blood bicarbonate concentrations via the exchange of acid-base equivalents at gills. Compensatory transport is usually activated within 20-30 min from disturbance and can reach elevated net acid-excretion rates within a few hours from acidosis induction (Claiborne, 1998; Evans et al., 2005). A transient compensatory alkalosis may thus result from an elevation of bicarbonate ions, as a compensatory response to acid-base disturbance (Cecchini et al., 2001). However, compensation to water acidification seems to be faster in marine rather than in freshwater species, in relation to differences in environmental water composition (Grottum and Sigholt, 1996).

Seabass exposed to carbon dioxide up to 55 mg CO_2l^{-1} (at 21°C) and induced water pH around 6.4, had restored blood pH values (7.5 at 21°C) within 3 hours (Marino et al., 2006a; Marino et al., 2006b). No significant differences in blood pH values (7.2-7.3) were observed in seabass exposed to approximately 5, 15, 30 and 50 mg CO_2 l^{-1} and induced water pH values of 7.6, 6.8, 6.5 and 6.4 for 45 days. This would suggest that there is a strong acid-base regulation in these fish, allowing them to maintain constant blood pH level at any given blood CO_2 concentration. Long-term exposure (45 days) to low water pH levels (6.4) can impair osmoregulatory balance, with significant plasma chloride losses (Marino et al., 2006) and can induce kidney damage (WEALTH, 2008).

Seabream are able to maintain blood pH values when placed in water at pH 7.0, without causing detrimental effects on growth, even at high TAN concentrations (Eshchar et al., 2003; Eshchar et al., 2006).

Alkalosis usually occurs with pH values higher than 8.5 and is less frequent than acidosis; fish are usually able to cope with an acute rise in pH values by modulating ammonia excretion and osmoregulation (Lemarié, unpublished).

4.1.4. Carbon dioxide

When supplementation of oxygen is applied and low specific water flow in intensive systems is used, there is a gradual build up of natural catabolites such as ammonia and carbon dioxide, and the pH is reduced (Person-Le Ruyet et al., 1997, Summerfelt et al., 2000), especially when water is re-used after treatment in recirculating ongrowing systems. The build-up of carbon dioxide concentration in water is considered to be the main factor responsible for the detrimental effects, rather than ammonia, but the role of the resulting reduced pH is unclear, especially in seawater (Fivelstad, 1999; Ishimatsu, 2004). Since supplementation of oxygen is not used in sea cage culture, hypercapnia is not a problem in such systems.
In seabass and seabream, an intake of 1 kg of commercial pelleted food leads to the production of 250 to 360 g CO_2 in water depending on species, physiological status and food composition. Although data are available on physiological consequences of hypercapnia (blood pH variation, respiratory and cardiovascular changes), few studies consider the effects of CO_2 on rearing growth performances (feed intake, specific growth rate, food conversion index). One study on seabass ranging from 40 to 120 g (Grottum and Sigholt, 1996) showed that the lethal concentration leading to 50% mortality after 96 h (LC 50-96 h) was close to 50.4 mm Hg (112.1 mg CO_2 l^{-1}) at 15 °C. Blood parameters measured exhibited significant decrease of blood chloride ion concentration. For seabass from 453 to 983 g, another study has shown that at a concentration of 75mg l^{-1}, fish became lethargic and exhibited darkening of the skin. At 110 mg CO_2 l^{-1}, fishes did not feed and swam erratically (Cecchini et al., 2001).

Recent studies on recirculated systems under experimental conditions (WEALTH, 2008; Marino et al., 2006), clearly indicate that both acute and chronic exposure to carbon dioxide (50-55 mg l^{-1}, pH 6.6 to 6.4) affected the stress response and immune system in seabass. Fish exposed to hypercapnia exhibited marked increase in blood PCO_2 values, followed by changes in acid-base status with an elevation in blood bicarbonate ions (from 14 to 52 mmol l^{-1}) and plasma chloride losses (from 167 to 125 mmol l^{-1}). Similar compensatory mechanisms were already reported in seabass exposed to graded environmental hypercapnia (Cecchini et al., 2001) and seabream exposed to moderate CO_2 concentrations (3.8 mmHg, pH 7.3). Alterations in seabream metabolic profiles were described as a consequence of the anaerobic metabolism induced by hypercapnia at tissue level (Michaelidis et al., 2007).

Chronic exposure to high carbon dioxide concentrations (50-55 mg CO_2 l^{-1}) also significantly affects the specific immune system in seabass (Marino et al., 2007), mainly reducing the respiratory burst activity of phagocytes and the total number of lymphocytes and also modifying the ratio of B and T lymphocytes in both head kidney and blood. In addition, the *in vitro* proliferation of head kidney lymphocytes was significantly affected by hypercapnic conditions (Romano et al., 2007). Increased CO_2 concentrations are also reported as the primary causes of kidney mineralization or nephrocalcinosis in some fish species, especially in freshwater (Smart et al., 1979; Fivelstad et al., 1999). It is also commonly observed in seabass and seabream reared in intensive land-based farms and recirculatedg systems. Under experimental conditions, a 45 day chronic exposure of seabass to environmental hypercapnia (50-55 mg l^{-1}) induced significant histopathological changes in the excretory kidney, with atrophy and degeneration of tubular epithelium (Marino et al., 2007). Under farming conditions, Beraldo and Galeotti (2006) reported that all seabass exposed for an entire production cycle (about two years) to 15 mg CO_2 l^{-1} displayed nephrocalcinosis. The pathological process became reversible by stripping CO_2 and improving water quality. Since nephrocalcinosis is a significant factor further practical studies are justified under commercial conditions on seabass and seabream farms to determine threshold CO_2 levels. Blancheton (2000) suggested for juveniles and adult seabass and seabream not exceeding CO_2

concentrations above 40 mg l^{-1} in recirculatedg and flow-through systems with supplement oxygenation.

4.1.5. Supersaturation

Surpersaturation occurs when the partial pressure of a gas dissolved in water becomes greater than the sum of the partial atmospheric pressure plus the hydrostatic pressure of that gas at any point of a water column. Supersaturation can be caused by oxygen injection systems, entrapment of air in piped supplies (leaks in pumps or valve systems), sudden decrease in pressure or sudden increase in temperature. Seabass and seabream tolerate moderate oxygen supersaturation (Saroglia et al., 1995; Scapigliati et al., 1999; Cecchini and Caputo, 2003). Super-saturation with N_2 is considered as the most hazardous, because it is never metabolized. The degree of supersaturation is the most important factor defining an eventual outcome, however that level depends on temperature, size of fish, pressure on incoming water and flow rate. It is not normally possible to establish a general threshold because the precipitating causes are multifactorial and variable. When a fish detects supersaturation, it generally goes deeper in the water column to compress the gases and thereby prevent nitrogen bubble formation in its blood. When nitrogen gas saturation of the water is at 102%, for example, the depth at which bubbles will not form in the blood of the fish, is 200 mm. As a guide, for every 1% increase in gas pressure, the fish have to swim 100 mm deeper in the water to equilibrate. If the depth of the tank does not allow the fish to equilibrate the total gas pressure, nitrogen supersaturation occurs and induces gas embolism formation in the blood vessels of the fish (Chamberlain et al. 1980).

In small vessels this can lead to rupture and haemorrhage, and even in larger vessels, the bubbles can obstruct blood flow. Fish may die without obvious signs, but those that survive may be blind, or suffer cerebral, renal or hepatic vascular rupture and haemorrhage, and often clear gas bubbles can be seen as bubbles below the cornea and epidermis (Wedemeyer, 1996). They are invariably compromised in one way or another, and do not thrive (Roberts and Shepherd, 1997). Cornacchia and Colt (1984) reported that 10 to 31 day old striped bass larvae (*Morone saxatilis*) developed over-inflated swim bladders and bubbles in the intestinal lumen at total gas pressure (TGP) % levels of 102.9% and 105.6%. At a TGP% of 105.6% there was 33% mortality in a 78-hour period. In other experiments, these authors found that 19 day old fish succumbed to a TGP% of 106.3% with 35% mortality in a 72-hour period. However, in 29 day old fish TGP% levels of 106.3% did not produce any mortality over a 72 hour period.

Most commercial farms are designed to avoid it, as it is primarily an engineering problem but when it occurs it has serious welfare implications as well as lower productivity (Harvey and Cooper, 1962).

4.1.6. Ammonia

Among water quality criteria, ammonia has been described as one of the most significant limiting factors for growth and survival in fish (Russo and Thurston, 1991; Tomasso, 1994). This constraint has been re-emphasized by the recent developments in water recirculation technologies and systems in marine fish farming (Handy and Poxton, 1993; Dosdat et al., 1996). Ammonia and urea are the two major end-products of nitrogen metabolism in fish (Forster and Goldstein, 1969). Mainly excreted through the gills, ammonia production by fish is primarily dependent on protein and energy feed intake (25-35 mg TA-N kg^{-1} feed ingested in seabass), and on the metabolic efficiency of the fish, which is species specific and is affected by increasing levels of ambient ammonia. Both the ionised (NH_4^+) and un-ionised (UIA-N, NH_3) forms of total ammonia nitrogen (TA-N) are toxic to fish, but the un-ionised form seems to be much more toxic. The equilibrium between the two forms is highly dependent on salinity and inversely related to pH, shifting to toxic forms when pH is increased (Handy and Poxton, 1993). Moreover, low environmental dissolved oxygen levels also increase the toxicity of ammonia both for seabass (Tudor et al., 1994) and seabream (Wajsbrot et al., 1991).

While acute toxicity tests are well documented, data concerning chronic ammonia toxicity to marine fish are relatively scarce, partly because of the technical difficulty in maintaining steady-state environmental parameters over a long period (US EPA, 1998). Acute and chronic ammonia toxicity (1-3 months of exposure) has been reported on turbot juveniles (Rasmussen and Korsgaard, 1996, Person-Le Ruyet et al., 1997a; 1997b; Person-Le Ruyet and Bœuf, 1998), seabass (Person-Le Ruyet et al., 1995, Lemarié et al., 2004) and seabream juveniles (Wajsbrot et al., 1993; Person-Le Ruyet et al., 1995) (Table 7). Above 0.9 mg l-1 UIA-N unionized ammonia nitrogen, there are risks of impairing the survival in seabass juveniles (Lemarié et al., 2004). The 0.26-mg l−1 UIA-N concentration can be considered as a safe long-term limit conditions in seawater (Dosdat, 2003).

Table 7. **Total and unionized ammonia LC50 and EC50**

	Average 96-h LC5O		55 day EC50*
	TA-N	UIA-N	TA-N
Seabass juveniles	40 mg l^{-1}	1.7 mg l^{-1}	22 mg l^{-1}
Seabream and tubot	57-59 mg l^{-1}	2.5-2.6 mg l^{-1}	

*55 day (EC 50): concentration at which growth is reduced by 50 % after 55 days
**Average 96-h LC5O: letal concentration for 50% of the individualsexposed for 96h

4.1.7. Salinity

Seabass and seabream are euryhaline fishes capable of tolerating both high saline waters (SW) and freshwater (FW) environments. Extreme tolerance levels for seabass are reported to be 0-5 to 60 (Jensen et al., 1998).) Optimum salinity for feeding and growth of 30 for seabass and between 18-20 for seabream was reported (Conides and Glamuzina 2006).

Despite its tolerance to wide salinity ranges, seabass proves to be sensitive to acute changes. The first studies on sea-bass acclimation to low salinity indicated that juveniles were not able to tolerate salinity lower than 3 (Dendrinos and Thorpe, 1985) and acute transfer from SW to FW (Cataudella et al., 1991), while it is capable to gradually acclimate to decreasing salinity values from 38 to 0 (Marino et al., 1994). In contrast, Eroldogan and Kumlu (2002) reported no mortality in juveniles seabass transferred from SW to FW, assuming a different degree of tolerance among seabass populations (Caccone et al., 1997). The origin of the particular seabass population (i.e. coastal lagoon, open marine waters) seems to have a significant role to explain different tolerance to low salinities under similar environmental conditions (Allegrucci et al., 1997).

Acute salinity changes in juveniles and adult seabass may result in highly increased metabolic rates (Dalla Via et al., 1989; 1998). Metabolic rates increase with decreasing salinity especially in warm waters (>20 °C). Similarly, increased metabolic rates were observed in seabream at low salinity (Tort et al., 1994; Conides and Glamuzina, 2006).
On the contrary, Chatelier et al. (2005) reported that adult seabass (510 g) exposed at constant temperature (14 °C), to acute reduction in water salinity down to freshwater (10, 5 and 0) did

not show alterations in metabolic and cardiac performances, basically in relation to the strong capacity to keep constant plasma osmolarity levels. Thus temperature seems to play an important role in determining the effects due to salinity changes.

The level of salinity has been extensively investigated in relation to growth performance (Dendrinos and Thorpe, 1985; Eroldogan and Kumlu, 2002; Eroldogan et al., 2004). Seabass juveniles reared in SW at 22.6 °C displayed better growth and FCE compared with seabass in FW, as a consequence of higher energy demand necessary to maintain osmoregulation in FW (Eroldogan et al., 2004). Better growth and growth indexes are observed in seabass juveniles reared at 10 and 20 (near to the iso-osmotic threshold -11mg l^{-1}- for the species) than at 30 and 40 (Eroldogan and Kumlu, 2002). Minor differences in growth under FW and SW conditions were observed in larger seabass (100 g).

Growth rate and survival in seabream larvae were higher at 25 than at 40 (Tandlera et al., 1995). Similar findings are reported for seabream juveniles with higher daily growth between 18 and 28 (Conides and Glamuzina, 2006).

Salinity is also reported to have an influence on the immune system. Humoral immunity of seabream, which is less tolerant of salinity than seabass, was reported to be negatively affected after acclimatization to low salinity water (6), brackish water (12) and high salinity water (55) (Cuesta et al., 2005).

4.1.8. Light/ Photoperiod

Light is a complex ecological factor whose components include colour spectrum (quality), intensity (quantity) and photoperiod (periodicity). The effects of light in fish change according to the species and the developmental stage (Boeuf and Falcon, 2001). In seabass photoperiod is recognized as a key factor affecting growth, development and reproduction.

In hatcheries, eggs and yolk sac larvae of seabass and seabream are maintained in darkness and/or very low light intensities. Exposure to light before first feeding is avoided in both species (Moretti et al., 1999), as light stimulates larval activity which results in rapid growth and poor yolk sac utilisation (Boeuf and Falcon, 2001). From first feeding onwards light is considered as a critical factor in the initiation of proper predatory activity. Long photoperiods stimulate growth and development. Minimum intensity of 800 lux within an optimal range of 1 000-3 000 lux is recommended for seabream. Conversely, in seabass the light intensity should be as low as 100 lux, with an optimal value of 500 lux (Moretti et al., 1999).

Until metamorphosis, photoperiod significantly affects growth, survival and condition factor of seabream larvae. It has been reported that for the first 20 days after hatching, continuous photoperiods (24h light) support the highest mean survival and high growth rate and condition factor (Tandler and Helps, 1985).

Juveniles seabream reared under various photoperiods (16L[light]:8D[dark] and 24L:0D) showed different growth rates. Under 24L:0D seabream converted less efficiently than seabream kept under 16L:8D and natural photoperiod. The lipid content of muscle was also lower and skin luminosity directly related to the number of hours of light exposure (Gines et al., 2004).

Photoperiod is also a key factor in the control of sex and age at puberty during the first annual reproductive cycle of seabass. Continuous light regimes are currently under investigation in order to avoid or reduce the proportion of early sexually maturing males. It has recently been demonstrated that long term exposure to continuous light induces important alterations on endocrine function and particularly in pituitary gonad axis activity in seabass. It also inhibited testicular development (Rodriguez et al., 2004). Felip et al. (2008) demonstrated that exposure to continuous light during pre-gametogenesis (4 months) and gametogenesis (6 months) reduced the number (3 vs 22%) of early maturing males at first sexual maturity. The effects of long photoperiod on growth during the first annual cycle of seabass are more variable. No detrimental effects were observed by Rodriguez et al. (2001) and by Felip et al. (2008), whereas Begtashi et al. (2004) observed loss of weight, loss of perivisceral fat and reduced hepatosomatic index. Photoperiod treatments are also used to advance or delay the spawning time in seabass and seabream and to facilitate out-of-season juvenile production. Spawning of seabass is delayed under constant photoperiod (Prat et al., 1999). However, temperature also modulates gametogenesis and affects spawning time. Both of these have to be combined to produce high quality eggs out of season.

4.1.9. Water renewal / Water exchange rate / Specific water flow

When the specific water flow (expressed in l h^{-1} kg^{-1} fish biomass) is reduced, the accumulation of excretion products from the fish as ammonia (NH_3), urea, carbon dioxide (CO_2), suspended solids and induced low pH may reach levels which impair growth performance and health status in fish. The specific water flow can also be expressed in grams of ingested food per cubic metre of water renewal (g m^{-3} water) (Fivelstad, 1999). This ratio exhibits the direct relationships between the feed ingested and the induced load of wastes in water. There are very few studies on the detrimental effects of these combined factors when water renewal is decreasing. When water renewal rate was 8.15 l h^{-1} kg^{-1} or 59 g feed m^{-3} water, there was a 10% reduction in feed intake and growth compared with control fish. It was considered that the principle factor responsible for the reduced performance was the corresponding 40 mg l^{-1} carbon dioxide concentration. In the largest flow-through farm (56 000 m^3 rearing tank capacity with a 110% water renewal per hour) in France, the specific flow rate in routine does not exceed 15 g of feed per cubic metre of water, which could be considered as a conservative value. In cage systems, the water renewal inside each cage will depend on the tidal or current exchange which is a function of siting, cage design, cage orientation, biofouling control and stocking density. Provided the oxygen requirements of the fish are met, then the flow rate in the cage will be adequate to dilute ammonia and CO_2 to non limiting levels (Muller Feuga, 1993; Chacon Torrès, 1988; Maldonaldo et al., 2005; Pérez, 2003).

In hatcheries, the fish biomass (larvae) is generally low during the larval stage and the water renewal in flow-through and in recirculating systems is enough to ensure the dilution of the fish waste products to non-limiting levels. Problems may arise when biomass increases at the end of this stage and management of flow rate is more critical (Lemarié pers. com.).

4.2. Biotic factors / Behavioural interactions

4.2.1. Stocking density

In seabass and seabream larval and pre-ongrowing stage, stocking density is expressed in number of eggs or individuals per litre (ind l^{-1}). The effects of rearing density and size grading on sex ratio in 30 families of seabass reared in the same tank from the fertilization stage onwards were investigated by Saillant et al. 2003. Two extreme density treatments (200 vs. 20

eggs l^{-1}) were applied. Growth was faster under low density from 49 to 191 days post-fertilization (p.f.) (27–10% relative difference in length) and was then equivalent between the two groups until the end of the treatments (414 days p.f.). Density had no effect on sex ratio, suggesting that the high larvae densities usually applied in aquaculture are not involved in the systematic excess of males reported in farmed seabass populations. Repeated size grading performed from 84 to 199 days p.f. had no effect on the sex ratio of the overall population studied showing that sex determination had not been affected by the treatment but was driven by genetic determinants and environmental parameters. Based on metabolic needs and estimated models including temperature, salinity, oxygen and fingerling's weight (from 1 to 5 g), Dalla via et al. (1998) estimated the optimal stocking densities for seabass and recommended for commercial culture conditions an optimum stocking density of one-tenth of the maximum density calculated by their model. They hereby confirmed empirically determined stocking densities of 0.5–2 ind l^{-1} (Giordani and Melotti, 1984; Saroglia and Ingle, 1992) or 0.02–0.2 ind l^{-1} (Ravagnan, 1992) reported for intensive bass culture conditions. The final fish weight between groups of the two highest and the lowest stocking densities (80, 165, 325 and 650 ind m^{-3}) was significantly different for seabass during the first 6 months of ongrowing (Papoutsoglou et al. 1998). The highest specific growth rate and the lowest food conversion ratio were observed for fish of the highest stocking density (which coincided with poorer water quality). No significant differences were found in haematocrit values, or in hepatosomatic indices in the groups of fish held at any stocking density.

In seabass and seabream ongrowing stages, the most basic formula to calculate stocking density is to divide the total biomass of fish by the available volume (in kg m^{-3}). However, there is a dispute as to the cause of the observed effects of increasing density, with water quality deterioration or an increase in inappropriate behavioural interactions being variously proposed. There are a limited number of studies investigating the behaviour and welfare of fish at farmed stocking densities, mainly because of the difficulties of observing fish under commercial conditions and due to the complexity of the interrelation between stocking density and water quality. In practice, the densities at which farmers keep their stock are based on experience and intuition, with codes of practice and handbooks being used as a guide to produce healthy, good quality fish (Ellis et al., 2002). In cage systems, such guidance is in the 10-20 kg m^{-3} range or more, depending upon fish size, site and cage characteristics (the water currents and net cage status determining the water renewal inside the cage, and accordingly the availability of oxygen). Higher densities (60-90 kg m^{-3}) are maintained in recirculation tanks or race-ways using adapted water flows with oxygenated seawater. It is suspected that in seabass farms in particular, larger tanks can favourably mitigate the stress effects observed in experimental trials of high stocking density by allowing fish to adopt shoaling displacements but there are no published studies to confirm it.

There are relatively few experiments on seabass with stocking density manipulation in which water quality was controlled and did not affect the results. Two experiments on the effects of stocking density (in flow-through and in recirculation systems respectively) were performed in 2004-2005 (WEALTH, 2008; Di Marco et al., 2007b). During 6 successive periods of 21 days each at constant water temperature (21°C) in 15 tanks of 1 m^3, seabass (75 g initial mean weight) were stocked in triplicate tanks at densities of 10, 20, 40, 70 and 100 kg m^{-3}. The water flow rates, ranging from 0.5 to 2.5 $m^3\,h^{-1}$ per tank with oxygenated seawater, were adjusted to the biomass to ensure similar specific water flows among the tanks whatever the stocking density and ensured the same water quality parameters at non-limiting levels in all tanks. Fish were fed at satiation once day. Biomass in excess was removed at day 21, 42, 63, 84, 105 and 126. The results were similar in both experiments and have shown that an early mortality (due to the stress of handling and transportation) affected the highest density more than the other ones. Swimming behaviour, based on visual control, showed large differences between the extreme treatments: at the lowest densities (10 and 20 kg m^{-3}), fish were distributed in the all volume of the tank and swam quietly in different directions. From 40 to 100 kg m^{-3} treatments, fish showed polarized displacements at higher swimming speed, turning around. Feed intake and growth rate were slightly lower (-10% and -14% respectively) in the 100 kg m^{-3}. No differences in the feed conversion ratio and no significant effects on stress (cortisol), susceptibility to nodavirus and other stress indicators (Na+, K+, glucose, pH, haematocrit) were found. No noticeable incidence of wounds or fin damage was seen in the high stocking densities. It can be concluded that, as with Boujard et al. (2002) in rainbow trout, that the reduced food intake was due to altered appetite at high densities level (100 kg m^{-3}) and not due to impaired food access or feed utilization. Terova et al. (2005) demonstrated that such a high rearing density (80 and 100 kg m^{-3}) affects seabass glucocorticoid receptor mRNA, whose abundance in the liver decreased inversely with blood cortisol levels

In contrast, and at relative lower densities compared with the experiment mentioned above, for both seabass and seabream, negative physiological and immune changes have been demonstrated when juveniles were exposed to different levels of stocking density whether in the form of chronic stressors or an acute crowding stressor. High stocking density produces crowding stress altering some physiological and biochemical parameters in seabream juveniles (Montero et al., 1999) and Barton et al. (2005) demonstrated that superimposition of additional stressors led to further reduction in their normal capacity to handle stressors. Their findings indicated for example that, stocking density increases modified lipid metabolism in order to help meet the increased energy demand. Similar conclusions were reached for ongrowing seabass by Di Marco et al. (2008). In their study, two consecutive experiments were carried out in order to investigate the physiological response of seabass to different stocking densities and to an acute stress challenge. Seabass were reared in recirculating tanks at three stocking densities (15, 30 and 45 kg m^{-3}) for 6 weeks, and then crowded at 100 kg m^{-3} for 15 min. Blood samples were analyzed for cortisol, glucose, non-esterified fatty acids (NEFA), total proteins, triglycerides and cholesterol concentrations. No differences in growth and survival were observed in seabass kept at 15, 30 and 45 kg m^{-3} for 6 weeks. Serum cortisol levels measured at 2 and 6 weeks were not significantly different among density groups. A significant increase in NEFA concentration was observed in seabass kept at 45 kg m^{-3} after 6 weeks, suggesting enhanced lipid mobilization. In response to an additional acute crowding stressor, the levels of cortisol and NEFA were significantly higher and of glucose significantly lower in seabass reared at 45 kg m^{-3} compared to fish kept at 15 and 30 kg m^{-3}. However, pre-stress levels were recovered after 24–48 h in all groups. Multivariate analysis did not reveal any discriminating variables among groups, indicating similar physiological conditions in the seabass experiencing different stocking densities and similar recovery patterns after crowding. Results indicate that stocking density at 45 kg m^{-3} for 6 weeks did affect the energetic status of seabass and their sensitivity to a subsequent crowding stressor. After exposure to adverse conditions the fish requires a recovey time which is related to the magnitude of the adverse effect (Di Marco et al., 2008).

4.2.2. Intraspecific interactions: aggression and competition

It is generally assumed that social interactions through competition for food and/or space can negatively affect growth and welfare of farmed fish. A sign of competition is an increase in the coefficient of variation of size over time due to the establishment of social ranks with dominant seabream getting more benefits under conditions of forced social interaction at low densities or of competition for access to food (Canario et al., 1998). Competition for access to food is induced either when ration is restricted (Jobling et al., 1999), rate of feed delivery reduced (Juell and Lekang, 2001) or when stocking density is increased (Canario et al., 1998). In many cultivated fish species, growth is inversely related to stocking density and this is mainly attributed to social interactions. The importance of aggression as a decisive factor in social hierarchies has been demonstrated by consistently superior performance of the most aggressive fish. However, most studies have been done in salmonids where intra-specific aggression can be an important welfare issue in larvae and pre-ongrowing mostly, leading for example to fin damage and cannibalism. The results of the few studies on seabass and seabream are summarized below and as with salmonids it seems that incidence of aggression will depend on a number of factors, including water flows and adequate feeding (Huntingford and Adams, 2005).

In relation to effects of stocking densities on seabass and seabream larval and post-larval stages, evidence of intraspecific interactions were given by Hatziathanasiou et al. (2002) and Kestemont et al. (2003). Under experimental conditions, results indicate that stocking density did not affect survival and growth of larvae and no cannibalistic phenomena were observed at this stage.

On the other hand, survival of seabass post-larvae was higher at 5 and 10 ind l^{-1} than at 15 and 20 ind l^{-1}, while growth performance fluctuated between the lowest value recorded in the group of 10 ind l^{-1} and the highest value in that of 5 ind l^{-1}. Feed intake in post-larvae was independent of stocking density. Cannibalism however was the main cause of death in seabass post-larvae at high densities (Hatziathanasiou et al., 2002).

In pre-ongrowing stage, the effect of stocking density on growth and size variability in sea-bream was tested by growing juveniles (between 1.3-14.4 g) at densities of 0.35 kg m^{-3}, 1.3 kg m^{-3} and 3.2 kg m^{-3}. Fish in the highest density group grew 25% slower than fish in the lowest density group (Canario et al., 1998). The coefficient of variation of weight (standard deviation/mean) did not change during the experiment. The effect of size variability on growth rate was tested by creating groups with coefficients of variation of weights ranging from 0.11 to 0.32 g, and growing them at a similar density (1.5 kg m^{-3}). No differences in growth rates between groups were found. Only the most heterogeneous group showed a large decrease in the coefficient of variation over time. Canario et al. (1998) therefore concluded that growth in gilthead seabream, although negatively correlated to stocking density, did not seem to be related to intraspecific competition as assessed by changes in size variability. Goldan et al. (2002) also found under experimental conditions that seabream displayed aggressive interactions almost exclusively during feeding. Rank in the hierarchy had a profound effect on the behaviour and growth of all group members. The dominant fish in each group carried out more aggressive acts and bit at food particles more often than the other group members. The dominant fish also had the highest relative specific growth rate. Direct competition for food is probably the major social mechanism regulating growth in small groups of juveniles of this species when food is limited and defendable. The relevance of these findings for the commercial culture of this species would only be seen at low rearing densities.

In contrast, for seabass, Papoutsoglou et al. (1998), reared juveniles around 6 g under closed circulated water system conditions at different stocking densities (and with significant differences in water quality among treatments with relatively poorer water quality occurring at the higher fish densities). Results suggest that shoaling behaviour of seabass may have been a factor in the observed differences in fish performance but no clear dominance hierarchies were observed.

In ongrowing systems, changes in growth, feed conversion, feed handling behaviour, swimming and aggression were investigated in gilthead seabream (32 g, reared at 12 kg m^{-3} held in recirculated water system) in response to imposed competition levels, manipulated by altering ration size and feed delivery rate (Andrew et al., 2004). They concluded that ration size influenced competition levels as feeding intensity, fish density under the feeder and swimming speeds increased during meals when rations were low. This pattern was also found for bream fed by hand in cages (Andrew et al., 2002), but on-demand feeding, which apparently reduces competition for feed, was also found to lead to an increase in swimming speed during meal times. Aggressive interactions were not found to increase by restricting ration (Andrew et al., 2004). In conclusion, feeding regime influences competition for feed amongst gilthead seabream and this in turn can influence feeding behaviour and feeding efficiency. When fed to over-satiation, competition was reduced and growth high, but waste was also high which resulted in a poor feed conversion. At feeding levels just below satiation, growth was high, as was feeding efficiency. This is consistent with a study by Hedenskog et al. (2002) on brown trout, and may be due to the possibility that aquaculture conditions such as high stocking densities limit agonistic interactions between fish. In contrast, for seabass, Di Poï et al. (2007) and Millot et al. (2008) both reported that juveniles (above 50 g) can display competition around feeding devices or meals assorted with differential dynamic growth and brain seretoninergic activity. It is however mostly related to scramble competition for feed and not accompanied by aggressive interactions and thus biting and fin damage are seldom observed in seabass.

4.2.3. Predation

Potential predators of seabream and seabass vary during the life cycle depending upon the farming system. The principal predators are fish-eating birds (Melotti et al., 1994; 1995), such as cormorant and heron, and marine mammals (seals, dolphins) and there may be some predation by piscivorous fish (such as Seriola sp.).

The impact of predators on farmed seabream and seabass also varies across the life stages. Eggs and larvae are kept in conditions isolated from the wild so that predation is not a risk at these stages. Industrial experience as well as expert knowledge, indicates that for the pre- and ongrowing stages predation is largely irrelevant in indoor tank systems, but it becomes a serious problem in any outdoor systems including tanks, ponds, lagoons and sea cages as soon as the fish are transferred for further ongrowing.

Any anti-predator behaviour or avoidance response has a behavioural cost for the animals, either directly in terms of energy expenditure, or as lost opportunities to feed then they have to spend time to hide from an unknown danger. Farmed fish have the ability to habituate to their farming situation, and respond less as they become accustomed to their farming conditions. They do however show characteristic ‘spook’ effects when predators such as cormorants have been attacking them and may not feed well for several days thereafter. Depending on the size of the fish, many will also suffer chronic but often severe traumatic wounds which become secondarily infected (EIFAC, 1988; Beveridge, 1987).

The effects of predators on farmed seabass and seabream in ponds and lagoons includes fish killed during the attack, fish injured by the attack, stress response in the remaining fish, fish escaping through for example damaged nets and damaged infrastructure. Industrial experience coupled with the high expenditure on predator control would suggest that a large number of fish are lost to or harmed by predators, i.e. a major welfare problem. However there has been no systematic collection of data on this subject. A variety of methods of preventing or minimising the damage caused by such attacks have been developed but there would appear to be considerable variability in the efficacy of these methods and a lack of any rigorous scientific investigation or clear practical advice for farmers nor have the welfare implication of methods of predator control been evaluated. To be killed by a predator does not result in prolonged suffering unless the attack is protracted or repeated prior to death of the injured fish. Predation is the eventual fate of most fish in the wild but that does not make it an acceptable fate for farmed fish. Those fish that were damaged during an attack but not killed, arguably, suffer most as a result of an attack since they are injured and have to undergo a period of recovery or decline and death. Attacks by predators can also cause a stress response in the whole population. Fish that escape following a predator attack may take some time to learn how to find natural food and their success in doing so will depend on many factors including the availability of natural food. A brief period without food is not necessarily a serious welfare issue. If large numbers of fish escaped and then died of starvation this would be a significant welfare issue. Fish that escape and survive may also impact on the health and viability of wild populations.

Predators are controlled by different means according to the particular farming situation. In general, however predators will be from protected species and there is often a severe constraint on the farmer in terms of his ability to deal with them. Fish holding facilities (outdoor tanks, ponds) are often covered with nets to deter bird predators or wires may be stretched across ponds or raceways.

Studies on predation by fish-eating birds indicated the convenience of adopting fish nets during seabass and seabream intensive rearing cycles, in the light of considerable losses both in numerical and quantitative terms and of indirect losses (Melotti et al., 1994, 1995). The impact of predation on seabream production, expressed as average survival rate, appears to decrease as the fish increased in size. The difference between covered and uncovered basin is around 10% of production in the first year of rearing and 6.5 % at the end of second year rearing. The morphology of seabream and larger size of adults probably do not encourage predation by birds, which direct their efforts towards younger fish. However, more significant in term of welfare is the number of fish damaged by birds, which are about 6 times more in uncovered than in covered ponds (3.6 vs 0.6% of fish population). In seabass culture, fish density in ponds (10 vs 20 kg m^3) significantly influenced the distribution of fish-eating birds and final survival rate during the first year of rearing (Melotti et al. 1994). Direct losses, due to the capture by fish-eating birds, and indirect losses, due to damage to farmed fish, are less relevant during the second year rearing. As for seabream, the protection of basins with nets significantly affects survival rate and the number of damaged fish. The use of protective nets, however, do not completely protect the fish from predators, although they represent a considerable obstacle to hunting.

Bird scarers may also be deployed on farms. In sea cages anti-predator nets are used to completely surround the cages to prevent predation by birds, dolphins, seals, tuna and great amberjack.

There is little scientific information on the extent of predation, although it is widely perceived as a serious issue within the industry and producer's codes of practice recommend anti-predator measures (Donati et al., 1998). The efficacy of anti-predator measures is also poorly documented (for seabass Melotti et al., 1994 and for seabream Melotti et al., 1995).

4.3. Food and Feeding

In order to fulfil the nutritional requirements of larval stages of seabass and seabream, first feeding uses live foods. For these reasons, dedicated live feed production facilities are an integral part of bass and bream hatcheries. Seabass are fed on enriched *Artemia nauplii* and weaning on microdiets occurs by day 40 (out of 80 days in total). Seabream larvae also require live food, both rotifer (*Brachionus* spp.) and *Artemia nauplii*. The availability of such small-sized preys (<150 µm) and high prey densities in rearing tanks (5-10 rotifer ml^{-1}) is an essential adjunct to the successful survival of larvae. The administration of live diets enriched with poly unsaturated fatty acids (Koven et al., 1989; 1992), mainly arachidonic acids (Bessonart et al., 1999; Koven et al., 2001), with phospholipids (Hadas, 1998) and with amino acids increases both survival and larval growth. Larval deformities have been attributed to low quality, leading to deficiency of essential micronutrients or shortage of high-energy elements.
In later stages, improving the digestibility and optimising feeding regimes are both factors in the improvement of feed utilization efficiency in farmed fish (Cho and Bureau 2001). In recent years knowledge of of the nutritional requirements of seabass and seabream has been improved considerably (Kaushik, 1998). The digestible protein and digestible energy balance (DP/DE) for seabass and seabream is the subject of some discussion. According to various authors it would appear to lie between 19-21 MJ kg^{-1} and 21-24 MJ kg^{-1} (Bavcevic and Lovrinov; 2006). Variations in the optimal DP/DE quoted in scientific publications would seem to reflect significant variation of DP/DE in the different commercial diets which were used. Fat content in feed for seabass and seabream is normally around 20%. Required enrichment with essential fatty acids (especially HUFA n 3) is usually obtained by incorporation of at least 7% of fish oil in the diet. Essential fatty acid (EFA) relative content in farmed seabass and seabream muscle fat was found to be similar to the relative content of EFA in fish oil. Vitamin-mineral premixes are standardized and given in higher concentration in certain special feeds which may also be enriched with immunostimulants, and used in specific circumstances to enhance immunobalance. There are still however serious concerns within the industry and in this respect expert opinion will agree that currently manufactured diet for seabass and seabream are often variable and may be deficient because of the lack of open formulation and the use of least cost formulation by the limited number of manufacturers. For proper nutrition of seabass under intensive conditions new diets of uniform quality based on full knowledge of ingredients and on seasonal variations in fish requirements are needed.

It is essential to know when, how much and by what means fish should be fed which in turn requires information on how fish behave under different feeding systems. Fish are able to regulate their daily food intake based on their nutrient and energy requirements (Kaushik and Medale 1994). This self-regulation, however, does not mean that they will make the most efficient use of the diet (Azzaydi et al., 1998), as the ration size that optimises feed efficiency is generally lower than that producing the highest growth rate (Talbot, 1993). Thus for example, higher feeding efficiencies are seen in European seabass if food access is restricted under certain circumstances (Azzaydi et al., 1998). Reducing their daily ration appears to compel fish to make the best use of the feed they have ingested without affecting their growth. It is assumed that fish will feed themselves at their preferred time (Boujard et al., 1996; Heilman and Spieler 1999) and in accordance with their nutritional requirements with demand feeding (Sanchez-Vazquez et al., 1998, 1999; Yamamoto et al., 2000a, b). Demand feeding has also proved useful for seabream production (Paspatis et al., 2000; Sanchez-Muros et al., 2003), and to study their feeding behaviour (Velazquez et al., 2004). For feeding seabass in culture conditions the self-feeding practice has been proved appropriate and is often better than other methods (Divanach et al., 1993; Azzaydi et al., 1998; Covès et al., 1998; Paspatis et al., 1999, 2003; Boujard et al., 2000; Andrew et al., 2002).

By comparing a number of automatic feeding systems for European seabass, Azzaydi et al. (1999) concluded that those in which the food is supplied at a defined period coinciding with the time of maximum appetite might produce comparable, and sometimes even better, results than self-feeding systems. Other authors have found improved growth when feeding is tailored to the animals feeding rhythm (Reddy et al., 1994; Boujard et al., 1995; Azzaydi et al., 1998; Hossain et al., 2001). For seabream Velazquez et al (2006) have studied the effects of time-restricted food access and ration restriction on demand-feeding behaviour and nutritional use of the diet, and also compared the nutritional efficiency of three different feeding systems: manual, automatic and modulated-automatic (daily ration distributed into three meals of 25%, 50% and 25% respectively). Restricting the amount of food modifies gilthead seabream self-feeding behaviour, with fish increasing the number of demands provided these are rewarded with food. However, demand-feeding activity does not increase if rewards are restricted to a certain time. Feeding gilthead seabream by hand versus automatically, and distributing the daily food ration in two or three equal or unequal-size daily meals, have no effect on the animals growth, nutritional use of the diet or body composition.

Although the beneficial effects of demand-feeding on fish have been largely demonstrated both in cages and tanks (see above), hand feeding is still the most widely used method along with automatic feeders. In the case of semi-extensive systems, fish rely on natural prey and if additional manufactured feed is offered to them, which can lead to differential access to feed, survival and growth of the fish population (Bégout-Anras et al., 2001).

4.3.1. Food deprivation and starvation

Fish in their natural environment alternate periods of feeding and fasting in response to several factors (e.g. feed availability, temperature, spawning migration, reproduction, etc.). Cultured fish normally should not experience such deprivation but this may happen under imposed conditions such as essential husbandry operations or stormy weather etc. To survive such food restrictions, fish mobilize their energy reserves, which impose metabolic adjustments that are species dependent. Intraspecific adjustments to these conditions also depend on other factors such as fish age, nutritional state, etc. (Navarro and Gutiérrez, 1995). On commencement of refeeding there may also be different responses between species. Recovery from food deprivation also depends on factors such as the environmental conditions, period of food deprivation and the previous feeding history (Navarro and Gutiérrez, 1995). Except in those cases in which food deprivation induces irreversible damage, in most fish species metabolic profile seems to return to pre-starvation values after a short refeeding period (Perez-Jimenez et al., 2007).

For seabass, there are a few studies focused on the influence of food deprivation on growth and some plasma metabolites (Stirling, 1976; Gutiérrez et al., 1991; Echevarría et al., 1997; Santulli et al., 1997). Until recently information on intermediary metabolism modifications due to starvation and refeeding was not existent and even now the influence of diet composition on such adaptations has been scarcely studied. Perez-Jimenez et al. (2007) demonstrated that ongrowing seabass experience rapid metabolic adjustments to both short terms starvation (9 days) and refeeding and that diet composition significantly influences the metabolic responses to these nutritional challenges. For seabream, it was shown that this species seems able to use its energy reserves, namely perivisceral fat, in order to counterbalance the nutrient shortage arising from feeding interruption for up to 13 days (Ferreira Pinto et al., 2007).

4.3.2. Probiotics and nutriomics / partial protein substitution and alternative sources protein

Probiotics are live single species of microorganisms or a mixture of species added to feeds after pelleting. By colonizing the fish gut, they are supposed to out compete detrimental microorganisms allowing the fish to spare metabolic energy and to stimulate growth (Tovar et al., 2002**).** Enzyme supplements are either single, purified or crude enzyme preparations added to feeds by spraying after extrusion (they are denatured at temperatures above 65°C) to enhance the digestion of complex carbohydrates and collagen.

No adverse consequence in terms of somatic growth or nitrogen utilisation have been observed in seabass fed with diets in which proteins from fish meal were decreased gradually from 100% to about 2% and replaced by plant protein sources. There was, however, a slight increase in fat deposition in fish fed diets with plant protein sources and there was a slight increase in nitrogen losses (from 83 to 103 g N/kg weight gain) and a significant reduction in total phosphorus losses (from 13 to 5 g P/kg weight gain). (Kaushik et al., 2004).

4.4. Husbandry and Management

Husbandry and management are crucial factors to ensure the health, welfare, high performance and premium quality of farmed seabass and seabream. Husbandry can be considered as all measures to take care of the fish and management as the strategic decisions regarding the production system.

4.4.1. Disturbance

Seabass and seabream are exposed to disturbances that may cause stress during the ordinary farming procedures. Potential stressors can be found in all stages of the production cycle and include feed distribution mode, handling and manipulation, cleaning routines, grading, crowding and confinement, 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.

4.4.2. Cleaning activities

The health status and performance of marine fish depends on a well-planned prevention including strict cleaning routines. However, cleaning procedures may disturb the fish. During larval rearing a disinfected siphon is used once or twice a day to remove the settled debris (uneaten food and faeces) and dirt from the bottom of the tanks. The floating debris and the superficial oily film trapped by surface floating skimmers are being removed with the use of a paper tissue at regular intervals (Moretti et al., 1999). Trapped larvae and dead post-larvae are removed daily. During weaning sediments at the bottom of the tank are removed by purging twice a day through the bottom valve and by the use of siphon. When the above clearing procedures are performed with great care by well-trained personnel, it does not cause significant disturbance to fish.

At ongrowing, an important factor is the husbandry of the pens; frequent net changing is essential (up to every 15-20 days during summer) to maintain water exchange at optimized levels, as well as weekly cleaning to remove fouling organisms. Several fish farms apply also periodical treatment with anti-fouling. The removal of dead and moribund fish by divers is done on a daily or week basis. Net change involves crowding and deterioration of water quality.

4.4.3. Grading

Grading is a particularly important exercise for intensively farmed fish. Even when eggs come from the same batch, post-larval fish and juveniles of both species grow at varying rates leading, in relation to the crowding conditions, to hierarchies, aggressive behaviour and cannibalism. Sorting is a common practice too in broodfish to obtain the appropriate sex ratio, in larvae for the inspection of functional swim bladder and in fry to check for skeletal deformities. The grading procedure involves (for all fish) harvesting, crowding, handling with a hand net, passing through a series of sorters of different size, and transfer into new hosting weaning tanks. Grading is repeated for intermediate sized animals and the smallest fish which have not been retained by both graders are concentrated and moved into a new weaning tank. The procedure is performed 2-3 times for seabass and seabream respectively during the weaning and pre-ongrowing phase.

Grading (0-2 times) may also take place during the ongrowing phase at the summer months and at a size of 25-40 g and around 100 g.

Seabass is a scaly fish with less skin mucus than salmonids and has 2 flat spines on the operculum and one on the dorsal fin. When on-grower fish are crowded and confined, handled and graded, there are some risks for mechanical injuries caused by unintentional stinging from the operculum and dorsal fin spines. Wounds and resulting bacterial infections may occur as well as mortality (Ghittino, 2003). The use of anaesthetics can prevent partly this problem, but usually, farmers avoid sorting fish during the ongrowing phase by growing more homogeneous batches of fry calibrated in hatcheries.

4.4.4. Crowding and confinement

Fish are exposed to confinement and short-term crowding several times during the whole stages of the production cycle. Crowding and confinement is a common practice and an essential process for handling of fish prior to transport between units, grading, vaccination, tagging, sexing and induced spawning of broodfish, and harvesting. Both seabass and seabream show the classical pattern of physiological responses reported for other intensively reared fishes following exposure to acute stressors such as physical confinement and short term crowding. In general, both species show increase in several stress indicators such as plasma cortisol, glucose and lactate. For example, plasma cortisol concentrations peak at 1 h after exposure to intense sort-term crowding or confinement stress and resting levels are obtained within 24 h following exposure to the stressor (Marino et al., 2001; Rotland et al., 2001; Ortuño et al., 2001; Fanouraki et al., 2008). Prolonged confinement of seabass at 70 kg m^{-3} for 24 h results in elevated plasma cortisol concentrations (up to 24 h) and an increased activity of the interrenal cells after 1 and 4 h of confinement (Rotland et al., 2003). Long term confinement (11 days) in seabream induced a biphasic cortisol response with peaks at 1h and at 2 and 3 days (Arends et al., 1999). However, it has to be noticed that seabass show higher plasma cortisol concentrations, higher cortisol content of head-kidney homogenates, and higher basal unstimulated release of cortisol from the head-kidney in unstressed fish when compared with other fish species (Rotland et al., 2001; Rotland et al., 2003; Fanouraki et al., 2008).

4.4.5. Transport between units

Egg harvest

Seabass and seabream are pelagic spawners producing floating eggs. Commercial hatcheries have adopted automated egg collector devices to harvest the floating eggs. Great care should be given to avoid mechanical and physical (thermal and salinity) shock during egg collection, transport and incubation that may result in mortalities or deformities in later life-stages. Egg introduction into the receptor tanks (dedicated incubation tanks or directly in the larval rearing tanks) is always accompanied by gradual acclimation to the new abiotic (mainly temperature) conditions.

Egg disinfection is an operation used in several hatcheries to prevent transmission of diseases from batches produced within the hatchery or transported from other hatcheries.

Egg cleaning with sterilised water and egg disinfection using iodine (50 - 100 ppm, 10 minutes) (VESO, 2005) and in some cases glutaraldehyde or hydrogen peroxide (similar doses) is performed in some but not in all hatcheries.

Transfer to larval rearing installations

Hatched eggs in the incubation tanks are transferred to the larval rearing tanks by harvesting pre-larvae either via the bottom valve of the incubator into several partially filled buckets or using a jug directly dipped in the incubator. Mechanical stress should be avoided by minimizing the difference between inner water level and water level in the bucket and by avoiding splashing. When eggs are incubated directly to the larval rearing tanks the only operation performed is careful cleaning of the hatching debris by the use of siphon and re-adjustment of water exchange and aeration protocol.

Transfer to pre-ongrowing facilities

Weaning usually take place in the larval rearing tanks. Prior to or just after the end of the weaning phase juveniles are transferred to the pre-ongrowing tanks. Fish at this phase are highly sensitive to transport stress and important precautions should be followed. The procedure includes careful cleaning of the larval rearing tank bottom, to avoid polluting the medium, transferring of fish early in the morning, prior to feed them. Then fish are gradually caught with a smooth net with special care to avoid direct touch and jumping or exposing of fish out of water. Finally, fish are weighed, placed in buckets of 50 l volume supplied with aeration and transferred to the weaning tanks, following acclimatising procedure to ensure that receiving abiotic conditions much those of the larval rearing tanks. When transfer completed fish should be fed to avoid cannibalism.

Fry transport

In pre-growth fry are raised from 1–2 g to 20–30 g (or in some areas even up to 60 g) in size. Fish are then transferred to the ongrowing installations. The most common way to transport fry is by special fibreglass tanks installed on trucks. Long distance transportation constructions are closed rectangular tanks with a volume of 1.6 to 2.5 m^{-3}, allowing 6 to 12 tanks on a vehicle. Short distance transportation can be performed in simplest round, flat bottom open tanks with a volume up to 1.5 m^{-3}. Stocking density for long transportation (2 to 3 days) does not usually exceed 25 kg m^{-3}. Few farms transfer fry in boats under continuous water exchange and at densities of 50 to 60 kg m^{-3}.

Transportation procedure includes starving of fish 24-48 h prior to transport, crowding of fish in the weaning tank, confinement, air exposure during transfer to the transportation tanks, monitoring of oxygen and pH, water renewal (for long transport), and transfer from the transportation tanks into the ongrowing installations (manually in buckets or automatically by the use of special pumps). An important precaution at arrival is to acclimatise fish to the receiving water rearing conditions (Moretti et al.1999, Pavlidis et al. 2003).

4.4.6. Sexing of brood stock

Sex ratio is an important factor for maximizing reproductive and spawning performance especially in the protandrous hermaphroditic gilthead seabream. Therefore, sexing of broodfish is a pre-requisite for optimum broodstock management. In most fish farms sexing is performed at the end of the spawning period by manual stripping. Males are identified or chosen when releasing sperm, and females when releasing eggs. The main disadvantages of the method are handling stress and the high incidence of fish releasing no gametes, *i.e.* high sexing uncertainty. Sex of fish may be assessed by urogenital catheterization biopsy, although this is an invasive stressfull procedure that may threaten fish health and reproductive performance (Martin-Robichaud and Rommens, 2001). Biopsies are taken by trained personnel from anaesthetized females using a flexible polyethylene sterile catheter (0.8 - 1.0 mm diameter), inserted through the genital pore to approximately the central portion of one of the gonads. The intra-gonad samples are then drawn by suction while the catheter is slowly withdrawn and are immediately examined under a phase contrast microscope. Sexing seabass broodstock is performed once during the whole life cycle, while sexing seabream adults has to be performed in males once per year due to possible sex change. The main disadvantage of this technique – besides handling stress – is the possibility of damage of the urogenital papilla area and/or gonadal injury in particular in seabream due to the presence of intersex gonads. A third method for sexing seabass is ultrasonogaphy (M. Pavlidis, personal communication), this method has been successfully used for other fish species (Martin-Robichaud and Rommens, 2001, Blythe, et al , 1994.) but has not yet been implemented by Mediterranean fish farmers for sexing fish. All sexing methods involve crowding (by closing water inlet, lowering the water level, dividing broodstock tank into two compartments by the use of a net barrier and keeping all fish in one compartment), netting, handling, and fish anaesthetisia. Anaesthetics are veterinary medicinal products and their administration should be done under veterinary supervision. There is only one anaesthetic licensed for use in fish for human consumption, MS222, which is considered less suitable than at least other unlicensed alternatives for seabass and seabream such as phenoxyethanol or clove oil (M. Pavlidis, personal communication).

4.4.7. Hormonal manipulation

All-year round spontaneous spawning of seabream and seabass broodfish is feasible, and the most common to achieve at an industrial scale, by the use of photoperiod and temperature manipulation. Induced breeding may be a choice of commercial seabass hatcheries for the synchronisation of gamete release and/or optimization of production. It can be also applied in both species for biotechnology (gametes preservation, hybridization) or genetic selection programmes. Induced breeding techniques include crowding, netting, handling, anaesthetising, and biopsy of fish. Following identification of sex and estimation of maturity stage, spermiated males and late- to post-vitellogenic stage females are selected and exposed to hormonal treatment. In the past the most common drugs used for induced spawning were the human chorionic gonadotrophin (hCG) and several analogues of the luteinising hormone-releasing hormone (LH-RH). Human CG has serious drawbacks (low female responsiveness, low egg quality and immunization reaction) and is to be replaced soon in commercial hatcheries by LH-RH. Technological progress resulted in the development of slow-release degradable polymers as vehicles for the administration of LH-RH analogues (Forniés et al., 2001). These methods proved to be more appropriate in multiple spawners including seabass and seabream. However, hormonal spawning induction, in the case of seabream, is not a common practice anymore while for seabass it is still in use by a small number of hatcheries for synchronisation of spawning (commercial experience). Proper training of personnel is essential to prevent physical damage of the broodstock. It has also to be mentioned that currently used products are not licensed to be used in EU and alternatives methods, based on temperature manipulation (abrupt changes within the spawning period), are becoming more the common practice. However these abrupt changes may result in stress leading either to mortality or reduced disease resistance in exposed fish. (see Section 4.1.1.).

4.4.8. Testing and sampling procedures for quality (swim bladder and deformities) and health monitoring

Larvae rearing quality control monitoring includes sampling at regular intervals of 30 to 50 larvae per tank and inspection under a stereoscope. X-rays are used to identify and characterised deformities and batch quality.

The importance of a proper swim bladder for a normal development and growth requires the sorting and elimination of specimens without swim bladder. As handling represents a considerable stress for fish testing for a functional swim bladder should be performed in coincidence with other grading or quality measuring controls. The method is based in the difference in buoyancy in hypersaline water; anaesthetized fish with functional swim bladder will float while the others will sink to the bottom (Moretti et al., 1999).

Quality control also affects juveniles. Sometimes some types of malformations are difficult to observe in larvae or early juveniles. Quality controls are usually more frequent in 0.5-2 g larvae, when fish are counted, graded and sometimes manually selected before they are grouped in the different shipment stocks to the ongrowing sites.

4.4.9. Larval deformities

During larval development anatomical deformities may occur in seabass and seabream larvae both in the wild and farm conditions. It is generally accepted that the overall malformation rate is higher in hatchery-reared than in wild larvae of seabass (Sola et al., 1998). The extent to which this reflects iatrogenic activity however cannot be measured and expert opinion is divided on this issue. Skeletal deformities affect splanchno-cranium, vertebral column, appendicular skeleton and the operculum. Spinal malformation, such as scoliosis and lordosis, missing or additional fin rays and pterygiophores, jaw deformities and fore-shortened operculum are the more frequently observed deformities in both species (Boglione et al., 1993). Most anomalies are recognizable from the very beginning of skeletal development (Marino et al., 1993), indicating that environmental, nutritional or genetic factors influence even the very early stages of larval development.

Larval development rate is a function of water temperature (Bertolini et al., 1991), and high temperatures during early larval development increased the frequency and gravity of larval deformities (Marino et al., 1991). Some dietary nutritional components also interfere with the normal larval development and affect larval quality. Dietary lipid fraction, mainly phospholipid concentration in live diets may increase spinal malformation rate (Cahu et al., 2003), whereas incorporation of 20 amino acids peptides in the diet leads to a reduction of spinal deformities. Among vitamins, diet containing an excess of vitamin A and polyunsaturated fatty acids (DHA, EPA) have been related to increased malformation rate affecting the cephalic region (Villeneuve et al., 2005).

Genetic factors along with environmental clues during larviculture seem to be involved in swim bladder anomalies, which mainly consist in the lack of primary inflation and swim bladder hyperinflation at later larval stages (Peruzzi et al., 2007). Larvae without swim bladders are characterised by shorter standard length, higher incidence of vertebrae anomalies and malformation of vertebral axis (Marino et al., 1993). However, vertebral column malformation, such as scoliosis and lordosis may also occur in larvae and fingerlings with functional swim bladders during the early weaning phase (Divanach et al., 1997) if water currents in tanks are higher than 10 cm s^{-1}. The first evidence of kyphosis deformities can be recorded in larvae 10 mm long in the form of light malformation or asymmetry of prehemal vertebrae (vertebrae 5 and 6). The maximum incidence is recorded in larvae 17-18 mm in length (Koumoundouros et al., 2002). Seabass larvae affected by malformation of the vertebral axis show lethargic behaviour and low swimming performance (Basaran et al., 2007). The incidence of the vertebral column deformity decreased over time due to the heavy mortality of the affected fish during metamorphosis (Koumoundouros et al., 2002). Lordosis significantly affects body shape in surviving seabass, particularly the posterior abdominal region (Sfakianakis et al., 2006).

Opercular deformities are the most commonly observed type of malformation, affecting up to 80% of the hatchery stocks (Barahona- Fernandes, 1982; Chatain, 1994; Verhaegen et al., 2007). The morphology and the biological performances of such fish are seriously affected, and under low oxygen levels, fish present lower resistance to death than normal fish (Paperna et al., 1978; Chatain, 1994). In intensively reared seabream larvae, the first evidence of malformation was observed at 6.1 mm total length (Koumondoros et al., 1997). The deformities were fully developed during metamorphosis (11-12 mm TL). Osteological analyses indicate severe cranial shifts, associated with twisting and folding of the operuculum and suboperculum (85.5%), but rarely with bone atrophy. Biometrical analyses demonstrate differences in fish size between de-operculated and normal fish, even though fish allometry was not different (Verhaegen et al., 2007). Opercular deformities are unilateral (81.4%) with similar right/left frequency (fluctuant asymmetry, Koumondoros et al., 1997). Recent studies suggested that opercular deformities may be reversible. Regeneration, however, proceeds in different ways in relation to the anatomical structure involved, and is not yet completed after 9 months rearing (Beraldo et al., 2003).

The literature on the causes of opercular deformities is contradictory. Vitamin deficiency syndrome (especially vitamin C) with hyperventilation (increase of operculum movements) associated with high feeding rates under intensive rearing conditions, unfavourable abiotic parameters and pollution have all been proposed as possible causes. The determinism of opercular deformities could be a multi-factorial with nutritional, metabolic, environmental and behavioural causes (Koumoundoros et al., 1997).

4.4.10. Mortality

Mortalities of fish during the production cycle can result from a variety of causes, including, disease, damage, predation and adverse environmental conditions. The new Fish Health Directive 2006/88 to be implemented by the Member States in 2008 contains obligations to report "increased mortality", while this is defined by the directive, most farms do not currently have any formal means to differentiate between expected mortalities and unexpected or increased mortalities. At present there is limited data on what the levels of mortality are in the various farming systems or what levels of mortality might be considered acceptable for the various farming systems and life stages. Very poor welfare (e.g. disease, poor growth and high mortality) is not cost-effective for the farmer, so even the farms that have relatively poor fish welfare have found a balance between welfare and productivity. However, in many cases high or increasing mortalities are an indication of disease and husbandry problems with serious economic and welfare implications. At present many farms rely on the experience of the farm manager to decide when mortalities require additional action. This is not a simple task since mortalities vary over time depending on a variety of factors such as life stage, temperature, farming system, presence of endemic diseases, etc.

4.5. Genetic selection impact on welfare

Domestication is defined as a process by which an animal population becomes adapted to humans and to the captive environment by genetic changes occurring over generations and environmentally-induced developmental events reoccurring at each generation (Price, 1984). In this sense, domestication can be viewed as both an evolutionary process and a developmental phenomenon (Price, 1998). One commonly used approach in studying the effects of domestication is to compare wild and domestic stocks of a given species (Desforges and Wood-Gush, 1976; Boice, 1980; Price, 1980). However, within the context of fish culture little is known about the influence of fish domestication and/or selection on behaviour and adaptation. In marine fish, selection has been applied only recently (one or two generations) and growth has been the major trait of interest. In particular, the European seabass and gilthead seabream industry has been based on empirical criteria for genetic selection and systematic genetic improvement programmes have only recently been implemented. Large scale, family based selection programmes started in Greece in 2002 and 2004 for seabream and seabass, respectively (Thorhald et al., 2007). The breeding animals were collected from locations in Greece and elsewhere, and 50 full- and half-sib families were produced and performance tested annually. Re-use of a limited number of breeders in subsequent year-classes allows for testing approximately 150 families per generation. The first genetically improved eggs were marketed in 2005. Selective breeding programmes are also carried out in France (Sola et al. 2007).

The effect of the genetically improved livestock on growth rate, incidence of deformities and external pigmentation is under validation and data are not yet available. Research of criteria that could characterize coping abilities and stress tolerance is essential to understand if domesticated fish adapt to their rearing environment and if breeders have a high welfare potential. Research is also necessary on determining if genetic selection for one trait could affect other life history traits and have a negative welfare impact. This research area still is in its infancy but such features will be even more important to select fish for breeding programmes which is an unavoidable activity in animal production. An initial study was set up to investigate differences in risk-taking behaviour of European seabass coming from wild breeders and from breeders selected for growth (Millot et al., submitted). Individual learning, memory and social learning abilities were also measured in seabass and it was found that fish displayed different potentials when they came from wild or selected breeders. Fish selected for growth showed slower learning but this remained constant over time and so could be a benefit for a selected strain which may potentially be less sensitive to different stressors and consequently better adapted to the changes in their rearing environment..

4.6. Impact of disease on welfare

Disease in farmed fish is generally closely linked with the husbandry and environmental conditions under which the fish are being reared and many pathogens are ubiquitous in the environment or in the fish's tissues but only manifest themselves in a clinical fashion if husbandry or environmental parameters facilitate their establishment. Thus although clinical disease can usually be considered as a welfare issue in its own right, it is also generally an indicator of an underlying husbandry or environmental deficiency. As with abiotic and biotic factors, disease may be a result of several interacting factors. An initial infection may weaken or compromise the host, leading on to secondary and tertiary infections. Infectious diseases may involve obligate pathogens that, except during transmission, infect a host, or facultative pathogens which are normally free-living, but which become pathogenic if the host is compromised. Disease susceptibility may also vary with the life-stage of the host. Non-infectious diseases may also occur.

The present report does not attempt to cover all diseases of seabream and seabass but instead will consider several diseases that may have important implications in terms of welfare at some stages of the production cycle in order to serve as examples of the ways in which disease can impact welfare. The following diseases are considered to be of particular significance because of their: i) severity of effect on physiological integrity of fish, ii) known frequency of occurrence in farming systems and iii) impact of preventive and/or curative measures.

4.6.1. Viral Nervous Necrosis (VNN) / Viral encephalopathy and retinopathy (VER) / Nodavirus infection

VNN is a viral disease caused by a Betanodavirus. Four types of the virus (SJNNV, RGNNV, BFNNV and TPNNV) have been described so far (Nishizawa et al., 1997) and from recent epidemiological studies seem that RGNNV and also SJNNV are the prevalent types in the Mediterranean area (Cutrin et al., 2007). It affects a large number of species and also can be detected in a wide number of species from the wild (Gomez et al., 2004). It can be found in

Europe, Asia, North America and Australia. In Mediterranean aquaculture, seabass is the main affected species although outbreaks have also been described in the Sciaenids family. The virus has also been isolated from seabream as a potential carrier status and from also can affect seabream larvae. The disease is in the list of the OIE notifiable diseases (OIE, 2003) but it is not included in the list of non-exotic diseases in Council Directive 2006/88/EU.

It affects mainly seabass larvae and juveniles where the highest mortality rates are described but can also affect ongrowing fish. The disease mainly affects the central nervous system and the retina and this is the reason because the main clinical signs include erratic swimming behaviour, with spasmodic, spiral and whirling swimming. In chronic forms, due to the lesions in the visual areas of the brain and in the retina, the fish becomes blind and usually bumps into the nets and cage structures, inducing severe corneal lesions with subsequent severe panophthalmitis and also frequently skin wounds. In some cases, swim bladder hyperinflation is also observed and affected fish are observed floating near the water surface. Nodavirus is highly neurotropic and produces vacuolar neuronal degeneration in different areas of the brain (medulla oblongata, hypothalamus, cerebellum, tectum opticum) together with gliosis and marked vascular congestion. It also affects the nuclear layers of the retina.

In acute and per-acute disease (usually seen in seabass larvae and post-larvae) mortalities can be up to 100%. In juvenile fish, acute forms typically can affect 40-50% of the stock and in ongrowing fish 10-25% mortality in the sub-acute or chronic forms are not unusual. In this case, affected moribund fish severely damaged and can be observed in the fish cages for several days. These fish weak are prone to associated parasitic infections for a long period.

Nodavirus infection in seabass usually develops at temperatures between 22-25 ºC (summer and early autumn) but outbreaks (usually not as virulent as at high temperatures) can also be detected at lower temperatures. Incubation period may vary from 4 to 30 days depending on the age and water temperature. Fish that survive seem to develop some immunity to re-infection in the following years, but can also become carriers.

Although some vaccine patents have been registered, no commercial vaccines are available. Experience with experimental vaccines in turbot and halibut indicate that protection levels achieved with these vaccines are still not satisfactory (Husgaro et al, 2001; Sommerset et al., 2005). No treatment is available.

Preventive measures for the control of the disease should be applied in hatcheries as a first step. Broodstock testing for Nodavirus carriers either by indirect ELISA method (Breuil and Romestand 1999) or RT–PCR (Password, F. 2001), 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. In cages, special cautions (distance, water flows, cage grouping, stock densities, not introducing very young fish during the period of high water temperatures) can be taken to prevent the transmission of the virus from the cages with +1 and +2 fish (if they are potential carriers) to the fry and juvenile fish. Control of fish from the wild can be also a preventive measure, but usually this is very difficult when fish are reared in cages. Mortality management is critical in the control of the disease. Dead or moribund fish should be immediately removed from the affected cages or ponds and safely disposed.

4.6.2. Monogenean infections

Monogenean parasites (skin and gill flukes) are a common and widespread problem in aquaculture and also for seabream and seabass farming. Seabass are mainly affected by Dipectanid species (mainly by *Diplectanum aequans* but also by *Diplectanum laubieri*) and in seabream the most problematic species is *Sparicotyle* (*Microcotyle*) *chrisophri* (Faisal and Imam, 1990), although *Furnestinia echeneis* is also usually detected in seabream farms. Other monogenean species can occasionally be found in theses two fish species but without a significant detrimental effect.

The pathological effect of these parasite species is mainly due to the attachment system of the adults of these species to the gills and also is due to the feeding activity (grazing and ingesting mucus and epithelial cells and/or by its haematophagous activity). *Diplectanum* species attach tightly to the gills through its specialised haptor with scales and hooks, and provoke a marked inflammatory reaction in the adjacent area of the gill (Dezfuli et al., 2007). *Sparicotyle* has a large number of clamps in its haptor and the attachment of the parasite is made by nipping single or groups of two gill lamellae. This attachment provokes damage on these lamellae and triggers a severe epithelial hyperplasia and inflammatory response around the area of the attachment. The final effects on the seabream or the seabass depend primarily on the number of the parasites attached on the gills and this is related to the size of the fish and the total respiratory surface. In juvenile fish, a lower number of parasites present on the gills can generate the same problems as in bigger fish with a higher number of parasites. In addition to the inflammatory response in the gills, the effect of the reduction of the respiratory surface area, the gill damage, and the effect of the parasites' feeding activity on the fish gill add to the severity of the disease. In addition the damaged tissue can facilitate the entrance of other pathogens (mainly bacteria and virus) i.e. a secondary external bacterial infection, mainly by *Tenacibaculum maritimum* but by Vibrionaceae. These bacteria gradually expand into the surrounding areas of the gills, leading to extensive necrotic lesions, causing a deterioration in the fish health for a long period of time.

One of the most important points in the epidemiology and the control of this disease is the life cycle of these parasites. All of them are oviparous and the eggs have attachment structures similar to tendrils. These structures allow the eggs to attach in the gills but also in other substrates, mainly in the fouling of the nets, cage structures, ropes and tank walls. From these eggs, the oncomiracidium, a swimming larva, hatches and infest new fish. If these eggs are not regularly and efficiently eliminated (net replacements, net cleaning) several parasite cycles overlap after few weeks, increasing in an exponential way the number of parasites within the fish gills. Sequential formalin or hydrogen peroxide (100-300 ppm) treatments have been traditionally used to treat the adults and the juvenile parasites in the fish (in cages using tarpaulins), but the eggs seem to be resistant to these treatments so fish easily become reinfested if eggs are not removed before or immediately after the treatment (Cecchini and Cognetti-Varriale, 2003). The development of the life cycle of these parasites is temperature-dependent (Cecchini, 1994, 1998).

Some oral treatments using classic antiparasitic products such as Praziquantel, Mebendazol and Bithionol have been tested, with limited success (Sitjà-Bobadilla et al., 2006). Some natural products such as caprilic acid and also allicin and rosemary oil seem to have some effect. The presence of the same parasites in wild fish and the potential transmission and reinfestation should not be underestimated (González-Lanza et al., 1991).

4.6.3. Winter syndrome

The so-called Winter Syndrome (WS) refers to a specific condition affecting exclusively seabream reared at low temperatures. The problem was described in the late 1980s and early 90s (Bovo, 1995). It is a multifactorial problem associated to the physiological and immunological disturbances due to the low tolerance of this fish species to commercial rearing conditions at low (below 15 ºC) temperatures (Padrós et al., 1996, Tort et al., 2004).

This condition is usually observed in seabream reared in cages or ponds in northern areas of the Mediterranean and during winter months where water temperatures can drop between 8 and 15ºC for several months. The disease mainly affects fish up to 100 g. Affected fish show a lethargic swimming and usually lie down by on the sides for several days. In ponds, affected fish are darker and present the typical "stress bands" (i.e. transversal bands of darker skin coloration in the fish body). In cages fish usually present a distended abdomen sometimes slightly haemorrhagic and corneal cloudiness is occasionally observed. These affected fish usually shows other secondary problems associated, such as monogenean infection and also necrotic lesions associated with *Tenacibaculum maritimum*. Fish are also anaemic. During the necropsy, hepatic pallor and an extreme distension of the intestines, filled with a watery fluid and mucous casts are very characteristic of WS. The main histopathological findings are: atrophy of the exocrine pancreas, hepatocyte degeneration, massive infiltration of the adipose tissue by eosinophylic granular cells, hyperplasia of mucous cells in the intestinal epithelium, and severe degeneration of white muscle fibres (Tort et al., 1998).

There are two forms of WS. The first form (called "first winter") typically takes place in December-March, with low but constant mortalities. The second one (called "second winter") appears during early spring, when temperatures rise to 15-16ºC. At this time, more sudden deaths are seen, sometimes up to 20-30% of the stock and in these cases *Pseudomonas anguiliseptica* can be frequently isolated (Berthe et al., 1995, Domenech et al., 1997; Domenech et al., 1999) usually from the brain associated with meningitis and encephalitis.

Some haematological and biochemical studies on plasma from the affected fish showed a drastic decrease of the haematocrit, alteration on the plasma:protein ratio, decrease of the free amino acids, increase of GOT, and decrease of the ATPase activity and the potassium levels (Gallardo et al., 2003). Cortisol levels are also increased and there is a decrease in circulating lymphocytes, lysozyme, complement activity and macrophage activity. Some studies have been done to test the physiological performance of seabream at low temperatures (Sala-Rabanal, 2003) and some results seem to mimic some of the physiological and immunological disturbances seen in the diseased fish. The same studies indicate that seabream stop feeding at 13ºC and at low temperatures fish cannot metabolise fat properly (Ibarz et al., 2007). Moreover, it has been observed that the extreme mobilisation of fat from the adipose tissue in the perivisceral and subcutaneous tissue due to the water temperature drop causes severe disturbances in liver metabolism. For this reason, it has been recommended to reduce progressively high-energy feeds (25-30% fat) during October and November in order to reduce the amount of fat reserves in the fish, to use low energy highly digestible diets supplemented with vitamins and immunostimulatory products to avoid feeding and reduce handling when temperatures are below 15 ºC.

4.6.4. Vibriosis

Vibriosis is one the most common bacterial problems in seabream and seabass farming but also in a wide range of fish species. In seabass, *Listonella* (*Vibrio*) *anguillarum* is probably the most common problem in hatcheries, in pre-ongrowing and ongrowing systems. In seabream, *L. anguillarum* infections are not so common. Other vibrioses such as *Vibrio alginolyticus* or *Vibrio harveyi* are relatively frequent in both fish species but usually these infections are stress-related problems or secondary infections associated with another pathogen. Outbreaks are typically associated with water temperature changes and in ongrowing conditions are usually seen in spring or autumn and usually last 8-15 days. Vibriosis can cause septicaemia and also can affect skin (haemorrhages and ulcers) and gills. Vibriosis outbreaks can be controlled by using broad-spectrum antibiotics (Oxytetracycline, Flumequine etc) but can be effectively controlled by immunoprophylaxis, however the use of vaccination depends on disease incidence in the farming area. Licensed Vibrio vaccines (mainly against *L. anguillarum*) are available in most Mediterranean countries, with acceptable efficacy. Double bath vaccination (first vaccination and booster) in 1-5 gram fish induces 6-7 months immunity. Intraperitoneal or intramuscular vaccination can be used for longer protection periods. Vibriosis can be a serious problem in hatcheries due to the fact that protection can only be achieved after vaccination at 2-3 grams.

4.6.5. Pasteurellosis

Pasteurellosis due to *Photobacterium damsela piscicida* is a common bacterial problem in both seabream and seabass in ongrowing conditions but it mainly affects seabream in the hatchery and nursery. The disease is expressed usually in the septicaemic form but can also develop into a chronic form. Outbreaks are observed in summer and autumn, usually at temperatures >21 ºC. The treatment is similar to that for vibriosis. In case of an outbreak, the disease is usually controlled by the use of approved antibiotics given with the feed. There are also available licensed vaccines against pasteurellosis but their efficacy is lower and immunity is shorter (3-4 months after double bath vaccination). With IP vaccination immunity can be slightly higher. Mortalities in hatchery or nursery can reach 50-60% in the most severe cases but in ongrowing conditions, cumulative mortalities can reach 5-20%.

4.6.6. Lymphocystis

Lymphocystis is a viral and self-limiting disease due to an Iridovirus that causes a relatively benign external disease in seabream usually in juveniles (2-15 grams). The infection does not affect seabass. The disease is characterized by the development of whitish nodules corresponding to massive hypertrophied fibroblasts in the fish skin that proliferate, conflux and form cauliflower-like structures. These structures (lymphocysts) spontaneously revert after 40-60 days (according to the water temperature). Although morbidity can be very high, mortality is usually low or very low, but can be a problem if fish are not appropriately managed during this period. This is due to secondary bacterial infections if the skin is damaged. During the period when cysts are present, fish can show abnormal swimming behaviour that can easily be induced by high production densities or by manipulations such as grading or counting (skin abrasion). Such stressors can delay the complete recovery of the fish.

4.7. Disease Control Measures

No veterinary medicinal products (VMP) are specifically authorized for seabream and seabass. This is mainly due to the low economic benefits for the pharmaceutical companies due to the high investment needed to authorise these products and the relative low return. Very few of these substances have been licensed specifically for fish so routinely, licensed products for other animal species (poultry, pigs, etc) are prescribed in accordance with the so-called "cascade" mechanism (Directive 2001/82/EC as amended), that allows the use of VMPs for other animal species when no licensed commercial products for that species is available. The VMPs more commonly used in Seabream and Seabass include active substances cited in Annex I (substances with maximum residue limits (MRL) for certain animal species) or Annex II (substances for which it is not considered necessary for the protection of public health to establish MRL values) of Council Regulation (EEC) No 2377/90. Very few substances in Annex I have specific MRLs for fish, so veterinary prescriptions of these products have to be done using the maximum withdrawal period as cited in Article 11 of Directive 2001/82/EC as amended. Antibiotics are usually administered as medicated feed and are produced by the fish feed companies. In hatcheries, due to the relative low biomasses and the specific characteristics of these facilities, antibiotics prescribed by veterinary practicioner are manually mixed together with fish feed and also occasionally administered by baths. Formalin and hydrogen peroxide are often used to control the bacterial load and parasites in hatchery tanks.

Table 8. **Authorized VMPs for seabass and seabream**

	Antibacterial	Antiparasitic	Antifungal	Anaesthetics	Others
GREECE	Oxytetracycline Oxolinic acid Trimethoprime-Sulphadiazine Amoxycillin	None	None	None	AQUAVAC Ergosan (all fish species)
ITALY	Oxytetracycline Clortetracyline Amoxycillin Flumequine Trimethoprim- Sulphadiazine (only salmon)	Bronopol 2-bromo-2-nitropropane-1,3-diol		Tricaine methanesulphonate (only for vaccination and research use)	AQUAVAC Ergosan (all fish species)
SPAIN	Oxytetracycline Amoxycillin Flumequine Trimethoprim- Sulphadiazine Florfenicol	Formalin (bath) Hydrogen peroxide (bath)	None	Benzocaine Tricaine methanesulphonate	

Source: Data collected by questionnaire at the consultation meeting (EFSA advisory forum and stakeholders representatives) on Animal Welfare aspects of Husbandry Systems for Farmed Fish held on 4 March 2008 in Parma.

4.7.1. Anaesthetics

The most common used anaesthetics are 2-phenoxyethanol (200-300 ppm), quinaldine dissolved in acetone (3-5 ppm) and MS 222 (20-50 ppm).

Annex II of Council Regulation (EEC) No 2377/90, includes the substances for which it is not considered necessary for the protection of public health to establish MRL values. The use of these substances is allowed for food producing species if the animal species is identified and its use is in accordance with the conditions set out, if any (e.g. specific route of administration). It should be noted that an entry in Annex II is not equivalent to the status “generally recognised as safe”. In fact only a sub-group of Annex II substances do fall under this category. The following fish anaesthetic products can be found in Annex II

- Benzocaine: but only for salmonids
- Tricaine mesilate / Tricaine methanosulphonate (MS-222). There is a licensed product (Alpharma MS-222)

Although practical experience shows that 2-phenoxyethanol, is one of the most common anaesthetics currently used in fish farming, it is not included in Annexes I, II or III in Council Regulation (EEC) No 2377/90 and hence is not allowed. Clove oil, a natural product obtained ed by distillation of the flowers, stems and leaves of the clove tree, can be used as an effective anaesthetic in seabream and seabass at almost 10-fold lower doses than 2-phenoxyethanol (Mylonas et al. 2005) but this substance is not authorized for use in aquaculture.

4.7.2. Vaccination

As in other fish species, vaccination has become essential for the prevention and control of several infectious diseases and the general considerations for the use of vaccines in seabass and seabream are the same as for salmonids and other fish species. However, some differences need to be taken into account. One main difference is that during larval development, seabass and seabream do not have a well-developed immune system and consequently (Dos Santos et al., 2000), fish can not be effectively vaccinated until they have around 1 g, although some authors claim that a partial immunity can be achieved in younger fish. Therefore, during the larval and weaning periods good prophylaxis and the use of chemical therapeutics are the only systems to prevent infectious disease.

Vaccines used in these two species are killed vaccines (bacterines) (Table 9) and most of them are applied in a bath (dip), although some of them can be given by intra-peritoneal injection. Dip vaccination is routinely used to protect juveniles for a relatively short period (4 months for pasteurellosis and 6-8 months for vibriosis). Sometimes this is enough to reduce the outbreaks as these two diseases affect mainly juveniles. However, in some farms, due to specific conditions (temperature, infection pressure) dip vaccination does not protect at a suitable level and in these cases IP injection is indicated.

Dip vaccination

For vaccination, juveniles are concentrated and confined in one part of a tank using nets or gratings and by lowering the water level. In this procedure, additional oxygen supply is paramount. Usually the fish have been fasted for 12 hours. In some farms, fish are slightly sedated before proceeding with the vaccination but if the vaccination process is done quickly usually it is not necessary to sedate the fish. Fish are then immersed in a bucket or other type of container with the vaccine using a 1:10 dilution in clear water at the same temperature as the tank. Oxygen is also sometimes added in the container. Special care is taken to avoid putting too many juveniles in the net each time. Fish are immersed for 30 seconds (dip) and immediately released into a fresh tank for recovery. Used vaccination solution is changed each time for a fresh solution after 100 kg of juveniles have been vaccinated.

IP vaccination

Vaccination by injection is usually intraperitoneal. Fish should be big enough (>40-50 grams) and vaccination is done most of the times manually. The IP vaccination procedure includes previous crowding and sedation procedures as in dip vaccination, although in this case the biomass is much larger. Seabass (*Dicentrarchus labrax* L.) injected intraperitoneally with monovalent (*Photobacterium damselae* subsp. *piscicida* or *Vibrio anguillarum*) and divalent (*Ph. damselae* subsp. *piscicida* and *V. anguillarum*) vaccine formulations, with or without adjuvants (mineral oil, liposome or alginate), had moderate lesions, indicating that in seabass, the pathological effects due to intraperitoneally injected vaccines are less severe than in other fish species. The divalent oil adjuvanted vaccine induced the most severe side effects, with macroscopic granulomas consistently present up to 11 months (Afonso et al. , 2005). The two registered vaccines for seabass and seabream (see Table 9) that can also be injected are water-based. Some autologous vaccines may be formulated including oil and other adjuvants but it constitutes a very small percentage of the vaccines used in these species therefore the problems related to adhesions and melanisation are much less observed than in salmon and trout (F. Padrós, pers. com.).

Table 9. **Authorized Vaccines for seabass and seabream**

	Vibriosis	Pasteurelosis	Vibriosis /Pasteurellosis
GREECE ITALY	Aquavac Vibrio (Vibriosis immersion, Injection – water based excipient)	Aquavac Photobac Prime (Pasteurellosis Immersion)	AQUAVAC Vibrio Pasteurella (Double injection vaccine - water based excipient)
GREECE	AQUAVAC Vibrio Oral (Vibriosis Oral administration)	AQUAVAC Photobac Boost (Pasteurellosis Oral administration)	ALPHA Dip (Double Immersion Vaccine)
GREECE	Vibrogen (Vibriosis immersion, Injection – water based excipient)		ALPHA JECT (Double Injection Vaccine – oil based excipient)
GREECE ITALY	VIBRI-FISHVAX (Vibriosis immersion, Injection – water based excipient)		
GREECE	Norvax® Vibrio (Vibriosis immersion, Injection – water based excipient)		

SPAIN	*Aquavac Vibrio* .Licensed only salmonids but used also for seabass. *Aquavac Vibrio Oral* for Vibriosis. Licensed for salmonids.	*Aquavac Photobacterium* Inactivaded bath vaccine for Pasteurelosis. *Aquavac Photobacterium Oral.* Inactivaded oral vaccine for Pasteurellosis.
SPAIN*	ICTHIOVAC-VR –Vibriosis water based excipient.Immersion. Licensed for turbot, but used also for seabream and seabass.	ICTHIOVAC-PD- Pasteurellosis water based excipient Immersion Licensed for seabream, but used usually also for seabass.

*Autologous / autovaccines are also frequently used in Spain

4.7.3. Biosecurity

A biosecurity plan in accordance to the World Organization for Animal Health Aquatic Animal Health Code (OIE 2007) means a plan that identifies significant potential pathways for the introduction and spread of disease in a zone or compartment, and describes the measures which are being, or will be, applied to mitigate the risks to introduce and spread disease, taking into consideration the recommendations in the Aquatic Code. The plan should also describe how these measures are audited, with respect to both their implementation and their targeting, to ensure that the risks are regularly re-assessed and the measures adjusted accordingly. Council directive 2006/88/EC on animal health requirements for aquaculture animals and the prevention and control of certain diseases determines the general framework for prevention and spread of listed diseases. Seabream and seabass are not included in the list susceptible species of any of the listed diseases (Annex II CD/2006/88) but the general principles of surveillance are applied to this type of aquaculture.

Health biosecurity in seabass and seabream production are usually designed to minimize the risk in the different types of facilities. Biosecurity measures tend to be stricter in hatcheries but more difficult to apply in pre-ongrowing and ongrowing facilities, speacially if they operate in cages or flow-through systems. Recirculation systems allow for stricter biosecurity measures. Biosecurity measures include water treatment, husbandry hygiene control of movements of i) fish, ii) materials used in the farm and iii) staff.

5. Risk Assessment approach to welfare of seabass and seabream

5.1. Introduction

Animal welfare problems are generally the consequence of animal environment changes resulting from management or production factors as well as environmental, genetic and disease factors and interactions thereof. Presently there are not any standards for animal welfare risk assessment, but previous studies exist where risk assessment for animal welfare has been explored (Anonymous, 2001; EFSA 2006). In this section, the application of risk assessment to the study of the welfare for seabass and seabream is described.

Risk assessment is a systematic, scientific-based process to estimate the magnitude of and exposure to a hazard and includes 4 steps: hazard identification; hazard characterisation; exposure assessment and risk characterisation. In food risk assessment terminology (*Codex alimentarius*), a hazard is a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect. The risk is a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food. Making a parallel to the *Codex alimentarius* risk assessment methodology, a hazard in animal welfare risk assessment is a factor with a potential to cause a negative animal welfare effect (adverse effect). A risk in animal welfare is a function of the probability of occurrence and the consequences of occurrence. Four parameters were scored to assess the importance of a hazard:

1. the probability of a given target population to be exposed to a particular hazard has been scored as <u>frequency of occurrence of the hazard</u>;
2. the <u>proportion of the population affected</u>;
3. the consequences of exposure have been scored by <u>severity of the effect</u> in the individual; and
4. the <u>duration of the effect</u>.

Mortality was additionally scored to provide more information on the consequences of the hazard

While hazards usually relate to negative welfare impacts, the risk assessment approach could be also extended to include positive welfare consequences (resulting in risk-benefit analysis). Factors which may result in improved welfare were not considered in this analysis.

The degree of confidence in the final estimation of risk depends on the <u>uncertainty</u> and variability. Information obtained from epidemiological, experimental, and laboratory animal studies have a level of uncertainty, related to, *inter alia*, the number of animals used, frequency of observations and the test characteristics. Uncertainty is increased when results are extrapolated from one situation to another (e.g. from experimental to field situations). Uncertainty also arises from incomplete knowledge. Uncertainty can be evaluated (and reduced) by carrying out further studies to obtain the necessary data or quasi-formally by using expert opinion or by simply making a judgment. Variability is a biological phenomenon (inherent dispersion) and is not reducible. The importance of welfare hazards will inevitably vary between farms and countries and over time. Reduction in variability is not an improvement in knowledge but instead reflects a loss of information. However, it is not always easy to separate variability from uncertainty. Uncertainty combined with variability is generally referred as total uncertainty.

The methodology described in this section is based in the approach used in previous EFSA scientific opinions on pig welfare (EFSA, 2007a, b, c) and the welfare of fish (salmon, trout, carp and eel). However, due to the number life stages of common seabass/bream in production systems, some changes were made. The final risk score used duration of the effect of the hazard, while in the previous reports duration of the hazard and duration of effect were used. In the present report, duration of the effect was scored as a proportion of the time span of the life stage under consideration (e.g. if the life stage lasts 60 days and the effect of the hazard persists for 10 days the score was 1/6). In the previous fish welfare reports the denominator used was the total remaining life potential lifespan after the hazard occurred. It is therefore not appropriate to use the final risk scores to make comparisons of the importance of hazards between species.

5.2. Steps of the Risk Assessment

5.2.1. Definition of the life stages and production systems and Hazard Identification

For the risk assessment of welfare of seabass and seabream the different production systems, as well as the different life stages were identified.

The life stages considered in the risk assessment were eggs, larvae, juveniles, ongrowers and broodstock. The different production systems vary depending on the life stage and are summarized in Table 3 (page 25).

A list of potential hazards to fish welfare and health of seabass and seabream was drawn up. A hazard table was built for each combination of life stage production system considered. A total of 12 hazard tables were considered for scoring. The list of potential hazards affecting each of the combination life stage production system was established and agreed by the working group. The main reason why the identified hazard may adversely affect welfare is noted in the ‘effect’ column (see hazard tables in Appendix A).

Different factors that may affect the welfare of farmed fish are for example, water temperature and stocking density. These factors may also more broadly be described as conditions that may have a direct impact on the welfare and health of fish. Subsequently, hazard (a detrimental factor) identification (clinical signs and physiological changes), its character, and the consequences of it occurring, are all important issues to be taken into account when making a risk assessment.

Different hazards may directly affect an animal or indirectly by changing the animals’ environment so that their ability to fulfil their basic needs is diminished, which can also lead to animal welfare problems. Since production factors can interact and welfare problems are generally due to multiple exposures to different factors, any positive or negative interactions with other factors should be reviewed. The applied RA methodology only allows concentrating on single factors without taking account the multi-factorial effect and the interactions between factors.

5.2.2. Hazard Characterisation

The objective of this step is to describe the consequences of the adverse effect in terms of duration and severity and also likelihood of effect. Severity was scored from 1 to 4 based on the guidance in Table 10. The severity score should be consistent with the description of the effect of the hazard.

Table 10. **Severity of the adverse effect.**

Evaluation	Score	Explanation
Limited	1	No or limited pain, malaise, frustration, fear or anxiety as evidenced by measures of the normal range of behavioural observations, physiological measures and clinical signs for >95% of the species or strain/breed
Moderate	2	Moderate changes from normality and indicative of pain, malaise, fear or anxiety
Severe	3	Substantial changes from normality and indicative of pain, malaise, fear or anxiety.
Very severe	4	Extreme changes from normality and indicative of pain, malaise, fear or anxiety, that if persist would be incompatible with life.

The proportion of the populations affected by the hazard should also be scored (Table 11). The entire population may be exposed to hazards associated with water quality parameters; however, not all the population will be affected, i.e. exhibit signs consistent with the description of the effect of the hazard.

Table 11. **Proportion of the population affected (likelihood of effect)**

Evaluation	Score	% of population affected by the hazard
Very low	1	1-20
Low	2	21-40
Moderate	3	41-60
High	4	61-80
Very High	5	81-100

In relation to the duration of the adverse welfare effect on the fish, it may, in some cases, only last for the period that the fish is exposed to the hazard. In other cases the impact of the hazard may last for the remainder of the fish’s life (e.g. low oxygen during the early life stages causing a deformity). The total length of time in days that the effect of the hazard will last in seabass and seabream was estimated in the duration column.

5.2.3. Exposure Assessment

The objectives of this step is to assess the probability that the hazard occurs during the particular life stage in the considered system.The probability that the hazard occurs should be scored from 1-5 based on the description of the probability

Table 12. **Probability that the hazard occurs (frequency of exposure)**

Evaluation	Score	Explanation
Extremely low	1	The hazard would be extremely unlikely to occur
Very low	2	The hazard would be very unlikely to occur
Low	3	The hazard would be unlikely to occur
Moderate	4	The hazard would occur with an even probability
High	5	The hazard would be very likely to occur

5.2.4. Uncertainty

The uncertainty value is an indication of the type of information available, that is whether there are different studies with differing conclusions, but also whether scientific information, published or unpublished, is available. A single uncertainty score (low, medium and high) gives the overall uncertainty of all the parameters (Table 13).

Table 13. **Uncertainty score**

Score	Evaluation	Explanation
1	**Low**	**Solid and complete data available; strong evidence in multiple references with most authors coming to the same conclusions**
2	**Medium**	**Some or only incomplete data available; evidence provided in small number of references; authors' conclusions vary from one to the other;** **Solid and complete data available from other species which can be extrapolated to the species considered**
3	**Max**	**Scarce or no data available; evidence provided in unpublished reports, or based on observation or personal communications; authors' conclusions vary considerably between them**

5.2.5. Mortality

The percentages of the individuals affected by the hazard which are likely to die as a result of exposure to the hazard were scored (Table 14). This parameter is not used in the risk estimate calculation.

Table 14. **Mortality score**

Evaluation	Score	% of population
Very low	1	1-20
Low	2	21-40
Moderate	3	41-60
High	4	61-80
Very High	5	81-100

5.2.6. Scoring process and calculation of the risk score

Experts were asked individually to complete the hazard tables. In general, a single expert initially completed tables for groups of hazards, and the results discussed and adjusted during a group discussion. The scoring was based on current scientific knowledge, published data, field observation and experience of seabass bream farming. Final scores were agreed by all WG members.

The methodology used assumes the linearity of the severity scores (2 days suffering from a score 2 effect is equivalent as 1 day suffering from a score 4 effect), the no interactions between the hazards and that the hazards are mutually exclusive. Because the previous assumptions are not verified the risk scores have to be interpreted with caution. Secondly, the risk scoring is semi-quantitative. Thus the scores allow a ranking but do not give meaningful absolute figures (e.g. a risk score of 12 should not interpreted as being twice as important as a hazard with a score of 6).

The severity and duration of the effect of the hazard, the probability of its occurrence and the proportion of the population affected are equally weighted in calculating the final risk score.

The score for each parameter is standardised by dividing the maximum possible score. The overall score is the product of the standardised scores:

[Probability that the hazard occurs /5] * [Proportion of the population affected/5] * [Severity of adverse effect /4] * [Duration of effect of the hazard/Duration of the hazard with the longest duration]

The risk scores within each life-stage have been ranked and tabulated with the uncertainty score and mortality score in the summary tables and graphs (see Appendix A). The uncertainty score gives an indication of the robustness of the ranking.

The risk assessment allows the ranking of hazards in order of importance to each life stage and allows the comparison between the same hazards among different production systems.

The score may also be used to highlight areas where further research is needed to improve the certainty of the scientific data.

5.3. Risk Assessment Discussion and Draft Conclusions

5.3.1. Eggs

In this life stage, in both flow-through and recirculated production systems all hazards occurred with low probability (scoring 1 or 2). Hazards considered at the egg stage were important because of embryonic abnormalities, which may persist through later life stages. It should be taken into consideration that welfare *per se* is not relevant at this life stage.

- Flow-through (Appedix A, Table 1)

In the flow-through system the highest ranked risks were sudden change in temperature and inappropriate transport between units, which would cause significant mortality, followed by low water renewal and a sudden change in dissolved oxygen. The high score for inappropriate transport was mainly attributable to the fact that a high proportion of the population was affected. The sudden change in temperature has the highest severity score.

- Recirculation (Appendix A, Table 2)

Three hazards stood out in the recirculation system: low water renewal, inappropriate transport and sudden change in temperature.
A range of deformities will arise from the listed hazards, some of which may result in mortality after days, others will resolve in time.

5.3.2. Larvae

- Flow-through and Recirculation systems (Appendix A, Table 3 and Table 4)

The highest ranked hazards (in ranked order) for larvae in flow-through and recirculation systems were:

1. Inadequate stocking density
2. Inappropriate water velocity
3. Inadequate feed formulation and storage conditions
4. VNN / VER / Nodavirus infection

It should be pointed out that there is a big scoring difference between the four highest scored hazard and the rest of the hazards in the list (~0.010 vs ~0.003).

- The inadequate stocking density hazards scored highly because they persist for 40 days and occurred relatively frequently
- Inappropriate water velocity scored highly mainly because it had a high severity score (3) and the effect lasted 21 days.
- Iinadequate feed formulation/storage conditions had a severity scores of 3.
- Disease (VER/VEN/nodavirus) was the fourth highest ranked hazard. These diseases only occur infrequently (probability score = 1) but affect a high proportion of the population with high severity. The outcome is frequently death, thus the mortality score is also high. The period of clinical disease, prior to mortality, is approximately 5 days.

5.3.3. Juveniles

This stage lasts about 50 to 90 days. Fish are growing rapidly and removal of fish generally occurs twice to maintain a consistent stocking density (kg/m^3). Three systems were considered: flow-through, recirculation, and extensive.

- Flow-through and recirculation systems (Appendix A, Table 5 and Table 7)

- In both systems poor tank hygiene receives a much greater score than any other hazard, attributable to a high severity score, a high proportion of the population affected and long duration (70 days).
- VNN/VER/nodavirus was an important hazard during the larval stage and remains important (second most highly ranked hazard) for juveniles in flow-through tanks and recirculation systems but not in extensive systems.
- Monogenean infections received high scores in tanks and recirculation systems. There is a lower probability of the disease hazards occurring in the recirculation systems, compared with the flow-through tanks. However, in recirculation systems the impact of monogeneans may be more severe. Poor biosecurity will lead to infection that will last 30 days.
- Abdominal adhesions due to vaccination (vaccine side effects) also ranked highly. Other adverse effects of vaccination by bath challenge were considered to be handling and

crowding, which were considered separately. Handling did not feature as a highly ranked hazard. However data presented in the Scientific Report clearly indicates that handling is a welfare hazard, especially because the lack of anaesthetic and repeated manipulations of juveniles during this period for different purposes.

- Extensive (Appendix A, Table 6)

In extensive systems, considered hazards were different to the other 2 systems. Overall fewer hazards were identified, (n=4) and the value of the highest ranked hazard (predation) was approximately half the value of the highest ranked hazard in the more intensive sytems.

- Predation was by a very wide margin the most important hazard. Its high score was due to the severity (mainly physical injury) and duration of the effect of the hazard.
- Extreme temperature was another important hazard because whilst the conditions occurred relatively infrequently, if affected a high proportion of the population and affected them for on average 14 days.

These hazards reflect the nature of the systems. There exists an inherently reduced capacity to influence the environmental parameters in extensive compared with other systems.

5.3.4. Ongrowers

Handling issues and inadequate feed formulation / storage were the most important hazards in sea cages, recirculated and flow-through tanks (Appendix A, Table 8, Table 9 and Table 11).

Inadequate feed formulation and storage scored highly because it affects a high proportion of the population, has a relatively high probability (3) and severity score (3) but above all because of a high estimated duration (150 days). 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 (2).

Clear difference exists between the extensive and the other systems. In extensive systems few hazards were identified for ongrowers compared with other systems. Almost no handling occurs in extensive systems at this stage. Feed formulation is not an important issue in extensive systems as natural feed represents an important complement. The main hazards for ongrowers in extensive systems are disease and predation (Appendix A, Table 10). In sea cages, protective nets normally prevent bird predation. Failure in the system leading to predation will be, in general, quickly corrected and so predation does not score highly. In more extensive systems protective nets are not practicable. In extensive and semi-intensive systems, algal blooms and lack of artificial oxygenation system may cause hypoxia. It did not however achieve a high score because it had a low probability score (1) and duration was estimated to be only 7 days.

The difference between the extensive and other systems reflect the nature of the inherently lower level of disease management and predator control that is attainable under extensive systems. However, these systems are better in terms of reduced disturbance and feed formulation and storage scoring a much lower.

5.3.5. Broodstock

The welfare of broodstock is particularly important since fish might be fertile for a number of years. Two hazards stood out: high stocking density and not using anaesthetics. The manipulation of broodstock without the use of anaesthetics occurs because anaesthetics are not licensed for seabass or bream in the EU. Manipulation without anaesthesia was considered to be a hazard with low severity (1) but affects all broodstock for approximately 63 days during this life stage. Similarly high stocking density received a low severity score (1) but since it lasted for 300 days the final score was high.

The following hazards attained considerably lower scores: inadequate feed formulation and storage conditions, inadequate feed size and improper sexing. Inaccurate sexing of broodstock may lead to an absence of males, and female seabream may retain eggs leading to reproductive dysfunctions and/or mortality (Appendix A, Table 12).

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GLOSSARY

Denticles	Denticles or placoid scales are small outgrowths which cover the skin of many cartilaginous fish including sharks. They are similar in structure to teeth, and teeth may have evolved from denticle-like structures in primitive fish
Eurytherm	Organism tolerating great temperature variations
Gonochoristic	Describes a sexually reproducing species in which there are at least two distinct sexes. The sex of an individual is genetically determined and does not change throughout its lifetime.
Hepatosomatic index (HSI)	Relative weight of liver (wet weight of liver in g / total body weight in g). Used to estimate the energy status of the fish. Often result a poor predictor of energy reserves.
Hypercapnia	A condition with elevated carbon dioxide concentration in the water.
Interrenal cells	The interrenal cells are the equivalent to the cells of the cortex of the suprarenal gland of higher Vertebrates. In fish in view of the anatomical position the term interrenal is preferred.
Mesocosm	Isolated ecosystem of a relatively great water volume, varying from one to 10 000 m^3. Used especially for the culture of live-food organisms in jars, tanks, plastic bags, ponds and enclosures.
Perivisceral	Surrounding the internal organs
Ongrowing	Colloquial term for the process of rising of organisms after the initial larval/juvenile stages to a marketable size.
Juveniles	From the end of the weaning phase tilll a size of 1-2.5g
Protandry	A state of hermaphroditism in which the male gonad matures before the female gonad.
Salinity	An expression for the concentration of soluble minerals (often restricted to salts of the alkali metals or of magnesium) and chlorides in water or soil; usually expressed as parts per thousand (ppt). Related to chlorinity by the formula S = 1.805 Cl + 0.030 where both S and Cl are expressed in ppt.
Vallicultura.	from the Latin *vallum*, meaning embankment, it was originally the simple and primitive lagoon sector, enclosed for fish-culture purposes. Nowdays the term valliculture is used for extensive and semintensive farming practices in coastal basins of the North Adriatic Sea (Italy).
Vomerine teeth	Teeth located in the vomer bone (along the palate)
Weaning	Process in which an animal's dependence on its mother, directly or indirectly (e.g. yolk sac) for food or protection comes to an end. In aquaculture, also used to refer to the transition from live food to processed feed for small larval fish.

ABBREVIATIONS

DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FCE	Feed Conversion Efficiency
GOT	Glutamic-Oxaloacetic Transaminase
HPI	hypothalamic pituirity interineal
HUFA	Highly Unsaturated Fatty Acids
MRL	Maximum residue level
PCO_2	Carbon dioxide partial pressure
SGR	Specific Growth Rate
TA-N	Total Ammonia Nitrogen
TGP	Total Gas Pressure
TL	Total lenght
VMPs	Veterinary medicinal products

Animal welfare aspects of husbandry systems for Farmed European seabass and gilthead seabream [1]

MINORITY OPINION

This minority opinion from Prof. Donald M. Broom is based on the view that the accepted Report and adopted Opinion are incomplete and that in order to answer the mandate from the European Commission, the introductory chapters on the welfare, biological functioning and farming of fish should be included.

[1] For citation purposes: Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on Animal welfare aspects of husbandry systems for farmed European seabass and gilthead seabream. *The EFSA Journal* (2008)844-Annex II, 1-19

SUMMARY

Fish are very diverse in their body form and have a wide range of sensory systems, some of which, such as electroreceptors and the lateral line system, are not shared by birds and mammals. As vertebrates, fish, birds and mammals share a similar general brain structure. Over and above this, however, comparative neuroanatomy highlights many differences among vertebrate groups; it also highlights differences in brain structure among species of fish. On the other hand, studies of brain function suggest a number of parallels between fish and other groups. Fish have nociceptors and these look like and have a similar response profile to those of birds and mammals. The question of whether fish experience the input of these receptors as pain remains controversial but experiments have shown the brain is active during such stimulation and that painkillers reduce prolonged behavioural and physiological responses. It is clear that the responses given by fish to nociceptive stimulation are more complex than simple reflexes, including significant shifts in behavioural priorities and the performance of anomalous behaviour. In this context, our working position is that juvenile and adult fish have the capacity to perceive painful stimuli and experience at least some of the adverse affective states that we associate with pain in mammals. Data suggest that the affective state of fear sometimes motivates behaviour in fish. The systems in mammals and birds that result in the production of adrenaline and cortisol have close anatomical and functional parallels in fish. Fish show physiologically and behaviourally similar freeze and flight responses and prolonged cortisol production is associated with immunosuppression.

WELFARE CONCEPTS

Attitudes to animal welfare encompass three aspects: what animals feel or experience; how animals are functioning; and how the subject animals compare with their 'natural' wild counterparts (Fraser, 1999) and these influence how animal welfare is understood. Feelings and experiences are part of animal functioning and have effects that may be assessed. However, observations on animals in the wild are not involved in welfare assessment, but give a guide as to their likely functioning when removed from the environment in which they have evolved.

Welfare is a characteristic of an individual animal and is concerned with the effects of all aspects of its genotype and environment on the individual (Duncan 1981). Broom (1986) defines welfare as follows: "the welfare of an animal is its state as regards its attempts to cope with its environment". According to this definition, an animal's welfare depends on the ease or difficulty of coping and also the extent of any failure to cope, which may lead to disease and injury. Furthermore, welfare also includes pleasurable mental states and unpleasant states such as pain, fear and frustration (Duncan 1996, Fraser and Duncan 1998). Such feelings cannot be measured directly but may be inferred from measurements of physiology and behaviour and are a component of coping systems (Cabanac 1979, Broom 1998, Panksepp 1998).

Whenever animals are overtaxed by environmental impacts, welfare is poor to some degree and aspect of animal welfare (MacIntyre et al., 2008).

When considering fish, application of these welfare concepts appear more difficult to develop and require specific consideration. There are several reasons for this. First, there is less knowledge of basic biology particularly of brain functioning in relation to awareness of pain and fear then for mammals or birds (Rose, 2002).._Fish are poikilothermic animals which live in an aquatic environment. Environmental factors have a major impact on fish biology and coping with environmental changes is a major task for fish. There are many publications, on the impact of external factors on fish physiology and behaviour: Such biological knowledge is a valuable source of information when assessing fish welfare (Iwama, 2007). In this context, the concept of 'needs' is central to discussions of animal welfareThe needs can be fulfilled by physiological changes and by carrying out certain behaviours. Such behavioural requirements are more difficult to evaluate as sophisticated experimental evidence is required to determine their strength (Hughes & Duncan 1988, Jensen & Toates 1993, Broom and Johnson 1993, Vestergaard 1996). Such experiments have rarely been conducted in fish even though a failure to meet such needs in some way may contribute to poor welfare.

Where welfare or health are referred to as good in this report, these words imply a state that is positive for an individual and by implication for the population as a whole, Where welfare or health are referred to as poor, a negative state is implied. The following sections will deal with the major recognisable adverse states in the fish species being studied with a review of the available scientific data and its interpretation.

CONCLUSION

The concept of welfare is relevant to all farmed animals, including farmed fish, and some aspects of fish welfare can be scientifically assessed. However, although the same methodology is relevant in studying the welfare of fish, birds and mammals, much less research has been carried out on fish.

WELFARE ASSESSMENT IN FISH

The scientific assessment of welfare is discussed by Huntingford *et al.*(2006) and and FSBI (2002).. Welfare assessment may be based upon a list of needs, for example measuring the hazards associated with the non-fulfilment of these needs. It may be assessed in various ways. Poor welfare can be assessed by how far an individual animal has deviated from what is normal for animals in a good environment (Morton and Griffiths, 1985), i.e. one that meets all of their needs. Normality is not necessarily that which is natural for wild fish and an assessment of deviation from normality must be based upon baseline studies of farmed fish in a satisfactory environment., taking into account their previous experiences e.g. (specific) rearing environment. To understand, compare and develop actions to improve fish welfare, defined protocols of welfare measures or indicators are needed.

Some welfare research involves measuring direct indicators of poor or good welfare while other research evaluates what is important to animals by studies demonstrating positive preferences and motivation (Dawkins 1990) and also aversion i.e. negative preferences and how hard an animal will work to avoid, as opposed to access, an environmental variable. Some such work on preferences and motivation has been conducted with fish, but there is not a large amount of data on these issues. Measures of physiological functioning, productivity, health and pathology and behaviour all form the basis of welfare assessment. As an example, measuring disease resistance or the functioning of the immune system offers one way of estimating the welfare "cost" of certain aquaculture conditions. Compromised immune performance can lead to disease outbreaks with associated direct negative welfare consequences. Moreover, lowered disease resistance is generally believed to be a consequence of maladaptive physiological stress, and disease challenge testing may therefore also be an indirect measure of such stress conditions.

Due to the complex causal relationships among the various needs of farmed fish and their behavioural and physiological consequences, it is impossible to find one single measurement or welfare indicator that will cover all possible welfare relevant effects of all possible rearing systems, farmed species and potential situations. Some of the methods used and evaluation of the results will be species and system specific. When the welfare of fish or other animals is assessed, sets of measures can be used, which might be physiological (Oliveira *et al.*, 1999, Ellis *et al.*, 2004), behavioural or pathological (see Huntingford *et al.*, 2006). Whilst a single measure could indicate poor welfare, a range of measures will usually provide a more accurate assessment of welfare because of the variety of coping mechanisms used by the animals (Koolhaas *et al.*, 1999, Huntingford and Adams 2005) and the various effects of the environment on individuals. Useful welfare indicators must to be valid reflections of welfare and repeatable. In addition to measures, which are the outputs of good husbandry, farm practices that help to ensure good welfare provide important indirect welfare indicators, independent of the condition of the fish. Such indicators of welfare through good practice include staff training, good husbandry protocols, monitoring and biosecurity systems, health plans and contingency plans. These complement measures of welfare outcomes by indicating ways by which poor welfare can be avoided.

Indicators that do not necessarily give information about individual fish are commonly used by fish farmers to assess changes at a population level. Indeed, many fish farms have strategies for real-time monitoring of such indicators, which include feed intake, growth rate and mortality. In the case of feed intake, the indicator is not the feed intake per se, but the deviation from an expected feed intake based on biomass and water temperature. Production variables of this kind have a place in welfare assessment and a failure of fish to feed and grow often indicates poor welfare. However, high performance levels (e.g. high feed intake and good growth) do not necessarily indicate good welfare. At a population level, changes in rate of mortality may be a useful indicator of poor welfare.

Indicators at the individual level cover all measurements of individual fish in a system, either by non-invasive monitoring in free-swimming fish, or with targeted sub-sampling of fish. Examples of individual measures are fin condition and parasite load. Representative sub-samplings are difficult in large farm systems, but can work well in smaller systems. The individual indicators commonly relate to the ability of the fish to maintain a normal physiological (and possibly behavioural) state, including the ability to mount effective immune responses.

INTRODUCTION TO THE BIOLOGY AND FUNCTIONING OF FARMED FISH SPECIES

1.1. Diversity of teleost fish forms and environmental adaptations

The three major groups of fish are: Agnatha (hagfish, lampreys), Chondrichthyes (sharks, rays, sturgeons) and Actinopterygii (bony fish with teleosts being the most prevalent). Most aquaculture finfish species are teleostean fish (Evans *et al.*, 2005). There are more than twenty thousand living species of teleosts that have been evolving over 500 million years, representing every aquatic environment and a vast range of physiological and behavioural traits.

Each species has developed a set of tolerance limits for each environmental factor, and within such ranges ecological interactions are further limiting the natural distribution and habitat selection (Randall *et al.*, 2002, Helfman *et al.*, 1997). The tolerance ranges are species specific and may be wide or narrow, developing the species into opportunistic generalists or specialists designed for long-lasting natural ecological niches. Individual fish have abilities to to cope with a changing environment, including large annual changes in e.g. water temperature and food availability. As a result of such plasticity, fish have been able to inhabit every conceivable aquatic environment, from a Tibetan lake at an altitude of 5,250m to the pacific depth at –8,370m. They are also extraordinary diverse in terms of numbers of species, body forms, life-styles and physiologies. Fish genomes are more varied and plastic in comparison with other vertebrates, owing to frequent genomic changes (Cossins and Crawford, 2005).

Teleost fish share many common morphological and physiological adaptations with other vertebrates, including many components of the neural and endocrine systems, immune system and the physiological stress cascade. However, some key systems such as the respiratory- and osmoregulatory systems differ markedly form land-living vertebrates due to the particular challenges imposed by living in water. Respiration (i.e. exchange of gases such as oxygen and carbon dioxide) takes mainly place over the gills (except in the early larval stages). The gills are also involved in uptake and excretion of ions and maintenance of osmoregulatory balance. The intimate physiological contact of all body fluid compartments and tissues through gills, skin and gastro-intestinal system with the external environment is a situation that can lead to major physiological challenges. Variations in water conditions (including oxygen levels, temperature, pathogen, salinity and water-borne pollutants) can have a direct and unavoidable impact on susceptible cells, tissues and organs. This close physiological contact is more easily defined and its impact more readily studied than in terrestrial species. Fish are sensitive sentinels of environmental challenge particularly pollution (Cossins & Crawford 2005).

Some fish species go through marked metamorphosis or habitat changes such as transfer from freshwater to sea-water that often represent critical periods with reduced capacity to withstand stressors or infectious diseases. The intimate contact with the water, including pathogens, represents a challenge in terms of barrier functions as a part of the disease defence. Breakdown of the integrity of these barriers, e.g. due to various forms of stress, may lead to increased susceptibility to infectious diseases. The development of acquired immune function often takes place after the metamorphosis from larval to juvenile form which represents a challenge on vaccination, in particular in marine farmed fish species that are exposed to a suite of pathogens from early life stages.

Fish in a natural habitat display complex swimming, feeding, anti-predator and reproductive behaviours, and such behavioural traits are linked to genotypical differences between species and individual animals, and are modified by phenotypical development and learning. In addition, several fish species undergo ontogenetic niche shifts during their lifespan, and consequent changes in behaviour, e.g. change in salmon from a territorial parr in the river to a schooling fish which migrates from freshwater to sea-water, and years later to a mature fish which migrates back to the river prior to spawning **(**McCormick *et al.*, 1998).

1.2. Environmental factors and fish physiology.

The main environmental factors which control spatio-temporal distribution of fish are temperature, salinity, light, oxygen, food, pollutants, hydrodynamics and substratum. Moreover, the physiological processes of fish are carried out under environmental conditions harsher and more restrictive in many ways than those experienced by terrrestrial animals (Wedemeyer, 1997). For example, the concentrations of the gases in the aquatic environment are highly variable compared with those in air. Oxygen depletion in water is not unusual and at times respiration can be difficult. All these reasons explain why coping with changes in environmental factors is a major ability for fish species that is relevant when considering fish welfare.

During the last 40 years, considerable research effort has been devoted to the effects of environmental factors on fish physiology-(Somero and Surarez, 2005).

Scientific information on the effects of environmental factors on physiological functions in fish, including development, growth, reproduction, excretion, osmoregulation, respiration and immunity are summarised in several text books on fish ecophysiology (Evans 1993, Rankin 1994, Bruslé and Guignard 2004). Teleost fish share with other vertebrates many common developmental pathways, physiological mechanisms and organ systems. The challenge imposed by aquatic life leads to major physiological roles for exchanging epithelia such as gills. This is not only related to the major physiological functions (i.e. respiration, osmoregulation, excretion, acid-base balance regulation) carried by the gill which then play a central role in a suite of physiological responses to environmental and internal changes but also to the huge surface exchange built up by the gill which are a major entry for many biotic or abiotic water compounds (Evans, 2005). An example of fish ecophysiologyis the study of the effect of xenoestrogen on sex differentiation on trout reared in cages (Jobling et al., 1998) which led to literature on the effect of endocrine disruptors (Sumpter and Johnson, 2005).

Literature on fish behaviour and analysis of behavioural responses exhibited by fish exposed to stressors are mostly devoted to fish in their natural environment (Schreck, Olla and davis, 1997). Fewer studies have looked at fish behaviour in production systems. Feeding behaviour (Volkoff and Peter, 2006), social interaction and hierarchies (Gilmour et al., 2005) are important in fish aquaculture.

CONCLUSION

Fish live in the aquatic environment and respond to harmful chemicals and many other stressors at intensity levels frequently far below those that can be perceived by terrestrial animals

1.3. Sensory systems in fish

Both conservation and innovation in the organisation of sensory systems occur across vertebrates. Fish perceive optical, positional, chemical, tactile, mechanosensory and electrosensory (lateral line), acoustic, and magnetic stimuli by receptors innervated by particular brain regions (Hodos & Butler 1997). Some basic patterns of sensory innervation are common to all vertebrates for the relay of sensory inputs from putative stressors in the environment to the brain, directly impacting on the fish's welfare.

The optical characteristics of water affect illumination intensity and spectral quality. This has led to evolution of the fish eye to cope with these challenges. Fish eye adaptations allow the efficient collection of light (Warrant & Lockett 2004) and other specialisations (Siebeck & Marshall 2001). They do not have eyelids or nictitating membranes and the large choroidal complexes are subject to pressure changes and to gaseous embolism. Thus the fish eye is particularly vulnerable to a variety of husbandry effects leading to poor welfare (Roberts 2001).

Sound and vibrations travel well in water and fish are highly responsive to and potentially easily disturbed by exposure to such systems. However, it is not clear whether or not salmonid fish are disturbed by such stimuli (Wysocki *et al*. 2007).

The ear of bony fish comprises three semi-circular canals, a utricle and a sacculae and lagena. The auditory receptors comprise a very variable set of sensory organs that perceive sound from the environment. The ascending auditory pathways in mammals and fish are similar. The vestibular system of vertebrates detects position and motion of the head and is important for equilibrium or balance and coordination of head, eye and body movements.

Fish have highly elaborate chemosensory detection of information from the environment including other fish. Chemicals detected by the fish and conveyed to the brain via cranial nerve I are involved in olfaction. Structural organisation of the peripheral olfactory organ is variable throughout fish species, although the ultrastructural organisation of the olfactory sensory epithelium is extremely consistent (Hara 1994). Olfactory signals such as those involved in reproduction and feeding may be processed independently through two distinct subsystems (Laberge & Hara 2001, Nikonov *et al.*, 2005). The neuronal components are similar to the olfactory systems of mammals except that there is no connection between respiratory structures and the olfactory system in fish. Chemical pollution and chemical signals such as alarm pheromones may often cause poor welfare in fish so consideration of the impact of olfactorily important chemicals in the fish environment can improve welfare.

The taste buds of vertebrates are the receptors of the gustatory or taste organ that may occur in the oropharyngeal cavity and elsewhere on the body surface (Hara 1994).

The lateral line system detects mechanosensory information and is found in all fishes and some amphibians but has been lost in reptiles, birds and mammals. The sensory organ consists of hair cells called neuromasts located in the lateral line canals or on the head and body. The lateral line system allows fishes to respond to water movements and other movements relatively close to the fish. This system alerts fish to prey, predators, school neighbours, water flow from environmental obstacles, and in salmon reproductive vibrations (Satou *et al.*, 1994) that facilitates orientation behaviour (Montgomery *et al.*, 1997).

Magnetoreceptors have not been identified with certainty in any animal, and the mode of transduction for the magnetic sense remains unknown. However, magnetite particles embedded in specific cells in the basal lamina within the olfactory lamellae of rainbow trout, *Oncorhynchus mykiss*, have been identified (Walker *et al.*, 1997). All fish can use their lateral line to detect local movement and electroreception is widespread in fish, including farmed species. The implications for welfare are starting to be considered (Spiess *et al.*, pers. comm.).

CONCLUSION:

Fish have a wide range of sensory systems, some of which, such as electroreceptors and the lateral line system are not shared by birds and mammals.

1.4. Comparative Brain Structure

As in all vertebrate brains, the fish brain consists of forebrain (i.e. telencephalon and diencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon). The pallium constitutes the exterior surface of the telencephalon, in mammals the neocortex is a greatly expanded part of the pallium. Thus, the general anatomy of the teleost (bony fish) brain is similar to that of other vertebrate brain, however, the fish brain is smaller relative to body size and less complex in structure than that of higher vertebrates (Kotrschal *et al.*, 1998). Moreover, among fish there is a marked inter species variation in brain anatomy, often reflecting sensory specialization, fundamental differences in embryonic development, and the degree of cell migration and proliferation and intraspecific variation in brain structure is evident (Butler 2000).

The fish brain grows continuously throughout life and appears to be highly responsive to the environmental conditions that the fish experiences as it develops (Ramage-Healey & Bass 2007, Dunlap *et al.*, 2006, Kihslinger & Nevitt 2006, Kihslinger *et al.*, 2006, Lema 2006).

In vertebrates specific brain structures have been associated with emotions and motivated behaviour. It is now indicated that the same function can be served by different structures in different groups of animals (e.g. cognitive functions in birds and mammals, Jarvis *et al.*, 2005) and structures that seem to be different may be more homologous than had previously been thought. Comparative anatomical studies have shed some light on the potential functional role of fish brain structures in relation to motivational and affective states. The issues are complex and there is considerable disagreement among specialists about the extent of commonality of brain function within the vertebrates. Fish do not have the extensive analytical cortex that mammals have and sensory processing is carried out in different regions of the brain according to the adaptations of the particular group of fishes. Fish do not have the extensive cerebral cortex that mammals have, this being smaller relative to body size and without the characteristic folded and layered appearance of the mammalian cortex. Additionally, sensory processing is carried out in different regions of the brain according to adaptations of the particular group of fishes (Rose 2002, Vogt 2003).

The possibility cannot be excluded that parts of the brain other than the cerebral cortex have evolved the capacity for generating negative emotional states in fish (Huntingford *et al.*, 2006). The concept of pain in vertebrates revolves around the perceived noxiousness of certain stimuli, and may have been conserved through evolution as a protective strategy.

At the level of the telencephalon, fish lack the higher cortical centres that have been demonstrated as necessary for full processing and experience of pain in mammals (Rose 2002). Extensive interconnections exist between the telencephalon, diencephalon and mesencephalon in fish (Rink & Wullimann 2004). Neural pathways that connect to various forebrain structures are of fundamental importance to consciousness and the perception of pain and fear in mammals (Willis & Westlund 1997). The pallium (the grey matter that covers the telencephalon) has thickened to various extents in different classes of vertebrates, and in mammals it consists of a laminated structure, the cerebral cortex (Striedter 1997). Unlike mammals, in the majority of modern fish species, the pallium is unlaminated (Vogt 2003), however there is evidence to suggest it has developed into a highly differentiated structure with respect to the processing of sensory information (Bradford 1995, Butler 2000). The telencephalon in fish contains several brain structures that are thought to be functionally homologous to those associated with pain and fear in higher vertebrates (Bradford 1995, Chandroo *et al.,* 2004, Portavella *et al.*, 2004), and this is known to be active during a potentially painful event (Dunlop and Laming 2004). Therefore, information about noxious stimuli, such as those resulting from tissue damage, in fish may be processed in a functionally homologous way, not yet fully characterised, to that involved in processing noxious stimuli in mammals. In mammals, the hippocampus, a telencephalic structure, is involved in memory and learning of spatial relationships whereas the amygdala, a structure which is also telencephalic, has long been known to be important in arousal and emotions, particularly fear responses (Carter 1996, Maren 2001). Recent studies have identified structures in the teleost telencephalon that appear to be homologous to the mammalian amygdala and hippocampus with alterations in fear, spatial learning and memory retrieval when these areas are lesioned (Portavella *et al.,* 2002). Another important structure in the fish brain, the hypothalamus, is thought to perform functions similar to those of the hypothalamus in other vertebrates. The hypothalamus is involved in various functions, including sexual and other social behavior, and is also responsible for the integration of both internal and external signals including those originating from those telencephalic areas that have been implicated in fear responses (Fox *et al.,* 1997, Portavella *et al.,* 2002, Chandroo *et al.,* 2004).

CONCLUSION

Our understanding of the extent to which brain structure and function in fish are comparable with other vertebrate groups is limited. As vertebrates, fish, birds and mammals share a similar general brain structure. Over and above this, however, comparative neuroanatomy highlights many differences among vertebrate groups; it also highlights differences in brain structure among species of fish. On the other hand, studies of brain function suggest a number of parallels between fish and other groups.

1.5. Sentience

Sentience refers, among other properties, to the ability to experience pleasurable and adverse states, a key issue when considering the welfare of any animal and a focus of public concern and there are discussions of this matter in relation to fish (Broom 2006, 2007, Yue *et al.* 2008).

Animals that have some cognitive ability at a certain stage of their development, start development without such ability. Hence it is relevant to consider at what time, during the life of a fish, their perceptual and cognitive abilities develop. It is likely that fish develop some cognitive ability only when they are able to perceive external stimuli. While little is known about the development of cognitive ability, we have some evidence concerning the stage of life at which the development of responsiveness to external stimuli starts (EFSA, 2005).

1.6. Pain

Pain is defined as an aversive sensation associated with tissue damage. As non-human animals are unable to communicate the experience of pain directly, a number of criteria have been defined to provide a guide as to whether an animal might be capable of experiencing pain (Bateson 1991, Broom 2001a, b, Sneddon 2004). These criteria include: (i) the existence of functional nociceptors (ii) the presence and action of endogenous opioids and opioid receptors (iii) the activation of brain structures involved in pain processing (iv) the existence of pathways leading to higher brain structures (v) the action of analgesics in reducing nociceptive responses vi) the occurrence of avoidance learning vii) the suspension of normal behaviour associated with a noxious stimulus.

Each of these areas will be considered in turn to assess how well fish fulfil these criteria and how their functioning compares to the nociception and pain systems of higher vertebrates.

Nociception is the detection of a noxious stimulus and is usually accompanied by a reflex withdrawal response away from that stimulus immediately upon detection. Noxious stimuli are those that can or potentially could cause tissue damage so stimuli such as high mechanical pressure, extremes of temperature and chemicals, such as acids, venoms, prostaglandins and so on, excite nociceptive nerve fibres. Martin & Wickelgren (1971) and Mathews & Wickelgren (1978) identified sensory neurones in the skin and mouth of a lamprey (*Petromyzon marinus*) during heavy pressure, puncture, pinching or burning, and found that the output was like that which would be recorded in a mammalian nociceptor when responding to a painful stimuli. Studies of the rainbow trout (*Oncoryhnchus mykiss*) have shown that nociceptors are present on the trout face and are innervated by the trigeminal nerve (Sneddon 2002, 2003a). These studies on nociceptor anatomy and physiology strongly support the hypothesis that the rainbow trout has the sensory equipment for detecting potentially painful stimuli. Studies of nerve responses, nerve and other tissue regeneration, behavioural responses and effects of analgesics indicate nociceptive function in the fins of salmonid and other fish (Becerra et al 1983, Geraudie and Singer 1985, Turnbull et al 1996, Chervova 1997).

Fish have the necessary brain areas for nociceptive processing to occur (e.g. pons, medulla, thalamus; Sneddon 2004).The functional possibility for high level processing, such as that carried out in the cortex in humans, is crucial in terms of pain perception. In term of anatomy the fish brain is far smaller relative to body size and simpler in structure than of a human. Moerover, fish lack cortical structure such the neocortex, which plays a key role in the subjective experience of pain in humans (FSBI 2002; Rose 2002). However, it is not impossible that parts of the brain other than the cerebral cortex have evolved the capacity of generating negative emotional states in fish (Huntingford et al. 2006).

In fish as in other vertebrates, nociceptive information is relayed to the brain from the periphery via two major tracts. The trigeminal tract conveys information from the head while the spinothalamic tract conveys information from the rest of the body. In fish the trigeminal has been shown to project to the thalamus as it does in other vertebrates (Goehler & Finger 1996, Finger 2000). The elasmobranch (Ebbesson & Hodde 1981) and teleost (Goehler & Finger 1996, Finger 2000) groups both have the same basic components of ascending spinal projections as higher vertebrates.

The possession of opioid receptors, endogenous opioids and enkephalins is one of the requirements to determine whether nociception can occur in an animal (Bateson 1991, Broom 2001a, b). These substances are involved in analgesia in the mammalian central nervous system and are produced in order to reduce pain internally. Met-enkephalin and leu-enkephalin are present in all vertebrates which have been tested and there are at least six opioid receptors described for teleost fish (Dores and Joss 1988, Dores *et al.*, 1989, Dores and Gorbman 1990, McDonald and Dores, 1991). Opioids elicit antinociception or analgesia through three distinct types of receptors in mammals (Newman *et al.*, 2000) and these have been identified in the zebrafish, *Danio rerio* (Stevens 2004). When goldfish are subjected to stressful conditions, there is an elevation of pro-opiomelanocortin, the precursor of the enkephalins and endorphins, just as there would be in humans (Denzer and Laudien, 1987). Goldfish which are given electric shock show agitated swimming but the threshold for this response is increased if morphine is injected and naloxone blocks the morphine effect (Jansen and Greene 1970). Work by Ehrensing *et al.*, (1982) showed that the endogenous opioid antagonist MIFI down-regulates sensitivity to opioids in both goldfish and rats. Opiate receptors and enkephalin like substances have also been found in various brain areas of goldfish, *Carassius auratus* (Finger 1981, Schulman *et al.*, 1981) and rainbow trout, *O. mykiss* (Vecino *et al.*, 1991). The distribution of enkephalins in the fish brain shows a similar pattern to that seen in higher vertebrates (Simantov *et al.*, 1977, Vecino *et al.*, 1992). In general it is clear that there are very many similarities amongst all vertebrates in their opioid systems.

A simple reflex response to a noxious stimulus can indicate nociceptive function, however, adverse affects on an animal's normal behaviour beyond a simple reflex may indicate a psychological component that is indicative of suffering, and suggests that the animal may be perceiving pain. Reflex responses occur instantaneously and within a few minutes but some of the responses of fish may be prolonged.(Sneddon 2006). A recent study investigated the behavioural response of rainbow trout that had been given subcutaneous injections of acetic acid and bee venom (algesics) to the lips (Sneddon *et al.*, 2003a). These fish showed an enhanced respiration rate for approximately 3 hours, did not feed within this period, and showed anomalous behaviours such as rubbing of the affected area on the aquarium substratum and glass and rocking from side to side on either pectoral fin (Sneddon 2003b, Sneddon *et al.*, 2003a). These, therefore, appear to represent changes in behaviour over a prolonged period as a result of nociceptive stimulation.

The ability of analgesics to modulate nociceptive responses is also indicative of pain perception since the selectively act on this system. The adverse behavioural responses seen in the rainbow trout, *O mykiss,* were quantified and when morphine was administered to fish injected with acid, there was a dramatic reduction in this rubbing behaviour as well as rocking behaviour and the enhanced respiration rate was also ameliorated (Sneddon 2003b, Sneddon *et al.*, 2003a). Further to this, acid injected fish did not show an appropriate fear response to a novel challenge supporting the idea that this painful stimulus dominates the fish attention (Sneddon *et al.*, 2003b). Studies have shown that goldfish are able to learn to avoid noxious, potentially painful stimuli such as electric shock (Portavella *et al.,* 2002, 2004). Learned avoidance of a stimulus associated with a noxious experience has also been observed in other fish species (Overmier & Hollis 1983, 1990) including common carp, and pike, avoiding hooks in angling trials (Beukema 1970a, b).

There are strong debates on the question of pain in fish with opposing views (Rose 2002, Derbyshire *et al.*, 2007, Sneddon 2004, 2006). For example, Derbyshire *et al.,* (2007) argue that the results from Sneddon's studies presented above can be interpreted as showing a remarkable capacity of trout to withstand oral trauma which would be expected as trout normally feed on potentially injurious prey such as crayfish, crabs and spiny fish. They also suggest that there is an important difference between knowledge about sensation and sentience (Derbyshire *et al.*, 2007). Rose (2002) argues that there are major neurobehavioral differences between fish and humans, particularly at the level of brain regions responsible for pain awareness in humans. In fish, in which the cerebral hemispheres were removed, leaving the brainstem and spinal cord intact, ome behaviour was still possible(Overmier and Hollis, 1983). Because the experience of fear and pain depends on cerebral cortical structures in mammals and these are absent in fish brains, Rose (2002) concluded that awareness of fear and pain is impossible in fish. However, evidence of an active nociceptor system in fish associated with effects of administration of noxious substances on normal behavioural repertoire has led to the inference that fish potentially have the capacity for long-term suffering (Chandroo et al. 2004, Sneddon 2006, Braithwaite and Boulcott 2007).

CONCLUSION

It has been convincingly demonstrated that fish have nociceptors and that these look like and have a similar response profile to those of birds and mammals. The question of whether fish experience the input of these receptors as pain remains controversial but experiments have shown the brain is active during this stimulation and that painkillers reduce prolonged behavioural and physiological responses. It is clear that the responses given by fish to nociceptive stimulation are more complex than simple reflexes, including significant shifts in behavioural priorities and the performance of anomalous behaviour. In this context, our working position is that juvenile and adult fish have the capacity to perceive painful stimuli and experience at least some of the adverse affective states that we associate with pain in mammals.

1.7. Fear

Fear serves a function that is fundamental to survival and is the activation of a defensive behavioural system that protects animals against actual or potentially dangerous environmental threats. In higher vertebrates, fear involves mainly the amygdaloid and hippocampal regions of the brain although other areas are also implicated. Studies in fish have shown that these responses also appear to be dependent upon cognitive mechanisms and homologous limbic brain regions in the telencephalon. The dorsomedial (Dm) telecephalon in fish has been implicated in emotional learning and is thought to be homologous to the amygdala in mammals (Bradford 1995, Butler 2000, Portavella *et al.,* 2004). In mammals the hippocampus is involved in memory and learning of spatial relationships and it is the dorsolateral (Dl) telencephalon in fish that is thought to be functionally homologous to the hippocampus. Dm lesions impaired acquisition of an avoidance response but had no effect on performance in a spatial learning task, while Dl lesions affected spatial learning but did not impair the acquisition of the avoidance response (Portavella *et al.,* 2002). Therefore Dm and Dl areas of the fish telencephalon share functional similarities with the amygdala and hippocampus, respectively, in mammals.

Studies on fear conditioning in mammals measure levels of freezing and startle behaviour (Fendt & Fanselow 1999). In fish, a number of different behavioural responses to potentially threatening stimuli have been described and include escape responses such as fast starts (Chandroo *et al.,* 2004, Domenici & Blake 1997, Yue *et al.,* 2004) or erratic movement (Cantalupo *et al.,* 1995, Bisazza *et al.,* 1998), as well as freezing and sinking in the water (Berejikian *et al.,* 1999, 2003). Such behaviours may serve to protect the individual from the threat and a number of studies have illustrated that these behaviours can be shown in response to conditioning. Many fish species also release chemical alarm substances when injured. These are thought to act as warning signals, as conspecifics show a behavioural fright response to these chemicals (Smith 1992, Lebedeva *et al.,* 1994, Brown & Smith 1997, Berejikian *et al.,* 1999). These alarm behaviours include dashing movements, vigorous movements in the aquarium substratum, and fast swimming towards hiding places, remaining there for an extended period. These behaviours are thought to be associated with predator evasion (Hamdani *et al.,* 2000).

Learned avoidance studies not only show that a consistent suite of behaviours are produced in response to fearful stimuli in fish but they also provide evidence that the displayed behaviour is not merely a reflex response. Learning to avoid an aversive stimulus in the future implies a cognitive process of recognising that the behavioural response will lead to the desired effect of avoidance (Yue *et al.,* 2004). This may support the suggestion that an affective state such as fear may serve to motivate behaviour in fish.

Learning is thought to be mediated in part by receptors in the brain that are activated by N-methyl-D-aspartic acid (NMDA). Administration of selective antagonists of NMDA receptors impair learning mechanisms such as associative learning and conditioned fear in mammals (Miserendino *et al.,* 1990, Sanger & Joly 1991, Kim *et al.,* 1991, Maren 2001). Experiments with goldfish have shown that intracranial administration of MK-801, an NMDA receptor antagonist, blocks specific aspects of Pavlovian fear conditioning in fish (Xu & Davis 1992, Xu 1997).

CONCLUSION

Fear often depends on cognitive and learning ability and fear responses by fish are described for various situations, suggesting that the affective state of fear sometimes motivates fish.

1.8. Stress responses

Selye (1973) defined stress as “the nonspecific response of the body to any demand made upon it”. Following a period of controversial debates about the definition of stress and stressors, all recent reviews on stress in teleost fish define this term as a condition in which the homeostasis is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli commonly defined as stressors (Wendelaar Bonga 1997, Iwama *et al.,* 1997, Barton 2002, Chrousos 1998, Wendemeyer *et al.,* 1990). The problems associated with Selye’s concept of stress are discussed by Broom and Johnson (2000) and there is debate about whether or not the concept should be limited to that which is detrimental to the fish. The response to stressors is often an adaptative mechanism that allows the fish to cope with stressors in order to maintain homeostasis. If the intensity of the stressors is overly severe or long lasting, physiological response mechanisms can become detrimental to fish welfare or maladaptative (Barton 2002, FSBI, 2002, Wendelaar Bonga 1997).

During the last 20 years, there has been extensive research devoted to the biology of stress in fish. Physiological and behavioural responses to a large variety of physical, chemical and biological stressors including those seen in aquaculture have been measured (for review see Wendelaar-Bonga 1997, Iwama *et al.,* 1997, Barton 2002, FBSI 2002, Conte 2004, Ashley 2007). Hypothalamic-pituitary-interenal (HPI) axis responses are generally considered as an adaptive strategy to cope with a perceived acute threat to homeostasis, for example poor water quality. Although fish are able to tolerate acute adverse water quality conditions, when they become too challenging or prolonged, fish cannot maintain homeostasis and experience chronic stress which in the long term can impair immune function, growth and reproductive function. Furthermore, chemicals may have toxic effects at the level of cell and tissue but, in addition, elicit an integrated stress response which may be specific to the toxicant.

The stress physiology of fish is directly comparable to that of higher vertebrates. Stress physiology is manifested by primary, secondary and eventually tertiary stress responses (see review Wedemeyer *et al.,* 1990, Wendelaar Bonga 1997, FSBI 2002, Ashley 2007). The primary stress response to short term potentially harmful situations involves, amongst other things, the release of catecholamines (adrenaline and noradrenaline) from the chromaffin cells into the circulating system. Simultaneously, activation of the hypothalamic-pituitary-interenal (HPI) axis is observed. The corticotrophin releasing factor (CRF) is released from the hypothalamus and acts on the pituitary resulting in the synthesis and release of adrenocorticotrophic hormone (ACTH) which in turn stimulates the synthesis and mobilisation of glucocorticoid hormones (cortisol) from the interrenal cells. Released catecholamines and cortisol will result in an activation of various physiological and behavioural mechanisms that constitute the secondary and possibly tertiary stress responses. The secondary changes include alteration of secretion of other pituitary hormones and thyroid hormones, changes in turn-over of brain neurotransmitters, mobilisation of energy by breakdown of carbohydrate and lipid reserve and by oxidation of muscle protein, improvement of respiratory capacity via increased heart stroke volume and increase blood flow to gills. As a consequence of this last effect, disruption of the hydromineral or osmoregulatory balance can be observed.

Primary and secondary stress responses are short-term effects of acute, short-lived challenges. When these responses are prolonged or repeated and fish has no way to avoiding or escape the challenge, a series of tertiary effects become apparent, including changes in immune function and disease resistance (Pickering 1992, Balm 1997), in growth (Barton *et al.,* 1987, Pickering *et al.,* 1991) and in reproduction (Pankhust and vander Kraak 1997, McCormick 1998, Schreck *et al.,* 2001).

Behavioural responses are often shown early in defence against adverse environmental changes, often triggered by the same stimuli that initiate the primary physiological stress responses. The exact behavioural response depends on the stressor in action. For example, the response to an approaching potential predator might be escape, whereas the response to an approaching competitor might be attack. The behavioural response to abiotic environmental stressors, such as inappropriate water temperature, oxygen or water current, includes a range of responses in movement pattern, spatial choice and social interactions, but these responses are poorly described in most fish species. In addition, individuals of the same species may differ in the nature and magnitude of their behavioural responses to various stressors. Such behavioural differences, together with the physiological variation with which they are associated, are referred to as coping strategies. Some individuals adopt what is called a proactive coping strategy, showing adrenaline-based fright and flight responses, while others adopt a reactive coping strategy, showing cortisol based “freeze” and hide responses (Korte *et al.,* 2005).

However it is not clear to what extent these are general strategies. These differences are correlated with variation in brain serotoninergic activity (Schjolden and Winberg 2007) and are also affected by the extent of exposure to stressors.

Chronic stress is a major factor in the health of fish (Conte, 2004). As in mammals, there is a clear link between stress and immune status arising mostly through the effects of cortisol which can suppress many aspects of the immune system (Wendelaar Bonga 1997). However, the relationship between stress and immune system goes in two directions since components of the immune system can influence stress responses through modification of the secretion of hormones (Ottaviani *et al.*, 1996, Balm 1997). While disease is not always connected to poor environmental conditions (Huntingford *et al.*, 2006), aquaculture practice presents many situations where stress and physical injury can increase susceptibility to naturally occuring pathogens (Ashley, 2007). For example, diseases associated with low temperatures over winter period have been described in a number of different species (Tort 1998b). Fin erosion is also an important problem in aquaculture which often occurs as results of aggressive interactions. Fin erosion may increase susceptibility to infections (Turnbull *et al.*, 1996). One example of the strong interaction between environmental stress and a serious infectious disease is the case of furunculosis. Many fish may carry the causative pathogen but clinical outbreaks occur normally after stressful events such as grading or transportation of fish. So predictable is the response that a predictive test for identifying carrier populations is the 'furunculosis stress test' where samples of healthy fish are injected with cortisone to identify individuals which might become clinical cases if stressed (Hiney *et al.*, 1994).

An acute stress response does not necessarily imply any harmful consequence as such a response may be important to the maintenance of homeostasis. However, mid- and long-term exposure to stressors generally leads to maladaptative effects and sometimes to chronic stress, which are associated with decreased welfare. Such effects have been described with chronic effects on growth, reproduction or immune function and disease resistance. So, while studies on stress responses do not necessarily give us a complete view of welfare in fish, deleterious effects of several components of the stress response observed after chronic exposure to stressors are indicative of poor welfare (Huntingford *et al.*, 2006, Ashley *et al.* 2007).

Measurements of the levels of both glucose and lactate in the plasma may sometimes be biomarkers of stress in fish (e.g. Arends *et al.*, 1999; Acerete *et al.*, 2004). Measures of the expression of stress related genes might also provide useful markers (e.g. Gornati *et al.*, 2004). Chronic stress has been also studied and exerts a strong effect on haematology (Montero *et al.*, 2001), metabolism (Mommsen *et al.*, 1999), neuroendocrine function (Dibastistta *et al.*, 2005b), and osmoregulation (Wendelaar Bonga, 1997). However, reliable indicators of chronic stress are still under investigation and will probably rely on a range of measurements.

Avoidance of the maladaptative consequences of prolonged stress is a central concern in aquaculture and assessments of potential methods to reduce stress responses is an active area of research (Ashley 2007). Thus, fish have been selectively bred for reduced emergency responses: High responding (HR) and low responding (LR) lines of rainbow trout have been generated by selection for consistently high or low cortisol response to a standard confinement test (Pottinger and Carrick 1999). In addition, these two strains of rainbow trout also show a divergence in sympathetic reactivity as a response to confinement (Schjolden and Winberg 2007). However, all testing was conducted under controlled laboratory conditions and the welfare and productivity of LR strains have not yet been compared under commercial conditions. Manipulation of fish diet has been also shown to play an important role in inter-renal sensitivity: For example, vitamin E added in the diet has been shown in sea bream to slow down elevation of plasma cortisol levels in response to a stressor and to increase survival rate

(Montero *et al.*, 2001). In African catfish (*Clarias gariepinus*), vitamin C fed during early development induced lower inter-renal gland activity (Merchie *et al.*, 1997).

Although much research has been devoted to stress biology in fish, major questions concern the development of new techniques for non-lethal and non-invasive sampling of physiology and behaviour of fishes which would allow measurement of stress outside a controlled laboratory environment (Scott and Ellis 2007), including meat quality measurements (Skjervold *et al* 1999). Cumulative stress responses at different life stages and methods for evaluating stress in relationship to fish performance have not been much studied.

If stressors and failure to cope persist, the final consequence is death. Mortality rate is therefore a useful welfare indicator as mentioned in Chapter 5. In fish species, there is variation amongst species in the mortality rate in the wild. Amongst salmonids, the egg is large so mortality in alevins and fry is lower than in some species with less food reserve available. When considering the mortality rate, that which occurs in the wild is not directly relevant as farmed fish should be cared for and protected from starvation, predation and avoidable disease. Taking into account the biological functioning of the fish species, mortality rate can give information about the extent of stress and poor welfare.

CONCLUSION

In common with all vertebrates, fish possess a suite of adaptative behavioural and physiological strategies that have evolved to cope with stressors. The systems in mammals and birds that result in the production of adrenaline and cortisol have close anatomical and functional parallels in fish except that the adrenaline and cortisol production are from the more diffuse chromaffin and inter-renal tissue rather than from a discrete adrenal gland. Fish show physiologically and behaviourally similar freeze and flight responses and prolonged cortisol production is associated with immunosuppression.

NEEDS OF FISH

A need is a requirement on the part of an animal to obtain a particular resource or to respond to a particular environmental or bodily stimulus. The exact set of needs for any given species is a consequence of its biology. In general needs are associated with all of the major biological functions of the animal. In aquaculture, the fish experience only a part of the range of natural variation in environmental factors. Some factors may be less variable than in the wild, e.g. food availability, while other factors vary more than in nature, e.g. oxygen concentration. In addition, while fish in nature may swim away from adverse or sub-optimal conditions, the farmed fish spatial and temporal environment, gives few options for individual preference. Nevertheless all farmed animals have needs and good welfare depends upon these being met to a greater or lesser degree.

However, there is variation in the importance of the various needs for the welfare of the individual. Needs range from resources whose absence results in rapid death to those whose presence improves welfare for a period, but lack of which would never result in death

The following list of needs is not in order of importance and reflects current knowledge. Some needs require being satisfied only at intervals of some hours or only when fish are at certain life stage, young or adult. The causes of some problems of fish are multifactorial and may be related to more than one need. The welfare risk assessment refers to hazards that are linked to the known needs of a particular species. Those hazards or factors have been identified for each species.

1 Need for adequate physical and chemical environmental conditions:

1A. To have access to appropriate oxygen concentration

All fish need oxygen of a certain partial pressure, the actual value varying according to species.

1B. To avoid harmful substances or environmental conditions in water

All fish need an appropriate aquatic environment. Inappropriate water conditions, for example too high salinity or carbon dioxide concentration, too much ammonia or other toxic chemicals, or suboptimal pH can harm fish.

1C. To have appropriate visual, olfactory and other environmental conditions

It may be that problems are caused to fish of particular species by inappropriate light, vibration, chemical stimuli, pressure changes, or electrical changes.

1D. To avoid extreme temperatures

Although fish are poikilotherms, adverse temperature conditions can harm fish for various reasons including impact on oxygen availability and demand, so they need to avoid them if possible. Body temperature modification in most fish, where it can occur at all, is behavioural.

1E. To osmoregulate

Fish need to maintain relative stability in the ionic composition and osmotic strength of their body fluids, for example when exposed to inappropriate salinity.

1F. To have space for movement

Fish require space to carry out various functions, such as food searching, social interactions and responses to threats, and crowding can lead to problems. The fish species vary greatly in what space they need.

2 Need to have appropriate social interactions

Some fish species shoal for much of their lives and good welfare may depend upon such behaviour. Other species are social for part of their lives or for none of their lives. Some fish need to avoid attacks by conspecifics.

3 Need to avoid predation

Many fish living in natural conditions are very vulnerable to predation. The biological functioning fish of most species is strongly adapted to maximise the chance of recognition of danger from predators and escape from it.

4 Need to feed for maintenance and growth

A variety of nutrients are needed by fish. Fish also need to avoid feed containing dietary toxins and anti-nutrients.

5 Need to maintain good health condition

Fish use various behaviours, anatomical adaptations, physiological responses and immune responses to combat pathogens. They need to avoid any physical or chemical impact that causes tissue damage.

RECOMMENDATION

Since there is evidence in fish for the range of abilities and functions associated with learning and cognition and with affective states such as pain and fear, the welfare of fish should be considered during all aspects of their husbandry.

Fish farming in Europe

World aquaculture has significantly increased during the last fifty years from a production of less than a million tonnes in the early 1950s to 59.4 million tonnes by 2004. Consumption of farmed fish is about 45.5 million metric tons whereas around 60 million tons are wild caught fish from both fresh and sea-water. The 70% of the total aquaculture production comes from the Chinese aquaculture, 22% from the Asian and the Pacific region whereas Europe contributed to approximately 4% of world farmed fish production (FAO;

http://www.fao.org/newsroom/en/news/2006/1000383/index.html).

Nevertheless Europe has the largest production of some species like Atlantic salmon, European sea bass and gilthead sea bream. Currently, Norway is the top producer in Europe, with an annual salmon production of more than 580,000 tonnes representing a 41% of increase in the production rate from 1998 to 2003. Other major producing countries of farmed fish in EEA are Spain, France and Italy. United Kingdom and Greece are also centres of fish farming activity and smaller quantities are produced in several other European countries (Table 1).

Table 1**. Finfish aquaculture production in EEA countries in 2005**

Country	2005	% growth 1995 - 2005
Norway	652306	135.30
United Kingdom	143012	64.50
Greece	80136	268.36
Spain	57346	100.74
France	50352	-23.11
Italy	47642	-27.49
Denmark	38732	-13.41
Poland	36607	45.78
Germany	35130	-22.02

Czech Republic	20455	9.51
Ireland	15384	15.68
Finland	14355	-17.24
Hungary	13661	45.95
Netherlands	8675	213.63
Iceland	8246	136.61
Romania	7284	-63.27
Sweden	4805	-20.20
Portugal	4115	137.31
Bulgaria	2971	-31.70
Austria	2420	-17.07
Cyprus	2315	419.06
Lithuania	2013	17.44
Slovenia	1335	72.04
Switzerland	1214	4.57
Belgium	1200	41.84
Slovakia	955	-40.94
Malta	736	-18.58
Estonia	553	75.56
Latvia	542	3.24
Total	1253283	63.29

(Source: Eurostat, 2008)

European finfish aquaculture species comprises a range of teleosts including salmonids like Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and arctic charr (*Salvelinus alpinus*), sea basses (mainly European sea bass *Dicentrarchus labrax*), sea breams (mainly gilthead sea bream *Sparus aurata*), carps (e.g. common carp, crucian carp, grass carp and silver carp), flatfish like turbot (*Psetta maxima*), halibut (*Hippoglossus hippoglossus*) and sole (*Solea vulgaris vulgaris or Solea solea*), European eel (*Anguilla anguilla*), catfish (*Clarius sp.*) and gadoids like Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) (Table 2).

Table 2. *Yearly production of the main farmed fish species in EEA in tonnes*

Species	1995	2000	2005
Atlantic salmon	347 861	589 606	733 332
Rainbow trout	258 168	286 629	261 805
Gilthead seabream	17 487	58 747	71 475

Common carp	75 000	72 178	69 557
European seabass	17 000	40 869	49 202
European eel	6 819	10 658	8 202
Atlantic cod	317	169	8 115
Turbot	2 978	4 785	6 838
Catfish	1 482	3 640	6 674
Silver carp	8 851	4 909	2 568
Atlantic halibut	-	35	1 445
Grass carp	1 334	1 526	1 090
Arctic charr	531	1 028	905
Haddock			72
Sole	30	23	11

*catfish (Clarius Spp. and Silurius spp)

Source: Eurostat, 2007

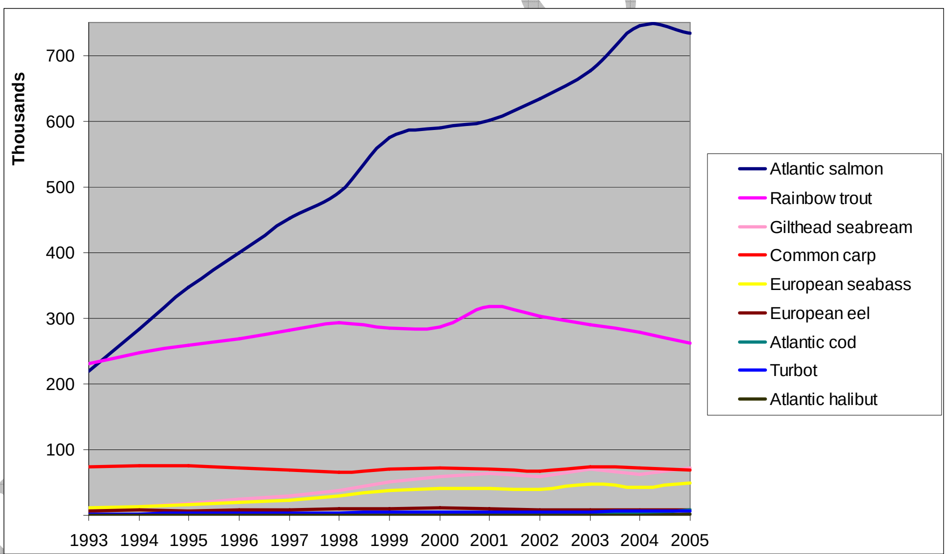

Figure 1: *Yearly production (thousands of tons) of the main farmed finfish species in EEA* (Source: Eurostat, 2007).