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## ■ Water quality and fish diseases

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## ■ Ποιότητα νερού και ασθένειες των ιχθύων

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**ABSTRACT.** Certain water quality parameters are known to cause serious problems to fish, especially when they are living under intensive farming conditions. These problems range from discomfort of fish to heavy mortalities and factors, such as the fish species, the time and level of exposure and the synergic effect of other coexisting stressful conditions, play an important role. In addition, when these parameters are outside the preferable for each fish species range, they can induce stress to fish, compromising their immune system and making them vulnerable to many opportunistic pathogens. Oxygen is, probably, the main limiting factor under farming conditions and when its levels are below the preferable range for any fish species, many morphological, as well as physiological alterations develop. Increased levels of carbon dioxide in the water usually coexist with decreased levels of oxygen and can cause respiratory acidosis and nephrocalcinosis. The latter is characterized by the development of granulomas in many internal organs and urolithiasis. Ammonia usually causes problems under intensive farming conditions, especially when the water is recirculated and the pH is high. Increased levels of ammonia in the water can cause extensive alterations in the gills and degenerative changes in the liver and kidney. Gas supersaturation of the water, depending on the level of saturation and the time of exposure, can result in the gas bubble disease. Formation of bubbles in the eyes, skin and gills and extensive necrotic areas in many organs due to gas emboli are the main findings caused by this disease. Many contaminants in the water can, also, create serious problems to fish. Fin erosion, epidermal hyperplasia or papilloma and degenerative and necrotic alterations in many internal organs are common findings observed in fish on many cases of water pollution. Many of the factors mentioned above, as well as others, such as nutrition and bad management, can result in abnormal development of the body of fish, when exposed at their early life stages. Due to farming conditions, cultured fish tend to exhibit increased rate of body malformation compared to wild ones. Careful design of the facilities, use of specialized equipment and, probably, application of genetic selection program can minimise or even eliminate the effects these water parameters have on the cultured fish.

**Keywords:** water quality, fish, diseases

**ΠΕΡΙΛΗΨΗ.** Είναι γνωστό ότι πολλές παράμετροι της ποιότητας του νερού μπορούν να δημιουργήσουν προβλήματα στην υγεία των εντατικώς εκτρεφόμενων ψαριών. Τα προβλήματα αυτά μπορεί να είναι μια απλή ανησυχία των ψαριών ή ακόμα και εκδήλωση υψηλής θνησιμότητας. Παράγοντες που παίζουν σημαντικό ρόλο στη σοβαρότητα της κατάστασης είναι το είδος του ψαριού, ο χρόνος και το επίπεδο έκθεσης, καθώς και η συνεργική δράση άλλων συνθηκών καταπόνησης που ενδεχομένως συνυπάρχουν. Επιπροσθέτως, όταν αυτές οι παράμετροι βρίσκονται έξω από τα ανεκτά για κάθε είδος ψαριού επίπεδα, μπορούν να προκαλέσουν καταπόνηση σε αυτά, με αποτέλεσμα να εξασθενήσει το αμυντικό τους σύστημα, κάνοντάς τα ευάλωτα σε πολλούς ευκαιριακά παθογόνους οργανισμούς. Το οξυγόνο αποτελεί τον κυριότερο περιοριστικό παράγοντα στην εκτροφή των ψαριών και όταν τα επίπεδά του είναι κάτω από τα επιθυμητά για τα ψάρια όρια, προκαλούνται πολλές μορφολογικές, αλλά και

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φυσιολογικές μεταβολές. Αυξημένα επίπεδα διοξειδίου του άνθρακα στο νερό συνήθως συνυπάρχουν με μειωμένη συγκέντρωση οξυγόνου και μπορεί να προκαλέσουν αναπνευστική οξείδωση και νεφροκαλσίνωση. Η τελευταία χαρακτηρίζεται από ανάπτυξη κοκκιωμάτων σε πολλά όργανα και ουρολιθίαση. Η αμμωνία προκαλεί προβλήματα κάτω από συνθήκες εντατικής εκτροφής και ιδιαίτερα όταν το νερό ανακυκλώνεται και το pH του είναι αυξημένο. Αυξημένα επίπεδα αμμωνίας στο νερό μπορούν να προκαλέσουν εκτεταμένες αλλοιώσεις στα βράγχια και εκφυλιστικές αλλοιώσεις στο ήπαρ και το νεφρό. Ο υπερκορεσμός του νερού με αέρια, ανάλογα με το επίπεδο κορεσμού και το χρόνο έκθεσης, μπορεί να οδηγήσει στο σχηματισμό φυσαλίδων στο δέρμα και τα βράγχια, καθώς και σε νεκρωτικές εστίες σε πολλά όργανα, εξαιτίας του σχηματισμού εμφράκτων λόγω φυσαλίδων στο αίμα. Η ύπαρξη ρυπαντών στο νερό μπορεί, επίσης, να δημιουργήσει σημαντικά προβλήματα στα ψάρια. Διάβρωση των περυνγίων, επιδερμική υπερπλασία ή ανάπτυξη θηλωμάτων, εκφυλιστικές και νεκρωτικές αλλοιώσεις σε πολλά εσωτερικά όργανα είναι συνήθη ευρήματα που παρατηρούνται σε πολλές περιπτώσεις περιβαλλοντικής ρύπανσης. Πολλοί από τους παράγοντες που έχουν αναφερθεί, καθώς και άλλοι, όπως η διατροφή και η κακή διαχείριση, μπορούν να οδηγήσουν στην εμφάνιση δυσμορφιών στα ψάρια, όταν αυτά εκθέτονται σε αυτούς τους παράγοντες στα πρώτα στάδια της ζωής τους. Ο προσεκτικός σχεδιασμός των εγκαταστάσεων, η χρήση εξειδικευμένου εξοπλισμού, αλλά και η εφαρμογή προγραμμάτων γενετικής επιλογής μπορούν να μειώσουν ή και να εξαλείψουν πολλά από τα προβλήματα υγείας που προκαλούν αυτοί οι παράγοντες του νερού.

*Λέξεις ευρητηρίας:* ποιότητα νερού, ψάρια, ασθένειες

## INTRODUCTION

**F**ish in their environment constantly interact with many biotic, as well as abiotic factors present in the ecosystem. When the amounts of dissolved oxygen, carbon dioxide, ammonia or other pollutants in the water are outside the preferable for each fish species range, then the health of fish is affected. These disorders usually cause negligible problems to wild fish, since they have the freedom to move into more favourable areas, whenever the environmental conditions are outside the acceptable range. Cultured fish, however, living in the restricted environment of a fish farm, are more sensitive to environmental factors, which are very often combined with other stressors. Depending on the factor, short-term or/and long-term exposure to these factors can cause high mortalities, especially in the weakest individuals. Stress induced by even the slightest variation of these environmental factors can compromise the immune system of fish and, as a result, opportunistic or pathogenic infectious agents can proliferate within the fish, provoking serious diseases (Huntingford et al. 2006). To many authors, as it will be discussed below, the overall health status of any population of organisms living in a certain aquatic environment is a reliable indicator of the conditions that prevail in the area.

This review summarizes the diseases of cultured fish associated with the main water quality parameters that can cause serious problems to fish under intensive farming conditions. Preventive measures, which can minimise or even eliminate the effects of those parameters, will be briefly discussed.

## REDUCED LEVELS OF DISSOLVED OXYGEN

Dissolved oxygen (DO) is a fundamental factor for the life of all aquatic organisms. In nature, the concentration of DO in the aquatic environment can be reduced by many biotic, as well as abiotic factors. The abiotic factors include: increased water temperature, salinity and reduced atmospheric pressure. On the other hand, the biotic factors refer to increased oxygen consumption by aquatic organisms. In most cases, a combination of both abiotic and biotic factors results in decreased oxygen levels in the water. For example, increased oxygen consumption by aquatic plants (algae) is noted whenever there is increased amount of nutrients in the water and/or intense sunlight (Noga 2000). The algae produce oxygen during the day, but they consume oxygen during the night and this leads to environmental hypoxia early in the morning (Noga 2000). Such hypoxic conditions are very common in the Mediterranean area, during summer, especially in closed lagoons where the water exchange is reduced. Under intensive farming conditions, increased stocking densities of fish can, also, result in increased oxygen consumption by the fish (Pichavant et al. 2001).

According to Fick's diffusion equation (Jobling 1994), oxygen is transferred inside the body of the fish through the gills and the skin. In general terms, the transfer is comprised of three steps: ventilation or water flow past the exchange sites (for example the gills), diffusive transfer between water and blood and, finally, blood flow through exchanging structures (Piiper 1998). In this transfer, the partial pressure of the oxygen

(tension) is more important than oxygen concentrations. Depending on the fish species, below a range of 50-70 % air saturation (which is roughly proportional to oxygen partial pressure in the water), oxygen uptake is affected (Jobling 1994). It should be noted that other factors which can affect the oxygen diffusion are respiratory surface area (gills and skin) and gas diffusion distance. These factors are usually affected by the lifestyle and habitat of fish (Perry and McDonald 1993).

Each fish species has different oxygen requirements. In general terms, prolonged reduced oxygen levels in the water result in many physiological, as well as functional and morphological alterations, such as: lower metabolism, alterations in ventilation rates, changes in circulatory parameters, increased number of circulating red blood cells, decreased levels of intraerythrocytic phosphates, adjustments of intraerythrocytic pH, mobilization of anaerobic energy pathways, changes in acid-base balance, decreased swimming capacity and, in some fish species, morphological adaptations (Medale 1985; Barton and Taylor 1996; Val et al. 1998). Hypoxia as a stressor can, also, influence the immune system (Henrique et al. 2002), making fish more susceptible to infectious diseases. Growth, food consumption and food efficiency can, also, be affected. For example, oxygen level of approximately 40% of saturation can significantly reduce the growth rate of sea bass, probably due to decreased food intake by the fish rather than decreased feed efficiency (Thetmeyer et al. 1999; Pichavant et al. 2001). Increased rate of accumulation of toxicants in the fish due to greater ventilation rate can also be a result of low oxygen levels, as Yediler and Jacobs (1995) showed investigating the accumulation of mercury in carp (*Cyprinus carpio*) exposed to hypoxia. Finally, it has been noted that, in sea bass, the total respiratory surface area is negatively correlated with oxygen availability in the water (Sargolia et al. 2002).

Under farming conditions, regular monitoring of the levels of DO is necessary and whenever it is required, the levels can be increased either by aeration or by direct diffusion of oxygen into the farm water. Using oxygenation, the levels of DO can be higher than the ones achieved when using aeration and thus the stocking capacity of the rearing facilities can increase, as Clark and Helfrich (2006) reported. Interestingly, the same authors, also, concluded that at 90% oxygen saturation there were no significant differences in growth rates,

feed conversion and overall survival, between rainbow trout cultured using aeration or oxygenation.

## NEPHROCALCINOSIS

Nephrocalcinosis has been reported in a wide range of farmed fish species, such as salmonids, halibut and some sparidae (Gómez 2000). It is a chronic condition affecting the kidney and it is characterized by the presence of calcium deposits within the renal tissue.

Although many aspects of the mechanisms via which this condition develops are still unknown, nephrocalcinosis is usually associated with increased levels of free CO<sub>2</sub> in the water (Fivelstad 2003). This usually occurs when fish are reared under intensive conditions in tanks. It should be noted that under these conditions, it is possible that other adverse conditions, also, coexist, such as decreased levels of DO and reduced pH.

It has been suggested (Roberts and Rodgers 2001) that one mechanism that leads to lesions in the kidney could be that the calcium hydrogen phosphate present in the urine can be precipitated, if pH of the urine increases, because its solubility decreases with increasing pH. There is evidence that such a change could occur in fish exposed to elevated CO<sub>2</sub> concentration. However, such a mechanism does not explain the formation of granulomas in the stomach, which are often observed. It is possible that more than one mechanism is involved in the appearance of the disease. For example, in chinook salmon, *Oncorhynchus tshawytscha*, the condition has, also, been connected with high levels of calcium coupled with phosphorous in the diet (Richardson et al. 1985).

The mortalities are usually low with no external signs observed in the affected fish (Harrison and Richards 1979). In some cases, however, the abdomen is distended due to ascites. The organs that are usually affected are the kidneys, the stomach and maybe the muscles (Harrison and Richards 1979). In the kidney, the lesions in most cases include calcium deposition in renal tubules and collecting ducts with little or no damage of the tubular epithelial cells. In severe cases, granulomas and extensive destruction of the renal tissue can be observed. The reported histopathological findings in the stomach include: areas of calcification and granulomas, mainly in the lamina propria, granulosa and submucosa, although the alterations can be occasionally found throughout the wall of the

stomach. Muscle changes are observed only in the most severely affected fish and they consist of calcareous bodies and granulomas, which in some cases occur in small clusters.

It should be noted that the rise in blood partial CO<sub>2</sub> pressure (P<sub>CO2</sub>), as a result of increased CO<sub>2</sub> levels in the water, causes acidosis (Hosfeld et al. 2008). This condition results in reduced oxygen transport to tissues, increased ventilation rate and bradycardia. Reduced food intake and reduced growth, as consequences of water induced hypercapnia, have also been reported. Sublethal effects of carbon dioxide exposure on Atlantic salmon smolts in freshwater include a transient increase in plasma cortisol, transient increase in haematocrit and a sustained reduction in plasma chloride level (Hosfeld et al. 2008).

Since the main factor associated with nephrocalcinosis in fish is the increased concentration of free CO<sub>2</sub> in the water, effective monitoring and removal of excessive CO<sub>2</sub> could prevent this condition. This can be achieved, firstly, by maintaining the fish at low stocking density and, secondly, by increasing the aeration of the water, especially when fish are reared in tanks.

## **DISEASES ASSOCIATED WITH WATER POLLUTION**

In the last decades, many studies have focused on the effects of the increasing pollution of the waters on many aquatic organisms and, of course, fish. Water pollution occurs when various harmful or potential harmful chemicals or particles, originated from industrial, agricultural or residential areas, end up in the aquatic ecosystem through the water or the wind. Many toxic chemicals may adhere to particles, which are then taken up by plankton and benthos animals and thus are bio-accumulated within the marine food chains.

Under farming conditions, in particular, some activities of the farmers may result in accidental short-term or long-term exposure of the fish to various chemicals, which can pose serious risks both to fish and the consumers. An interesting example has been presented by Varvarigos (2007) who reported mortalities due to the use of net antifoulants containing toxic copper compounds. In that case, young fish appeared more sensitive and sea bass more susceptible than sea bream.

Schnitzler et al. (2008) found that cultured sea bass

tends to exhibit a significantly higher amount of pollutants, especially PCBs, in their muscles, on fresh weight basis, compared to wild sea bass. The authors concluded that the use of commercial feeds may play an important role, since, due to increased lipid content of the feeds used, the cultured fish tend to accumulate more fat in their bodies, where the pollutants are usually bio-accumulated. The same authors, also, reported that in the Mediterranean Sea wild, marine animals, such as fish and mammals, tend to exhibit higher concentrations of persistent organic pollutants in their tissues compared to the levels measured in the same species in the Atlantic.

For most contaminants, the effects observed are not related with sex, age, fish age or length (Au 2004). However, some abiotic or biotic factors can play a significant role in the severity of the respective diseases. Stressed fish, for example, generally appear more susceptible to environmental pollution (Vethaak and Rheinallt 1992).

The effects of most types of pollutants on fish and, generally, all the aquatic animals appear to be non-specific and many authors suggested that the study of those effects is indicative of the general quality of the environment (Au 2004). In most cases of environmental pollution, the contaminants affect many organs of the fish simultaneously. Changes in swimming activity and non-specific lesions in the gills, skin and fins are easily observed and are often used as indications of contaminated environments. The most common of these symptoms include fin erosion and epidermal hyperplasia or papilloma. Au (2004) has reviewed the alterations observed in the aquatic organisms and these are summarized below.

In the gills, epithelial hyperplasia and hypertrophy with lamellar fusion, telangiectasia, edema with epithelial lifting and epithelial desquamation are common histopathological findings.

Abnormal development of the operculum and the skeleton has, also, been reported for many substances, such as organochlorine compounds, chlorinated hydrocarbons and pulp mill effluents. In some fish species, as in the northern pike, for example, these abnormal developments can be sex-related.

In the kidney, degenerative changes in tubular epithelium, dilation of tubules, protein or cellular casts within tubular lumen, tubular necrosis and/or epithelial

desquamation and necrosis of interstitial hematopoietic tissues are common findings in a number of cases of marine pollutions.

In the liver, necrotic areas, neoplasms, megalocytic hepatitis, nuclear pleomorphism in the hepatocytes and hydropic vacuolation can, also, be seen and, in some cases, they can be associated with certain seasons, age and sex.

Finally, environmental pollution can affect the quality of the gametes and the development of the embryos (Au 2004).

Guidelines for the safe use of all chemical substances have already been set by many relevant organizations. In some cases, choosing the appropriate area to set up a farm and the detailed planning of the facilities can, also, help with the prevention of accidental exposure of the fish to various pollutants, which can enter the farm via the incoming water or the use of contaminated feeds.

### **GAS BUBBLE DISEASE (GBD)**

This condition is caused by increased pressure of gases in the water. Since the first report on this disease in the late 1800's, much information has been published on the effects of gas supersaturation on cultured freshwater fish species, mainly salmon and trout, but very little on the marine species (Gunnarsli et al. 2008). Lesions can be induced when either the incoming water is supersaturated with gasses or when supersaturation occurs within the farm. According to Noga (2000), possible causes include: trapping in air in pressurized water pipes, use of supersaturated (especially in nitrogen or carbon dioxide) ground water, excessive plant growth, especially in ponds, malfunctioning water pumps and rapid heating of the water. Accidental supersaturation of the water with oxygen can, also, occur when pure oxygen is used to oxygenate the farm water.

Excess nitrogen rather than excess oxygen is usually the cause of gas emboli, although oxygen levels of over 125% of saturation may cause serious problems (Noga 2000). However, it should be noted that today, despite the fact that the use of degassers in many fish farms has minimized the occurrence of the acute form of GBD, the chronic responses of fish to moderate gas supersaturation can still lead to significant losses (Gunnarsli et al. 2008).

Whenever the gas concentration in the water is above 100% of saturation, then diffusion of gases into the swim bladder occurs. However, GBD develops at higher supersaturation. As Colt (1986) reports, the process of bubble formation within the fish tissues may be influenced by many factors, such as surface tension of the blood, number and size of nucleation sites for bubble growth, compliance characteristics of the vascular system, gas diffusion rates through the animals' integument, functional properties of the swim bladder and lesions in the choroidal rete in the eye.

The effects of gas supersaturation on the fish depend on many factors, such as water depth, temperature, fish size, the duration of exposure, the level of supersaturation, the species and the life stage of the affected fish (Colt 1986). For example, it has been reported that, when gas supersaturation of the water occurs, the fish that swim near the surface are more susceptible to this condition, due to low hydrostatic pressure near the water surface. For this reason, higher mortalities are observed in hatcheries, where fish cannot escape to lower depths. Susceptibility to GBD is reduced as the fish grow older and bigger (Gunnarsli et al. 2008). Fastening, also, seems to increase susceptibility of fish to GBD (Colt 1986). In some cases, gas supersaturation in natural waters may be associated with certain seasons, especially with spring and summer (Colt 1986).

Some fish species, for example Chinook salmon (*O. tshawytscha*), have the ability to detect the level of gases in the water and to avoid unsuitable depths with low hydrostatic pressure (Johnson et al. 2005).

Exposure of the fish to moderate supersaturation, depending on the species, may require a long period of time before the first deaths appear. Prolonged exposure to moderate levels of supersaturation (<110% of saturation) can lead to death. The clinical signs include severe inflation of the swim bladder in small fish, ocular lesions, immunosuppression, reduced growth, bubbles in the intestinal tract and reduced swimming ability (Gunnarsli et al. 2008). Especially, the lesions in the eye include development of exophthalmus and subsequent blindness, usually associated with gas embolism within or around the choroid. Behavioural abnormalities, such as hyperactivity and loss of equilibrium due to lesions in the related organs, can, also, occur. The increased susceptibility of the fish to infectious diseases (due to induced stress) and the

presence of necrotic areas in many tissues are considered to play significant role in the resulting mortalities. Oxygen concentration of about 120% of saturation for a week provoked bubble formation in the tissues around the eyes in cultured glass eels *Anguilla anguilla* (Angelidis, personal observations). In that case, the eels were unable to immerge and remained under the water surface, which aggravated their condition. Due to their constant effort to swim deeper, they were exhausted rapidly, refused to eat and died within a few days. In the same case, deaths were, also, observed among the adult eels which were kept in supersaturated water (over 130% of saturation) for about two weeks. The mortality remained low ( $\pm 0.1\%$  per day), but it lasted for almost a month. Histological investigation of the gills showed gill necrosis.

When the level of supersaturation is significantly high, then the fish die quite soon, even within minutes (hyperacute form) or most commonly within a few days, due to anoxia resulting from obstruction in the blood flow (Huchzermeyer 2003).

Histopathological findings of GBD include edema and epithelial degeneration of the secondary lamellae, destruction of the buccal and intestinal mucosa and, in the most chronic forms, degeneration of the tubular epithelium in the kidneys and multiple necrotic areas in many organs, such as liver and brain (Noga 2000).

In order to prevent GBD, constant monitoring of the dissolved amounts of the gases in the water and the use of degassers are strongly suggested. The use of degassers and the construction of reservoirs, where the water can remain for some time, in order for the gases to equilibrate with atmospheric air, are common methods. Some degassers, for example, the column aerators, can remove the excess gases, while increasing the dissolved oxygen to over 91% saturation (Marking et al. 1983).

## AMMONIA POISONING

Ammonia and urea are the two main end-products of nitrogen metabolism in fish (Forster and Goldstein 1969). Ammonia is mainly excreted through the gills and the amount that is excreted from the fish mainly depends on the amount of protein in the feed (Hargreaves and Tucker 2004) and the metabolic efficiency of the fish. Apart from ammonia that is excreted from fish, ambient ammonia usually increases via decomposition of organic matter (i.e. faeces, uneaten feeds, etc), which occurs in the sediment,

especially in ponds. During a process called “nitrification”, various bacteria oxidize ammonia and thus lowering its concentration, in a two-step process, first, to nitrite ( $\text{NO}_2^-$ ) and subsequently to nitrate ( $\text{NO}_3^-$ ). Factors affecting the nitrification rate are: the total ammonia concentration, the water temperature and the available dissolved oxygen (Hargreaves and Tucker 2004). Under intensive rearing conditions and, particularly, when the water is recirculated and the fish are cultured in high densities, ammonia concentrations may reach high levels which can have negative effects on fish survival and growth (Person Le Ruyet et al. 2003).

The un-ionized ammonia (UIA-N) is the toxic form for the aquatic organisms. The  $\text{NH}_4^+$  is considered non-toxic. As Benli et al. (2008) mentioned, the toxicity of UIA-N is attributed to the fact that this form of ammonia can readily pass through the cell membranes in the gills due to its lipid solubility and lack of charge, whereas it is quite difficult for the  $\text{NH}_4^+$  to penetrate the cell membranes of the organisms.

The percentage of total ammonia (TAN) present as UIA-N can be calculated by the aqueous ammonia equilibrium, which is strongly dependent on the temperature, pH and salinity (Lemarié et al. 2004). The high buffer capacity of seawater compared to freshwater tends to keep the pH relatively high, resulting in a larger fraction of the ammonia present in the water as UIA-N (Wajsbrodt et al. 1991).

The sensitivity of fish to increased levels of ammonia is influenced by many other factors. In general terms, sensitivity to ammonia decreases with age (Wajsbrodt et al. 1993). Interestingly, when the  $\text{O}_2$  in the water is depleted through fish respiration, the sensitivity to ammonia decreases, especially when the water exchange is, also, reduced (Tudor et al. 1994). In this case, as  $\text{CO}_2$  increases, pH is lowered and this, in turn, reduces the relative quantity of UIA-N. On the other hand, when environmental hypoxia occurs due to other reasons, then reduced DO increases the sensitivity of fish to ammonia. For example, it has been found that sea bream juveniles, maintained in water with oxygen concentration below 85 % of saturation, exhibited increased sensitivity to ammonia and, below 40 % saturation, increased mortalities were observed within a few hours (Wajsbrodt et al. 1991).

Although there are many studies on the effects of ammonia on freshwater fish after both short and long-

term exposure, there are relatively few studies on the effects of ammonia on marine fish (Lemarié et al. 2004). From these studies, it appears that some fish species are particularly sensitive to ammonia. For example, juvenile sea bass is considered more sensitive than turbot (Person-Le Ruyet et al. 1995). In addition, marine fish seem to be less sensitive to long-term exposure to ammonia than most salmonids (Lemarié et al. 2004).

Wajsbrodt et al. (1993) reported almost no histopathological findings in the gills of sea bream after exposing them to up to 13 mg l<sup>-1</sup> TAN. However, the livers of fish, maintained in this concentration of ammonia, exhibited some degree of atrophy in the hepatocytes, which contained large PAS-positive eosinophilic inclusion bodies. In Nile tilapia (*Oreochromis niloticus*) telangiectasis and hyperplasia of the gill epithelium, accompanied by congestion and haemorrhages, and degenerative changes in the livers and kidneys have been reported (Benli et al. 2008).

We should keep in mind that ammonia can be stressful for the fish even at low concentrations, something that can have adverse effect on the overall performance of fish and, especially, their resistance to diseases (Kalogianni et al. 2003; Lemarié et al. 2004).

Many techniques can control the level of ammonia in the aquatic environment of a fish farm. These include: appropriate stocking density, appropriate feeding regime, use of bio-filters in re-circulating systems, low water pH, which in turn reduces the percentage of TAN as UA-N, addition of ion-exchange materials, such as zeolites and, finally, increased water exchange ratio (Hargreaves and Tucker 2004).

### ABNORMAL BODY DEVELOPMENT

It is, now, well-known that wild-caught fish generally exhibit significantly lower levels of body malformations and almost never severe ones, compared to their cultured counterparts (Boglione et al. 2001). Only wild fish found in heavily polluted areas tend to exhibit increased incident of body malformations. The causes for the various forms of abnormal body development, under farming conditions, are many. Although many steps have been taken to improve the rearing conditions for some marine species whose culture started only a few years ago, the percentage of fish that exhibits such malformations can still be quite high, something that can

have a negative effect on the quality of the produced fish and the production cost. In the previous two decades, the reported incidences of some malformations in reared sea bream and red sea bass were in some cases as high as 90% of the population (Boglione et al. 2001). Since then, many steps have been taken to improve the rearing methods and thus reducing the percentage of deformed fish. For example, the removal of the oily film, formed on the surface of the water of the rearing tanks due to live feeds used in larval rearing, almost eliminated the problem of the lack of inflated swim bladder (Chatain 1997).

Malformations are economically important, firstly, because they require manual sorting and, secondly, due to the fact that the affected fish do not perform as well as the not deformed fish. And, of course, deformed fish have lower market value.

Many authors have studied the relationship between skeletal deformities and various environmental factors, such as light, temperature, salinity, mechanical shock during embryo or larval development, tank currents and type of rearing system. For example, lordosis in sea bass and sea bream, mainly affecting vertebrae 14–15, has been related with the hydrodynamics in tanks, particularly the water current intensity (Chatain 1994). Inadequate light intensity, as well as inappropriate temperature, depending on the species, and salinity in rearing water have been reported as causes of skeletal malformations (Johnson and Katavic 1984). Some genetic factors may, also, play an important role in the appearance of some types of deformities, as Afonso et al. (2000) have observed in certain sea bream families. Nutritional imbalances, such as deficiencies in vitamins, aminoacids or essential fatty acids in the diets of broodstock or larvae, may, also, alter the development of reared larvae (Gapasin and Duray 2001; Fernandez et al. 2008; Roo et al. 2009). Finally, some pathogenic bacteria can induce high rates of malformations, for example *Flavobacterium psychrophilum* (Madsen and Dalsgaard 1999) in farmed rainbow trout (*Oncorhynchus mykiss*).

It should be noted that, in some cases, for reasons not well defined, there is seasonal variation in the appearance of some deformities, for example some opercular malformations in sea bream (Galeotti et al. 2000).

The most deformed fish usually die quickly, but most of the affected fish live for a period of time,

depending on the form of the malformation. Regardless of their form, these malformations lower the performance of reared fish, for example the swimming ability, the conversion index, the growth rate, the survival and their susceptibility to stress and various pathogens. For example, due to absent or deformed operculum, the affected fish are more vulnerable to various bacterial pathogens, which can attach the exposed gills easier (Galeotti et al. 2000).

Malformations can be observed in many areas of the body, such as the head (e.g. deformed jaws, aplastic or deformed operculum, deformed eyes), the spinal column (e.g. lordosis, scoliosis, kyphosis), the fins or internal organs, such as absent or abnormal swimbladder (Andrades et al. 1996; Kingsford et al. 1997; Galeotti et al. 2000; Cahu et al. 2003; Fernandez et al. 2008; Roo et al. 2009). In some cases, the development of certain malformation tends to be correlated with the appearance of some other

malformations, for example the abnormal development of the spinal cord with the absence of functional swimbladder (Andrades et al. 1996), although it should be noted that more factors are involved in the development of spinal deformities (Kihara et al. 2002).

Since most of the malformations are associated with the rearing procedures (i.e. the use of artificial diets and some water parameters), adoption of appropriate farming methods, suitable for each fish species, will reduce the percentage of cultured fish that exhibit such body malformation. When the problem is suspected to be associated with certain broodstock, then the application of a genetic program, which will eliminate some genetic traits, will improve the situation. However, in cases where the exact aetiology is still unknown, extensive research is required to identify the cause. ■

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