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ABNORMALITIES IN FINFISH MARICULTURE: AN OVERVIEW OF THE PROBLEM, CAUSES AND SOLUTIONS

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Abstract

Abnormalities of shape or color and bony / body deformities whether related to lack of swimbladder or not, are still a major problem for many Mediterranean finfish sea farms. Despite their high economical consequences, they remain difficult to eliminate, due to basic gaps of knowledge about their causes and sometimes management errors in the rearing technique.

After a review of abnormalities in aquaculture, the authors targeted the mechanisms of their apparition and aimed to the main gaps of knowledge and the possible solutions. Until accurate cost effective conditions for prevention are found, strategies of early assessment and sorting are recommended. The role of the EU research in these actions is emphasized.

1- Introduction

Abnormalities are natural or induced irreversible deviations from a standard (e.g. morphological, ethological, physiological) of quality. Often due to gaps of knowledge in some critical segments of the rearing processes they are common in intensive aquaculture, especially when the rearing process is relatively new. The economical consequences are important because the abnormalities may downgrade both the image of the product and the biological performances of reared fish, negatively affecting both the market value and the production cost.

In intensive Mediterranean finfish mariculture, the problem of morphoanatomical abnormalities was very important during the 70s and 80s, when the research priorities were targeted more on quantitative rather than qualitative production. Up to 90 % of the production was sometimes discarded. The recent and impressive progress in rearing methods, nutrition and disease control significantly solved many of the problems associated with the scale of production. But paradoxically, it is possible to find solutions only for few morphoanatomical abnormalities. The aim of improving the quality of reared products in order to lower production costs and to meet markets preferences of conformity with wild standards, is a new priority.

The aim of this paper is to review the importance of the problem of morphoanatomical abnormalities in finfish culture, focusing on Mediterranean species, their causes and solutions.

2- Types of abnormalities

The main morphoanatomical abnormalities observed in fish can be summarized into five categories (pigmentation, scales, shape, skeletal, and swimbladder), according to the affected part of the body (Fig. 1). They are reported in both wild and reared species (Table I), with a variable range of expression.

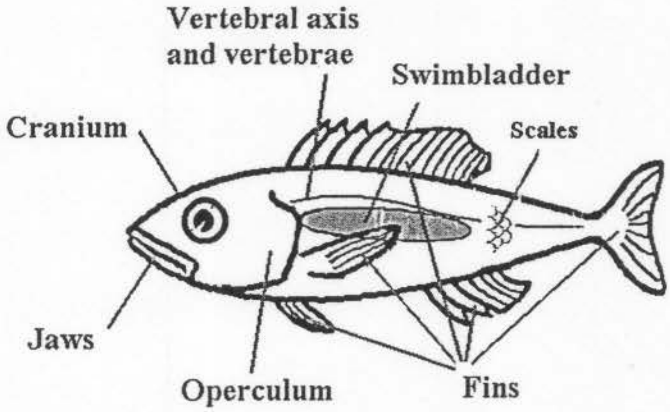

Fig. 1: Main types of abnormalities usually encountered in finfish mariculture.

2.1 Shape

Shape is the first visual criteria of species recognition and the image of wild type is often a requisite for marketing. All fish species, especially farmed *Sparus aurata* and *Dicentrarchus labrax*, may develop shape abnormalities. When the abnormalities are very important such as in dwarfism or Swollen Abdomen Syndrome (body deformations associated to huge vertebral abnormalities), they are so negative that the market for fish cannot be exploited correctly. When light, they are generally ignored. However, in some geographic areas, shape begins to be empirically recognized as a marker of origin and experienced consumers are able to identify the 'wild-type' fish, considering the other types (short and abnormally shaped fish) as reared products of lower quality.

Unfortunately, few studies actually consider this important quality factor. Rearing conditions were proved to induce differences of external morphology in fish (Loy et al. 1996, Corti et al 1996). Shape changes related to different rearing conditions were ascertained by analysis using Bookstein's Shape Coordinates (Loy et al. 1996) and an application of the Thin-Plate Splines Regression Analysis (Corti et Al. 1996). In sea bass, shape was described in different salinity experimental conditions using geometric morphometrics (Loy et al., 1996). External morphological differences between reared and wild individuals were also defined: small heads and prominent ventral profiles characterized the reared bass; while relatively bigger heads and 'rectilinear' ventral profiles were related to the wild ones. As the head dimensions can be imputed to size differences (growth allometry factor), ventral profiles can be considered peculiar to rearing conditions (Loy et al., 1996). In sea bream, the integration of morphoanatomic and morphometric examinations has correlated some shape characteristics to some class of skeletal anomalies: a wider caudal peduncle was associated to hemal and caudal vertebrae anomalies, while characteristic deformations of the postcranial and trunk regions were correlated to lordosis and vertebral fusion in the same regions (Loy 1996, Loy et al., 1996).

Shape deviations often appear during early developmental stages. Divanach (1985) and Matsuoka (1987) reported significant differences in body shape between reared and wild juveniles of *Sparus aurata* and *Pagrus major*, respectively, while in juvenile *Sparus aurata*, Koumoundouros et al. (1994) ascertained differences of allometric growth as a result of different rearing conditions. However, the possible effect of these differences on the shape or biological performance of the bigger fish has not yet been studied.

2.2 Pigmentation

Many species of fish, especially flatfishes, may develop severe pigment abnormalities in wild (Gartner, 1986; Honma, 1990) or farming conditions (Gatesoupe and Luquet, 1982; Seikai 1985, 1987; Fukusho et al., 1987; Sugiyama et al., 1985; Parker and Klar, 1987; Dickey-Collas, 1993; Lagardère et al., 1993; Dedi et al., 1995). These anomalies mainly involve partial or total albinism, ambicoloration and xanthochroism. In cultured *Dicentrarchus labrax* and *Sparus aurata,* such abnormalities are rare, but a few cases of albinism and ambicoloration have been recorded.

Light deviations of pigmentation from the wild standard (which are usually reversible), are more frequent. They involve the melanism of *Pagrus pagrus*, the lack of the bright reddish band on the gill cover and the yellowish inter-orbital band of *Sparus aurata*, the frequently darker pigmentation of *Sparus aurata* and *Dicentrarchus labrax*. As a result of inappropriate rearing conditions (light intensity, limiting food ingredients, diseases, stress, etc.) or special post-harvesting manipulation (use of cold water and/or of ice), these deviations, if not of high intensity, up to now, have not significantly affected the market value of the mediterranean fish.

2.3 Scales

Scale abnormalities are frequent in many fish species, especially carps. They involve absence or loss of scale with or without nacreous-like scaleness body (Yamamoto, 1977), much smaller (Tomita,1992) or bigger scales than the normal size, or abnormal orientation patterns (Browder et al., 1993). In cultured *Dicentrarchus labrax* and *Sparus aurata*, slightly disoriented patterns of the lateral line, often associated with vertebral deformities, are common. In sea bream, loss of scales associated with lymphocystis and/or aggressiveness are known, but not documented. With this species, abnormal reorganization of scales after regeneration is probable (Bereither-Hahn and Zylberberg 1993), but not confirmed.

2.4 Swimbladder

As all physoclistous fish, *Sparus aurata* and *Dicentrarchus labrax* may suffer swimbladder anomalies. The most important for sea farmers is the non inflation of this organ during the larval phase, as the absence of functional swimbladder has high negative repercussions on vertebral / skeletal integrity (Paperna, 1978; Daoulas et al., 1991; Kitajima et al., 1991; Chatain,1994a; Andrades et al., 1996), biological ranges, growth rate, resistance to stress, conversion index and survival (Chatain, 1987; Chatain and Dewavrin, 1989). At the beginning of Mediterranean finfish mariculture in the 70s - 80s, this anomaly was so important that up to 90% of the sea bass and sea bream fry population ought to be sorted and discarded. Nowadays, swimbladder inflation is easily solved by the combined use of surface skimmers (Foscarini, 1988; Chatain and Ounais-Guschemann, 1990; Sweetman, 1992) and appropriate abiotic conditions during the critical stage of development. Furthermore, the small part of larval population with uninflated swimbladder can be easily sorted away by floating test during weaning or pre-growing phase, thus completely eliminating the problem from the ongrowing phase (Chapman *et al.,* 1988a; Chatain and Corrao, 1992; Boglione *et al.,* 1995).

Inflated swimbladder can also follow an abnormal development leading to over-inflation (Swimbladder Stress Syndrome) (Bagarinao and Kungvankij, 1986) under inadequate environmental conditions or stress due to disturbances of abiotic factors (Johnson and Katavic, 1984; Katavic, 1986; Foscarini, 1988), handling and transportation (Carmichael and Tomasso, 1984, in Katavic, 1986). Although, due to its important repercussions on early and high mortality, this syndrome affects the productivity of hatcheries more than the quality of the fry.

2.5 Skeleton

2.5.1 Fins

Fins anomalies are extremely well documented in both wild and reared fish and some of them (mainly in *Carassius* sp.) have been used in ornamental aquaria.

The total or partial absence of fin formation is the most severe deformity of both the dorsal fin (saddleback syndrome of Tave et al., 1983) and anal fin (Hussain, 1979). According to Tave et al. (1983) the saddleback syndrome in *Sarotherodon aureus* may range from the lack of only one spine to the complete lack of dorsal fin, including the pterygiophores associated to the missing spines and rays. Saddleback syndrome has been observed both in wild (Gartner, 1986; Valente, 1988; Browder et al., 1993; Lemly, 1993; Honma, 1994) and reared fish (Tave et al., 1983; Tave, 1986). In sea bream hatcheries these anomalies are noted but not considered severe. In a *Dicentrarchus labrax* hatchery, however, a severe case of saddleback syndrome has been reported (Thomas, 1995). The formation of additional dorsal fin (Ishikawa, 1990; Raadik, 1993), the duplication of dorsal fin (Komada, 1980) and the concave dorsal fin (Matsusato, 1986) are other rarer anomalies of single fins. They have not yet been noticed in bass and bream culture.

Caudal fin deformities are frequent in both reared and wild fish. In aquaculture they involve absence of tail, partial tail (single-lobed), double or triple tail or lobes (Tave et al., 1982; Bondari, 1983; Dunham et al., 1991), formation of extra fin rays (Ishikawa, 1990) and size reduction or roundness of the tips (Mazik et al., 1987). In the wild, they involve absence of tail (Honma and Noda, 1987; Honma, 1990; Honma, 1994) or compression (Lemly, 1993). In reared *Dicentrarchus labrax*, Daoulas et al. (1991) reported the presence of curved rays on single fins. In hatchery reared *Sparus aurata*, the caudal fin anomalies can affect up to 65% of the population (Koumoundouros et al., 1995) and involve compression, duplication or lateral twist. Deformation of caudal fin rays is also reported in *Sparus aurata* with uninflated swimbladder (Paperna, 1978). When not serious, the caudal fin deformities do not negatively affect the market acceptability of the fish. They can however, either induce secondary vertebral deformations or lower the biological performances (growth, conversion index) of the fish due to their effect on the swimming efficiency.

Incomplete formation, lack or asymmetry of the pectoral and pelvic fins, was reported in a wide variety of reared (Komada, 1980) or wild fish (Chandrasekaran, 1979; Berra and Au, 1981; Kusuma and Neelakantan, 1981; Graham et al., 1986; Shrivastava and Desai, 1986; Brown and Scott, 1987; Cabrera et al., 1988; Valente,1988; Blouw and Boyd, 1992; Browder et al., 1993; Lemly, 1993; Dutta and Kour, 1994). In cultured *Sparus aurata* and *Dicentrarchus labrax* such deformities are rare, but Paperna (1978) reported the presence of curved pectoral fins in *Sparus aurata* without functional swimbladder.

All mentioned abnormalities are always accompanied by considerable deformations (fusion, bifurcation, twists, extra formation or abnormal size and shape) of the various elements supporting the fins. However, deformations of these elements are extremely frequent in reared and wild fish, without necessarily relating to macroscopically severe deformities (Gartner, 1986; Matsusato, 1986; Matsuoka, 1987; Daoulas et al., 1991; Marino et al., 1993; Boglione et al., 1993).

2.5.2 Spinal column

Deformities of the spinal column are well documented in a variety of fish species under both rearing (Hickey et al., 1977; Sato et al., 1978; Komada, 1980; Sato et al., 1983; Matsusato, 1986; Soliman et al., 1986; Albrektsen et al., 1988; Zitzow and Millard, 1988; Teskeredzic et al., 1989; Dabrowski et al., 1990; Harris and Hulsman, 1991; Kitajima et al., 1991; MacConnell and Barrows, 1993) and wild conditions (Hickey et al., 1977; Komada, 1980; Berra and Au, 1981; Bucke et al., 1983; Matsusato, 1986; Honma and Noda, 1987; Bengtsson et al., 1988a; Reash and Berra, 1989; Honma, 1990; Lom et al., 1991; Treasurer, 1992; Browder et al., 1993; Lemly, 1993). They involve scoliosis (lateral curvature), lordosis (V-shaped curvature in the sagittal plan) and kyphosis (opposite V-shaped curvature) of the vertebral column and sometimes, in the worst cases, various combinations of these three deformities.

Spinal deformities of various degrees of severity are common in reared *Sparus aurata* and *Dicentrarchus labrax* (Paperna, 1978; Barahona-Fernandes, 1982; Francescon et al., 1988; Daoulas et al., 1991; Saroglia and Scarano, 1992; Balebona et al., 1993; Boglione et al., 1993; Chatain, 1994a 1994b; Boglione et al., 1995; Andrades et al., 1996). Lordosis is always correlated with the absence of functional swimbladder (Chatain 1994a) but this association is not exclusive as Boglione et al. (1995) showed. In many farms, *Dicentrarchus labrax* with functional swimbladder could be affected of a high occurrence of vertebral/spinal abnormalities especially lordosis. The abnormalities of the spinal axis can seriously affect the shape of fish and therefore decrease their commercial value. They also affect their biological performances even when not severe and perfect fish fry from mesocosm hatchery always grow better than their homologues from intensive systems.

The abnormalities of the spinal column are always correlated to abnormalities of a wide range of vertebrae. These abnormalities involve a) dislocation, fusion, shortening, deformation or lack of the centra, b) dislocation, compression, deformation, lack or extra formation of the hemal and neural arches and apophysis, and c) dislocation, shortening, deformation, lack or separation of the ribs (Komada, 1980; Matsuoka, 1987; Dabrowski et al., 1990; Daoulas et al., 1991; Boglione et al., 1993; Boglione et al., 1995; Dedi et al., 1995). The most severe is the extensive fusion of vertebrae which seriously affect the shape of the body. According to Boglione et al. (1993), vertebrae present a higher incidence of abnormality in reared and wild *Dicentrarchus labrax*, than fins or spinal axis. However, when not correlated to severe spinal abnormalities they usually do not decrease the quality of the fish.

2.5.3 Head

Cephalic deformities are frequently found in both hatchery produced (Hickey et al., 1977; Komada, 1980; Soliman et al., 1986; Matsuoka, 1987; Harris and Hulsman, 1991; Lagardère et al., 1993; Mac Connell and Barrows, 1993) and wild fish (Hickey et al., 1977; Silverman and Silverman, 1979; Komada, 1980; Berra and Au, 1981; Honma and Noda, 1987; Honma, 1989; Honma, 1990; Lindesjöö and Thulin, 1992; Browder et al., 1993; Lemly, 1993; Honma, 1994; Lindesjöö et al., 1994). They mainly appear on the gill cover and the jaws. The generally admitted classification are six different types based on morphoanatomic criteria, each one presenting a wide range of expression.

a. Gill cover anomalies are unilateral (generally) or bilateral deformities of the opercular complex bones which consist of reduction, twist or folding of the operculum, suboperculum and sometimes the preoperculum, often associated with malformations of the gill arches (Koumoundouros et al., 1996). They are found frequently in reared *Sparus aurata* and to a lesser extent in *Dicentrarchus labrax* (Paperna, 1978; Barahona-Fernandes,1982; Francescon et al., 1988; Chatain, 1994).

b. Pugheadeness is a deformity which affects skull, jaws and eyes and results in a reduction of the frontal skull and the upper jaw bones. In *Sparus aurata* and *Dicentrarchus labrax* it was reported by Barahona-Fernandes (1982), Daoulas et al. (1991) and Chatain 1994b).

c. Crossbite is a deformity in which the lower jaw is skewed off center or displaced laterally so that it appears bent, or crossed and not oriented parallel to the upper jaw. According to Hickey et al. (1977) two different types of crossbite (full crossbite and semi-crossbite) can be defined in respect to the orientation of the axis of deformity. Crossbites were reported in *Sparus aurata* and *Dicentrarchus labrax* by Barahona-Fernandes (1982), Daoulas et al. (1991) and Chatain (1994b).

d. Lower jaw reduction is a deformity leading to a head appearance close to the full crossbite of Hickey et al. (1977). In *Sparus aurata* and *Dicentrarchus labrax* it was reported by Barahona-Fernandes (1982) and Chatain (1994b).

e. Ventrally projected hyobranchial skeleton is a deformity which locks the gill covers in an open position due to their anatomical relationships. There is no bibliographic report of the occurrence of this deformity in *Sparus aurata* and *Dicentrarchus labrax,* but in some Greek and Italian farms we observed it mainly in sea bass (Personal observations).

f. Pike Jaw Deformity (PJD) is a deformity mainly reported in wild *Esox lucius* (Lindesjöö and Thulin, 1992) which involves the upward bending of the anterior part of the parasphenoid and the frontal bones. In *Sparus aurata* jaw deformities were also reported by Francescon et al. (1988) and Polo et al. (1991), but apart from the official pre-mentioned five categories.

Cephalic anomalies strongly affect the morphological appearance of the commercial fish and lower their market value. They also often decrease the respiratory efficiency of the on-growing fish. In reared *Sparus aurata* they decrease the survival and the growth rates of the fish.

3- Known causative factors

3.1- Swimbladder inflation

Although not totally understood, the cause of this abnormality is the best known. Larvae of physoclistous fish initially inflate their swimbladder with an air bubble transferred through the digestive tract and the pneumatic duct, which later degenerates (Steen, 1970). This process cannot be performed too late or too early (Kitajima et al., 1981 in Chatain and Ounais-Guschemann, 1990; Battaglene and Talbot, 1990; Bailey and Doroshov, 1995). In *Sparus aurata* and *Dicentrarchus labrax* normal swimbladder development is performed in two stages, initial inflation (4.0-5.0 mm and 5.5-6.5 mm total length - TL, respectively), and expansion (12 mm TL to 40-50 mm TL) (Chatain, 1986).

Swimbladder inflation depends on many factors. It requires the access of the larvae to the water-air interface and the subsequent gulping of air. This operation is restricted by the presence of oily films on the water surface (Kitajima et al., 1981 in Bailey and Doroshov, 1995; Chapman et al., 1988; Foscarini, 1988; Battaglene and Talbot, 1990; Chatain and Ounais-Guschemann, 1990; Battaglene et al., 1994) and subsequently by all factors generating oily films, mainly feeding conditions. It depends also on larval vigor (Kitajima et al., 1991; Tandler et al., 1995) and on abiotic factors such as salinity (Chapman et al., 1988b; Battaglene and Talbot, 1990, 1993 in Bailey and Doroshov, 1995; Tandler et al., 1995), light intensity (Weppe et Joassard, 1987; Battaglene and Talbot, 1990; Battaglene et al., 1994), photoperiod (Ronzani Cerqueira and Chatain, 1991), water turbulence (Foscarini, 1988; Chatain and Ounais-Guschemann, 1990; Battaglene and Talbot, 1993) and temperature (Hadley et al., 1987). Unfavorable ranges of these conditions and/or factors which restrict the larval access to the water surface may inhibit initial swimbladder inflation.

3.2 Other abnormalities

The causes of other anomalies are less understood. Phenotype is a result of genome, environment and 'developmental noise' (Scheiner, 1993). It is quite obvious that diversities in the genetic pool can determine variations in the developmental pattern. Developmental noise is a factor which can theoretically induce phenotype differences in genetically identical individuals developing in identical environments. In this context, morphological variability in a genetically related population can supply a 'size' of the developmental noise. In this regard, Soulè (1982) maintains that an augmentation of the phenotypic variability is a characteristic of biologic systems subjected to stress (like intensive rearing conditions, for instance) and that the developmental noise reveals itself as a reduction of the intracellular order. The second factor, the environment, includes the influences exercised by external conditions, such as biotic and abiotic factors.

Abnormalities can be induced during the embryonic and post-embryonic periods of life through mechanisms not yet well understood. Evidence is also emerging that anomalies can be induced during the larval periods (Cataudella et al., 1996, Koumoundouros et al. 1994, 1995, 1996). Notochord deformations during larval development were in part responsible for the formation of spinal deformities in juvenile and on-growing *Sparus aurata* (Andrades et al., 1996). Many factors causing anomalies by altering the biological process of ontogenesis in fish have been investigated. The most significant causative agents of osteological, shape and pigmentation defects in fish can be divided into two principal groups: epigenetic and genetic factors. Among epigenetic factors, environmental parameters are considered, such as:

1. abiotic determinants (light intensity, pH, O_2, CO_2, radiation, salinity, temperature, turbulence or flow rate of water, ammonia ions, tank volumes,...)
2. biotic determinants (nutritional requirements, stocking density, handling, hormonal or photothermic stimulation, parasites, bacterial and viral infections, algal biotoxines, mechanical trauma,...)
3. xenobiotic determinants (algaecides, fungicides, herbicides, insecticides, industrial effluents, heavy metals, organochlorine contaminants, pesticides, oils, ...).

Each intrinsic factor of genetic origin (mutation, hybridisation, inbreeding) or connected to the regulation of gene expression which could be responsible for impaired organogenesis of anatomical structures and therefore for teratological malformations in fish, are considered as genetic factors. Furthermore, because too many etiological factors have been suspected but no demonstrated, it is difficult to single out 'one cause-one effect', especially in the large variety of fish species affected by anomalies.

In wild populations, the unusual high frequencies of malformations in some geographic areas are considered by many to have been caused by environmental factors, such as pollution. Therefore, the frequency of observed abnormalities could be considered as useful index of pollution (Carls and Rice, 1990; Dahlberg, 1970; Haya, 1989; Lindsejöö *et al.*, 1994; Sloof, 1982, Weis and Weis, 1989; Westernhagen *et al.*, 1988; Whittle *et al.*, 1992; Wiegand, 1989).

It is generally accepted that skeletal deformities can be environmentally induced in two ways: (a) by alteration of biological processes necessary for maintaining the biochemical integrity of bone, or (b) neuromuscular effects, which lead to deformities without a chemical change in vertebral composition. Among the xenobiotic substances, heavy metals are suspected to cause skeletal anomalies: in particular, Cd, Pb, Zn and Cu which induce scoliosis, lordosis, deformed jaws, reduced or absent fins, shortened operculum in *Abramis brama* (Sloof, 1982). Exposure of Pacific herring eggs to 10 ppm cadmium results in a decrease of four important enzymes (NAD- and NADP-malic enzymes, propionyl-CoA-carboxylase and phosphoenol-pyruvate carboxykinase) (Mounib *et al.*, 1975). Aquaculturists should pay a particular attention to the effects of algaecides and anti-fouling substances. The former is described by Hickey (1973), as inductors of structural abnormalities in embryos, meanwhile the latter contains a certain percentage of heavy metals or of cytotoxic substances as tributyltin (TBT) (Fent, 1992). Cadmium and zinc can affect bone biochemical composition by interfering with mineralization and can also affect neuromuscular activity. Mercury and lead primarily affect the neuromuscular system. Lead possibly competes for sites normally occupied by other divalent ions, such as calcium (Valentine, 1975). Deformed jaws, fins, vertebrae and vertebral column are often observed in fish showing a high concentration of heavy metals in muscle and liver (Bengtsson *et al.,* 1988c). While the concentrations of these elements in marine fishes are not well documented, their presence can certainly be inferred from the high concentrations found in various marine sediments (Valentine, 1975).

In reared fishes, most authors suspect the influence of biotic requirements on anomalies onset, particularly nutritional deficiencies. Diets with different amount of aminoacids, minerals, phospholipids, PUFA and vitamins have been tested to reduce frequency of anomalies in reared fish, but no clear results have been obtained. For instances, vertebral axis deviations (scoliosis, kyphosis and lordosis) frequencies have been reduced by increasing (in different experiments) dietary quantities both of tryptophan, or phospholipids, or Vitamin C or by diminishing Vitamin A quantities. Shortened operculum occurrence was high both in *Ictalurus punctatus* (Lim and Lovell, 1978) and in *Ciclasoma urophtalmus* (Chaves De Martinez, 1990) nourished with insufficient quantities of Vitamin C, but the same anomaly has been also ascribed to genetic factors in *Salmo gairdneri* (Aulstad and Kittelsen, 1971), to heavy metals (Doimi, 1989; in *Abramis brama*, Sloof, 1982), to industrial effluents in *Paralabrax nebulifer* (Meade *et al.,* 1969), to insecticides (Sloof, 1982), to insufficient quantities of fatty acids and vitamins in *Dicentrachus labrax* larval diets (Barahona-Fernandez, 1978) and rearing conditions (*Sparus aurata*, Cataudella *et al.,* 1996). Despite the above suggestions, it can be stated that the mechanism underlying the opercular abnormalities is still unknown.

Vitamin A excess provokes lordosis and scoliosis (Dedi et al., 1995). Vitamin C is essential for collagen formation, takes part in transformation of folic acid in folinic acid, in noradrenaline and some hormones (oxytocin and antidiuretic) production. In organisms, it assumes the function of antioxidant agent (Goodman et al., 1992) and seems to have some role in immunologic activity (Li and Lovell, 1985). In particular, dietary essentiallity of ascorbic acid (Vit. C) results from absence of the enzyme L-gluconolactone oxidase which is required for biosynthesis of ascorbic acid (Soliman et al., 1986). Reduced quantity of vitamin C is considered the cause (beyond the above described operculum reductions) of deformed caudal complex (Agrawal and Mahajan, 1980), of dental deformations (Agrawal et al., 1978), of deformed gills, spines and fins (Andrew and Murai, 1975), of deformed ribs and vertebral spines (Barahona-Fernandez, 1978), fused vertebrae (Spanò, 1996), scoliosis

Table 1: Causative factors for anomalies in Mediterranean marine reared fishes.

Cause	Author	Species	Effect
Alimentary deficiencies			
Generic	De Veen, 1969	*Platessa platessa*	depigmentation
	Dickey-Collas, 1993	*Pleuronectes platessa*	depigmentation
	Lagardère et al., 1993	*Solea solea*	jaws and pigmentation anomalies
	Robin et al, 1996.	*Dicentrachus labrax*	shortened opercles/abnormal swim bladder
Micropellets	Menu, 1994	*Dicentrachus labrax*	vertebral anomalies
	Menu, 1994	*Sparus aurata*	Jaws anomalies
	Modica et al., 1993	*Sparus aurata*	urinary calculi and abnormal swimbladder
lack of live algae	Barahona-Fernandez, 1978	*Dicentrachus labrax*	anomalie larvali
PUFA	Barahona-Fernandez, 1978	*Dicentrachus labrax*	absence of swimbladder
	Dickey-Collas, 1993	*Pleuronectes platessa*	pigmentation
	Koven et al.,1990	*Sparus aurata*	absence of swimbladder
Vitamin A (defect)	Robin et al, 1996.	*Scophthalmus maximus*	depigmentation
Vitamin C (defect)	Barahona-Fernandez, 1978	*Dicentrachus labrax*	skeletal anomalies
	Chatain, 1994	*Dicentrachus labrax Sparus*	skeletal anomalies
	Godeluc, 1983	*Dicentrachus labrax*	lordosis and scoliosis
	Spanò, 1996	*Sparus aurata*	fused vertebrae
Vitamin D (excess)	Chatain, 1994	*Dicentrachus labrax Sparus*	skeletal anomalies
Abiotic parameters			
generic	Weppe and Bonami, 1983	*Dicentrachus labrax Sparus*	abnormal swimbladder (tumour)
low rate of water supply	Sugiyama et al., 1985	*Pleuronectiformes*	albinism and reversal larvae
Light	Barahona-Fernandez, 1978	*Dicentrachus labrax*	larval anomalies
	Johnson and Katavic, 1984	*Dicentrachus labrax*	Gas bubble disease
	De Veen, 1969	*Pleuronectiformes*	abnormal pigmentation
Salinity	Lee and Menu, 1981	*Mugil cephalus*	deformed vertebral axis
	Johnson and Katavic, 1984	*Dicentrachus labrax*	Gas bubble disease
Temperature	Bertolini et al., 1991	*Dicentrachus labrax*	notochordal anomalies
	De Veen, 1969	*Pleuronectiformes*	abnormal pigmentation
	Marino et al., 1991	*Dicentrachus labrax*	notochordal anomalies
	Johnson and Katavic, 1984	*Dicentrachus labrax*	Gas bubble disease
	Polo et al.,1991	*Sparus aurata*	cranial and vertebral anomalies
	Shelbourne, 1968	*Pleuronectiformes*	anomalous pigmentation
Hydrodynamic conditions	Chatain,1994	*Dicentrachus labrax, Sparus*	lordosis
Turbulence	Kentouri,1985	*Sparus aurata*	vertebral deformation
Genetic factors			
Hybridization	Barahona-Fernandez, 1982	*Dicentrachus labrax*	combined cranial and spinal anomalies
Xenobiotic substances			
Heavy metals	Johnson and Katavic, 1984	*Dicentrachus labrax*	Spinal anomalies and urinary calculi
Various causes			
inducted by other anomalies	Chatain, 1987 and 1994	*Dicentrachus labrax, Sparus*	lordosis associated to absence of swimbladder
	Paperna, 1978	*Sparus aurata*	lordosis associated to absence of swimbladder
	Tesseyre, 1979	*Dicentrachus labrax*	lordosis associated to absence of swimbladder
Density	Devauchelle and Chopin, 1982	*Dicentrachus labrax, Sparus*	notochordal deviations, bicephaly,
	De Veen, 1969	*Pleuronectiformes*	abnormal pigmentation
	Lagardére et al., 1993	*Solea solea*	jaws and pigmentation anomalies
Oil films	Chatain,1986, 1989	*Dicentrachus labrax, Sparus*	abnormal swimbladder
	Chatain and Ounais-Guschemann,	*Sparus aurata*	abnormal swimbladder
Rearing conditions	Barahona-Fernandez, 1978	*Dicentrachus labrax*	larval anomalies
	Boglione et al., 1994	*Dicentrachus labrax*	skeletal anomalies
	Divanach, 1985	*Sparus aurata*	shape
	Cataudella et al., 1995	*Dicentrachus labrax*	skeletal anomalies
	Cataudella et al., 1996	*Dicentrachus labrax, Sparus*	skeletal anomalies
	Loy et al., 1995	*Dicentrachus labrax*	skeletal anomalies
	Loy, 1996	*Dicentrachus labrax*	prominent ventral profile
	Divanach et al. 1996	*Dicentrachus labrax*	lordosis
Environmental factors	Barahona-Fernandez, 1978	*Dicentrachus labrax*	asymmetric anomalies
Unknown factors	Andrades et al., 1996	*Sparus aurata*	lordosis
	Balebona et al., 1993	*Sparus aurata*	lordosis
	Daoulas et al., 1991	*Dicentrachus labrax*	swimbladder, vertebral anomalies
	Francescon et al., 1988	*Sparus aurata*	cephalic, opercle and vertebral anomalies
	Giavenni and Doimi, 1983	*Dicentrachus labrax*	abnormal swimbladder
	Koumoundouros et al., 1995;	*Sparus aurata*	Tail abnormalities
genetic?	Paperna, 1978	*Sparus aurata*	skeletal and swimbladder (tumour) deformity

Furthermore, it has been suggested that detoxification process in fish might result in competition for vitamin C between collagen metabolism in bone and enzyme systems such as mixed-function oxidases (MFO), involved in the detoxification or metabolization of organic chemicals in the liver (Mayer *et al.*, 1978; Street *et al.*, 1971; Wagstaff and Street, 1971). Consequently a change in the biochemical compositions of bones might result from disturbance of the detoxification process. The hypervitaminosis D is considered the cause of urinary *calculi* by Roberts and Shepherd (1986) but Modica *et al.*. (1993) identified the cause in an excess of calcium and phosphorus in enriched *Artemia* nauplii or in the water. In the same study, *calculi* were found to be made of apatite crystals, $Ca_5(PO_4)_2$, linked to Mg, Cl and S. Vitamin K seems also to be implicated in the onset of skeletal malformations (Roberts and Shepherd, 1986).

The importance of highly unsaturated n-3 fatty acids for marine fish larvae has been widely demonstrated (Koven *et al.*, 1992, Watanabe, 1983; Rodriguez *et al.*, 1994; Cataudella *et al.*, 1995; Chavez *et al.*, 1995) and various publications also suggest the importance of n-6 fatty acids (Bell *et al.*, 1992; Castell *et al.*, 1994; Henderson *et al.*, 1985; Lie *et al.*, 1992; Robin, 1995), with a specific incorporation of arachidonic acid in phosphatidylinositols. In lipids of marine fish, n-3 polyunsaturated fatty acids (PUFAs) are predominant, with a ratio of n-3/n-6 PUFA of 10 to 15:1 (Ackman, 1980). Watanabe (1982) has reviewed the EFA requirements of fish. Compared with freshwater fish, marine fish have a poor capacity for elongation and desaturation of both linolenic and linoleic acids (Kanazawa *et al.*, 1979). For these animals, the requirement of highly unsaturated fatty acids of the n-6 series has not yet been defined. It is quite difficult to study as it is presumed to be relatively low. The crucial role of polyunsaturated fatty acids is in the build-up of membranes structures and temperature acclimation. The anomalies most frequently attributed to insufficient quantity of PUFA in fish diet are the not-activated swimbladder (in *Dicentrachus labrax*: Barahona-Fernandez, 1978; in *Sparus aurata*: Koven *et al.*, 1990), anomalous pigmentation (in *Pleuronectes platessa*: Dickey-Collas, 1993) and skeletal deformities (in *Sparus aurata*: Koven *et al.*, 1990; Watanabe, 1983; in *Clupea harengus*: Navarro and Sargent, 1992). On the other hand, in other experiments no effects of recommended doses of PUFA have been observed on frequency of skeletal anomalies in *Sparus aurata* (Spanò, 1996).

Others biotic requirements are less cited causes of fish deformations. Protozoa infections (Myxosporidian) have been detected in *Perca fluviatilis* with spinal curvature (Langdon, 1987), with compressed vertebrae and lordosis (Lom et al., 1991), or with scoliosis (Treasurer, 1992) and in *Pomoxis annularis* with scoliosis (Baumann and Hamilton, 1984). Parasites cysts have been isolated in malformed vertebrae (Treasurer, 1992) and spores of *Triangula percae* have been found in brains of deformed fish (Langdon, 1987). The association between infection and spinal curvatures led the authors to consider Myxosporids as the agents of these anomalies.

Stocking density, manipulation, mechanical trauma and different rearing conditions are other biotic causes invoked by many authors. The latter, in particular, have been evidenced in *Sparus aurata*, mostly as operculum anomaly (Cataudella et al., 1996), and in the sea bass it is possible to define hatchery-specific anomalies (Boglione et al., 1994). The presence of an oil film at the interfaces air-water has been invoked as the cause for abnormal swimbladder, as already described above. High turbulence and low water change flow can cause lordosis (Backiel et al., 1984; Chatain, 1994b; Kentouri, 1985). High currents during the weaning stage can induce lordosis in sea bass with functional swim bladder (Divanach et al. 1996).

It is well known that abiotic parameters induce skeletal anomalies. Temperature and salinity different from the species optimal range are considered to cause anomalous jaws, vertebral axis, vertebrae and fins, and depigmentation in Pleuronectiformes. Evidences are emerging of a malformative effect of photo and thermo-period induction of reproduction in *Sparus aurata*: larvae show the absence of caudal complex (including caudal vertebrae) and augmented skeletal deformities frequencies (Boglione, pers. com.). Oxygen depletion, excess of carbondioxyde, acidity and radiation could be also considered as malformation inductors.

Evidences of inheritance of skeletal anomalies are quite rare. Rosenthal and Rosenthal (1950) proved that lordosis is the result of recessive alleles. Other evidences in favor of genetic origin of some anomalies are given by Gordon (1954) and Nelson (1971) and in Japanese sea bream *(Pagrus major)* by Taniguchi et al., 1984. Lodi (1978) considered fused vertebrae in *Poecilia reticulata* as inheritable. In *Salmo salar*, a specific spinal deformity was found to be genetically inheritable by McKay and Gjerde, 1986; in the guppy *(Poecilia reticulata*) fused vertebrae are due to a new dominant autosomal gene (palla, P1) (Lodi, 1978) meanwhile in the same species 4 types of vertebral curvatures have been recognized, which proportionate incidences indicated the possibility of high inbreeding level. The strain differences suggested the existence of polymorphism of a malformed gene(s) (Ando et al., 1995). Furthermore, in zebrafish *(Danio rerio)* the no-tail mutant phenotype is caused by a mutation in the zebrafish homologue of the murine T gene (Schulte-Merker, 1995). The eye deformity (ey-1) and fused vertebra (fu-6 and fu-7) mutants were found in the medaka (*Oryzias latipes*) by Tomita (1991). A homozygotic morphogenetic mutant (Da) in medaka develops ventralization of dorsal counterparts in the caudal region (Ishikawa, 1990). An autosomal recessive lethal mutation in tilapia (*Oreochromis niloticus*) caused the caudal deformity syndrome (CDS) consisting of an upward curvature of the spine in the caudal region with varying degrees of reduction of the caudal fin (Mair, 1992). Genetic factors have often been cited as the causes of caudal fin deformation (Andrades et al., 1996; Aulstad and Kittelsen, 1971), as well as lordosis and fused vertebrae (Baumann et al., 1984; Berra and Au, 1981; Moore et al., 1976; Taniguchi et al., 1984; Tomita, 1991).

4- Elements of solutions

In respect to the present state of knowledge on the causes of fish abnormalities, four concrete types of actions are proposed to overcome this problem in intensive fish mariculture:

4.1 Adequate rearing conditions

This obvious action remains essential. At every stage of culture, the application of the "bio-logics" of rearing (Divanach 1985), are a requisite for the integration of the complex biological conditions of life and harmonious development. The initial adequacy site - objective, the further attempt to match the technical/human supply to the biological demand, the respect of biological ranges, seasonal cycles and biorhythms, the avoidance of stress, are basic elements of this strategy. Intensification has to be thought in a new context. With well mastered species such as *Dicentrarchus labrax* and *Sparus aurata*, whose intensive culture is easy, attempts to break records of intensification can result in errors of strategy which can lead to total loss of batch quality. With the new and unwell known candidate species for aquaculture, the use of the mesocosm hatchery technique which leads to mass production of high quality products, is a new cost effective hatchery tool (Dhert et al., 1996, Papandroulakis et al., 1996).

The main recommended actions for prevention of abnormalities are reported in the table 2 .

Table 2 .Recommended actions for prevention of abnormalities in fish culture

Rearing phase	Recommended actions / Important factors
At every phase	1- Quality of water / site. Clear breeding environment from pesticides fertilizers, metallic ions, hypochlorite and other pollutants 2- Respect of biological ranges / Avoidance of stress conditions
Broodstock maintenance	1- Inbreeding prevention 2- Diet quality (HUFA, Vitamins, etc) and quantity
Incubation/ prelarval stage	1- Transportation at low density 2- Avoidance of salinity, temperature and mechanical shocks 3- Use of low light intensity 4- Quality assessment and elimination of poor quality batches
Larval rearing	1- Appropriate conditions of swimbladder inflation 2- Enrichment of rotifers and Artemia with essential fatty acids and vitamins 3- Avoidance of salinity, temperature and mechanical shocks 4- Avoidance of water turbulence and high currents 5- Quality assessment and elimination of poor quality batches
Weaning and pregrowing	1- Avoidance of forced swimming 2- Enriched Artemia / fresh and well balanced artificial diets 3- Selection and elimination of abnormal fry 4- Quality assessment and subsequent verifications
Ongrowing	1- Fresh and well balanced diet 2- Monitoring of pollution

4.2 Early detection/evaluation of abnormalities

Whatever the gaps of knowledge on causes are, the negative effects of abnormalities in farms could be significantly reduced by early identification and accurate evaluation of their potential impact so as to provide elements of decision for possible rapid elimination of the batch. Some laboratories in Europe are well equipped for this purpose and can provide services to check quality of fry and postulate on their future performances/quality.

The methods currently used for quality assessment of the fish in commercial hatcheries are: 1) the direct morphological examination of transparent larvae under stereoscope or by naked eye. The disadvantage of these methods is that they cannot be accurate early enough especially for skeletal spinal deformations which may occur during weaning. The direct morphological examination of the fish is appropriate for detection of the externally observable abnormalities, but the skeletal deformities can not be examined if they are not fully developed, or of a low severity. 2) In toto staining, as for example the double staining with alcian blue and alizarin red S (Simons and Van Horn 1971, Dingerkus and Uhler 1977) insures an accurate and fast detection of skeletal deformities when just originating (Marino et al. 1993; Koumoundouros et al., 1996), even during the first month and still of low intensity

(Koumoundouros et al. 1994 and 1995). The application of this method however requires precise developmental and anatomical knowledge i.e. highly experienced staff. Furthermore its higher cost and time requirements make it unsuitable after metamorphosis when other cheaper methods are possible. 3) The soft X ray radiographic / photographic analysis is a very accurate method for evaluation of swimbladder and skeletal abnormalities, but its application is possible only after fish metamorphosis (late weaning and pre-growing phase) when the skeleton is completely ossified. Furthermore, it must always be combined with the external examination of the fish, for detection of either skeletal deformities which are not apparent on the lateral projections (i.e., opercular deformities, crossbite, and scoliosis), or pigment and scale anomalies.

More accurate methods of fry quality assessment by analysis of specific morphological characteristics (Boglione et al 1993) which may be automated by image analysis (Wimberger 1993, Naudin et al 1996), would allow every farmer to build up its own data base and check its production. Although morphometry represents the method for the direct evaluation of shape divergence from the wild standard, its application is inappropriate at the beginning of deformities when their effect is not yet fully expressed. Further standardization of this method could significantly lower its high cost and time requirements.

4.3 Accurate and easy methods for sorting of abnormal animals

When identified, abnormal fish have to be sorted before commercialization. Until now, the only existing method for easy, quick, cheap and massive elimination of abnormal fish is the floating test after anesthesia in 55 - 70 ppt saline water used for selection of fish with functional swimbladder (Chapman et al., 1988a; Chatain and Corrao, 1992; Boglione et al., 1995). All the other abnormalities require individual hand grading. If the batches with a low rate (less than 10 %) of very visible anomalies (such as tail, opercular complex, or heavy lordose) can be sorted rather easily and cheaply during the flotation test, those with a medium rate (40 - 60 %) of low visible anomalies such as stage 2 and 3 of vertebral deformation (Divanach et al 1996) present a heavier economical impact. They need careful observation by transparency with low efficiency of 2-4 thousand fish/hour/man, for efficient sorting.

Some prototypes of sorting devices based on the differences of behavior / metabolic resistance between normal and abnormal fish already exist (such as the difficulty to swim against strong water current for fish with tail/fin abnormality, the sensibility to anoxia for fish with opercular complex abnormality or absence of swimbladder, the sensibility to low temperature for fish with heavy lordosis, etc). But further research is needed to develop more accurate techniques of separation. However, the total automation of sorting by use of computerized image analysis that do not match with a delimited number of quality criteria, would be a far more significant progress.

4.4 Better knowledge of the determinism of the abnormalities

Whatever the quality of sorting techniques, it is not satisfactory that Mediterranean finfish producers continue to discard part of their production. The prevention of abnormalities at hatchery level is a more ambitious and realistic objective. As the exact determinism of many abnormalities remains unknown despite the variety of possible active factors, the definition of their causal organization is the first requisite. A Cartesian cost-effective research strategy could be developed in 5 steps: 1) etiological field studies with extensive multi-parametric monitoring, to screen the circumstances of apparition (or the absence) and to pinpoint the predisposing factors of abnormalities in the wide range of rearing conditions provided by the commercial/experimental Mediterranean hatcheries; 2) identification of the prodroms (ie the early symptoms) to define both the earlier moments of apparition (ie the important stages / conditions to be investigated) and the possibilities of reversibility at this moment; 3) elaboration of a scenario for a possible causal organization; 4) realization of specific experimentation to test the validity of the previous hypothesis; 5) definition of the more cost-effective technology to apply the result at a commercial level.

As this research implicates multidisciplinary approaches in a wide geographic range of Mediterranean regions, European concerted actions /collaborations will be necessary.

5 Conclusion

Abnormalities in Mediterranean finfish mariculture are a complex problem with many concausative factors, and are generally due to gaps of knowledge about their development. But in many cases they are also due to insufficient management and/or lack of "biotechnologic" in the rearing strategy. Basic and applied research, control of the environmental quality of sites, assessment of the quality of techniques and fish, transfer of results and know-how, are the necessary tools for a solution.

Research targets should focus on the following topics: 1) Determination of the standards of quality and of a scale (gradient) of abnormalities; 2) Development of cheap, quick, simple and accurate methods for mass selection of abnormal fish. 3) determination of the prodroms and of the earlier stages affected; 4) Identification of the causative agents/organization.

Morpho-anatomy is one of the most important tool for this quality assessment within a number of disciplines. The application of a multidisciplinary approach will accelerate the improvement of this sector reducing the cost of the production. As the market demand tends towards wild phenotypes, fish shape analysis could be an important new component in the setting up a rational quality control system. The use of mesocosm hatchery techniques to produce juveniles with wild shape phenotype could complete this set of tools and allow producers to meet the preferences of consumers.

As the problem is Mediterranean, its fast solution implies collaborative action and net work between specialized laboratories. As a consequence, the role of the EU research in the harmonization, organization and financing of such research programs is primordial.

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