Chapter 8
Skeletal deformities and juvenile quality

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Chapter contents

Abstract: Mass rearing of Sparidae juveniles is at present a reality, but relatively low survival rates (20–30%) and suboptimal quality too often characterize commercial juvenile production. Therefore, one of the bottlenecks of the Sparidae aquaculture industry is the presence of morphological deformities, which result in major economic losses due to mortalities, reduced growth, or unmarketability of the final product. Deformed fingerlings need to be manually selected and eliminated, but current sorting methods do not eliminate completely the presence of deformities in the ongrowing phase, and further sorting is needed before fish are marketed for food. Different types of skeletal deformities, body malpigmentation, malformed scales or lateral line, anomalous shape, abnormalities in the olfactory organ, eye cataract, and inflammation are reported in cultured Sparidae fishes. The causes reported for these morphological anomalies are many and only partially understood. Basically, there are as many causative factors as there are biological fields: genetic, nutritional, physiological, physical, chemical, biomolecular, and environmental causes have all been blamed for inducing pigmentation or skeletal anomalies in fish but, at the end, it is still possible to state that the same typology of skeletal anomaly may be due to different causes, but also that different causes may produce the same type of anomaly. Up to now, the available data seem to indicate that anomalies are the consequence of so many influential and interacting factors, that interdisciplinary studies combining anatomic, genetic, and biomolecular data, with physiologic data on larval welfare conditions will probably be necessary.

Key words: causative factors; developmental anomalies; pigmentation; scales; shape; skeletal deformities

8.1 Introduction

Mass rearing of Sparidae juveniles is necessary to ensure a predictable supply of high-quality fish for the ongrowing phase of commercial aquaculture. However, relatively low survival rates (20–30%) and often

suboptimal quality characterize juvenile production of many cultured species. Therefore, one of the bottlenecks of the finfish aquaculture industry is the presence of morphological deformities, which result in major economic losses due to mortalities, reduced growth, or unmarketability of the final product. Juveniles with skeletal deformations are manually selected and eliminated, and as many as 15–50% of gilthead sea bream (*Sparus aurata*) juveniles with deformities are culled out of the productive cycle at the end of the hatchery phase (Boglione *et al*. 2001). Nonetheless, current sorting methods do not eliminate the presence of deformities in the ongrowning phase, and further sorting is needed before fish are marketed for food. Body pigmentation that differs from the wild also represents a problem in some Sparidae, including the red porgy (*Pagrus pagrus*) (Van der Salm *et al*. 2004), red sea bream (*Pagrus major*) (Watanabe 1993) and gilthead sea bream (Devauchelle and Chopin 1982). Malformed scales or lateral line (Tomita 1992; Bereiter-Hahn and Zylberberg 1993; Browder *et al*. 1993; Andrades *et al*. 1996; Corrales *et al*. 2000; Carrillo *et al*. 2001; Jawad *et al*. 2008), anomalous shape (Divanach *et al*. 1996; Loy *et al*. 1999; Verhaegen *et al*. 2007), abnormalities in the olfactory organ (Mana and Kawamura 2002), eye cataract, and inflammation (Carrillo *et al*. 1999) are also reported in both wild and cultured Teleosts.

As mentioned above, malformations are economically important, not only because they require manual sorting, but also because they lower the performance of deformed fish, for example, swimming ability, feed conversion ratio, growth rate, survival, and susceptibility to stress, pathogens or bacteria (Balbebona *et al*. 1993; Andrades *et al*. 1996; Divanach *et al*. 1996; Hilomen-Garcia 1997; Koumoundourous *et al*. 1997b; Boglione *et al*. 2001, 2003; Cahu *et al*. 2003; Matsuoka 2003; Lall and Lewis-McCrea 2007; Le Vay *et al*. 2007; Castro *et al*. 2008). Furthermore, a cultured fish having a different shape or coloration than its wild counterpart induces diffidence in consumers and may prevent commercialization. The occurrence of deformed juveniles varies greatly depending on the specific farm and the rearing methodology applied: in gilthead sea bream juveniles, obtained from the same egg batch but reared under intensive or semi-intensive conditions, very different percentages of individuals have been observed to be affected by severe cephalic and vertebral axis anomalies, which affect the external shape of the fish (Boglione *et al*. 2001). The incidence of individuals with at least one skeletal anomaly can exceed 80% in hatchery-reared gilthead sea bream (Andrades *et al*. 1996; Beraldo *et al*. 2003; Verhaegen *et al*. 2007). Matsuoka (1987) reported that specimens with either abnormal bone or abnormal fin pterygiophore arrangements reached 94 and 100% in two hatchery-reared groups of red sea bream. Boglione *et al*. (2003) reported occurrences of individuals with at least one skeletal malformation ranging from 88 to 100% in reared sharpsnout sea bream (*Diplodus puntazzo*) compared to 19–21% in wild individuals, and 44–80% in reared common pandora (*Pagellus erythrynus*) juveniles compared to 12–24% in wild animals. Also, Boglione *et al*. (2005) reported 83 and 100% of deformed individuals in reared red porgy and common dentex (*Dentex dentex*), respectively. About 27% of gilthead sea bream larvae at hatching showed different types of axial deformation related to notochord alterations during embryogenesis. Finally, about 22% died soon after hatching, but 5% survived and reached juvenile and adult stages (Andrades *et al*. 1996).

Developmental anomalies are reported also in wild Sparidae (Boglione *et al*. 1993, 1994, 2001, 2003; Tsukamoto 1993, 1997, 1999; Uchida *et al*. 1993; Kingsford and Atkinson 1994; Koumoundouros *et al*. 1997a, 1999, 2001c, Koumoundouros 2008; Carrillo *et al*. 2001; Palma *et al*. 2001; Mana and Kawamura 2002; Favaloro and Mazzola 2003, 2006; Matsuoka 2003; Sfakianakis *et al*. 2005; Kouttouki *et al*. 2006; Verhaegen *et al*. 2007; Le Vay *et al*. 2007), as a consequence of the teratogenic effects of some xenobiotics (Table 8.1) or toxins produced by other marine organisms (Table 8.2), radiation, temperature, or salinity variations (Table 8.3). However, up to now, these deformities occur much less frequently compared to cultured fish (above-cited authors, and Marino *et al*. 1993; Santamaria *et al*. 1994; Hosoya and Kawamura 1998; Gavaia *et al*. 2002; Beraldo *et al*. 2003; Kause *et al*. 2007; Castro *et al*. 2008). According to Tave and Handwerker (1994), animals with deformations have difficulty surviving under wild conditions, and the hypothesis of a severe selection pressure against malformed

Table 8.1 Xenobiotic (epigenetic) factors reported as causes of skeletal anomalies in fish

Causative factor	Description	References
Antibiotics	ATB enrofloxacin (a fluoroquinolone antibiotic) causes skeletal anomalies in fry and parr of salmons	Gil-Martens *et al*. 2009
Hormones	Estrogens (17α-ethynylestradiol EE$_2$) regulate bone cells involved in bone homeostasis	Oursler *et al*. 1993
	Mummichog, *Fundulus heteroclitus*, exposed for 60 days to 17α-methyltestosterone (MT) showed manly lordosis, scoliosis, anal swelling, jaw, facial (wider or bent upward) and head abnormalities (bump on top of the head), haemorrhaging, and varying degrees of malformed fins and fin rays, with the caudal fin being longer and squarer than the usual rounded shape. Up to 92.1–100% abnormal fish in the 10,000 ng/L group, with an average of 3.67 (2.54–4.79) abnormalities compared to only 1.15 (0.73–1.57) in the 1000 ng/L MT-treatment group, and only one abnormal fish in the control group	Boudreau *et al*. 2005
	Hormones, especially estradiol-17β, are important elements in mobilization and/or utilization of Ca^{++} via scale resorption, as shown in juvenile and maturing rainbow trout, *O. mykiss*. Estradiol-17β induces osteoclastic activity associated with the scales whose cells have the E2-estradiol receptor	Hall 2005
	Skeletal development was affected significantly in 17α-ethynylestradiol-exposed fathead minnow (*Pimephelas promelas*): vertebral malformations were observed in up to 62% of fish in a non monotonic dose–response	Warner and Jenkins 2007
Heavy metals	*Fundulus heteroclitus* embryos exposed to inorganic mercury at concentrations of 0.03 or 0.1 mg/L at the early blastula stage, showed unsuccessful axis formation and a significant proportion of cyclopic embryos developed	Weis and Weis 2006
	F. heteroclitus embryos which developed in lead at concentrations of 1 and 10 mg/L were normal in appearance until hatching, at which time they exhibited lordosis or were unable to uncurl from the position they had while still inside the chorion	Weis and Weis 2006
	Affect bone development	Fernández and Gisbert 2009
Herbicide	Herbicides affect bone development in fish	Fernández and Gisbert 2009

(*Continued*)

Table 8.1   (*Continued*)

Causative factor	Description	References
Metals	Short Selenium exposition of *Pogonichthys macrolepidotus* during somite development provoked pericardial oedema and deformities of skeletal tissues (loss of tail, lordosis, scoliosis, and kyphosis), dysplasia, hyperplasia, and metaplasia of skeletal tissues in the deformed fish	Teh *et al*. 2002
	Manganese and zinc supplementation in *Artemia* caused vertebral deformity in red sea bream (*P. major*) larvae	Nguyen *et al*. 2008
	Effect of zinc or manganese enrichment in Artemia on red sea bream (*P. major*): reduced growth; short-body dwarfism, disturbance of bone formation; anorexia, cataracts, fin erosion; reduced Zn content in body and bone, reduced bone Ca; high mortality	Nguyen *et al*. 2008
PCB	Polychlorinated biphenyls (PCB) affect bone development in fish	Fernández and Gisbert 2009
	PCB may be a cause of bone anomalies in fry and parr of salmons	Gil-Martens *et al*. 2009
Pesticides	Pesticides affect bone development in fish	Fernández and Gisbert 2009

fish in the wild has been proposed (Andrades *et al*. 1996; Koumoundouros *et al*. 2001c; Favaloro and Mazzola 2003), but not yet demonstrated.

The problem of skeletal deformations in cultured fishes takes on greater importance, considering that effective methods for avoiding their onset and for culling out the affected individuals are not yet available. Even severe skeletal anomalies may actually occur throughout the entire life cycle and their onset is often masked, since it begins with slight aberrations of the internal elements, which then develop into more severe abnormalities affecting the external body shape (Divanach *et al*. 1997; Koumoundouros *et al*. 1997b, 2001c, 2002; Loy *et al*. 1999, 2000; Boglione *et al*. 2001, 2003; Sfakianakis *et al*. 2003, 2004, 2006; Gjerde *et al*. 2005). The methods currently used in hatcheries to cull out deformed juveniles differ as regards to two main deformation typologies: swim bladder and skeletal anomalies. Individuals with defective swim bladder are easily sorted, as they do not float when anaesthetized in hypersaline (60 psu) medium. In the case of skeletal deformities, obviously only individuals with externally detectable anomalies can be identified for culling by manual sorting after anaesthesia. Currently, the commercialization of juveniles for ongrowing farms entails x-raying a sample lot, just to prove the presence of normally inflated swim bladders and the absence of externally undetectable skeletal deformations. However, skeletal deformities—and consequently shape anomalies—can develop all along the entire fish life cycle. Cataudella and Boglione (1996) screened, for skeletal deformities, two lots of gilthead sea bream juveniles from different hatcheries, before stocking and after 15 months in sea cages, under the same rearing conditions in the same farm. In both lots, an increase was observed in older individuals of the number of deformities on each malformed individual, a lowering of the incidences of several severe deformities, and the appearance or augmentation of others (fusions among cranial or prehemal vertebrae,

Table 8.2 Various factors reported in literature as causes of skeletal anomalies in fish

Causative factor	Description	References
Association with other anomalies	Skeletal malformations have been associated with noninflation of the swim bladder	Chatain 1994a; Jacquemond 2004
	D. labrax, *S. aurata*: lordosis associated to absence of swim bladder	Chatain 1987; 1994a
Idiopathic causes	In *D. labrax,* swim bladder, vertebral anomalies	Daoulas *et al*. 1991
	Lordosis in *S. aurata*	Balebona *et al*. 1993
	As lordotic gilthead sea bream larvae are present before swim bladder inflation, then, in addition to noninflation of the swim bladder, other causes during embryonic development may be implicated in the appearance of lordosis and other abnormalities	Andrades *et al*. 1996
	Some skeletal malformations are thought to originate during the labile egg and larval phase, becoming progressively evident in older individuals	Norcross *et al*. 1996
	A substantial variability was noted between the replicas and a lack of statistical importance between the results obtained, probably due to the multiplicity of factors influencing bladder inflation	Martin-Robichaud and Peterson 1998
Mechanical factors	Physical damage caused by wall-nosing of larvae and postflexion larvae may be responsible for breakages at the symphysial joint, observed in jaw malformation of postflexion striped trumpeter larvae. Wall-nosing has been associated with tank color and light conditions in larval rearing tanks, and modified lighting conditions, including increased use of green water culture, may resolve this problem in striped trumpeter culture	Bristow and Summerfelt 1994
	Development of "gaping jaws" in *H. hippoglossus* larvae appears to be related to abrasion of the head by contact with the rearing container, which should not be as significant a factor when the larvae are reared in large silos as when they are reared in small containers. It is also related to penetration of the eroded tissues by organisms present in the water, which would probably not normally be pathogenic	Morrison and MacDonald 1995
	Growth-generated strains and pressures plays a role in limb morphogenesis	Henderson and Carter 2002
	There is a relationship between muscle growth and bone growth	Parfitt 2004

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Table 8.2 (*Continued*)

Causative factor	Description	References
	The mineralization process during endochondral ossification is stimulated by mechanical loads	Tanck *et al*. 2004
Pathogens, parasites	Flexibacter, *Myxobolus cerebralis salmons* is considered a causative factors of skeletal deformities in fry and parr of salmons	Gil-Martens *et al*. 2009
	Pathogens such as the myxosporidian *Myxosoma sp.* may induce congenital or postnatal skeletal deformations in fish	Treasurer 1992
	In walleye (*Stizostedion vitreum*) larvae, delayed swim bladder inflation could be due to infection by bacterial aerocystitis in the swim bladder resulting from the ingestion of contaminated surface films	Marty *et al*. 1995
	Bacterial invasion of physically damaged oral membranes in Atlantic halibut has been associated with jaw malformation in cultured larvae	Morrison and MacDonald 1995
	The 4.8% of severely deformed rainbow trout affected by the disease *rainbow trout fry syndrome* (RTFS), caused by the bacterium *Flavobacterium psychrophilum,* indicate a possible role of *F. psychrophilum* in the aetiology of the observed deformities	Madsen and Dalsgaard 1998
	Diagenean trematode (likely *Apophallus donicus*) cysts in the body were directly associated with 86.5% of 592 primary precaudal deformities detected in chiselmouth (*Acrocheilus alutaceus*). Infected wild fish with a *Myxobolus* sp. (likely *Myxobolus cyprini*) exhibited a high incidence of vertebral deformities (70–93%): extra spines, lordosis, fused vertebrae, and increased vertebral density. Confirmation obtained by exposure to *Apophallus donicus* under controlled laboratory conditions	Villeneuve *et al*. 2004
	Parasites can affect bone development	Fernández and Gisbert 2009
Toxins	The dietary uptake of one suite of dinoflagellate-produced neurotoxins, commonly called paralytic shellfish poisoning (PSP) toxins, is known to cause acute fish kills. Morphological abnormalities were observed in zebrafish (*D. rerio*) larvae following exposures to sublethal, dissolved saxitoxin (STX), a PSP: uninflated swim bladder, pronounced dorsal curvature of the anterior–posterior body axis and craniofacial deformities in larvae with severe pericardial and yolk sac oedema. Oedema and dorsal body curvature were not observed in control larvae	Lefebvre *et al*. 2004

Table 8.3 Environmental (epigenetic) factors causing skeletal anomalies in fish

Causative factor	Description	References
Broodstock and egg quality	Brood age, nutritional and health status, husbandry are some of the causes of spinal deformities in salmons	Gil-Martens *et al*. 2009
	Maternal effect, egg quality have effects on bone abnormalities in Atlantic salmon	Wargelius *et al*. 2009
Density	In *Solea solea*: dentigerous and shape-modified premaxillae and mandibles on the ocular side and lack of pigmentation on parts of the ocular side of the head	Lagardére *et al*. 1993
	In common sea bream (*P. pagrus*), high stocking densities may also cause unnatural pigmentation	Van der Salm *et al*. 2004
	Opercular deformities, pectoral and pelvic fins, epural fusion anomalies in dusky grouper reared at higher densities (28 larvae/L)	Boglione *et al*.
	Affects bone development	Fernández and Gisbert 2009
	High rearing densities in fry and parr of salmons	Gil-Martens *et al*. 2009
Fast growth conditions	Scoliosis and caudal abnormalities in Japanese flounder (*P. olivaceus*) appeared in fish that were subjected to high temperatures to accelerate growth	Hosoya and Kawamura 1998
	Hypertrophic vertebrae are a result of accelerated organogenesis while the body maintains a normal size, in Atlantic halibut	Lewis and Lall 2004
	Vertebrae of fish with a low mineral content were more compressed than those of fish with a high mineral content. This was related to an increase in the angle between the wall of the cone of the vertebrae and the cranial–caudal axis, which occurred around transfer to seawater. The fish with a low mineral content grew slower than the fish with a high mineral content	Fjelldal *et al*. 2007
	Fast growth in salt water on smolt 0+ Atlantic salmon	Gil-Martens *et al*. 2009
	Fast growth conditions (light treatment, high water temperature, and feed administration) augmented bone abnormalities in Atlantic Salmon	Wargelius *et al*. 2009
Generic environmental factors	Certain deformities arise especially in captivity because of the artificial rearing and management systems	Leatherland and Woo 1998
	Possible role of environmental factors in the aetiology of spinal deviations in fish	Carls and Rice 1990

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Table 8.3 (*Continued*)

Causative factor	Description	References
	Environmental factors, such as water temperature, density of eggs, heavy metal contamination, or hydrodynamic conditions have also been cited to cause malformations during development	Faustino and Power 1998; Kranenbarg *et al*. 2005a; Sfakianakis *et al*. 2004
	Gross and micromorphological abnormalities in the olfactory systems of red and black sea bream from cultured (cage) stocks are a consequence of certain procedural condition(s) in mariculture facilities	Mana and Kawamura 2002
	Spinal deformity shows a higher degree of environmental sensitivity in cod, *Gadus morhua*	Kolstad *et al*. 2006
	Gilthead sea bream: no significant heritabilities were observed for neither lordosis or lacking operculum, and only a weak indication of genetic control was revealed for lacking operculum. Therefore, the emphasis should be put on environmental variables to control them at farm facilities	Castro *et al*. 2007
	The occurrence of developmental disorders in farm animals should be controlled by means of improved management practices	Kause *et al*. 2007
	Most phenotypic variation observed for lordosis and lack of operculum in gilthead sea bream is due to environmental factors	Castro *et al*. 2008
	Incubation and rearing temperature; manipulation, handling and treatment in fry and parr of salmons	Gil-Martens *et al*. 2009
	O_2, hypersaturation, high CO_2, low flow rate in fry and parr of salmons	Gil-Martens *et al*. 2009
	Rearing conditions in Atlantic salmon	Wargelius *et al*. 2009
Handling stress	Grading and transport can cause bone anomalies in fry and parr of salmons	Gil-Martens *et al*. 2009
Hydrodynamism/ water turbulence/ water suppy rate	Swim bladder inflation in Australian bass *Macquaria novemaculeata*	Battaglene and Talbot 1990
	Swim bladder inflation in *S. aurata*	Chatain and Ounais-Guschemann 1990
	Lordosis in *D. labrax* and *S. aurata*	Chatain 1994a
	High currents during the weaning stage can induce lordosis in sea bass with functional swim bladder	Divanach *et al*. 1996
	Swim bladder inflation depends also on water turbulence	Divanach *et al*. 1996
	Lordosis induction in juvenile red sea bream, *P. major*, by high swimming activity	Kihara *et al*. 2002
	Vertebral deformations in lordotic sea bass	Kranenbarg *et al*. 2006

Table 8.3 (*Continued*)

Causative factor	Description	References
	In *Dentex dentex*, skeletal deformities might be affected by tank hydrodynamics	Gimenez and Estevez 2008
	Affect bone development	Fernández and Gisbert 2009
	Increased larval motility due to high water current undoubtedly affects the loading regime on the bones. In *Danio rerio*: increased larval activity accelerated the onset of ossification in the tail fin skeleton. In sea bass (*Dicentrarchus labrax*) increased mechanical loading affects bone formation: lordosis appeared to change the local loading regime of vertebrae which in turn resulted in a change in bone formation	Kranenbarg *et al*. 2009
Intensive conditions	Shortened operculum occurrence in *S. aurata*, has been also ascribed to intensive conditions	Cataudella and Boglione 1996
	Common dentex larvae in extensive and intensive: significant differences were observed in the incidence of skeletal deformations, both for percentage of deformed larvae and for some deformation types such as those related to vertebral column and to the caudal complex. Initial larval density, initial prey density and quality, and tank hydrodynamics are the factors suspected to affect skeletal deformities	Gimenez and Estevez 2008
Light	Illumination is among the culture factors which can induce malpigmentation in flatfish	Yamamoto *et al*. 1992
	Swim bladder inflation depends on light intensity and photoperiod	Battaglene and Talbot 1990; Battaglene *et al*. 1994; Ronzani Cerqueira and Chatain 1991
	Skeletal malformations in Atlantic halibut larvae	Bolla and Holmefjord 1988
	Australian bass larvae had higher inflation rates when held under dark conditions, while continuous light exposure almost totally inhibited inflation	Battaglene and Talbot 1990
	Exposure to fluorescent light can cause the staining form of hypermelanosis in juvenile Japanese flounder	Seikai 1991
	Deformations in Teleost larvae are attributed to inappropriate light intensity and spectra	Liu *et al*. 1994
	Shorter photoperiods resulted in higher inflation rates in striped bass larvae, which have temporary physostomous swim bladders	Martin-Robichaud and Peterson 1998

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Table 8.3 (*Continued*)

Causative factor	Description	References
	Environmental factors, such as light have been reported to be involved in the origin of neurocranial, spinal and apendicular aberrations in fish	Cobcroft *et al*. 2001; Sfakianakis *et al*. 2004; 2006
	Affects bone development	Fernández and Gisbert 2009
Oil film	Swim bladder inflation is restricted by the presence of oily films on the water surface and subsequently by all factors generating oily films, mainly feeding conditions	Battaglene and Talbot 1990; Chatain and Ounais-Guschemann 1990; Battaglene *et al*. 1994
	The presence of an oil film at the interfaces air–water has been invoked as the cause for abnormal swim bladder	Divanach *et al*. 1996
O₂/CO₂	Oxygen depletion and excess of carbondioxyde could be considered as malformation inductors in fish	Divanach *et al*. 1996
	Hypoxic conditions induce centrum defects in red sea bream *P. major*	Sawada *et al*. 2006; Hassell *et al*. 2008
	Affects bone development in fish	Fernández and Gisbert 2009
pH	Affects bone development in fish	Fernández and Gisbert 2009
Mechanical stress	Mechanistic explanation of vertebral deformations in lordotic sea bass	Kranenbarg *et al*. 2006
Radiation	Affect bone development in fish	Fernández and Gisbert 2009
Salinity	Swim bladder inflation depends on salinity	Battaglene and Talbot 1990; Tandler *et al*. 1995
	Salinities different from the species optimal range are considered to cause anomalous jaws, vertebral axis, vertebrae and fins, and depigmentation in Pleuronectiformes	Divanach *et al*. 1996
	Skeletal malformations in Atlantic halibut *Hippoglossus hippoglossus*	Bolla and Ottesen 1998
	Skeletal malformations in black bream *Acanthopagrus butcheri*	Haddy and Pankhurst 2000
	Salinity have been reported to be involved in the origin of neurocranial, spinal and apendicular aberrations in fish	Cobcroft *et al*. 2001; Sfakianakis *et al*. 2004; 2006
	Adequate salinity during larvae rearing largely dictate the successful transformation of larvae to juveniles during metamorphosis which, in turn, determines juvenile quality	Koven 2003
	Affects bone development in fish	Fernández and Gisbert 2009
Substratum	Hatchery-reared juvenile Atlantic halibut in tanks provided with sandy substrata had the lowest occurrence of pigment abnormalities	Ottesen and Strand 1996

Table 8.3   (*Continued*)

Causative factor	Description	References
	In hatchery-reared juvenile Atlantic halibut inability to bury is significantly more important than light exposure in causing blind-side hypermelanosis	Iwata and Kikuchi 1998
Tank characteristics	In walleye, wall-nosing (whose consequence is jaw malformation) has been associated with tank color and light conditions in larval rearing tanks, and modified lighting conditions, including increased use of green water culture, may resolve this problem in striped trumpeter culture	Bristow and Summerfelt 1994
	Improved inflation rates resulted when larvae were reared in black tanks in striped bass	Martin-Robichaud and Peterson 1998
	In red porgy, white backgrounds, particularly in combination with high densities (i.e., during transport), might interfere with the (down-)regulation of the HPI axis activity normally associated with prolonged stressful conditions	Rotllant *et al*. 2003
	Affect bone development in fish	Fernández and Gisbert 2009
Temperature	Jaw malformation in cultured Atlantic halibut larvae due to inappropriate incubation temperature of eggs and yolk-sac larvae	Pittman *et al*. 1990; Lein *et al*. 1997
	Temperature different from the species-specific one induces developmental anomalies in sea bass (*D. labrax*) embryos and larvae	Bertolini *et al*. 1991
	Temperature variation induces cranial and vertebral anomalies in *S. aurata*	Polo *et al*. 1991
	Temperature different from the species optimal range is considered to cause anomalous jaws, vertebral axis, vertebrae and fins, and depigmentation in Pleuronectiformes	Divanach *et al*. 1996
	Scoliosis and caudal abnormalities in Japanese flounder (*P. olivaceus*) appeared in fish that were subjected to high temperatures to accelerate growth	Hosoya and Kawamura 1998
	The timing of initial swim bladder inflation is temperature dependent in striped bass	Martin-Robichaud and Peterson 1998
	Effects on development, survival, and deformations in Atlantic halibut larvae are attributed to inappropriate temperature	Ottesen and Bolla 1998
	Deformations in striped trumpeter (*Latris lineata*) larvae are attributed to inappropriate temperature	Bermudes and Ritar 1999
	Skeletal malformations in tilapia, *Oreochromis mossambicus*	Wang and Tsai 2000

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Table 8.3   (*Continued*)

Causative factor	Description	References
	Temperature effects on the origin of neurocranial, spinal, and apendicular aberrations in fish	Cobcroft *et al*. 2001; Sfakianakis *et al*. 2004; 2006
	A number of studies have shown that manipulating temperature during larviculture can result in higher quality juveniles	Koven 2003
	Rearing temperature influenced the occurrence of metamorphosis-related morphological abnormalities in spotted halibut	Aritaki *et al*. 2004
	In brown sole *Pseudopleuronectes herzensteini*, four morphological types (normal, two pseudoalbino types, and ambicolorate fish) varied according to the rearing temperatures	Aritaki and Seikai 2004
	75% of total observed abnormalities, mainly in the area of the caudal fin, were observed in *P. erythrinus* reared at the higher of the tested temperature regimes (23°C)	Sfakianakis *et al*. 2004
	Temperature effects higher than the water current effect on lordosis in sea bass	Sfakianakis *et al*. 2006
	In sea bass, the frequency of fish with abnormal branchiostegal rays and fluctuating asymmetry of the branchiostegal rays was significantly higher when embryonic and larval phase was conducted at 15°C than at 20°C water temperature	Georgakopoulou *et al*. 2007
	Affects bone development in fish	Fernández and Gisbert 2009
	Higher temperature (16 vs. 10°C) deformed vertebrae in Atlantic Salmon: also affected vertebrae changed (16°C: mainly affecting vertebrae from 36 to 51th; 10°C: 37–45th)	Wargelius *et al*. 2009

deformed prehemal vertebrae, lordosis in hemal vertebrae and dislocation of glossohyal). Furthermore, in one lot the dental prognathism, initially affecting 29% of juveniles, was observed in 57% of market-size sea bream after 15 months. Therefore, sorting practices carried out at the end of the hatchery phase do not guarantee the absence of skeletal deformations in ongrowing fish.

8.2   Morpho-functional ontogenesis in Sparidae

Skeletal differentiation represents a fundamental event in larval ontogenesis: bones and cartilages provide support for the body, protection for major organs, a storehouse for calcium and phosphorus, and host the metabolic regulation of mineral homeostasis, as well as provide a means of attachment for ligaments and muscles (Hall 2005). One central principle of studies in ecomorphology is that morphology limits the performance or

capabilities of an individual and, hence, its patterns of resource use (Arnold 1983; Long 1995; Wainwright 1991; Costa and Cataudella 2006; Antonucci *et al*. 2009). For these reasons, studies carried out on the ontogenetic pattern of skeletal elements involved in locomotion (vertebral column and fins) and feeding (splanchnocranium), indirectly allow information to be gleaned on the swimming and feeding capabilities of larvae and juveniles of reared species. Furthermore, as skeletal anomalies in reared juveniles are almost exclusively found in the trunk, fins and splanchnocranium, the study of the development of these skeletal elements could represent a useful tool for the early identification of possible anomalies during larval development. Consequently, knowledge of the skeletogenic pattern of reared species could represent an important contribution to the optimization of larval rearing since (a) it would be useful to know when and which skeletal structures involved in swimming (vertebral column and fins) and feeding (splanchnocranium) are available for fish during their larval development, (b) it could help if potential critical developmental phases could be identified, and (c) it would extend our knowledge of the biology of a reared species.

In fish, there are many different calcium-containing tissues (Hall 2005). Consequently, the mineralization process of the various bones in fish is different: in the class Actinopterygii, the most advanced of the bony fishes (such as Sparidae), the two main types of bone formation are the chondral or substitute bone, and connective tissue or dermal or membrane bone (Harder 1975), which are indistinguishable when fully developed. A number of intermediate types also exist, and in the same structure (e.g., viscerocranium, vertebral axis) different bones may have a different ontogenetic development (Table 8.4). Unfortunately, the current literature on skeletal development in reared Sparidae refers only to skeletal elements, and not to histological differentiation. In fish, different bones are present (Table 8.5), which differ in the original germ layer, mode of histogenesis and in evolutionary history (Hall 2005). As many as five mineralization processes are described in fish, but existing literature on Sparid skeletogenesis refers only to two general types: cartilage replacement bone, which has a cartilaginous precursor, and dermal bone, which develops within or just beneath the skin without any cartilaginous precursor, and no differences have been reported between dermal and membrane bone, for instance. A greater understanding of the development of cartilage and bone would be important in determining factors and conditions that perturb these processes. In Tables 8.6 and 8.7, data on different bones in red sea bream (Matsuoka 1985), common dentex (Koumoundouros *et al*. 2000, 2001a), common pandora (Sfakianakis *et al*. 2004; Boglione *et al*. 2006), gilthead sea bream (Koumoundouros *et al*. 1997a; Faustino and Power 1998, 1999, 2001; Saka *et al*. 2008), white sea bream (*Diplodus sargus*) (Koumoundouros *et al*. 2001b),

Table 8.4 Definition of the skeleton in fish

Generic subdivision	Definition	System	Elements	Embriological origin
Exoskeleton	Skeletal system that forms in contact with the ectoderm or endoderm	*Appendicular*	Fin inner supports	Neural crest
		Dermal	Dermal bone, scales, gill rakers, teeth, jaws	
Endoskeleton	Skeletal system that does not form in contact with ectoderm or endoderm	*Axial*	Neurocranium Vertebrae Notochord residuals	Mesoderm, neural crest
		Extraskeletal	Sesamoids, myoseptal tendons, intramuscular bones	

Table 8.5   Type of bone and modalities of ossification in fish (modified from Hall 2005)

Type of bone		Definition	Mode of ossification
Chondral bone	*Endochondral bone*	Type of bone that develops in a *preformed cartilage* model: the majority of the **endoskeleton**	Endochondral ossification (replacement bone)
	Perichondrial bone	Subtype of chondral bone that ossifies from the perichondral connective tissue	Perichondrial ossification
Membrane bone		Type of bone that develops *without contact with the ectoderm or endoderm,* but which is *not preformed in cartilage*	Intramembranous ossification (indirect ossification)
Subdermal bone		Neoformations that develop without contact with the ectoderm or endoderm: sesamoid bones, **pathological ossifications**	
Perichordal bone		Type of bone that forms in the connective tissue sheath surrounding the notochord	Perichordal ossification
Dermal bone		Type of bone that is *not preformed in cartilage* and that *develops in contact with ectoderm or endoderm*: the majority of the **exoskeleton**	Intramembranous ossification

sharpsnout sea bream (Sfakianakis *et al*. 2005), and red porgy (Çoban *et al*. 2009) are given for all the skeletal systems.

The origin of the bone in each skeletal structure appears to remain constant in the Sparidae studied, as well as the development of structures within a given fin (Faustino and Power 1999; Çoban *et al*. 2009). Some differences seem to exist when the differentiation sequence of the first appearance of the different main skeletal structures is taken into consideration. As highlighted in Table 8.8, some common as well as some different sequences emerge, mainly inherent dorsal and anal fin and vertebral axis events (notochord flexion included). It has to be underlined that the data in the table are reported without any reference to the size (total or standard length) or number of days posthatching of the individuals: the rearing temperatures were quite different for the various studies, and one of the main factors affecting osteological development is temperature. On the other hand, the aim of this comparative table was to compare differentiation sequences—and not timing or length—at which different Sparidae develop the same skeletal element, for each skeletal structure.

Before suggesting some theories explaining the ecological meaning of the observed differences, several considerations must be made. Firstly, more specific investigations are necessary to confirm the reported ossification sequence in wild individuals and with specific markers for bone and cartilage. In all these studies, in fact, with the exception of that of Faustino and Power (1998), who also performed an assay for bone-specific alkaline phosphatase activity, the applied histological methods used to demonstrate the presence of cartilaginous and bony structures were not entirely specific: alizarin red S stains areas of calcium salt deposition (Humason 1962; Pearse 1985), but is not a specific dye for hydroxyapatite, the main mineral phase of bone (Zerekh 1993) and may indicate merely the deposition of calcium salts in nonossifying embryonic connective tissue (Faustino and Power 1998). Alcian blue dye is an even less specific one, which stains acid mucopolysaccharides and glycosaminoglycans that are present also in tissues other than cartilage. Furthermore, its use entails the use of acetic acid, which can demineralize lightly ossified elements, thus masking the very beginning of the

Table 8.6   Ossification typology of cranium bones in Sparidae (modified from Faustino & Power 2001)

Dermal bones	Cartilage replacement bones
Neurocranium	
Frontal	Basioccipital
Infraorbitals	Basisphenoid
Lacrimal	Epiotic
Nasal	Ethmoid
Parasphenoid	Exoccipital
Parietal	Intercalar
Vomer	Lateral ethmoid
	Prootic
	Pterotic
	Pterosphenoid
	Sclerotics
	Sphenotic
	Supraoccipital
Suspensorium	
Ectopterygoid	Hyomandibular
Endopterygoid	Metapterygoid
Dentary	Palatine
Maxillary	Quadrate
Premaxillary	Symplectic
Opercular series	
Interopercle	
Opercle	
Preopercle	
Subopercle	
Jaws	
	Angular
	Retroarticular
Hyoid arch	
Branchiostegal rays	Basihyal
Urohyal	Ceratohyal
	Epihyal
	Hypohyal, lower
	Hypohyal, upper
	Interhyal
Branchial arches	
	Basibranchial
	Ceratobranchial
	Epibranchial
	Hypobranchial
	Pharyngobranchial

Table 8.7 Ossification typology of vertebral and fin bones in Sparidae

	Ossification typology	
Structure	Membrane bone	Cartilage replacement bone
Vertebral column	Vertebrae centra, epineural ribs	Neural arches and spines parapophyses, pleural ribs, hemal arches and spines
Dorsal and anal fins	Dorsal and anal rays	Dorsal and anal pterygophores, supraneurals
Caudal fin	Principal and concurrent rays and uroneurals	All elements but lepidotrichia
Pectoral fin and girdle	Rays Distal pterygophores (actinosts) Girdle elements	Propterygium Proximal pterygophores
Pelvic fin and girdle	Metapterygium rays	Basypterygium

ossification process. Other reasons that prevent us from inferring any explanation on ethoecological basis are as follows: (i) these studies are largely conducted on cultured individuals, and it is not known if the observed skeletogenic sequence is an ecoevo character or an epigenetic one; (ii) some data are lacking for a number of elements/events in some species, thus altering the sequence; and (iii) skeletal development is modular at the level of individual skeletal systems, individual skeletal elements, cellular condensations in bone formation, gene networks and epigenetic control (Hall 2009). Nonetheless, it is possible to identify some trends: mouth, gills, pectoral girdle, and caudal fin elements seem to be the first skeletal structures to begin differentiating in all the Sparidae studied; pelvic girdle/fin and ribs seem to be the last ones to begin differentiation; notochord flexion occurs after the beginning of differentiation of vertebral arches, but not in red porgy; the beginning of differentiation of dorsal and anal fins is generally postponed to the beginning of vertebral centra development, but not in common pandora and white sea bream. If differences exist in the skeletogenic sequence among Sparidae, they refer to the start of the notochord flexion and vertebrae ossification and of the dorsal and anal fins differentiation.

In Table 8.9, the sequence of the final achievement of total lepidotrichia is reported in the different species. By analyzing the differences compared with what was reported in Table 8.8, it is possible to point out some important generalizations. The caudal fin, which is one of the first skeletal structures to differentiate, is the first fin to achieve the final number of lepidotrichia, unlike the pectoral girdle and fin, which is the first fin to show some differentiated elements, but is the last one to achieve the final number of rays. Dorsal and anal fins, which in some Sparidae begin differentiation at the same time, terminate the achievement of the rays at different times. In these fins, the sequencing of differentiation between soft and hard rays is not always the same in the different Sparidae, and there are no clearly distinguishable patterns. The fin differentiation pattern may be related to the locomotor capabilities of larvae and juveniles. The caudal fin is the fin most involved in transmitting muscular force for propulsive tail strokes, which are commonly used by fish in escape or attack responses (Hale 1999). Koumoundouros *et al.* (2009) used the critical swimming speed as a measure of swimming performance during ontogenesis in gilthead sea bream, and linked the precocious caudal fin development to the pelagic larval niche, where the needs for prolonged swimming against water currents dominate. On the other hand, the prolonged and delayed pectoral fin development was linked to the transition to the demersal juvenile environment, where manoeuverability is considered an advantage.

Table 8.8 Skeletogenic sequence of the first appearance of elements in Sparidae

Species	Authors	Sequence						
Sparus aurata	Faustino and Power 1998, 1999, 2001	Mouth, gills, and pectoral girdle	Caudal	Notochord flexion	vertebrae ossification	Dorsal and anal	Pelvic	Ribs
Pagrus major	Matsuoka 1987	Pectoral / mouth and gills	Caudal	*Lack data on notochord flexion*	Anal	Pelvic	Dorsal	*Lack data on ribs*
Archosargus rhomboidalis	Houde and Potthoff 1976	Mouth and gills (pectoral)	Caudal	Notochord flexion	Dorsal and anal			
Diplodus puntazzo	Sfakianakis *et al.* 2005	*Lack data on cranium* (pectoral)	Caudal		Dorsal and anal		Ribs	
Pagellus erythrynus	Sfakianakis *et al.*2004	*Lack data on cranium*	Dorsal (Vertebral column)	Anal	Notochord flexion	Vertebrae ossification	Pelvic	
Pagrus pagrus	Çoban *et al.*2009	*Lack data on cranium*	Notochord flexion (Vertebral column)	*Lack data on vertebrae ossification* / Vertebrae ossification	Vertebral column	Anal		*Lack data on ribs*
Dentex dentex	Koumoundourous *et al.* 1999, 2000, 2001a	Mouth, gills, and pectoral girdle	Vertebral column / Dorsal and anal	Dorsal and anal	Vertebrae ossification			Ribs
Diplodus sargus[a]	Koumoundourous *et al.* 2001b	*Lack data on cranium*	Pectoral / Dorsal and anal	Notochord flexion	Vertebrae ossification			

When some elements start to differentiate at the same time, they are put together.

Table 8.9 Skeletogenic sequence of final number of lepidotrichia achievement in fins in Sparidae

Species	Authors	Sequence					
Sparus aurata	Faustino and Power 1998, 1999	Caudal	Dorsal	Anal	Pelvic	Pectoral	
Pagrus major	Matsuoka 1987	Caudal	Pectoral	Dorsal, anal, and pelvic fins			
Diplodus puntazzo	Sfakianakis et al. 2005	Caudal	Anal	Pelvic fin	Dorsal	Pectoral	
Pagellus erythrynus	Sfakianakis et al. 2004	Caudal	Dorsal	Anal	Pelvic	Pectoral	
	Çoban et al. 2009	Caudal	Dorsal	Anal	Pectoral	Pelvic fin	
Pagrus pagrus	Machinandiarena et al. 2003	Caudal, dorsal, and anal fin			Pectoral and pelvic fin		
Diplodus sargus	Koumoundourous et al. 2001b	Caudal	Anal	Dorsal	Pectoral		
Dentex dentex	Koumoundourous et al. 2000, 2001a	Caudal	Dorsal[a]	Pelvic fin	Pectoral		

[a]indicates that some data were extrapolated from figures in the article; when some elements start to differentiate at the same time, they are put together.

The precocious presence of the caudal fin in Sparidae, which plays a major role in forward propulsion in fish larvae (Kohno *et al*. 1984), gives them the capability of actively capturing (and selecting) preys through fast-start movements. The later differentiation of the pectoral fins may facilitate grazing. In particular, the differentiation of a very small pectoral fin element, the propterygium (i.e., the distal cartilage of the uppermost pectoral ray) is particularly important: the two *arrector* muscles actually insert on it, allowing expansion of the pectoral fin surface by pulling anteriorly on the first ray, accelerate and decelerate the fin, and assist in controlling the dorsoventral movement of the fin (Lauder and Drucker 2004). The same authors emphasized the critical role played by the leading edge of the pectoral fin, which leads the remaining rays during the fin beat cycle. The propterygium differentiation helps the Sparid larvae to remain stationary in vortex streets, in yawing turns, braking, hovering, in generating locomotor forces, and in benthic station holding (Lauder and Drucker 2004). The earliest propterygium differentiation occurs in white sea bream and gilthead sea bream larvae, soon after the first neural and hemal arch differentiation; while in common dentex, common pandora, red porgy, and sharpsnout sea bream it occurs just before pelvic fin differentiation (Faustino and Power 1999; Sfakianakis *et al*. 2004, 2005; Koumoundouros *et al*. 2001b; Çoban *et al*. 2009). Current knowledge of these Sparidae larvae and juvenile ecology in the natural environment is so scanty that no interpretation of the observed differences in the achievement of the expansion of the pectoral fin surface capacity can be formulated.

In higher Teleosts, the anterior spinous dorsal fin is used to slice through water whilst the soft, posterior, part plays an anti-pitch function during forward movement, or acts in synergy with the anal one, in braking, crawling along the underwater-floor, aiding in propulsion during slow swimming or in backing-up motions, allowing the fish to turn sharply (Harder 1975; Koumoundouros *et al*. 2001a).

At present, very little is known about the function of the pelvic fins, as no experimental or quantitative hydrodynamic analyses of the function of these fins have been conducted yet (Lauder and Drucker 2004): some observations are available on the active use the fish make of their pelvic fins as control surfaces during manoeuvers (Drucker and Lauder 2003).

8.3   Skeletal deformities in reared Sparidae

The presence or absence of deformed skeletal elements in reared fish is very often an underestimated problem: the selection practices currently implemented during the rearing cycle are effective in culling out only the individuals with externally detectable deformations, such as serious lordosis, kyphosis, kypholordosis, scoliosis, shortened opercula, cranial deformity, and malpigmentation. As mentioned in the introduction, manual sorting at the end of the hatchery phase gives no guarantee that market-size fish will be deformity-free. Skeletal anomalies can appear throughout the fish's life, as growth in these continuously growing vertebrates involves incessant bone modeling and remodeling under the influence of genetic and epigenetic factors.

According to Koumoundouros *et al*. (2001c), Boglione *et al*. (2003), and Sfakianakis *et al*. (2004, 2006), the current research regarding morphological quality assessment and verification seems to take into insufficient consideration internal deformities in juveniles, in the conviction that abnormal internal elements do not necessarily affect external morphological features. But, they can.

With the exception of deformities of the gill cover (Koumoundouros *et al*. 1997a) and the noninflation of the swimbladder (Kitajima *et al*. 1994), skeletal deformities have a continuous expression ranging from slight aberrations of the internal anatomy, to severe internal abnormalities with significant effects on the external body shape (Divanach *et al*. 1997; Koumoundouros *et al*. 1997b, 2001c, 2002; Loy *et al*. 1999, 2000; Sfakianakis *et al*. 2003, 2006; Gjerde *et al*. 2005). A list of references available in the literature on skeletal deformities affecting different anatomical structures in Sparid species is shown in Tables 8.10, 8.11 and 8.12. In the following sections, a detailed description is provided for each deformity, grouped according to the body region they affect.

8.3.1 *Spinal column*

The spinal column, or vertebral column, or vertebral axis, is a part of the axial endoskeleton, which embryologically originates from the mesoderm and neural crest cells. The mineralization process involves the formation of both endochondral bone and perichordal bone. Deformities of the spinal column are well documented in

Table 8.10 Deformities in the vertebral axis and vertebrae in reared and/or wild sparids published since 1990

Species	Author
Acanthopagrus latus	Leu and Chou 1996
Archosargus rhomboidalis	Browder *et al*. 1993
Dentex dentex	Boglione *et al*. 2000, 2003 Rueda and Martínez 2001 Gimenez and Estevez 2008
Diplodus argenteus	Browder *et al*. 1993
Diplodus puntazzo	Favaloro and Mazzola 2000 Boglione *et al*. 2000 Boglione *et al*. 2003 Favaloro and Mazzola 2003 Favaloro and Mazzola 2006
Diplodus sargus	Sfakianakis *et al*. 2003
Lagodon rhomboides	Browder *et al*. 1993
Pagellus erythrinus	Boglione *et al*. 2000 Boglione *et al*. 2003 Sfakianakis *et al*. 2004
Pagrus major	Kihara *et al*. 2002 Hattori *et al*. 2003 Matsuoka 2003 Hattori *et al*. 2004 Sawada *et al*. 2006 Nguyen *et al*. 2008
Pagrus pagrus	Boglione *et al*. 2000 Roo *et al*. 2009
Sparus aurata	Balebona *et al*. 1993 Chatain, 1994 Santamaria *et al*. 1994 Andrades *et al*. 1996 Koumoundouros *et al*. 1997a Loy *et al*. 1999 Afonso *et al*. 2000 Boglione *et al*. 2000 Boglione *et al*. 2001 Castro *et al*. 2008 Fernandez *et al*. 2008

Table 8.11   Deformities in the splanchnocranium in reared and/or wild Sparidae published since 1990

Species	Author
Archosargus rhomboidalis	Browder *et al*. 1993
Dentex dentex	Boglione *et al*. 2000, 2003 Gimenez and Estevez 2008
Diplodus argenteus	Browder *et al*. 1993
Diplodus puntazzo	Boglione *et al*. 2000 Favaloro and Mazzola 2000 Boglione *et al*. 2003
Lagodon rhomboides	Browder *et al*. 1993
Pagellus erythrinus	Boglione *et al*. 2000 Boglione *et al*. 2003
Pagrus auratus	Jawad and Hosie 2007
Pagrus major	Matsuoka 2003
Pagrus pagrus	Boglione *et al*. 2000 Roo *et al*. 2009
Sparus aurata	Polo *et al*. 1991 Koumoundouros *et al*. 1997b Loy *et al*. 1999 Boglione *et al*. 2000 Galeotti *et al*. 2000 Boglione *et al*. 2001 Beraldo *et al*. 2003 Verhaegen *et al*. 2007 Castro *et al*. 2008 Fernandez *et al*. 2008

reared Sparidae (Santamaria *et al*. 1994; Boglione *et al*. 1995, 2000, 2003; Andrades *et al*. 1996; Loy *et al*. 1999; Afonso *et al*. 2000; Favaloro and Mazzola 2000, 2003; Kihara *et al*. 2002; Hattori *et al*. 2003, 2004; Matsuoka 2003; Sfakianakis *et al*. 2004; Castro *et al*. 2008; Fernández *et al*. 2008; Gimenez and Estevez 2008; Nguyen *et al*. 2008; Roo *et al*. 2009) (Table 8.10). These deformities involve scoliosis (lateral curvature), lordosis (V-shaped dorsoventral curvature of the sagittal plan including the vertebral column and the spinal cord) and kyphosis (Λ-shaped curvature) of the vertebral column and, in the worst cases, various combinations of these three deformities (Figure 8.1). Recently, Afonso *et al*. (2000) described the LSK syndrome, a consecutive repetition of Lordosis-Scoliosis-Kyphosis (LSK), from the head to the caudal fin in gilthead sea bream.

During embryonic and early larval development, axial deviations, which could lead to further malformations in the vertebral column, result from defective development of the notochord and the perinotochordal connective sheet (Santamaria *et al*. 1994). Alteration of collagen metabolism has been related to notochordal malformation in *Xenopus laevis* tadpoles (Schultz *et al*. 1985; Riggin and Schultz 1986). The mechanism of notochord malformation during embryonic development is still not clear. European sea bass (*Dicentrarchus labrax*) with lordosis display chondroid instead of acellular bone at the articular surfaces of the affected vertebrae (Kranenbarg *et al*. 2006).

Table 8.12 Deformities affecting fins and other anatomical structures in reared and/or wild Sparidae published since 1990

Target	Species	Author
Fins	*Archosargus rhomboidalis*	Browder *et al*. 1993
	Dentex dentex	Boglione *et al*. 2000 Koumoundouros *et al*. 2001a Gimenez and Estevez 2008
	Diplodus argenteus	Browder *et al*. 1993
	Diplodus puntazzo	Boglione *et al*. 2000 Favaloro and Mazzola 2000 Boglione *et al*. 2003 Favaloro and Mazzola 2003 Favaloro and Mazzola 2006
	Diplodus sargus	Sfakianakis *et al*. 2003
	Lagodon rhomboides	Browder *et al*. 1993
	Pagellus erythrinus	Boglione *et al*. 2000 Boglione *et al*. 2003 Sfakianakis *et al*. 2004
	Pagrus major	Matsuoka 2003
	Pagrus pagrus	Boglione *et al*. 2000 Roo *et al*. 2009
	Sparus aurata	Loy *et al*. 1999 Koumoundouros *et al*. 1997a Boglione *et al*. 2000 Boglione *et al*. 2001 Fernandez *et al*. 2008
Scales	*Archosargus rhomboidalis*	Browder *et al*. 1993
	Lagodon rhomboides	Browder *et al*. 1993
	Lagodon rhomboides	Corrales *et al*. 2000
Shape	*Sparus aurata*	Loy *et al*. 1999 Verhaegen *et al*. 2007
Eye	*Sparus aurata*	Carrillo *et al*. 1999
Lateral line	*Sparus aurata*	Carrillo *et al*. 2001
Terminal tract of urinary duct	*Diplodus puntazzo*	Boglione *et al*. 2003
	Pagellus erythrinus	Boglione *et al*. 2003
	Sparus aurata	Boglione *et al*. 2001

(a)

(b)

(c)

(d)
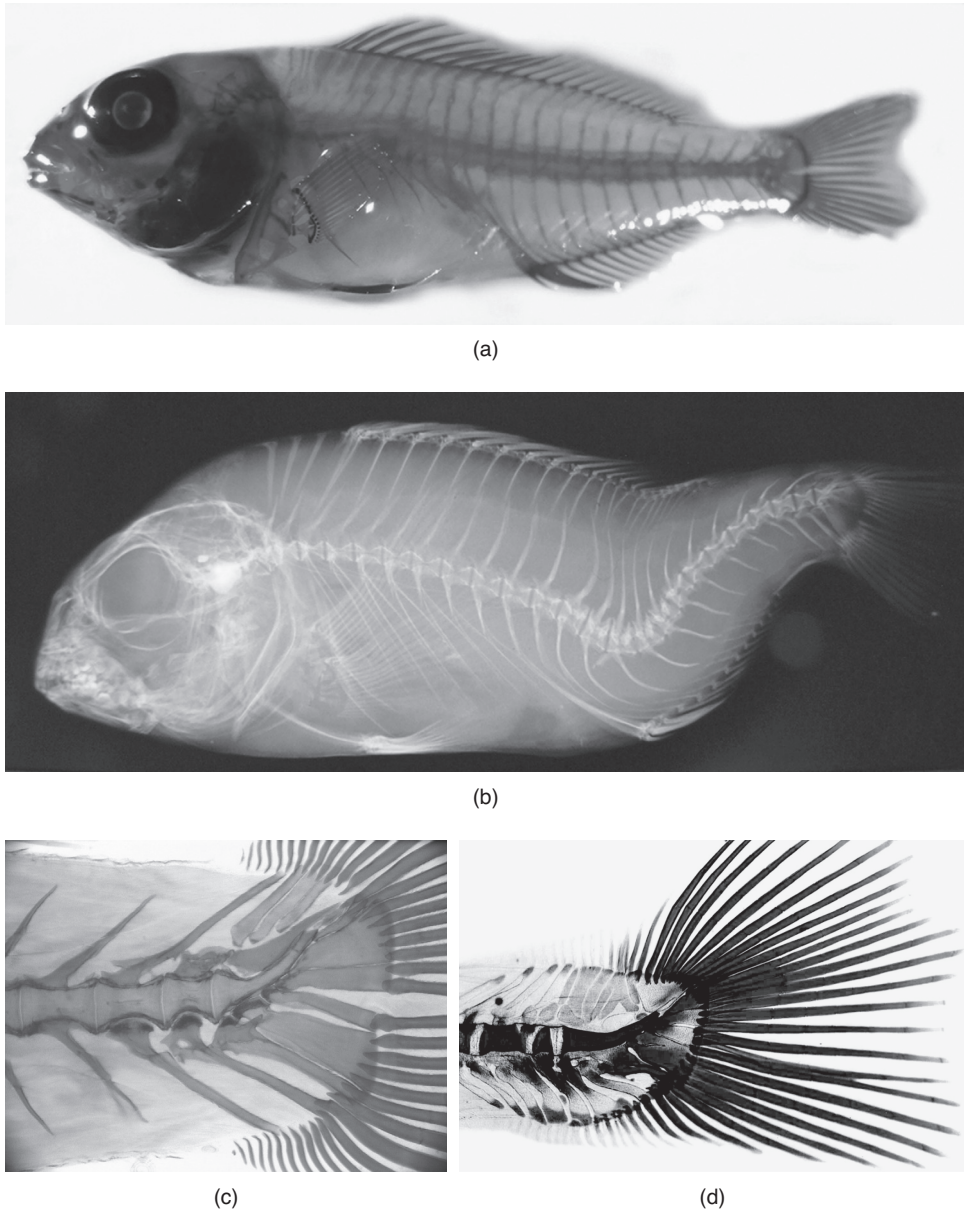
Figure 8.1 (a) *In toto* staining of a wild gilthead sea bream (*Sparus aurata*) juvenile without any deformity. (b) X-ray of a reared gilthead sea bream with a heavy lordo-kyphosis affecting the external shape of the fish (stump body). (c) Normal caudal vertebrae and fin in gilthead sea bream. (d) Gilthead sea bream caudal region showing another hypoplasmic fin located at its dorsal part. The main characteristic of this deformity is the formation of extranumerary dorsal principal rays articulated on extra epurals. (A color version of this figure appears in the color plate section.)

(a)　　　　　　　　　　　　　(b)　　　　　　　　　　　　　(c)
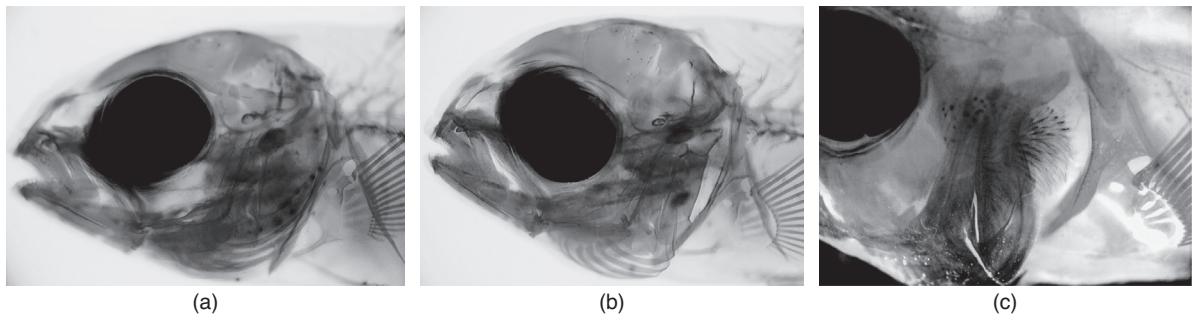
Figure 8.2 Gilthead sea bream at 72 days posthatching (DPH) showing a normal (a) and reduced (b) left operculum, and (c) more advanced reduction of operculum with consequent exposure of the branchial arches. (A color version of this figure appears in the color plate section.)

Chondroid bone is a skeletal tissue intermediate between bone and cartilage and appears to meet the demand for an accelerated local growth rate, combined with the need for shear resistant support (Huysseune 2000). In cultured gilthead sea bream, lordosis affects primarily vertebrae 10–16, which generally correspond to the hemal region of the vertebral column (Andrades *et al*. 1996). Lordosis in the past was always considered as being linked to the absence of a functional swim bladder (Chatain 1994a), but this association is now being questioned since lordotic adults may possess an inflated and functional swim bladder, and larvae with a lordotic curvature of the notochord are detectable before the period in which the swim bladder inflates in gilthead sea bream (Andrades *et al*. 1996). Lordotic larvae may well have an impaired ability to inflate the swimbladder at the appropriate time; if so, the non-inflation of the swim bladder would be a consequence rather than of a cause of lordosis. According to Andrades *et al*. (1996), the survival of the lordotic larvae is higher than that of other deformed larvae, probably because, in contrast to other grosser deformities, lordosis in larvae does not interfere totally with swimming and feeding behavior.

Spinal deformities of various degrees of severity are common in reared gilthead sea bream (Table 8.10), and lordosis, and shortened or deformed operculum (Figure 8.2) has been considered the most significant abnormality in gilthead sea bream by Beraldo *et al*. (2003) and Verhaegen *et al*. (2007). Many authors also reported severe spinal caudal malformations in the reared yellowfin sea bream (*Acanthopagrus latus*), western Atlantic sea bream (*A. rhomboidalis*), sharpsnout sea bream, white sea bream, common dentex, common pandora, red sea bream, and red porgy (see Table 8.10). The severe deformities of the spinal column always involved abnormalities over a relatively wide range of vertebrae, which can appear fused and deformed mostly in the region of maximal axis curvature.

Also vertebrae deformities may be present regardless of the presence of axis deviation. They include (a) dislocation, fusion, shortening, deformation or lack of the centra (Hattori *et al*. 2004; Sawada *et al*. 2006); (b) dislocation, compression, deformation, lack or extra formation of the hemal, and neural arches and apophysis (Nguyen *et al*. 2008), and (c) dislocation, shortening, deformation, lack, or separation of the ribs (Boglione *et al*. 1993, 1995). The most severe is the extensive fusion of vertebrae, which can affect seriously the shape of the body, causing it to become shortened. However, in Atlantic salmon (*Salmo salar*) it has been demonstrated that the onset of vertebrae fusion does not inevitably lead to fish with deformed vertebral columns (Witten *et al*. 2006). A compressed vertebra is characterized by a normal central body and deformational changes in the anterior and posterior rims of the compact bone (Witten *et al*. 2005).

8.3.2   *Head*

The head is composed by the neuro- and splanchnocranium. The neurocranium is rarely reported as being affected by anomalies in Sparidae, while the splanchnocranium, which originates from neural crest cells and it is mostly preformed in cartilage, has been found to be the target of severe anomalies affecting the exterior appearance of the fish (Table 8.11). Cephalic deformities are frequently found in hatchery reared Sparidae (Lagardère *et al.* 1993; Koumoundouros *et al.* 1997b; Boglione *et al.* 2000, 2001, 2003; Favaloro and Mazzola 2000; Galeotti *et al.* 2000; Beraldo *et al.* 2003; Matsuoka 2003; Verhaegen *et al.* 2007; Castro *et al.* 2008; Gimenez and Estevez 2008; Fernández *et al.* 2008; Roo *et al.* 2009). Several forms of mouth malformation have been reported in fish (Figure 8.3), including crossbite, pugheadedness, sucker mouthed, prognatism, or reduction of dentale (Boglione *et al.* 2001, 2003), double mouth (Swan 1968), open-jaw (Crouch *et al.* 1973), lower jaw deformity syndrome (Bruno 1990; Hughes 1992; Sadler *et al.* 2001; Lijalad and Powell 2009), and gaping in larvae (Pittman *et al.* 1990; Morrison and MacDonald 1995). Fish affected by lower jaw deformity syndrome experience severe distortion of the articulation with the jaw locked in a permanently open mode as a result of ankylosis of the mandibular bones (Bruno 1990; Hughes 1992). The generally admitted classification covers six different types based on morphoanatomic criteria, each one presenting a wide range of expressions, as described below:

(i)   Gill cover anomalies are unilateral (generally) or bilateral deformities of the opercular complex bones that consist of reduction, twisting or folding of the operculum, suboperculum, and sometimes of the preoperculum. Shortened opercular plates are the most frequent deformities observed in reared gilthead sea bream (Chatain 1994a; Koumoundouros *et al.* 1997b; Galeotti *et al.* 2000; Beraldo *et al.* 2003; Verhaegen *et al.* 2007), together with lordosis.

(ii) Pugheadedness is a deformity that affects skull, jaws, and eyes, and leads to a reduction of the frontal skull and the upper jaw bones. It has been reported in gilthead sea bream (Chatain 1994b) and in wild snapper (*Pagrus auratus*) in New Zeland (Jawad and Hosie 2007).

(iii) Crossbite is a deformity in which the lower jaw is skewed off centre 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 semicrossbite) can be defined according to the orientation of the axis of deformity. Crossbites have been reported in gilthead sea bream (Chatain 1994b).

(iv) Lower jaw (dentale) reduction is a deformity leading to a head appearance similar to the full crossbite of Hickey *et al.* (1977) and has been reported in gilthead sea bream (Chatain 1994b) and red sea bream (Matsuoka 2003).

(v)   Pike Jaw Deformity (PJD) is a deformity described first in wild northern pike (*Esox lucius*) (Lindesjóó and Thulin 1992), which involves the upward bending of the anterior part of the parasphenoid and the frontal bones. In gilthead sea bream, similar jaw deformities have been reported (Francescon *et al.* 1988; Polo *et al.* 1991).

8.3.3   *Fins*

The total or partial absence of a fin is the most severe observed deformity of both the dorsal fin (in association with the "saddleback syndrome" of Tave *et al.* 1983) and the anal fin (Hussain 1979). Saddleback syndrome is anatomically attributed to the lack of one or all spines and rays, as well as associated pterygiophores, and is also associated with a characteristic depression in the anterior dorsal profile (Figure 8.4). Saddleback syndrome has been associated with caudal fin deformities in reared common dentex (Koumoundouros *et al.* 2001c) and white sea bream (Sfakianakis *et al.* 2003).

(a)

(b)

(c)
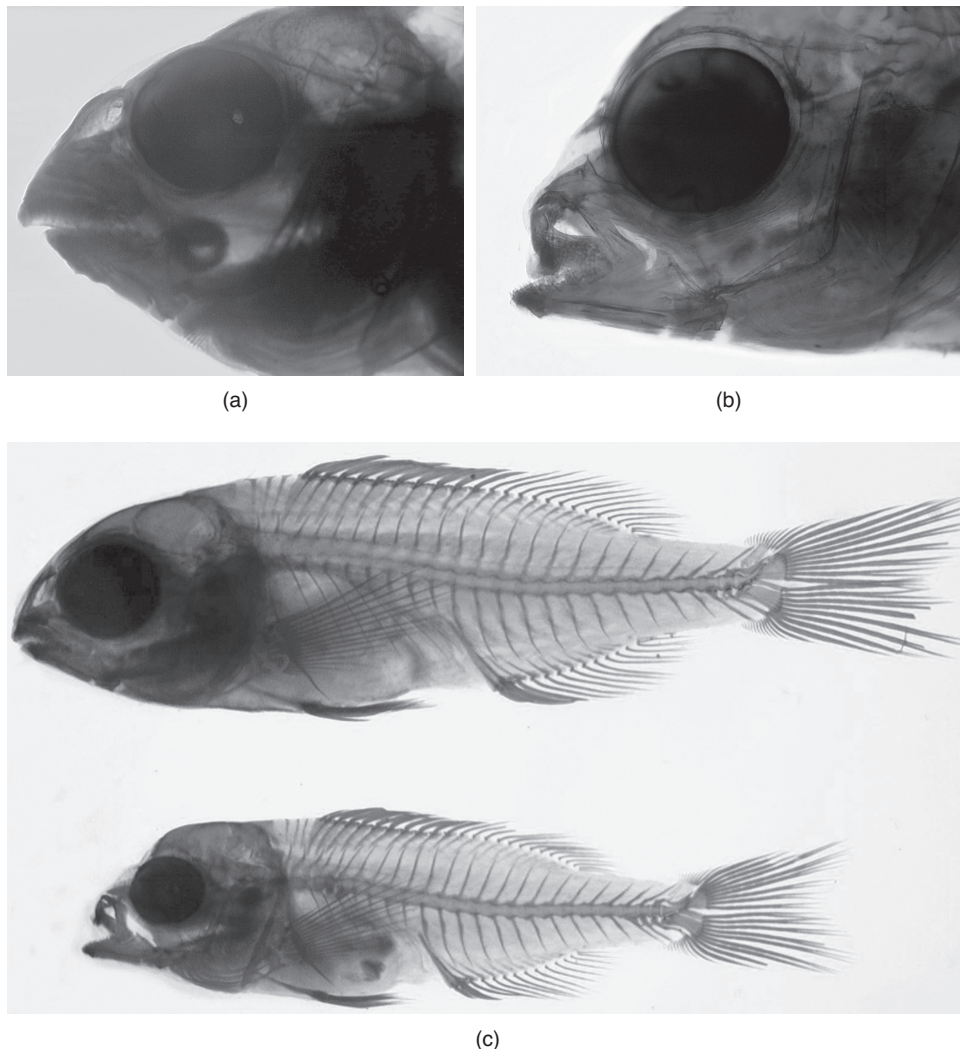
Figure 8.3   (a) Gilthead sea bream showing deformed premaxillary and dental. Also the mesethmoid is deformed (compare with normal head profile in c, and Figs. 8.1a and 8.2a). (b) Another example of deformed (twisted) premaxillary. This juvenile shows also an elongated dental. (c) Gilthead sea bream juveniles from the same batch and rearing tanks, at 115 days posthatching (DPH). The upper individual is normal, while the lower (and smaller) one has many deformations affecting the left premaxillary (twisted), the anteriormost prehemal vertebrae (fused and deformed vertebral bodies) and curved parahypurals in the caudal fin.

Caudal fin deformities are frequent in both reared and wild Sparidae (Table 8.12). In aquaculture, the most severe deformities involve the absence of tail (Koumoundouros *et al.* 1997a), partial tail (single-lobed), double (Koumoundouros *et al.* 1997a) or triple tail or lobes (Tave *et al.* 1982), formation of extra fin rays (Ishikawa 1990), and size reduction or roundness of the tips (Mazik *et al.* 1987). The caudal fin deformities include all the deformations and fusions affecting each of the bones making up the caudal fin plate, including the last vertebra (urostyle). In hatchery reared gilthead sea bream, the caudal fin anomalies can affect up to 65% of

(a)   (b)

(c)
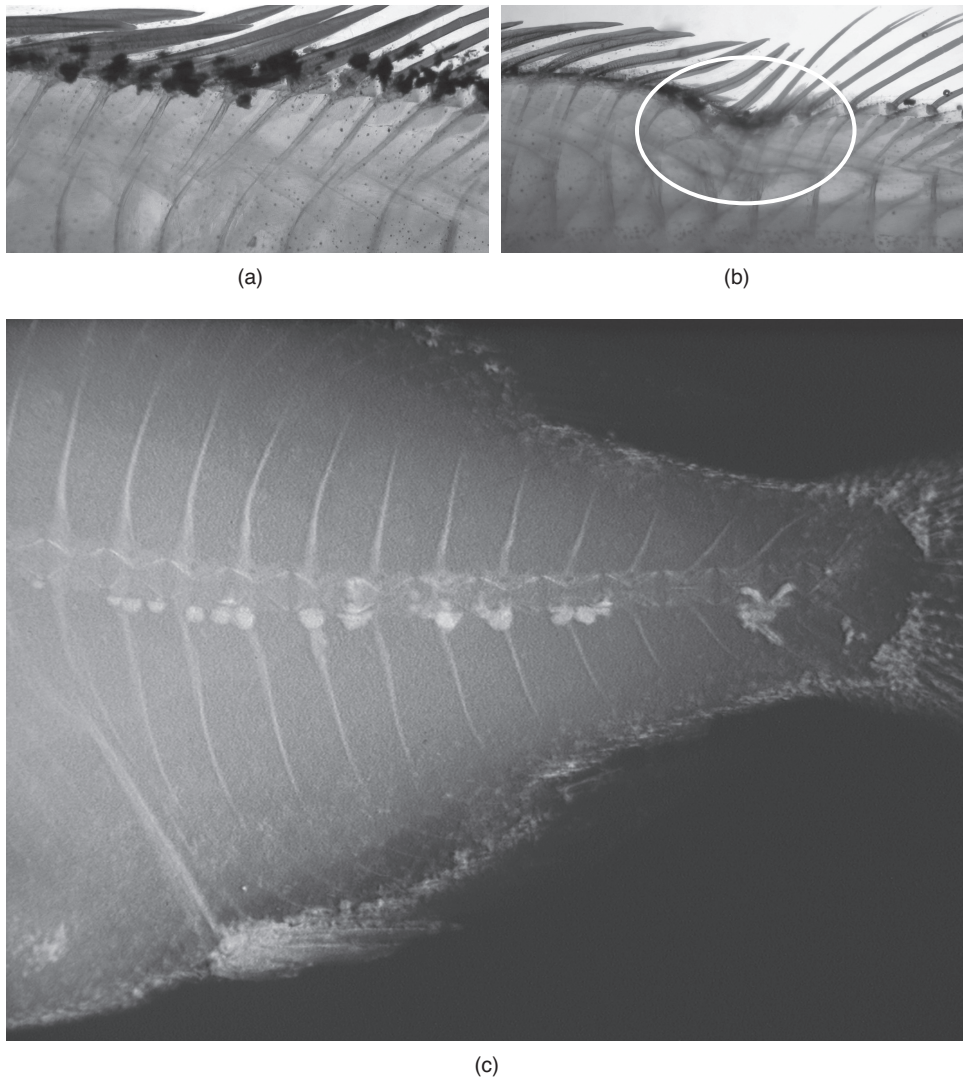
Figure 8.4 (a) Normal common pandora (*Pagellus erythrinus*) dorsal profile. (b) Dorsal profile affected by "saddleback" syndrome in common pandora. The circle highlights the deformed pterygophores underlying the saddleback. (c) Vertebral column of a red porgy (*Pagrus pagrus*) juvenile showing hypercalcified hemal arches (white blotches).

individuals (Koumoundouros *et al*. 1995) and involve compression, duplication, or lateral twist (Koumoundouros *et al*. 1997a); deformation of caudal fin rays is also reported in gilthead sea bream with uninflated swimbladder (Paperna 1978). In sharpsnout sea bream the malformations of the caudal complex are more extensive in adults than in juveniles and seem to be connected to the rearing environment (Favaloro and Mazzola 2000, 2003).

In cultured gilthead sea bream, Paperna (1978) reported the presence of curved pectoral fins in fish, lacking a functional swim bladder. Costa *et al*. (2005), by analyzing the level of asymmetries in different lots of wild and
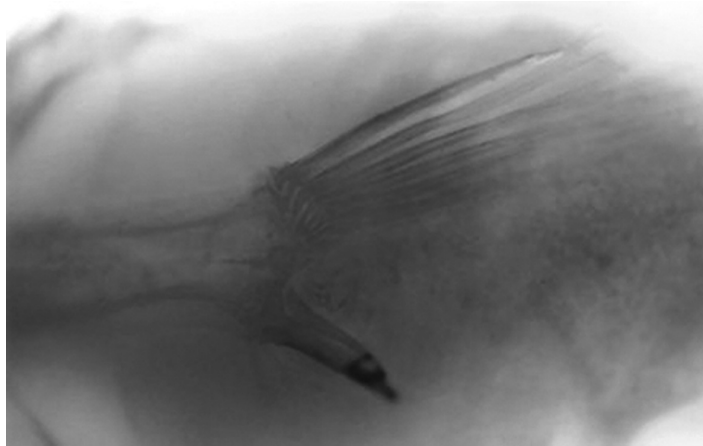
Figure 8.5 Asymmetry in median fin element. In this sharpsnout sea bream (*Diplodus puntazzo*) the rays of the left pelvic fin are completely fused together (Boglione *et al*. 2000).

reared Sparidae, found a significantly higher degree (p<0.001) than expected in one lot of reared gilthead sea bream, in reared and wild common pandora lots, and in one reared lot of sharpsnout sea bream. Interestingly, the semi-intensively reared lots of gilthead sea bream showed the same lower than expected occurrence of asymmetries as wild individuals.

Fin abnormalities (Table 8.12, Figure 8.5) may affect each of the elements that can be fused, bifurcated, twisted, or abnormally sized and shaped. However, deformations of these elements are extremely frequent in reared and wild fish, without necessarily being related to macroscopically severe deformities (Gartner 1986; Matsusato 1986; Matsuoka 1987; Boglione *et al*. 1993; Loy *et al*. 1999; Boglione *et al*. 2000, 2001).

8.4   Other developmental anomalies

8.4.1   *Shape*

Shape is the first visual criterium of species recognition and the image of the wild phenotype is often a marketing reference, although all fish species may develop shape abnormalities (Loy *et al*. 1999). When the abnormalities are very important, such as in dwarfism or swollen abdomen syndrome (body deformations associated with huge vertebral abnormalities), they impact the external shape of the fish so strongly that they cannot be marketed. In southern Europe, some experienced consumers are able to identify and choose the "wild-type" fish from the reared ones, in the absence of any externally detectable anomalies. Unfortunately, a few studies actually consider this important quality factor. Rearing conditions were found to induce differences in the external morphology of fish. Shape changes related to different rearing conditions have been ascertained by geometric morphometric tools (Figure 8.6) (Corti *et al*. 1996; Loy *et al*. 2000; Costa 2004; Verhaegen *et al*. 2007; Ambrosio *et al*. 2008). In cultured gilthead sea bream, Costa (2004) observed shape differences linked to skeletal anomalies. Verhaegen *et al*. (2007), in the same species, found that deoperculated specimens could be detected much earlier using the geometric morphometrics approach. This information, when combined with the implementation of automated systems for shape detection and description, such as elliptic Fourier analysis (Menesatti *et al*. 2008, Costa *et al*. 2009) or the Bezier method (Loy *et al*. 2000), could thus be a reliable, time- and cost-effective method for the quality assessment of reared fish stocks.
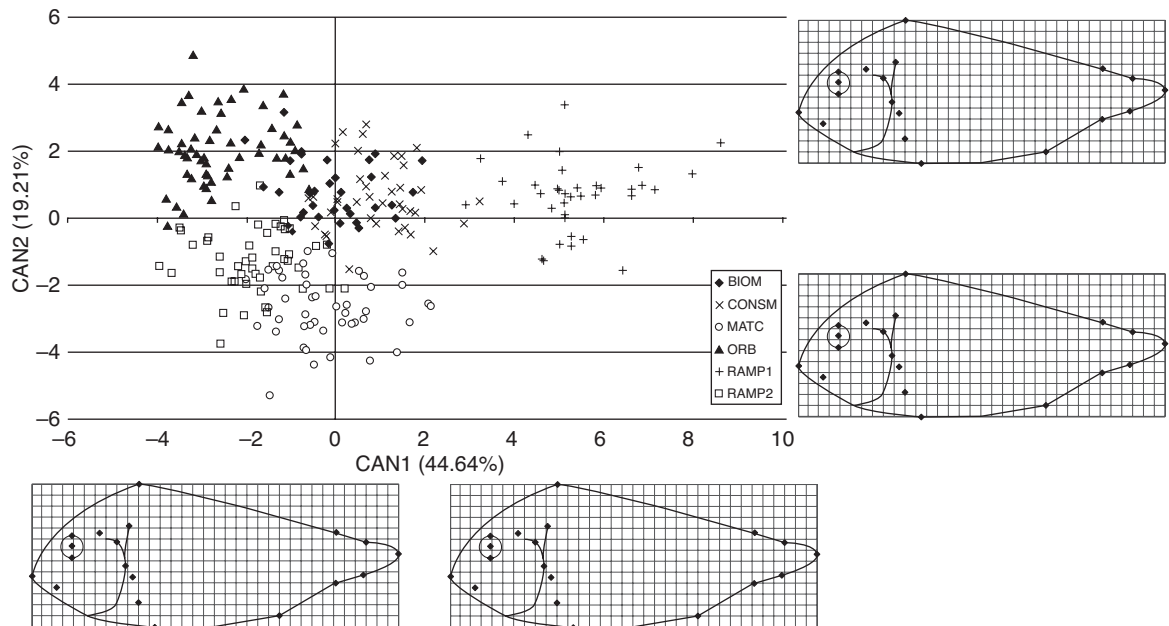
Figure 8.6 An example of the application of geometric morphometry to study the rearing conditions effects on external shape of gilthead sea bream: canonical variates analysis on the shape of individuals belonging to lots reared under different conditions. On the right and lower sides, the splines relative to the extremes of CAN2 and CAN1 axis, respectively, are reported (from Costa 2004).

8.4.2 Pigmentation

Pigmentation anomalies involve mainly partial or total albinism, ambicoloration, and xanthochroism (Jawad *et al*. 2006/07) and affect mostly flatfishes. Slight deviations of pigmentation from the wild standard (which are usually reversible) are more frequent. They involve the melanism of red porgy*,* and the absence of the bright reddish color on the gill cover and yellowish interorbital band, and the frequently darker pigmentation of reared gilthead sea bream. It has been reported that this species often displays much darker colors when stressed, showing characteristic vertical stripes (Ruane *et al*. 2005). As a result of inappropriate rearing conditions (e.g., light intensity, limiting food ingredients, diseases, and stress) or special postharvesting manipulation (use of cold water and/or of ice), these deviations, unless intense, have so far not affected significantly the market value of the Mediterranean fish (Divanach *et al*. 1996).

8.4.3 Scales

Scale abnormalities have only recently been monitored in a number of fishes (Table 8.12). They involve the absence or loss of scales with or without nacreous-like scaleless body (Yamamoto 1977), much smaller (Tomita 1992) or bigger scales than the normal size, or display of an abnormal orientation pattern (Browder *et al*. 1993; Corrales *et al*. 2000) and an abnormal scale distribution (Carrillo *et al*. 2001; Jawad *et al*. 2006/07). In cultured gilthead sea bream*,* slightly disoriented patterns of the lateral line scales are common, often associated with vertebral deformities. Also, loss of scales associated with lymphocystis and/or aggressiveness is known, but not documented. In this species, abnormal reorganization of scales after regeneration is probable (Bereiter-Hahn and Zylberberg 1993), but not confirmed. From the economic point of view, the lack of scales may affect market

acceptability by lowering the color brightness of the commercial product and the correlative visual impression of its freshness.

Up to now, it has deemed that these abnormalities do not lower the biological performance of fish, even if the absence of scales or an anomalous lateral line is a first symptom of some disorder. Secondarily, scales reduce water resistance (Harder 1975), while the lateral line scales in some species and development stages host some free neuromasts on the surface and are involved in the functioning of the trunk lateral line system. According to Carrillo *et al*. (2001), the absence of some sectors of the body lateral line increases the possibility of deficient sensory reception. Deformities of the body lateral line are in some cases linked to vertebral deformities, although only a few authors have studied them in any detail (Andrades *et al*. 1996; Carrillo *et al*. 2001; Jawad *et al*. 2006/07). According to Andrades *et al*. (1996), examination of the lateral line is a useful method for identifying low lordotic curvatures in juvenile fish. Carrillo *et al*. (2001) observed that lateral line anomalies in gilthead sea bream could be used as an alternative method for distinguishing wild from hatchery-reared fish, and to obtain relevant information on fishery management that cannot be gathered by other methods.

8.4.4 Other anomalies

Hypervitaminosis D was considered the cause of urinary calculi by Roberts and Shepherd (1986), but in gilthead sea bream Modica *et al*. (1993) identified the cause as being an excess of calcium and phosphorus in enriched *Artemia nauplii* or in the water. In the same study, calculi were found to be made up of apatite crystals ($Ca_5(PO_4)_2$) linked to Mg, Cl, and S. More recently, some authors considered urinary calculi as stress indicators in European sea bass and gilthead sea bream larvae (Moretti *et al*. 1999). Koumoundouros *et al*. (2004) cited an incidence of urinary calculi of 30–40% in fish reared in mesocosms.

Carrillo *et al*. (1999) described different types of ocular abnormalities in reared gilthead sea bream and discussed their association with the social behavior of the fish. In aquaculture, ocular pathologies and abnormalities are frequently used as an indicator of fish health.

8.5   Effects of deformities on fish performance and quality

As indicated above, malformations can affect different aspects of fish morphology, such as pigmentation, shape, scales, skeleton, and swim bladder (Divanach *et al*. 1996; Castro *et al*. 2008). Skeletal malformations in particular affect marine larviculture of many important species other than Sparidae (salmonids, European sea bass, flatfish, etc.), with effects on survival, production costs, and marketability of the fish (Andrades *et al*. 1996; Howell *et al*. 1998; Cobcroft *et al*. 2001). As many as 50–60% of hatchery juveniles present at least one severe, externally detectable skeletal malformation. In the aquaculture industry, losses due to malformations also impact ongrowing farms, where malformed market size fish have to be discarded or sold at lower prices.

Skeletal deformities are considered to lack species specificity and develop in particular during the hatchery phase (Sfakianakis *et al*. 2006). However, evidence exists that there is a continuous capacity in fish for developing deformations throughout their life cycle. Other than in the gilthead sea bream example illustrated in the Introduction, also in Atlantic halibut (*Hippoglossus hippoglossus*), more severe skeletal abnormalities appear with increases in length, particularly in the prehemal region of the vertebral column. Therefore, with increasing length, the occurrence and severity of the abnormalities seems to increase (Lewis & Lall 2004).

Cephalic anomalies affect strongly the morphological appearance of the commercial fish and lower their market value. It has been suggested that malformations of the mouth, as manifested in young larvae, have a lethal character: mouth deformities could cause starvation in fish due to impaired feeding ability (Pittman *et al*. 1989; Cobcroft *et al*. 2001). Fish affected by lower jaw (dentale) reduction cannot adduct their mandible and thus buccal-opercular pumping of water across the gills is likely to be impaired, showing a limited capacity to

recover from tests of exhaustive swimming (Lijalad & Powell 2009). Jaw malformations are not always lethal, although extensive attrition during the juvenile phase may be attributed to the progressive inability of malformed fish to feed either on live prey or inert particulate diets (Morehead *et al*. 1999; Cobcroft *et al*. 2001).

The abnormalities of the spinal axis can affect seriously the shape of fish and, therefore, decrease their commercial value. Thus, the reduction of the incidence of larval deformities would reduce the economic cost of production, both in hatcheries and in the outgrowing production sectors, and ameliorate the image of aquaculture products to consumers. In reared gilthead sea bream, spinal deformities decrease the survival and growth rate (Divanach *et al*. 1996). The high level of spinal malformations observed in hatchery gilthead sea bream represents an actual bottleneck for the development of Mediterranean aquaculture (Andrades *et al*. 1996). Unlike other larger deformities, lordosis in larvae does not interfere with swimming and feeding behavior completely and is not deleterious (Andrades *et al*. 1996), thus allowing the survival of affected individuals to marketable size.

The possibility of obtaining some economic remuneration from having reared lordotic individuals could be to use such fish for filet processing. But also the derived products for the food industry are affected in the processing chain by these abnormalities (Gjerde *et al*. 2005). Fin damage may also affect the perceived product quality (Winfree *et al*. 1998 and references therein).

8.6   Causative factors of skeletal deformities

There is a great number of scientific papers available on skeletal deformities in fish; therefore, the present review cannot be considered exhaustive of all the scientific knowledge accumulated on this topic and some work has been left out, reporting only papers published after 1990.

In spite of the strong impact of skeletal deformities on hatchery production (Castro *et al*. 2008), the causative factors are only partially understood. Basically, there are as many causative factors as there are biological fields: genetic, nutritional, physiological, physical, chemical, biomolecular, and environmental causes have all been blamed for inducing pigmentation or skeletal anomalies in fish (Tables 8.1, 8.2 and 8.3 as well as 8.13, 8.14 and 8.15). In some cases, sibling juveniles reared in different conditions show a different typology and incidence of skeletal anomalies, which suggests that an epigenetic control is exerted over skeletal development and growth. In other cases, genetic factors have undoubtedly a causative effect. In general, it is possible to state that the same typology of skeletal anomaly may be due to different causes, while different causes may produce the same type of anomaly.

Skeletal deformities in fish are of prime economic importance to aquaculture. Consequently, the majority of available studies use quantitative or qualitative data on skeletal or pigmentation deformities to test the validity of experimental diets, rearing protocols and technical innovations. Very often, studies have been conducted on a commercial scale, where many other epigenetic and genetic factors interact among themselves, as well as with the factor introduced for experimental validation. The use of deformity rate as an indicator for new innovations in aquaculture, mostly in the hatchery sector, is an approach much appreciated by the industry and for this reason has received a number of research grants. So far only the problem of swim bladder inflation has been solved (Chatain & Ounais-Guschemann 1990). However, despite the presence of surface skimmers to eliminate the oil film from the water surface, the mass production of gilthead sea bream with uninflated swim bladders sometimes still occurs.

As previously described, the majority of the available literature on skeletal deformities in fish is based on *in toto* staining or x-rays, which have the advantage of showing every malformation of skeletal elements, even those externally undetectable, in a large number of samples and at relatively low cost. This approach can ensure a sound overall analysis based on a statistically significant number of analyzed individuals so as to reduce "background noise." By "background noise" it is meant the accessory items of instability that are introduced when experimental tests are conducted on fish, such as rearing conditions (color, volume, and shape of the tank, light conditions, stocking density, hydrodynamics, water change, temperature, salinity, oxygen/carbon dioxide

Table 8.13   Nutritional (epigenetic) factors reported in literature as causes of skeletal anomalies in fish

Causative factor	Description	References
Aminoacids	Vertebral axis deviations (scoliosis, kyphosis and lordosis) frequencies have been reduced by increasing (in different experiments) dietary quantities of tryptophan	Divanach *et al*. 1996
	A dietary deficiency for one aminoacid could have major implications for larval development	Cahu *et al*. 2003
Generic nutritional factors	The possible role of nutritional factors in the aetiology of spinal deviations in fish has been extensively invoked	Hinton *et al*. 1992
	Skeletal malformations have been associated with nutritional deficiencies	Bruno and Poppe 1996
	Nutritional factors can cause alterations in the normal development of the skeleton leading to structural abnormalities in adult fish	Hilomen-Garcia 1997; Cahu *et al*. 2003; Lall and Lewis-McCrea 2007
	Larval nutrition appears to be a key factor and enrichment of larval diets with fatty acids and vitamin A can greatly reduce malpigmentation rates in hatchery-reared flatfish; however, levels sufficient to prevent pigmentation defects frequently cause other abnormalities	Bolker and Hill 2000
	Vertebrae and spinal anomalies (hypnosis, lordosis, scoliosis, platyspondyly) and neck bend (stargazer) are the most common nutrient linked disorders	Lall and Lewis-McCrea 2007
	Nutritional deficiency affects bone development in fish	Fernández and Gisbert 2009
Inert diet	Early feeding with micropellets is considered the cause in *S. aurata* for abnormal swim bladder	Modica *et al*. 1993
	Early feeding of *S. aurata* and *D. labrax* with micropellets causes jaws anomalies	Menu 1994
Minerals deficiency	Deficiencies of minerals such as phosphorus, magnesium, manganese, or zinc in the diets have produced skeletal abnormalities in salmonids	Ogino *et al*. 1978; Watanabe *et al*. 1984; Satoh *et al*. 1983
	Mineral imbalance of phosphorus in smolts and breeders of salmons	Gil-Martens *et al*. 2009
	Mineral imbalance of Ca is the cause of skeletal anomalies in smolts and breeders of salmons	Gil-Martens *et al*. 2009
	Low (0.5% dry matter basis) phosphorus diet in 4.6 g halibut for 14 weeks provokes abnormal haemal and neural spines in the haemal region and scoliosis in the cephalic and haemal vertebrae in halibut. Vertebrae and operculum are more sensitive to low and high dietary phosphorus intake	Lall and Lewis 2009

Table 8.13 (*Continued*)

Causative factor	Description	References
Oxidized lipid	Oxidized marine fish oil (peroxide value, 7.5 mEq kg^{-1}) in 4.6 g halibut for 14 weeks: dose-dependent scoliosis, spanning the cephalic/prehaemal regions as well as the anterior haemal region of the vertebral column. Lordosis, fused vertebrae, supernumerary and bifurcated neural spines. Increase in liver and muscle malondialdehyde concentration. Reduced liver ascorbic acid concentration	Lall and Lewis-McCrea 2007
Peptides (hydrolysate)	Spinal malformations were scarce in sea bass groups fed peptide diets (2–6%) and significantly lower than in the control group (24%)	Zambonino Infante *et al*. 1997
	In sea bass larvae: only 2% of the larvae, fed a diet incorporating 58% hydrolysate (CPSP) in substitution to fish meal, exhibited skeletal (spinal or jaw) malformations, while the malformation rate reached 13% in the group fed the diet without hydrolysate. Incorporation of di- and tripeptides instead of CPSP led to similar results: malformation rate decreased with increasing hydrolysate incorporation. Nevertheless, dietary incorporation of high hydrolysate levels has a negative effect on growth	Cahu *et al*. 2003
Phospholipid	Abnormal pigmentation rate was increased by a deficiency of dietary glycerophospholipids in marble sole larvae, *Limanda yokohamae*	Kanazawa 1993
	Vertebral axis deviations (scoliosis, kyphosis and lordosis) frequencies have been reduced by increasing (in different experiments) dietary quantities of phospholipids	Divanach *et al* , 1996
	Phosphatidylinositol prevents skeletal deformities in *Cyprinus carpio*	Geurden *et al*. 1997; 1998
	A dietary level of 1.6% phosphatidylinositol, used from first feeding onwards, is convenient for preventing deformities during sea bass development	Cahu *et al*. 2003
PUFA	Non-activated swim bladder and skeletal deformities in *S. aurata*	Koven *et al*. 1990
	S. aurata: absence of swim bladder	Koven *et al*. 1990
	Nutritional imbalances involving essential fatty acids have resulted in skeletal deformities in different species	Hinton *et al*. 1992; Divanach *et al*. 1996; Sfakianakis *et al*. 2006
	Skeletal deformities in *Clupea harengus*	Navarro and Sargent 1992

(*Continued*)

Table 8.13   (*Continued*)

Causative factor	Description	References
	The *Pleuronectes platessa* juveniles fed continuously on unenriched *Artemia* during their larval period showed 29.0% normal pigmentation whereas those fed solely on enriched *Artemia*, showed 63% normal pigmentation	Dickey-Collas 1993
	Hypomelanosis on the ocular side in marble sole larvae, *L. yokohamae*, was found in larvae fed DHA-deficient microdiets	Kanazawa 1993
	Direct provision of HUFA to larvae is critical to normal development and can significantly affect the health of hatchery-reared finfish larvae and deficiencies may result in malpigmentation	Watanabe 1993; Sargent *et al*. 1995
	Poor pigmentation was also related to low n-3 HUFA and especially very low DHA contents in polar lipids of turbot larvae	Rainuzzo *et al*. 1994
	The dietary ratio DHA:EPA has been considered as a determinant of pigmentation in turbot larvae, especially during premetamorphic stages	Reitan *et al*. 1994
	Forty-day-old milkfish fed HUFA/vitamin C-enriched live food had significantly lower incidence of opercular deformity, mainly cleft branchiostegal membrane (8.4–14.7%), compared with those given HUFA-enriched (15.8–23.5%), or unenriched 27.3–33.5% live food	Gapasin *et al*. 1998
	Incorporation of HUFA in *Artemia* was shown to diminish opercular deformities in milkfish	Gapasin *et al*. 1998
	Dietary induced malpigmentation observed in turbot fed Arachinoid acid enriched live-prey	Estévez *et al*. 1999
	Pigmentation anomalies are now known to be caused by impairment of neural development due to inadequate dietary provision of HUFAs	Bolker and Hill 2000; Koven 2003
	Opercular deformity was reduced by 50% in the group receiving DHA enriched live preys, compared to the control group (17% and 33%, respectively) in milkfish *C. chanos*	Gapasin and Duray 2001
	Studies on Atlantic halibut, turbot and Japanese flounder report higher metamorphic success, in terms of pigmentation, eye migration and general development, when copepods, rather than enriched *Artemia*, were fed to larvae	Koven 2003
	EFA-deficient diets produced abnormal eggs of low hatchability and high larval deformities	Watanabe and Vassallo-Agius 2003

Table 8.13   (*Continued*)

Causative factor	Description	References
	DHA content in rotifers on the occurrence of skeletal deformities in red porgy *P. pagrus*: elevated levels of bone abnormalities (mostly fused vertebrae) associated to low DHA content in the live prey. Although DHA content in the rotifers used for feeding was the only difference, a 50% reduction in the number of deformed fish for each type of deformity studied was obtained when the larvae fed higher DHA levels	Roo *et al*. 2009
Underfeeding	Underfeeding can increase fin erosion in Artic charr	Damsgård *et al*.1997
	Fin damages were high in feed-deprived cod	Hatlen *et al*. 2006
	No effects on deformities but does have an effect on vertebral shape	Gil-Martens *et al*. 2009
Vitamin A	Retinoic acid also exerts teratogenic effects on the pharyngeal skeleton when applied to embryos even after the end of cranial neural crest cell migration into the pharyngeal arches	Ellies *et al*. 1997
	Vitamin A levels sufficient to prevent albinism lead directly to overdoses of RA that can cause skeletal defects	Suzuki *et al*. 1998; Takeuchi *et al*. 1998; Suzuki *et al*. 1999; Suzuki 2000; Haga *et al*. 2002
	Vitamin A has been reported as a dietary component regulating pigmentation success in flatfish	Miki *et al*. 1990; Kanazawa 1993; Estevez and Kanazawa 1995
	Color abnormality on the ocular side of the flounder could effectively be reduced by feeding the larvae with rotifers enriched with 50,000 IU of vitamin A in 1 L of culture medium, but this vitamin A concentration caused bone deformity	Miki *et al*. 1990
	Feeding *Artemia* enriched with more than 40 mg vitamin A palmitate produced a negative effect on growth of larval flounder as well as a high incidence of bone deformity (mainly compressed vertebrae, intervertebral gaps, but also lordosis, scoliosis, vertebrae central fusions in almost all the vertebrae)	Dedi *et al*. 1995
	50 IU g^{-1} levels of vitamin A palmitate in *Artemia* cause skeletal abnormalities in turbot	Estevez and Kanazawa 1995
	Retinoic acid (RA), which induces duplications along the proximodistal axis in regenerating limbs, affects regeneration of the caudal fin in zebrafish	Geraudie *et al*. 1995

(*Continued*)

Table 8.13 (*Continued*)

Causative factor	Description	References
	In Teleost, exogenous RA applied at the shield stage induces severe malformations in the mandibular arches by anteriorizing the expression domain of *Hox* genes	Alexandre *et al*. 1996; Suzuki *et al*. 1998
	Vertebral axis deviations (scoliosis, kyphosis and lordosis) frequencies have been reduced by diminishing vitamin A quantities in diet	Divanach *et al*. 1996
	50 IU g^{-1} levels of vitamin A palmitate in *Artemia* cause skeletal abnormalities in larval flounder (*Paralichthys olivaceus*)	Dedi *et al*. 1997; Takeuchi *et al*. 1995
	Excess vitamin A levels and low calcium content of the water can also induce caudal complex abnormalities in Japanese flounder	Hosoya and Kawamura 1998
	Embryonic Japanese flounder treated with RA exhibit skeletal deformities in craniofacial structures, vertebrae, and fins	Suzuki *et al*. 1998; Suzuki 2000; Haga *et al*. 2002
	The high concentrations of various vitamin A compounds added into the *Artemia* culture medium were toxic to flounder, and the vertebrae became compressed, resulting in reduced growth	Takeuchi *et al*. 1998
	Incidence of vertebral deformity in flounder increased in correspondence with increased level of retinoic acid in *Artemia*. Vertebral deformity observed in this study was similar to that of hypervitaminosis A in juvenile flounder	Haga *et al*. 1999
	In *P. olivaceus*, it was demonstrated that the early and high dietary retinoic acid levels represses the expression of Hoxd-4 provoking jaw malformations and higher incidence of bone deformities	Suzuki *et al*. 1999
	The alteration of retinoids pathway induces skeletal malformations	Uni *et al*. 2000 ; Haga *et al*. 2002b; 2003
	Immersion of 8-day-old flounder larvae in different retinoic acid receptors (RAR/RXR) resulted in characteristic malformations in the lower jaw	Haga *et al*. 2001
	All-trans retinoic acid (ATRA) administered during 4–8 days to flounder caused a high number of abnormal vertebrae mainly in the caudal area	Haga *et al*. 2002b
	Exposition to synthetic retinoids of Japanese flounder larvae: Am80 [a retinoic acid receptor (RAR)a/h-selective retinoid] induced lower jaw deformities in all fish, whilst to methoprene acid [MA, a retinoid X receptor (RXR)-selective retinoid] induced lower jaw deformities in less than 20% of the fish. Am80 caused drastic deformities in the upper jaw as well as the lower jaw, while MA did not affect the upper jaw	Haga *et al*. 2003

Table 8.13 (*Continued*)

Causative factor	Description	References
	Studies on Atlantic halibut, turbot and Japanese flounder report higher metamorphic success, in terms of pigmentation, eye migration and general development, when copepods (which have higher levels of vitamin A), rather than enriched *Artemia*, were fed to larvae	Koven 2003
	In 87-days-old sea bass (4.4 $\pm$ 0.41 g) fed with 1 g retinoic acid (RA)/kg (dry weight), 78.8% were malformed versus 24.9% in the group fed on 0.05 RA/kg, with significative differences in neuro- and splanchnocranium anomalies. A linear relationship was found among the jaw and opercle deformations and the RA rate in larvae	Villeneuve 2005
	Dietary retinol had a significant effect on the development of deformities in all the body parts of sea bass but it depends to a great extend on the skeletal elements under consideration.	Mazurais *et al*. 2009
	In juvenile Atlantic halibut, toxicity of vitamin A resulted in scoliosis within two regions, the cephalic/prehaemal region and the anterior haemal region of the vertebral column	Lall and Lewis-McCrea 2007
	Retinoic acid exposure (at any dose tested) of larval Summer Flounder, *P. dentatus* increased the frequency of skeletal defects in jaws, fins, pectorals, hypurals, and vertebrae compared with controls	Martinez *et al*. 2007
	Increasing RA led to a greater frequency of hyper pigmentation among larval Summer Flounder, *P. dentatus*, that completed metamorphosis	Martinez *et al*. 2007
	Vitamin A deficit in Senegal sole early juveniles: vertebral and caudal fin deformities augmented; neural spines, haemal spines, caudal fins, fusions of hypurals 1–2, epurals in bone derived cell; endochondral bone much more sensitive than intramembraneous bone	Fernández and Gisbert 2009
	Solea senegalensis, fed vitamin A supplementations, showed marked increase in deformities	Gavaia *et al*. 2009
	High vitamin A (52,837 IU kg^{-1}) in 4.6 g Atlantic halibut (*Hippoglossus hippoglossus*) for 14 weeks: scoliosis spanning the cephalic/prehaemal and anterior haemal regions of the vertebral column, fused vertebrae, compressed vertebrae. Significant increase in liver retinol concentration	Lall and Lewis 2009

(*Continued*)

Table 8.13   (*Continued*)

Causative factor	Description	References
Vitamin C	Vitamin C influences skeletal development in finfish	Kanazawa *et al*. 1992; Gapasin *et al*. 1998
	Opercular abnormalities, associated with distortion of gill filament cartilages, are characteristic of scorbutic fish and are the result of a decalcification	Dabrowski *et al*. 1990
	Shortened operculum occurrence was high in *Ciclasoma urophtalmus* nourished with insufficient quantities of vitamin C	Chaves De Martinez 1990
	Abnormal pigmentation was further increased by a deficiency of dietary retinol in marble sole larvae, *L. yokohamae*	Kanazawa 1993
	In *D. labrax* and *S. aurata,* vitamin C deficiency is associated to skeletal anomalies	Chatain 1994b
	Vertebral axis deviations (scoliosis, kyphosis, and lordosis) frequencies have been reduced by increasing (in different experiments) dietary quantities of vitamin C	Divanach *et al*. 1996
	Forty-day-old milkfish, *C. chanos*, fed HUFA/vitamin C-enriched live food had significantly lower (P<0.05) incidence of opercular deformity mainly cleft branchiostegal membrane (8.4–14.7%) compared with those given HUFA-enriched 15.8–23.5%.or unenriched 27.3–33.5% live food	Gapasin *et al*. 1998
	Rainbow trout fed the chitin-enriched diet, the low vitamin C diet, the high vitamin C diet, and the control diet had the highest deformity levels, ranging from 8.9 to 12.5%, while the group fed the glucan-enriched diet had the lowest level of deformities (4.8%). In all groups examined, the deformities were spread over the whole vertebral column. The deformities in the group fed the low vitamin C diet were more severe than those found in the other groups	Madsen and Dalsgaard 1998
	Vitamin C deficiency in smolts and breeders of salmons is considered a cause of skeletal anomalies in fish	Gil-Martens *et al*. 2009
	No vitamin C supplement in 4.6 g halibut for 14 weeks: scoliosis and lordosis primarily in the haemal vertebrae and fused and compressed vertebrae	Lall and Lewis 2009
Vitamin D	Winding of the abdominal vertebrae in Japanese flounder fed on 0.05 mg 1,25-dihydroxyvitamin D_3/100 g are especially in the middle part of the abdominal vertebrae, where a higher incidence of winding was observed compared to fusion. Severe and complete hypermelanosis was observed only in this group	Haga *et al*. 2004

Table 8.13   (*Continued*)

Causative factor	Description	References
Vitamin K	In mummichog *F. heteroclitus*, fed a phylloquinone-supplemented diet (as a vitamin K source in fish feed) showed significantly lower occurrence of vertebral fusion, vertebral deformity, and vertebral row irregularity and no effect on neural and haemal spine abnormalities such as fusion, extraossification and a combination of the two	Udagawa 2001
	The minimum levels of vitamin K that promotes a normal skeletal growth and mineralization has been recently determined for haddock	Roy and Lall 2007
	Vitamin K deficiency inhibits mineralization and enhances deformity in vertebrae of haddock (*Melanogrammus aeglefinus*)	Roy and Lall 2007

concentration, catabolites, etc.) during the experiment, or the species or the fish size chosen for the tests, or the initial morphological quality of the fish used for the test. Rearing conditions may themselves be a cause of homeostasis alteration and, thus, be synergistically concurrent in inducing deformities. The differences in response may be due to species or fish size/age. Also, if the initial lot already shows a high prevalence of deformed individuals, it may be difficult to interpret some responses. Basic *ad hoc* experiments to investigate the mechanisms underlying the onset of skeletal anomalies in fish seem to be required, but so far only a few have been carried out. Almost all the experiments are necessarily performed in obligated "unnatural conditions" (aquaria, static conditions), which are particularly liable to cause stress in undomesticated animals, such as all fishes except carps (Balon 2004). This is probably the reason why it is not yet possible to identify a single cause for each anomaly and vice versa, notwithstanding the large amount of scientific work undertaken since the first publications on skeletal deformities in reared fish (Santerre 1976; Barahona-Fernandes and Girin 1977; Paperna 1978).

8.6.1   *Epigenetic causes related to rearing conditions*

These causes include the effects of broodstock age, nutritional and health status, egg quality, stocking density, fast growth conditions—such as 24 hours light regime, high water temperature, and high feed level—handling stress, hydrodynamism/water turbulence/water supply rate, intensive versus semi-intensive hatchery conditions, light, mechanical factors, oil films on water surface, levels of O_2/CO_2, pH, physical trauma/mechanical stress, pathogens, parasites, radiation, salinity variation, typology of substratum (mainly for flatfish), tank characteristics (volume, shape, color, material), variation of temperature and toxins (Tables 8.2 and 8.3). The most extensively studied environmental conditions in larval rearing in the last decade have been higher or inappropriate water dynamics in the tanks, high stocking densities, and fast growth conditions. These parameters are considered the triggering causes of secondary, induced, physiological alterations of homeostatic processes during bone developmental or physiological processes caused by distressing rearing conditions. Concerning high stocking densities, fast growth conditions, inappropriate water dynamics, mechanical factors, and physical trauma/mechanical stress, they can all be included in a group of causative factors relating to the "mechano-regulatory pathway" (Prendergast *et al*. 1996) of interactions between biophysical stimuli and tissue phenotype. It is assumed that the relationship between the mechanical forces on cells and the biochemical environment generated by cell

Table 8.14 Physiological (epigenetic) factors reported in literature as causes of skeletal anomalies in fish

Causative factor	Description	References
Distress	In *P. pagrus*, stress may cause development of unnatural pigmentation	Van der Salm *et al*. 2004
	Sole larvae with a darker color had significantly higher cortisol levels than sole with lighter color	Ruane *et al*. 2005
	Differences in color of the Senegalese sole larvae at 20 DAH are related to different levels of stress in larvae	Engrola *et al*. 2009
Inflammation	Inflammation alters the normal pattern of bone growth and can lead to spinal deformities	Gratacos *et al*. 1994, Raisz 1999, Raisz and Lorenzo 2006
	In Atlantic salmon, observed deformities corresponded to vertebral fusions located at or in proximity to the injection point. The injection may cause local inflammation which can disturb bone growth resulting in a spinal deformity at a later stage	Gil-Martens *et al*. 2009
	MMP-13 gene is overexpressed in chronic inflammation and showed a significant upregulation in all structures (notochord, compact bone and trabecular bone) of the compressed vertebrae. MMP-13 could be involved in the aetiology of expanding deformity, as the chronic inflammation could be involved in the primary aetiology of the compressed deformity	Wargelius *et al*. 2009
Immunological responses	Vaccinations, infections, and autoimmunity are considered causes of bone abnormalities in Atlantic Salmon	Wargelius *et al*. 2009
Thyroid hormones	Treatment with doses of triiodothyronine (T_3: 0.1 or 0.01 μM) influenced skeletal ontogeny of the larvae and intriguingly, skeletal anomalies affecting the caudal fin and cranium, reminiscent of those frequently reported in aquaculture populations were observed	Power *et al*. 2001
Vaccination	Vaccination can cause abdominal adhesions and skeletal deformities that can lead to decreased growth	Ellis 1997; Cipriano and Bullock 2001, Jutfelt *et al*. 2006; Gil-Martens *et al*. 2009

expressions ultimately generates a relationship between the tissue and its mechanical function. Therefore, tank conditions causing hyperstimulation of muscles induce an increased mechanical load on adjacent vertebrae causing vertebrae or axis deformities in several Teleosts (Kranenbarg *et al*. 2005). Mechanical strengths interact in the modeling and remodeling of all skeletal elements. For example, Prendergast *et al*. (1997) found that tension forms fibrous connective tissue, shearing forms cartilage and compression forms bone.

There is much evidence to suggest that the total number of deformed larvae in many cultured fish and the percentage of larvae with deformities related to the vertebral column, caudal complex, jaw, and anal fin are

Table 8.15 Genetic factors reported in literature as causes of skeletal anomalies in fish

Causative factor	Description	References
Ascertained heritability	Caudal deformity syndrome (CDS), an autosomal recessive lethal mutation in the tilapia, *Oreochromis niloticus*, affecting the terminal tract of the spinal column, has been reported as simple Mendelian inheritance	Mair 1992
	The existence of an underlying genetic component is based on the observation that lordotic larvae of cultured gilthead sea bream (*S. aurata*) could proceed from surviving lordotic fish of the previous generation	Andrades *et al*. 1996
	The magnitude of the estimated heritabilities for deformity *(humpback* anterior or posterior dorsal fin or a shortened tail) in *S. salar* shows that vertebral deformity is determined by a substantial additive genetic component	Gjerde *et al*. 2005
	Moderate to low heritabilities for external color and spinal deformities (0.21 ± 0.02 and 0.12 ± 0.02, respectively) were ascertained in gilthead sea bream	Thorland *et al*. 2006
	When there was a management failure and incidence was unusually high, heritability for skeletal deformations in farmed rainbow trout *O. mykiss* was elevated. Consequently, the occurrence of developmental disorders in farm animals should be controlled by selective breeding when traits have genetic variation	Kause *et al*. 2007
	Paternally and maternally inherited factors may contribute to the expression of swim bladder anomalies in sea bass along with major environmental clues	Peruzzi *et al*. 2007
	In gilthead sea bream no significant heritabilities were observed at both lordosis and lacking operculum and only a weak indication of genetic control was revealed for lacking operculum	Castro *et al*. 2008
	The susceptibility for the presence/absence of column deformations in 11,038 gilthead sea bream (*S. auratus*) from 18 sires and 19 dams showed a high additive genetic component of the body deformities, suggesting the presence of a gene with important influence for trait	Afonso *et al*. 2009
	Lordosis and consanguinity levels in brood stock of gilthead sea bream affect larva quality increasing the incidence of deformed larvae	Afonso *et al*. 2009
	Lordosis-Scoliosis-Kyphosis (LSK) syndrome was statistically associated with the family structure, under controlled crosses and mass spawning in gilthead sea bream (*S. auratus*), suggesting a possible genetic origin	Afonso *et al*. 2009

(*Continued*)

Table 8.15   (*Continued*)

Causative factor	Description	References
	Reported estimations of heritability for skeletal abnormalities (lordosis and lack of operculum) in gilthead sea bream (*S. auratus*) fingerlings, on an experimental scale	Navarro *et al*. 2009b
Generic genetic factors	Genetic factors are invoked for skeletal malformations	Afonso *et al*. 2000; Shikano 2005
	Skeletal malformations have been associated with genetic effects	Sindermann 1990
	Genetic factors have often been cited as the causes of caudal fin deformation, as well as lordosis and fused vertebrae	Andrades *et al*. 1996; Tomita 1991
	Several reports have suggested that both simple Mendelian genes and polygenic determinants could explain the incidence of skeleton deformities	Andrades *et al*. 1996; Afonso *et al*. 2000; Gjerde *et al*. 2005; Shikano 2005
	Several reports have suggested that both simple Mendelian genes and polygenic determinants could explain the incidence of body pigmentation anomalies	Kause *et al*. 2003; Shikano 2005
	Different families or cohorts of farmed salmonids have shown a variable response to specific environmental stressors, suggesting underlying genetic variation to these characters	Kause *et al*. 2007
	The defects of the fins and scales are due to the role of *Eda* (mutation in zebrafish *edar* gene) signalling in organizing epidermal cells into discrete signalling centres of the scale epidermal placode and fin fold	Harris *et al*. 2008
	Genetic factors affect bone development	Fernández and Gisbert 2009
	Inbreeding and triploidosis are among the causes for skeletal deformities in smolts and breeders of salmons	Gil-Martens *et al*. 2009
	Genetic segregation of an LSK deformity was found in gilthead sea bream (*S. auratus*), under mass spawning	Navarro *et al*. 2009a
Genetic modifications	Most genetic correlations between deformations recorded in different generations of farmed salmonids were weakly to strongly positive, and also negative correlations between generations were present. The results emphasize that genetic architecture of binary traits can be influenced by trait expression	Leatherland and Woo 1998
	Transgenic salmon exhibited cranial, jaw, and opercular deformities	Kang and Devlin 2003
Mutation	The eye deformity (*ey-l*) and fused vertebra (*fu-6* and *fu-7*) mutants were found in the medaka, *O. latipes*	Tomita 1991
	A homozygotic morphogenetic mutant (*Da*) in medaka develops ventralization of dorsal counterparts in the caudal region	Ishikawa 1990

Table 8.15 (*Continued*)

Causative factor	Description	References
	An autosomal recessive lethal mutation in tilapia *(O. 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
	The strain differences suggested the existence of polymorphism of a malformed gene(s) in the guppy *P. reticulata*	Ando *et al*. 1995
	In zebrafish (*D. rerio*) the *no-tail* mutant phenotype is caused by a mutation in the zebrafish homologue of the murine T gene	Schulte-Merker 1995
	A slight association with inbreeding has been reported on skeleton abnormalities in gilthead sea bream, *S. aurata*	Astorga *et al*. 2003
	Zebrafish touchtone/nutria mutants exhibit severe growth retardation and gross alterations in skeletal development in addition to embryonic melanophore and touch–response defects. These alterations include accelerated endochondral ossification but delayed intramembraneous ossification, as well as skeletal deformities. The touchtone/nutria phenotype results from mutations in *trpm7*	Elizondo *et al*. 2005
	Lordosis and consanguinity levels in brood stock of sea bream affect larval quality, increasing the incidence of deformed larvae: crossing with different levels of consanguinity and family background	Afonso *et al*. 2009
Selective breeding	In Finland, selective breeding has been suggested by veterinarians to be a potential factor increasing the frequency of skeletal deformations in farmed rainbow trout	Koski 2000
	The prevalence of skeletal deformities throughout the development of different populations (all-female triploid, mixed-sex triploid, all-female diploid and mixed-sex diploid) of Tasmanian Atlantic salmon (*S. salar*) was significantly higher in triploid populations, with jaw deformity, including lower jaw deformity syndrome (LJD), highest in triploid FW smolt. Short opercula were observed in up to 22% of triploids and 16.6% of diploids. Up to 60% of triploids and 4% of diploids suffered from the absence of primary gill filaments (gill filament deformity syndrome) (GFD) during FW development prior to SW transfer, and up to 50% of triploid FW smolt and 60% of triploid SW smolt	Sadler *et al*. 2001
	The genetic correlation between 50.8 ± 16.5 gr (BW) rainbow trouts and deformation rate observed when BW $= 1080 \pm 263$ gr was positive, indicating that when selection is applied on weight recorded during tagging, selection may tend to increase the frequency of deformations	Kause *et al*. 2005

(*Continued*)

Table 8.15   (*Continued*)

Causative factor	Description	References
Triploidy	Triploid rainbow trout embryos and larvae have a higher incidence of deformities (macrocephalia, lordosis and twisted body) than diploids	Myers and Hershberger 1991
	Lower jaw deformities in triploid Atlantic salmon are considered due to the triploid condition itself rather than to the induction shock applied to eggs	Sutterlin and Collier 1991, McGeachy *et al.* 1996; Sadler *et al.* 2000
	High numbers of deformities in triploid red sea bream contributed to their lower survival	Sugama *et al.* 1992
	A high incidence of deformities in triploid European sea bass larvae is described, which died just after hatching	Zanuy *et al.* 1994
	15–20% incidence of spinal deformities in triploid all-female rainbow trout (*O. mykiss*) (no data for diploid controls)	Madsen *et al.* 2000
	Atlantic salmon triploids were more affected than diploids by skeletal, opercular and gill filament malformations and presented a reduced gill surface area but they found impossible to separate both effects from triploidy itself and shock damage	Sadler *et al.* 2000
	In European catfish (*Silurus glanis*), all heat shocks treatments for triploidization tested produced a high percentage of malformations, up to 80%	Linhart *et al.* 2001
	The overall incidence of external vertebral axis deformities, although higher in triploids than in diploids Atlantic salmon (*S. salar*) early in development, is lower in triploids at slaughtering. A higher rate of eye cataracts in triploids was recorded	Oppedal *et al.* 2002; Cotter *et al.* 2002

higher in intensive rearing tanks than in semi-intensive tanks (Boglione *et al.* 1993, 1994, 1998a, 1998b, 1998c, 2000, 2001, 2008; Cataudella *et al.* 1995, 1994a; Divanach *et al.* 1996; Koumoundouros *et al.* 1997a, 1997b; 1999, 2000; Loy *et al.* 1995, 1999, 2000; Sola *et al.* 1998; Divanach and Kentouri 1983, 2000; Shields 2001; Papandroulakis *et al.* 2004; Sfakianakis *et al.* 2004, 2005; Gimenez & Estevez 2008), although Koumoundouros *et al.* (2004) cited an incidence of urinary calculi in 30–40% of fish reared using the semiextensive mesocosm method. Also, Gimenez and Estevez (2008) found that the incidence of deformities affecting opercula, caudal, and dorsal fins in common dentex reared in intensive *versus* those reared in mesocosm conditions, did not differ.

8.6.2   *Epigenetic factors: xenobiotics*

Antibiotics, hormones, heavy metals, herbicides, metals, ammonia, PCBs, pesticides, and pollutants have been reported to induce malformations in fish (Table 8.1). Numerous studies have shown that exposure to some xenobiotics causes vertebral damage through effects on the neuromuscular system or on collagen and bone metabolism and composition (Fent 1992). Deformed jaws, fins, vertebrae, and vertebral column are often observed in fish with high concentrations of heavy metals in muscle and liver. In the past, these causative factors were invoked almost exclusively to account for skeletal anomalies in wild fishes. Some 50% of the literature

dealing with xenobiotics was published in the last 9 years. In these more recent papers, a series of tests was conducted on reared fish exposed to certain hormones or heavy metals, or fed diets containing different metals, to see if there was any relationship with the onset of skeletal anomalies. Among Sparid fish, manganese and zinc enrichment in *Artemia* caused vertebral deformity (Nguyen *et al.* 2008) and reduced growth, short-body dwarfism, disturbance of bone formation, anorexia, cataracts, fin erosion, reduced zinc content in body and bone, reduced calcium in bone and high mortality (Nguyen *et al.* 2008) in red sea bream larvae.

8.6.3   *Epigenetic factors: nutrition*

Aminoacids, indirect nutritional deficiencies, inert diet, mineral deficiencies, oxidized lipids, peptides (hydrolysates), phospholipids, PUFAs, underfeeding, and vitamins A, C, D, and K have been documented to cause malformations in fish (Table 8.13). Since the first paper published on the toxic effects of vitamin A hypervitaminosis in rainbow trout (*Oncorhynchus mykiss*) (Hilton 1983), the search for the optimal doses of vitamin A enrichment has been the main topic of recent studies, but most of them deal with flatfishes. Another topic investigated in recent studies is that of vitamin A action mechanisms, starting from the effect of hypervitaminosis on accelerating chondrocyte maturation and stimulation of osteoclasts (Vasan and Lash 1975). The consequences described consisted of a delay in the production of bone matrix. This accelerates the development of the vertebral column through precocious mineralization, which can cause skeletal deformities including vertebral curvatures (Dedi *et al.* 1995, 1997; Estevez and Kanazawa 1995), vertebral compression (Dedi *et al.* 1995, 1997; Takeuchi *et al.* 1998), vertebral fusion (Dedi *et al.* 1995, 1997; Takeuchi *et al.* 1995), and jaw deformities (Haga *et al.* 2003). Fresh insight comes from the observation that the exposure of flatfish larvae to different synthetic retinoids produces deformations in different bones (Haga *et al.* 2003), with endochondral bone being much more sensitive than intramembraneous bone to vitamin A dietary imbalances (Fernández and Gisbert 2009). Mazurais *et al.* 2009 found that the dietary retinol levels for the optimal development of European sea bass larvae depend to a great extent on the skeletal elements under consideration, for example, jaw and hyoid deformities were reduced for retinol levels lower than 9402 IU kg^{-1} DM (mg per kg of dry matter), whilst vertebral and fin elements deformities decreased when retinol levels were higher than 9402 IU kg^{-1} DM. The concomitant presence of diversified differentiation level of skeletal elements at the moment of retinol administration could be the reason for such dissimilar effects on different bones. This means that any indication on optimal level of vitamin A for a given species is invalidated and that an age-effect on the response of European sea bass to dietary retinol could be hypothesized. The biological activity of retinoids, which are vitamin A-related compounds, is thought to be mediated mainly by retinoic acid (RA) and to be involved in the regulation of cell differentiation, proliferation, and morphogenesis (Haga *et al.* 2002). The effects of excessive RA on the developing central nervous system include posteriorization, loss of anterior hindbrain, and transformation of the anterior hindbrain. The hindbrain is a source of neural crest cells that contribute to connective tissue, cartilage and bone of the craniofacial skeleton, odontoblasts of the teeth, and to neurons and cranial ganglia. *Homeobox* (*Hox*) genes provide positional information for neural crest cells and regulate their differentiation and are involved in the formation process of the anteroposterior axis (Conlon 1995).

Other recent papers address vitamin D and K involvement in causing skeletal or pigmentation anomalies. Vitamin D$_3$ is metabolized and is biologically functional in the form of 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$), which positively regulates calcium absorption in marine fish living in a calcium-rich environment (Haga *et al.* 2004). When a diet supplemented with excess of vitamin D$_3$ compounds was administered during vertebral morphogenesis, vertebral deformities were apparently introduced in some species and not in others (Haga *et al.* 2004). The inconsistency of these and other data justifies the hypothesis of size- and T-dependence effects of hypervitaminosis of vitamin D.

As far as vitamin K is concerned, the studies of its effects on developmental anomalies in fish are scarce, but recent studies open up new insights into the inherent biomolecular mechanisms (Udagawa 2001; Roy and

Lall 2007). *Matrix (γ-carboxyglutamic acid) Gla protein* (*Mgp*) and *bone Gla protein* (also known as *Bgp* or *osteocalcin*) are small extracellular matrix proteins, originally isolated from vertebrate bone and belong to the family of Ca^{2+}-binding vitamin K-dependent proteins. Recent work indicates that *Bone Gla* protein is required for adequate maturation of the hydroxyapatite crystal in bone (Pinto *et al.* 2001), and it is an endogenous calcification inhibitor (Gavaia *et al.* 2006). In gilthead sea bream, it was found that the molecular organization of the *Sparus* BGP (*spBGP*) gene is similar to mammalian *BGP* genes, and its expression throughout development follows the onset of calcification occurring only in bone, and showing extensive homology to its mammalian homolog (Pinto *et al.* 2001). Bone formation by osteoblasts, mineralization of bone matrix, and bone remodeling involving bone resorption by osteoclasts are three important steps for bone development and growth in fish (Witten *et al.* 2000). Bone demineralization is an early sign of vitamin K deficiency, which may progress over time to bone fractures and structural deformities when deficiency is continued for 8–20 weeks (Roy *et al.* 2002). The minimum levels of vitamin K that promote normal skeletal growth and mineralization were not yet determined for sparids (Roy and Lall 2007).

8.6.4   *Epigenetic factors: physiology*

Distress, inflammation, immunological responses, thyroid hormone growth regulation, and vaccinations have been associated with other anomalies (Table 8.14). Distress has been blamed for malpigmentation in red porgy (Van der Salm *et al.* 2004), and the inflammation processes observed in deformed vertebrae have been considered the etiological factor for altered bone growth and expansion of the deformity (Gratacos *et al.* 1994; Raisz 1999; Raisz and Lorenzo 2006; Wargelius *et al.* 2009). Some experimental approaches have been designed to test the capacity to induce skeletal anomalies of xenobiotics, forced hydrodynamic conditions, temperature and salinity variations. Some of these studies were carried out with reared species (as reported above), but most of them used freshwater fish, particularly zebrafish (*Danio rerio*). This is due to the ease with which zebrafish may be reared and reproduced in artificial conditions, to the short time it takes to reach sexual maturity, and to the extensive availability of known mutants in numerous genes involved in early ontogenesis development regulation. Zebrafish belongs to the minnow family (*Cyprinidae*), which is typically characterized by a relatively high phenotypic plasticity. Ferreri *et al.* (2000) had already highlighted that the high occurrence (87.2%) of deformed individuals in wild fish from the Ganges river system was comparable to that observed in zebra fish from broods of commercial origin for aquaria (93.4%). Furthermore, wild zebrafish presented higher frequencies of some anomalies than reared ones, for example, hemal supernumerary elements, opening of the neural arch or spine bifurcation, caudal shift of insertion of hemal arches, vertebral body anomalies, axis anomalies, and dorsal and anal anomalies. This characteristic phenotypic plasticity of zebrafish may have the drawback of increased risk of unsound results obtained in experimental tests, as the control seems to be too variable to validate them in any statistical analysis. Some authors have also stressed this problem in the case of other species, signalling the presence of a substantial background level of skeletal anomalies, so that a high frequency of defects (e.g., vertebral abnormalities) was also observed in controls (Martinez *et al.* 2007). Further, the small number of eggs laid by a zebrafish female compared to other fish could not help to bypass this problem by increasing the number of individuals. Even more detailed studies at histological or cellular level performed on this species are also at risk, as the actual "normal" conditions might be difficult to establish. Therefore, it is necessary to accurately describe the rearing conditions and morphological quality of the zebrafish lot used for experimental tests, also for the new field of research applied to human skeleton problems, using zebrafish as a model.

8.6.5   *Genetic factors*

Studies have dealt mainly with genetic monitoring, seeking a correlation between genotype and altered phenotype (Table 8.15). A series of studies have been conducted to ascertain the heritability of deformations, the existence

of gene mutations in deformed fish, the effects on the phenotype of genetic modifications, of selective breeding and of triploidy. Scientific papers on the presence of genetic drift in reared stocks, published during the 1980s, have been abandoned in the last 20 years.

Studies on genetic causes for deformations in fish are still ongoing, with new horizons opened up by the development of highly polymorphic genetic markers, such as microsatellites, which have provided an essential tool for estimating the genetic basis of body malformations (O'Reilly & Wright 1995; Ferguson & Danzmann 1998; Castro *et al*. 2004, 2006, 2007). The genetic basis of susceptibility to environmentally generated deformities in reared fish was not fully investigated in the past and recently published papers attempted to fill this knowledge gap.

Although little attention has been paid to their genetic basis, several reports have suggested that both simple Mendelian genes and polygenic determinants could explain the incidence of body pigmentation (Kause *et al*. 2003; Shikano 2005) and skeletal deformities (Andrades *et al*. 1996; Afonso *et al*. 2000; Gjerde *et al*. 2005; Shikano 2005). A series of papers has recently been published on the heritability of some skeletal deformities in gilthead sea bream. For example, Thorland *et al*. (2006) found moderate to low heritabilities for external color and spinal deformities (0.21 ± 0.02 and 0.12 ± 0.02, respectively). Castro *et al*. (2008) reported that in gilthead sea bream no significant heritabilities were observed in the case of either lordosis or reduced operculum and only a weak indication of genetic control was revealed for reduced operculum. Afonso *et al*. (2000) initially found that the LSK syndrome was statistically associated with the family structure, under controlled crosses, and mass spawning in gilthead sea bream, suggesting a possible genetic origin. In a later paper (Afonso *et al*. 2009), lordosis and consanguinity levels in gilthead sea bream brood stock were shown to affected larval quality, increasing the incidence of deformed larvae, and proved that the susceptibility for the presence/absence of column deformations in 11,038 fish from 18 sires and 19 dams showed a highly additive genetic component of body deformities, suggesting the presence of a gene with important influence for trait. Also Navarro *et al*. (2009b) reported estimates of heritability for skeletal abnormalities (lordosis and lack of operculum) in gilthead sea bream fingerlings, on an experimental scale, and a slight association with inbreeding was reported on skeleton abnormalities in gilthead sea bream by Astorga *et al*. (2003).

8.7   Discussion and conclusions

Some advances have been made in the reduction of skeletal anomalies in reared fishes. For example, safe levels of vitamin A were investigated in flatfish and related to the developmental problems during skeletogenesis in Japanese flounder (Dedi *et al*. 1995; Takeuchi *et al*.1995) and to the pigmentation success in turbot (*Scophthalmus maximus*) (Estevez and Kanazawa 1995). The minimum levels of vitamin K that promote a normal skeletal growth and mineralization were recently determined for haddock (*Melanogrammus aeglefinus*) (Roy and Lall 2007). Also, the incorporation of vitamin C and HUFA in *Artemia* was shown to diminish opercular deformities in milkfish (*Chanos chanos*) (Gapasin *et al*. 1998). The supplementation of phosphorus (Uyan *et al*. 2007), and the substitution of native fish meal proteins with fish meal hydrolysate (Zambonino Infante *et al*. 1997), led to a decrease in deformities in fish. Finally, surface skimmers in the tanks reduced the occurrence of larvae with uninflated swim bladder (Chatain Ounais-Guschemann 1990).

However, an analysis of the literature consulted reveals the possibility that a species and/or a developmental stage effect might exist and may be summed together with all the other epigenetic factors that interact in the onset of skeletal anomalies. The hypothesis of a species-specific response to environmental stress due to captivity was recently formulated by Almeida *et al*. (2008) who analyzed levels of fluctuating asymmetry, morphological abnormalities, and ectoparasites in goldfish (*Carassius auratus*) and two varieties of carp (*Cyprinus carpio*) in wild and captive conditions. Further, the existence of critical developmentally sensitive periods is well known in larval flatfishes, in which specific sensitive periods or "windows" have been identified, during which

juvenile pigmentation can be enhanced or perturbed by dietary manipulations (Næss & Lie 1998; Bolker & Hill 2000); the morphogenesis of specific skeletal elements is presumably also differentially sensitive to disruption at successive stages (Martinez *et al*. 2007). The latter authors also hypothesized an ongoing vulnerability to retinoic acid overdose across a range of developmental stages rather than interference with a single stage or process.

Another open problem regards the fact that, when *in toto* staining analyses are carried out, very few completely normal fish are found even among controls, which indicates a very high background level of skeletal defects, particularly vertebral abnormalities (Martinez *et al*. 2007). In such a framework, the use of control groups in any experimental test should probably be considered useless, owing to the high background noise constituted by the unnatural rearing conditions. The rearing conditions include perhaps too many factors that differ from the optimal ones of the species, which interact both among themselves and with the factor being tested. The risk is that of not obtaining unbiased and sound results, applicable to all the rearing conditions, developmental stages, or species. To end, it would probably be better to obtain and use the existing data on occurrences of skeletal deformities or other anomalies in wild juveniles or adults of the same studied species as a control group, if the aim is to solve the problem of deformities in reared fish.

Up to now, the available data seem to indicate that anomalies are the consequence of so many influential factors acting and interacting among themselves, that interdisciplinary studies combining anatomic, genetic, and biomolecular with physiologic data on larvae welfare conditions will probably be necessary. Vertebral malformations may now be the norm in hatchery lots. It seems that all reared fish should be considered as "stressed" fish, in which epigenetic and genetic factors strive to buffer the environmental noise effects, but are not always successful. Intensive feeding and the lack of predators allow the deformed individuals to live on and that is a problem for the farmers.

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