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Skeletal descriptors and quality assessment in larvae and post-larvae of wild-caught and hatchery-reared gilthead sea bream (*Sparus aurata* L. 1758)

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Abstract

Gilthead sea bream from different Mediterranean hatcheries show a consistent number of skeletal anomalies, mainly in the hemal and caudal body regions. Four hundred and twenty-two hatchery-reared sea bream from Italy, France and Spain were checked for skeletal malformations and meristic counts. The same skeletal descriptors were examined in 72 wild-caught sea bream. Correspondence analysis (CA) was performed to rank groups of hatchery-reared sea bream according to their skeletal abnormalities. The position of each group on the first correspondence axis with respect to the wild-caught specimens was used as a larval quality indicator. Wild phenotype similarity was also tested performing meristic counts and inter-groups meristic differences were illustrated. Results highlighted a quality gap between wild-caught and hatchery-reared specimens, with the only exception being sea bream larvae that were reared in semi-intensive conditions. This larval monitoring method is proposed as a tool for evaluating hatchery larval quality at a morpho-anatomical level.

Keywords: *Sparus aurata*; Gilthead sea bream; Anomalies; Larvae; Larval quality

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1. Introduction

In marine fish culture, the problem of an insufficient supply of fry has been overcome (Matsuoka, 1987; Caggiano, 1988; Chatain, 1994a), and a general improvement of hatchery rearing, and selection techniques has been achieved (Chatain, 1986; Chatain and Ounais-Guschemann, 1990; Chatain and Corrao, 1992). The increase in Mediterranean aquaculture production of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) fry is greatly impaired, however, by a high incidence of skeletal malformations (Barahona-Fernandes, 1982; Caggiano, 1988; Balbelona et al., 1993; Boglione et al., 1993; Koumoundourous et al., 1997a). In particular, Barahona-Fernandes (1982) reported that an abnormal operculum was found in up to 90% of the 100-day-old *D. labrax*, reared in hatcheries. Other authors have reported quite similar incidences (80%) in hatchery-reared gilthead sea bream (Paperna, 1978; Francescon et al., 1988; Chatain, 1994c; Andrades et al., 1996). Komada (1980) reported that hatchery-reared *Plecoglossus altivelis* had a malformation frequency ranging from 100 to 500 times more than their wild counterparts. Matsuoka (1987) reported how specimens with either abnormal bone or abnormal fin pterigiophore arrangements amount to 94% and 100% in two hatchery-reared groups of red sea bream (*Pagrus major*). Although not reported in the literature, inquiries at various farms indicated that the rate of discarded abnormal gilthead sea bream ranged between 15% and 50% according to farm and country (P. Divanach, personal communication). Malformations are economically important, as they require manual sorting (Koumoundourous et al., 1997b), and they lower the performances of hatchery-reared fish (i.e., swimming ability, conversion index, growth rate, survival, and susceptibility to stress, pathogens, bacteria) (Balbelona et al., 1993; Barahona-Fernandes, 1982; Andrades et al., 1996; Hilomen-Garcia, 1997; Matsuoka, 1987; Koumoundourous et al., 1997b; Jofre, 1988; Paperna et al., 1977). Furthermore, they have a negative effect on the final step of commercialisation because hatchery-reared fish have a different shape from the wild-caught ones (Koumoundourous et al., 1997b; Loy et al., 1998), and are therefore ruled out by the consumers. So the potential impact of the problem has been well reported and morpho-anatomical parameters (i.e., skeletal malformations) can be very useful for production management because they refer to chronology and conformity of development (Chatain, 1994a).

There is a considerable amount of literature on wild and reared fish abnormalities today (as reviewed by Divanach et al., 1996). Both genetic (Aulstad and Kittelsen, 1971; Barahona-Fernandes, 1982; Taniguchi et al., 1984; Poynton, 1987; Ishikawa, 1990; Mair, 1992) and epigenetic causes have been suggested as a possible source of such alterations (Sola et al., 1998). In particular, temperature (Boglione et al., 1989; Purcell et al., 1990; Bertolini et al., 1991; Marino et al., 1991; Polo et al., 1991; Wiegand et al., 1989), light (Bolla and Holmefjord, 1988), salinity (Lee and Menu, 1981), pH (Steingraeber and Gingerich, 1991), low oxygen concentrations (Hubbs, 1959), inadequate hydrodynamic conditions (Backiel et al., 1984; Kentouri, 1985; Chatain, 1994b), feeding quality (Kanazawa, 1985; Mazik et al., 1987; Zitzow and Millard, 1988; Robin et al., 1996) and parasites (Lom et al., 1991; Treasurer, 1992) have been reported as sources of skeletal malformations in farms.

Only a few studies have attempted to compare hatchery-reared larvae and juveniles to their wild-caught counterparts from an osteological point of view (Matsuoka, 1987). In Perciform fish, such studies have been carried out on sea bass (*D. labrax*) (Boglione et al., 1993; Marino et al., 1993) and red sea bream (*P. major*) (Matsuoka, 1987). There are very limited data on wild-caught gilthead sea bream malformations in the literature, and also very little information related to meristic count variations. Many authors have reported malformations on hatchery-reared juveniles and adults. These include axial (Paperna, 1978; Caggiano, 1988; Francescon et al., 1988; Polo et al., 1991; Balbelona et al., 1993; Chatain, 1994b; Kiriakos et al., 1994, Andrades et al., 1996, Koumoundourous et al., 1997a), opercular (Paperna, 1978; Francescon et al., 1988; Menu, 1994; Koumoundourous et al., 1997b), caudal complex (Paperna, 1978; Koumoundourous et al., 1995, 1997a) and upper and lower jaw (Caggiano, 1988; Menu, 1994) anomalies.

The present study provides a preliminary set of data on malformations and meristic characters of larvae and juveniles of wild-caught and cultured gilthead sea bream, which allows a morphological approach to be used in quality assessment. Such an approach, based on the comparison between wild-caught and hatchery-reared fish, is important for two reasons. From a commercial point of view, it could be useful in the identification of wild-like finfish aquaculture production. Secondly, the identification of high quality juvenile production may reduce the demand for wild juveniles used for sea-ranching or extensive purposes.

The present study does not try to investigate the possible causes of the observed abnormalities, but tests the use of morphological alterations as descriptors of the overall larval quality of a farm production cycle. As monitoring of skeletal anomalies in wild fish may be useful in evaluating marine pollution levels (Sloof, 1982; Westernhagen et al., 1988; Loganathan et al., 1989; Whittle et al., 1992), we propose that the monitoring of skeletal anomalies be used to measure aquaculture production quality.

2. Materials and methods

A total of 494 larvae and juveniles of gilthead sea bream were monitored, 72 of which were wild-caught specimens, while the remaining 422 were obtained from commercial hatcheries located on the Mediterranean Sea. The wild juveniles were caught along the Italian coast (Lazio, Tyrrhenian Sea) (Table 1).

Larvae from groups 2, 3 and 4 were obtained from eggs produced by wild-caught broodstock and subsequently reared under intensive conditions in Italy. “F” group came from an intensive French hatchery and “S” came from a Spanish one. Individuals from group 1 were obtained from thermo-photoperiodically induced wild-caught spawners. Groups 5 and 6 represent two reproductions from a hatchery in northern Italy, which were carried out in 1994 and 1995, respectively. In both replicates, larvae were reared in semi-intensive conditions.

After fixation (5% formalin in phosphate buffer, pH 7.2, 0.1 M), each individual was observed on their left side with the aid of a stereomicroscope (wild) in order to detect any abnormalities, measure standard length (SL) and perform meristic counts. Measure-

Table 1
Characterisation of hatchery-reared and wild-caught gilthead sea bream samples

Code	Origin	Rearing conditions	*n*	Mean SL (mm)	Range (mm)	s.d.
Hatchery-reared						
1	north-east Italy	intensive	25	18.88	11–21.5	2.18
2	insular Italy	intensive	13	46.38	40.5–51	3.44
3	southern Italy	intensive	39	39.70	20–57.5	13.5
4	insular Italy	intensive	26	19.30	17–21.5	1.47
5	north-east Italy (valle) 1995	semi-intensive	66	24.77	14.5–36	5.07
6	north-east Italy (valle) 1996	semi-intensive	121	15.44	13–19	1.46
F	France	intensive	50	42.28	25.5–68	11.4
S	Spain	intensive	82	44.88	30–69	8.87
Total reared			422	29.44	11–69	14.47
Wild-caught						
Wild	Tyrrhenian Sea		72	19.88	9.5–49	8.05

ments were performed using a micrometric glass for smaller individuals (approximation superior 0.5 mm) and with a digital caliper (Micron Metrology) for those bigger than 60 mm SL. SL was measured from the tip of the snout to the distal edge of the hypural bones. Larger specimens (SL > 60 mm) were X-rayed (4 min/5 mA/80 kW), while smaller specimens were stained for bone and cartilage according to Dingerkus and Uhler (1977).

Abnormalities were classified with a letter indicating the region and a number indicating the anomaly (Table 2). The skeletal terminology used here is according to Harder (1975). The vertebral column was divided into four regions, based on distinct morphological features. Thoracic vertebrae were split into cranial (1st–2nd, centra showing epipleural ribs only during first developmental periods) and pre-hemal (from 3rd–10th, centra with epipleural and pleural ribs after first developmental stages). Also for other Perciform species such as *P. major* (Matsuoka, 1987), *Archosargus rhomboidalis* (Houde and Potthoff, 1976), *Microspathodon crysurus* (Potthoff et al., 1987) and *D. labrax* (unpublished data), it is possible to differentiate thoracic vertebrae using this method, during early larval development. Vertebrae, which were characterised by an hemal arch which is closed by an hemal spine, usually called hemal vertebrae, were divided into hemal (11th–21st) and caudal (22nd–24th) categories in the following way:

- the 22nd vertebra has neural and hemal processes which are longer and which widen more in their distal portion than the preceding ones,
- the 23rd vertebra has a shorter centra than hemal vertebrae and also has a specialised neural arch,
- the 24th vertebra is the urostyle.

In the cephalic region, only splanchnocranium anomalies were observed. These abnormalities were reported as a single bone malformation and not in the traditional way

Table 2
List of considered anomalies

Region
A. Cephalic (1st–2nd vertebra)
B. Pre-hemal (3rd–10th vertebra)
C. Hemal (11th–21st vertebra)
D. Caudal (22nd–24th vertebra)
E. Pectoral fin
F. Anal fin
G. Caudal fin
H. Dorsal spines
I. Dorsal soft rays
Anomalies
1. Lordosis
2. Kyphosis
3. Vertebral fusion
4. Vertebral malformation
5. Malformed neural arch and/or spine
6. Malformed hemal arch e/o spine
7. Malformed ray (deformed, absent, fused, supernumerary)
8. Malformed pterygophores (deformed, absent, fused, supernumerary)
9. Malformed hypural (deformed, absent, fused, supernumerary)
10. Malformed epural (deformed, absent, fused, supernumerary)
11. Malformed caudal distal cartilage (deformed, absent, fused, supernumerary)*
12. Swim-bladder anomaly
13. Presence of calculi in the urinary ducts
14. Malformed dentale
15. Malformed maxillary and / or pre-maxillary
16. Dislocation of glossohyal
17. Malformed left opercle
18. Supernumerary vertebra

* not considered in this study.

which is too general (i.e., pugheadness, crossbite), where a single term included several malformations of different bones.

The following data on anomalies were collected:

1. total number of anomalies according to the different origin of the specimens;
2. frequency of each anomaly in each group;
3. frequency of individuals affected by each observed anomaly in each group.

A correspondence analysis (CA) (Benzecri, 1973) was performed on the anomaly data (matrix 494×39). The sea bream groups were found to be different mostly for frequencies of anomalies rather than for types of malformations. A CA was performed on the group frequency data, plotting individuals as supplementary objects in the ordination, i.e., in a way that does not affect the structure of the correspondence axes. This was done because a group can be better described by the relative incidence of a set of abnormalities and by the frequency of normal individuals rather than by averaging the

characteristics of its abnormal individuals. Nevertheless, it is also useful to represent the heterogeneity of the subsets of individuals that compose each group. Furthermore, to establish a value of standard quality, a new descriptor (i.e., a binary "absence of anomalies" variable) was introduced, so that all the observed individuals, including those which showed no abnormalities, could be analysed.

The MRPP (Multi-Response Permutation Procedure) was applied to the anomalies data matrix as it provides a non-parametric approach for testing the hypothesis of no difference between two or more groups of entities. In particular, it tests the hypothesis that intra-group mean distances are smaller than expected. No assumptions (such as multivariate normality and homogeneity of variances) are necessary when using the MRPP that particularly makes it suitable when analysing count data. A good explanation of the method can be found in Biondini et al. (1985).

The non-parametric Mann–Whitney's *U*-statistics was used to test the hypothesis of equal mean number of anomalies per specimen in reared and in wild groups.

The following meristic counts were carried out:

1. total vertebrae (including the urostyle),
2. anal and dorsal rays (divided into spinous and soft rays),
3. principal caudal fin rays (divided into upper principal caudal rays (UPCR) and lower principal caudal rays (LPCR)),
4. pectoral fin rays (left side only).

Meristic data variability was represented using box and whisker plots (STATISTICA for Windows, Stat. Soft. 1996).

3. Results

3.1. Skeletal anomalies

As many as 3183 anomalies were detected and each anomaly listed in Table 2 has been recorded. Severe anomalies, like kyphosis (type 1), lordosis (type 2), vertebrae fusion and deformation (types 3 and 4, respectively), splanchnocranium anomalies (types 14, 15, 16 and 17) and calculi (type 13) accounted for 24.3% ($n = 774$) of the total. Frequencies of individuals with at least one severe anomaly varied among groups, as shown in Fig. 1, but wild-caught specimens had less anomalies than hatchery-reared ones (Figs. 1 and 2). The Mann–Whitney *U*-test showed that this difference was highly significant ($U = 1061.5$, $P < 0.001$, $n_{\text{reared}} = 422$, $n_{\text{wild}} = 72$) (Fig. 2).

The main types of surveyed bone abnormalities were as follows:

1. *Caudal complex* ("G" and "D" anomalies). The caudal portion was the part of the body that had most malformations (Fig. 3), accounting for the 48.5% of anomalies observed in hatchery-reared larvae and 78.8% in the wild-caught group. Up to 91% of hatchery-reared and 29% of wild-caught sea bream showed at least one anomaly in this region. In particular, caudal fin anomalies (G7, G9 and G10, Fig. 4A) which were considered as slight malformations, were the most frequent in sea bream. They made up

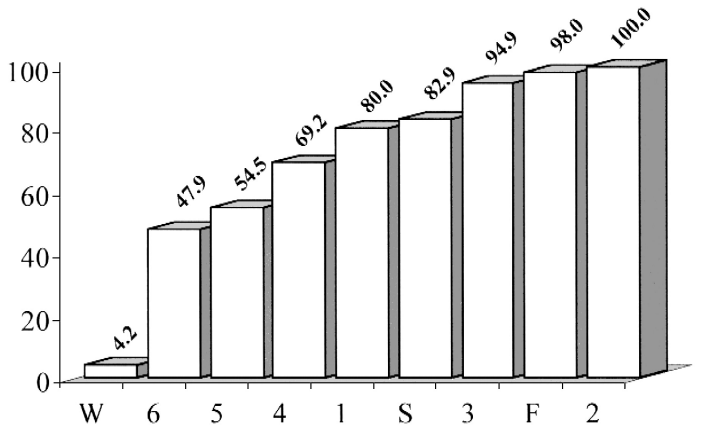

Fig. 1. Frequencies (%) of individuals with at least one serious anomaly (kyphosis, lordosis, vertebrae fusion or deformation, splanchnocranium deformities) in each observed group of gilthead sea bream. Data are referred to the total of individuals of each group.

28.9% of hatchery-reared and 57.6% of wild-caught anomalies, affecting 85.8% and 22.2% of hatchery-reared and wild-caught individuals, respectively. Many single or multiple hypuralia fusions (G9, Fig. 4A) occurred both in wild-caught (42.4% of total anomalies) and hatchery-reared (14.1%) fish. In several cases more than one hypural element was fused to the urostyle. Epurals were highly variable both in number and

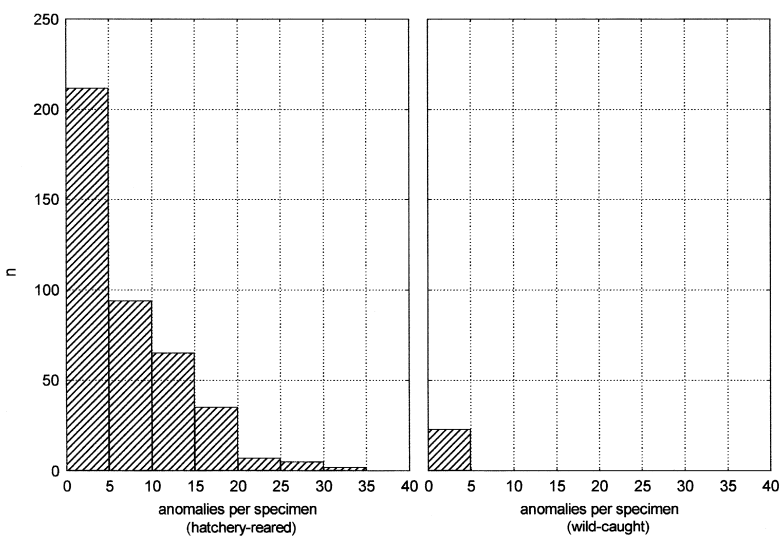

Fig. 2. Number of anomalies per specimen in malformed hatchery-reared and wild-caught sea bream. The difference in the mean number of anomalies per specimen between the two groups was highly significant (Mann–Whithey *U*-test).

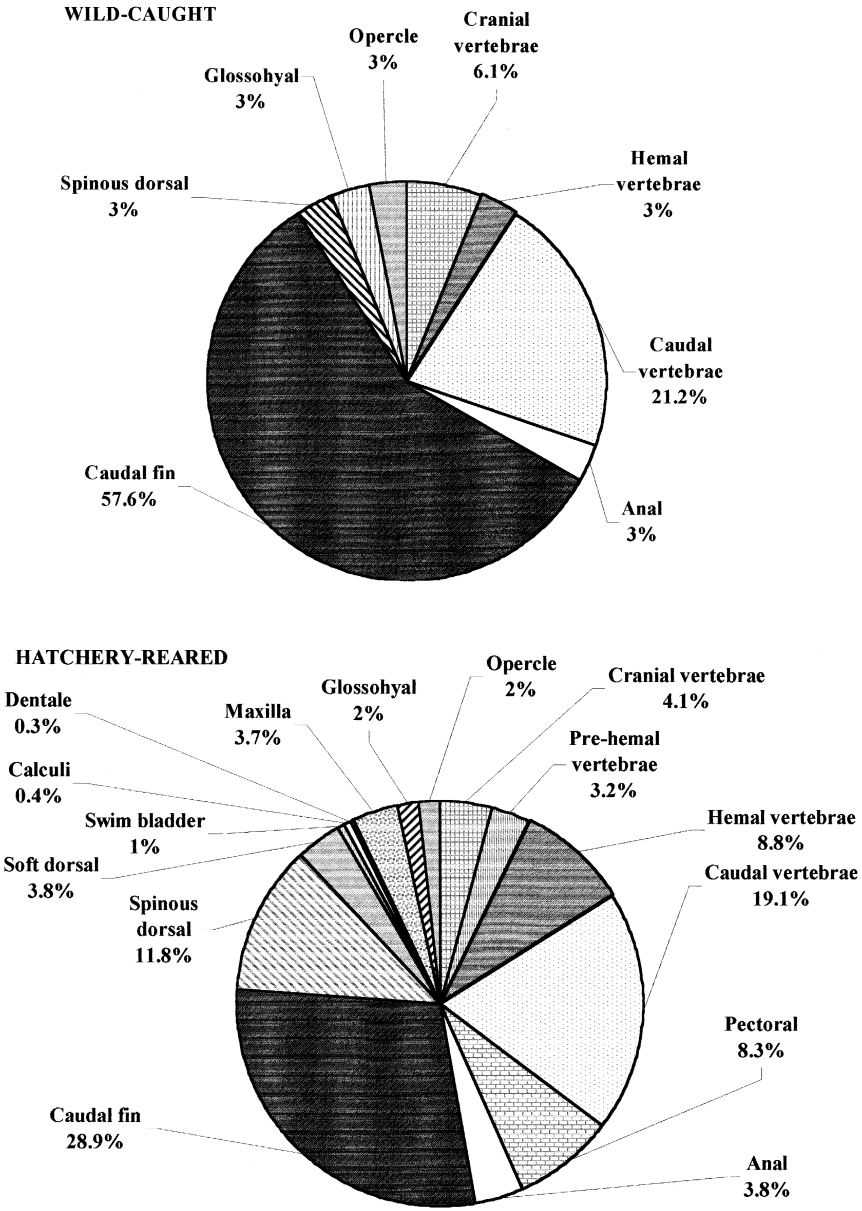

Fig. 3. Distribution of anomalies among different anatomical regions of hatchery-reared and wild-caught specimens.

shape (G10). Supernumerary elements, absence of one element (Fig. 4A), proximal and distal branching, fusions between different epurals were observed. Principal caudal fin rays chiefly showed two kinds of malformation (G7): the first one was a concomitant

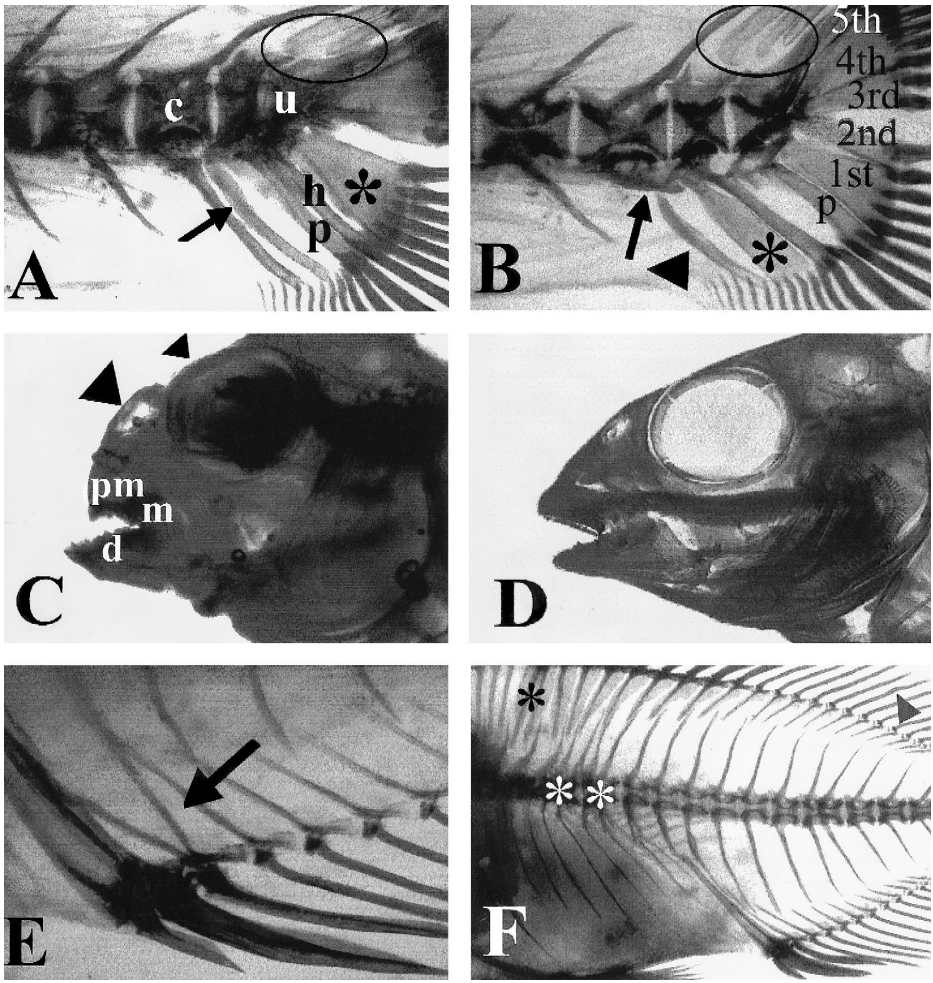

Fig. 4. (A) Abnormal and supernumerary hemal spine (D6: arrow), absence of one epural (G10: circle; see photo B for normal conditions) and fusion between 1st and 2nd hypuralia (G9: asterisk). Last caudal vertebra (c) preceding urostyle (u) is shortened (D4) and its hemal arch (h) is fused with parahypural (p) arch. (B) The second vertebra on the left presents a deformed and fused hemal arch (arrow), one supernumerary (asterisk) and one detached (arrow head) hemal spine (D6). The circle indicates a normal epural arrangement. p = parahypural; 1st = first hypural; 2nd = second hypural; 3rd = third hypural; 4th = forth hypural; 5th = fifth hypural. (C) Anomalies of pre-maxillary (pm), maxillary (m) (anomaly 14), dentale (13: d) and of neurocranium (arrow heads) completely altered the head profile of this hatchery-reared juvenile. (D) Normal head profile of gilthead sea bream juvenile. (E) Anal V-shaped pterygophore (F8: arrow). The following pterygophores are normal. (F) Some Pre-hemal (B3: white asterisks) and hemal vertebrae (C3: the fourth and fifth vertebrae from the right) are fused. The preceding and the following vertebrae are normally shaped. A dorsal soft ray is slightly deformed ("I"7: arrow head) and two dorsal pterygophores are fused (H8: black asterisk).

reduction in size and length of the distal section, while the second one was the presence of bone notches. Anomalies of the caudal vertebral region (even if only formed of three

vertebrae) accounted for 19.1% of the total number of observed anomalies in hatchery-reared groups and 21.2% in the wild-caught group (Fig. 3). In particular, severe anomalies of caudal vertebrae (D1, D2, D3 and D4) are quite common: they made up 7.2% and 9.1% of total hatchery-reared and wild-caught anomalies, respectively. Shortened (Fig. 4A), triangular shaped (D4) and fused centra (D3), straight urostyle, distally branched neural or hemal spine (D5 and D6, respectively) and supernumerary hemal spines (mainly between the 22nd and 23rd hemal processes) were observed (Fig. 4A and B). Often these supernumerary elements were detached from the corresponding centra (Fig. 4B).

2. *Vertebral column and elements* (anomalies 3–8). Some cases of lordosis (anomaly 1 observed in 61 hatchery-reared and 3 wild-caught sea bream) and kyphosis (anomaly 2 observed on 38 hatchery-reared fish, none in wild-caught fish), but no case of scoliosis, were detected. The most common vertebral anomalies found were shortened and fused centra (Fig. 4F). In some cases, fusions involved three adjacent vertebral elements (Fig. 4F), which slightly affected the external shape of the fish. Anomalies of neural and hemal processes consisted of reduced, distally branched, twisted or wholly lacking neuroapophysis or hemapophysis. An incomplete formation of the neural or hemal arches was often observed. Cranial neuroapophysis occasionally showed a distal enlargement.

3. *Dorsal* (anomalies H and I) *and anal* (anomalies F) *fins*. Pterygophores showed some length reduction, abnormal shape (Fig. 4E) and fusions mainly between the distal or proximal section of two close elements (Fig. 4F). Fin ray anomalies mainly consisted of a reduction in length, size and number, and rarely fusions.

4. *Upper and lower jaw*. Pre-maxillary and maxillary malformations usually occurred together. A reduction in length (even if only on one side) was the most common anomaly observed on dentale, pre-maxillary and maxillary (Fig. 4C and D).

5. *Opercular series*. Malformations involved opercular and subopercular bones and branchiostegal rays. The most common opercular and subopercular anomalies consisted of a reduction of their distal section, and a bending toward the opercular chamber.

3.1.1. Wild-caught group

A total number of 23 wild-caught sea bream (31.9% of the group) showed malformations (Table 3), but the majority of malformed fish (78.8%, Fig. 1) presented only slight anomalies, mainly affecting the caudal fin. Severe anomalies such as kyphosis, vertebral fusions, uninflated swim bladder and *calculi* in the terminal tract of the urinary ducts were not recorded and only 14 out of a total of 39 types were observed. One wild-caught sea bream presented two lordosis cases, one affecting the cranial and the other one the hemal and caudal vertebrae.

3.1.2. Hatchery-reared groups

Every group of hatchery-reared larvae contained 100% of abnormal fish (with at least one malformation), except for group 6 (98.3%, Table 3). In addition to the types of abnormalities checked in wild-caught specimens, hatchery-reared fishes showed more severe anomalies such as *calculi* in the urinary ducts (0.4%), severe lordosis (1.9%), kyphosis (0.4%), malformed or fused vertebrae (14.29%), uninflated swim bladders

Table 3
Frequencies (%) of individuals affected by each anomaly in each group

Group	1	2	3	4	5	6	F	S	Wild
n =	25	13	39	26	66	121	50	82	72
A1	16.0	7.7	33.3	15.4	1.5	1.7	0.0	0.0	6.0
A2	4.0	0.0	0.0	0.0	0.0	0.0	2.0	1.2	0.0
A3	0.0	0.0	5.1	0.0	0.0	0.0	0.0	2.4	0.0
A4	28.0	30.8	43.6	19.2	0.0	0.0	12.0	4.9	0.0
A5	24.0	0.0	17.9	15.4	3.0	4.1	6.0	9.8	6.0
B1	0.0	0.0	5.1	0.0	0.0	0.0	0.0	0.0	0.0
B2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0
B3	0.0	7.7	15.4	3.8	0.0	0.0	10.0	8.5	0.0
B4	4.0	7.7	23.1	0.0	1.5	0.0	12.0	12.2	0.0
B5	0.0	7.7	7.7	3.8	1.5	0.8	4.0	8.5	0.0
C1	4.0	7.7	12.8	3.8	3.0	0.0	20.0	11.0	6.0
C2	0.0	0.0	2.6	0.0	0.0	0.0	8.0	1.2	0.0
C3	0.0	0.0	5.1	3.8	0.0	0.0	4.0	1.2	0.0
C4	4.0	7.7	30.8	3.8	4.5	2.5	20.0	14.6	0.0
C5	8.0	7.7	15.4	7.7	6.1	0.0	12.0	9.8	0.0
C6	12.0	7.7	23.1	11.5	7.6	18.2	20.0	29.3	0.0
D1	0.0	0.0	5.1	0.0	0.0	0.0	2.0	2.4	6.0
D2	0.0	0.0	2.6	0.0	0.0	0.0	4.0	0.0	0.0
D3	0.0	0.0	12.8	3.8	1.5	0.0	4.0	2.4	0.0
D4	32.0	0.0	64.1	26.9	22.7	7.4	68.0	41.5	12.1
D5	24.0	0.0	74.4	15.4	3.0	1.7	54.0	14.6	18.1
D6	28.0	23.1	79.5	38.5	31.8	4.1	84.0	45.1	6.0
D18	8.0	0.0	23.1	0.0	1.5	0.8	12.0	2.4	0.0
E7	72.0	0.0	2.6	50.0	0.0	0.0	18.0	28.0	0.0
F7	44.0	0.0	10.3	3.8	4.5	3.3	10.0	14.6	0.0
F8	16.0	23.1	17.9	7.7	3.0	2.5	12.0	9.8	6.0
G7	68.0	7.7	12.8	61.5	0.0	5.8	32.0	23.2	6.0
G9	32.0	69.2	94.9	23.1	87.9	69.4	88.0	54.9	78.5
G10	32.0	30.8	66.7	38.5	40.9	19.0	62.0	43.9	12.1
H7	8.0	7.7	5.1	7.7	4.5	16.5	20.0	54.9	0.0
H8	44.0	30.8	59.0	38.5	13.6	4.1	34.0	34.1	6.0
I7	28.0	7.7	10.3	19.2	9.1	1.7	20.0	17.1	0.0
I8	8.0	0.0	23.1	11.5	3.0	2.5	8.0	4.9	0.0
12	0.0	0.0	10.3	0.0	0.0	0.8	18.0	2.4	0.0
13	0.0	0.0	0.0	0.0	0.0	0.0	10.0	9.8	0.0
14	8.0	7.7	2.6	3.8	0.0	0.0	4.0	4.9	0.0
15	4.0	61.5	25.6	11.5	15.2	37.2	34.0	26.8	0.0
16	8.0	7.7	7.7	19.2	15.2	3.3	36.0	8.5	6.0
17	28.0	7.7	10.3	46.2	1.5	3.3	22.0	3.7	6.0
No anomalies	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	68.1

(0.5%) and lower jaw (5.3%) anomalies. Hypural fusion was the most common abnormality (14.1%). The anomalies recorded in each single group of hatchery-reared gilthead sea bream did not show any hatchery-specific distribution, as pointed out in the CA results. For the distribution of anomalies in each group, see Table 4.

Table 4
Distribution of anomalies in each group

	1		2		3		4		5		6		F		S		Wild	
obs	$n = 299$	%	$n = 60$	%	$n = 535$	%	$n = 265$	%	$n = 237$	%	$n = 316$	%	$n = 620$	%	$n = 818$	%	$n = 33$	%
A1	4	1.3	1	1.7	13	2.4	4	1.5	1	0.4	2	0.6	0	0.0	0	0.0	1	3.0
A2	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	1	0.1	0	0.0
A3	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0	0	0.0	0	0.0	2	0.2	0	0.0
A4	9	3.0	5	8.3	28	5.2	6	2.3	0	0.0	0	0.0	6	1.0	5	0.6	0	0.0
A5	7	2.3	0	0.0	9	1.7	4	1.5	2	0.8	5	1.6	3	0.5	8	1.0	1	3.0
B1	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
B2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.1	0	0.0
B3	0	0.0	1	1.7	7	1.3	1	0.4	0	0.0	0	0.0	7	1.1	14	1.7	0	0.0
B4	1	0.3	1	1.7	15	2.8	0	0.0	2	0.8	0	0.0	7	1.1	16	2.0	0	0.0
B5	0	0.0	1	1.7	4	0.7	1	0.4	1	0.4	1	0.3	3	0.5	15	1.8	0	0.0
C1	1	0.3	1	1.7	5	0.9	1	0.4	2	0.8	0	0.0	10	1.6	9	1.1	1	3.0
C2	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	4	0.6	1	0.1	0	0.0
C3	0	0.0	0	0.0	6	1.1	1	0.4	0	0.0	0	0.0	2	0.3	2	0.2	0	0.0
C4	1	0.3	3	5.0	18	3.4	2	0.7	4	1.7	3	0.9	26	4.2	26	3.2	0	0.0
C5	3	1.0	2	3.3	15	2.8	4	1.5	4	1.7	0	0.0	12	1.9	9	1.1	0	0.0
C6	3	1.0	3	5.0	11	2.1	4	1.5	5	2.1	26	8.2	17	2.7	29	3.5	0	0.0
D1	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0	0	0.0	1	0.2	2	0.2	1	3.0
D2	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	2	0.3	0	0.0	0	0.0

D3	0	0.0	0	0.0	5	0.9	1	0.4	1	0.4	0	0.0	2	0.3	2	0.2	0	0.0
D4	14	4.7	0	0.0	51	9.5	10	3.8	16	6.7	9	2.8	56	9.0	51	6.2	2	6.1
D5	8	2.7	0	0.0	53	9.9	5	1.9	2	0.8	2	0.6	41	6.6	12	1.5	3	9.1
D6	7	2.3	3	5.0	57	10.6	11	4.1	25	10.5	5	1.6	70	11.3	52	6.4	1	3.0
D18	2	0.7	0	0.0	10	1.9	0	0.0	1	0.4	1	0.3	6	1.0	2	0.2	0	0.0
E7	87	29.1	0	0.0	3	0.6	66	24.9	0	0.0	0	0.0	24	3.9	82	10.0	0	0.0
F7	12	4.0	0	0.0	4	0.7	5	1.9	9	3.8	4	1.3	5	0.8	20	2.4	0	0.0
F8	4	1.3	3	5.0	19	3.5	2	0.7	2	0.8	3	0.9	16	2.6	11	1.3	1	3.0
G7	66	22.1	2	3.3	10	1.9	74	27.9	0	0.0	19	6.0	48	7.7	56	6.8	3	9.1
G9	10	3.3	10	16.7	82	15.3	7	2.6	78	32.9	112	35.4	81	13.1	63	7.7	14	42.4
G10	10	3.3	4	6.7	33	6.2	11	4.1	33	13.9	24	7.6	40	6.4	38	4.6	2	6.1
H7	2	0.7	3	5.0	2	0.4	2	0.7	3	1.3	29	9.2	15	2.4	182	22.2	0	0.0
H8	13	4.3	5	8.3	29	5.4	10	3.8	11	4.6	8	2.5	17	2.7	40	4.9	1	3.0
I7	18	6.0	1	1.7	4	0.7	6	2.3	12	5.1	4	1.3	25	4.0	16	2.0	0	0.0
I8	2	0.7	0	0.0	12	2.2	3	1.1	2	0.8	5	1.6	7	1.1	4	0.5	0	0.0
12	0	0.0	0	0.0	4	0.7	0	0.0	0	0.0	1	0.3	9	1.4	2	0.2	0	0.0
13	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	0.8	8	1.0	0	0.0
14	2	0.7	1	1.7	1	0.2	1	0.4	0	0.0	0	0.0	2	0.3	4	0.5	0	0.0
15	1	0.3	8	13.3	10	1.9	3	1.1	10	4.2	45	14.2	17	2.7	22	2.7	0	0.0
16	2	0.7	1	1.7	3	0.6	5	1.9	10	4.2	4	1.3	18	2.9	7	0.9	1	3.0
17	9	3.0	1	1.7	4	0.7	15	5.7	1	0.4	4	1.3	15	2.4	4	0.5	1	3.0
Σ	598	100	120	100	1070	100	530	100	474	100	632	100	1240	100	1636	100	66	100

n = number of cases; obs = total number of observed anomalies in each group; % = relative frequency (percentage of each anomaly type out of the total number of anomalies inspected, per group).

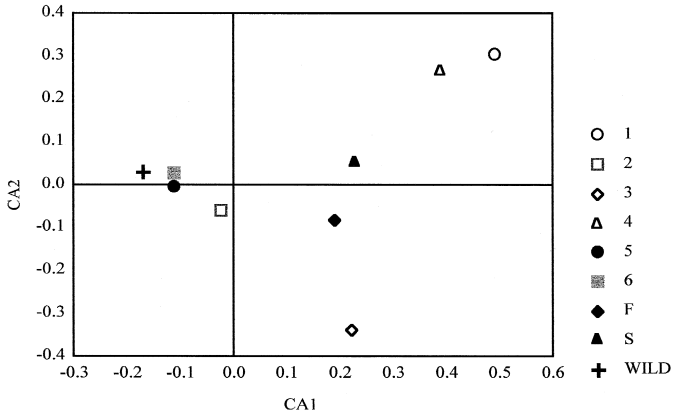

Fig. 5. CA: Ordination of group centroids (see text for explanations).

3.1.3. Correspondence analysis

The ordination of the nine group centroids (which indicate mean patterns of abnormality) in the space defined by the first two correspondence axes (CA1 and CA2, that constituted 10.4% and 8.4% of the overall variance) is shown in Fig. 5, whereas the ordination of descriptors is shown in Fig. 6. Descriptors and group centroids were plotted on separate figures, but they actually share the same reduced space, so that their ordinations could be superimposed. In this ordination the axes scale was changed, because group-points are much closer to the axes origin than most of the descriptor points. Both descriptors and group centroids are arranged within a triangular area. The "absence of anomalies" point is located at the negative end of this area along CA1, whereas almost all the points that describe abnormalities have positive CA1 co-ordinates.

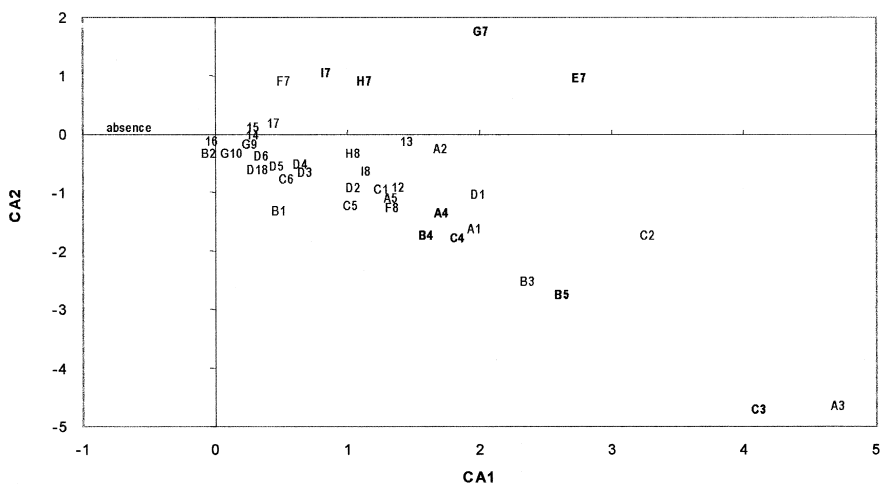

Fig. 6. CA: Ordination model of descriptor points (see text for explanations).

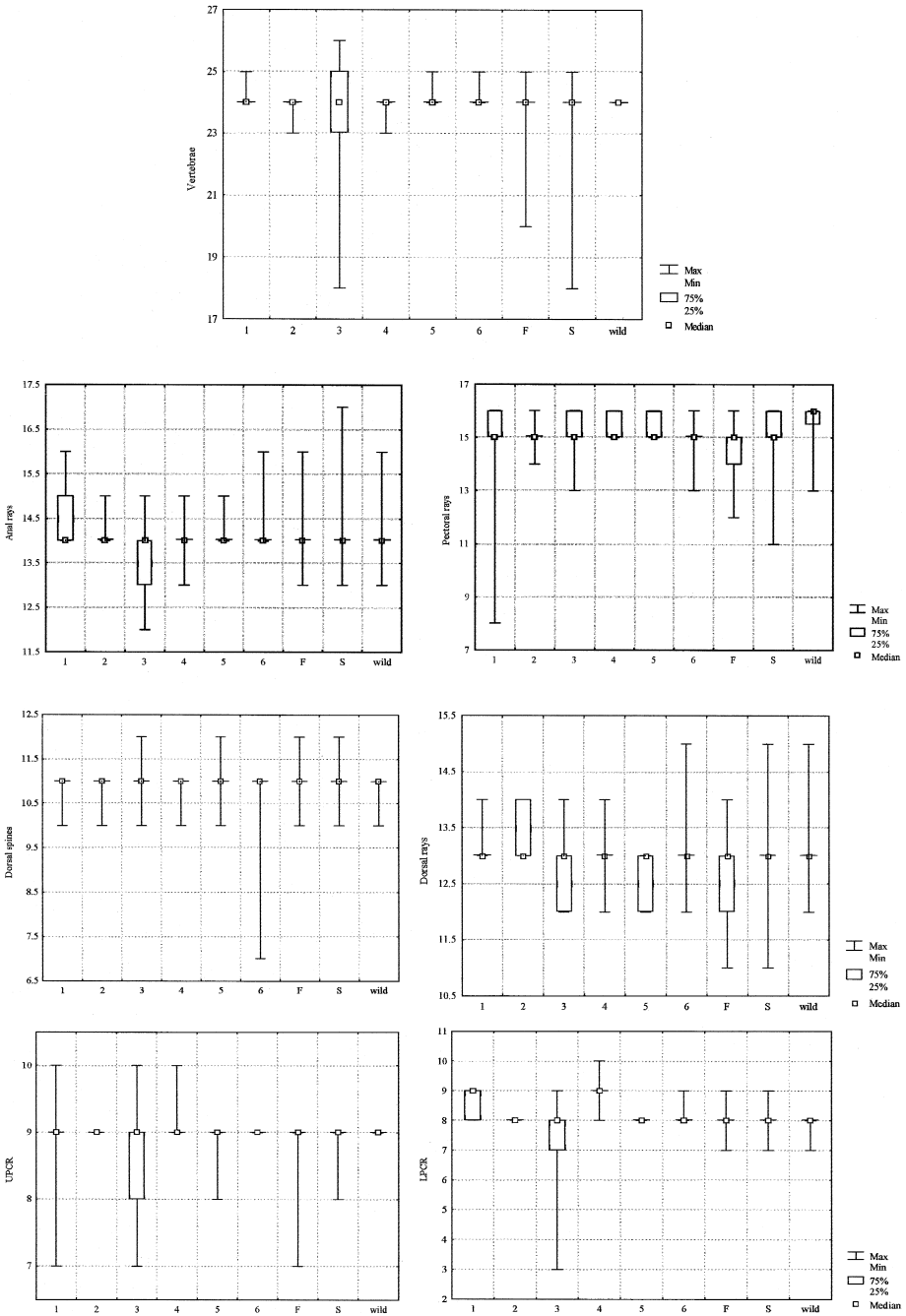

Fig. 7. Box and whisker plots of meristic counts.

Sea bream groups are arranged in the same way, though they occupy a much smaller area than individuals. The wild-caught sea bream group is the leftmost and closest to the "absence of anomalies" variable, whereas the average frequency of abnormalities tends to increase proportionally to the CA1 co-ordinate. The relative position of groups, with respect to anomalies, effectively summarise their main characteristics.

3.2. Meristic characters

For each meristic character the median, range and ranged-quartile values are indicated in Fig. 7. The most variable meristic counts were found to be the LPCR, pectoral rays, dorsal soft rays and anal rays. The number of vertebrae, dorsal spines and UPCR had the lowest variability range.

3.2.1. Wild-caught group

These specimens showed the lowest variability in each meristic count. Vertebrae (24) and principal caudal fin rays (9 + 8) were the most canalised elements (Fig. 7). Furthermore, no hatchery-reared groups exhibited values of pectoral rays (quartile range and median) similar to the wild-caught ones.

3.2.2. Hatchery-reared groups

Some characters such as vertebrae (range: 18–26), anal (12–17), pectoral (8–17), lower principal caudal fin rays (LPCR: 3–10) and dorsal soft rays (11–15) were highly variable, while the UPCR (7–10), and dorsal spines (7–12) were found to be less variable. Hatchery 3 showed the largest differences when compared to other groups, including the wild-caught ones (Fig. 7).

The MRPP results showed that the mean intra-group distance for reared and wild specimens is significantly lower than expected when both groups were drawn from a single population ($R = 0.045$, $P < 0.001$). Moreover, wild specimens were much more homogeneous in terms of anomalies than reared individuals, i.e., their intra-group mean distance ($d_{\text{wild}} = 0.684$) was lower than the reared intra-group mean distance ($d_{\text{reared}} = 4.992$). The same procedure was also applied to the reared group alone. The mean intra-group distance for the eight reared subsets was lower than expected when all the subsets were drawn from a single population ($R = 0.140$, $P < 0.001$). This result implies that some anomalies are subset-specific, i.e., they are due to rearing conditions.

4. Discussion

In gilthead sea bream, the presence of heavy skeletal anomalies has been mainly documented using gross external analysis (Paperna, 1978; Francescon et al., 1988) or radiographs (Balbelona et al., 1993; Chatain, 1994b; Andrades et al., 1996). However, such methods are not successful when looking for malformations at early developmental stages. In these cases, osteological techniques for staining bones and cartilages of small vertebrates are well-established methodologies (Matsuoka, 1987; Boglione et al., 1993; Marino et al., 1993; Koumoundourous et al., 1995, 1997a,b) and represent a powerful tool to highlighting phenotypic differences.

The present study clearly demonstrates the poor skeletal and meristic quality of hatchery-reared *S. aurata* juveniles, compared to wild-caught samples. Previous indications on gilthead sea bream meristic characters (Bianchi, 1984; Fischer et al., 1987) reported some values (hard dorsal fin rays = 11, soft dorsal fin rays = 12–13; hard anal fin rays = 3, soft anal fin rays = 11) on wild-caught specimens which have been confirmed in the present paper. Meristic count analysis showed a high variability in hatchery-reared larvae when compared to the wild-caught group. This has already been documented for red sea bream (Matsuoka, 1987) and sea bass (Boglione et al., 1993; Marino et al., 1993). Therefore, it seems that rearing conditions could produce greater meristic variations. Fowler (1970) and Taning (1952) suggested that the vertebral number is established very early during development (i.e., before hatching), while fin rays are sensitive to thermal variation during the larval period.

In the present study, wild-caught gilthead sea bream presented a low number of malformations and were never affected by any severe anomalies (spinal curvatures, urinary calculi, swim bladder abnormality, twisted fin rays) and only about 4% of the inspected individuals showed one or two vertebral body deformations. Higher frequencies than those we observed in wild-caught individuals have been observed only in polluted waters (Sloof, 1982; Westernhagen et al., 1988; Loganathan et al., 1989; Whittle et al., 1992) or in freshwater which is subjected to significant variations of environmental parameters (such as temperature, i.e., Hubbs, 1959). The hypothesis of a severe selection pressure against malformed fish in the wild has been proposed (Vladimirov, 1975; Shelbourne, 1964), but not demonstrated. Our results differ both in frequencies and types of anomalies, from those of Francescon et al. (1988), who documented only axial malformations in 1% of a wild-caught *S. aurata* sample ($n = 516$). However, such a discrepancy could be due to different investigation techniques (gross external morphology versus in toto staining), sampling size (516 versus 72) and length of the individuals (11.2–15.9 mm versus 9.5–49 mm). In the red sea bream (*P. major*), Matsuoka (1987) reported that wild-caught fish had a number of abnormal bones per individual which was 10 times lower than hatchery-reared individuals, and never exhibited severe anomalies. Similar results have been reported for sea bass (Boglione et al., 1993; Marino et al., 1993) and herring (*Clupea harengus*) (Balbotin et al., 1973).

In both wild-caught and hatchery-reared gilthead sea bream, the caudal complex was affected by the highest number of malformations. Koumoundourous et al. (1997a,b), already pointed out this peculiarity, but, even if many caudal anomalies have been identified in both studies, a doubled or laterally twisted caudal fin was not observed in the present study. Tail malformations in hatchery-reared gilthead sea bream (Paperna, 1978) and other teleosts (Matsuoka, 1987; Ishikawa, 1990; Dunham et al., 1991; Mair, 1992; Boglione et al., 1993; Marino et al., 1993) have already been documented. In some cases, causes were ascertained (Ishikawa, 1990; Mair, 1992), but only for specific caudal anomalies like the ventralisation of dorsal caudal elements or the absence of the caudal fin. In channel catfish (*Ictalurus punctatus*), a reduction in caudal fin abnormalities was achieved by supplying a proper level of vitamin C to the diet of the fry (Mazik et al., 1987). In the same species, a lack of inheritance for the "taillessness" anomaly was documented by Dunham et al. (1991). The identification of a single cause for caudal

complex abnormalities is hard to achieve. Nevertheless, it is worth stressing that in vertebrates, the posteriormost somites are the last to develop and the most sensitive to the environment (Fowler, 1970). On the other hand, the fact that the caudal region is more susceptible than other regions to malformations has not been documented in sea bass and other teleosts, supporting the hypothesis that "caudal region sensitivity" could be a specific gilthead sea bream feature.

In addition to the above-mentioned types of malformation, 14.45% of hatchery-produced larvae were affected by evident lordosis (with a consequent deformation of the vertebrae affected). Chatain (1994b) pointed out that lordosis in gilthead sea bream and sea bass primarily occurred in the backbone region (vertebrae 14–15), under continuous muscular stress, and stated that this axial anomaly depends on hydrodynamic tank conditions. The same cause was suggested for carp (*Cyprinus carpio*) reared in fast running water (Backiel et al., 1984). Kitajima et al. (1994), in a study on red sea bream (*P. major*), Japanese sea bass (*Lateolabrax japonicus*) and amberjack (*Seriola aureovittata*), reported that larvae failing to gulp air at the surface (and thus unable to inflate swim bladder), develop lordotic deformations as a consequence of unnatural upward swimming. They propose water currents, food quality and oily film on the water surface as factors to be carefully considered to prevent such problems. However, the presence of lordotic *S. aurata* larvae has been reported before swim bladder inflation (Andrades et al., 1996), suggesting that different causes may be responsible for axial malformations in hatchery-reared individuals. Kanazawa (1985) emphasised that larval fish, during periods of rapid growth, require an abundance of phospholipids for new cell component formation and that, if lecithin was added to the diet, there was a reduction of 63% to 5% of scoliosis in larval ayu (*Plecoglossus altivelis*).

CA applied to frequency data provided an effective discrimination of the different groups according to their distinctive malformation patterns, even though this application was somewhat different from previous research into hatchery-reared sea bass (*D. labrax*) (Boglione et al., 1994). In fact, sea bass from different hatcheries were easily identified on the basis of a CA performed on qualitative data (presence/absence of specific anomalies) because this species has many anomalies that are hatchery-specific. On the contrary, in gilthead sea bream the differences in larval quality between hatcheries depend on the frequency of occurrence of the skeletal anomalies (quantitative data) rather than on their presence/absence. Even though each group of hatchery-reared sea bream is very heterogeneous in terms of abnormalities of individuals, the distance of its centroid from the point that represents the wild-caught group can be considered as a measure of larval quality. Given the properties of the correspondence axes, this measure cannot be expressed on an absolute quantitative scale, but it is very reliable in terms of group ranking. For instance, the highest quality groups among those considered in this paper are groups 5 and 6, which are the closest to the centroid of the wild-caught individuals and showed a lower number of malformations. It should be stressed that these groups, as already outlined in the Materials and Methods, have been reared under semi-intensive conditions, in large tanks (60 m^3), with green water and particular hydrodynamic characteristics. It is interesting to note that other authors have already documented better morphological results for gilthead sea bream larvae reared under semi-intensive conditions (Koumoundourous et al., 1997a,b).

The present data underline the evaluation capacity of the larval monitoring method, based on meristic count analysis performed in parallel with malformation examination, in quantifying larval quality in hatchery production. It can be performed early (within 50–100 days from hatching, according to the species), it is fast and very simple to perform and gives reliable result. The overall analysis can be performed in the hatchery lab itself, as it does not require expensive equipment (a stereoscope and a computer) or harmful agents (using only KOH, Alizarin red, Alcian blue, glycerol, ethanol). The only limitation could be that a thorough knowledge of fish skeletal anatomy is required.

To sum up, the use of larval monitoring for the identification of juveniles which are suitable (on a morpho-anatomical basis) for seeding lagoons is necessary, after a preceding genetic characterisation of breeders to obtain autochtonous juveniles. This approach is particularly necessary considering the reduced availability and high cost of wild-caught fry, and it may also reduce the fishery effort. Furthermore, taking into account that the observed quality gap between wild-caught and hatchery-reared specimens points out the existence of considerable improvement margins for gilthead sea bream-rearing techniques, the approach proposed in this paper could represent a prerequisite to the optimisation of rearing protocols.

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References

Andrades, J.A., Becerra, J., Fernández-Llebrez, P., 1996. Skeletal deformities in larval, juvenile and adult stages of cultured gilthead sea bream (*Sparus aurata* L.). Aquaculture 141 (1–2), 1–11.

Aulstad, D., Kittelsen, A., 1971. Abnormal body curvatures of rainbow trout *Salmo gairdneri* inbred fry. J. Fish. Res. Board Canada 28 (12), 1918–1920.

Backiel, T., Kokurewicz, B., Ogorzalek, A., 1984. High incidence of skeletal anomalies in carp, *Cyprinus carpio*, reared in cages in flowing water. Aquaculture 43, 369–380.

Balbelona, M.C., Morinigo, M.A., Andrades, J.A., Santamaria, J.A., Becerra, J., Borrego, J.J., 1993. Microbiological study of gilthead sea bream (*S. aurata* L.) affected by lordosis (a skeletal deformity). Bull. Eur. Assoc. Fish Pathol. 13 (1), 33.

Balbotin, F., De Silva, S.S., Ehrlich, K.F., 1973. A comparative study of anatomical and chemical characteristics of reared and wild herring. Aquaculture 2, 217–240.

Barahona-Fernandes, M.H., 1982. Body deformation in hatchery reared European sea bass *Dicentrarchus labrax* (L.). Types, prevalence and effect on fish survival. J. Fish Biol. 21, 239–249.

Benzecri, J.P., 1973. L'Analyse des Données. L'Analyse des Correspondances vol. 2 Dunod, Paris, France, p. 628.

Bertolini, B., Boglione, G., Cataudella, S., Finoia, M.G., Marino, G., Monaco, G., 1991. Temperature induced developmental anomalies in sea bass (*Dicentrarchus labrax*) embryos and larvae. Acta Embryol. Morphol. Exp. 12 (1), 77–79.

Bianchi, G., 1984. Study on the morphology of five Mediterranean and Atlantic Sparid fishes with a reinstatement of the genus *Pagrus*, Cuvier, 1817. Cybium 8 (4), 31–56.

Biondini, M.E., Bonham, C.D., Redente, E.F., 1985. Secondary successional patterns in a sagebrush (*Artemisia tridentata*) community as they relate to soil disturbance and soil biological activity. Vegetatio 60, 25–36.

Boglione, C., Marino, G., Bertolini, B., Saroglia, M., Cataudella, S., 1989. Morphological observations on body abnormalities in embryos and larvae of sea bass (*Dicentrarchus labrax*) reared at different temperatures. Eur. Aquacult. Soc., Spec. Publ. 10, 276.

Boglione, C., Marino, G., Bertolini, B., Rossi, A., Ferreri, F., Cataudella, S., 1993. Larval and postlarval monitoring in sea bass: morphological approach to evaluate finfish seed quality. In: Barnabé, G., Kestemont, P. (Eds.), Prod. Env. and Quality. Bordeaux Aquaculture '92, Gent, Belgium. Eur. Aquacult. Soc., Spec. Publ. vol. 18 pp. 189–204.

Boglione, C., Marino, G., Ferreri, F., Finoia, M.G., Scardi, M., Cataudella, S., 1994. Anatomical aspects for seed quality assessment in sea bass (*D. labrax*): hatchery and wild populations. In: Kestemont, P., Sevila, F., Williot, P. (Eds.), Measures for Success. Cemagref-Edition.

Bolla, S., Holmefjord, I., 1988. Effect of temperature and light on development of Atlantic halibut (*Hippoglossus hippoglossus*) larvae. Aquaculture 74, 355–358.

Caggiano, M., 1988. La riproduzione artificiale dei pesci marini: risultati e problematiche. Pesce 5 (4), 47–49.

Chatain, B., 1986. La vessie natatoire chez *Dicentrarchus labrax* et *Sparus auratus*: I. Aspects morphologiques du developpement. Aquaculture 53, 303–311.

Chatain, B., 1994a. In: Kestemont, P., Muir, J., Sevila, F., Williot, P. (Eds.), Quality Assessment of Marine Fish Larvae and Juveniles. Cemagref, Edition.

Chatain, B., 1994b. Abnormal swimbladder development and lordosis in sea bass (*D. labrax*) and sea bream (*S. aurata*). Aquaculture 119, 371–379.

Chatain, B., 1994. Estimation et amélioration des performances zootechniques de l'elevage larvaire de *Dicentrarchus labrax* et de Sparus auratus. These Doctorale es Sciences, Universite d'Aix-Marseille II, 199 pp.

Chatain, B., Ounais-Guschemann, N., 1990. Improved rate of initial swim bladder inflation in intensively reared *Sparus auratus*. Aquaculture 84, 345–353.

Chatain, B., Corrao, D., 1992. A sorting method for eliminating fish larvae without functional swimbladders. Aquaculture 107, 81–88.

Dingerkus, G., Uhler, L.D., 1977. Enzyme clearing of Alcian blue stained whole small vertebrates, for demonstration of cartilage. Stain Technol. 52 (4), 229–232.

Divanach, P., Boglione, C., Menu, M., Koumoundouros, G., Kentouri, M., Cataudella, S., 1996. Abnormalities in finfish mariculture: an overview of the problem, causes and solutions. Sea-bass and Sea Bream Culture: Problems and Prospects, Verona, Italy, October 16–18. European Aquaculture Society, Oostende, Belgium, pp. 45–66.

Dunham, R.A., Smithermank, R.O., Bondari, K., 1991. Lack of inheritance of stumpbody and taillessness in channel catfish. Prog. Fish-Cult. 53, 101–105.

Fischer, W., Schneider, M., Bauchot, M.L. (Rédacteurs), 1987. Fiches FAO d'identification des espèces pour le besoins de la peche. (Revision 1). Méditerranée et Mer Noire. Zone de peche 37, vol. II. Vertebres, Vol. 2. FAO, Rome, pp. 761–1530.

Fowler, J.A., 1970. Control of vertebral number in teleosts — an embryological problem. Q. Rev. Biol. 45, 148–167.

Francescon, A., Freddi, A., Barbaro, A., Giavenni, R., 1988. Daurade *S. aurata* L. reproduite artificiellement et daurade sauvage. Expériences paralleles en diverses conditions d'elevage. Aquaculture 72, 273–285.

Harder, W., 1975. Anatomy of Fishes. in: E. Scweizerbart'sche Verlagsbuchhandlung (Eds.), Stuttgart, 612 pp.

Hilomen-Garcia, G.V., 1997. Morphological abnormalities in hatchery-bred milkfish (*Chanos chanos* Forsskal) fry and juveniles. Aquaculture 152, 155–166.

Houde, E.D., Potthoff, T., 1976. Egg and larval development of the sea bream *Archosargus rhomboidalis* (Linnaeus): Pisces, Sparidae. Bull. Mar. Sci. 26 (4), 506–529.

Hubbs, C., 1959. High incidence of vertebral deformities in two natural populations of fishes inhabiting warm springs. Ecology 40, 154–155.

Ishikawa, Y., 1990. Development of caudal structure of a morphogenetic mutant (Da) in the Teleost fish, medaka (*Oryzias latipes*). J. Morphol. 205, 219–232.

Jofre, J., 1988. Aspectos generales de patologia infecciosa. In: CAICYT (Eds.), Patologia en Acuicultura, pp. 1–29.

Kanazawa, A., 1985. Essential fatty acid and lipid requirement of fish. Nutrition and Feeding in Fish. Academic Press, London, pp. 281–298.

Kentouri, M., 1985. Comportement larvaire de 4 sparides Mediterraneens en élevage *S. aurata, Diplodus. sargus, Lithognathus mormyrus* et *Puntazzo puntazzo* (Poissons Teleosteens). Thèse D'etat, Univ. Sci Technol. Languedoc, Montepellier.

Kiriakos, Z., Koumoundourous, G., Divanach, P., Kentouri, M., 1994. Prelarval and larval description of notochord development as a criterion for early detection of tail abnormalities in gilthead sea *bream (S. aurata)*. Measures for success. (Abstracts) Eur. Aquacult. Soc., Spec. Publ. 21, 117–118.

Kitajima, C., Watanabe, T., Tsukashima, Y., Fujita, S., 1994. Lordotic deformation and abnormal development of swim bladders in some hatchery-bred marine physoclistous fish in Japan. J. World Aquacult. Soc. 25 (1), 64–77.

Komada, N., 1980. Incidence of gross malformations and vertebral anomalies of natural and hatchery *Plecoglossus altivelis*. Copeia 1, 29–35.

Koumoundourous, G., Gagliardi, F., Divanach, P., Stefanakis, S., Kentouri, M., 1995. Osteological study on the origin and development of the abnormal caudal fin in gilthead sea bream (*S. aurata*) fry. Quality in Aquaculture. Eur. Aquacult. Soc., Spec. Publ. vol. 23 European Aquaculture Society, Gent, Belgium, pp. 16–18, 413 pp.

Koumoundourous, G., Gagliardi, F., Divanach, P., Boglione, C., Cataudella, S., Kentouri, M., 1997a. Normal and abnormal osteological development of caudal fin in *S. aurata* L. fry. Aquaculture 149, 215–226.

Koumoundourous, G., Oran, G., Divanach, P., Stefanakis, S., Kentouri, M., 1997b. The opercular complex deformity in intensive gilthead sea bream (*Sparus aurata* L.) larviculture. Moment of apparition and description. Aquaculture 149, 215–226.

Lee, C.S., Menu, B., 1981. Effects of salinity on egg development and hatching in grey mullet *Mugil cephalus* L. J. Fish Biol. 19, 179–188.

Loganathan, B.G., Tanabe, S., Tatsukawa, R., Ogawa, K., Miyako, G., 1989. Temporal changes of morphological abnormalities and parasitic infestation in fishes from the river Nagaragawa, Jpn. Nippon Suisan Gakkaishi 55 (5), 769–774.

Lom, J., Pike, A., Dykova, I., 1991. *Myxobolus sandrae* Reuss, 1906, the agent of vertebral column deformities of perch *Perca fluviatilis* in Northeast Scotland. Dis. Aquat. Org. 12, 49–53.

Loy, A., Boglione, C., Cataudella, S., 1998. Geometric morphometrics and morpho-anatomy: a combined tool in the study of sea bream (*S. aurata*) shape. J. Appl. Ichthyol. 14, 104–110.

Mair, G.C., 1992. Caudal deformity syndrome (CDS): an autosomal recessive lethal mutation in the tilapia, *Oreochromis niloticus* (L.). J. Fish Dis. 15, 71–75.

Marino, G., Boglione, C., Finoia, M.G., Bronzi, P., Monaco, G., Bertolini, B., Cataudella, S., 1991. Effects of incubation temperature on embryonic development and hatching in *Dicentrarchus labrax* L. eggs. In: Lavens, P., Sorgeloos, P., Jasper, E., Ollevier, F. (Eds.), LARVI '91. Symposium on Fish and Crustacean Larviculture, Gent, Belgium, 27–30 Agosto 1991. Eur. Aquacult. Soc., Spec. Publ. vol. 15 pp. 230–232.

Marino, G., Boglione, C., Bertolini, B., Rossi, A., Ferreri, F., Cataudella, S., 1993. Observations on the development and anomalies in the appendicular skeleton of sea bass, *D. labrax* L. 1758, larvae and juveniles. Aquacult. Fish. Manage. 24, 445–456.

Matsuoka, M., 1987. Development of skeletal tissue and skeletal muscle in the red sea bream, *Pagrus major*. Bull. Seikai Reg. Fish. Res. Lab. 65, 1–102.

Mazik, P.M., Brandt, T.M., Tomasso, J.R., 1987. Effects of dietary vitamin C on growth, caudal fin development and tolerance of aquaculture-related stressors in channel catfish. Prog. Fish-Cult. 49, 13–16.

Menu, B.Effects of micropellets used as a first larval feed, on the rate of skeletal abnormalities in sea bass and sea bream. Measures for success, 1994. (Abstract) Eur. Aquacult. Soc., Spec. Publ. 21, 160–161.

Paperna, I., Colorini, A., Gordin, H., Kissil, G., 1977. Disease of *S. aurata* in marine culture at Elat. Aquaculture 10, 195–213.

Paperna, I., 1978. Swimbladder and skeletal deformations in hatchery bred *S. aurata*. J. Fish Biol. 12, 109–114.

Polo, A., Yùfera, M., Pascua, E., 1991. Effects of temperature on egg and larval development of *S. aurata* L. Aquaculture 92, 367–375.

Potthoff, T., Kelley, S., Saksena, V., Moe, M., e Young, F., 1987. Description of larval and juvenile yellowtail damselfish, *Microspathodon chrysurus*, Pomacentridae, and their osteological development. Bull. Mar. Sci. 40 (2), 330–375.

Poynton, S.L., 1987. Vertebral column abnormalities in brown trout, *Salmo trutta*, L. J. Fish Dis. 10, 53–57.

Purcell, J.E., Grosse, D., Grover, J.J., 1990. Mass abundance of abnormal Pacific herring larvae at a spawning ground in British Columbia. Trans. Am. Fish. Soc. 119, 463–469.

Robin, J.H., Menu, B., Gouillou, M.F., Alexandre, J.C., Wilson, R., 1996. La qualité des juvéniles: nutrition et environnement des larves. J. Nutr. Poissons INRA/IFREMER Saint Pée sur Nivelle, (21–22 février 1996, Recueil des communications, 5 pp.

Shelbourne, J.E., 1964. The artificial propagation of marine fish. In: Russel, F.S. (Ed.), Adv. Mar. Biol. vol. 2 Academic Press, New York, pp. 1–383, 870 pp.

Sola, L., De Innocentiis, S., Rossi, A.R., Crosetti, D., Scardi, M., Boglione, C., Cataudella, S., 1998. Genetic variability and fingerling quality in wild and reared stocks of European sea bass. In: Bartley, D., Basurco, B. (Eds.), Cah. Options Mediterr. vol. 34 pp. 273–280.

Sloof, W., 1982. Skeletal anomalies in fish from polluted surface waters. Aquat. Toxicol. 2, 157–173.

Steingraeber, M.T., Gingerich, W.H., 1991. Hatching, growth, ion accumulation, and ossification of brook trout (*Salvelinus alpinus*) alevins in acidic soft waters. Can. J. Zool. 69, 2266–2276.

Taniguchi, N., Azuma, K., Umeda, S., 1984. Difference due to parents in incidence of vertebral malformation in artificially-bred sea bream. Bull. Jpn. Soc. Sci. Fish. 50 (5), 787–792.

Taning, A.V., 1952. Experimental study of meristic characters in fishes. Biol. Rev. Cambridge Philos. Soc. 27 (2), 169–193.

Treasurer, J., 1992. Vertebral anomalies associated with *Myxobolus* sp. in perch, *Perca fluviatilis* L., in a Scottish loch. Bull. Eur. Assoc. Fish Pathol. 12 (2), 63.

Vladimirov, V.I., 1975. Critical periods in the development of fishes. J. Ichthyol. 15, 851–868.

Westernhagen, H., Dethlefsen, V., Cameron, P., Berg, J., Furstenberg, G., 1988. Developmental defects in pelagic fish embryos from the western Baltic. Helgol. Meeresunters. 42, 13–36.

Whittle, D.M., Sergeant, D.B., Huestis, S.Y., e Hyatt, W.H., 1992. Foodchain accumulation of PCDF isomers in the Great Lakes aquatic community. Chemosphere 25 (1–2), 181–184.

Wiegand, M.D., Hataley, J.M., Kitchen, C., Buchanan, L., 1989. Induction of developmental abnormalities in larval goldfish (*Carassius auratus*) under cool incubation condition. J. Fish Biol. 35, 85–95.

Zitzow, R.E., Millard, J.L., 1988. Survival and growth of lake whitefish (*Coregonus clupeaformis*) fed only formulated dry diets. Aquaculture 69, 105–113.