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REVIEW

Comparison of Meristic Variations and Bone Abnormalities between Wild and Laboratory-Reared Red Sea Bream

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Abstract

To obtain basic information for the production of healthy and vigorous larvae and juveniles, a comparative study on meristic variations and bone abnormalities between wild and laboratory-reared red sea bream, *Pagrus major*, was undertaken. Although 95% of the specimens of wild juveniles had a definite number of dorsal fin rays, reared juveniles exhibited extensive variations, and the latter had fewer pectoral fin rays than the former. Bone abnormalities included the transformation of spines to soft rays, shrinkage of lower jaw, pug-headness, shrinkage of anterior centra, fusion of vertebrae, abnormal pterygiophores, etc. Detailed observations of cleared and double-stained specimens revealed that wild juveniles had an average of 0.2 abnormal bones per specimen, whereas, the reared ones had an average of 2.04–21.4 abnormal bones per specimen, depending on the groups. Therefore, reared fishes showed many abnormalities, although they appeared to be normal based on external observation. The causes of these abnormalities have not been elucidated, except for the lordosis. Abnormalities of reared fishes seemed to affect bones and cartilage as well as other tissues, such as the deformity of the inter-nostril epidermis in one case. It is likely that various defects associated with rearing conditions induce a variety of abnormalities. Aquaculturists should attempt to produce larvae and juveniles which are similar in morphological, behavioral, physiological and biochemical characteristcs to wild ones.

Discipline: Aquaculture
Additional key words: *Pagrus major*, fin ray

Introduction

Blaxter[5] emphasized, in his review, the importance of morphological, behavioral, physiological and biochemical comparative studies between wild and reared fishes of the same species. Anraku and Azeta[2] compared the chemical components of wild and reared red sea bream juveniles, *Pagrus major*, and reported that the amount of calories per one individual of reared specimens was 10–40% higher than that of wild specimens. Fig. 1 shows specimens of cleared and stained juveniles from wild and reared red sea bream with almost the same size. These 2 specimens were apparently different even in external proportion, suggesting that detailed morphological comparative studies, including studies on internal organs between wild and reared stocks should be conducted.

There have been many reports on meristic variations in teleosts, especially in the number of vertebrae[11], because meristic characters are useful for the identification of species and populations. However, there have

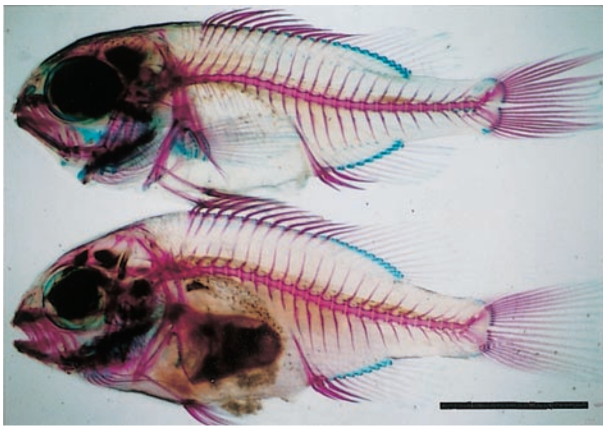

Fig. 1. Photograph of cleared and stained specimens of wild and reared red sea bream
Top: Wild specimen, 20.0 mm TL. Bottom: Reared specimen, 20.0 mm TL. Scale bar indicates 5 mm.

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been few detailed comparative studies on meristic variations in many characters between wild and reared specimens of the same species. More than one thousand papers have been published on the anomalies of fishes, mainly in adults (see, Dawson[7–9]). Information on the anomalies of reared fishes has been gradually increasing[3,16,17,25–28]. The majority of such information is related to serious anomalies which can be observed even based on external morphological characters. There are few detailed examinations on slight anomalies of internal characters, particularly from the viewpoint of comparison between wild and reared fishes of the same species.

This study aimed at analyzing the meristic variations and bone abnormalities in both wild and reared red sea bream juveniles.

Materials and methods

Specimens examined consisted of 2 groups of wild fishes and 4 groups of reared fishes. Wild group A: 100 specimens (range 21.0–51.5 mm in total length (TL)) collected in Shijiki Bay (Nagasaki prefecture) in 1977. Wild group B: 59 specimens (range 12.3–28.6 mm TL) collected in the coastal area of Kumihama (Kyoto prefecture) in 1980 and 1981. Reared group A1: 50 specimens (range 14.0–20.9 mm TL) reared at the Fisheries Research Station, Kyoto University in 1981. Reared group A2: 50 specimens (range 8.15–12.5 mm TL) reared from eggs spawned by the same parental stock as that of Reared A1, but reared in another tank. Reared group B: 50 specimens (range 12.2–20.1 mm TL) reared at the Fisheries Farming Center B in 1981. Reared group C: 25 specimens (range 31.0–72.0 mm TL) reared at the Fisheries Farming Center C in 1977.

Specimens fixed in 10% formalin solution were cleared and stained by the bone staining methods of Hollister[15] for the Wild A group and Taylor[30] for the Reared C group, and by the bone and cartilage staining method of Dingerkus and Uhler[10] for the other groups. These 3 methods did not affect the examination of meristic variations and bone abnormalities.

Observations were made under a stereomicroscope. For meristic characters such as the numbers of vertebrae, pleural and dorsal ribs and fin rays, counts were performed. The number of vertebrae was counted in all the groups. In the reared groups, accurate counting of the number of vertebrae was difficult because a large number of specimens had fused centra. Gabriel[13] suggested that the counting of the arch elements was a more reliable method than the counting of centra. Therefore, a centrum with 2 neural spines and/or 2 haemal spines was considered to be formed by fusion and counted as 2, even if the centrum itself did not show any indication of fusion in appearance. The numbers of pleural and dorsal ribs were counted in relatively larger specimens of the Wild A and Reared C groups, because of the late attainment of their full complements. The number of fin rays was counted in the 2 wild groups and 3 reared groups, except for the Reared A2 group in which the fin rays were still in the course of formation. Two rays articulated with the most posterior distal radial in the dorsal and anal fins were counted as 1 in this study. The numbers of pectoral and pelvic fin rays were counted on the left body side. Bone abnormalities were examined in all the groups.

Bone description and terminology recommended by Matsuoka[23] were adopted in the current study.

Results

1. Meristic variations

The number of vertebrae in all the spariform fishes was 10+14=24 (abdominal vertebrae+caudal vertebrae = total vertebrae)[1]. In the Wild A group, 98 specimens (98%) had 24 (10+14) vertebrae. Two specimens (2%) had 25 (10+15) vertebrae, including 1 specimen (1%) with a fused caudal centrum counted as 2. All the specimens in the Wild B group had 24 (10+14) vertebrae. In the Reared A1 group, 48 specimens (96%) had 24 (10+14) vertebrae, including 10 specimens (20%) with a fused centrum. Two specimens (4%) had 23 (10+13) vertebrae. The reared A2 group consisted of relatively smaller specimens, and in some of the specimens a few pleural centra were still in the course of formation. Most of the specimens seemed to have 24 (10+14) vertebrae, based on arch elements, but 36 specimens (72%) had 1 or more fused centra. All the specimens in the Reared B group had 24 (10+14) vertebrae, including 1 specimen (2%) with a fused centrum. In the Reared C group, 23 specimens (92%) had 24 (10+14) vertebrae, including 4 specimens (16%) with a fused centrum. Two specimens (8%) had 24 (9+15) vertebrae.

All the 17 specimens (range 27.0–51.5 mm TL) in the Wild A group had 8 pairs of pleural ribs. In 25 specimens (range 31.0–72.0 mm TL) of the Reared C group, 21 specimens had 8 pairs of pleural ribs. Three specimens had 7 pairs and 1 specimen had 7 and 8 pleural ribs on either body side. The number of dorsal ribs was more variable in the wild specimens than other meristic characters. In the Wild A group, 13 specimens had 12 pairs of dorsal ribs. Three specimens had 11 and 12 dorsal ribs, and 1 specimen 12 and 13 on either body side. On the other hand, only 2 specimens had 12 pairs of dorsal ribs in the Reared C group. Others had 11 pairs (11 specimens), 11 and 12 (11 specimens), and 12 and 13 (one specimen) on either body side.

Fig. 2 shows the variations in the number of dorsal fin rays. In the 2 wild groups, the majority of the specimens (96% in the Wild A group and 94.9% in the Wild B group) had a fin ray formula of XII-10 (12 spines-10 soft rays). A few specimens with XI-10, XII-9, XII-11 or XIII-9 rays were observed. In contrast, the number of dorsal fin rays in the reared groups was considerably variable and specimens with XII-10 rays did not account for more than 50% (the minimum was 20% in the Reared B group). These variations in appearance included the following 2 abnormalities of development in the dorsal fin rays. One involved an extra small spine which was often formed, just anteriorly to the original first one (arrow in G of Fig. 4). The other abnormality involved the original most posterior spine which often exhibited an intermediate structure between the spine and soft ray, i.e. a thin and long spine or a soft ray with a few segmentations only on the distal tip (A in Fig. 4). In some of the specimens, the ray was still undifferentiated to spine with a sharply pointed tip or appeared as a soft ray with segmentation even at the size where differentiation should have usually been completed. Such undifferentiated rays (denoted by a circle in the Reared A1 and Reared B groups in Fig. 2) were not observed in the Reared C group which consisted of larger specimens, where they probably grew to become intermediate rays. Recounting of the dorsal fin rays was performed to distinguish such abnormalities of development from normal meristic variations: the extra small spine located anteriorly to the original first one was not included and the original most posterior spine was counted as a spine even if it was an intermediate ray or was undifferentiated (arrows in Fig. 2). The number of specimens with XII-9 rays, indicating a decrease in the number of soft rays, ranged from about 20 to 40% in the reared groups.

Fig. 3 shows the variations in the numbers of anal, pectoral and caudal fin rays. Variations in the number of anal fin rays in the reared groups were considerably fewer than those of the dorsal fin rays, except for the

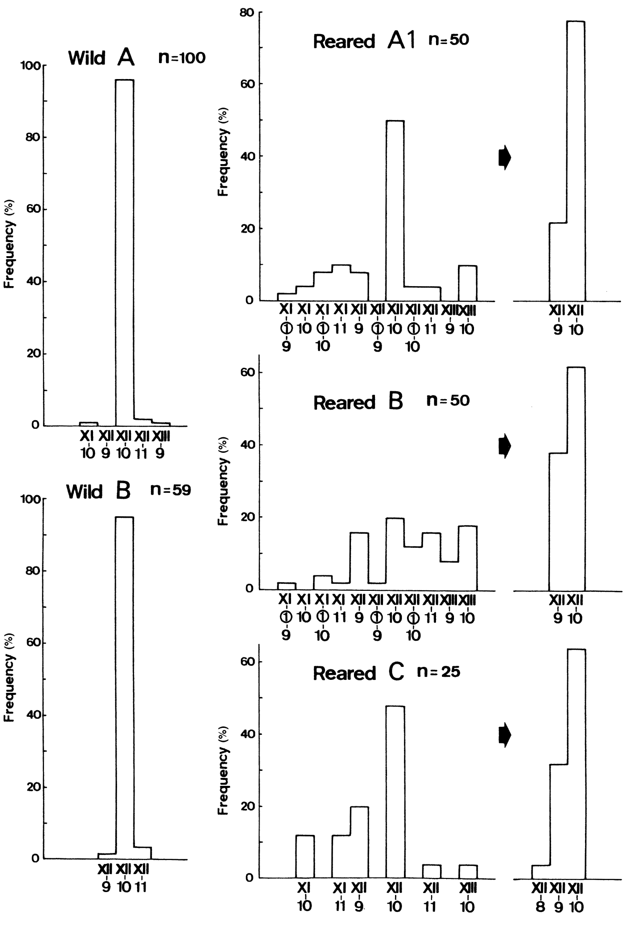


Fig. 2. Frequency distribution (%) of number of dorsal fin rays in respective wild (A, B) and reared (A1, B, C) groups of red sea bream

XII-10 denotes 12 spines-10 soft rays. Number in circle indicates undifferentiated ray. Arrows show recounted frequency distribution to eliminate the influence of abnormal rays in reared groups.

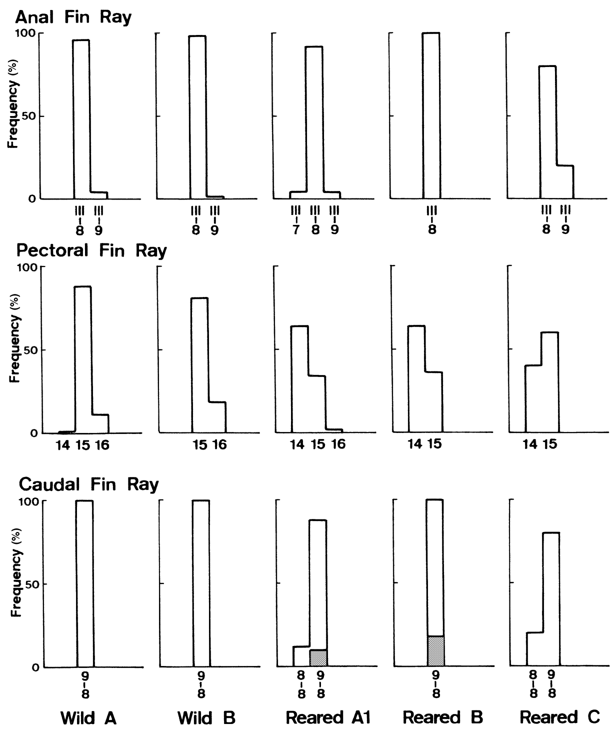
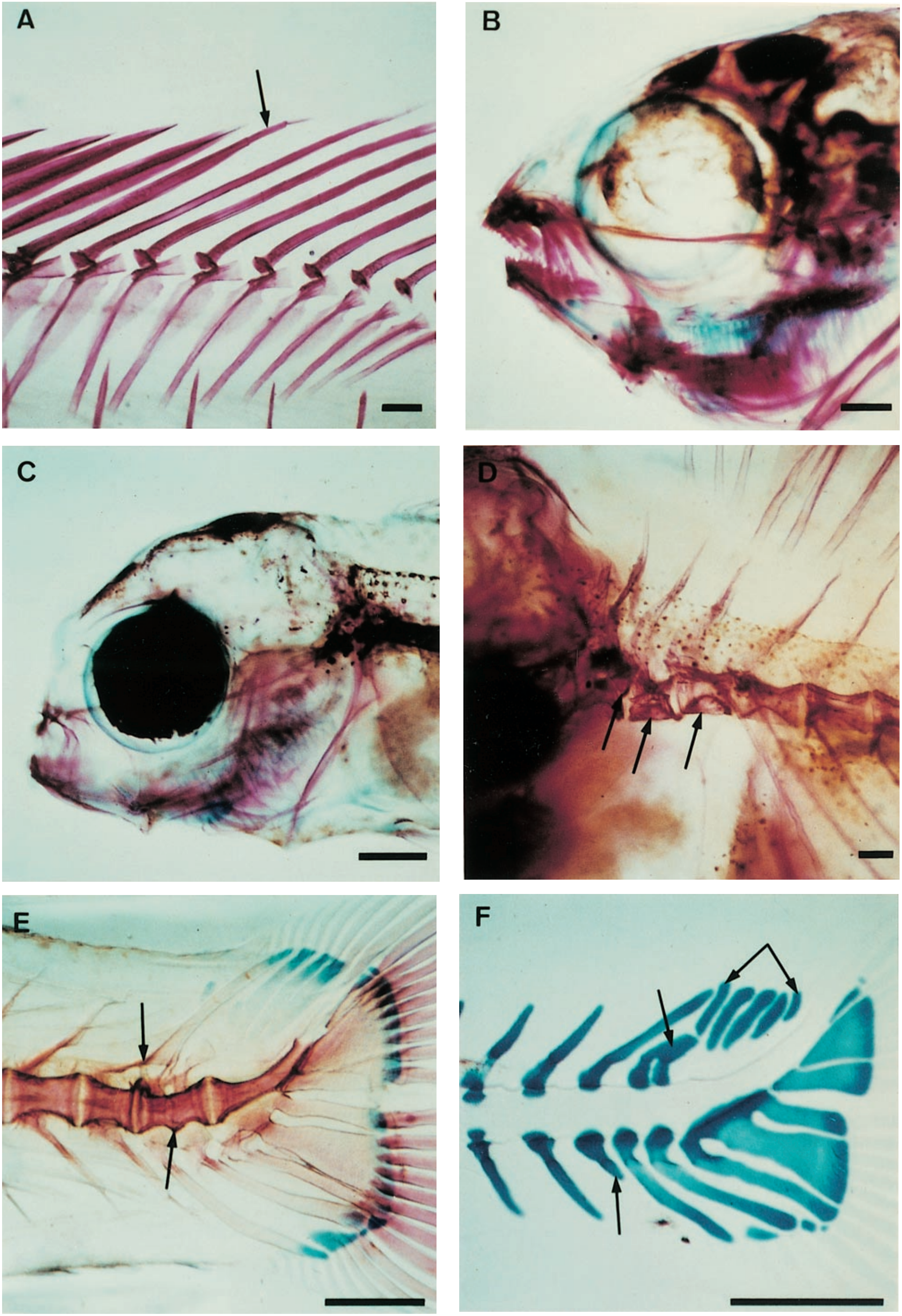


Fig. 3. Frequency distribution (%) of numbers of anal, pectoral and caudal fin rays in respective wild (A, B) and reared (A1, B, C) groups of red sea bream

III-8 denotes 3 spines and 8 soft rays. 9–8 indicates 9 soft rays in the upper lobe and 8 soft rays in the lower lobe. Stippled bar shows the frequency of fusion between rays in caudal fin.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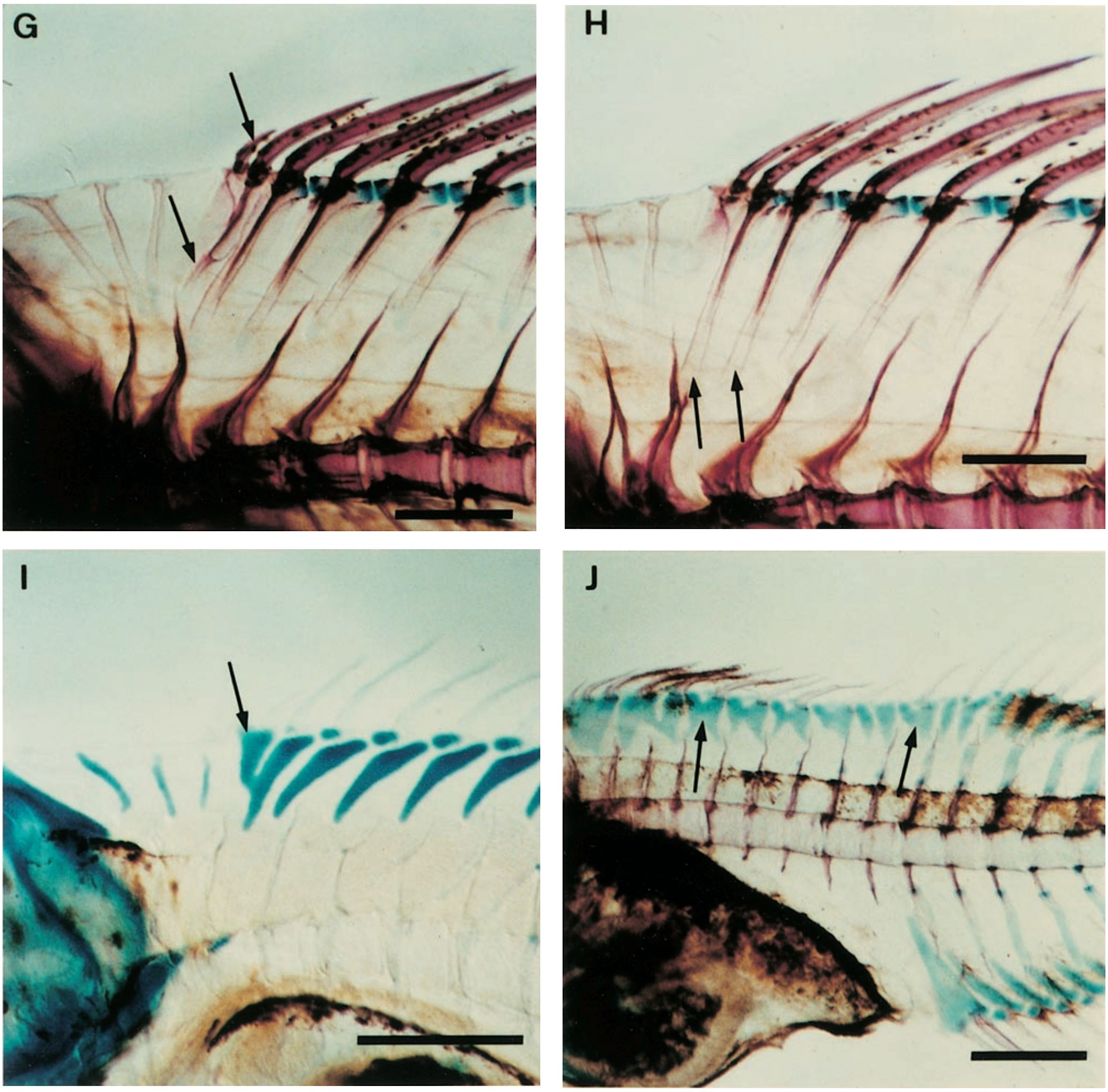

Fig. 4. Photographs showing abnormal bones in cleared and stained specimens of reared red sea bream

A : 47.0 mm TL with intermediate ray having a few segmentations on only the distal tip (arrow).
B : 16.6 mm TL with shortened lower jaw.
C : 9.70 mm TL with both shortened upper and lower jaws.
D : 21.1 mm TL with deformed anterior 3 centra (arrows).
E : 17.1 mm TL with fused caudal centra and fused neural arches (arrows), etc.
F : 8.75 mm TL with fused neural arches, shortened haemal spine and excessive formation of epurals (arrows).
G : 16.0 mm TL with branched first proximal radial and an extra small spine (arrows).
H : 16.8 mm TL with completely separated first proximal radial (arrows).
I : 8.10 mm TL with a large process from first proximal radial (arrow).
J : 7.50 mm TL with deformed and fused proximal radials in dorsal fin-supports (arrows).
Red areas, bones; blue areas, cartilage. Scale bars indicate 0.5 mm.

Reared C group. Specimens with III-8 rays accounted for 96% in the Wild A group, 98.3% in the Wild B group, 92% in the Reared A1 group and 100% in the Reared B group, while the Reared C group contained 20% specimens with III-9 rays. The number of pectoral fin rays in the wild groups was more variable than the number of other fin rays. Specimens in 11.1% of the Wild A group and 18.6% of the Wild B group had 16 rays, although a large number of specimens had 15 rays. In the reared groups, however, specimens with 15 rays accounted for only 34% in the Reared A1 group, 36% in the Reared B group and 60% in the Reared C group: the other specimens had 14 rays. The number of pelvic fin rays did not vary in both wild and reared groups, in which all the specimens had I–5 rays, although Kohno et al.[20] described only 5 rays even in the largest specimen examined. The number of principal caudal rays did not vary in the wild groups, in which all the specimens had 17 (9 soft rays in the upper lobe + 8 soft rays in the lower lobe) rays. In the reared groups, 12% of the specimens in the Reared A1 group and 20% in the Reared C group had 16 (8 + 8) rays. In the Reared A1 group, 10% of the specimens and in the Reared B group, 18% of the specimens showed a fused ray counted as 2 (stippled bars in Fig. 3).

2. Bone abnormalities

The main types of bone abnormalities observed were as follows:

(1) Upper and lower jaws
Both the upper and lower jaws or only the lower jaw were shortened, along with a reduction or deformation of the dentary, premaxillary, maxillary and palatine (B and C in Fig. 4). Shortening of only the upper jaw was not frequently observed.

(2) Centrum
The first centrum was reduced, showing a triangular shape from the lateral view, along with a deformation of several posterior centra (D in Fig. 4). Successive 2 or 3 centra were deformed in the anterior region as well as in other regions of the vertebrae. The fusion of the centra mainly involved the posterior caudal vertebrae. The degree of fusion differed: the fused face between 2 centra was recognizable in some cases (E in Fig. 4), but a somewhat long centrum with 2 neural and/or 2 haemal spines was formed without a distinct fused face in other cases. A reduced triangular centrum with only a haemal spine was often formed just anteriorly to the urostyle.

(3) Neural and haemal arches and spines
The tip of the neural and haemal spines was branched anteriorly and posteriorly. Adjoining neural or haemal spines were fused to each other (E and F in Fig. 4). The neural and haemal spines were shortened (F in Fig. 4), and occasionally both arch and spine were completely lacking. The fusion, shortening and lack of the neural and haemal arches and spines were frequently associated with a fusion or reduction of the centra.

(4) Predorsal
Shortening, branching and fusion of the predorsals were observed. Increase or decrease in the number of predorsals was also seen. These changes did not appear to be normal meristic variations but extreme cases due to branching or fusion.

(5) Dorsal and anal pterygiophores
The first proximal radial of the dorsal fin was branched at the tip (G in Fig. 4) or sometimes completely divided into 2 proximal radials that showed a normal appearance (H in Fig. 4). In many cases, branching of the first proximal radial was accompanied by the formation of an extra small spine (arrow in G of Fig. 4), just anteriorly to the original first one, so that the branched first proximal radial had 3 spines with secondary articulation and 1 spine with serial articulation. The deformed first proximal radial could be distinguished from the normal one even in the cartilaginous state because a relatively large process or separation of cartilage from the first proximal radial occurred (I in Fig. 4). The first proximal radial of the anal fin was not deformed unlike that of the dorsal fin. Deformation and fusion of the dorsal and anal pterygiophores were occasionally observed, especially in a reared group in which all the specimens showed many deformed proximal radials in the trunk region (J in Fig. 4).

(6) Parhypural and hypural
The parhypural and hypural 1 and 2 were fused, mostly between the parhypural and hypural 1, and between the hypural 1 and 2. The bases of the parhypural and hypural 1 and 2 were normally fused by cartilage at early stages, but the above cases resulted from the fusion of ossified structures. The hypural 5 was frequently lacking.

(7) Epural
Shortening, branching and fusion of the epurals were observed. The increase or decrease in the number of epurals did not appear to be due to normal meristic variations, but was probably caused by branching or fusion, like in the predorsals.

(8) Branchiostegal ray
The first and second branchiostegal rays were fused at the basis. A thick ray was often formed by complete fusion of these rays. Increase and decrease in the number of branchiostegal rays were also

observed and included as bone abnormality.

(9) Others

In a few cases, shortening, separation and lack of pleural ribs were observed. In rare cases, the tips of the pleural ribs, except for the eighth pair, were ossified and sharply pointed, but usually the tips were covered by cartilage. The most ventral actinost of the pectoral fin was deformed, sometimes with an orifice. The accessory cartilage Ac 4 was fused with Ac 3 or Ac 5, and Ac 4 and Ac 5 were, respectively, separated into small pieces of cartilage.

Bone abnormalities were seldom observed in the head skeleton and the pectoral and pelvic fin-supports, except for the shortening of the upper and lower jaws and the deformation of the most ventral actinost.

Fig. 5 shows the number of abnormal bones classified into 12 types for 100 individuals in the wild and reared groups, respectively. In the Wild A group, there

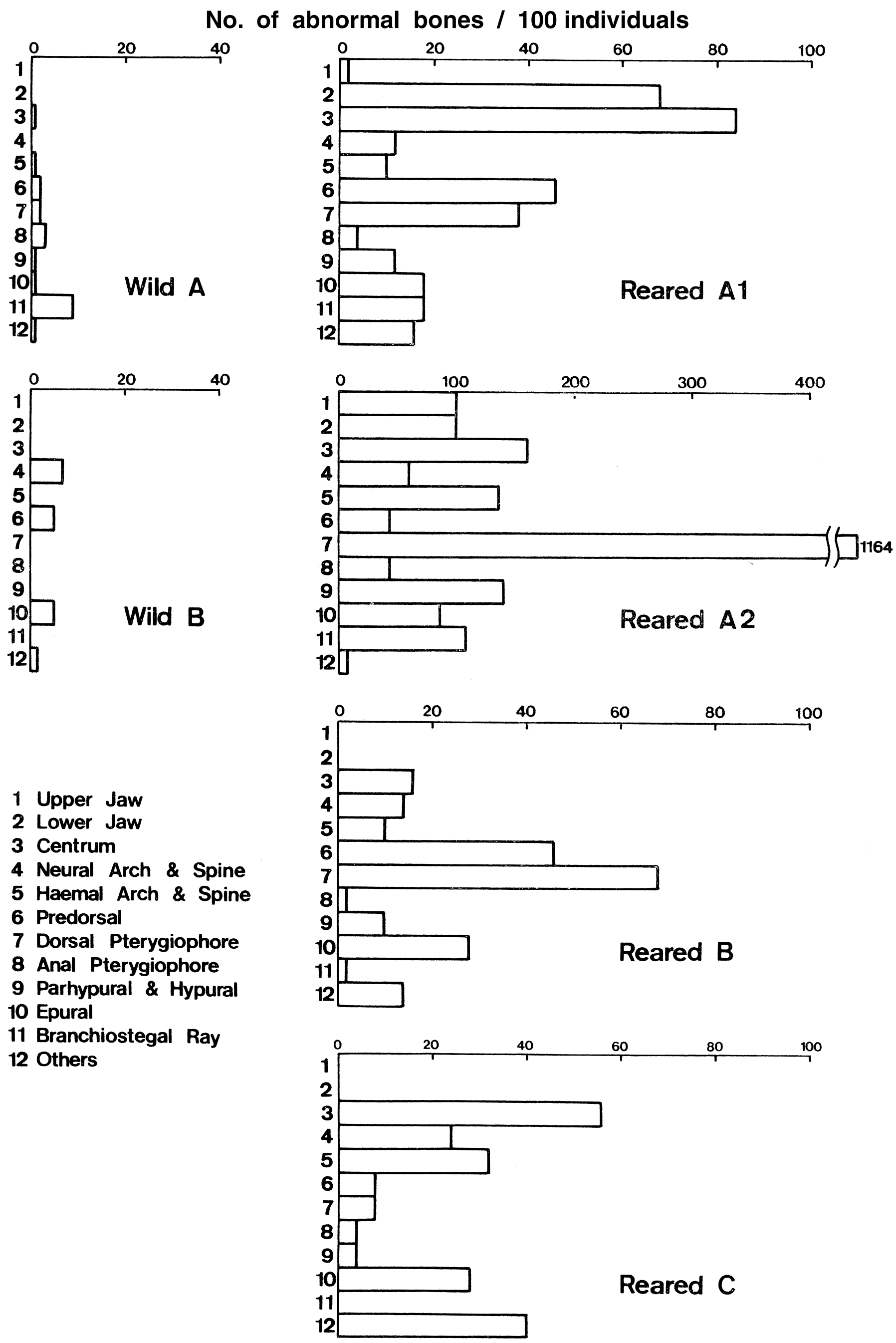

Fig. 5. Number of abnormal bones classified into 12 types for 100 individuals in respective wild (A, B) and reared (A1, A2, B, C) groups of red sea bream

were 9 cases of abnormal (including meristic variations in appearance) branchiostegal rays in which 6 cases had 7 rays. Other bone abnormalities were very few. In the Wild B group, abnormal bones affected the neural arch and spine (6.8 cases), predorsal (5.1 cases), epural (5.1 cases) and others (1.7 cases). The number of abnormal bones in all the reared groups was considerably higher than that in the wild groups. In the Reared A1 group, abnormal bones were particularly concentrated on the lower jaw (68 cases), centrum (84 cases), predorsal (46 cases) and dorsal pterygiophore (38 cases). Specimens in the Reared A2 group, grown from eggs spawned by the same parental stock as that of the Reared A1 group, showed numerous abnormal bones of almost all types. Abnormalities of the dorsal pterygiophore were observed in 1,164 cases. All the specimens examined had shortened upper and lower jaws and malformations mainly involved the centrum (160 cases), haemal arch and spine (136 cases), parhypural and hypural (130 cases), branchiostegal ray (108 cases), etc. In the Reared B group, a large number of abnormalities occurred in the predorsal (46 cases), dorsal pterygiophore (68 cases) and epural (28 cases). In the Reared C group, abnormal bones involved mainly the centrum (56 cases), neural arch and spine (24 cases), haemal arch and spine (32 cases), epural (28 cases), and others (40 cases, mostly in the pleural ribs).

Fig. 6 shows the frequency of the specimens having at least 1 abnormal bone and the average number of abnormal bones per individual in the wild and reared groups, respectively. The results in the 2 wild groups were very similar, in which about 15% of the specimens had abnormal bones and the average number of abnormal bones was about 0.2 per individual. On the other hand, the percentage of specimens with abnormal bones in the reared groups reached 96% (Reared A1), 100% (Reared A2), 84% (Reared B) and 76% (Reared C). The average number of abnormal bones was 3.28 (Reared A1), 2.10 (Reared B), 2.04 (Reared C) with an extremely high value of 21.4 (Reared A2) per individual.

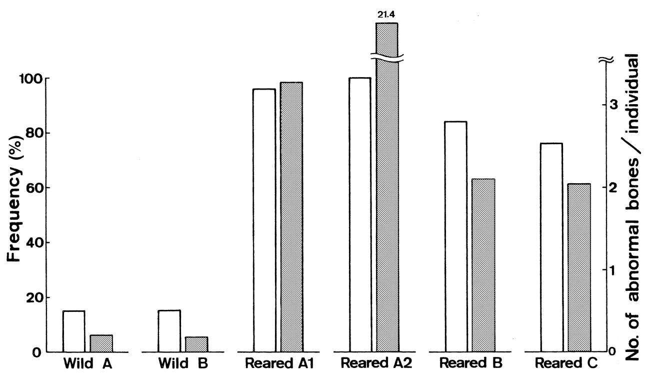

Fig. 6. Frequency (%) of specimens with at least one abnormal bone (open bar) and average number of abnormal bones per individual (stippled bar) in respective wild (A, B) and reared (A1, A2, B, C) groups of red sea bream

Discussion

Fowler[11] pointed out in his review that laboratory-reared fishes generally displayed more variations in the meristic characters than natural populations of the same species. Lau and Shafland[22] noted that reared specimens of *Centropomus undecimalis* displayed more meristic variations than wild specimens, although no quantitative analysis was undertaken. The present data clearly show that the reared red sea bream exhibited considerably more variations in the numbers of vertebrae, pleural and dorsal ribs, and fin rays than the wild specimens.

In reared specimens, accurate counting of the number of vertebrae is rather difficult because of fusion and reduction. Taniguchi et al.[29] reported a higher incidence of reared red sea bream with 23 vertebrae (maximum, 19.6%) than in the current study. In this respect, their results may have included fusion or reduction of the centrum. Wide variations in the number of dorsal fin rays were partly associated with abnormal rays such as extra small spine and intermediate ray. The fused rays were often observed in the caudal fin. Therefore, it is suggested that meristic variations in these characters in the reared specimens may, to a certain extent, be due to abnormal formation of the bone and ray, in addition to normal variations.

The number of pectoral fin rays in the reared specimens tended to be lower than that in the wild specimens, although abnormal rays were not observed in the former. Recounting of the number of dorsal fin rays after eliminating the influence of abnormal rays also showed a similar tendency. Lower meristic counts in reared fishes than wild fishes were also reported in the vertebrae of *Gadus callarias*[6] and the scales of *Salmo kamloops*[24]. Tateishi and Ikeda[31] observed that the reared red sea bream had a lower average number of pectoral fin rays than the wild ones, and Tateishi et al.[32] tried to use this characteristic to identify artificially released red sea bream from wild ones. Lower counts of some meristic characters in reared fishes may be due to environmental differences, e.g. temperature, between wild and rearing conditions, as reviewed by Fowler[11] and Barlow[4].

The high incidence of morphological abnormalities in reared fishes has been described by many authors. For example, Barahona-Fernandes[3] reported that about 90% of the specimens of reared *Dicentrarchus labrax* had opercular abnormalities. Komada[21] compared wild and reared specimens of *Plecoglossus altivelis* and indicated that the frequency of external morphological abnormalities in reared specimens was about 100–500 times higher than that in wild ones and the frequency of deformed vertebrae in the former was about 8–15 times higher. In the red sea bream examined here, external morphological abnormalities, except for the shortened jaws in reared specimens, were seldom observed. The majority of the specimens appeared "normal". However, detailed examination of the bones on cleared and stained specimens revealed that the frequency of specimens with abnormal bones in the reared groups was at least 5 times higher than that in the wild groups. The number of abnormal bones per individual in the reared groups was at least 10 times higher. It should be noted that there were numerous skeletal abnormalities even in the externally "normal" reared specimens.

It was demonstrated that the lordosis found in the red sea bream was related to swimbladder deformation mainly caused by nutritional deficiency[12,18,19,33]. The lordosis is due to a secondary deformation of the centra[27]. On the other hand, the majority of the skeletal abnormalities observed seemed to be directly due to abnormal bone formation such as fusion, reduction or excessive formation. The cause of such abnormal bone formation remains to be determined. Taniguchi et al.[29] suggested the possibility of genetic influence on skeletal abnormalities of the red sea bream. In the present study, the fishes in the Reared A1 and A2 groups were reared from eggs spawned by the same parental stock, in respective small tanks (30 L). The reared A2 group, however, showed a considerably higher incidence of abnormal bones than the Reared A1 group. Therefore, it is suggested that rearing conditions may influence more significantly abnormal bone formation than genetic factors. The fishes in the Reared B and C groups were reared in large tanks (50, 100 t). The incidence of abnormal bones in these groups was relatively lower than that of the fishes in the Reared A1 and A2 groups. Therefore, tank size may influence abnormal bone formation to some extent. The highest incidence of abnormal bones among the 12 types was different in the 4 reared groups (Fig. 5). It is assumed that various factors in the respective rearing conditions, e.g. nutrition, quality of rearing water, fish density, tank size, temperature, may be related to the different types of abnormal bone formation.

Abnormalities of reared fishes appeared to affect bones and cartilage as well as other tissues, such as the deformity of the inter-nostril epidermis[14] is one case. Through the improvement of the rearing conditions, aquaculturists should attempt to produce larvae and juveniles which are similar in morphological, behavioral, physiological and biochemical characteristics to wild ones.

References

1. Akazaki, M. (1962) Studies on the spariform fishes-Anatomy, phylogeny, taxonomy and ecology. *Spec. Rep. Misaki Mar. Biol. Inst., Kyoto Univ.*, **1**, 1–368 [In Japanese with English summary].
2. Anraku, M. & Azeta, M. (1973) Difference of body components between artificially reared and natural sea bream: larva and young. *Bull. Seikai Reg. Fish. Res. Lab.*, **43**, 117–131 [In Japanese with English summary].
3. 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.
4. Barlow, G. W. (1961) Causes and significance of morphological variation in fishes. *Syst. Zool.*, **10**, 105–117.
5. Blaxter, J. H. S. (1975) Reared and wild fish—how do they compare? *10th Europ. Symp. Mar. Biol.*, **1**, 11–26.
6. Dannevig, A. (1932) Is the number of vertebrae in the cod influenced by light or high temperature during the early stages? *J. Cons. Perm. Int. Explor. Mer.*, **7**, 60–62.
7. Dawson, C. E. (1964) A bibliography of anomalies of fishes. *Gulf Res. Rep.*, **1**, 308–399.
8. Dawson, C. E. (1966) A bibliography of anomalies of fishes. Supplement 1. *Gulf Res. Rep.*, **2**, 169–176.
9. Dawson, C. E. (1971) A bibliography of anomalies of fishes. Supplement 2. *Gulf Res. Rep.*, **3**, 215–239.
10. Dingerkus, G. & Uhler, L. D. (1977) Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technol.*, **52**, 229–232.
11. Fowler, J. A. (1970) Control of vertebral number in teleosts—an embryological problem. *Q. Rev. Biol.*, **45**, 148–167.
12. Fujita, S. & Kitajima, C. (1978) Occurrence of body deformation in larvae and juveniles of red sea bream. *Mar. Sci., Mon.*, **10**, 721–727 [In Japanese].
13. Gabriel, M. L. (1944) Factors affecting the number and form of vertebrae in *Fundulus heteroclitus*. *J. Exp. Zool.*, **95**, 105–147.
14. Goto, M. (1986) On the morphological abnormality of nostril in cultured red sea bream. *Saibai Giken*, **15**, 87–88 [In Japanese].
15. Hollister, G. (1934) Clearing and dyeing fish for bone study. *Zoologica*, **12**, 89–101.
16. Houde, E. D. (1971) Developmental abnormalities of flatfish *Achirus lineatus* reared in the laboratory. *Fish. Bull.*, **69**, 537–544.
17. Kitajima, C. (1978) Acquisition of fertilized eggs and mass-culture of juveniles of red sea bream, *Pagrus major*. *Spec. Rep. Nagasaki Pref. Inst. Fish.*, **5**, 1–92 [In Japanese with English summary].
18. Kitajima, C. et al. (1977) Relation between curvature of vertebral column and hatchery-reared undeveloped swimbladder in red sea bream, *Pagrus major*. *Bull. Nagasaki Pref. Inst. Fish.*, **3**, 23–32 [In Japanese].
19. Kitajima, C. et al. (1981) Relationship between uninflated swim bladders and lordotic deformity in hatchery-

reared red sea bream *Pagrus major*. *Nippon Suisan Gakkaishi*, **47**, 1289–1294 [In Japanese with English summary].
20. Kohno, H. et al. (1983) Development of swimming and feeding functions in larval *Pagrus major*. *Jpn. J. Ichthyol.*, **30**, 47–60.
21. Komada, N. (1980) Incidence of gross malformations and vertebral anomalies of natural and hatchery *Plecoglossus altivelis*. *Copeia*, **1980**, 29–35.
22. Lau, S. R. & Shafland, P. L. (1982) Larval development of snook, *Centropomus undecimalis* (Pisces: Centropomidae). *Copeia*, **1982**, 618–627.
23. Matsuoka, M. (1987) Development of skeletal tissues and skeletal muscles in the red sea bream. *Bull. Seikai Reg. Fish. Res. Lab*., **65**, 1–114.
24. Mottley, C. M. (1931) The effect of temperature on the number of scale in trout. *Science*, **74**, 316.
25. Shimizu, H. & Fujita, S. (1985) Bone abnormality of hatchery-reared *Oplegnathus fasciatus. Aquiculture*, **33**, 149–156 [In Japanese with English summary].
26. Takashima, F. (1976) Anomalies in hachery reared ayu, *Plecoglossus altivelis*-II. Malformation in the larva. *J. Tokyo Univ. Fish.*, **62**, 99–112[In Japanese with English summary].
27. Takashima, F. (1978) Vertebral malformation in hatchery-reared red seabream, *Chrysophrys major. Nippon Suisan Gakkaishi*, **44**, 435–443 [In Japanese with English summary].
28. Takashima, F. et al. (1976) Anomalies of hatchery reared ayu, *Plecoglossus altivelis*-I. Malformation of the skeletal system found in the fry. *J. Tokyo Univ. Fish.*, **62**, 91–97 [In Japanese with English summary].
29. Taniguchi, N. et al. (1984) Difference due to parents in incidence of vertebral malformation in artificially-bred red seabream. *Nippon Suisan Gakkaishi*, **50**, 787–792 [In Japanese with English summary].
30. Taylor, W. R. (1967) An enzyme method of clearing and staining small vertebrates. *Proc. U. S. Nat. Mus.*, **122**, 1–17.
31. Tateishi, K. & Ikeda, Y. (1985) Difference in the number of pectoral fin rays between laboratory reared and wild red sea bream (Preliminary study). *In* Abstracts of oral presentation at annual meeting of Japan. Soc. Sci. Fish., Kyusyu Branch, Nagasaki January, 1985, 4 [In Japanese].
32. Tateishi, K. et al. (1985) Catch ratio of artificially-released red sea bream identified from wild one by the number of pectoral fin rays. *In* Abstracts of oral presentation at annual meeting of Jpn. Soc. Sci. Fish., Kagoshima, October, 1985, 23[In Japanese].
33. Watanabe, T. (1978) Nutritional value of lipid in living foods for fish larvae. *In* Fish Culture and Food Lipid. ed. Japan Soc. Sci. Fish., Koseisha Koseikaku, Tokyo, 93–111 [In Japanese].