Belg. J. Zool. - Volume 127 (1997) - issue 2 - pages 115-122 - Brussels 1997

Received : 12 February 1997

PRESENCE OF DOUBLE SPINES ON THE SECOND PREURAL CENTRUM OF THE TURBOT (SCOPHTHALMUS MAXIMUS L., PLEURONECTIFORMES: SCOPHTHALMIDAE)

BRUNO CHANET (¹) AND FRANCE WAGEMANS (²)
(¹) Ressources halieutiques – Poissons marins – Faculté des sciences,
Université de Bretagne Occidentale, 6 avenue Le Gorgeu, BP 809, 29285 Brest cedex, France.
(²) Service de morphologie fonctionnelle, Institut de Zoologie,
22 quai de van Beneden, B-4020 Liège, Belgium.
e-mail: chan2837@eurobretagne.fr

Abstract. The literature concerning the presence of double spines on the second preural centrum in flatfishes is being reviewed and the different hypotheses proposed to account for this phenomenon are presented. A study using laboratory-reared specimens of the development of the caudal endoskeleton of the turbot on gives new data to explain the presence of this feature. Such double spines seem to be the result of a fusion during the ontogeny between the third preural centrum and the second preural centrum.

Key words: Pleuronectiformes, turbot, caudal endoskeleton, development.

INTRODUCTION

The caudal endoskeleton of pleuronectiform fishes has been studied by a large number of authors (BARRINGTON, 1937; MONOD, 1968; HENSLEY & AHLSTROM, 1984; CHAPLEAU, 1993 and further references therein). Normally, the second preural centrum possesses one neural spine and one haemal spine (Fig. 1), these spines often being broader than the other ones. But many authors showed the frequent occurrence of double spines on this centrum, i.e. the second preural centrum (PU2) possesses two neural spines and/or two haemal spines (CHABANAUD, 1937; BARRINGTON, 1937; FUTCH, 1977; HENSLEY & AHLSTROM, 1984). This feature is common in Pleuronectiformes (HENSLEY & AHLSTROM, 1984) and different hypotheses have been proposed to explain it:

1) these double spines are the result of the fusion of one epural and one hypural with respectively the neural spine and the haemal spine of the centrum (COLE & JOHNSTONE, 1902).

2) these double spines are the result of the fusion of the two last neural arches together on the one hand, and of the two last haemal arches on the other hand (BARRINGTON, 1937).

3) these double spines are the result of the fusion of the two last preural centra (HENSLEY & AHLSTROM, 1984, following ROSEN, 1973).

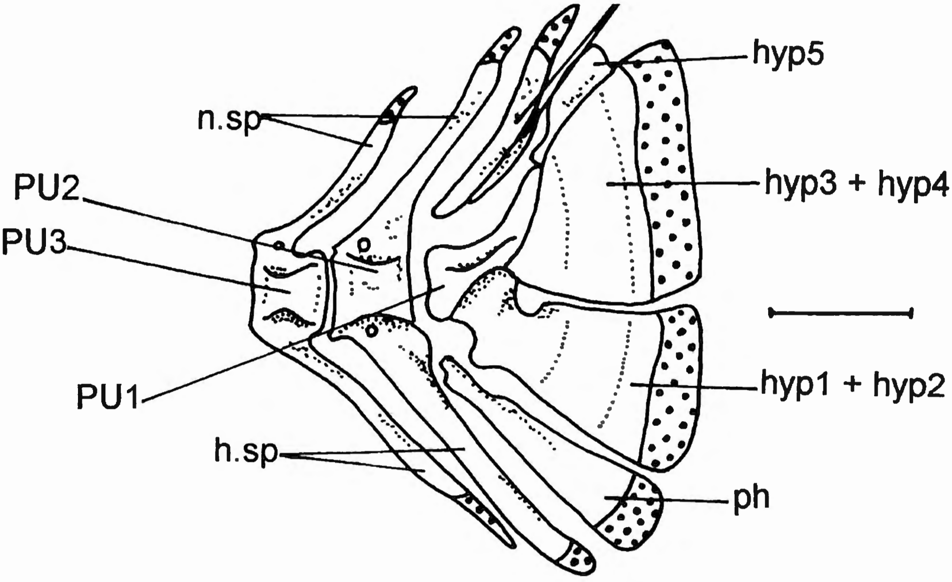

Fig. 1. – Caudal endoskeleton of a young turbot (*Scophthalmus maximus*), 29th day of development (SL=16 mm), left lateral view. The centrum of PU2 bears one neural spine and one haemal spine. The scale indicates 1 mm. The areas with black circles are cartilaginous regions. [ep: epural; h.sp: haemal spine; hyp: hypural; n.sp: neural spine; PU: preural centrum].

HENSLEY & AHLSTROM (1984) stressed that a detailed survey of this feature was needed. Recently, we have had the opportunity to study the development of the caudal endoskeleton of a pleuronectiform fish: the turbot, *Scophthalmus maximus* L., 1758, Scophthalmidae. Some specimens showed double spines on the second preural centrum. The purpose of this paper is to present our results and to give data in order to corroborate or to invalidate these hypotheses and thus to try to explain the occurrence of these double spines.

MATERIAL AND METHODS

Scophthalmus maximus fry were raised in the aquaculture-station of France Turbot-NATA (Noirmoutiers, France) at 15°C. Batches of 40 fry were sampled on days 0 to 31 and batches of 30 fry on days 33 to 61 post hatching. The fry were fixed in a $CaCO_3$ buffered 10% formalin solution and were cleared in trypsin. Some of them were stained with alcian blue to reveal the cartilages and others with alizarine, which stains calcified bones, according to Taylor and Van Dyke's method (1985). It was possible to stain the most young stages simultaneously with alizarine and alcian. Finally, the fry were stored in glycerin. A 6 month-old specimen was cleared in trypsin, stained with alizarine and stored in glycerin according to Taylor and Van Dyke's method. The specimens, 270 larval and juvenile turbots, have been studied with a binocular Wild M10 Leica dissecting microscope at 8x magnification, and a drawing tube. The length from the tip of the snout to the posterior margin of hypural elements(standard length - SL) was measured for each specimen.

RESULTS

The caudal skeleton elements can be detected as early as the 14th day after hatching. At this stage, they are merely cartilaginous buds. They develop progressively and their ossification begins on the 22nd day. This process first occurs in the centra, at the base of the spines and in the median part of the hypurals, consequently, the latter still have both extremities, proximal and distal, made of cartilage (Figs 1-2-3). This cartilaginous proximal part is gradually replaced by bone, whereas the cartilaginous distal part remains for a longer time. 123 of our specimens (45%) possess only one haemal spine and one neural spine on the PU2 centrum at every stage of the development (Figs 1-4-5). They are long, slender; the neural spines are postero-dorsally directed, whereas the haemal spines are postero-ventrally orientated. 55% of the studied specimens show double spines on the second preural centrum (Figs 2-3). On these specimens, the PU2 centrum bears two neural

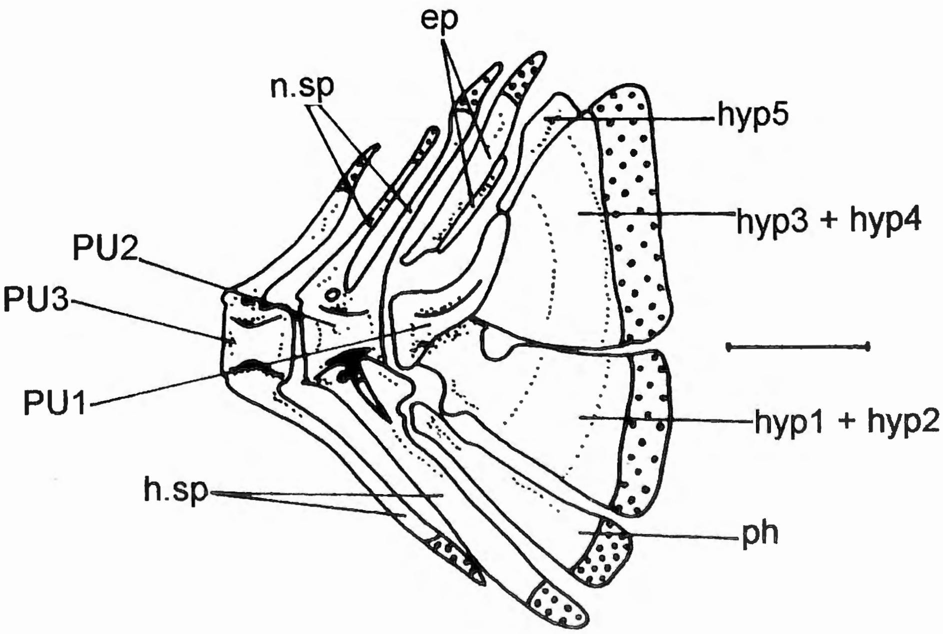

Fig. 2. – Caudal endoskeleton of a young turbot (*Scophthalmus maximus*), 29th day of development (SL=17 mm), left lateral view. The centrum of PU2 bears double neural spines and a broader haemal spine. The scale indicates 1 mm. The areas with black circles are cartilaginous regions. [ep: epural; h.sp: haemal spine; hyp: hypural; n.sp: neural spine; PU: preural centrum].

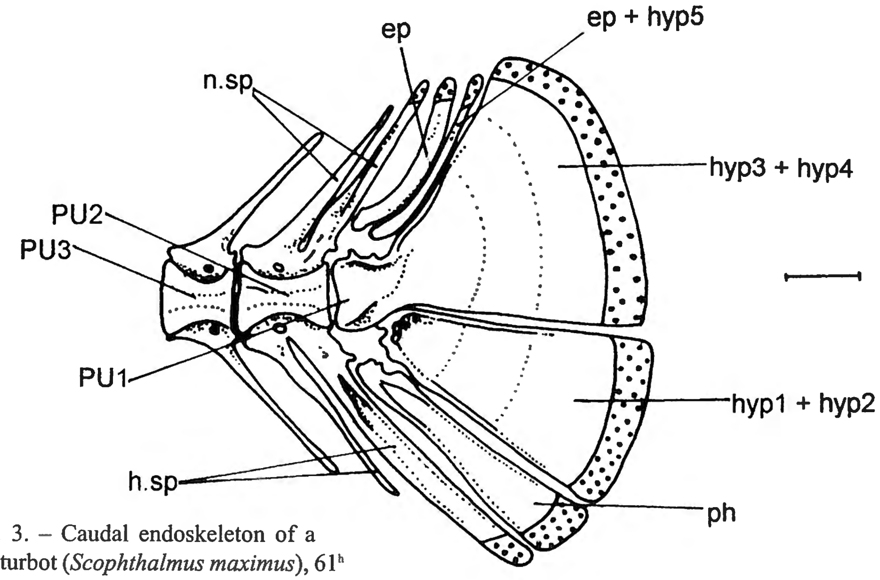

Fig. 3. – Caudal endoskeleton of a young turbot (*Scophthalmus maximus*), 61^{h} day of development (SL =28 mm), left lateral view. The centrum of PU2 bears double neural and haemal spines. The scale indicates 1 mm. The areas with black circles are cartilaginous regions. [ep: epural; h.sp: haemal spine; hyp: hypural; n.sp: neural spine; PU: preural centrum].

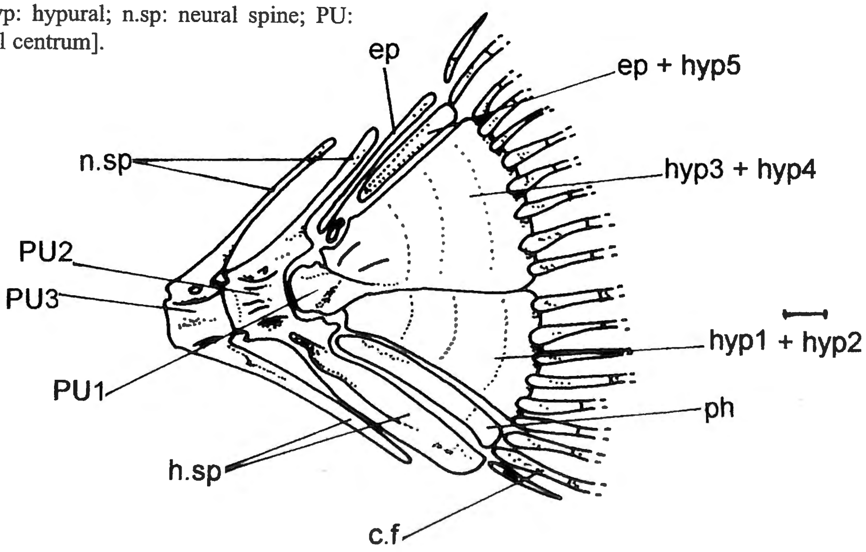

Fig. 4. – Caudal endoskeleton of a young turbot (*Scophthalmus maximus*), 6 month of development (SL= 68 mm), left lateral view. The centrum of PU2 bears one neural spine and one haemal spine. The scale indicates 1 mm. The areas with black circles are cartilaginous regions. [c.f: caudal fin ray; ep: epural; h.sp: haemal spine; hyp: hypural; n.sp: neural spine; PU: preural centrum].

spines and two distinct haemal spines or two neural spines and a very broad haemal spine (Figs 2-3). The bases of these double spines are very close together. These specimens are not only different from the others by the presence of these double spines, but they also differ in the number of vertebrae. The specimens bearing one neural spine and one haemal spine on the PU2 centrum possess 10-11 precaudal vertebrae and 20 caudal vertebrae. These numbers are constant, but each specimen which possesses double spines on the second preural centrum has only 19 caudal vertebrae. 25 of the studied specimens (9%) show no double spines and have 19 caudal vertebrae (Fig. 5). Moreover, the caudal endoskeleton of all the specimens is built on the same pattern. They possess five hypurals, one parhypural, and two epurals. Hypurals 1 and 2, on the one hand, and hypurals 3 and 4 on the other hand, are fused in two distinct plates (Figs 1-2-4) and the upper hypural plate (formed by the fusion of hypurals 3 and 4) is fused with the centrum of PU1. Before day 60, each of our young specimens had two epurals (Fig. 2) whereas in older fish, the more posterior epural becomes fused with hypural 5 (Fig. 4), as FUTCH (1977) described it in *Trichopsetta ventralis* (GOODE AND BEAN) 1885 (Bothidae).

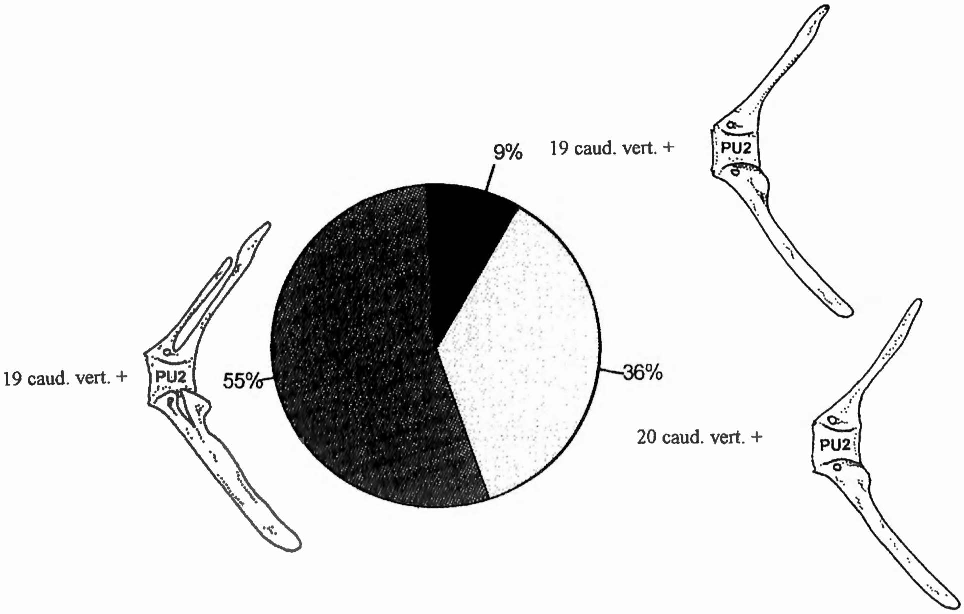

Fig. 5. – Representation of the numerical percentage of the number of the caudal vertebrae related to the number of neural and haemal spines on the centrum of PU2 in *Scophthalmus maximus*.

DISCUSSION

The presence of double spines on the second preural centrum has been mentioned by many authors, with several explanations proposed. COLE & JOHNSTONE (1902: 194) noticed in the plaice (*Pleuronectes platessa* L. 1758, Pleuronectinae) that: «the posterior shafts (of the second preural centrum spines) so closely resemble the succeeding epural and hypural respectively as to suggest that one epural above and an hypural below have fused to the laminate portions, which latter are undoubtedly similar to and perhaps represent the neural and haemal spines in front. As, however, we have no positive evidence for such a fusion, the spines in question are here described as simple neural and haemal spines». BARRINGTON (1937:468), in his study about the development of the tail in the plaice and the cod (*Gadus morrhua* L. 1758, Gadidae), showed that: «the penultimate vertebra (= second preural centrum) comes to bear two dorsal and two ventral arches as a result of their fusion during development». HENSLEY & AHLSTROM (1984:676) reviewed the relationships of flatfishes and considered that the presence of two neural spines on the second preural centrum is the result of « a fusion of this vertebra with an anterior one bearing a spine». Actually, they followed ROSEN (1973:499): «the fusion of the preural caudal centra may explain the presence of two neural spines on the PU2 centrum». ROSEN (1973) stressed that this kind of event is frequent within higher euteleostean groups, but HENSLEY & AHLSTROM (1984) provided no ontogenetic data to corroborate this vertebral fusion. Are these double neural spines or double haemal spines anomalies? The former authors stressed the fact that this feature was not so rare and that a detailed survey of their occurrences was needed. A review of the bibliography shows that this feature has been already observed by several authors. CHABANAUD (1937:377) represented the caudal skeleton of *Samaris cristatus* GRAY 1831 (Samaridae), with two neural spines on the PU2 centrum. In the same article (:378), a caudal skeleton of *Solea solea* (= *Solea vulgaris* QUENSEL 1806, Soleidae) is shown with double neural and haemal spines. Usually in this species, the centrum bears only one neural spine and one haemal spine (FABRE-DOMERGUE & BIÉTRIX, 1905; CHAPLEAU & KEAST, 1988). When BERG (1941) described *Eobothus vialovi* BERG 1941, a probable fossil flatfish from the Lower Eocene of Uzbekistan (CHANET & SCHULTZ, 1994), he mentioned two haemal arches and two haemal spines on the PU3 and PU2 centra. In 1969, AMAOKA, in his study about the Japanese sinistral flatfishes, showed doubles spines (haemal and neural) on the PU2 centrum of three bothid species: *Taeniopsetta ocellata* (GÜNTHER 1880), *Parabothus coarctatus* (GILBERT 1905) and *Laeops kitaharae* (SMITH & POPE 1906). HENSLEY (1977:696) mentioned a particular larva of *Engyophrys senta* GINSBURG 1933 (Bothidae): «one 7.0 mm larva has two neural spines (unossified) associated with the area of the notochord where the second preural centrum develops». SAKAMOTO (1984) showed that some flatfish species possessed two haemal spines on the PU2 centrum: one Pleuronectinae (*Hippoglossus stenolepis* SCHMIDT 1904), one Rhombosoleinae (*Ammotretis elongatus* MC CULLOCH 1914) and one Samaridae (*Samariscus latus* MATSUBARA & TAKAMUKI, 1951). FUTCH (1977) indicated that one specimen of *Trichopsetta ventralis* (Bothidae) showed double neural spines on the PU2 centrum. MUNROE (1996) explained the presence of multiple neural spines (from one to four) on the caudal centra of one reversal specimen of *Symphurus vanmelleae* CHABANAUD 1952 (Cynoglossidae) as the result of fusion between caudal vertebrae.

Our data show that, in the turbot, the presence of double spines on the PU2 centrum seems to be the result of a fusion between two centra: the antepenultimate (PU3) and the penultimate (PU2) vertebrae, as ROSEN (1973) and HENSLEY & AHLSTROM (1984) thought. This phenomenon is not just the result of the fusion between the arches as BARRINGTON (1937) indicated. And what about a probable capture of one hypural and one epural element as COLE & JOHNSTONE (1902) proposed? If such a mechanism occurred the numbers of epurals and hypurals would have been affected and the fishes bearing double spines would have possessed a peculiar caudal endoskeleton. But, none of the specimens we have examined shows such differences. Although young turbot have two epurals, older fish have five hypurals and one epural, and hypural 5 present in the adult is the result of fusion between the posterior epural and hypural 5.

The study of the development of the turbot gives arguments to say that the occurrence of double spines on the second preural centrum is the result of the fusion of the third preural centrum and the second preural centrum. Such an hypothesis seems to be valid to explain the presence of these double spines in the turbot. But, because it is possible to think that the double spines present on the penultimate centrum of the plaice (COLE & JOHNSTONE, 1902; BARRINGTON, 1937) or *Trichopsetta ventralis* (FUTCH, 1977) may be the result of different mechanisms occurring during the development of these fishes, this hypothesis has to be confirmed by ontogenetic studies on some other flatfish species. One can argue that these abnormalities are the consequence of the fact that the studied specimens were not reared in natural conditions but in laboratory conditions. Many authors reported numerous incidences of morphological and skeleton abnormalities associated with aquacultural practices for flatfishes (HOUDE, 1971; HEAP & THORPE, 1987; LAGARDÈRE *et al.*, 1993). In field sampled specimens, however the presence of double spines on the PU2 centrum is not rare. Pending new evidence, the simplest solution to explain the presence of double spines on the PU2 centrum in flatfishes is to suppose that these features have been formed by the same mechanism as proposed here for the turbot.

ACKNOWLEDGEMENTS

We have the opportunity here of thank: Mr Leclercq (France Turbot, Noirmoutiers, France), Mrs Lebègue (NATA, Noirmoutiers, France) for the rearing of the specimens, P. Vandewalle (Institut de Zoologie, Liège, Belgium), F. Meunier (MNHN, Paris, France) for their advice, D. Baron (Brest, France) for editing our English first text, Prof. E. Schokaert (Limburgs Universitair Centrum, Diepenbeek, Belgium) and two anonymous reviewer who greatly improved the quality of this article. FW is a researcher at the Fonds pour la Recherche dans l'Industrie et l'Agriculture.

REFERENCES

AMAOKA, K. (1969) – Studies on the sinistral flounders found in the waters around Japan.- Taxonomy, anatomy and phylogeny. *J. Shimonosoki Univ. Fish.*, **18**: 65-340.

BARRINGTON, E.J.W. (1937) – The structure and development of the tail in the plaice (*Pleuronectes platessa*) and the cod (*Gadus morrhua*). *Quart. J. Microsc. Sci.*, **79**: 447-469.

BERG, L.S. (1941) – *Eobothus vialovi* n. sp. An Eocene flatfish from the western Than-Shan. – *Bull. Acad. Sci. URSS, cl. Sci. Biol.*, **3**: 480-485.

CHABANAUD, P. (1937) – L'extension prorse périphérique, la contraction axiale post-hypophysaire et l'anisoconie rhachidienne des téléostéens dyssymétriques. *Bull. Soc. zool. Fr.*, **62**: 368-385.

CHANET, B. & O. SCHULTZ (1994) – Pleuronectiform fishes from the Upper Badenian (Middle Miocene) of St. Margarethen (Austria). *Ann. Naturhist. Mus. Wien*, **96A**: 95-115.

CHAPLEAU, F. (1993) – Pleuronectiform relationships: a cladistic reassessment. *Bull. Mar. Sci.*, **52** (1): 516-540.

CHAPLEAU, F. & A. KEAST (1988) – A phylogenetic reassessment of monophyletic status of the family Soleidae, with comments on the suborder Soleoidei (Pisces, Pleuronectiforms*). Can. J. Zool.*, **66**: 2797-2810.

COLE, F.J. & J. JOHNSTONE (1902) – *Pleuronectes*: the Plaice. *Trans. Liverpool Mar. Biol. Soc.*, **8**: 145-396.

FABRE-DOMERGUE E. & E. BIETRIX (1905) – Développement de la sole (*Solea vulgaris*). Introduction à l'étude de la pisciculture marine. Vuibert et Nony, Paris 247 pp.

FUTCH, C.R. (1977) – Larvae of *Trichopsetta ventralis* (Pisces: Bothidae), with comments on intergeneric realtionships within the Bothidae. *Bull. Mar. Sci.*, **27** (4): 740-757.

HEAP, S.P. & J.P. THORPE (1987) – A preliminary study of comparative growth rates in O. group malpigmented and normally pigmented turbot, *Scophthalmus maximus* (L.), and turbot-brill hybrids *S. maximus, S. rhombus* (L.). *Aquaculture*, **60**: 251-264.

HENSLEY, D.A. (1977) – Larval development of *Engyophrys senta* (Bothidae), with comments on intermuscular bones in flatfishes. – *Bull. Mar. Sci.*, **27**: 681-703.

HENSLEY, D.A. & E.H. AHLSTROM (1984) –Pleuronectiforms: Relationships. In: Ontogeny and Systematics of Fishes (Moser H. G., W. J. Richards, A. W. Kendall, M. P. Fahay, S. L. Richardson and D. M. Cohen, eds.). *Sp. Pub., Am. Soc. Ichth. Herp.*, **1**: 670-687.

HOUDE, E.D. (1971) – Developmental abnormalities of the flatfish *Achirus lineatus* reared in the laboratory. *U. S. Fish. Bull.*, **69**: 537-544.

LAGARDÈRE, F., M. BOULHIC & T. BÜRGIN (1993) – Anomalies in the cephalic area laboratory-reared larvae and juveniles of the common sole, *Solea solea*: oral jaw apparatus, dermal papillae and pigmentation. *Env. biol. fish.*, **36**: 35-46.

MONOD, T. (1968) –Le complexe urophore des poissons téléostéens. *Mém. Inst. Afr. N.*, **81**: 1-705.

MUNROE, T.A. (1996) – First record of reversal in *Symphurus vanmelleae* (Pleuronectiformes: Cynoglossidae), a deep-water tongue fish from the tropical eastern Atlantic. *Cybium,* **20(1)**: 47-53.

ROSEN, D.E. (1973) – Interrelationships of higher euteleostean fishes. In Interrelationships of fishes, P.H. Greenwood, R.S. Lies and C. Patterson, eds. *Zool. J. Linnean Soc.*, suppl. 1, **53**: 397-536.

SAKAMOTO, K. (1984) – Interrelationships of the family Pleuronectidae (Pisces: Pleuronectiformes). *Mem. Fac. Fish. Hokkaïdo University*, **31**: 95-215.

TAYLOR, W.R. & G.C. VAN DYKE (1985) – Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium,* **9**: 107-121.