Histological and cytological studies on the developing thymus of sharpsnout seabream, *Diplodus puntazzo*

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**ABSTRACT**

The structure of the developing thymus of the marine teleost, *Diplodus puntazzo*, was studied by light and transmission electron microscopy. The first anlage of the thymus developed by d 20 postfertilisation (p.f.) as a group of undifferentiated cells dorsal to the epithelium of the branchial chamber. The organ increased significantly in size around d 51–66 p.f. and differentiation of cortex and medulla occurred concomitantly. On the basis of their localisation, 4 main types of epithelial cell were distinguished: (1) limiting, adjacent to the connective capsule; (2) medullary and cortical reticular cells; (3) nurse cells, located in the corticomedullary boundary; (4) Hassall-like corpuscles. The majority of medium to large blast-like lymphoid cells were localised in the medulla, while small lymphocytes were housed in the cortical region. These morphological features were maintained at later stages. However, in juveniles in the medulla we observed reticular epithelial cells with cysts and rare Hassall-like corpuscles. The study was designed to obtain more information concerning the histology of the developing thymus of sharpsnout seabream and give a concise description of the differentiation of epithelial cells and lymphoid cells in the thymic parenchyma.

**Key words:** Teleost fishes; thymic development.

**INTRODUCTION**

The major function of the thymus in vertebrates, such as birds and mammals, is to provide the appropriate microenvironment within which cells of the T lineage can develop, proliferate, mature, generate their antigen receptor repertoire and leave the thymus to enter the pool of recirculating lymphocytes which help to protect the animal against pathogens (Ritter & Crispe, 1992). Teleost fishes show basic aspects of the immune system of other vertebrates such as lymphoid tissues (thymus, head kidney, spleen and mucosa-associated lymphoid tissue) (reviewed by van Muiswinkel, 1995). The thymus of fish, as in all vertebrates, probably plays an important role in the development of a functional immune system as was demonstrated from early thymectomy experiments (Salienidi, 1973; Nakanishi, 1986). As in all jawed vertebrates, fish thymus is composed mainly of Ig-negative lymphoid cells (Scapigliati et al. 1995) within a network of reticular epithelial cells, generally organised into a cortex and medulla (for review, see Manning, 1994). Among the fish Ig-negative cells, putative T cells appear to be involved in cell-mediated immune reactions such as proliferation induced by T-cell mitogens (Sizemore et al. 1984), mixed leucocyte reactions (Miller et al. 1985) and allograft rejection (Botham & Manning, 1981).

The structure of the teleost thymus has been studied in several fish species, but there is considerable controversy as to the degree of heterogeneity of the thymic epithelial and nonepithelial components (Castillo et al. 1991; Pulsford et al. 1991; Zapata et al. 1996; Flano et al. 1996) and their functional properties (Gorgollo, 1983; Zapata & Cooper, 1990; Aviles-Trigueros & Quesada, 1995). A single type of epithelial cell forming the parenchymal framework was described in *Rutilus rutilus*, *Gobio gobio* and *Sicyases sanguineus* (Zapata, 1981; Gorgollón, 1983). Two types were described as ‘stellate’, forming the thymus...
Fig. 1. Thymus development in *Diplodus puntazzo*. (a) At d 20 p.f. the thymus anlage (arrow) is located in the dorsal-caudal portion of the gill chamber. Pappenheim staining. × 750. (b) On electron microscopy, the cells of the thymic anlage appear undifferentiated and show a large euchromatic nucleus, electron-dense cytoplasm with long cytoplasmic extension. Bar, 1 µm. Inset. Numerous ribosomes and large
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Materials and Methods

Larvae and juveniles of sharpsnout seabream (from d 5 postfertilisation (p.f.) to 1-y-old) were reared in 6 m³ (larvae) or 28 m³ (juveniles) fish tanks at 20 ± 3 °C, in natural day/night regimes, at the La Rosa fish farm, Grosseto, Italy, and sampled to study thymic development.

The fish were killed with an overdose of tricaine methasulphonate (1 mg/ml) and whole fish or dissected thymuses were fixed and embedded. Bouin’s or Wood’s fixatives (Wood, 1963) and paraffin embedding were utilised for histology. Serial 7 µm sections were stained with haematoxylin and eosin, May-Grünwald/Giemsa (Pappenheim method) or Mallory’s trichrome.

Cell measurements were obtained with a computer-assisted image analysis system [Leitz Aristoplan microscope, TK-1070E colour video camera (JVC, Japan) interfaced through TARGA 16 plus (AT&T) with a 486 PC, and Image ProPlus software package (Media Cybernetics, Silver Spring, MD, USA)].

For electron microscopy, a mixture of 2% glutaraldehyde, 1% osmium tetroxide and 1% potassium bichromate in 0.1 M sodium cacodylate buffer (van Diepen et al. 1991) was used to fix 1 mm³ specimens. After fixation, the specimens were dehydrated through a series of graded alcohols, embedded in Epon 812 resin (Fluka, Switzerland) and polymerised at 60 °C for 12 h. Ultrathin sections (70 nm) were prepared with a Reichert Ultracut microtome, stained with uranyl acetate and lead citrate and examined with a 1200 JEOL EX II electron microscope.

Results

Development of the thymus

The first indication of thymic development in Diplodus puntazzo was observed at d 20 p.f. (Fig. 1a). It appears as a paired anlage which extends 30–35 µm craniocaudally (fish length ~ 0.25 cm), on the dorsal-caudal portion of both gill chambers, above gill arch IV. The thymic anlage at this stage and later, at d 26 p.f. (66–77 µm length), is mainly constituted by groups of cells lying in intimate association with the pharyngeal epithelium, which separates the anlage from the external gill chamber. Electron microscopy (Fig. 1b), showed a large euchromatic nucleus with a prominent nucleolus, electron-dense cytoplasm with long cytoplasmic extensions, numerous ribosomes and large mitochondria with vesicular cristae. No lymphoid characteristics or epithelial characteristics, such as intermediate filaments, were observed. A few neutrophil-like granulocytes were localised in the parenchyma (data not shown).

At d 51 p.f. the thymus anlage measured 182–186 µm in length (~ 1 cm fish length) and it

mitochondria fill the cytoplasm. Bar, 200 nm. (c) At d 66 p.f. the thymus protrudes into the branchial chamber and is separated from the external environment by the pharyngeal epithelium (arrows). Medullary (m) and cortical (c) regions by the different density of lymphoid elements in the parenchyma can be distinguished (separation indicated by superimposed lines). Semithin section, toluidine blue stain, × 325. (d) At d 86 p.f. the thymus medulla (m) is localised from the subcapsular zone to the central part of the organ (separation indicated by superimposed lines). c, Cortex; b, gill chamber. Pappenheim staining, × 140. (e) At d 91 p.f., connective trabeculae (arrow) penetrate into the thymus and incompletely divide the organ in lobes. Medullary region, × 275.
The thymus of juveniles is subdivided in medullary (M), corticomedullary (arrows) and cortical (C) portions. Pappenheim staining, ×75. (b) Two Hassall-like corpuscles (arrows) situated in the medullary zone in a juvenile thymus. ×700. (c) A rare myoid cell (arrow) is localised in the medullary area of juvenile thymus. ×800. (d) TEM of thymus at d51–66 p.f. showing limiting epithelial cells of perivascular type (asterisk) surrounding the endothelium of a vessel (E). Arrows, basal lamina. Bar, 1 μm. (e) Limiting epithelial cells (L) of subcapsular type in the thymus of a larva, lying on basal lamina (arrows) and connecting with medullary reticuloepithelial cells (R). Bar, 2 μm.

appeared enriched by lymphoid cells. The outer region of the thymus showed small lymphocytes (3 ± 1.2 μm in diameter, n = 100) occupying the parenchyma, whereas the inner portion possessed lymphocytes of larger size (3.96 ± 1.3 μm, n = 100). In the inner region of the thymus connective tissue capsule delimited the thymus in combination with a layer of limiting epithelial cells (LEC) which was in intimate association with thymic cells. Small blood vessels were present in the outer region of the thymus. Macro-
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phages were occasionally observed scattered in the parenchyma.

At d 66 p.f. the thymus was 3 times larger (780–786 µm in length, fish length 1.04–1.07 cm) than in the previous stage and thymus extended into the gill chamber. It was covered on its external aspect by branchial epithelium. The compartmentalisation of thymic parenchyma into the inner (medulla) and outer (cortex) regions became recognisable by the differing density of lymphoid elements (mean lymphocyte diameter 2.8±0.6 µm cortex, 4.44±1.0 µm medulla, n=100) (Fig. 1c).

At d 86 p.f. the thymus measured 820–1176 µm in length (fish length ~2 cm) and protrusion into the gill chamber (Fig. 1d). Numerous epithelial cells and lymphocytes were present in the thymic parenchyma (Fig. 1d) and numerous small lymphocytes filled in the cortex (3±0.6 µm in diameter, n=100), whereas infrequent epithelial cells were evident. Occasionally, apoptotic-like cells were observed in this zone. Lymphocytes of larger size (4±0.6 mm, n=100) were observed in the medulla.

At d 91 p.f. the thymus measured 1134–1536 µm in length (fish length ~2.50 cm). Connective tissue trabeculae penetrated into the thymus and subdivided the organ incompletely into lobes (Fig. 1c). At this stage the medullary region was more evident because of the presence of large epithelial cells. In the medulla the lymphocytes appeared larger (mean lymphocyte diameter 4.3±0.7 µm, n=100) with respect to the small cortical lymphocytes (mean cellular diameter 2.69±0.11 µm, n=100) that were tightly packed close to the epithelium of the branchial chamber.

In juveniles (8 mo–1 y.p.f., fish size 15–20 cm) and in adults (2 y sexually mature, fish length 25–30 cm) the thymus measured ~1 cm and was divided into 3 lobes which, in turn, were subdivided into a medulla (mean lymphocyte diameter 4±0.5 µm, n=100), corticomedullary junction (mean lymphocyte diameter 3.3±0.72 µm, n=100) and cortex (mean lymphocyte diameter: 3±0.11 µm, n=100) (Fig. 2a). Numerous lymphocytes filled the parenchyma (where they were intermingled with large epithelial cells. Hassall-like corpuscles (Fig. 2b) and myoid cells (Fig. 2c) were occasionally observed in the medullary zone.

**Development of thymic epithelial cells**

TEM analysis revealed a heterogeneity of epithelial components in the thymus. In order to unify the terminology used in mammals and birds (van de Wijngaert et al. 1984; Romano et al. 1996), D. puntazzo epithelial cells were named in terms of similarity in morphology and localisation. We distinguished in the juvenile thymus of sharpsnout seabream, 4 different types of epithelial cells: (1) limiting cells (LECs), (2) cortical and medullary reticular epithelial cells (c-RECs and m-RECs, (3)) nurse-like cells (NLCs), and (4) Hassall-like corpuscles (HLCs).

The differentiation of epitheliocytes in thymus was first observed around d 50 p.f., when limiting cells (LECs) became localised in subcapsular and perivascular areas (Fig. 2d,e). Characteristically, these cells displayed a basal lamina which separated them from the connective tissue. LECs showed an ovoid euchromatic nucleus with condensed chromatin close to the nuclear membrane. A variably electron-dense cytoplasm showed well developed smooth and rough endoplasmic reticulum, small round mitochondria, some vesicles (~200 nm in diameter) with a floccular content, intermediate filaments and numerous cytoplasmic interdigitations with adjacent LECs. From d 81 p.f. onwards, when the first connective tissue septa were established, another type of LEC was observed, the peritrabecular epithelial cell. Peritrabecular cells, together with subcapsular limiting cells, separated the thymus from the connective tissue and the external environment. Perivascular limiting cells were localised around blood vessels separating the endothelium from the thymic parenchyma (Fig. 2d).

The outer portion of the thymus (cortex) was not demarcated by LECs during ontogenesis or in the adult. Thus the thymic parenchyma was in direct contact with the pharyngeal epithelium (PE). An extensive infiltration with lymphoid elements is seen in the PE and no junctions were observed between thymic epithelial and PE cells. In the thymus of larvae from d 51 p.f. onwards cortical reticular-epithelial cells (c-RECs) were observed (Fig. 3a). They were characterised by electron-lucent cytoplasm, irregular shape with long cytoplasmic processes connected to each other by demosomes to form a reticular network. A large euchromatic nucleus (5.5–6 µm o.d.) with one or more nucleoli was often located eccentrically. The cytoplasm showed small elongated mitochondria, Golgi apparatus, rough endoplasmic reticulum, intermediate filaments, vesicles and numerous ribosomes. The vesicles were variable in diameter (0.4–1.7 µm o.d.) and had a floccular or, occasionally, floccular/granular content. In adults the thymus displayed a more compact parenchyma than in larvae and the RECs were situated closer to lymphocytes (Fig. 3b). m-RECs, first appeared at ~ d 51–66 p.f.
Fig. 3. (a) Cortical reticular-epithelial cell (E) in the thymus of a larva characterised by an irregular shape with long cytoplasmic processes and a large euchromatic nucleus. The vesicles (arrows) show floccular content or floccular/granular content. Bar, 2 µm. (b) The parenchyma in juveniles appears more condensed than in larvae and the cortical reticular-epithelial cells (E) are more closely opposed to lymphocytes. Arrows, vesicles. Bar, 2 µm.
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(69x45) thymus development (Abelli et al. 1996), and the first M
Manning, 1981) it contains lymphocytes only after develop (Grace & Manning, 1980; Botham &
fish species the head kidney is the first organ to
differences occur between species (reviewed by Tatner,
inhomogeneous content (from 0.6
to 4 µm diameter) (Fig. 4b). The cyst was always
located at one pole of the cell surrounded by
intermediate filaments and was constituted by
in-vaginations of smooth reticulum, which formed
vesicles and indentations. In the medulla, HLCs
(~ 40 µm in diameter) which were observed only in
juveniles (from 1-y-old onwards) (Fig. 2c). Numerous
flattened epithelial cells were rich in intermediate
filaments. There was cellular debris in the centre of the
corpuscles.

From the same age (i.e. d 51 p.f.), at the medullary-
cortical boundary, it was observed that the nurse-like
cells (NLC) (Fig. 5) contained many small lymphocytes. The membrane of the NLC appeared to
completely surround the enclosed lymphocytes. The
nucleus appeared irregular with a prominent nu-
cleolus. The cytoplasm displayed free ribosomes,
numerous small mitochondria, rough and smooth
endoplasmic reticulum, some small lysosome-like
vesicles and intermediate filaments.

DISCUSSION

In recent years some histological descriptions of the
ontogenetic development of the lymphoid organs
have been made for teleostean fishes, although
differences occur between species (reviewed by Tatner,
1996; Zapata et al. 1996). The fish thymus, which may
be considered a key organ of the immune system,
seems to be the major location for T cells (Romano et
al. 1997a). Likewise, the fish thymus appears to be a
primary lymphoid organ for T cells as was shown in
sea bass (Abelli et al. 1996) and carp (Romano et al.
1997b). The ontogeny of the thymus of sharpsnout seabream, as in other fish species (Abelli et al. 1994;
Zapata & Cooper, 1990) developed from the phar-
yngeal pouch as groups of undifferentiated cells.
Then, after few days the thymus acquired an obvious
lymphoid appearance. Although in the majority of
fish species the head kidney is the first organ to
develop (Grace & Manning, 1980; Botham &
Manning, 1981) it contains lymphocytes only after
thymus development (Abelli et al. 1996), and the first
T cells are seen before the appearance of B cells during
ontogeny (Romano et al. 1997b). It remains to be
elucidated whether the first lymphoid organ originates
from lymphoblasts in situ or from foci of early
haematopoietic stem cells.

Differences in thymus histology and development
among fish species seem to be related to 3 points. The
first concerns the extension and localisation of cortical
and medullary regions, the others to the different
types of epithelial cell (Chilmonczyk, 1992) and the
presence/absence of LECs to delimit the gland from
the pharyngeal epithelium (Zapata et al. 1996).
Although the presence of a thymic epithelial network
in a variety of fish species is well established (Zapata,
1981; Castillo et al. 1990, 1991; Josefsson & Tatner,
1993; Abelli et al. 1994, 1996), a uniform classification
of thymic epithelial elements and their possible
functional role(s) is still lacking. Epithelial sub-
populations in fish thymus were demonstrated by the
presence of tonofilaments in the cytoplasm of sea bass
and carp by a polyclonal antikeratin antibody
(Froehly & Deshaux, 1986) and by histoenzymatic
reactions (Castillo et al. 1990). Some authors have
suggested the existence of 7 types of epithelial cell,
classified on the basis of histochemical and his-
tological staining (cf. Zapata et al. 1996). However,
electron microscopy revealed the existence of only 2
types as dark or pale epithelial cells (in Solea solea;
Pulsford et al. 1991) or 3 types of cell including
limiting cells (Castillo et al. 1991). Our histological
and ultrastructural studies on the thymus of D.
puntazzo confirm the heterogeneity of the epithelial
subpopulations previously observed in fish
(Gorgollón, 1983; Castillo et al. 1990). The mor-
phology of the epithelial cells was related to different
cell types on the basis of their localisation and
cytological characteristics (reviewed by Zapata, 1996).
Thus we identified (1) limiting epithelial cells (LEC)
located in the subcapsular, perivascular and peri-
trabecular zones; (2) reticular epithelial cells (REC)
situated in medullary and cortical zone; (3) cystic
cells, located in the medullary compartment; (4)
thymic nurse-like cell (TNLC) situated in the cortico-
medullary boundary; and (5) medullary Hassall’s
corpuscles. Only in adult specimens did we observe
rare Hassall’s corpuscles formed apparently by kera-
tinised medullary RECs.

The differentiation of epithelial components in D.
puntazzo occurred at ~ d 40–66 p.f.; at the same time
the thymus increased 3 times with respect to the
growth in length of the larva (unpublished data). Similar observations have been made on the thymus
of ciclids, where an increase in vascularisation and in
Fig. 4. (a) Cluster of medullary reticular epithelial cells (MR) in d 86 p.f. larva. There are fewer cytoplasmic vesicles than in cortical RECs. Bar, 2 µm. (b) A medullary reticular epithelial cell shows a cyst formation (asterisk). The cyst, located at one pole of the cell, is constituted by invaginations of smooth reticulum. V, vesicles. Bar, 1 µm.
Fig. 5. Corticomedullary boundary at d 51 p.f. showing a nurse-like cell (NC) surrounding lymphocytes (L) by cytoplasmic processes (arrows). Bar, 1 µm.

the presence of stromal components may explain the nonlinear relationship increment (Fishelson, 1995). For the embryological origin(s) of the epithelial cell populations of the thymus, Castillo et al. (1991) suggested that they may arise from the epithelium of the digestive tract or from the inner sides of the operculum, with an origin from endodermal and ectodermal elements. A similar double origin occurs for instance in mammals and chickens (Kendall, 1991). The precise origin(s) of the epithelial cells remains an open question. They play an important role in creating the milieu within which T lymphocytes can differentiate, as occurs in mammals (Ritter & Crispe, 1992) and birds (Kendall, 1991). For instance, it is still not clear if fish epitheliocytes produce thymic hormones or if they undergo a ‘transformation’ induced by hormonal factors originating in situ or from other tissues (Frohely & Deschaux, 1986). The first epithelial components to develop in *D. puntazzo* seem to be the LECs. Their morphology was similar to that described in other fish species (Castillo et al. 1990; Zapata & Cooper, 1990; Abelli et al. 1994). LECs in rainbow trout exhibited a slightly different enzymatic pattern in comparison with other epithelial components (Castillo et al. 1990); this observation could indicate some special metabolic activity. In the thymus of *D. puntazzo*, some perivascular spaces are expanded throughout the parenchyma and are iso-
lated from it by a perivascular LEC. The evidence of an epithelial thymus-blood barrier has been demonstrated in mammals (reviewed by Ritter & Crispe, 1992) and suggested by studies in fish (Tatner & Manning, 1982; Chylmonczyk, 1992; reviewed by Zapata, 1996) as a necessary isolation of the thymic microenvironment during the selection of thymocytes. Moreover, the thymus of many fish species, including *D. puntazzo*, is delineated by a connective capsule that never completely surrounds the organ. In fact, in larvae and often in juveniles the outer zone of the thymus is in direct contact with the pharyngeal epithelium and, consequently, with the external environment. Although not much is known about antigen-processing in pharyngeal epithelium, indications are available for antigen-uptake in channel catfish (Lobb & Clem, 1987), and it can be hypothesised that a similar process close to the thymus could lead to the maturation of thymocytes. Recently, the permeability of the pharyngeal epithelium of rainbow trout fry and adults was studied (Castillo et al. 1998). The use of trout fry of different ages immersed in a 0.5% solution of ferritin showed that the gills but not the thymus are involved in antigen trapping during development. Surprisingly, only when very immature (4-d-old) fry were exposed to ferritin for prolonged periods, did it appear in the thymic parenchyma, but not inside the epitheliocytes, suggesting that the ferritin particles passively cross the pharyngeal epithelium (Castillo et al. 1998).

The RECs were organised in the parenchyma of *D. puntazzo* to form a wide network separating 3 compartments: medulla, cortex and corticomедullary boundary. The presence of the 3 compartments observed from d 66 p.f. onwards differed from the situation in other fish species where there was only a separation of the parenchyma into outer and inner zones (Gorgollón, 1983; reviewed by Zapata et al. 1996), or, simply, into medulla and cortex (reviewed by Chlmonczyk, 1992). Questions can be raised about the function/s of these cells in the thymus parenchyma. An indication may come from a previous study in carp and sea bass thymus where the authors showed the presence of hormonal factors in RECs (Frohely & Deschaux, 1986). These hormonal substances seem to be necessary in mammals in order to stabilise the thymic microenvironment in which the lymphocytes develop and differentiate (c.f. Ritter & Crispe, 1992).

In the cortical-medullary boundary zone of the *D. puntazzo* thymus numerous epithelial cells formed thymic nurse-like cells (TNLCs) similar to those previous described in situ as a lymphocyte-epithelial cell association in other fish species with cortical or corticomедullary localisation (Pulsford et al. 1991; Flaño et al. 1995). In mammals thymic nurse cells (TNC) have a cortical localisation and are characterised by the presence of numerous enclosed lymphocytes (c.f. Ritter & Crispe, 1992). Although in vitro these lymphocytes are completely surrounded by TNC membranes, the presence of small gaps in the membranous system permits CD4+/CD8+ thymocytes to enter and exit from these structures (Ritter et al. 1981). Thus in mammals, TNCs represent likely candidates for the positive selection of thymocytes. In fish, the lack of markers to assess the presence of specific CD complexes or class I and class II major histocompatibility complexes prevents the determination of the role of these cells in the maturation of thymocytes.

The m-RECs with cysts in *D. puntazzo* displayed small intracytoplasmic cavities filled with numerous filaments which accumulated close to the cysts, as described in cling fishes (Gorgollón, 1983). Their degeneration was related to the formation of Hassall’s corpuscles (Gorgollón, 1983; Zapata & Cooper, 1990). Hassall’s bodies are generally lacking in fish thymus (Zapata et al. 1996), although they have been reported in some fish species (Good et al. 1966; Gorgollón, 1983; Fishelson, 1995). Hassall’s corpuscles were observed in the thymus of cyclid fishes from larval stages to ageing animals as formed by inner and outer rings of epitheliocytes, with intermingled macrophages and central cellular debris (Fishelson, 1995). In *D. puntazzo* we observed rare medullary Hassall’s corpuscles with the same characteristics as those described by Fishelson (1995). However, their presence seems to be related to growth because we observed Hassall’s corpuscles only from juvenile stages onwards.

A nonepithelial stromal cell, the myoid cell, was occasionally observed in the thymus of *D. puntazzo*. Myoid cells have been described in a few teleostean species as ciclids (Gorgollón, 1983) and in higher vertebrates as round or oval cells with a cytoplasm containing myofilaments organised in sarcomere-like structures around the nucleus. Although their description is an agreement with our light microscopy observations, further studies are needed to clarify their role in the thymic microenvironment. The functional significance of this type of cell is still uncertain even in higher vertebrates (reviewed by Zapata, 1996).

Thymocytes were evenly distributed in the parenchyma intermingled with medullary and cortical RECs. However, differences in thymocytes morph-
ology and size were evident between thymic regions. For example, medullary thymocytes were larger than cortical thymocytes. Moreover, medullary thymocytes displayed a large nucleus and more cytoplasm as in sea bass (Abelli et al. 1994, 1996). Similar apoptotic-like cells were found in the cortex of the developing thymus of D. puntazzo again as observed in sea bass (Abelli et al. 1998). From these morphological observations it can be suggested that the different regions of the fish thymus could play similar roles in the maturation of thymocytes as in mammals and birds.

In conclusion, this study is a first step towards knowledge of the basic morphology of the microenvironment of the thymus of the teleost fish D. puntazzo where the peculiar presence of a well developed corticomedullary boundary could be interesting for future studies on lymphocyte selection mechanisms. Further studies are required to explore the organisation of other lymphoid organs and to analyse the function of the thymic microenvironment.

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