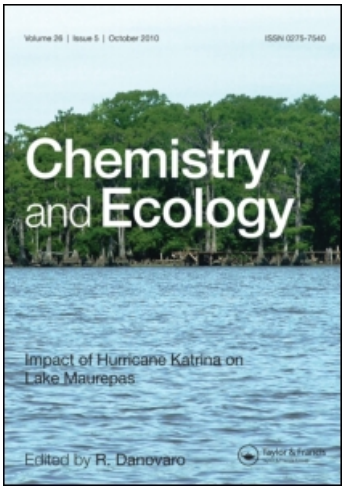

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ADAPTATION OF FISH LYMPHOMYELOID ORGANS TO POLAR WATER

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Lymphomyeloid organs of three common Antarctic fish species, *Trematomus bernacchii*, *Trematomus nicolai* and *Chionodraco hamatus*, were analysed.

Contrary to species living in temperate sea water, the thymus of polar fishes were flattened, incompletely lobated and scarcely distinguishable by normal histology into cortical and medullary regions. Functional regionalisation, however, was suggested by differences in the sizes of thymocytes from the outer to the inner thymus zone. Another particularity was observed in the thymus of *Trematomus* species: next to lymphocytes, numerous erythroid cells circulated and differentiated in the parenchyma. Only two main types of epithelial cells could be found by cytological analysis: (i) limiting cells that surround the haematopoietic tissue and (ii) reticular cells that constitute the frame where the lymphoid and erythroid cells can proliferate and differentiate. The reticular cells could not be distinguished in cortical and medullary subtypes as observed in temperate-water fish. Numerous Hassall's corpuscles, probably with a scavenging role, were also observed in the thymus.

The head kidney housed haematopoietic tissue, lacked any excretory tubules, and had a huge blood supply, characteristic of polar fish species. It appeared mainly lymphopoietic in *C. hamatus* but contemporary erythropoietic and lymphopoietic in *Trematomus* species. The ultrastructural analysis revealed the presence of both reticular and limiting epithelial cells. Reticular epithelial cells (REC) characteristically showed numerous vesicles with a granular content and cell debris. Numerous lymphoblasts, lymphocytes and plasma cells were observed among the REC. Erythropoiesis occurred in all polar species analysed, but in *C. hamatus* the erythroblasts did not differentiate because they had a fast senescence.

The spleen appeared mainly erythropoietic, with scarcely developed areas of white pulp, in *Trematomus* species; the erythropoiesis was scarcely evident in *C. hamatus*. Small vascular ellipsoids showed numerous melano-macrophages in *Trematomus*, while large haematopoietic areas were organised around the capillaries in *C. hamatus*. Utrastructural analysis revealed, in all species examined, two main types of epithelial cells: reticular, close to the ellipsoids, and limiting-subcapsular, which surround the organ. A large blood supply and extended capillary frame were also observed in polar species. The possible adaptation of lymphoid organs to the low temperatures of polar water is discussed.

Keywords: Thymus; Head kidney; Spleen; Antarctic fish; Teleost

1 INTRODUCTION

In the Antarctic region, the Notothenioidea comprise 90–100 known species that have maintained a genetic isolation because of the physical barrier of the 'Antarctic water circulation' (Eastman and De Vries, 1996). Throughout evolution, these polar Notothenioidea had a great

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adaptive radiation within two main families: Nototheniidae and Channichtyidae. These fishes have lived toward extreme conditions and have developed special adaptations of physiology and tissue/organ anatomy. It is well known that polar fishes possess high concentration of ions in body fluids (Eastman and De Vries, 1996) and anti-freeze circulating substances, produced in the liver (Chen *et al.*, 1997) by AFGP gene expression (Logsdon and Doolittle, 1997). Moreover, polar fishes have a low heart rate (O'Brien *et al.*, 2000) and, in general, a low metabolism (Focardi *et al.*, 1995) and special body enzymes adapted to work at low temperature (Goldspink, 1995). In the 'ice-fish' as *Chionodraco hamatus* (Channichtyidae) the absence of haemoglobin and circulating erythrocytes allows blood circulation even at very low pressure (Ito *et al.*, 1995; Bargelloni *et al.*, 1998).

In the process of adaptation it appears likely that the immune system also underwent important changes, such as the capacity to elicit responses at low temperature, but only scarce information is available on the structural organisation and function of lymphoid organs in polar fishes (O'Neill, 1989; Romano *et al.*, 1997a). The general organisation of the fish immune system involves lymphoid organs such as thymus, head and trunk kidney, spleen and MALT (skin, gills and gut) (Romano, 1998). Recent studies of our group on polar fish species indicated some difference of lymphomyeloid tissues compared with warm/temperate species (Romano *et al.*, 1997a, 2000, 2002). Antarctic fishes have less developed higher vascularisation of lymphomyeloid organs and a peculiar distribution of lymphoid and erythroid areas. Moreover, the total serum immunoglobulin titre seems to be significantly higher (Scapigliati *et al.*, 1997) and the structure of immunoglobulins appears mutated in variable regions, in double sulphur links and in length of chains constant region. These differences greatly contribute to acquired molecular flexibility and capacity to work efficiently in high-viscosity fluids (Coscia *et al.*, 2000).

Since Antarctic teleosts appear to be good candidates for the study of an immune system organised to work at low temperature, this study is focused on a general comprehensive histological and cytological view of tissue structure of the main lymphomyeloid organs (thymus, head kidney (HK) and spleen) of some common Notothenioidea species (*Trematomus bernacchii, Trematomus nicolai* and *C. hamatus*). Morphological features are also compared with known structures of temperate fish to understand specialisations of polar species better.

2 MATERIALS AND METHODS

2.1 Animals

In the species examined, the number of specimens was equally divided for histology and electron microscopy studies.

The Osteichthyes species *T. nicolai* (Boulanger, 1920, Notothenidae, $N = 6$, 16 cm in length, ~4–6 years old, calculated on the basis of scale rings), *T. bernacchii* (Boulanger, 1920, Notothenidae, $N = 6$, 25 cm in length, ~4–5 years old, calculated on the basis of scale rings) and *C. hamatus* (Lönnberg, 1905, Channichtyidae, $N = 8$, 30 cm in length, ~3–4 years old, calculated on the basis of scale rings) were collected during the summer period (November/February) in the Antarctic continent, Terranova Bay coast (74°42′ S, 164°07′ E), in the first few meters under the water surface layer.

2.2 Histology

The thymus, HK and spleen of polar fish (at least three specimens for each species) were fixed *in loco* with Bouin fixative (for 7 h at 4 °C), then washed for 12 h with 70% alcohol. The

specimens were immersed again in 70% alcohol and sent to Italy. In our laboratory, the organs were dehydrated through a series of graded alcohols, cleared in xylene and embedded in paraffin.

Serial sections (7 μm thickness) were obtained with a Reichert-OME microtome. De-waxed sections were rehydrated and stained by Mallory's trichrome, May-Grünwald/Giemsa according to Pappenheim (Mazzi, 1977) or haematoxylin–eosin. Sections were mounted and examined with a Zeiss Axiophot microscope. Photographs were taken with a Zeiss camera loaded with film Kodak T-MAX 100 ISO or Agfa PAN F 50 ISO.

2.3 Electron Microscopy and Histology

The thymus, HK and spleen (at least three specimens per organ for each species) fragments of 1 mm^3 were fixed *in loco* for 1 h with a mixture of 2% glutaraldehyde, 1% osmium tetroxide and 1% potassium bichromate in 0.1 M sodium-cacodylate buffer. After fixation, the specimens were immersed in 70% alcohol and sent to our laboratory. The samples were dehydrated through a series of graded alcohol and embedded in EPON 812 resin (Fluka Chemie, Switzerland). Semithin sections (0.5 μm thickness) were used for histological observation after toluidine-blue staining. Ultrathin sections were prepared with a Reichert Ultracut microtome, stained with uranyl acetate and lead citrate and examined with a JEOL 1200 EX II transmission electron microscope.

2.4 Quantitative Analysis

Cell measurements from semithin sections 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)).

3 RESULTS

3.1 Thymus

In the Antarctic fish species examined, the thymus was found as a paired organ localized in the dorsal portion of both gill chambers. It had a flat shape and could be divided into a subcapsular region, where the inner portion of the organ was lined by a single layer of limiting epithelial cells, and an outer portion (peripharyngeal region), in close contact with the pharyngeal mucosa. The connective tissue partially penetrated from the subcapsular region into the thymic parenchyma and failed to completely lobulate the thymus in all species (Fig. 1(a)). The capillaries in the thymus parenchyma were surrounded by another type of limiting epithelial cells (perivascular-limiting epithelial cells, Fig. 1(b)) that were connected to the reticular epithelial cells (REC) to constitute the framework of the organ (Fig. 1(c)).

By electron microscopy analysis, the limiting epithelial cells possess thin and flat shape (Fig. 1(d)), while the REC appeared stellate-shaped (Fig. 1(e)). The REC have cytoplasmic processes filled by intermediate filaments and small vesicles. The nucleus of REC was irregular, with nucleolus.

Lymphoblasts and lymphocytes, packed among the REC cytoplasmic processes, distributed differently in the outer and inner regions of the thymus. Larger size lymphoblasts (cell diameter: 4.70 ± 0.60 μm in *T. bernacchii*; 4.58 ± 0.53 μm in *T. nicolai*; 4.22 ± 0.47 μm in *C. hamatus*) were mainly localized in the subcapsular/inner region; smaller size lymphocytes (Fig. 1(f)) (cell diameter: 2.70 ± 0.70 μm in *T. bernacchii*; 2.55 ± 0.40 μm in *T. nicolai*; 2.78 ± 0.43 μm in *C. hamatus*) were localized in the pharyngeal/outer region.

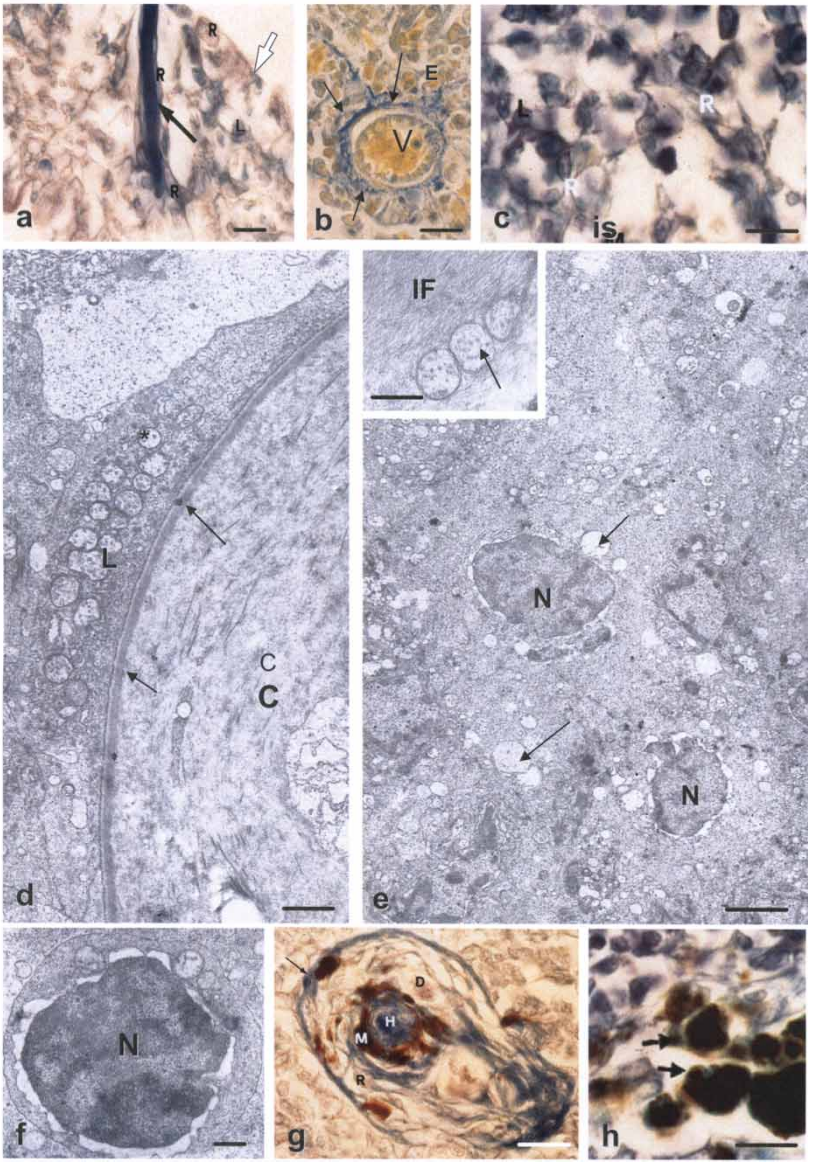

FIGURE 1 Histology and cytology of thymus in polar fish species. (a) Subcapsular limiting epithelial cells in *C. hamatus* (R, white arrow) surround the thymus in the inner portion. Epithelial layer (R) limiting the connectival trabeculae (black arrow), which comes from the capsule (that was removed to see the epithelial layer of the gland). Mallory's trichrome staining, (L) lymphocyte, bar: 8 µm. (b) Limiting epithelial cells (→) that surround the capillary (V) are connected with REC (*T. nicolai*). Mallory's trichrome staining, (E) erythrocytes, (L) lymphocytes, bar: 15 µm. (c) Reticular epithelial cells (R) form in the parenchyma a network where lymphocytes (L) can grow and differentiate (*C. hamatus*). IS, intercellular space, Mallory's trichrome staining, bar: 10 µm. (d) An electron micrograph shows a limiting-subcapsular epithelial cell (L). This cell appears thin and contains a thick basal lamina (→). In the cytoplasm numerous vesicles with floccular or granular content are shown (*T. bernacchii*). Bar: 1 µm. (e) Electron micrograph shows two REC (identifiable by nucleus, N). In the cytoplasm numerous vesicles (→) and intermediate filaments (IF, in the inset) are observable (*T. bernacchii*). Bar: 2 µm, bar in the inset: 500 nm. (f) Electron micrograph of a cortical lymphocyte (identifiable by nucleus, N) in *T. bernacchii*. Bar in the inset: 500 nm. (g) Close to the thymus external region the Hassall's-like corpuscles (H) are shown by light microscopy (*C. hamatus*). Numerous rings of epithelial cells (pale blue) surround the core of the corpuscle (H). Among the rings of epithelial cells, melano-macrophages (M) and cellular debris (D) can be observed. Mallory's trichrome staining, bar: 15 µm. (h) Light microscopy picture shows numerous melano-macrophage cells (→) that are grouped in the parenchyma of *Trematomus* spp. (*T. nicolai*). Pappenheim staining, bar: 10 µm.

In the outer region, numerous Hassall's-like bodies were observed in *T. nicolai*, less numerous, but larger in size, in *T. bernacchii* and *C. hamatus* (Fig. 1(g)). Melano-macrophages were scattered in the parenchyma, as small groups in *T. nicolai* (Fig. 1(h)) or isolated cells in *C. hamatus*.

3.2 Head Kidney

The kidney extended cranio-caudally as bilateral organ, in extra-peritoneal location. Distinct regions of the kidney can be distinguished: HK that lacked renal tubules but was filled with haematopoietic tissue (Fig. 2(a)) and opisthonephros, housing the excretory tubules. Evident

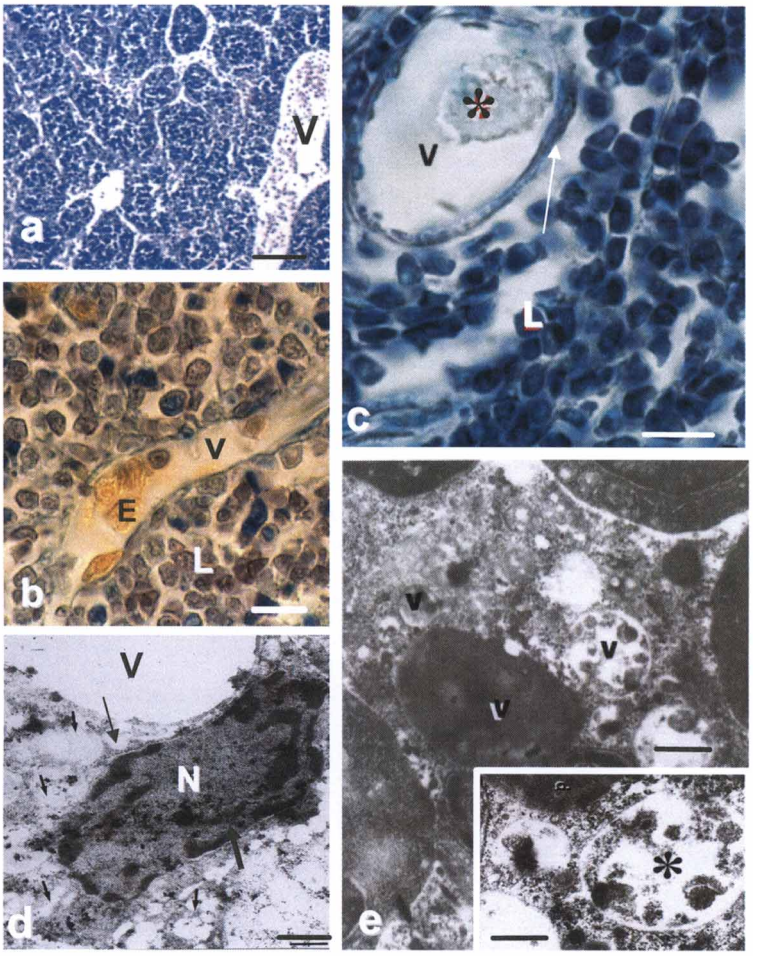

FIGURE 2 Histology and cytology of HK in polar fish species. (a) The haematopoietic HK of *C. hamatus* possesses a well-developed vascular system that divides the parenchyma into lobes. V, vessels. Pappenheim staining, bar: 70 µm. (b) The HK *T. bernacchi* also shows a vascular system widely distributed, L, lymphocyte; E, erythrocyte; V, vessel, bar: 10 µm. Mallory's trichrome staining. (c) *C. hamatus* light microscopy shows the perivascular epithelial cells (→) dividing the renal parenchyma from the endothelial cells of blood vessels (V). Pappenheim staining, (L) lymphocyte, (G) granulocyte, bar: 10 µm. (d) An electron micrograph shows the limiting-perivascular epithelial cell of *C. hamatus* (N, nucleus). The cell is provided of vesicles with different size (→). Capillary lumen, V. Bar: 1 µm. (e) An electron micrograph shows the REC of *T. bernacchii* containing numerous vesicles with different content (V). Bar: 1 µm. A magnification of a vesicle (∗) with floccular/granular content is shown in the inset, bar: 500 nm.

differences were observed among species as regard the quality of tissue: it was lymphopoietic in *C. hamatus* and haematopoietic (lymphopoietic and erythropoietic) in *T. nicolai* and *T. bernacchii* (Figs. 2(a) and (b)).

The renal parenchyma was surrounded by a capsule constituted by connective tissue and a single layer of flat epithelial cells (subcapsular/limiting epithelial cells). These possess a thick basal lamina close to the connective tissue. By electron microscopy analysis, these cells show a regular, heterochromatic nucleus and sparse cytoplasmic vesicles (diameter 30–60 nm) containing floccular material.

The parenchyma was completely divided into lobes by a developed vascular system, more extended in *C. hamatus* as compared with *Trematomus* species (Fig. 2(a)). In some large vessels, cells of the adrenal homologue were also observed (not shown). Numerous lymphoid cells were inside the lobes in *C. hamatus*, while in *Trematomus* spp. there were also granulocytes and erythrocytes (Fig. 2(b) and (c)). Around the numerous capillaries, limiting epithelial cells were observed by ultrastructural analysis (perivascular, Fig. 2(d)). These cells showed many analogies with the subcapsular epithelial type, but the cytoplasmic vesicles were more numerous and large (diameter 0.6–2 μm) and no evident basal lamina was observed. Inside the lobes, the

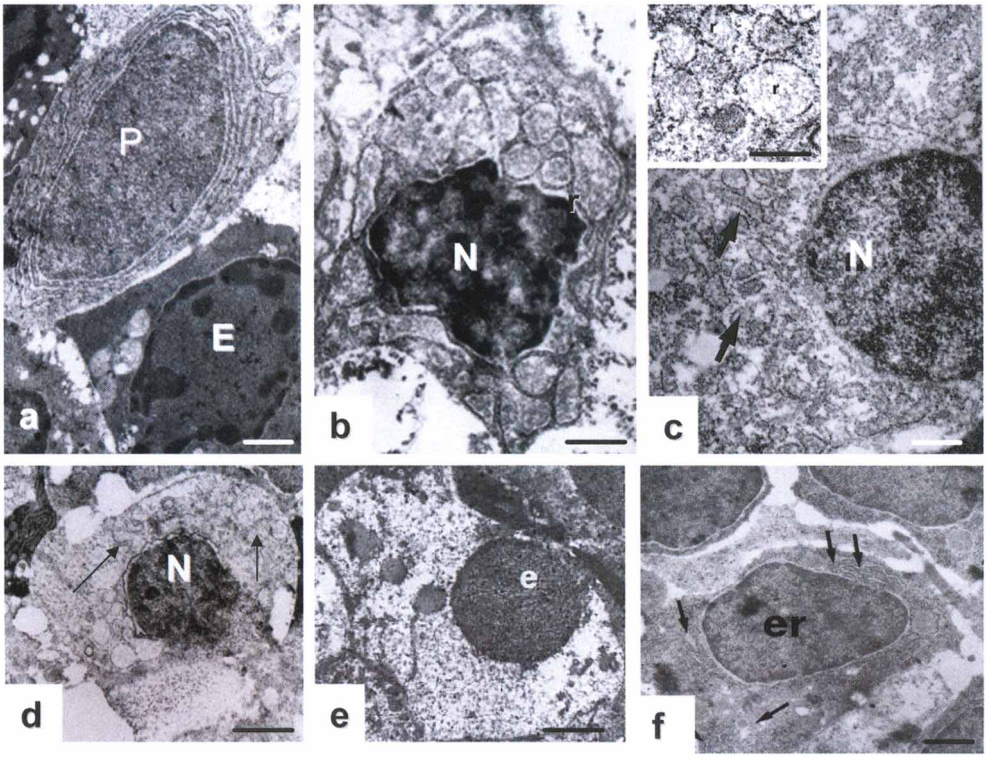

FIGURE 3 Cytology of HK erythrocytes in polar fish species. (a) An electron micrograph shows a plasma cell (P) and an immature erythrocyte in the HK of *C. hamatus*. Bar: 1 μm. (b) In a second step of maturation, the immature erythrocyte of *C. hamatus* shows a heterochromatic nucleus (N) with enlarged nuclear cisternae. In the cytoplasm the well-developed RER is filled by granular material (r), bar: 2 μm. (c) A successive step of erythrocyte maturation in *C. hamatus* shows small cisternae of RER (→). N, nucleus; bar: 500 nm. A magnification of RER (r) is shown in the inset, bar: 400 nm. (d) The erythrocyte of *C. hamatus* in a successive step of maturation: RER becomes degraded (→) like the nucleus (N). Bar: 4 μm. (e) *C. hamatus* electron micrograph of a mature-senescent erythrocyte (e) shows the dissolving nucleus and the destruction of cytoplasm organelles. Bar: 4 μm. (f) A micrograph of an immature erythrocyte of *T. bernacchii* (er) shows a well-developed RER and some vesicles (→) in the cytoplasm. Bar 3 μm.

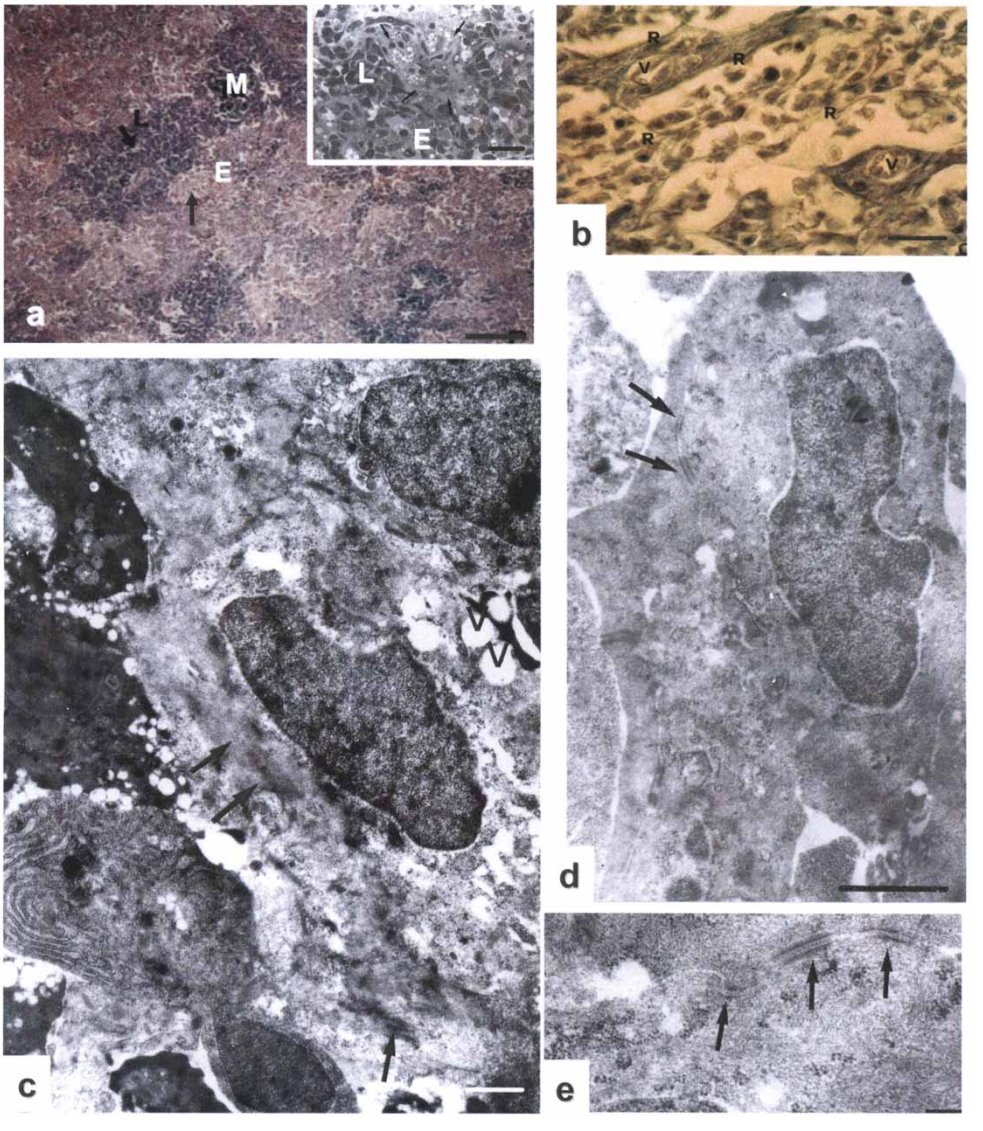

FIGURE 4 Histology and cytology of spleen in polar fish species. (a) Light microscopy picture shows the splenic parenchyma of *T. bernacchii* filled by small areas of lymphoid tissue (L) that can be distinguished from the abundant red pulp (E). The haematopoietic areas develop around the ellipsoids (→). M, melano-macrophage centre, Pappenheim staining, bar: 50 µm. In the inset, a semithin section of *T. nicolai* shows a group of REC (arrows) close to an ellipsoid. The lymphoid (L) and erythroid (E) elements are intermingled. Toluidine-blue staining, bar: 10 µm. (b) In the splenic parenchyma small areas of lymphoid tissue of *C. hamatus* (→) are localised around the vessel (V). R; REC, and V, capillaries. Pappenheim staining, bar: 20 µm. (c) An electronic micrograph shows the REC of stellate-shape type, with irregular nucleus and cytoplasm filled of intermediate filaments (→) and vesicles (V) (*C. hamatus*). Bar: 2 µm. (d) A transmission electronic micrograph shows the REC of round-shape type, with regular, euchromatic nucleus and cytoplasm enriched of medium size vesicles (*T. bernacchii*). Desmosomes (→), bar: 2 µm. (e) A micrograph shows a particular of desmosome junction (→) between REC. Bar: 200 nm.

REC connected with each other and with the limiting epithelial cells to form a loose stromal network. The renal REC of *T. bernacchii* were thinner, with long cytoplasmic extensions, and the cytoplasmic vesicles contained more heterogenous material: dense, granular or floccular (Fig. 2(e)). In *C. hamatus*, REC had cytoplasmic intermediate filaments and vesicles. These

latter had either granular/floccular content, with crystalline material disposed eccentrically to the major vesicle axis or cellular debris (like phagosomes, not shown).

The erythrocytes were similar in both species only in the immature stages (Figs. 3(e) and (f)). In fact, in the first stages of maturation they had developed rough endoplasmic reticulum (RER) and electron-dark cytoplasm (Fig. 3(a)). After that stage, in *Trematomus* species the erythrocytes showed different density of cytoplasm (Fig. 3(f)), corresponding to the haemoglobin concentration, as demonstrated also by histological staining (data not shown). In *C. hamatus*, different from *Trematomus* species, the erythrocytes did not reach mature stages but showed cytological features of degradation, such as: (a) the nucleus with dense chromatin, the cytoplasm less dark, filled with enlarged portions of RER with granular content (Fig. 3(b)); (b) the cisternae of RER small with sparse content (Fig. 3(c) and inset); (c) the cisternae of RER degraded (Fig. 3(d)) and (d) the erythrocyte degraded with a destruction of the nucleus and all cytoplasmic organelles (Fig. 3(e)).

3.3 Spleen

Anatomically, the spleen was localized in middle ventral position close to the first intestinal loop in all species. It was surrounded by connective tissue layers intermingled with epithelial cells (more numerous in *Trematomus* spp. compared with *C. hamatus*) that constituted a splenic capsule. The parenchyma was well distinguishable in large areas of red and white pulp in *T. bernacchii*, while these areas intermingled in *T. nicolai* (Fig. 4(a)). In *C. hamatus*, scarce erythroid cells stained pale blue (Pappenheim staining) due to lack of haemoglobin and were observed among the epithelial framework (Fig. 4(b)). The lymphoid cells formed large clusters around the ellipsoids.

In all the species, the splenic vascular network was highly developed and arterial blood capillaries were surrounded by a layer of perivascular epithelial cells with thickened endothelial cells encapsulated by connective tissue (ellipsoid). By electron microscopy analysis, the perivascular epithelial cells appeared similar to those described in other lymphoid organs. Perivascular cells of ellipsoids were joined by desmosomes with REC constituting the framework of the splenic parenchyma. By cytological analysis, REC showed two morphologies: (a) rounded shape (Fig. 4(d) and (e)), with regular, euchromatic nucleus and cytoplasm enriched with medium/large size vesicles (diameter range: 0.5–4 μm) and (b) stellate-shape (Fig. 4(c)), with irregular nucleus and cytoplasm filled with intermediate filaments and smaller vesicles (diameter range: 0.2–0.5 μm) – larger in *C. hamatus* (0.5–1 μm). The stellate-type of splenic REC, most common in *C. hamatus*, is generally associated to lymphoid cells. In *T. bernacchii* and *T. Nicolai*, type (b) REC formed clusters connected with large desmosomes (Fig. 4(a)-inset and (e)), while in *C. hamatus* they formed a more lapse framework (Fig. 4(b)).

4 DISCUSSION

The study of the immune system of polar fish can be an interesting field of research to understand how the immune system development and response to xenoantigens can occur at extreme, cold environmental temperature. This study represents a comprehensive outlook on histological and cytological organisation of the main lymphoid organs (thymus, HK and spleen) of the three Antarctic species, in order to compare the structural evolution of these organs between species of the families Nototheniidae (*T. bernacchii* and *T. nicolai*) and Channichtyidae (*C. hamatus*), generally considered as groups divided early throughout

evolution of polar fish. In addition, we compared this information with the available data for temperate-/warm-water fish species.

4.1 Thymus

The fish thymus plays an important role in the development of a functional immune system, as demonstrated in *Sebasticus marmoratus* by early thymectomy (Nakanishi, 1986). The thymus of temperate-water fish is mainly composed by cells of the T-lineage, lying within a network of REC, generally organized within a cortex and a medulla (Romano, 1998). Otherwise, the thymus of the *Chionodraco* and *Trematomus* species did not display clear cortex and medulla. However, the different size of thymocytes in the inner and outer thymic regions likely indicates a functional division of the gland as it occurs in temperate species. The lack of regionalisation in polar species may reflect the peculiar organisation of the epithelial framework where only an intermediate typology between cortical and medullary REC was observed. In temperate-water fishes, like sea bass, carp and sheephead sea bream, the thymus is organized into compartments housing different epithelial cell types, such as medullary- and cortical-reticular cells, nurse cells, limiting cells (subcapsular, perivascular or peritrabecular) and Hassall's-like corpuscles (Romano *et al.*, 1999a, b). In *Trematomus* species, especially in *T. nicolai*, the thymus seems to be involved in unconventional erythropoietic functions. The hypothesis can be advanced that the thymus, which retained a superficial position, thus being particularly exposed to freezing, could modify the vascular framework (and circulation of the anti-freeze fluid) with a simplified type of epithelial cells and the reduction of free spaces. Perivascular-limiting epithelial cells form an envelope around the capillary endothelium and likely constitute a blood barrier as in other vertebrate species (Ritter and Crispe, 1992) including fish (Romano, 1998). It is to be noticeable that in fish (as in mammals) the cortical–medullary border seems to be a key zone for the maturation of T-lymphocytes and is a zone particularly vascularized (Ritter and Crispe, 1992; Romano *et al.*, 1996; 1999b). Further studies by using specific markers (*i.e.* TcR$\alpha\beta$, CD8, CD4) on thymus of polar species, should be performed to understand where and how the thymocytes differentiate.

Hassall's-like corpuscles could also have a related role in the peculiar organisation of thymus in polar fishes. The Hassall's corpuscles, considered in mammals and birds sites of cell destruction (Parnham, 2000), are considerably large and well developed in polar fish species. In comparison with other species studied in temperate/warm water (Romano *et al.*, 1997a; 1999b), in polar fish these structures were numerous and enriched of melano-macrophages, cells implicated in scavenging and recycling materials (Zapata *et al.*, 1996). Thus, the abundance of these structures in the thymus could be related in scavenging thymocytes recognizing self-antigens and/or old erythrocytes.

4.2 Head Kidney

The fish kidney appears as a multi-functional organ endowed with endocrine function (Abelli *et al.*, 1996a), myelopoietic and immune functions (Zapata *et al.*, 1996). The HK of polar fishes is filled with myelopoietic tissue without renal tubules (Romano *et al.*, 1997a). A large vascularisation of the organ and different predominance of lymphopoiesis or erythropoiesis was observed in the HK of the three polar species, *T. nicolai, C. hamatus* and *T. bernacchii*, confirming previous observations (Romano *et al.*, 2000). These results are in keeping with species differences already observed in the haematopoietic system of warm sea teleosts (Zapata and Cooper, 1990; Zapata *et al.*, 1996). Interestingly, erythropoiesis also occurs (both in HK and spleen) in *C. hamatus*. This species does not possess

haemoglobin-bearing circulating erythrocytes, but the erythroid elements are still present in the HK and spleen and generally scavenged before reaching full differentiation, as documented in the present study. The phagocytosis of old erythrocytes or debris is probably exerted by melano-macrophages (Yu *et al.*, 1971), observed as scattered cells (*C. hamatus*) or in small groups (*Trematomus* spp.). An additional role of melano-macrophages as antigen-presenting cells was hypothesised for fish of temperate water (Ellis, 1980) but further investigation is needed.

The REC, forming the framework of parenchyma (Zapata, 1979; 1981; Meseguer *et al.*, 1991), in polar fish contain vesicles with phagosome-like (*C. hamatus*) or secretory-like (*T. nicolai* and *T. bernacchii*) typology. In temperate-water fish species, the secretory epithelial cells were described (Meseguer *et al.*, 1991; 1995), but the vesicles in *Trematomus* species differ in quantity, size and granular/floccular content. The presence of intermediate filaments in 'phagocytic' reticular cells in *Chionodraco* indicates their epithelial nature, thus, their possible role in antigen processing, exposure and instruction of B-cells (Zapata and Cooper, 1990). The perivascular cells of polar species were in close contact with endothelial cells (without any evident basal lamina), forming a barrier of partial selective diffusion between the blood and haematopoietic tissue. This type of cells had more numerous cytoplasmic vesicles (diameter 0.6–2 μm) than the limiting-subcapsular cells. The content of these vesicles could have a role in chemotactic and/or of erythropoiesis stimulation (Diago *et al.*, 2000), in vessel contraction (Morales *et al.*, 1990) and adrenal gland regulation.

4.3 Spleen

The spleen develops later and remains predominantly erythroid in most teleost species (Zapata, 1982; Rowley *et al.*, 1988). In some species, it was possible to distinguish red and white pulp areas (e.g. sea bass, Abelli *et al.*, 1996b). Different from *T. bernacchii* and *T. nicolai*, where the spleen showed intermingled lymphoid and erythroid areas (not marked in the latter species), the white pulp areas in *C. hamatus* are well developed and separated. Inside or close to the lymphoid areas, small melano-macrophage centres were found in both polar species examined in this study. In another polar fish (*Harpagifer antarcticus*) the melano-macrophage cells were absent in the spleen and infrequent in the HK (O'Neill, 1989), thus, suggesting species differences among polar fishes. The cytological analysis of the spleen revealed heterogeneity of epithelial components: reticular cells (round- and stellate-shape), perivascular and subcapsular cells are organized to form a delimitation (subcapsular) and a special framework (ellipsoids) where leucocytes and erythrocytes can grow and differentiate. In temperate–water fish species, the organisation of the spleen appeared similar, with differences in presence/absence of clearly identifiable red and white pulp areas (Botham and Manning, 1981; Micale and Perdichizzi, 1990; Quesada *et al.*, 1994; Abelli *et al.*, 1996b). As reported for other teleost species, REC connect to each other to form a framework in the parenchyma. Around capillaries, reticular cells are organized in ring-cluster of concentric cells (ellipsoid, Sailendri and Muthukkappan, 1975; Quesada *et al.*, 1994; Romano *et al.*, 1997a) where haematopoietic centres start to organize. The spleen is considered as a central organ for thrombopoiesis in juvenile carp (Romano *et al.*, 1997b) and a secondary lymphoid organ for B- and T-lymphocytes, macrophages and granulocytes (Romano, 1998). The REC of polar fishes showed two typologies: a first one more frequent in the ellipsoid and a second one constituting the splenic framework. In temperate/warm sea teleost species, only one type was described (Quesada *et al.*, 1994; Alvarez *et al.*, 1996). Morphological differences in polar species could reflect a special function (*i.e.* mitogeny stimulation, chemoattraction for circulating lymphocytes, erythropoiesis stimulation). The difference in quality and quantity of cytoplasmic vesicles between the epithelial types apparently strengthens this suggestion.

5 CONCLUSIONS

The adaptations of lymphoid organs to low temperature in polar species could be an original field of study, indicating how the immune system can work under extreme conditions.

In general, a stronger immune response is exerted by species living in warm water than in cold water (Rijkers, 1982; Stolen *et al.*, 1984). Thus, it could be easily hypothesized that low temperature as well as general physiological processes affect poikilothermic species (Eastman and De Vries, 1996). Polar fishes have indeed slower development, metabolism and physiology (O'Neill, 1989; Focardi *et al.*, 1995; Eastman and De Vries, 1996). Even the production of specific immunoglobulins is slower but still present (Scapigliati *et al.*, 1997). Considering that the number of pathogens that can resist to low temperature is quite restricted, the immune responses of polar fishes can result consequently. Marked adaptive changes regard immunoglobulin structures that are in *Trematomus* (Coscia *et al*., 2000). Furthermore, the serum titre resulted higher, probably to compensate the slow immune response (Scapigliati *et al.*, 1997). Moreover, in polar fishes the high blood supply, available by greatly extended vascularization in lymphoid organs – particularly in the HK – could facilitate the oxygen exchange, by enhancing the circulation of erythrocytes and compensating the slow circulation rate. It could also be hypothesized that the high vascularisation can favour the circulation of anti-freezing fluids, leucocytes and free immunoglobulins. Special considerations are needed for *C. hamatus*, in fact in the 'ice-fishes' (*i.e. Chionodraco* spp.) circulating erythrocytes are absent (Eastmen and De Vries, 1996). The occurrence of erythroblasts in *C. hamatus*, originally observed in HK and spleen in this study, is accompanied by degradation signs during the differentiation process as destruction of cell organelles. The peculiar process of erythroid differentiation reflects the incomplete evolution in Channichtyidae species from a erythrocyte-provided Notothenioidea ancestor. The extreme polar environment has favoured erythrocyte elimination and a faster circulation of a viscous blood in the high blood supply of the ice-fish.

In conclusion, comparing with temperate-/warm-fish species, numerous morphological changes were revealed in lymphomyeloid organs of polar species. The most significant adaptive changes were: (1) high vascularization of lymphomyeloid organs; (2) erythropoiesis in all organs; (3) simplified cyto-architecture of thymus; (4) heterogeneity of epithelial cells in HK and spleen that constitute the organs milieu showed vesicles with different contents; (5) occurrence and fast senescence of erythrocytes in *Chionodraco* species. Further physiological studies are needed to better understand if these morphological changes could reflect specialized immunological responses.

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References

Abelli, L., Gallo, V. P., Civinini, A. and Mastrolia, L. (1996a). Immunocytochemical and ultrastructural evidence of adrenal chromaffin cell subtypes in sea bass *Dicentrarchus labrax* (L). *General and Comparative Endocrinololy*, **101**, 113–122.

Abelli, L., Picchietti, S., Romano, N., Mastrolia, L. and Scapigliati, G. (1996b). Immunocytochemical detection of thymocyte antigenic determinants in developing lymphoid organs of sea bass *Dicentrarchus labrax* (L.). *Fish & Shellfish Immunolology*, **6**, 493–505.

Alvarez, F., Flano, E., Castillo, A., Lopez.Fierro, P., Razquin, B. and Villena, A. (1996). Tissue distribution and structure of barrier cells in the haematopoietic and lymphoid organs of salmoids. *Anatomical Records*, **245**, 17–24.

Bargelloni, L., Marcato, S. and Patarnello T. (1998). Antarctic fish hemoglobins: Evidence for adaptive evolution at subzero temperature. *Proceedings of National Academy of Science USA*, **95** (15), 8670–8675.

Botham, J. W. and Manning, M. J. (1981). The histogenesis of the lymphoid organs in the carp *Cyprinus carpio* L. and ontogenetic development of allograft reactivity. *Journal of Fish Biology*, **19**, 403–414.

Chen, L., De Vries, A. L. and Cheng, C. H. C. (1997). Cold adaptation parameters derived from cDNA sequencing and molecular modelling of evastase from antarctic fish *Notothenia neglecta*. *Proceedings of Natural Academy of Sciences (USA)*, **94**, 3817–3822.

Coscia, M. R., Morea, V., Tramontano, A. and Oreste, U. (2000). Analysis of a cDNA sequence encoding the immunoglobulin heavy chain of the Antarctic teleost *Trematomus bernacchii*. *Fish Shellfish Immunology*, **10**, 343–357.

Diago, M. L., Lopez-Fierro, P., Razquin, B. and Villana, A. (2000). Spontaneous in vitro angiogenesis in trout pronephric stroma cell line (TPS), and in TPS-haematopoietic co-cultures. *Fish Shellfish Immunology*, **10**, 21–31.

Eastman, J. T. and De Vries, A. L. (1996). Antarctic fishes. *Scientific American*, **255**, 96–103.

Ellis, A. E. (1980). Antigen-trapping in the spleen and kidney of the plaice, *Pleuronectes platessa*. *Journal of Fish Diseases*, **3**, 413–426.

Focardi, S., Fossi, M. C., Lari, L., Casini, S., Leonzio, C., Meidel, S. K. and Nigro, M. (1995). Induction of MFO activity in the Antartic fish *Pagothenia bernacchi.* Preliminary results. *Marine Environmental Research*, **39**, 97–100.

Goldspink, G. (1995). Adaptation of fish to different environmental temperature by qualitative and quantitative changes in gene expression. *Journal of Thermal Biology*, **20**, 167–174.

Ito, N., Komiyama, N. H. and Fermi, G. (1995). Structure of deoxyhaemoglobin of the antarctic fish *Pagothenia bernacchii* with an analysis of the structural basis of the root effect by comparison of the liganded and unliganded haemoglobin structures. *Journal of Molecolar Biology*, **250**, 648–658.

Logsdon, J. M. and Doolittle, W. F. (1997). Origin of antifreeze protein genes: A cool tale in molecular evolution. *Proceedings of National Academy of Science USA*, **94**, 3485–3487.

Mazzi, V. (1977). In Piccin (ed.), *Manuale di tecniche istologiche e istochimiche–Studio speciale dei tessuti e degli organi*, Italy, pp. 357.

Meseguer, J., Esteban, M. A. and Agulleiro, B. (1991). Stromal cells, macrophages and lymphoid cells the head kidney of sea bass (*Dicentrarchus labrax* L.): an ultrastructural study. *Archives of Histology and Cytology*, **54**, 299–309.

Meseguer, J., Lopez-Ruiz, A. and Garcia-Ayala, A. (1995). Reticulo-endothelial stroma of the head kidney from the seawater teleost gilthead seabream (*Sparus aurata* L.): An ultrastructural and cytochemical study. *Anatomical Records*, **241**, 303–309.

Micale, V. and Perdichizzi, F. (1990). A quantitative and histolochemical study on melano-macrophage centres in the spleen of the teleost fish *Diplodus annularis* L. *Journal of Fish Biology*, **37**, 191–197.

Morales, E. L., Meseguer, J., Lozano, M. T. and Agulleiro, B. (1990). Ultrastructure of the nephron of grey mullets (Mugil cephalus and Liza saliens Risso 1810). *Anatomischer Anzeiger*, **170**, 49–61.

Nakanishi, T. (1986). Effects of X-radiation and thymectomy on the immune response in the marine teleost *Sebasticus marmoratus*. *Developmental and Comparative Immunology*, **11**, 519–528.

O'Brien, K. M., Xue, H. and Sidell, B. D. (2000). Quantification of diffusion distance within the spongy myocardium of hearts from antarctic fishes. *Respiratory Physiology*, **122** (1), 71–80.

O'Neill, J. G. (1989). Ontogeny of the Iymphoid organs in an intarctic teleost, *Harpagifer antarcticus* (Notothenioidei: Perciformes). *Developmental and Comparative Immunology*, **13**, 25–33.

Parnaham, P. (2000). In *The immune system – current trends.* Gardaland Publishing, N.Y./London.

Quesada, J., Villena, M. I. and Navarro, V. (1994). Ontogeny of the sea bass spleen (*Dicentrarchus labrax*): A light and electron microscopic study. *Journal of Morphology*, **221**, 161–176.

Rijkers, G. T. (1982). Kinetics of urnoral and cellular immune reactions in fish. *Developmental and Comparative Immunology* (Suppl. 2), 93–100.

Ritter, M. A. and Crispe, I. N. (1992). In Rickwood, D. and Male, D. (eds.), *The thymus.* IRL Press, Oxford Univ. Press, pp. 1–85.

Romano, N., Casini, P., Abelli, L., Mastrolia, L. and Aita, M. (1996). Influence of partial decerebration and hypophyseal allograft on differentiation of thymic epithelial cells in chick embryos, an ultrastructural study. *Anatomy and Embryology*, **193**, 593–600.

Romano, N., Baldassini, M. R., Terribili, F. R., Abelli, L., Mastrolia, L. and Mazzini, M. (1997a). Histological observations on lymphoid organs of the Antarctic fish *Trematomus bernacchii* (Teleostei:Nototheniidae). *Polar Biology*, **18**, 358–362.

Romano, N., Taverne-Thiele, J. J., Maanen, J. C. and Rombout, J. H. W. M. (1997b). Distribution of leucocyte subpopulations in developing carp (*Cyprinus carpio* L.): Immunocytochernical studies. *Fish Shellfish Immunology*, **7**, 439–453.

Romano, N. (1998). Ontogeny of the immune system of fish using specific markers, *PhD thesis*, Agricultural University of Wageningen (ed.), Wageningen (NL), Poesen, K. & Looijen, B.V. Press, pp. 1–168.

Romano, N., Fanelli, M., Del Papa, G. M., Scapigliati, G. and Mastrolia, L. (1999a). Histological and cytologycal studies on the developing thymus of sharpsnout seabream, *Diplodus puntazzo. Journal of Anatomy*, **194**, 39–50.

Romano, N., Taverne-Thiele, J. J., Fanelli, M., Baldassini, M. R., Abelli, L., Mastrolia, L., van Muiswinkel, W. B. and Rombout, J. H. M. W. (1999b). Ontogeny of the thymus in a teleost, *Cyprinus carpio* L.: Developing thymocytes in the epithelial microenvironment. *Developmental and Comparative Immunology*, **23**, 123–137.

Romano, N., Ceccariglia, S., Mazzini, M. and Mastrolia, L. (2000). Histological studies on lymphoid organs of the Antarctic fish *Trematomus nicolai* and *Chionodraco hamatus* (Teleostei:Nototheniidae). *Polar Biology*, **23**, 321–328.

Romano, N., Ceccariglia, S., Mastrolia, L. and Mazzini, M. (2002). Cytology of lymphomyeloid head kidney of Antarctic fishes *Trematomus bernacchii* (Nototheniidae) and *Chionodraco hamatus* (Channichtyidae). *Tissue and Cell*, **34**, 63–72.

Rowley, A. F., Hunt, T. C., Page, M. and Mainwaring, G. (1988). Fish, in Rowley, A. F. and Ratcliffe, N. A. (eds.), *Vertebrate blood cells.* Cambridge Univ. Press, Cambridge, pp. 19–27.

Sailendri, K. and Muthukkappan, V. R. (1975). The immune response of the teleost, *Tilapia mossambica* to soluble and cellular antigens. *Journal of Experimental Zoology*, **191**, 371–381.

Scapigliati, G., Chausson, F., Cooper, E. L., Scalia, D. and Mazzini, M. (1997). Qualitative and quantitative analysis of serum immunoglobulins of four Antarctic fish species. *Polar Biology*, **18**, 209–213.

Stolen, J. S., Gahn, T., Kasper, V. and Nagle, J. J. (1984). The effect of environmental temperature of the immune response of a marine teleost, *Paraichthys dentatus. Developmental and Comparative Immunology*, **8**, 89–98.

Yu, M. L., Kiley, C. W., Sarot, D. A. and Perlmutter, A. (1971). Regulation of haemosiderin to erythropoiesis in the blue gourami (*Trichogaster tricopterus*). *Journal of Fisheries Research Board Canada*, **28**, 47–48.

Zapata, A. (1979). Ultrastructural study of teleost fish kidney. *Developmental and Comparative Immunology*, **3**, 55–65.

Zapata, A. (1981). Lymphoid organs of teleost fish. II. Ultrastructure of renal lymphoid tissue of *Rutilus rutilus* and *Gobio gobio*. *Developmental and Comparative Immunology*, **5**, 685–690.

Zapata, A. (1982). Lymphoid organs of teleost fish. III. Splenic Iymphoid tissue of *Rutilus rutilus* and *Gobio gobio. Developmental and Comparative Immunology*, **6**, 87–94.

Zapata, A. and Cooper, E. L. (1990). *The Immune system: Comparative Histophysiology – The bone marrow and its equivalents.* Wiley, J. and Sons, USA, pp. 35–71.

Zapata, A., Chibá, A. and Varas, A. (1996). Iwama, G. and Nakanishi, T. (eds.), *The fish immune system: Organism, pathogen and environment – Cell and tissue of the immune system of fish.* Academic Press, San Diego (CA-USA), pp. 255–287.