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THYMIC DEVELOPMENT IN TWO SPECIES OF MARINE TELEOST; AN ANTARCTIC SILVERFISH, *PLEURAGRAMMA ANTARCTICUM* BOULENGER, AND A WARMER-WATER SEA BASS, *DICENTRARCHUS LABRAX* (LINNAEUS)

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Abstract: The paired thymic organs of post-larval, juvenile and adult specimens of the Antarctic silverfish, *Pleuragramma antarcticum*, and sea bass, *Dicentrarchus labrax*, were examined histologically. In both species the paired thymic anlagen were infiltrated by small lymphocytes at the earliest post-larval stages. This was more advanced in *D. labrax*, with the formation of trabeculae from the sub-epithelial connective tissues (SECT).

A more advanced thymic development was noted for juvenile *D. labrax*, trabeculae were prominent and thymic zonation was distinct. In this species there was a greater infiltration of epithelial mucous cells and macrophages into the inner thymic area. A further distinction, not observed in *P. antarcticum*, was the presence of melano-macrophage centres and large myoid cells, indicating a more advanced thymic involution in the warmer-water species.

Thymic progression at the adult stage differed in the two species. *D. labrax* did not show an increased involution of the thymic zones; however, an increased trabecular and SECT development divided the thymus into discrete lobules. In contrast, the thymic trabeculae were lost in *P. antarcticum* and the organ regressed, leaving a few aggregations of small lymphocytes in the outer thymic zone.

1. Introduction

Temperature exerts perhaps the most pervasive influence on the ecology and physiology of fishes. Their ability to respond to a challenge by a foreign organism, or immunogen, by producing a specific immune response, is temperature dependent (O'NEILL, 1987). In general, eurythermal species acclimated to low temperatures show a substantial decrease in their immunological competence. Those species which adapted themselves to low environmental temperatures over evolutionary time, even the low and stable temperatures of the Antarctic, have adapted their immunological response (O'NEILL, 1987).

Immunocompetence in vertebrates depends on the development of the lymphopoietic organs. Of these organs the thymus shows significant morphological/histological changes, not only in its development, but with old age (COOPER *et al.*, 1983), stress (GHONEUM *et al.*, 1986), season, sex and reproduction (HONMA and TAMURA, 1984; NAKANISHI, 1986). Changes that result from temperature adaptation have still to be examined.

In this preliminary study, the paired thymic organs of the cold-water and stenothermal Antarctic silverfish, *Pleuragramma antarcticum*, were compared with those of the warmer-water European sea bass, *Dicentrarchus labrax*.

2. Materials and Methods

Four broadly comparable life-stages of *P. antarcticum* and *D. labrax* were examined (Table 1). Larval/yolk sac stages of *P. antarcticum* were unavailable. On capture the fish, with the exception of post-larval and adult *P. antarcticum*, were fixed whole in a 0.1 M phosphate buffer, pH 7.4, containing 4% paraformaldehyde-0.2% picric acid, for 24 h at 4°C. The fixative was prepared by the KARNOVSKY (1965) method and the picric acid, used to enhance fixative penetration (STEFANINI *et al.*, 1967) and preservation of tissue structure (TAKAMIYA *et al.*, 1979) was added just before use. A fresh solution, omitting picric acid, was used to store the specimens. The *P. antarcticum* post-larvae were received in alcohol and the adults in formalin-sea water, these were then processed and stored in the fixative used for the other specimens. Juvenile and adult specimens were decalcified in 16% formic acid (BDH) for 12 h at 4°C. The tissues were dehydrated through ethanol, infiltrated with two changes of glycol methacrylate monomer (BDH) and embedded in glycol methacrylate (JB4; Polysciences). Sections were cut at 2 μm (AS500 semi-thin microtome; Anglia Scientific), using 35 mm Ralph-glass knives (Histoknifemaker; Reichert-Jung), floated out on double distilled water, transferred to chromic acid etched microscope slides and dried down at 70°C. A modified Lee's stain, aqueous 1% (w/v) methylene blue and 1% (w/v) basic fuchsin, added 1 : 1 to 0.2 M phosphate buffer, pH 7.4, was used for routine staining.

Table 1. Capture data.

Species	Stage	Weight (g)	Number	Capture site
P. antarcticum	early post-larval	0.01–0.04	4	Weddell Sea (70°35′S, 09°23′W)
	late post-larval	0.4–0.5	4	(as above)
	juvenile	13.1–24.8	6	South Shetland Islands (62°05′S, 58°15′W)
	adult	43–46	3	Weddell Sea (70°35′S, 09°23′W)
D. labrax	30 day post-hatch	0.01–0.013	5	hatchery
	60 day post-hatch	0.08–0.12	4	hatchery
	unspawned maiden	530–1010	5	Southampton Water (50°49′N, 01°18′W)
	adult	1100–2300	3	(as above)

3. Results

The two species, *P. antarcticum* and *D. labrax*, demonstrated an identifiable thymus at all the stages examined. The thymus was a bilaterally paired organ, located on the dorsal body wall of the branchial cavity and near the dorsal articulation of the operculum. Only in the maiden and adult *D. labrax* was the organ visible macroscopically, as a pale-cream and opaque area under the epithelium.

The development of the vertebrate thymus commences with the infiltration of the pharyngeal epithelium by lymphocytes (thymocytes). Thymocytes had infiltrated and replicated within the epithelium, with the formation of an epithelio-reticular network, in the earliest post-larval stages of both species (Figs. 1 and 2). This process was at its earliest stages in the early post-larval *P. antarcticum* (Fig. 1). With increased thymocyte numbers, the thymus distended into the branchial cavity and, in *P. antarcticum*, was poorly protected by the posterior-dorsal edge of the post-larval operculum (Fig. 1). In both species the thymus was protected from the efferent current of the respiratory water flow by the pharyngeal epithelium alone.

The sub-epithelial connective tissues (SECT), which underlie the thymus, increased in thickness and become more prominent with age. The development of the SECT and its infiltration, as trabeculae, into the thymic tissue was more advanced in *D. labrax* and commenced at 30 days post-hatch (Fig. 2). Although SECT was apparent in late post-hatch *P. antarcticum*, no trabeculae had formed (Fig. 3). Trabeculae were well developed in the 60 day post-hatch *D. labrax* (Fig. 4) and had divided the lateral margins of the thymus from the pharyngeal epithelium. Such a separation was restricted in *P. antarcticum* to the anterior-dorsal margin of the juvenile thymus (Fig. 5) and the trabeculae were not as prominent as those of the maiden *D. labrax* (Fig. 6). Only in the adult *D. labrax* (Fig. 7) did the SECT divide the thymus completely from the pharyngeal epithelium and into discrete lobules, though the superficial position of the organ was maintained.

A greater distinction of thymic zones was observed in *D. labrax* (Fig. 6). The earliest stage of outer and inner thymic zonation in this species was found in the 60 day post-hatch specimens (Fig. 4). Zonation was observed in all juvenile (Figs. 5 and 6) and adult (Figs. 7 and 8) stages of both species. The inner zone of the thymus was characterized by an expansion of reticular cells, both from the SECT and epithelium, while in the outer zone tightly packed groups of small thymocytes were found in the interstices of "stretched" epithelio-reticular cells.

The inner thymic zone of the juvenile and adult specimens, infiltrated by a sub-epithelial reticulum from the SECT, demonstrated further features associated with the involution/aging of the thymus. Mucous cells, of epithelial origin, infiltrated the thymus, though these were more prominent in *D. labrax* (Fig. 9). Cysts, which may have been mucous cell associated, were seen in one juvenile specimen of *P. antarcticum* (Fig. 5). Individual, light-pigmented and active, macrophages were scattered throughout the outer and inner thymic zones of adult *P. antarcticum* and both maiden and adult *D. labrax* (Fig. 9). This was in contrast to the aggregations of melano-macrophages found in maiden and adult *D. labrax* (Fig. 10). Myoid cells, with characteristic concentric rings of muscle fibrils, were present in maiden and adult *D. labrax* (Fig. 11), though not in the Antarctic species. Although a blood vascular system infiltrated the thymus of 60 day post-larval *D. labrax* (Fig. 4) and juvenile *P. antarcticum* (Fig. 5), these vessels were restricted to the trabeculae. In the maiden and adult *D. labrax* capillaries, with thin endothelial walls (Fig. 10), were seen unassociated with trabeculae and within the inner zone of the thymus.

Thymic progression at the adult stage differed in the two species. In *D. labrax* (all female) an increased involution within the thymic zones was not evident, even

though the SECT had separated the thymic tissues from the epithelium and formed discrete thymic lobules (Fig. 7). In contrast, the structure of the adult (both male and female) thymus in *P. antarcticum* had regressed in size and the thymic trabeculae had been lost (Fig. 8). Aggregations of small thymic lymphocytes remained, mainly as clusters within the outer zone, surrounded by a proportionally larger volume of epithelial and SECT derived reticulum, which also contained scattered and active macrophages.

4. Discussion

In the earliest developmental stages of the two teleost species, examined in this study, a thymus had already developed. The initial development of the lymphoid organs in warmer-water teleost species can occur from as early as 22 days pre-hatch (ELLIS, 1977; *Salmo salar*) and upto 11 days post-hatch (HAFTER, 1952; *Astynax mexicanus*). While in viviparous teleosts, thymic development can occur up to 3 months pre-parturition (BLY, 1985; ENGEN, 1968; TAMURA *et al.*, 1981). Although the initial infiltration of lymphocytes and the development of the thymus were not as advanced in the Antarctic species, when compared to the warmer-water *D. labrax*, this need not necessarily predict a post-hatch onset of thymic development in *P. antarcticum*. Indeed, post-hatch development may not be an indicator of temperature dependence, when such large variations in thymic infiltration exist within the warmer-water teleosts.

Features of the development, progression and involution of the thymic organs examined were characteristic of those recorded in other teleosts. The expansion of the thymus in warmer-water teleosts, both marine (BLY, 1985; TAMURA and HONMA, 1977; *D. labrax* in this study) and freshwater (BOTHAM and MANNING, 1981; CHILMONCZYK, 1983, 1985; GRACE and MANNING, 1980; TATNER and MANNING, 1982), is more advanced and results in a more lobulate organ than that seen in the post-larvae of *P. antarcticum*. An inner and outer zonation of the thymus was found in both species but differentiated earlier in *D. labrax* than in the Antarctic species.

One of the most remarkable aspects of the teleost thymus is the superficial nature of the organ, exposed to the branchial water currents. In many teleost species only a thin layer of mucosal epithelial cells separates the thymus from the external environment. Even the protection of the operculum was deficient in the post-larval *P. antarcticum*. The fragility of this epithelial barrier may be increased by the presence of pores, as was observed by CHILMONCZYK (1985) in juvenile rainbow trout, *Salmo gairdneri*. A SECT developed in both species, though this was more advanced in the warmer-water *D. labrax* and started to insert between the lateral edges of the thymus and pharyngeal epithelium at 60 days post-hatch, compared to the later juvenile stage in *P. antarcticum*. In the adult *D. labrax* the SECT had partitioned the thymus, forming discrete lobules. This process may be the start of the partition of the thymus from its superficial position, as observed in the anglerfish, *Lophius piscatorius*, (FÄNGE and PULSFORD, 1985) and the higher vertebrates. No extra-thymic accumulations of lymphoid tissue, equivalent to those observed by BLY (1985), GORGOLLON (1983), HAFTER (1952) and SAILENDRI and MUTHUKKARUPPAN (1975b), were present in either the Antarctic or warmer-water teleost.

In the early stages of development, the blood vascular system infiltrated the thymus but was retained within the SECT trabeculae. This may provide the thymic barrier to blood-borne antigen, which was detected in the thymic cortex of mammals (RAVIOLA and KARNOVSKY, 1972) and considered to be present in teleosts (CHILMONCZYK, 1983; FÄNGE and PULSFORD, 1985; ZAPATA, 1981). However, in adult *D. labrax* and *P. antarcticum* there were blood capillaries with thin endothelial walls, even within the outer thymic zones of tightly packed thymocytes. The isolation of the thymus from antigenic stimulation, as shown by antigen challenge and lymphocyte circulation experiments in plaice, *Pleuronectes platessa*, (ELLIS, 1980; ELLIS *et al.*, 1976) and the observation of thymus-blood barriers, must be called into question. Macrophages were evident in the thymus of maiden *D. labrax* and adult *P. antarcticum*. Even melano-macrophage aggregations, more associated with antigen-processing in the spleen and pro-nephric kidney (AGIUS, 1985; AGIUS and COUCHMAN, 1986), were found in the thymus of the warmer-water *D. labrax* and by GORGOLLON (1983) in adult clingfish, *Sicyases sanguineus*. Although the active appearance of the phagocytic cells may be a consequence of the destruction of thymic cells in the involution process, antigen processing and presentation cannot be ruled out. Indeed, plasma cells have been reported within the teleost thymus, not restricted to the trabeculae and connective tissue (FÄNGE and PULSFORD, 1985; ZAPATA, 1981), and formed in response to immunisation (ORTIZ-MUNIZ and SIGEL, 1971; SAILENDRI and MUTHUKKARUPPAN, 1975a). Although thymic lymphocytes in some teleost species demonstrate surface-bound immunoglobulins, with the use of a hapten-carrier system, WARR *et al.* (1977) were unable to detect hapten-reactive cells (antibody-producing and B-equivalent), only carrier-reactive cells (T-equivalent) were found within the thymus of goldfish, *Carassius auratus*. This would suggest that the reported thymic plasma cells are immigrant cells, rather than clones produced by *in situ* antigenic stimulation.

Epithelial mucous cells were prominent in the older specimens and indicated a thymic involution. Their infiltration into the inner and outer zones of the thymus could be temperature, as well as age, related. The infiltration was greater in adult *D. labrax* than in the stenothermal Antarctic species. The hypertrophy of a few individual mucous cells was noted in the adults of both species; however, only in one juvenile specimen of *P. antarcticum* was a cyst observed. Such structures have been associated with age involution in freshwater (HAFTER, 1952; SAILENDRI and MUTHUKKARUPPAN, 1975b; ZAPATA, 1981) and marine teleosts (FÄNGE and PULSFORD, 1985; GORGOLLON, 1983; TAMURA and HONMA, 1977), though mucous cysts were also prominent in thymic hyperplasia presented by *S. gairdneri* (MCARDLE and ROBERTS, 1974).

Myoid cells occur in the thymus of many vertebrate groups, from elasmobranchs (ZAPATA, 1980) to humans (ITO *et al.*, 1969). Only in the warmer-water *D. labrax* were myoid cells found in the inner thymic zone, similar in structure to those of other vertebrate groups and other teleosts (CHILMONCZYK, 1983; FÄNGE and PULSFORD, 1985; GORGOLLON, 1983; HAFTER, 1952; RIZKALLA, 1969; ZAPATA, 1981). Although the myoid cells were of different sizes and developmental stages, the migration and phagocytosis of degenerating cells, recorded by FÄNGE and PULSFORD (1985) in *L. piscatorius*, were not observed. The myoid cells are associated with thymic involution, though their function has not been elucidated. They are probably derived from the thymic

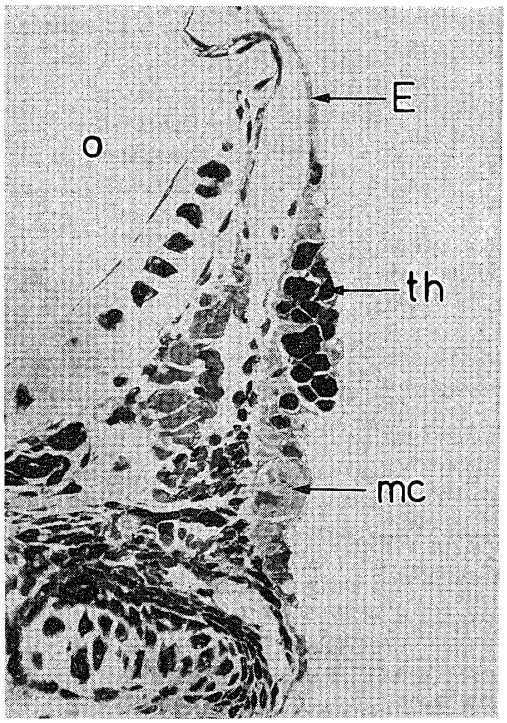

Fig. 1. T.S. mid-thymus of early post-larval P. antarcticum (×*525*)*; thymocytes* (*th*) *have infiltrated the mucosal epithelium* (*E*) *of the early thymus. mc, epithelial mucous cell; o, otic bulla.*

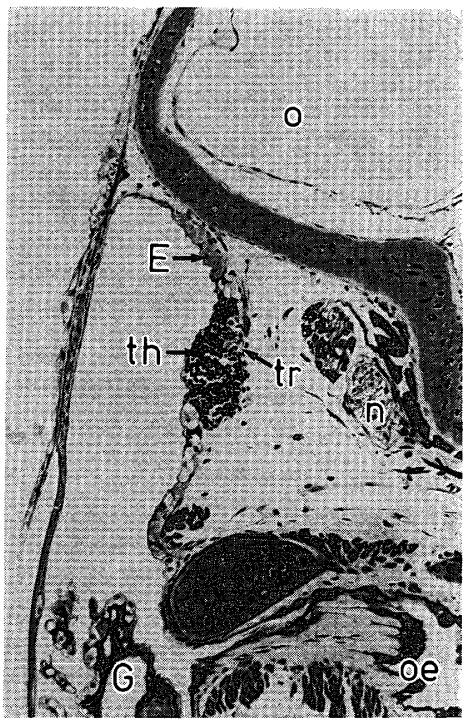

Fig. 2. T.S. mid-thymus of 30 day post-hatch D. labrax (×*260*)*; large numbers of thymocytes* (*th*) *have infiltrated the mucosal epithelium* (*E*) *and trabeculae* (*tr*) *have started to form. G, gill; n, nerve; o, otic bulla; oe, oesophagus.*

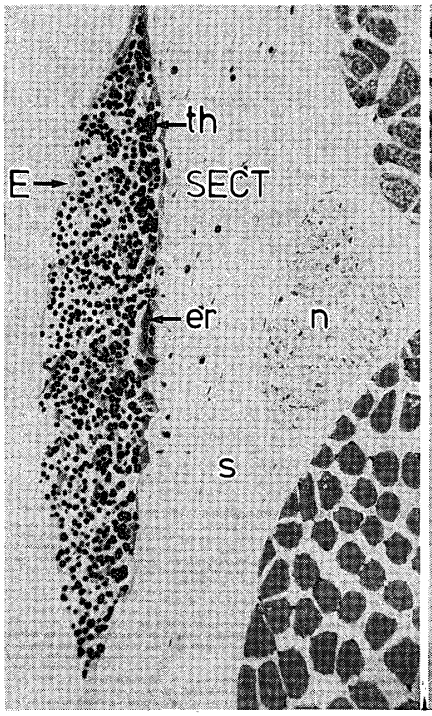

Fig. 3. T.S. mid-thymus of late post-larval P. antarcticum (×*290*)*; increased numbers of thymocytes* (*th*)*, within an epithelial-reticular network* (*er*)*, swell the thymus. E, mucosal epithelium; n, nerve; s, sinus; SECT, sub-epithelial connective tissue.*

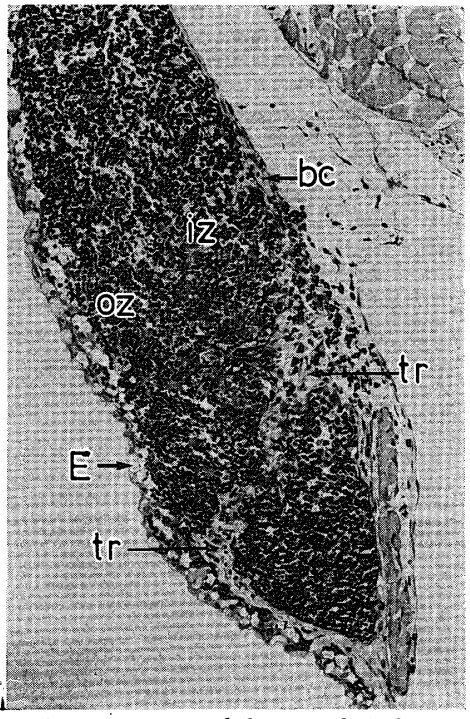

Fig. 4. T.S. mid-thymus of 60 day post-hatch D. labrax (×*269*)*; at this early stage a zonation of the thymus is observed and a substantial trabecular infiltration* (*tr*) *surrounds and partitions the lateral margin. bc, blood capillary; E, mucosal epithelium; iz, inner zone; oz, outer zone of closely packed thymocytes.*

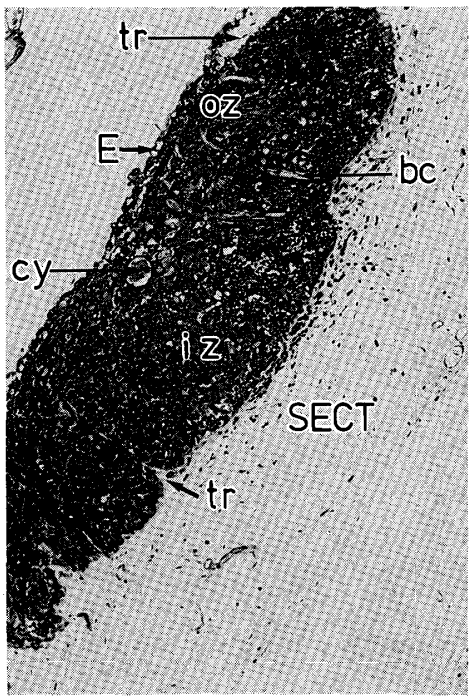

Fig. 5. T.S. mid-thymus of juvenile P. antarcticum *(×135); there is an expansion of the thymus away from the epithelium (E) at the anterior-dorsal edge, with infiltration of sub-epithelial connective tissue (SECT), forming trabeculae (tr), and the appearance of an inner thymic zonation (iz). bc, blood capillary entering the intrabecula; cy, epithelial cyst; oz, dense thymocyte aggregations in the outer thymic zone.*

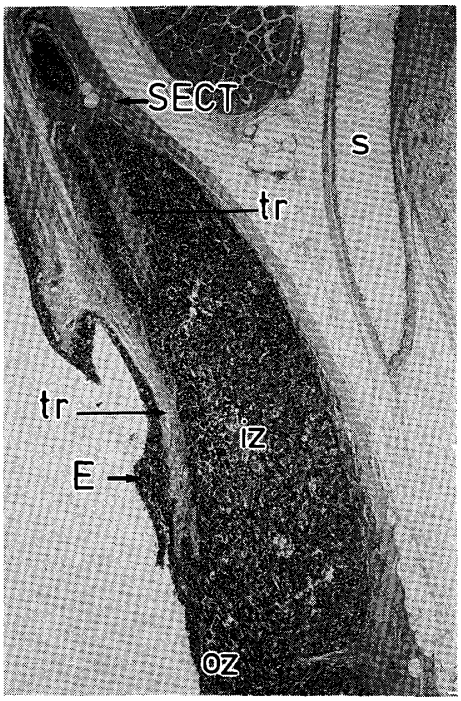

Fig. 6. T.S. mid-thymus of maiden D. labrax *(×50); the sub-epithelial connective tissue (SECT) forms a well defined structural component, dividing the thymic margin from the mucosal epithelium (E). iz, inner zone infiltrated by SECT and epithelial tissue (see Figs. 9, 10 and 11); oz, outer zone of closely packed thymocytes; s, sinus; tr, trabecula.*

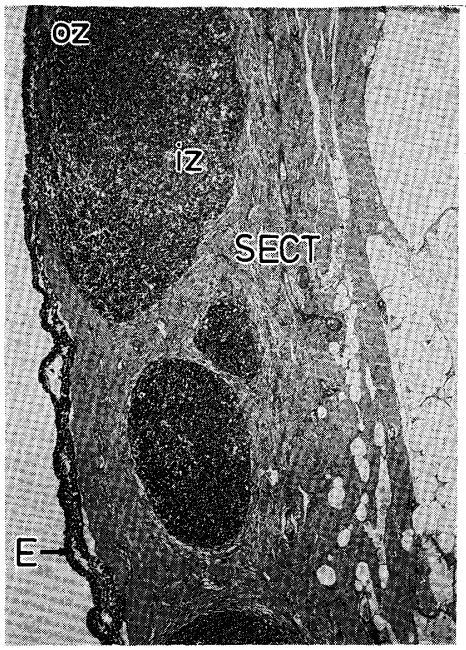

Fig. 7. T.S. mid-thymus of adult D. labrax *(×46); the sub-epithelial connective tissue (SECT) has separated the thymic tissues from the mucosal epithelium (E) and divided the organ into lobules. iz, inner zone; oz, outer zone.*

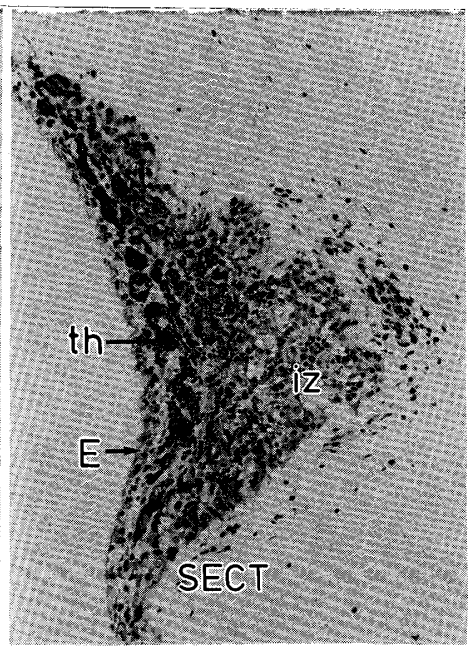

Fig. 8. T.S. mid-thymus of adult P. antarcticum *(×195), showing the marked regression. E, mucosal epithelium; iz, inner zone with few thymocytes; SECT, sub-epithelial connective tissue; th, thymocyte aggregations in the outer thymic zone.*

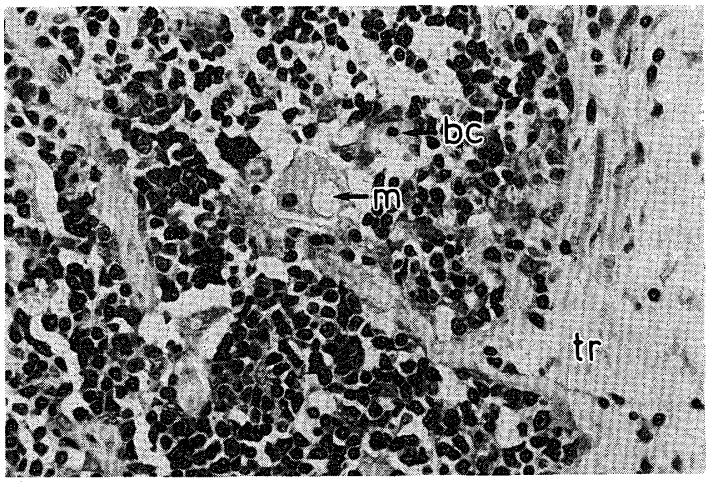

Fig. 9.

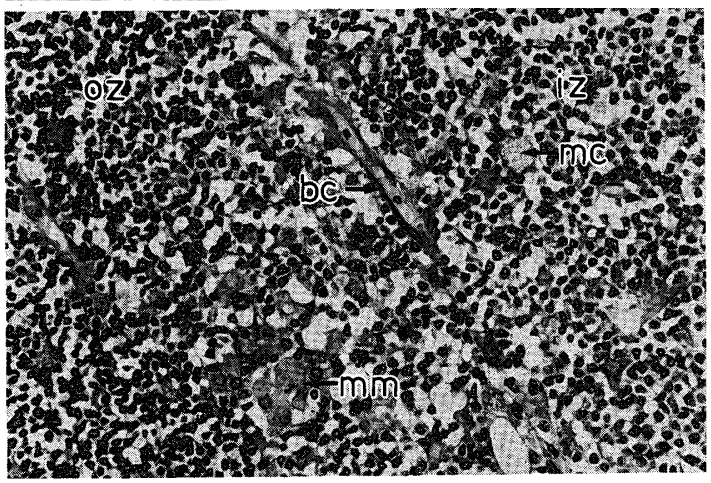

Fig. 10.

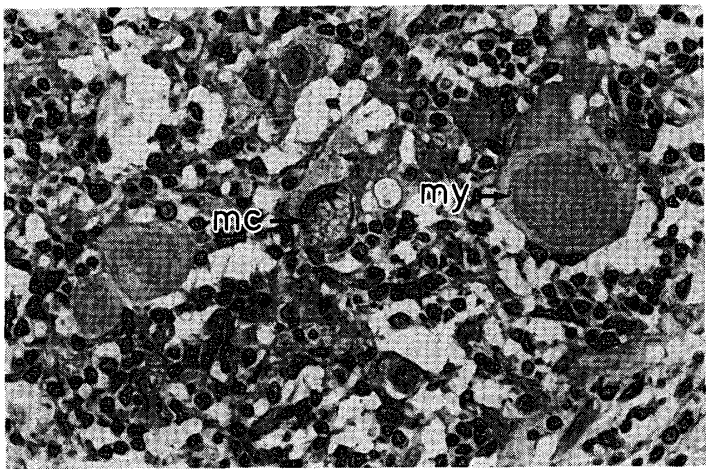

Fig. 11.

Fig. 9. Inner thymic zone (iz) of maiden D. labrax (×*840*)*; active macrophage (m) associated with trabecula (tr) process. bc, erythrocyte in fine capillary.*

Fig. 10. Inner thymic zone (iz) of maiden D. labrax (×*505*)*; mucous cell (mc) melano-macrophage aggregation (mm) and endothelial blood capilary (bc). oz, outer thymic zone.*

Fig. 11. Inner thymic zone of maiden D. labrax (×*1193*)*; myoid cell (my). mc, mucous cell.*

epithelio-reticular system and have an association with myasthenia gravis, an autoimmune disease (RIMMER, 1980; COOPER and TOCHINAI, 1982).

An age-related involution of the thymus was seen in both species; however, a gross depletion of the structural elements of the thymus was noticed in *P. antarcticum* alone. In addition to age-related involution, seasonal- and reproductive cycle-related changes (HONMA and TAMURA, 1984; TAMURA *et al.*, 1981; NAKANISHI, 1986) and structural involution, induced by corticosteroid treatments (CHILMONCZYK, 1982; GHONEUM *et al.*, 1986), have been reported. The influence of season, reproductive cycle and environmental stress on the development of the thymus of Antarctic teleosts has yet to be assessed. In their natural Antarctic environment such studies pose practical problems, but these may be overcome by studying laboratory-reared specimens.

There can be little doubt that an adaptation of the disease defence mechanisms to a cold-water environment has taken place within the Antarctic fishes. Just as certain biochemical systems can be shown to adapt (HAZEL and PROSSER, 1974), it would be expected that the immune response and the lymphoid organs, which provide the sites of initiation and the seat of the immune response, would also adapt to low temperatures. The thymus of *P. antarcticum* indicated a basic development and anatomical structure equivalent to that of warmer-water species, demonstrating an adaptation to low environmental temperature. However, certain developmental aspects within this Antarctic species may indicate a slower ontogeny. These were the initially slow development of the thymus and a reduced thymic involution, as shown by the small numbers of mucous cells or the lack of melano-macrophage centres and myoid cells, when compared to those species adapted to warmer-water environments.

Acknowledgments

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