ISSN 0113-2261

New Zealand Fisheries Research Bulletin No. 28

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1987

MAF Fish

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New Zealand Fisheries
Research Bulletin No. 28
1987

Published by MAFFish
Wellington
1987

ISBN 0-477-08045-6

MAF Fish

MAFFish is the fisheries business group of the New Zealand Ministry of Agriculture and Fisheries. It was established on 1 April 1987 and combines the functions of the old Fisheries Research Division and Fisheries Management Division and the fisheries functions of the old Economics Division.

The *New Zealand Fisheries Research Bulletin* series continues the *Fisheries Research Bulletin* series.

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Set in 10 on 11 Times

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ABSTRACT

Hine, P. M., Wain, J. M., and Boustead, N. C. 1987: The leucocyte enzyme cytochemistry of fish. *New Zealand Fisheries Research Bulletin No. 28.* 74 p.

Observations are made on the leucocyte enzyme cytochemistry of 158 fish species (2 agnathans, 24 elasmobranchs, 6 holocephalans, 13 elopomorphs, 3 clupeiforms, 14 salmoniforms, 10 stomiiforms, 6 scopelomorphs, 12 paracanthopterygians, 68 acanthopterygians). Peroxidase was not found in blood granulocytes of holocephalans, was uncommon and weak in eosinophils of elasmobranchs, variable in neutrophils and eosinophils of elopomorphs and clupeiforms, and rare and weak in eosinophils of higher teleosts. Alkaline phosphatase was strong in holocephalan fine granulocytes and occurred intermittently in teleost neutrophils, but in elasmobranchs was most often seen in lymphocytes of dogfish (Squaliformes). Acid phosphatase was common in all leucocytes and identified granules as lysosomes. Esterases were strong and intra- and extracellular in elasmobranch granulocytes, often strong in elopomorphs, but of variable strength in higher groups. General trends are discussed in relation to cell type and to occurrence in major taxa.

INTRODUCTION

The understanding of fish pathology requires an understanding of fish immune systems and the identity, function, and interrelationships of the leucocytes that constitute that system. Recent studies have concentrated on humoral aspects of fish immunology; there have been few studies on cellular aspects. This may be due to difficulties in working with fish blood, particularly with leucocytes (Ellis 1977). Fish leucocytes are not as highly developed and are less differentiated than mammalian leucocytes; thus, they are difficult to distinguish from each other, particularly large lymphocytes from monocytes (Bell 1976, Ellis 1976, Cannon *et al.* 1980).

The extensive knowledge of mammalian haematology makes it almost impossible for the fish haematologist to think outside mammalian terms, and interpretations are, therefore, in those terms. This has resulted in the use of often inappropriate mammalian nomenclature based on leucocyte origin (e.g., myelocyte; myelo = bone marrow) or tinctorial properties (e.g., neutrophil, eosinophil, basophil). However, all fish lack bone marrow, and granulocyte tinctorial properties do not always fit mammalian terminology, which causes confusion in cell identification (Kindred 1971, Ellis 1977). Furthermore, nomenclature implies function, and fish leucocytes may not have the same functions as their mammalian counterparts. The lack of abscess formation in fish (Roberts 1978, Ellis 1981), the inconsistent occurrence of mast cells and anaphylaxis (Ellis 1982), and the predominance of eosinophils in the blood of many primitive fish do not suggest absolute functional similarity.

In medicine leucocyte enzyme cytochemistry is used as a routine tool to differentiate between cell types (Hayhoe and Quaglino 1980), determine activation state (Cohn 1978, Karnovsky and Lazdins 1978, North 1978), differentiate between T and B lymphocytes in humans (Catovsky and Enno 1977), differentiate within T cell subpopulations (Davey, Dock, and MacCallum 1980, Zicca *et al.* 1981), and identify non-T and non-B lymphocytes (Grossi *et al.* 1982). Furthermore, enzyme cytochemistry may suggest function: peroxidase, lysozyme, and lysosomal hydrolases are associated with death and destruction of pathogens (Neeman, Lahav, and Ginsburg 1974, Spitznagel 1977, Karnovsky *et al.* 1981), and neutral proteases are associated with tissue destruction (Baggiolini *et al.* 1980); though the functions of alkaline phosphatase (Wilson, Smith, and Peters 1983) and esterases (Luppa and Andra 1983) are still poorly understood.

Therefore, the application of the enzyme cytochemical techniques used in medicine may help in the differentiation of fish leucocytes (including their subpopulations, maturation stages, and activation states) and may suggest leucocyte function, or the presence of disease. However, it is necessary to first establish normal enzyme patterns in fish leucocytes, so that indicator enzymes for these characteristics can be determined.

In fish, monocytes or macrophages are phagocytic (Finn 1970, Finn and Nielsen 1971, Finn and Nielson 1971, Hatai 1972, McKinney *et al.* 1977, Phromsuthirak 1977, Bach, Chen, and

Chapman 1978, Lester and Budd 1979, Mori 1980, Song and Kou 1981, Woodhead 1981, Griffin 1983, Hyder, Cayer, and Pettey 1983, MacArthur *et al.* 1984, MacArthur and Fletcher 1985, Parish, Wrathmell, and Harris 1985) and eosinophils are phagocytic in some species (Weinreb and Weinreb 1969, Fishman, Daniele, and Pietra 1979), but neutrophils may be phagocytic (Weinreb and Weinreb 1969, Finn and Nielson 1971, Hatai 1972, Phromsuthirak 1977, Lester and Budd 1979, Morrow and Pulsford 1980, Mori 1981, Elarifi 1982, Griffin 1983, Hyder, Cayer, and Pettey 1983, MacArthur *et al.* 1984, MacArthur and Fletcher 1985) or may not be (Ellis 1976, McKinney *et al.* 1977). Therefore, it is likely that different species or larger taxa differ in the events and participation of cells in inflammation and immune reactions. This is not surprising because of the antiquity of fish, the large number of species (about 21 700 (Nelson 1984), more than all other vertebrates combined), their radiation into diverse habitats, and their predisposition to pathogens readily transmitted in the aquatic environment. Although there have been many studies of inflammatory conditions in fish (Jakowska and Nigrelli 1953, Weinreb 1958 and 1959, Finn and Nielsen 1971, Finn and Nielson 1971, Joy and Jones 1973, McQueen *et al.* 1973, Roberts *et al.* 1973, Roberts 1975a, Timur, G., Roberts, and McQueen 1977, Timur, M., Roberts, and McQueen 1977, Fishman, Daniele, and Pietra 1979, MacArthur *et al.* 1984, MacArthur and Fletcher 1985, Ellis 1986), there are few in relation to the diversity of fish.

This is not intended as a definitive study of the blood of the fish examined. Details of appearance in Wright's-stained preparations are cursory, and a few descriptions are based on one fish and many are based on two. Because disease alters enzyme patterns (Hayhoe and Quaglino 1980), special care must be taken to ensure that normal healthy fish are selected when the sample size is small. Consequently, data based on one or two fish have been treated conservatively, and conflicting or dubious results have been excluded. Fish with external signs of disease were also discarded, as were poor blood films in which lymphocytes and thrombocytes were clumped. Ostariophysid fish were excluded because they have already been studied widely (Catton 1951, Yuki 1957, Fey 1966a, 1966b, 1966c, Haider 1968, Varo 1970, Bielek 1981, Garavini and Martelli 1981, Mori 1981); however, this was also true of salmonids (Catton 1951, Yuki 1957, Fey 1966a, 1966b, 1966c, Haider 1968, Finn and Nielsen 1971, Finn and Nielson 1971, Conroy 1972, Blaxhall and Daisley 1973, Johansson 1973, Lester and Budd 1979, Ezeasor and Stokoe 1980, Bielek 1981), but they were included for comparison with alepocephalids and galaxiids.

Leucocyte enzyme studies should be carried out at the ultrastructural level, but this would severely limit the number of species that could be studied, and studies on human neutrophils have only recently resolved the sequence of granule genesis (Brederoo, van der Meulen, and Mommaas-Kienhuis 1983). Therefore, this study reports what enzymes are, or are not, present in relation to cell type and does not identify granules. However, granulocyte and granule morphology are important in the identification of cells, and details of differential population staining are given for eosinophils in which fine granules can be discerned between coarse granules.

The identification of fish granulocytes is confused because of:

1. The reliance on tinctorial properties over granule or cell morphology (*see* Ellis (1977) for a review);
2. The interpretation of tinctorial properties to fit the mammalian system (Kelényi and Németh 1969);
3. The interpretation of enzyme cytochemistry to fit the mammalian system (Kelényi and Németh 1969);
4. The assumption that fish granulocyte morphology is comparable within fish in general or within groups of fish. This is particularly not the situation in older fish groups such as elasmobranchs (Morrow and Pulsford 1980, Hyder, Cayer, and Pettey 1983).

If fish leucocytes can be compared with those in mammals, similarities in function are important (Ellis 1977, Morrow and Pulsford 1980, Hyder, Cayer, and Pettey 1983). Tinctorial properties do not suggest function, but enzymes may suggest both function and lineage. However, it is not safe to base identification on enzyme cytochemistry alone; therefore, identification here is based on the following, in descending order of priority:

1. Cell morphology in Wright's-stained films, particularly the position, shape, and chromatin pattern of the nucleus; holocephalan and elasmobranch granulocytes were identified under the electron microscope;
2. Granule size and morphology;
3. Granule appearance in Periodic Acid-Schiff (PAS)-stained films;
4. Whether granule degradation and degranulation are apparent;
5. Tinctorial properties in Wright's-stained films;
6. Enzyme cytochemistry profile.

Granulocytes were identified by use of Wright's-, PAS-, and peroxidase-stained films (the latter two highlighted granules).

Basophils were rarely seen, but two granulocytes were common in teleost blood films. These were fine granulocytes (here called neutrophils), with a one- or two-lobed (or rarely polymorphonuclear) nucleus with marginated chromatin, which were usually neutrophilic or weakly basophilic and coarse granulocytes (here called eosinophils), with a one- or two-lobed, eccentric, often pycnotic, nucleus and large spherical or oval granules that were sometimes colourless in Wright's stain, were very large in degenerating cells, or were sometimes absent from degranulated cells. The use of the terms neutrophil and eosinophil, though indicating a similarity to their mammalian counterparts, is not intended to suggest strict functional similarity. Agnathan, holocephalan, and elasmobranch granulocytes could not be correlated with those of teleosts and higher vertebrates at the light or electron microscope level. Consequently, different terminology is used for these cells; in agnathans, they are granulocytes; in holocephalans, fine and coarse granulocytes; and in elasmobranchs, fine and coarse eosinophilic granulocytes and neutrophilic granulocytes. Elasmobranch granulocytes sometimes showed tinctorial properties not expected on the basis of morphology and were classified on the basis of ultrastructural studies (Hine and Wain 1987a, 1987b, 1987c). In these situations morphology at the light microscope level was a more reliable identifier than tinctorial properties. In general, holocephalan coarse granulocytes are equivalent to the eosinophils of higher vertebrates, but other granulocyte types cannot be compared directly (Hine and Wain in press). In contrast to Fey's (1966b) consistent observation of basophils, basophils were rarely seen and are not included here. In addition, unless specified, immature cells of indeterminate lineage occasionally seen in peripheral blood are not included.

MATERIALS AND METHODS

Blood from severed caudal vessels of moribund fish taken from fresh water by electric fishing and at sea by trawling or longlining was collected in heparinised capillary tubes, and air-dried films were prepared. Imprints of excised anterior kidney and spleen of some teleosts, and postorbital tissue, thymus, and spleen of holocephalans (Fänge and Sundell 1969), were also prepared.

Commercial kits (Sigma (Σ) Histozym ™) for peroxidase (perox.), alkaline phosphatase (alk. phos.), acid phosphatase (ac. phos.), β-glucuronidase (β-gluc.), α-naphthyl acetate esterase (ANAE), α-naphthyl butyrate esterase (ANBE), and naphthol AS-D chloracetate esterase (AS-D) gave good reproducible results, and alk. phos., ac. phos., ANAE, and AS-D kits were used routinely. Peroxidase was also demonstrated with either o-tolidine (Inoko and Itoga 1974), 4-chloro-1-naphthol (Elias 1980), or paraphenylenediamine-pyrocatechol (Hanker *et al.* 1977) as substrates. Other techniques used successfully were: β-gluc. (Lorbacher, Yam, and Mitus 1967), β-galactosidase (Yarborough *et al.* 1967), N-acetyl-β-glucosaminidase (N-ac-β-gluc.) (Hayashi 1965), ANBE (Lawrence and Grossman 1980), AS-D (Li, Lam, and Yam 1973, Moloney, McPherson, and Fliegelman 1960, Yam, Li, and Crosby 1971), acetyl-L-tyrosine-α-naphthyl esterase and tosyl-L-lysine-α-naphthyl esterase (Honda *et al.* 1983), and PAS (Barber and Mills Westermann 1975). For most species, only perox. (Hanker *et al.* 1977), alk. phos. (Σ), ac. phos. (Σ), ANAE (Σ), AS-D (Σ), and β-gluc. (Lorbacher, Yam, and Mitus 1967) were used. Wright's stain was used routinely.

Potassium cyanide (Yam, Li, and Crosby 1971), sodium azide (Bujak and Root 1974), and 3-amino-1,2,4 triazole (Romeo *et al.* 1973) were used as inhibitors for perox.; tartaric acid as an inhibitor for ac. phos. (Yam, Li, and Lam 1971); sodium fluoride as an inhibitor for non-specific esterases (Braunsteiner and Schmalzl 1970); and urea as an inhibitor for alk. phos. (Grozdea, Vergnes, and Martin 1983). As heparin may inhibit N-ac-β-gluc. (West, Dunphy, and Moore 1983), peritoneal fluid containing inflammatory macrophages induced by zymosan injected intraperitoneally in short-finned eels (*Anguilla australis*) (authors' unpublished data), and collected in heparinised tubes, was tested for N-ac-β-gluc. and was positive, which showed that no inhibition had occurred. Human and trout blood films were used as controls. Typical examples of each cell type per species which showed a distinct reaction were photographed.

Details of the appearance of Wright's-stained leucocytes are brief and are intended only to give other workers a guide to cell identity and the relative occurrence of granulocytes and monocytes. Differential counts could not be made because of the small sample size and, in some species (e.g., *Idiacanthus niger*), small blood volume (less than 0.1 ml). Where blood is described as thin, indicating a low haematocrit reading, the observation is based on the number of cells in relation to the blood volume in the films. The method of scoring staining intensity is similar to that used in mammals (Braunsteiner and

Schmalzl 1970, Yam, Li, and Crosby 1971) and other fish species (Fey 1966a, 1966b, 1966c), and scoring blind from numbered slides gave good reproducible results.

Classification is according to Nelson (1984). Numbers of species in each major taxonomic group examined for the main enzymes are given in Table 1.

TABLE 1: Numbers of species in each major taxonomic group examined for the main enzymes

	Perox.	Alk. phos.	Ac. phos.	β-gluc.	ANAE	AS-D	PAS
Agnatha	2	2	2	2	2	1	2
Elasmobranchii	24	20	19	19	20	17	19
Holocephali	6	6	5	4	5	5	6
Elopomorpha	13	10	11	4	9	9	8
Clupeimorpha	3	0	1	3	1	1	1
Salmoniformes	14	11	14	8	13	12	11
Stomiiformes	10	6	8	6	9	9	7
Scopelomorpha	6	3	5	6	5	5	5
Paracanthopterygii	12	11	11	7	9	9	11
Acanthopterygii	68	51	53	38	41	39	45
	158	120	129	97	114	107	115

Key to abbreviations

Enzymes and inhibitors

Ac. phos.	Acid phosphatase
Alk. phos.	Alkaline phosphatase
ANAE	α-naphthyl acetate esterase
ANBE	α-naphthyl butyrate esterase
AS-D	Naphthol AS-D chloroacetate esterase
at	3-amino-1,2,4 triazole
ATNE	Acetyl-L-tyrosine-α-naphthyl esterase
β-galact.	β-galactosidase
β-gluc.	β-glucuronidase
KCN	Potassium cyanide
N-ac-β-gluc.	N-acetyl-β-glucosaminidase
NaF	Sodium fluoride
NaN_3	Sodium azide
PAS	Periodic Acid-Schiff
Perox.	Peroxidase
ta	Tartaric acid
TLNE	Tosyl-L-lysine-α-naphthyl esterase

Reaction product intensity

−	Negative
+	Weak positive
++	Moderate positive
+++	Strong positive

Bold symbols show the most common intensity
Parentheses mean negative or the positive result shown
Arrows show the range of results

Granule staining (Wright's stain)

w	Weak
s	Strong
b	Basophilic
n	Neutrophilic
e	Eosinophilic
c	Colourless

Abundance (Ab)

1	Rare
2	Uncommon
3	Common
4	Very common
5	Abundant

Cell type

G	Granulocytes
N	Neutrophils
FG	Fine granulocytes
CG	Coarse granulocytes
FEG	Fine eosinophilic granulocytes
CEG	Coarse eosinophilic granulocytes
NG	Neutrophilic granulocytes
E	Eosinophils
M	Monocytes
L	Lymphocytes
T	Thrombocytes

Other abbreviations

(Ag1)	Species number
Ag	Agnathans
E	Elasmobranchs
H	Holocephalans
El	Elopomorphs
C	Clupeiforms
S	Salmoniforms
St	Stomiiforms
Sc	Scopelomorphs
P	Paracanthopterygians
A	Acanthopterygians
(7)	No. of fish examined
nl	No. of nuclear lobes.
MEL	Mean erythrocyte length (μm)
MD	Mean depth at which taken (m)
N/S	Not seen
N/D	Not determined
N/T	Not tested
*	Photograph in plates

RESULTS

Superclass: Agnatha

Order: Petromyzontiformes

(Ag1) *Geotria australis* (Petromyzontidae)
Lamprey
G: n→wb, nl 1, Ab 4. MEL 15.1; freshwater

	Ammocoetes (10)		Macrophthalmia (6)		Adults (4)	
	G	L	G	L	G	L
Perox.	–	–	(+)?*	–	–	–
Alk. phos.	+→+++*	–	+	–	++→+++*	
Ac. phos.	–	–	–	–	(+)	–
Ac. phos. + ta	–	–	–	–	–	–
β-gluc.	–	–	–	–	–	–
ANAE	–→++	+	+	–	–	–
ANAE + NaF	(+)	–	–	–	–	–
ANBE	–	–	–	–	N/D	N/D
AS-D	–	–	–	–	→++*	+*
PAS	+→+++	+→+++	N/D	N/D	+→+++*	+++*

Remarks: Figs. 1–9. Granulocytes with fine or coarse granules not easily differentiated (Fig. 1); clumped, apparently perox. granules (Fig. 2) may represent lysed macrophthalmia granulocytes; granulocyte alk. phos. in fine granules in ammocoetes (Fig. 3), but in both fine (Fig. 4) and coarse (Fig. 5) granules in adults; both granule types weakly ac. phos.; granulocyte and lymphocyte AS-D (Figs. 6 and 7) and PAS (Figs. 8 and 9).

Order: Myxiniformes

(Ag2) *Eptatretus cirrhatus* (Eptatretidae)
Hagfish (4)
G: n→wb, nl 1–2, Ab 3. MEL 23.8; MD unknown

	G	L
Perox.	–	–
Alk. phos.	–→++	–
Ac. phos.	+→+++*	–→+++
β-gluc.	–	–→++
ANAE	–	–
PAS	+→+++	+→+++*

Remarks: Figs. 10 and 11. Erythrocytes with cyanide-resistant perox. inclusions often associated with trypanosomes; granulocyte alk. phos. inhibited by urea; monocyte-like cells ac. phos. –→++ in granules of irregular size; thrombocyte ac. phos. –→++ and erythrocyte ac. phos. ++ in granules.

Superclass: Gnathostomata

Class: Chondrichthyes

Subclass: Elasmobranchii

Order: Hexanchiformes

(E1) *Heptranchias perlo* (Hexanchidae)
Sharpsnouted sevengill shark (3)
NG: wb→n, nl 1, Ab 3. FEG: c→we, nl 1, Ab 3. MEL 21.4; MD unknown

	FEG	NG	M	L	T
Perox.	–	–	–	–	–
β-gluc.	–	–	–	–	–

Remarks: Erythrocyte perox. ++ diffuse.

Order: Lamniformes

(E2) *Isurus oxyrinchus* (Isuridae)
Mako shark (1)
FEG: n, nl 1–5, Ab 3–4. CEG: c, nl 1–2, Ab 3. MEL 30.0; MD unknown

	FEG	CEG	M	L	T
Perox.	–	–	–	–	–
Alk. phos.	–	–	–	–	–

Remarks: Erythrocyte perox. ++ diffuse.

(E3) *Cephaloscyllium isabella* (Scyliorhinidae)
Carpet shark (4)
FEG: wb→we, nl 1, Ab 3–4. MEL 30.0; MD 74.

	FEG	M	L	T
Perox.	–	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	–	–	–	–
β-gluc.	–	–	–	–
ANAE	+→+++*	–	–	–
AS-D	++	–	++→+++*	–
PAS	+→+++	–	+→++	+→++

Remarks: Figs. 12–13. FEG nucleus irregular, but not distinctly lobed; erythrocyte perox. ++ diffuse.

(E4) *Apristurus* sp. (Scyliorhinidae)
Deepwater catshark (4)
FEG: we→wb, nl 1, Ab 3. NG: n, nl 1–3, Ab 3. M: Ab 2. MEL 29.8; MD 1103

	FEG	NG	M	L	T
Perox.	–	–	–		–
Alk. phos.	–	–	–	–	–
Ac. phos.	++→+++	+→+++	+→++	++→+++	++→+++
β-gluc.	–	–	–	–	–
ANAE	++→+++	+→+++	–	+→++	–
ANAE + NaF	++→+++	+→+++	–	+→++	–
ANBE	+++	++→+++	+++	+→+++	+→++
AS-D	++→+++	++→+++	++→**+++**	++→+++	++
AS-D + NaF	++→+++	++→+++	++→**+++**	–	–
PAS	++→+++	+→+++	N/D	(+)	(+)

Remarks: Granulocyte AS-D granular, but monocyte AS-D in heavy intracellular deposits; erythrocyte perox. +→++ diffuse.

(E5) *Mustelus lenticulatus* (Carcharhinidae)
Rig (4)
FEG: b→n, nl 1–4, Ab 3–5. CEG: b→e, nl 1–3, Ab 3. M: Ab 2–3. MEL 20.3; MD 129

	FEG	CEG	M	L	T
Perox.	–	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	–→+++*	–→+++	+→+++	+→+++	+→+++
β-gluc.	–	–	–	–	–
ANAE	–→++*	–	–	+→+++	+→++
ANBE	+	–	–	–→++	–
PAS	+→++	+→++*	–	N/D	N/D

Remarks: Figs. 14–16. Erythrocyte perox. ++→+++ diffuse; CEG PAS between coarse granules.

(E6) *Gollum attenuatus* (Carcharhinidae)
Slender smoothhound (2)
FEG: n→we, nl 1, Ab 2. NG: c→n, nl 1, Ab 3. MEL 31.4; MD unknown

	FEG	NG	M	L	T
Perox.	–	–	–	–	–
β-gluc.	–	–	–	–	–

Remarks: Erythrocyte perox. +→++ diffuse.

(E7) ***Galeorhinus australis*** **(Carcharhinidae)**

School shark (4)

FEG: e, nl 1–5, Ab 4. CEG: we→e, nl 1, Ab 2–3. NG: n, nl 1–5, Ab 2–3. M: Ab 2. MEL 23.6; MD 135

	FEG	CEG	NG	M	L	T
Perox.	−	−	−	−	−	−
Alk. phos.	−	−	−	−	−	−
Ac. phos.	−	(+)	−	(+)	+	+
Ac. phos. + ta	−	−	−	−	−	−
β-gluc.	−	−	−	−	−	−
ANAE	+→++	+→++	+→++	−	−	−
ANAE + NaF	+	+	+→++	−	−	−
AS-D	−→+++	−→+++*	−→++	−	+→++	+
PAS	+→++	+	+→++	N/D	++	++*

Remarks: Figs. 17–20. Erythrocyte perox. ++ diffuse.

(E8) ***Prionace glauca*** **(Carcharhinidae)**

Blue shark (1)

FEG: wb→n, nl 1–4, Ab 3. CEG: e, nl 1–2, Ab 2–3. M: Ab 2–3. MEL 23.5; MD 20

	FEG	CEG	M	L	T
Perox.	−	−	−	−	−
Ac. phos.	+	+++	(+)	(+++)	(++)
ANAE	+→++	−	−	−	−
AS-D	−	−	+→++	(+++)	(+++)
PAS	++→+++	++	−	−	−

Remarks: CEG ac. phos. in fine granules and over some coarse granules; lymphocyte and thrombocyte ac. phos. and AS-D usually −; CEG PAS in fine granules between coarse granules; erythrocyte perox. +→++ diffuse.

Order: Squaliformes

(E9) ***Oxynotus bruniensis*** **(Squalidae)**

Prickly dogfish (3)

NG: sb, nl 1, Ab 4. CEG: b, nl 1, Ab 2. MEL 33.5; MD 862

	CEG	NG	M	L	T
Perox.	N/D	(+)?	−	−	−
Alk. phos.	−	−	−	(+)	−
Ac. phos	+	+→+++	−	−→+++*	−
Ac. phos. + ta	+	+→+++	−	−→+++	−
β-gluc.	−	−	−	−	−
ANAE	+++*	+++	−	−	−
ANBE	+++	+++	++	−→++	N/D
AS-D	−→++	−→++	−	−	−
PAS	N/D	++*	−	+++*	++→+++

Remarks: Figs. 21–24. CEG ac. phos. in fine granules between coarse granules; lymphocyte alk. phos. (rarely +++); NG ac. phos. usually +→++, lymphocyte usually −, spleen macrophage +++; blast ANBE usually +→++, lymphocyte usually − or +, spleen macrophage +++; ANBE always diffuse; blast AS-D +→++, spleen macrophage +++; erythrocyte perox. + diffuse.

(E10) ***Squalus acanthias*** **(Squalidae)**

Spotted spiny dogfish (4)

FEG: b→we, nl 1, Ab 3–4. NG: b→n, nl 1–2, Ab 3. MEL 23.3; MD 52

	FEG	NG	M	L	T
Perox.	−	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	+++	++→+++	−	(+)	(+)
β-gluc.	−	−	−	−	−
ANAE	N/D	++→+++*	−	−	−
AS-D	−	+→++	−	−	−
PAS	++	+→++	(+)	(+)	(+)

Remarks: Figs. 25–27. NG ac. phos.; ANAE and AS-D around granules.

(E11) *Squalus blainvillei* (Squalidae)
Grey spiny dogfish (4)
FEG: wb→we, nl 1–2, Ab 3–4. NG: b→n, nl 1–2, Ab 3. MEL 25.2; MD 176

	FEG	NG	M	L	T
Perox.	−	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	++→+++	++→+++	−	(+)	(+)
β-gluc.	−	−	−	−	−
ANAE	++→+++	++→+++	−	−	−
AS-D	−	+→++*	−	−	−
PAS	++	+→++	(+)	+→++	(+)

Remarks: Fig. 28. NG AS-D diffuse around cell membrane (Fig. 28); erythrocyte perox. ++ diffuse.

(E12) *Deania calcea* (Squalidae)
Shovelnose spiny dogfish (7)
FEG: wb→we, nl 1, Ab 1–2. CEG: se, nl 1, Ab 3–4. NG: wb, nl 1, Ab 2–3. MEL 28.5; MD 897

	CEG	NG	M	L	T
Perox.	(+)?	−	−	−	−
Alk. phos.	−	−	−	+→++	+→++
Ac. phos.	++→+++*	−→+++	+→++	−→++	−→++
β-gluc.	−	−	−	−	−
ANAE	−→+++	+→+++*	−	−	−
ANBE	+++	++→+++	++→+++	−	−
AS-D	−	(+)	−	−	−
PAS	+→+++	+→+++	(+)	++	N/D

Remarks: Figs. 29–30. CEG perox. (+); normally + in Leydig's organ and +→++ in spleen; alk. phos. urea resistant; CEG precursors ac. phos. −; ac. phos. diffuse around coarse granules stained intensely (Fig. 29); CEG and NG ANAE intra- and extracellular (Fig. 30), CEG ANAE produced by fine granules between coarse granules; CEG ANBE similar to ANAE, but not extracellular; CEG PAS between coarse granules; erythrocyte perox. ++ diffuse.

(E13) *Scymnodon plunketi* (Squalidae)
Plunket's shark (2)
NG: wb→e, nl 1, Ab 3–4. M: Ab 2. MEL 33.7; MD 973

	NG	M	L	T
Perox.	−	−	−	−
Alk. phos.	−	−	−	−
Ac. phos.	+→+++	+→++	+→++	(+)
Ac. phos. + ta	−	−	−	−
ANAE	+++*	−	−	−
ANAE + NaF	+++	−	−	−
ANBE	+++	+→++	+++	+→++
AS-D	+→+++	−	−	−
PAS	++→+++	+	++→+++	+++

Remarks: Fig. 31. Spleen NG β-gluc. ++→+++ in fine granules or diffuse; large lymphoid cells in spleen cyanide-resistant perox. +→++ diffuse; erythrocyte perox. ++→+++ diffuse.

(E14) *Centroscymnus crepidater* (Squalidae)
Deepwater dogfish (4)
FEG: wb→we, nl 1, Ab 1–2. NG: b→wb, nl 1–3, Ab 3–4. M: Ab 3. MEL 35.2; MD 1017

	NG	M	L	T
Perox.	(+)?	−	−	−
Alk. phos.	−	−	−	−
Ac. phos.	+→+++	++→+++	−→+++	+++
Ac. phos. + ta	++	++	−	−
ANAE	+→+++	−	−	−
ANBE	+→+++	+→++	−→++	−
AS-D	+→+++	−	−	−
PAS	++→+++	−	+→++*	+→++*

Remarks: Figs. 32–34. Erythrocyte perox. ++ diffuse; incidental observations on two other species of *Centroscymnus* showed alk. phos. − in *C. coelolepis*, but moderate to strong in lymphocytes and thrombocytes of *C. owstonii* (Figs. 32 and 33).

(E15) *Centrophorus squamosus* (Squalidae)
Deepwater spiny dogfish (5)

CEG: e, nl 1, Ab 3. NG: b, nl 1, Ab 3–4. MEL 29.6; MD 877

	CEG	NG	M	L	T
Perox.	−	−	−	−	−
Alk. phos.	−	−	−	++→+++	−
Ac. phos.	+++	+→+++*	−	−	−
β-gluc.	−	−	−	−	−
ANAE	+→++	+→+++	−	−	−
AS-D	−	−→++	−	−	−
PAS	++	+→++	−	+→+++	+→++

Remarks: Fig. 35. Alk. phos. urea resistant; erythrocyte perox. +→++ diffuse.

(E16) *Etmopterus lucifer* (Squalidae)
Lucifer dogfish (4)

FEG: e→c, nl 1, Ab 3. NG: wb→n, nl 1, Ab 3–4. MEL 28.0; MD 798

	FEG	NG	M	L	T
Perox.	(+)	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	−→+++	−→+++	−	++→+++	++→+++
β-gluc.	−	−	−	−	−
ANAE	−	++→+++	−	−	−
AS-D	−	−	−	−	−
PAS	+	+→++	−	+→++	+→++

Remarks: NG ac. phos. around and between granules; NG PAS under cell membrane.

(E17) *Etmopterus baxteri* (Squalidae)
Baxter's lantern dogfish (9)

FEG: se, nl 1–2, Ab 4. NG: wb→b, nl 1, Ab 3. MEL 35.5; MD 972

	FEG	NG	M	L	T
Perox.	−	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	+++	−→+++	−	−	−
Ac. phos. + ta	+++	−→+++	−	−	−
β-gluc.	−	++*	−	−	−
ANAE	++	++→+++*	−	−	−
ANAE + NaF	++	++→+++*	−	−	−
ANBE	−	+→+++	++	−→+++	−→++
AS-D	+→++	+→+++	+→++	+	+
AS-D + NaF	+→++	−	−	−	−
PAS	+→++	+→++	(+)	+→++*	+→++

Remarks: Figs. 36–39. FEG precursors in Leydig's organ perox. +→++; NG ac. phos. and AS-D around granules; spleen NG β-gluc. ++ (rarely +++) (Fig. 38); erythrocyte perox. +→++ diffuse; NG PAS under cell membrane and between granules.

(E18) *Dalatias licha* (Squalidae)
Seal shark (3)

FEG: we, nl 1–5, Ab 3–4. NG: b→n, nl 1, Ab 3–4. MEL 26.6; MD 819

	FEG	NG	M	L	T
Perox.	−	(+)?	−	−	−
Alk. phos.	−	−	−	+→+++*	−
Ac. phos.	++→+++	+→+++*	−	++	+→++
ANAE	N/D	+→++	−	−	−
PAS	+→++	+→++	+→++	++	++

Remarks: Figs. 40–41. Lymphocyte alk. phos. urea resistant; erythrocyte perox. ++ diffuse; NG perox. could not be unequivocally demonstrated.

Order: Rajiformes

(E19) *Torpedo fairchildi* (Torpedinidae)
Electric ray (2)
FEG: wb→e, nl 1, Ab 3. NG: wb, nl 1, Ab 3. MEL 31.0; MD 73

	FEG	NG	M	L	T
Perox.	–	–	–	–	–
Alk. phos.	–	–	–	–	–
β-gluc.	–	–	–	–	–
ANAE	+	+→++	–	+	–
AS-D	–	–	–	–	–

Remarks: NG nucleus irregular, but not distinctly lobed; erythrocyte perox. + diffuse.

(E20) *Raja nasuta* (Rajidae)
Rough skate (8)
FEG: we, nl 1–4, Ab 3. CEG: e, nl 1, Ab 5. NG: wb, nl 1, Ab 2–3. MEL 24.3; MD 103

	FEG	CEG	NG	M	L	T
Perox.	–	+	–	–	–	–
β-gluc.	–	–	–	–	–	–

Remarks: Erythrocyte perox. +→++ diffuse.

(E21) *Raja innominata* (Rajidae)
Smooth skate (5)
FEG: we, nl 1–4, Ab 3. CEG: e, nl 1, Ab 5. NG: wb, nl 1, Ab 2–3. MEL 25.4; MD 122

	FEG	CEG	M	L	T
Perox.	–	+	–	–	–
Perox. + KCN	–	+→++	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	–	+→+++*	–	+	–
β-gluc.	–	–	–	–	–
ANAE	+→++	–→++	–	+→++	++
AS-D	–	–	–	++	+→++
PAS	+	+→++	–	–	–

Remarks: Fig. 42. CEG ac. phos., ANAE, and PAS in fine granules between coarse granules; erythrocyte perox. +→++ diffuse.

(E22) *Pavoraja spinifera* (Rajidae)
Prickly deepsea skate (4)
FEG: we, nl 1, Ab 1–2. CEG: e, nl 1–2, Ab 5. NG: wb, nl 1, Ab 2. MEL 29.5; MD 944

	CEG	NG	M	L	T
Perox.	(+)	–	–	–	–
Alk. phos.	+→++*	–	–	–	–
Ac. phos.	+→++	+→++*	–	+→++	–
β-gluc.	–	–	–	–	–
ANAE	+	+→++	–	–	–
AS-D	+	–	–	–	–
PAS	+	++	–	N/D	N/D

Remarks: Figs. 43–44. All CEG enzymes occurred in fine granules between coarse granules, and PAS also membrane-associated; large (< 50 μm) trypanosomes in peripheral blood.

(E23) Undescribed skate (Rajidae)
Long-nosed pale deepsea skate (5)

FEG: we, nl 1–2, Ab 2–3. CEG: e, nl 1–2, Ab 5. NG: wb, nl 1, Ab 2–3. MEL 24.9; MD 1073

	CEG	NG	M	L	T
Perox.	+	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	−	+→++	−	++	−
β-gluc.	−	−	−	−	−
ANAE	++	+→++	−	−	−
ANBE	(+)	+→++	+→++	(+)	(+)
AS-D	+→++	+→**+++**	−	++	+
PAS	+→++	++→+++	−	−→++	+→++

Remarks: Figs. 45–47. Erythrocytes contained many ac. phos. granules (Fig. 45); CEG AS-D diffuse between coarse granules; monocyte AS-D in heavy intracellular deposits; skates contained large trypanosomes (54 μm long) with a kinetoplast ac. phos., ANAE, and ANBE.

(E24) *Myliobatis tenuicaudatus* (Myliobatidae)
Eagle ray (2)

FEG: we, nl 1–5, Ab 3. CEG: e, nl 1–2, Ab 3. NG: wb→n, nl 1, Ab 2. M: Ab 2. MEL 20.1; MD 81

	FEG	CEG	M	L	T
Perox.	−	(+)	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	−	+	−	−	−
β-gluc.	−	−	−	−	−
ANAE	+	−→++	−	−	−
PAS	+→++	++	N/D	++→+++	N/D

Remarks: CEG ac. phos. in fine granules between coarse granules.

Subclass: Holocephali

Order: Chimaeriformes

(H1) *Callorhinchus milii* (Callorhynchidae)
Elephant fish (12)

FG: n, nl 1–3, Ab 4. CG: se, nl 1, Ab 2. MEL 14.2; MD 45

	FG	CG	L
Perox.	−	−	−
Alk. phos.	+++*	−	−
ANAE	−	−	−
AS-D	−	−	−
PAS	++	(+)	N/D

Remarks: Fig. 48. Fine and coarse granulocytes and lymphoid cells were the only morphologically differentiated types readily identifiable in H1–4; thymic fine granulocyte PAS ++, but cells seldom seen; fine granulocyte PAS +++ in post-orbital tissue.

(H2) *Chimaera phantasma* (Chimaeridae)
Giant ghost shark (2)

FG: n, nl 1, Ab 2–3. CG: e, nl 1, Ab 3–4. MEL 23.3; MD 987

	FG	CG	L
Perox.	−	−	−
Alk. phos.	++→+++*	−	−
Ac. phos.	+→+++*	++→+++*	(+)
β-gluc.	−	−	−
ANAE	+→++	+→+++	+→++
ANBE	−	−	−
AS-D	+→++*	N/D	N/D
PAS	++	(+)	−

Remarks: Figs. 49–51. Leucocytes in tissues also perox. −; in thymus, post-orbital tissue, and spleen fine granulocyte alk. phos. also ++→+++, but, though coarse granulocyte alk. phos. − in thymus and spleen, weak reactivity was seen between coarse granules in post-orbital tissue; incidental observations on alk. phos. in the blood and post-orbital tissue of two undescribed deepwater black and brown *Chimaera* spp. showed fine granulocyte alk. phos. +++; non-specific staining for both phosphatases was heavier in thymus than spleen; leucocyte ac. phos. was the same in tissues and blood; tissue lymphocyte ANAE could not be determined because of heavy non-specific staining.

(H3) *Hydrolagus novaezelandiae* (Chimaeridae)

Dark ghost shark (10)

FG: b, nl 1, Ab 3. CG: se, nl 1, Ab 3. MEL 17.5; MD 364

	FG	CG	L
Perox.	−	−	−
Alk. phos.	+++*	−	−
Ac. phos.	+→+++*	(+)*	−
Ac. phos. + ta	−	−	−
β-gluc.	−	−	−
ANAE	−	−	−
AS-D	−	−	−
PAS	++	+	−

Remarks: Figs. 52–53. Fine granulocyte ac. phos. and alk. phos. and also strong in post-orbital, thymus, and spleen imprints, but cells (thought to be fine granulocyte precursors) in post-orbital tissue were weaker ac. phos., −→++, but became stronger as cells developed; coarse granulocyte ac. phos. in spleen weak and inconsistent, and in post-orbital and thymus imprints showed no activity; spleen phagocytes +++ for tartrate-resistant ac. phos., fluoride-inhibited ANAE, and possibly AS-D, +→+++, though the latter could not be distinguished easily from coarse granulocytes.

(H4) *Hydrolagus* sp. (Chimaeridae)

Pale ghost shark (4)

FG: b, nl 1, Ab 2. CG: se, nl 1, Ab 4. MEL 20.2; MD 824

	FG	CG	L
Perox.	−	+	−
Alk. phos.	+→+++	−	+→+++
Ac. phos.	+	(+)	−
ANAE	+→++	+→++	−
ANBE	++→+++	+	−→++
AS-D	+	+	−
PAS	++→+++	+→+++	−

Remarks: Coarse granulocyte perox. +→++ in post-orbital tissue and spleen; alk. phos. urea resistant; lymphocyte alk. phos. ++→+++ in spleen; fine and coarse granulocyte ANAE +→++ in post-orbital tissue, but both ANAE ++→+++ in spleen; coarse granulocyte PAS diffuse between coarse granules and in peripheral coarse granules.

(H5) *Harriotta raleighana* (Rhinochimaeridae)

Long-nosed chimaera (4)

(*see* Hine and Wain (in press)) G: b, nl 1, Ab 2. Fine granules. MEL 20.6; MD 959

	G	L
Perox.	−	−
Alk. phos.	−	−
Ac. phos.	−	−
β-gluc.	−	−
PAS	++	−

Remarks: Fig. 54. Post-orbital imprints showed the same staining as blood given above, but thymic granulocytes were tartrate-inhibited ac. phos. + and spleen lymphoid cells were alk. phos. and tartrate-inhibited ac. phos. (+); coarse granulocytes in thymus were ANAE − or +++ and PAS ++→+++; these coarse granulocytes were tartrate-inhibited ac. phos. +++ in spleen (Fig. 54).

(H6) ***Rhinochimaera pacifica*** **(Rhinochimaeridae)**
Long-nosed chimaera (4)
G: sb, nl 1, Ab 2–3. Fine granules. MEL 20.2; MD 925

	G	L
Perox.	−	−
Alk. phos.	−	−
Ac. phos.	−	(+)
β-gluc.	−	−
ANAE	−	−
ANBE	+→++	++→+++
AS-D	(++→+++)	−
PAS	+→+++	−

Remarks: Post-orbital granulocyte ANBE +→++ diffuse; coarse granulocytes in post-orbital tissue and spleen ac. phos. ++→+++ in coarse granules, and post-orbital and thymic fine granulocyte and thymic lymphocyte ac. phos. +; post-orbital coarse granulocyte ANAE +→++ in coarse granules, post-orbital and thymic fine granulocyte ANAE + in fine granules, but no activity was seen in spleen; fine granulocytes in thymus, post-orbital tissue, and spleen, and coarse granulocytes in spleen had granular staining AS-D ++→+++ in two fish, but − in the other two.

Class: Osteichthys

Infradivision: Elopomorpha

Order: Anguilliformes

(El1) ***Anguilla anguilla*** **(Anguillidae)**
European eel (12)
N: n, nl 1–2, Ab 3. E: N/S. M: Ab 3. MEL 12.7; freshwater

	N	M	L	T
Perox.	++→+++	(++)	−	−
Ac. phos.	+++	+→++	−→++	(+)
ANAE	+++	N/D	−→++	−→++
AS-D	+++	N/D	++	−

Remarks: Alk. phos. reported from neutrophils (Fey 1966b); for *Anguilla* spp. *see* Hine *et al.* (1986).

(E12) *Anguilla australis* (Anguillidae)

Short-finned eel (145)

N: n, nl 1–2 (rarely 3), Ab 3. E: c→e, nl 1, Ab 2–3. M: Ab 3. MEL 13.5; freshwater

	N	M	E	L	T
Perox.	(++→+++)	(+)	++→+++	–	–
Perox. + KCN	–	N/D	++→+++	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	+→+++	+→++	+++	–→++	+→++
Ac. phos. + ta	+→+++	(+)	N/T	–→++	+
β-galact.	–	–	–	–	–
β-gluc.	–	–	–	–	–
N-ac-β-gluc.	–	–	–	–	–
ANAE	–→+++	+→+++	++→+++	–→++	+→++
ANAE + NaF	–→+++	+→+++	N/T	–→++	(+)
ANBE	+→+++	+→++	++→+++	–→++	–
ANBE + NaF	(+)	–	N/T	–	–
AS-D	–→++	–→+++	++→+++	–→++	–
ATNE	(+)	–	N/T	–	–
TLNE	(+)	–	N/T	–	–
PAS	++→+++	+	+++	+→++	–→++

(E13) *Anguilla dieffenbachii* (Anguillidae)

Long-finned eel (94)

N: n, nl 1–2 (rarely 3), Ab 3. E: N/D. M: Ab 3. MEL 14.4; freshwater

	N	M	L	T
Perox.	(++→+++)	(+)	–	–
Perox. + KCN	–	N/D	–	–
Alk. phos.	–	–	–	–
Ac. phos.	++→+++	+→++	(+)	–
Ac. phos. + ta	+→+++	(+)	(+)	–
β-galact.	–	–	–	–
β-gluc.	–	–	–	–
N-ac-β-gluc.	–	–	–	–
ANAE	++→+++	+→+++	+→++	–→++
ANAE + NaF	+→+++	+→+++	+→++	(+)
ANBE	+	–→++	–→++	–
ANBE + NaF	–	–	–	–
AS-D	+→+++	+→+++	–→++	–
PAS	++→+++	+	(+)	(+)

Remarks: Although eosinophils were often seen their enzyme cytochemistry was not determined except for cyanide-resistant perox. ++→+++.

(E14) *Conger verreauxi* (Congridae)

Conger eel (5)

N: n→wb, nl 1–2, Ab 3. E: c→e, nl 1, Ab 2–3. M: Ab 3. MEL 15.1; MD 20

	N	M	E	L	T
Perox.	(++)	–	+++	–	–
Alk. phos.	+→+++*	–	–	–	–
Ac. phos.	+→++	++	–	–→+++	(+)
ANAE	+→++	–	N/D	–	–
AS-D	–	–	N/D	+→++	–

Remarks: Fig. 55.

(E15) *Bassanago bulbiceps* (Congridae)
Swollenhead conger eel (2)
N: we granules in wb matrix (immature) to n granules in sb matrix (mature), nl 1, Ab 4. E: c, nl 1, Ab 2. M: Ab 1. MEL 18.6; MD 911

	N	M	E	L	T
Perox.	(++)	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	+→++	+	+	+→++	−
ANAE	−	−	−	−	−
AS-D	−	−	−	−	−
PAS	−→+++	−	(+)	N/D	N/D

Remarks: Immature neutrophil perox. −, mature cells perox.; neutrophil PAS strength increased with cell maturation; eosinophil ac. phos. in coarse granules.

(E16) *Diastobranchus capensis* (Synaphobranchidae)
Basketwork eel (2)
N: e granules in sb matrix (immature) to se granules in sb matrix (mature), nl 1, Ab 3. E: c, nl 1, Ab 2. M: Ab 1. MEL 17.4; MD 963

	N	M	E	L	T
Perox.	(++→+++)	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	−→++	−	−	(+)	(+)
β-gluc.	−	−	−	−	−
ANAE	−	−	−	−	−
AS-D	−	−	−	−	−
PAS	+→+++	−	+++	−	−

Remarks: Similar to E15 overall and in perox. pattern; neutrophil ac. phos. usually −→+ (rarely ++); much extracellular ANAE in films, but its origin was not determined; melano-macrophages in anterior kidney contained cyanide-inhibited perox.

(E17) *Simenchelys parasiticus* (Simenchelyidae)
Snubnosed eel (4)
N: e in b matrix, nl 1, Ab 3. E: N/S. M: Ab 3. MEL 15.4; MD 1027

	N	M	L	T
Perox.	−	−	−	−
Alk. phos.	−	−	−	−
Ac. phos.	+→++	+→++	+→++	N/D
ANAE	+→++*	−	−	N/D
AS-D	+→++	−	(+)	−
PAS	+→+++	(+)	−	−

Remarks: Fig. 56. Neutrophil PAS usually ++.

(E18) *Serrivomer samoensis* (Serrivomeridae)
Sawtooth eel (2)
N: n, nl 1–2, Ab 4. M: Ab 3. MEL 21.5; MD 1013

	N	M	E	L	T
Perox.	−	−	++	−	−
Ac. phos.	(+)	N/D	N/D	N/D	N/D

Remarks: Eosinophils not seen in Wright's-stained films; very low haematocrit reading meant few cells were seen.

(E19) *Avocettina infans* (Nemichthyidae)
Black snipe eel (2)
N: e, nl 1, Ab 5. E: N/S. M: Ab 3. MEL 20.8; MD 1013

	N	M	L	T
Perox.	−	−	−	−
Alk. phos.	−	−→++	(+)	−
Ac. phos.	+→++	N/D	N/D	N/D
PAS	++→+++	(+)	−	−

Remarks: Monocyte alk. phos. usually − or +; large blood cells; low haematocrit reading.

(El10) ***Nettenchelys parviceps*** **(Nettastomatidae)**
Duckbill eel (1)
N: wb, nl 1–2, Ab 3. E: c, nl 1, Ab 1. M: Ab 2. MEL 19.0; MD 907

	N	M	L	T
Perox.	–	–→++	–	–

Remarks: Observations limited by single specimen.

(El11) ***Eupharynx pelecanoides*** **(Eupharyngidae)**
Gulper (1)
N: we, nl 1–2, Ab 5. M: Ab 3. MEL 25.0; MD 1170

	N	M	L	T
Perox.	–	–	–	–
Alk. phos.	–	–	–	–
AS-D	++	+→++	N/D	N/D

Remarks: Very thin blood composed largely of neutrophils, which outnumbered erythrocytes; neutrophil AS-D in uniform, medium-sized granules, whereas monocyte AS-D in irregular granules; large blood cells.

Order: Notacanthiformes

(El12) ***Halosaurus pectoralis*** **(Halosauridae)**
Halosaur (2)
N: we→wb, nl 1, Ab 4. E: N/S. M: Ab 2. MEL 16.5; MD 858

	N	M	L	T
Perox.	–	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	++→+++	+→++	–→++	+
ANAE	+→++	–	–	–
AS-D	+→++	–	–	–
PAS	++→+++	(+)	–	–

Remarks: Fig. 57. Erythrocytes large and contained ANAE, AS-D, and PAS inclusions (Fig. 57).

(El13) ***Notacanthus sexspinis*** **(Notacanthidae)**
Spineback (26)
N: e, nl 1, Ab 4. E: N/S. M: Ab 3. MEL 16.4; MD 945

	N	M	L	T
Perox.	–	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	++→+++	–	+	–
Ac. phos. + ta	+	–	–	–
β-gluc.	–	–	–	–
ANAE	+→++	–→++	–→++	–→++
ANAE + NaF	+→++	–→++	–→++	–→++
PAS	+→+++	+	+	+

Remarks: Neutrophils in anterior kidney were weaker for ANAE, +, but stronger for PAS, ++→+++; lymphocytes in spleen were stronger for ac. phos., +→++, and PAS, +→++, but weaker for ANAE, (+); a strongly perox. granulocyte was seen occasionally in anterior kidney, but was inhibited by cyanide; erythrocytes contained many AS-D ++ inclusions.

Infradivision: Clupeomorpha

Order: Clupeiformes

(C1) *Sprattus antipodum* (Clupeidae)
Sprat (4)
N: n→we, nl 1, Ab 5. E: c→b, nl 1, Ab 1. M: Ab 3. MEL 12.7; MD surface

	N	M	E	L	T
Perox.	−	−	−	−	−
Ac. phos.	−→++	−	+	+	+
Ac. phos. + ta	−	−	−	−	−
β-gluc.	−	−	−	−	−
ANAE	−	−	−	−	−
AS-D	−	−	−	−	−
PAS	++	−	−	−	−

Remarks: In anterior kidney eosinophil ac. phos.-ta +→++; macrophages in anterior kidney contained granular and diffuse β-gluc.

(C2) *Sardinops neopilchardus* (Clupeidae)
Pilchard (5)
N: wb, nl 1, Ab 2. E: c, nl 1, Ab 2. M: Ab 2. MEL 10.2; MD < 30

	N	M	E	L	T
Perox.	(++)	−	−	−	−
Perox. + KCN	−	−	−	−	−
β-gluc.	−	−	−	−	−
ANBE	(+)	−	−	−	−

Remarks: Anterior kidney eosinophil cyanide-resistant perox. +; neutrophil ANBE diffuse; anterior kidney phagocyte ANBE ++→+++ in heavy deposits.

(C3) *Engraulis australis* (Engraulididae)
Anchovy (4)
N: we, nl 1, Ab 2. E: c, nl 1, Ab 3. M: Ab 2. MEL 12.2; MD < 20

	N	M	E	L	T
Perox.	−	−	−	−	−
β-gluc.	−	−	−	−	−

Remarks: Anterior kidney eosinophil cyanide-resistant perox. +.

Infradivision: Euteleostei

Superorder: Protacanthopterygii

Order: Salmoniformes

(S1) *Bathylagus antarcticus* (Bathylagidae)
Deepsea smelt (2)
N: wb, nl 1–2, Ab 3. E: N/S. M: Ab 2–3. MEL 23.0; MD 1100

	N	M	L	T
Perox.	++	−	−	−
Perox. + KCN	−	−	−	−
Ac. phos.	−	−	−	−
β-gluc.	−	−	−	−
ANAE	−	−	−	−
AS-D	++	−	−	−

(S2) *Rouleina* sp. (Alepocephalidae)
Black slickhead (10)
N: b, nl 1, Ab 4. E: sb, nl 1, Ab 3. M: Ab 3. MEL 24.7; MD 890

	N	M	E	L	T
Perox.	++	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	(+)	−	−	−	−
β-gluc.	−	−	−	−	−
ANAE	−	−	−	−	−
AS-D	−	−	−	−	−
PAS	+→+++*	(+)	+++*	−	−

Remarks: Figs. 58–60. With Wright's stain eosinophils had strongly basophilic cytoplasm containing colourless coarse granules with some loss of granule contents (Fig. 58); all blood cells were large, similar to those of elasmobranchs, and the haematocrit reading was often low; spleen eosinophil perox. ++ (Fig.59).

(S3) *Talismania* sp. (Alepocephalidae)
Slickhead (2)
N: sb, nl 1, Ab 3. E: sb, nl 1, Ab 5. M: Ab N/D. MEL 25.7; MD 998

	N	M	E	L	T
Perox.	++	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	−	−	−	+→+++*	−
Ac. phos.	+→++	−	(+)	(+)	−
PAS	++→+++*	−	+++*	−	−

Remarks: Figs. 61–62. Large blood cells; low haematocrit reading; note the abundance of eosinophils in blood; eosinophil ac. phos. in a few fine granules.

(S4) *Alepocephalus* sp. (Alepocephalidae)
Large-scaled brown slickhead (3)
N: sb with e granules, nl 1–2, Ab 3. E: b matrix with c granules, nl 1, Ab 4. M: Ab 3. MEL 19.8; MD 1034

	N	M	E	L	T
Perox.	++→+++	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	+→+++*	−	−→++*	−	−
Ac. phos.	++	−	(+)	−	−
β-gluc.	−	−	−	−	−
ANAE	+→++	−	−	−	−
AS-D	+	−	−	−	−
PAS	++→+++	(+)	+→++	−	−

Remarks: Figs. 63–64. Large blood cells; in blood eosinophil alk. phos., ac. phos., and PAS in small granules between large coarse granules; eosinophil alk. phos. urea resistant; in neutrophil alk. phos. staining usually concentrated in a central dense point (Fig. 63).

(S5) ***Alepocephalus australis*** **(Alepocephalidae)**
Small-scaled brown slickhead (4)
N: wb, nl 1, Ab 2. E: wb, nl 1, Ab 5. M: Ab 2. MEL 26.2; MD 1093

	N	M	E	L	T
Perox.	+→++	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	+→++	–	(+)	–	++
ANAE	++→+++	–	++→+++	–	–
ANBE	+→++	++→+++	–	–	–
AS-D	+→++	–	–	–	–
PAS	++→+++	–	+++	–	–

Remarks: Large blood cells; occasionally spleen eosinophils stain deep burgundy with Wright's stain; eosinophil ac. phos. occurred as 1–2 fine granules between coarse granules; note the dominance of eosinophils among blood granulocytes; myxosporean trophozoites in blood.

(S6) ***Persparsia kopua*** **(Searsiidae)**
(6)
N: n→wb, nl 1–2, Ab 3. E: c→b, nl 1, Ab 2. M: Ab 2. MEL 26.0; MD 520

	N	M	E	L	T
Perox.	+→++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Perox. + NaN$_3$	+→++	–	–	–	–
Perox. + at	+→++	–	–	–	–
Ac. phos.	++	N/D	(+)	++	+
Ac. phos. + ta	–	–	–	–	–
β-gluc.	–	–	–	–	–
ANAE	–→++	–	(+)	–	–
ANBE	–	–	–	–	–
AS-D	–	–	–	–	–
PAS	++→+++	–	+→++*	–	–

Remarks: Fig. 65. Eosinophil ac. phos., ANAE, and PAS in fine granules between coarse granules.

(S7) ***Retropinna retropinna*** **(Retropinnidae)**
Common smelt (18)
N: wb→b, nl 1–2, Ab 3. E: N/S. M: Ab 2–3. MEL 11.5; freshwater

	N	M	L	T
Perox.	++→+++	–	–	–
Perox. + KCN	–	–	–	–
Perox. + NaN$_3$	++→+++	–	–	–
Perox. + at	++→+++	–	–	–
Ac. phos.	–	–→++	+→++	–
Ac. phos. + ta	–	–	–	–
β-gluc.	–	–	–	–
ANAE	–	–	–	–
ANBE	–	–	–	–
AS-D	–	–	–	–
PAS	+→++	(+)	–	–

(S8) ***Galaxias brevipinnis*** **(Galaxiidae)**
Koaro (2)
N: n→wb, nl 1, Ab 4. E: N/S. M: Ab 3. MEL 12.0; freshwater

	N	M	L	T
Perox.	++→+++	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	(+)	(+)	(+)	–
ANAE	–	–	–	–
ANBE	–	–	–	–
AS-D	–	–	–	–

Remarks: Neutrophil precursors in anterior kidney also lacked alk. phos.

(S9) *Galaxias divergens* (Galaxiidae)

Dwarf galaxias (4)

N: n→wb, nl 1, Ab 2–3. E: N/S. M: Ab 4. MEL 14.1; freshwater

	N	M	L	T
Perox.	+++	+	–	–
Perox. + KCN	–	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	+	+→++	+→++	–
ANAE	–	–	–	–
AS-D	–	–	–	–
PAS	+	–	–	–

Remarks: Immature erythrocyte cytoplasm weakly diffuse for cyanide-resistant perox.

(S10) *Galaxias maculatus* (Galaxiidae)

Inanga (5)

N: n, nl 1, Ab 3. E: N/S. M: Ab 4–5. MEL 11.9; freshwater

	N	M	L	T
Perox.	+++	+→++*	–	–
Alk. phos.	–	–	–	–
Ac. phos.	–	(+→++)	(+)	–
ANAE	–	–	–	–
ANBE	–	–	–	–
AS-D	–	–	–	–
PAS	+	–	–	–

Remarks: Deep granular basophilia in monocyte cytoplasm with perox.

(S11) *Salmo trutta* (Salmonidae)

Brown trout (78)

N: n→wb, nl 1–4, Ab 3. E: N/S. M: Ab 3. MEL 16.0; freshwater

	N	M	L	T
Perox.	+++	(+)	–	–
Perox. + KCN	–	–	–	–
Perox. + NaN_3	++→+++	–	–	–
Perox. + at	++→+++	–	–	–
Alk. phos.	–→++	–	–	–
Ac. phos.	(+)	–→++	+→+++	–
Ac. phos. + ta	(+)	–	–	–
β-galact.	–	–	–	–
β-gluc.	(+→++)	(+→++*)	(+→++)	(+)
N-ac-β-gluc.	–	–	–	–
ANAE (Yam*)	(+)	–	–	(+)
ANAE (Σ)	–	–	(+→++)	–
ANBE	–	–	–	–
AS-D	–	–	(+→++)	–
ATNE	(+)	–	–	–
TLNE	(+)	–	–	–
PAS	+→++	–	–	–

*Yam, Li, and Crosby 1971.

Remarks: Figs. 66–67. Anterior kidney blast perox. (+) with Hanker *et al.*'s (1977) technique, – with Inoko and Itoga's (1974) technique; anterior kidney neutrophil precursor perox. (from the terminology of Blaxhall and Daisley (1973)), myelocyte –→+++, metamyelocyte +→+++, neutrophil +++ (Hanker *et al.* 1977); myelocyte –→++, metamyelocyte +→++, neutrophil +++ (Inoko and Itoga 1974); blood neutrophil perox. +→+++ with Elias's (1980) technique; at ph 9.0, blood neutrophil perox. +++, monocyte +, lymphocyte +, thrombocyte – (Hanker *et al.* 1977); anterior kidney neutrophil alk. phos. +→+++, spleen neutrophil (+); alk. phos. urea inhibited; anterior kidney neutrophil ac. phos. ++→+++, monoblast ++ (+ ta was –), monocyte +→++, lymphocyte ++→+++, and phagocyte +++; however, in spleen, neutrophil ac. phos. –→++, monocyte +→++, lymphoblast +++, large lymphocyte ++, small lymphocyte –, and phagocyte +++; seven trout contained weakly β-gluc. blood leucocytes (Fig. 66), but positive spleen macrophages phagocytising erythrocytes were common in most fish (Fig. 67); in anterior kidney all cells were ANAE – (Yam, Li, and Crosby 1971), but with *Σ* kit were + in anterior kidney and spleen monocytes; blood lymphocyte ANAE (Σ), large lymphocyte

−, small lymphocyte +→++, but with AS-D large lymphocyte +→++, small lymphocyte −; β-gluc. was very variable between individual fish, being absent in many; ATNE- and TLNE-stained neutrophils were rare and very weak.

(S12) *Salmo gairdnerii* (Salmonidae)

Rainbow trout (8)

N: n→wb, nl 1–5, Ab 3. E: N/S. M: Ab 3. MEL 15.1; freshwater

	N	M	L	T
Perox.	++→+++	(+)	−	−
Perox. + KCN	−	−	−	−
Alk. phos.	+→+++	−	−	−
Ac. phos.	(+)	+→+++	+→+++	−
Ac. phos. + ta	(+)	−	−	−
ANAE	−	−	−	−
AS-D	−	(+)	−	−
PAS	+→++	−	−	−

Remarks: Figs. 68–69. Peritoneal eosinophil ANAE (Fig. 68) and AS-D (Fig. 69) −→+++ in fine granules or diffuse between coarse granules; often moderately to strongly stained; blood neutrophils had longitudinal ridge of alk. phos. in centre of nucleus and its lobes.

(S13) *Oncorhynchus tshawytscha* (Salmonidae)

Quinnat or chinook salmon (14)

N: n→wb, nl 1–4, Ab 3. E: N/S. M: Ab 2. MEL 15.1; freshwater

	N	M	L	T
Perox.	++→+++	−→++	−	−
Perox. + KCN	**+**→++*	−	−	−
Alk. phos.	+→+++	−	−	−
Ac. phos.	+	++→+++	++→**+++***	++→+++
β-galact.	−	−	−	−
β-gluc.	−	−	−	−
N-ac-β-gluc.	−	−	−	−
ANAE	−	(+)	(+)	N/D
AS-D	−	(+)	−	−
PAS	+	(+)	−	−

Remarks: Figs. 70–72. Blasts in blood perox. +→++ (Hanker *et al.* 1977), PAS **+**→++, and β-galact., β-gluc., N-ac-β-gluc., and AS-D −; spleen lymphocytes with distinctive ac. phos. staining pattern (Fig. 71); peritoneal eosinophil ANAE and AS-D ++→+++; alk. phos. figures in the neutrophil nucleus (Fig. 72).

(S14) *Oncorhynchus nerka* (Salmonidae)

Sockeye salmon (3)

N: n, nl 1–4, Ab 2–3. E: N/S. M: Ab 2. MEL 16.2; freshwater

	N	M	L	T
Perox.	+→++	N/D	−	N/D
Perox. + KCN	−	−→++	−	N/D
Alk. phos.	+→+++	−	−	−
Ac. phos.	**+**→++	+→++	++→+++	N/D
β-gluc.	−	−	−	−
ANAE	−	−	−	N/D

Remarks: Perox. cells resembling monocytes seen when incubated with cyanide in one fish, they may also have been degranulated eosinophils with residual activity; neutrophil ac. phos. rarely seen and confined to 1–2 granules; neutrophil alk. phos. in surface granules.

Superorder: Stenopterygii

Order: Stomiiformes

(St1) ***Gonostoma elongatum*** **(Gonostomatidae)**
Lightfish (2)
N: wb→n, nl 1, Ab 3. E: c→wb, nl 1, Ab 3. M: Ab 4. MEL 15.4; MD mesopelagic

	N	M	E	L	T
Perox.	+→++	−	++	−	−
Perox. + KCN	−	−	+	−	−
Alk. phos.	+→++	−	−	−	−
Ac. phos.	−	+	(+)	−	−
ANAE	−	−	−	−	−
AS-D	+→++	+	−	−	−
PAS	+→++	−	+++	−	−

Remarks: Large blood cells; low haemotocrit reading; eosinophil ac. phos. in fine granules; monocytes contained one large and several small granules.

(St2) ***Argyropelecus gigas*** **(Sternoptychidae)**
Giant hatchetfish (3)
N: wb, nl 1–4, Ab 3. E: N/S. M: Ab 3. MEL 16.5; MD 850

	N	M	L	T
Perox.	++	−	−	−
Perox. + KCN	−	−	−	−
ANAE	−	−	−	−
AS-D	−	−	−	−
PAS	++	+	−	−

Remarks: Large blood cells; moderate to low haematocrit reading; diffuse staining of erythrocyte nuclear membrane with ANAE.

(St3) ***Argyropelecus hemigymnus*** **(Sternoptychidae)**
Common hatchetfish (2)
N: wb, nl 1–4, Ab 3. E: N/S. M: Ab 3. MEL 22.5; MD 1170

	N	M	L	T
Perox.	++	−	−	−
Perox. + KCN	−	−	−	−
Alk. phos.	−	−	−	−
Ac. phos.	++	(+)	++	−

(St4) ***Maurolicus muelleri*** **(Sternoptychidae)**
Pearlside (elongate hatchetfish) (4)
N: wb→n, nl 1–3, Ab 3. E: N/S. M: Ab 2. MEL 7.0; MD 250

	N	M	L	T
Perox.	+→++	−	−	−
Perox. + KCN	−	−	−	−
Perox. + NaN_3	+→++	−	−	−
Perox. + at	+→++	−	−	−
Ac. phos.	+	−	−	−
β-gluc.	−	−	−	−
ANAE	−	−	−	−
AS-D	−	−	−	−
PAS	(+)	−	−	−

Remarks: Figs. 73–76. Neutrophil ac. phos. in 1–10 fine granules; nucleated erythrocytes rare, cytoplasm becomes compartmentalised with a strongly basophilic border becoming free of cell to form spindle-shaped haemoglobin-filled sacs and leaving a pycnotic nucleus (Figs. 74–76); nucleated erythrocytes rarely contained one or two ac. phos. cytoplasmic granules.

(St5) *Photichthys argenteus* (Photichthyidae)

Lighthouse fish (8)

N: wb→b, nl 1–2, Ab 3–4. E: c, nl 1, Ab 3. M: Ab 3. MEL 14.3; MD 497

	N	M	E	L	T
Perox.	++	–	+→++	–	–
Perox. + KCN	–	–	+→++	–	–
Perox. + NaN_3	++	–	++	–	–
Perox. + at	++	–	++	–	–
Alk. phos.	+→++	–	–	–	–
Ac. phos.	++→+++	–→++	–→++	++	–
β-gluc.	–	–	–	–	–
ANAE	–	–	–	–	–
AS-D	–	–	–	–	–
PAS	+→++	+	+→++	–	–

Remarks: Large cells; low haematocrit reading; monocyte ac. phos. in a focal concentration, eosinophil ac. phos. in small granules between large granules.

(St6) *Chauliodus sloani* (Chauliodontidae)

Viperfish (4)

N: wb→n, nl 1–2, Ab 3. E: N/S. M: Ab 3–4. MEL 19.5; MD mesopelagic

	N	M	L	T
Perox.	++→+++	–	–	–
Perox. + KCN		–	–	–
Alk. phos.	–→++	–	–	–
Ac. phos.	+→++	+	+	–
β-gluc.	–	–	–	–
ANAE	+→+++*	–	–	–
ANBE	–	–	++	–
AS-D	(+)	–	–	–
PAS	++→+++	(+)	–	–

Remarks: Figs. 77–79. Erythrocytes large, but leucocyte size more typical of teleosts (Fig. 77); low haematocrit reading; many myxosporeans in blood of one fish, and large multinucleate cells, possibly contaminants, in blood of another (Fig. 78); eosinophils, when seen, alk. phos. +++ around coarse granules (Fig. 79), and ac. phos. + between coarse granules.

(St7) *Stomias boa* (Stomiatidae)

Scaly dragonfish (2)

N: wb, nl 1–2, Ab 3. E: N/S. M: Ab 3–4. MEL 17.6; MD 100

	N	M	L	T
Perox.	+→++	–	–	–
Perox. + KCN	–	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	++	+→++	+→++	–
ANAE	+→+++	–	–	–
ANAE + NaF	–	–	–	–
AS-D	–	–	–	–

Remarks: Large blood cells; low haematocrit reading; neutrophil ANAE very labile in immersion oil.

(St8) *Borostomias antarcticus* (Astronesthidae)
Snaggletooth (1)
N: wb, nl 1–2, Ab 3. E: c in b matrix, nl 1, Ab 3–4. M: Ab 3. MEL 22.5; MD 790

	N	M	E	L	T
Perox.	++	–	++→+++	–	–
Perox. + KCN	–	–	++	–	–
Perox. + NaN$_3$	+→++	–	++→+++	–	–
β-gluc.	–	–	–	–	–
ANAE	–	–	–	–	–
AS-D	–	–	–	–	–

(St9) *Malacosteus niger* (Malacosteidae)
Blunthead dragonfish (2)
N: e in wb matrix, nl 1, Ab 3. E: c→e in very b matrix, nl 1, Ab 3–4. M: Ab 3–4. MEL 23.1; MD 980

	N	M	E	L	T
Perox.	+++	–	+	–	–
Perox. + KCN	+→++*	–	(+)	–	–
Perox. + NaN$_3$	++	–	(+)	–	–
Perox. + at	++→+++	–	(+)	–	–
Ac. phos.	+→++	+	–	–	–
β-gluc.	–	–	–	–	–
ANAE	–	–	–	–	–
ANBE	N/D	–	–	–	–
AS-D	–	–	–	–	–
PAS	+→++	(+)	+→++	–	–

Remarks: Fig. 80. Thin blood; low haematocrit reading; eosinophil PAS in coarse granules.

(St10) *Idiacanthus atlanticus* (Idiacanthidae)
Starry dragon (5)
N: b→n, nl 1–2, Ab 3. E: c in b or wb matrix, nl 1, Ab 3–5. M: Ab 3. MEL 21.8; MD 926

	N	M	E	L	T
Perox.	++→+++	–	++*	–	–
Perox. + KCN	–	–	+→++	–	–
Perox. + NaN$_3$	++	–	++→+++	–	–
Alk. phos.	–→+++	–	–	–	–
Ac. phos.	+→+++*	–→++*	+*	–	–
Ac. phos. + ta	–	–	–	–	–
β-gluc.	–	–	–	–	–
ANAE	–	–	–	–	–
AS-D	–	–	–	–	–
PAS	++	–	++	–	–

Remarks: Figs. 81–82. Eosinophil ac. phos. between and around coarse granules (Fig. 82).

Superorder: Scopelomorpha

Order: Aulopiformes

(Sc1) ***Chlorophthalmus nigripinnis*** **(Chlorophthalmidae)**
Cucumber fish (2)
N: wb→b, nl 1, Ab 4. E: N/S. M: Ab 3. MEL 15.0; MD 166

	N	M	L	T
Perox.	+→++	–	–	–
Alk. phos.	+→+++	–	–	–
Ac. phos.	+	+→++	(+)	(+)
β-gluc.	–	–	–	–
PAS	++→+++	(+)	–	–

(Sc2) ***Paralepis atlantica prionosa*** **(Paralepididae)**
Barracudina (1)
N: se, nl 1–5, Ab 4. E: sb→se, nl 1–2, Ab 3. M: Ab 3. MEL 14.2; MD 500

	N	M	E	L	T
Perox.	++→+++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Perox. + NaN_3	–	–	–	–	–
Perox. + at	+→++	–	–	–	–
Ac. phos.	+*	+	+*	–	–
Ac. phos. + ta	–	–	–	–	–
β-gluc.	+	+→++	–	–	–
ANAE	++*	(+)	–	–	–
ANAE + NaF	–	–	–	–	–
ANBE	+→++*	–	+→++*	–	–
ANBE + NaF	–	–	–	–	–
AS-D	+→++	–	–	–→++	–
AS-D + NaF	–	–	–	–	–
PAS	++→+++	(+)	+	–	–

Remarks: Figs. 83–86. Neutrophil perox. similar in anterior kidney and spleen except with aminotriazole, which caused partial inhibition in kidney neutrophils, ++, but almost complete inhibition, (+), in spleen neutrophils; eosinophil ac. phos. in central cell between coarse granules (Fig.84); β-gluc. diffuse, but often strong, ++→+++, in anterior kidney macrophages; eosinophil ANBE diffuse throughout cell (Fig. 86), but often concentrated under cell membrane or around nucleus; lymphocyte AS-D usually –.

Order: Myctophiformes

(Sc3) ***Diaphus*** **sp. (Myctophidae)**
Lanternfish (2)
N: n→wb, nl 1–2, Ab 3. E: N/S. M: Ab 3. MEL 13.0; MD mesopelagic

	N	L	T
Perox.	++→+++	–	–
Alk. phos.	–	–	–
Ac. phos.	+→++	–→++	+
β-gluc.	–	–	–
ANAE	–	–	–
ANBE	–	–	–
AS-D	+→++	–	–
PAS	+→++	–	–

Remarks: A few macrocytic erythrocytes seen in one fish.

(Sc4) *Lampanyctus* sp. (Myctophidae)
Lanternfish (2)
N: wb→b, nl 1–2, Ab 4. E: see remarks. M: Ab 3–4. MEL 18.9; MD mesopelagic

	N	M	E	L	T
Perox.	++→**+++**	–	–	–	–
Alk. phos.	–	–	N/D	–	–
Ac. phos.	+→+++	+→++	+	++	+→++
β-gluc.	–	–	N/D	–	–
ANAE	–	–	N/D	–	–
ANBE	+	–	N/D	–	–
AS-D	–→++	++	N/D	(+)	(+)
PAS	+→**++**	–	N/D	–	–

Remarks: Eosinophils only seen with certainty in perox. and ac. phos. films; neutrophils small and condensed; eosinophil ac. phos. between coarse granules; thrombocyte ac. phos. in apical deposits or granules.

(Sc5) *Lampanyctodes hectoris* (Myctophidae)
Lanternfish (4)
N: wb, nl 1–2, Ab 2. E: N/S. M: Ab 2. MEL 11.7; MD 275

	N	M	L	T
Perox.	+→++	–	–	–
Perox. + KCN	–	–	–	–
β-gluc.	–	–	–	–
ANAE	–	–	–	–
AS-D	–	–	–	–

(Sc6) *Symbolophorus* sp. (Myctophidae)
Lanternfish (2)
N: wb, nl 1, Ab 3. E: N/S. M: Ab 3. MEL 13.5; MD 150

	N	M	L	T
Perox.	+→++	–	–	–
Perox. + KCN	–	–	–	–
Perox. + NaN_3	+	–	–	–
Perox. + at	–	–	–	–
Ac. phos.	+→++	+→++	+→++	+
Ac. phos. + ta	–	–	–	–
β-gluc.	–	–	–	–
ANAE	(+)	–	–	–
AS-D	–	–	–	–
PAS	++	–	–	–

Remarks: Eosinophils seen in PAS films + diffuse between coarse granules.

Superorder: Paracanthopterygii

Order: Gadiformes

(P1) *Pseudophycis bachus* (Moridae)
Red cod (3)
N: we, nl 1, Ab 3. E: N/S. M: Ab 4. MEL 12.8; MD 153

	N	M	L	T
Perox.	+→++	−	−	−
Alk. phos.	(+)	−	+→++	−
Ac. phos.	++	+→++	++→+++	++
ANAE	++→+++	N/D	N/D	N/D
AS-D	−	−	−	−
PAS	+→+++	(+)	−	−

(P2) *Mora moro* (Moridae)
Ribaldo (2)
N: sb, nl 1, Ab 5. E: c, nl 1, Ab 3. M: Ab 3. MEL 14.8; MD 888

	N	M	E	L	T
Perox.	++→**+++**	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	**+**→++	**+**→++	+→++	−→++	−
β-gluc.	−	−	−	−	−
ANAE	(+)	(+)	−	(+)	−
AS-D	−	(+)	−	−	−
PAS	+	−	N/D	−	−

Remarks: Eosinophil ac. phos. in fine granules between coarse granules; macrocytic erythrocytes.

(P3) *Melanonus gracilis* (Melanonidae)
Black pelagic cod (1)
N: wb, nl 1–4, Ab 3. E: N/S. M: Ab 4. MEL 13.1; MD 1049

	N	M	L	T
Perox.	+→++	−	−	−
Perox. + KCN	−	−	−	−
Alk. phos.	+→+++	−→++	−	−
Ac. phos.	−	−	−	−
PAS	+	+	−	−

(P4) *Merluccius australis* (Merlucciidae)
Hake (3)
N: wb→n, nl 1–2, Ab 3–4. E: c, nl 1, Ab 2–3. M: Ab 3–4. MEL 11.4; MD 849

	N	M	E	L	T
Perox.	+++	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	−→++	−	−	**++**→+++	−
Ac. phos.	(+)	−→++	−	++	+→++
β-gluc.	−	−	−	−	−
ANAE	−	−	−	−	−
ANBE	−	−	−	−	−
AS-D	−	−	−	−	−
PAS	+→+++	(+)	(+)	−	−

Remarks: Eosinophils often degranulated, which may account for the lack of eosinophil enzymes; lymphocyte alk. phos. urea inhibited; extracellular ANAE and AS-D reaction product was common around blood cells, but its origin could not be determined.

(P5) *Macruronus novaezelandiae* (Merlucciidae)
Hoki (4)
N: n→we, nl 1–4, Ab 4–5. E: c, nl 1, Ab 2. M: Ab 3–4. MEL 11.4; MD 846

	N	M	L	T
Perox.	+→+++	–	–	–
Alk. phos.	++→+++*	++→+++*	–	–
Ac. phos.	+→++	++	+→++	+→++
β-gluc.	–	–	–	–
ANAE	–	–	–	–
AS-D	–	–	–	–
PAS	++→+++	–	–	–

Remarks: Fig. 87. Neutrophil and monocyte alk. phos. urea resistant; eosinophils too scarce to determine cytochemistry.

(P6) *Lyconus* sp. (Merlucciidae)
(2)
N: wb, nl 1–2, Ab 5. E: N/S. M: Ab 2–3. MEL 12.2; MD 1121

	N	M	L	T
Perox.	–→++	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	+→++	++	+→++	+→++
β-gluc.	–	–	–	–
ANAE	–	–	–	–
AS-D	–	–	–	–
PAS	++	(+)	–	–

Remarks: Much non-specific extracellular product in AS-D films.

(P7) *Trachyrincus longirostris* (Macrouridae)
Unicorn rattail (3)
N: n, nl 1, Ab 3. E: N/S. M: Ab 2–3. MEL 13.2; MD 942

	N	M	L	T
Perox.	++	–	–	–
Perox. + KCN	–	–	–	–
Alk. phos.	–	–	+++	–
Ac. phos.	+	–	–	–
ANAE	++	–	–	–
AS-D	–	–	–	–
PAS	+→++	–	–	–

Remarks: Fig. 88. Note the unusual strongly urea-resistant alk. phos. lymphocytes (Fig. 88); macrocytic erythrocytes occasionally present; neutrophil ANAE staining irregular.

(P8) *Coelorinchus biclinozonalis* (Macrouridae)
Twobanded rattail (2)
N: n, nl 1, Ab 3. E: N/S. M: Ab 3. MEL 12.9; MD 651

	N	M	L	T
Perox.	++	–	–	–

(P9) *Genypterus blacodes* (Ophidiidae)
Ling (4)
N: b, nl 1, Ab 3. E: c, nl 1, Ab 4. M: Ab 3. MEL 13.2; MD 582

	N	M	E	L	T
Perox.	++	–	–	–	–
Alk. phos.	++→+++*	–	–*	–	–
Ac. phos.	++	+→++	+→++	+→++	–→++
β-gluc.	–	–	–	–	–
ANAE	–	–	–	–	–
ANBE	+	–	–	–	–
AS-D	–	–	–	–	–
PAS	+→++	+	N/D	–	–

Remarks: Fig. 89. Neutrophil alk. phos. urea resistant; eosinophil ac. phos. in fine granules between coarse granules.

(P10) *Brotulataenia crassa* (Ophidiidae)
Blue cusk eel (2)
N: n, nl 1, Ab 3. E: b, nl 1, Ab 2. M: Ab 5. MEL 17.9; MD 1170

	N	M	E	L	T
Perox.	++→+++	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	+→++	+→++	(+)	+→++	+
β-gluc.	–	–	–	–	–
ANAE	–	–	–	–	–
ANBE	–	–	–	–	–
AS-D	+→++	++	+*	+	N/S
PAS	++	–	+→++	–	–

Remarks: Fig. 90. Eosinophil AS-D (Fig. 90) and PAS diffuse between coarse granules.

Order: Lophiiformes

(P11) *Chaunax pictus* (Chaunacidae)
Deepsea frogfish (1)
N: wb, nl 1, Ab 3. E: N/S. M: Ab 2–3. MEL 17.7; MD 889

	N	M	L	T
Perox.	+→++	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	–	–	–	–
PAS	++	–	–	–

Remarks: Low haematocrit reading.

Order: Gobiesociformes

(P12) *Diplocrepis puniceus* (Gobiesocidae)
Orange clingfish (4)
N: wb→n, nl 1, Ab 4. E: N/S. M: Ab 3. MEL 10.8; MD 1

	N	M	L	T
Perox.	+++	–	–	–
Perox. + KCN	–	–	–	–
Perox. + NaN_3	++→+++	–	–	–
Perox. + at	++→+++	–	–	–
Alk. phos.	–→+++	–	–	–
Ac. phos.	(+)	++→+++	++→+++	+→++
Ac. phos. + ta	–	–	–	–
β-gluc.	–	–	–	–
ANAE	–	–	–	–
ANBE	–	–→++*	–	–
AS-D	–	+→++	(+)	–
AS-D + NaF	–	+→++	(+)	–
PAS	++→+++	–	–	–

Remarks: Fig. 91.

Superorder: Acanthopterygii

Order: Beryciformes

(A1) ***Melamphaes microps*** **(Melamphaidae)**
Bigscale fish (2)
N: wb, nl 1–2, Ab 2–3. E: N/S. M: Ab 3. MEL 25.5; MD 1100

	N	M	L	T
Perox.	++	–	–	–
Perox. + KCN	–	–	–	–
β-gluc.	–	–	–	–
ANAE	+	–	–	–
AS-D	–	–	–	–

(A2) ***Poromitra oscitans*** **(Melamphaidae)**
Deepsea cresthead (2)
N: wb, nl 1–2, Ab 2–3. E: e, nl 1, Ab 2. M: Ab 3. MEL 19.3, MD 1170

	N	M	E	L	T
Perox.	++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	–→++	++	+→++	+→++	+→++
β-gluc.	–	–	–	–	–
ANAE	–	–	–	–	–
ANBE	–	–	–	–	–
AS-D	–	–	–	–	–
PAS	+→++	–	+→++	–	–

Remarks: Eosinophil ac. phos. irregularly distributed between coarse granules; PAS also between coarse granules.

(A3) ***Diretmoides parini*** **(Diretmidae)**
Black discfish (2)
N: wb→n, nl 1–2, Ab 4. E: c, nl 1, Ab 3. M: Ab 3. MEL 18.7; MD 895

	N	M	E	L	T
Perox.	+→++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Alk. phos.	–	–	–	+→++	–
Ac. phos.	++	++	+	–	–
β-gluc.	–	–	–	–	–
ANAE	+→++	–	–	–	–
AS-D	+→++	–	–	–	–
PAS	++→+++	–	+→++	–	–

Remarks: Eosinophil PAS in peripheral deposits and in fine granules between coarse granules; neutrophil ANAE (rarely +++).

(A4) ***Hoplostethus atlanticus*** **(Trachichthyidae)**
Orange roughy (20)
N: wb, nl 1, Ab 4. E: se granules in sb matrix, nl 1, Ab 2–3. M: Ab 2–3. MEL 13.4; MD 859

	N	M	E	L	T
Perox.	+→+++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	++	++	–→++*	++	++
β-gluc.	–	–	–	–	–
ANAE	+++*	+→++	–	+→++	–
ANAE + NaF	++→+++	+→++	–	+→++	–
ANBE	+→+++	++→+++	–	–	–
AS-D	+→++*	–	–	–	–
PAS	++→+++	(+)	–	–	–

Remarks: Figs. 92–94. Eosinophil ac. phos. in fine granules between coarse granules (Fig. 92); neutrophil ANAE extremely strong obscuring all cell detail (Fig. 93) until leached into immersion oil overnight, intra- and extracellular; neutrophil AS-D confined to small areas of cytoplasm (Fig. 94).

(A5) ***Hoplostethus mediterraneus*** **(Trachichthyidae)**
Silver roughy (2)
N: wb, nl 1, Ab 3. E: se, nl 1, Ab 4–5. M: Ab 4. MD unknown

	N	M	E	L	T
Perox.	++	−	−	−	−
Alk. phos.	++→+++*	−	−	−	−
Ac. phos.	+→++	++→+++*	−	++→+++*	++→+++*
β-gluc.	−	−	−	−	−

Remarks: Figs. 95–97. Unlike A4, silver roughy had strong neutrophil alk. phos. (Fig. 95) and many circulating eosinophils.

(A6) ***Centroberyx affinis*** **(Berycidae)**
Red snapper (2)
N: wb, nl 1, Ab 4. E: c, nl 1, Ab 3. M: Ab 3. MEL 11.1; MD 33

	N	M	E	L	T
Perox.	++→+++	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	+++	−	−	−	−
Ac. phos.	(+)	++→+++	+→++	++→+++	+→++
PAS	+++	(+)	++→+++	−	−

Remarks: Eosinophil ac. phos. in fine granules between coarse granules.

(A7) ***Beryx splendens*** **(Berycidae)**
Alfonsino (3)
N: n, nl 1, Ab 3. E: N/S. M: Ab 2. MEL 13.9; MD 889

	N	M	L	T
Perox.	++	−	−	−
Alk. phos.	++	−	−	−
Ac. phos.	+→++	++	++→+++	++→+++
β-gluc.	−	−	−	−
ANAE	−	−	−	−
ANBE	+→++	−	−	−
AS-D	+→+++	+→++	(+)	(+)
PAS	++→+++	−	−	−

Remarks: Neutrophil alk. phos. urea resistant; neutrophil ANBE activity partially in nucleus and partially diffuse in cytoplasm.

Order: Zeiformes

(A8) ***Zeus faber*** **(Zeidae)**
John dory (22)
N: wb, nl 2, Ab 2. E: se→e, nl 1–2, Ab 4. M: Ab 3. MEL 15.3; MD 108

	N	M	E	L	T
Perox.	+→+++	−	(+)	−	−
Perox. + KCN	−	−	(+)	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	−→++	+	(+)	(+)	(+)
Ac. phos. + ta	−	−	−	−	−
β-gluc.	−	−	−	−	−
ANAE	−	−	−	−	−
AS-D	−	−	−	−	−
PAS	++	(+)	−	(+)	−

Remarks: Neutrophil precursors in the anterior kidney perox. +→+++, ac. phos. +→++; eosinophil ac. phos. in fine granules between coarse granules; eosinophils yellow, which suggests weak perox.

(A9) *Cyttus traversi* (Zeidae)

Lookdown dory (2)

N: wb, nl 1, Ab 3. E: N/S. M: Ab 3. MEL 14.8; MD 357

	N	M	L	T
Perox.	++→+++	–	–	–
Alk. phos.	+→++	+→++	–→+++	–
Ac. phos.	+++	+→++	+→+++	+→++
β-gluc.	–	–	–	–

(A10) *Neocyttus rhomboidalis* (Oreosomatidae)

Spiky oreo (2)

N: wb, nl 1, Ab 2. E: N/S. M: Ab 2. MEL 15.6; MD 912

	N	M	L	T
Perox.	+	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	+→++	+→++	++	++
β-gluc.	–	–	–	–
ANAE	+	(+)	+	–
AS-D	–	–	–	–

(A11) *Pseudocyttus maculatus* (Oreosomatidae)

Smooth oreo (2)

N: n, nl 1, Ab 3. E: N/S. M: Ab 2. MEL 16.5; MD 1009

	N	M	L	T
Perox.	+→++	–	–	–
Perox. + KCN	–	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	+→++	+→++	–→++	–
β-gluc.	–	–	–	–
ANAE	–	–	(+)	–
AS-D	(++)	–	(+)	–
PAS	++	–	–	–

Remarks: Lymphocyte ac. phos. usually weak +.

(A12) *Allocyttus* sp. (Oreosomatidae)

Black oreo (2)

N: wb→n, nl 1–2, Ab 3–4. E: N/S. M: Ab 2–3. MEL 15.7; MD 720

	N	M	L	T
Perox.	+→++	–	–	–
Perox. + KCN	–	–	–	–
Perox. + NaN_3	+→++	–	–	–
Perox. + at	+	–	–	–
Ac. phos.	+→++	+	+	–
Ac. phos. + ta	–	–	–	–
ANAE	+→++	–	–	–
ANAE + NaF	–	–	–	–
ANBE	–→+++	–→++	–	–
ANBE + NaF	–	–	–	–
AS-D	–	–	–	–

Remarks: Neutrophil ANBE usually moderate ++, monocyte ANBE usually weak +; erythrocyte ANBE, –→++, diffuse in cytoplasm.

Order: Gasterosteiformes

(A13) *Macrorhamphosus scolopax* (Macrorhamphosidae)
Snipefish (2)
N: purple, nl 1, Ab 3. E: N/S. M: Ab 2. MEL 10.0; MD 107

	N	M	L	T
Perox.	++	−	−	−
β-gluc.	−	−	−	−

Order: Scorpaeniformes

(A14) *Helicolenus percoides* (Scorpaenidae)
Jock stewart (2)
N: wb→b, nl 1, Ab 2. E: c→wb, nl 1, Ab 2. M: Ab 2. MEL 12.5; MD 403

	N	M	E	L	T
Perox.	+→++	−	−	−	−
β-gluc.	−	−	−	−	−

Remarks: Granulocytes fragile.

(A15) *Trachyscorpia capensis* (Scorpaenidae)
Cape scorpionfish (2)
N: b, nl 1, Ab 2. E: c, nl 1, Ab 1. M: Ab 2. MEL 17.7; MD 890

	N	M	L	T
Perox.	+→++	−	−	−
Perox. + KCN	−	−	−	−
Alk. phos.	+→++	−	+→++	−
Ac. phos.	+→+++	(+)	−→+++	−→++
ANAE	+	+→++	(+)	−
AS-D	−	−	−	−
PAS	+→++	−	−	−

Remarks: Too few eosinophils seen to score; neutrophils fragile.

(A16) *Chelidonichthys kumu* (Triglidae)
Red gurnard (8)
N: wb→n, nl 1, Ab 3. E: se, nl 1, Ab 4. M: Ab 4. MEL 12.0; MD 119

	N	M	E	L	T
Perox.	++→+++	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	(+)	−	−	+→++*	−
Ac. phos.	++→+++	++→+++	−→++	−→+++*	−
Ac. phos. + ta	−	−	−	−→++	−
β-gluc.	−	−	−	−	−
ANAE	+→+++	−	−	−	−
ANAE + NaF	−	−	−	−	−
AS-D	−	++→+++*	−	(++)	(+)
PAS	+++	−	−	−	−

Remarks: Figs. 98–99. Eosinophil ac. phos. in fine granules between coarse granules; with ac. phos. and AS-D, two types of lymphocytes apparent, either moderately to strongly + or −, with no intermediate weakly stained cells; anterior kidney neutrophil alk. phos. −→+++, ac. phos. ++→+++ totally inhibited by ta, ANAE ++→+++; anterior kidney lymphocyte alk. phos. ++→+++ (Fig. 99), tartrate-resistant ac. phos. ++→+++, AS-D ++→+++; *Myxidium* (Myxosporea) spore pairs in the blood of three fish; large, well-differentiated leucocytes, apparent lymphocyte subpopulations, and easy maintenance of fish make gurnard good models for research on inflammation and immunity.

(A17) *Pterygotrigla picta* (Triglidae)
Spotted gurnard (2)
N: b, nl 1, Ab 2. E: c or se, nl 1, Ab 4. M: Ab 3. MEL 10.5; MD 276

	N	M	E	L	T
Perox.	+→++	−	−	−	−
β-gluc.	−	−	−	−	−

Remarks: Note more eosinophils than neutrophils.

(A18) ***Lepidotrigla brachyoptera* (Triglidae)**
Scaly gurnard (2)
N; wb, nl 1, Ab 2. E: c or se, nl 1, Ab 5. M: Ab 2. MEL 12.1; MD 112

	N	M	E	L	T
Perox.	+→++	−	−	−	−
β-gluc.	−	−	−	−	−

Remarks: Note abundance of eosinophils and uncommon neutrophils.

(A19) ***Congiopodus leucopaecilus* (Congiopodidae)**
Southern pigfish (3)
N: b, nl 1, Ab 2. E: se, nl 1–2, Ab 4. M: Ab 3–4. MEL 10.2; MD 50

	N	M	E	L	T
Perox.	++	−	+	−	−
Alk. phos.	(+)	−	−→++*	−	−
Ac. phos.	−	++	++	−	−
ANAE	+→++	−→++	+→++	−→++	−→++
AS-D	−	−	−	−	−
PAS	+	−	+→++	−	−

Remarks: Fig. 100. Eosinophil alk. phos. around coarse granules (Fig. 100) (rarely +++); monocyte, lymphocyte, and thrombocyte ANAE usually −; note eosinophils very common, neutrophils uncommon.

(A20) ***Neophrynichthys angustus* (Psychrolutidae)**
Pale toadfish (2)
N: b→n, nl 1, Ab 3–4. E: Ab 1. M: Ab 2. MEL 16.3; MD 800

	N	M	L	T
Perox.	+→+++	−	−	−
Alk. phos.	−	−	−	−
Ac. phos.	(+)	−	−	−
β-gluc.	−	−	−	−
ANAE	−	−	−	−
AS-D	−	−	−	−
PAS	+→++	(+)	−	−

Remarks: Large cells; low haematocrit reading; sporozoans in erythrocytes of one fish.

(A21) ***Psychrolutes* sp. (Psychrolutidae)**
Blobfish (2)
N: n→wb, nl 1, Ab 3. E: N/S. M: Ab 2–3. MEL 17.6; MD 1101

	N	M	L	T
Perox.	+→++	−	−	−
Alk. phos.	−	−	−	−
Ac. phos.	−	+	(+)	−
β-gluc.	−	−	−	−
ANAE	−	−	−	−
AS-D	−	−	−	−
PAS	++	−	−	−

Order: Perciformes

(A22) ***Polyprion oxygeneios*** **(Percichthyidae)**
Hapuku (2)
N: wb, nl 1, Ab 4. E: c in sb matrix, nl 1, Ab 4. M: Ab 3. MEL 11.6; MD 151

	N	M	E	L	T
Perox.	+→++	–	–	–	–
β-gluc.	–	–	–	–	–

(A23) ***Caesioperca lepidoptera*** **(Serranidae)**
Butterfly perch (2)
N: b, nl 1, Ab 3. E: c, nl 1, Ab 3. M: Ab 3. MEL 10.8; MD 92

	N	M	E	L	T
Perox.	++→+++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	–	–	+→++	→++	–
PAS	++	–	–	–	–

(A24) ***Callanthias allporti*** **(Serranidae)**
Splendid perch (2)
N: b, nl 1–2, Ab 4. E: c, nl 1, Ab 2. M: Ab 3. MEL 12.0; MD 92

	N	M	E	L	T
Perox.	+++	–	–	–	–
Ac. phos.	++→+++	–	–	++→+++	–
PAS	+→++	(+)	–	–	–

A25) ***Epigonus robustus*** **(Apogonidae)**
Cardinalfish (2)
N: wb, nl 1, Ab 3. E: N/S. M: Ab 3. MEL 12.1; MD 1021

	N	M	L	T
Perox.	+→++	–	–	–

(A26) ***Caranx georgianus*** **(Carangidae)**
Trevally (5)
N: wb, nl 1, Ab 4. E: N/S. M: Ab 3. MEL 11.1; MD 42

	N	M	L	T
Perox.	+→++	–	–	–
Perox. + KCN	–	–	–	–
Alk. phos.	++→+++	–	–	–
Ac. phos.	++→+++	++→+++	(++→+++)	N/D
β-gluc.	–	–	–	–
ANAE	+→++	(+)	–	–
AS-D	++→+++	+→++	–	–

Remarks: Two distinct types of lymphocyte with ac. phos. moderate to strongly granular or –.

(A27) ***Trachurus declivis*** **(Carangidae)**
Jack mackerel (4)
N: wb, nl 1, Ab: 1–2. E: we, nl 1, Ab 1–2. M: Ab 1–2. MEL 11.5; MD 104

	N	M	E	L	T
Perox.	++→+++	–	(+)	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	++→+++	+→++	–	++→+++	++→+++
β-gluc.	–	–	–	–	–
ANAE	–	–→++	–	+	–
AS-D	–	–	–	–	–
PAS	++	–	–	–	–

Remarks: Low leucocyte levels may be due to cell fragility and lysis.

(A28) ***Seriola lalandi*** **(Carangidae)**
Kingfish (2)
N: wb→n, nl 1–2, Ab 4. E: we, nl 1, Ab 3. M: Ab 4. MEL 10.7; MD 439

	N	M	E	L	T
Perox.	++	–	–	–	–
Alk. phos.	+→++	–	–	–	–
Ac. phos.	(+)	+→+++	+	+→++	+→++
ANAE	+→++	–	–	–	–
AS-D	–	–→++	–	–	–
PAS	+→++	(+)	(+)	–	–

(A29) ***Brama brama*** **(Bramidae)**
Ray's bream (1)
N: b, nl 1, Ab 3. E: c, nl 1, Ab 2. M: Ab 3. MEL 12.7; MD 1014

	N	M	E	L	T
Perox.	++	–	–	–	–

(A30) ***Chrysophrys auratus*** **(Sparidae)**
Snapper (15)
N: b, nl 1, Ab 1. E: se→e, nl 1, Ab 4. M: Ab 3. MEL 12.6; MD 102

	M	E	L	T
Perox.	–	++→+++	–	–
Perox. + KCN	–	–	–	–
Alk. phos.	–	–→++	–	–
Ac. phos.	++	++→+++	+→++	+
Ac. phos. + ta	–	–	(+)	–
β-gluc.	–	–	–	–
ANAE	–	(+)	(+→++)	+
ANAE + NaF	–	–	–	–
AS-D	–	–	–	–
PAS	(+)	+++	–	–

Remarks: Eosinophils develop from basophilic precursors in blood; two groups of lymphocytes apparent with ANAE.

(A31) ***Upeneichthys lineatus*** **(Mullidae)**
Red mullet (1)
N: b, nl 1, Ab 3. E: c, nl 1, Ab 3. M: Ab 3. MEL 10.4; MD 33

	N	M	E	L	T
Perox.	++→+++	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	+→++	+→++	–	++	–
ANAE	+→+++	(+)	–	–→++	–
AS-D	–	–	–	–	–
PAS	++→+++	+→++	++→+++	–	–

Remarks: Many immature or degenerating erythrocytes in films; neutrophil ac. phos. in a few small granules.

(A32) ***Scorpis violaceus*** **(Kyphosidae)**
Blue maomao (2)
N: b, nl 1–2, Ab 3. E: Ab 1. M: Ab 3. MEL 11.4; MD 98

	N	M	L	T
Perox.	++	–	–	–
Perox. + KCN	–	–	–	–
Alk. phos.	+→+++	–	–	–
Ac. phos.	+→++	++	+→+++*	+→++
PAS	++	(+)	–	–

Remarks: Lymphocyte ac. phos. formed two or three distinct patterns.

(A33) *Zanclistius elevatus* (Pentacerotidae)
Longfin boarfish (6)

N: wb, nl 1, Ab 2. E: se, nl 1, Ab 4. M: Ab 3. MEL 12.3; MD 120

	N	M	E	L	T
Perox.	(+→++)	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	−	−	++→+++*	−	−
Ac. phos.	++	(+)	++	−→++	−
β-gluc.	−	−	−	−	−
ANAE	++→+++	+→++	+++	(+)	−
AS-D	−	−	+++*	−	−
PAS	+	(+)	+→+++	−	−

Remarks: Figs. 101–102. Neutrophil perox. − had central nuclei, which suggested immaturity, and neutrophil perox. + had eccentric nuclei, which suggested maturity; the two types were equally abundant; note high enzyme content, particularly esterases in eosinophils (Fig. 102), and greater abundance of eosinophils than neutrophils in blood.

(A34) *Pentaceros decacanthus* (Pentacerotidae)
Yellow boarfish (4)

N: wb, nl 1, Ab 3. E: se, nl 1, Ab 4. M: Ab 3. MEL 12.1; MD 531

	N	M	E	L	T
Perox.	(++)	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	+→+++	−	−	+	−
Ac. phos.	+→+++	+→++	−	++	−
β-gluc.	−	−	−	−	−
ANAE	++→+++	++	+→++	++	−
AS-D	+→++	−	+++*	−	−
PAS	+→++	−	++→+++	−	−

Remarks: Fig. 103. Only about 5% of neutrophils perox.; eosinophil PAS in coarse granules.

(A35) *Chromis dispilus* (Pomacentridae)
Demoiselle (2)

N: sb, nl 1, Ab 3. E: Ab 1. M: Ab 3. MEL 11.3; MD 33

	N	M	L	T
Perox.	++→+++	−	−	−
Perox. + KCN	−	−	−	−
Alk. phos.	−	−	−	−
Ac. phos.	++→+++	+→++	+→+++	−
PAS	++	−	−	−

Remarks: Two groups of lymphocytes apparent with ac. phos. +→++ and +++; although eosinophils were too scarce to score for enzymes, eosinophil PAS ++→+++.

(A36) *Nemadactylus douglasi* (Cheilodactylidae)
Porae (2)

N: wb, nl 1–2, Ab 4. E: N/S. M: Ab 3. MEL 11.5; MD 33

	N	M	L	T
Perox.	++	−	−	−
β-gluc.	−	−	−	−

(A37) ***Nemadactylus macropterus* (Cheilodactylidae)**
Tarakihi (2)
N: wb→n, nl 2, Ab 5. E: e→we, nl 1, Ab 2. M: Ab 5. MEL 9.1; MD 145

	N	M	L	T
Perox.	++	–	–	–
Alk. phos.	–	–→++	–	–
Ac. phos.	(+)	++→+++	++→+++	++
ANAE	–	–	–	–
AS-D	–	–	–	–
PAS	++→+++	(+)	–	–

Remarks: Neutrophils and monocytes abundant, eosinophils too scarce to determine cytochemistry.

(A38) ***Aldrichetta forsteri* (Mugilidae)**
Yellow-eyed mullet (2)
N: b, nl 2, Ab 4. E: N/S. M: Ab 4

	N	M	L	T
Perox.	+→++	–	–	–
PAS	++→+++	–	–	–

(A39) ***Bodianus vulpinus* (Labridae)**
Red pigfish (1)
N: wb, nl 1, Ab 3. E: N/S. M: Ab 4. MEL 12.4; MD 33

	N	M	L	T
Perox.	+++	–	–	–
β-gluc.	–	–	–	–

(A40) ***Pseudolabrus celidotus* (Labridae)**
Spotty (2)
N: wb, nl 1, Ab 5. E: c→we, nl 1, Ab 2. M: Ab 3. MEL 9.5; MD 50

	N	M	E	L	T
Perox.	++	–	–	–	–
Alk. phos.	++→+++	+	++*	–	–
Ac. phos.	(+)	++	–	–	–
ANAE	–	–	–	–	–
AS-D	–	–→++	–	–	–
PAS	+→+++	(+)	(+)	–	–

Remarks: Fig. 104. Eosinophil alk. phos. in fine granules between coarse granules (Fig. 104).

(A41) ***Coris sandageri* (Labridae)**
Sandagers wrasse (2)
N: wb, nl 1–2, Ab 3. E: N/S. M: Ab 3. MEL 12.6; MD 33

	N	M	L	T
Perox.	++	–	–	–
Alk. phos.	++→+++	–	–	–
β-gluc.	–	–	–	–
PAS	++→+++	–	–	–

(A42) *Parapercis colias* (Mugiloididae)

Blue cod (2)

N: we, nl 1, Ab 3. E: c→wb→we, nl 1, Ab 2. M: Ab 4. MEL 10.8; MD 61

	N	M	E	L	T
Perox.	+→++	+→++*	–	–	–
Perox. + KCN	–	–	–	–	–
Ac. phos.	++→+++	+→++	–	(+)	–
PAS	+++*	(+)	–	–	–

Remarks: Figs. 105–106. Neutrophils had clear area in central cytoplasm when stained with PAS (Fig. 106).

(A43) *Cheimarrichthys fosteri* (Mugiloididae)

Torrent fish (9)

N: n, nl 1, Ab 3. E: c, nl 1, Ab 2. M: Ab 2–3. MEL 10.5; freshwater

	N	M	E	L	T
Perox.	+++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	+→++	–→++	N/D	+→+++	+→+++
β-gluc.	–	–	–	–	–
ANAE	–	–	N/D	–	–
AS-D	–	–	N/D	–	–
PAS	+	–	–	–	–

Remarks: In anterior kidney large blast-like cells lacked perox., but developing neutrophils contained weak to moderate perox., +→++; anterior kidney neutrophil ac. phos. strong, ++→+++.

(A44) *Chiasmodon niger* (Chiasmodontidae)

Black swallower (1)

N: wb, nl 1–2, Ab 3. E: N/S. M: Ab 3. MEL 14.6; MD 200

	N	M	L	T
Perox.	+→+++	–	–	–
Ac. phos.	+	–	–	–
β-gluc.	–	–	–	–
ANAE	+→++	–	–	–
ANAE + NaF	–	–	–	–
AS-D	–	–	–	–

(A45) *Genyagnus monopterygius* (Uranoscopidae)

Spotted stargazer (2)

N: b, nl 1, Ab 4. E: N/S. M: Ab 3. MEL 12.8; MD 66.

	N	M	L	T
Perox.	++→+++	–	–	–
Perox. + KCN	–	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	+→++	++→+++	++→+++	+→++
ANAE	–	–	–	–
AS-D	–	–	–	–

(A46) *Kathetostoma giganteum* (Uranoscopidae)
Stargazer (2)
N: b, nl 1, Ab 3. E: N/S. M: Ab 3. MEL 12.0; MD 271

	N	M	L	T
Perox.	+→++	–	–	–
Perox. + KCN	–	–	–	–
Alk. phos.	+→+++	–	–	–
Ac. phos.	–	–	+→++	+→++
ANAE	+→++	–	–	–
AS-D	(+)	–	–	–

(A47) *Gobiomorphus cotidianus* (Eleotrididae)
Common bully (5)
N: n, nl 1, Ab: 3. E: N/S. M: Ab 2. MEL 13.0; freshwater

	N	M	L	T
Perox.	+++	(+)*	–	–
Alk. phos.	–→++	+	–	–
Ac. phos.	+→++	–→++	(+)	(+)
ANAE	–	–	–	–
ANBE	–	–	–	–
AS-D	–	–	–	–
PAS	+→++	–	–	–

Remarks: Alk. phos. urea inhibited.

(A48) *Gobiomorphus huttoni* (Eleotrididae)
Redfinned bully (4)
N: n→wb, nl 1, Ab: 3. E: N/S. M: Ab 3. MEL 12.0; freshwater

	N	M	L	T
Perox.	+++	–	–	–
Perox. + KCN	–	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	+→++	++	+→+++	+→++
ANAE	+	(+)	+→++	+→++
ANBE	–	–	–	–
AS-D	–	–	–	–
PAS	+→++	–	–	–

(A49) *Thyrites atun* (Gempylidae)
Barracouta (2)
N: n, nl 1, Ab 3. E: N/S. M: Ab 3. MEL 12.4; MD 107

	N	M	L	T
Perox.	+→++	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	+→++	++→+++	+→+++	+→+++
β-gluc.	–	–	–	–
ANAE	–	–	–	–
PAS	++→+++	(+)	–	–

(A50) ***Rexea solandri* (Gempylidae)**
Gemfish, southern kingfish (2)
N: sb, nl 1, Ab 3. E: N/S. M: Ab 3. MEL 13.0; MD 47

	N	M	L	T
Perox.	++→+++	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	+→++	++→+++	+→++	+→+++
β-gluc.	–	–	–	–
PAS	+→++	–	–	–

(A51) ***Lepidopus caudatus* (Trichiuridae)**
Frostfish (2)
N: sb, nl 1, Ab 4–5. E: e, nl 1, Ab 2–3. M: Ab 3. MEL 13.4; MD 115

	N	M	E	L	T
Perox.	++→+++	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	+→+++	++	–	+→++	+→++
β-gluc.	–	–	–	–	–

(A52) ***Scomber australasicus* (Scombridae)**
Blue mackerel (2)
N: b, nl 1, Ab 4. E: c, nl 1, Ab 3. M: Ab 3. MEL 10.7; MD 67

	N	M	E	L	T
Perox.	++→+++	–	(+)	–	–
Perox. + KCN	(+)	–	N/D	–	–

Remarks: Eosinophils yellow diffuse staining.

(A53) ***Thunnus alalunga* (Scombridae)**
Albacore (2)
N: n, nl 1, Ab 3–4. E: we, nl 1–2, Ab 3. M: Ab 3. MEL 12.0; MD unknown

	N	M	E	L	T
Perox.	++→+++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Perox. + NaN_3	++→+++	–	–	–	–
Perox. + at	++→+++	–	–	–	–
Alk. phos.	+→++	–	–	–	–
Ac. phos.	++→+++	++	++	+→++	+→++
ANAE	–	–	–	–	–
AS-D	++→+++	–	–	–	–
PAS	++→+++	–	++→+++	(+)	(++)

Remarks: Only some eosinophil granules ac. phos.; PAS in eosinophil coarse granules.

(A54) *Thunnus albacares* (Scombridae)
Yellowfin tuna (2)

N: n, nl 1, Ab 3. E: e, nl 1–2, Ab 4. M: Ab 2–3. MEL 12.2; MD unknown

	N	M	E	L	T
Perox.	++→+++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Perox. + NaN$_3$	++→+++	–	–	–	–
Perox. + at	++→+++	–	–	–	–
Alk. phos.	+→++	–	–	–	–
Ac. phos.	++→+++	+→++	++	–→++	(+)
ANAE	–	–	–	–	–
AS-D	–→++	–	–	–	–
PAS	++→+++	–	++→+++	–	–

Remarks: As A53.

(A55) *Thunnus maccoyii* (Scombridae)
Southern bluefin tuna (1)

N: b, nl 1, Ab 3. E: se, nl 1, Ab 4. M: Ab 2. MEL 12.8; MD unknown

	N	M	E	L	T
Perox.	++	–	–	–	–
ANAE	–	–	+→++	–	–
PAS	++→+++	–	+→++	–	–

Remarks: Eosinophil PAS in and between coarse granules.

(A56) *Katsuwonus pelamis* (Scombridae)
Skipjack tuna (2)

N: n, nl 1, Ab 2. E: we, nl 1, Ab 2–3. M: Ab 2. MEL 12.3; MD unknown

	N	M	E	L	T
Perox.	++→+++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Perox. + NaN$_3$	++→+++	–	–	–	–
Perox. + at	++	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	++→+++	+→++	+→++	–→++	–→++
ANAE	+++	–	+	–	–
AS-D	++	–	–	–	–
PAS	+→+++	–	+→++	–	–→++

Remarks: As A53. Very many blasts and many thrombocytes in blood.

(A57) *Seriolella brama* (Centrolophidae)
Common warehou (2)

N: b, nl 1–4, Ab 5. E: we, nl 1, Ab 2. M: Ab 3. MEL 11.2; MD 69

	N	M	L	T
Perox.	+→+++	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	+→+++	(+)	+→++	–
β-gluc.	–	–	–	–
PAS	+→++	(+)	–	–

Remarks: Fig. 107. Spirochaetes in blood of one fish; neutrophils with refractile inclusion (Fig. 107); anterior kidney neutrophil and macrophage ac. phos. variable, but often strong, +→+++, as with spleen neutrophil ac. phos., ++→+++.

(A58) ***Seriolella punctata*** **(Centrolophidae)**
Silver warehou (2)
N: wb, nl 1, Ab 3. E: N/S. M: Ab 2. MEL 12.2; MD 107

	N	M	L	T
Perox.	+→++	−	−	−
Alk. phos.	−	−	−	−
Ac. phos.	+→+++	(+)	+→++	+→++
β-gluc.	−	−	−	−

Remarks: Neutrophils with refractile inclusion; macrocytic erythrocytes present.

(A59) ***Hyperoglyphe antarctica*** **(Centrolophidae)**
Bluenose (1)
N: b, nl 1–3, Ab 3. E: c, nl 1, Ab 2. M: Ab 2. MEL 12.3; MD 350

	N	M	E	L	T
Perox.	+→++	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	++→+++	(+)	−	+→++	++
β-gluc.	−	−	−	−	−
ANAE	−	−	−	−	−
AS-D	−	−	−	−	−
PAS	+→++	−	−	−	−

Remarks: Neutrophils with refractile inclusion; lymphocyte ac. phos. in four or five peripheral granules; very many lymphocytes in blood.

(A60) ***Centrolophus niger*** **(Centrolophidae)**
Rudderfish (4)
N: b→wb, nl 1–2, Ab 3–4. E: c, nl 1, Ab 2. M: Ab 2–3. MEL 13.8; MD 907

	N	M	E	L	T
Perox.	++→+++	−	−	−	−
Perox. + KCN	−	−	−	−	−
Alk. phos.	−	−	−	−	−
Ac. phos.	+→++	+→++	−	+→+++	−
β-gluc.	−	−	−	−	−
ANAE	(+)*	(+)	−	(+)	(+)
AS-D	−	−	−	−	−
PAS	+→++	(+)	−	−	−

Remarks: Fig. 108. Neutrophils with refractile inclusion lacking enzymes (Fig. 108).

(A61) ***Tubbia*** **sp. (Centrolophidae)**
(2)
N: wb, nl 1–2, Ab 3. E: N/S. M: Ab 3. MEL 10.2; MD 892

	N	M	L	T
Perox.	++→+++	−	−	−
Perox. + KCN	−	−	−	−
Alk. phos.	−	−	−	−
Ac. phos.	(+)	(+)	−	−
PAS	++	−	−	−

Remarks: Neutrophils with refractile inclusion; neutrophil ac. phos. in a few fine granules.

Order: Pleuronectiformes

(A62) ***Rhombosolea leporina*** **(Pleuronectidae)**
Yellow-belly flounder (8)
N: n→wb, nl 2, Ab 4. E: we granules in sb matrix, nl 1, Ab 2. M: Ab 3. MEL 8.9; MD 45

	N	M	E	L	T
Perox.	++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	+→++	+→++	N/D	+	(+)
β-gluc.	–	–	–	–	–
ANAE	–	–	–	–	–
AS-D	–	–	–	–	–
PAS	++→+++	(+)	–→++	–	–

Remarks: Eosinophil PAS in fine granules between coarse granules.

(A63) ***Rhombosolea plebeia*** **(Pleuronectidae)**
Sand flounder (2)
N: n→wb, nl 1, Ab 4. E: we granules in sb matrix, nl 1, Ab 2. M: Ab 3. MEL 10.3; MD 40

	N	M	E	L	T
Perox.	+→++	–	–	–	–
Alk. phos.	–	–	–	–	–

(A64) ***Colistium nudipinnis*** **(Pleuronectidae)**
Turbot (2)
N: n→wb, nl 1, Ab 4. E: N/S. M: Ab 3. MEL 12.1; MD 235

	N	M	L	T
Perox.	++	–	–	–
Alk. phos.	–	–	–	–
Ac. phos.	+→++	+→++	–→++	(+)
β-gluc.	–	–	(+→++)*	–

Remarks: Fig. 109. Lymphocytes usually –; β-gluc. granular or diffuse (Fig. 109).

(A65) ***Pelotretis flavilatus*** **(Pleuronectidae)**
Lemon sole (6)
N: n→wb, nl 1, Ab 4. E: e, nl 1, Ab 1. M: Ab 3. MEL 9.4; MD 82

	N	M	E	L	T
Perox.	+	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	+→++	++→+++	N/D	++→+++	++
ANAE	–	–	–	–	–
AS-D	–	–	–	–	–
PAS	++	–	+	–	–

Remarks: Heavy extracellular staining with ANAE, origin not determined; eosinophil PAS diffuse between granules.

(A66) ***Peltorhamphus novaezeelandiae*** **(Pleuronectidae)**
New Zealand sole (6)
N: n→wb, nl 1, Ab 3. E: c, nl 1, Ab 3. M; Ab 3. MEL 10.3; MD 58

	N	M	E	L	T
Perox.	+→++	–	–	–	–
Alk. phos.	–	–	–	–	–
Ac. phos.	+→++	+→++	++	++	–
ANAE	++→+++	++	+++	–	–
AS-D	–	–	–	–	–
PAS	++	(+)	+	–	–

Remarks: Eosinophil PAS in fine granules between coarse granules.

Order: Tetraodontiformes

(A67) *Parika scaber* (Balistidae)
Leatherjacket (7)
N: n→wb, nl 1, Ab 3. E: we→e, nl 1, Ab 2. M: Ab 1. MEL 9.8; MD 81

	N	M	E	L	T
Perox.	++→+++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Alk. phos.	+→++	–→++	++	–	–
Ac. phos.	(+)	–→++	++	–→++	–
β-gluc.	–	–	–	–	–
ANAE	+	++	++→+++	–	–
AS-D	–	++→+++	++→+++	–	–
PAS	+++	(+)	+	–	–

Remarks: Eosinophil alk. phos. and PAS in fine granules between coarse granules; AS-D diffuse around eosinophil coarse granules; neutrophil alk. phos. (rarely +++).

(A68) *Allomycterus jaculiferus* (Diodontidae)
Porcupine fish (4)
N: wb, nl 1, Ab 3. E: se, nl 1, Ab 4. M: Ab 2–3; MD unknown

	N	M	E	L	T
Perox.	+→+++	–	–	–	–
Perox. + KCN	–	–	–	–	–
Alk. phos.	+→+++	–	–	–	–
Ac. phos.	(+)	+→+++	–	+→++	–
Ac. phos. + ta	–	–	–	–	–
ANAE	+	–	–→+++	–	–
AS-D	+→+++	+→++	+→++	+→+++	+
PAS	++→+++	–	+→++	–	–

Remarks: Neutrophil alk. phos. usually ++; eosinophil PAS in fine granules between coarse granules.

Figs. 1–42:

1. *Geotria australis* (Ag1) adult G, fine and coarse granules, Wright's × 1250; 2. *Geotria australis* (Ag1) macrophthalmia G, perox. × 1008; 3. *Geotria australis* (Ag1) ammocoete G, alk. phos. × 1492; 4. *Geotria australis* (Ag1) adult G, fine granules alk. phos. × 1241; 5. *Geotria australis* (Ag1) adult G, coarse granules, alk. phos. × 1764; 6. *Geotria australis* (Ag1) adult G, AS-D × 1411; 7. *Geotria australis* (Ag1) adult L, AS-D × 1748; 8. *Geotria australis* (Ag1) adult G, PAS × 1513; 9. *Geotria australis* (Ag1) adult L, PAS × 1649; 10. *Eptatretus cirrhatus* (Ag2) G, ac. phos. × 900; 11. *Eptatretus cirrhatus* (Ag2) L, PAS × 874; 12. *Cephaloscyllium isabella* (E3) FEG, ANAE × 1441; 13. *Cephaloscyllium isabella* (E3) L, AS-D × 801; 14. *Mustelus lenticulatus* (E5) FEG, ac. phos. × 605; 15. *Mustelus lenticulatus* (E5) FEG, ANAE × 626; 16. *Mustelus lenticulatus* (E5) CEG, PAS × 561; 17. *Galeorhinus australis* (E7) CEG, AS-D, − coarse granules × 1238; 18. *Galeorhinus australis* (E7) CEG, AS-D, + and − granules × 1185; 19. *Galeorhinus australis* (E7) CEG, AS-D + and − cells × 766; 20. *Galeorhinus australis* (E7) T, PAS × 1445; 21. *Oxynotus bruniensis* (E9) L, ac. phos. × 908; 22. *Oxynotus bruniensis* (E9) CEG, ANAE × 681; 23. *Oxynotus bruniensis* (E9) NG, PAS × 698; 24. *Oxynotus bruniensis* (E9) L, PAS × 933; 25. *Squalus acanthias* (E10) FEG, Wright's × 908; 26. *Squalus acanthias* (E10) NG, Wright's × 631; 27. *Squalus acanthias* (E10) two NG showing strong (arrow) and moderate ANAE × 610; 28. *Squalus blainvillei* (E11) NG, AS-D × 1009; 29. *Deania calcea* (E12) CEG, ac. phos. × 933; 30. *Deania calcea* (E12) NG, ANAE × 488; 31. *Scymnodon plunketi* (E13) NG, ANAE × 787; 32. *Centroscymnus owstonii* L, alk. phos. × 1166; 33. *Centroscymnus owstonii* T, alk. phos. × 1009; 34. *Centroscymnus crepidater* (E14) L and T, PAS × 832; 35. *Centrophorus squamosus* (E15) NG, ac. phos. × 1108; 36. *Etmopterus baxteri* (E17) NG, ANAE × 723; 37. *Etmopterus baxteri* (E17) NG, ANAE + NaF × 706; 38. *Etmopterus baxteri* (E17) NG, spleen β-gluc. × 908; 39. *Etmopterus baxteri* (E17) NG and L, PAS × 505; 40. *Dalatias licha* (E18) NG, ac. phos. × 1009; 41. *Dalatias licha* (E18) L, alk. phos. × 1209; 42. *Raja innominata* (E21) CEG, ac. phos. × 1100.

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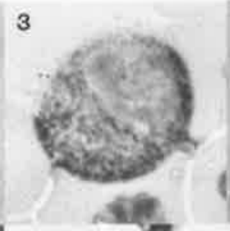
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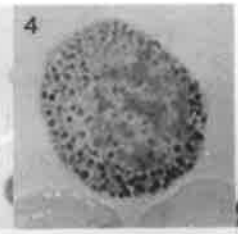
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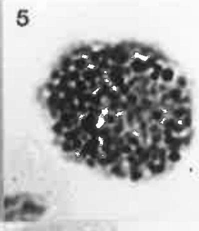
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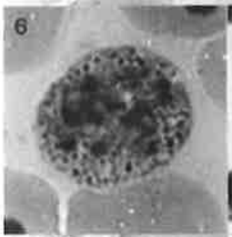
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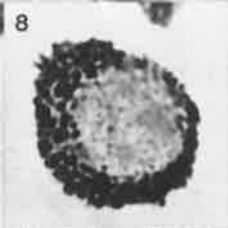
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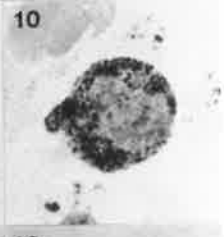
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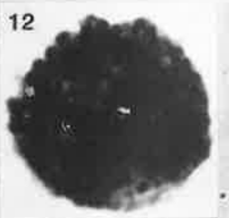
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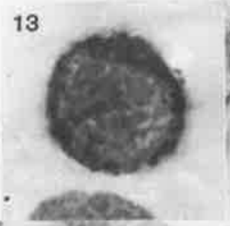
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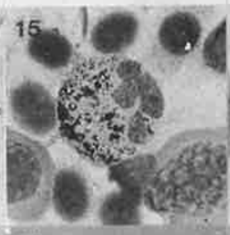
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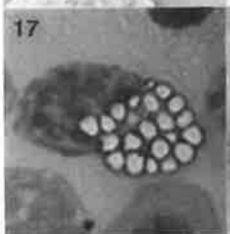
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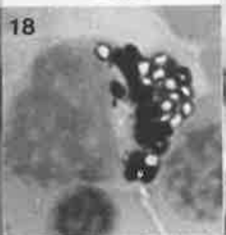
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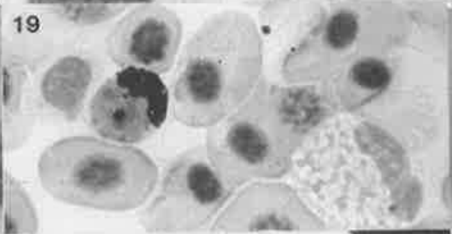
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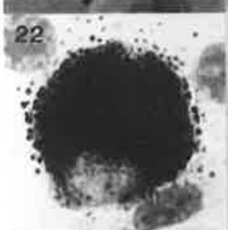
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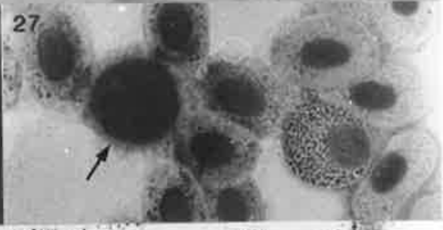
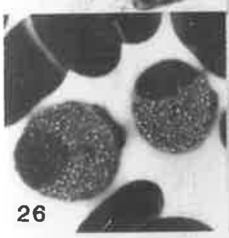
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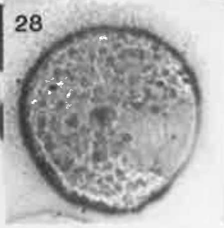
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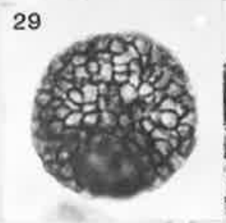
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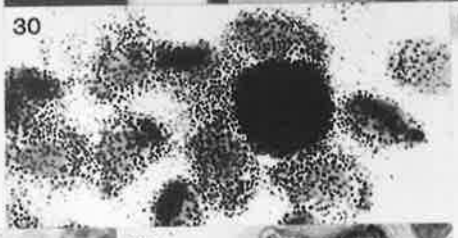
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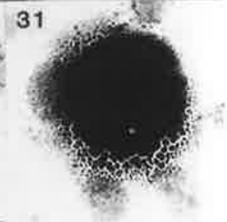
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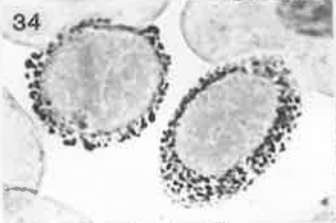
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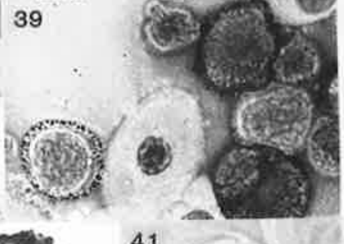
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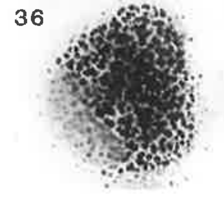
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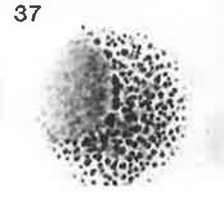
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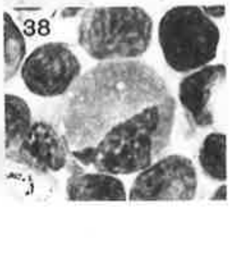
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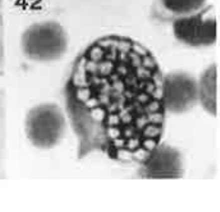
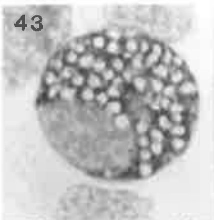


Figs. 43–79:

43. *Pavoraja spinifera* (E22) CEG, alk. phos. × 1066; 44. *Pavoraja spinifera* (E22) NG, ac. phos. × 1094; 45. Undescribed skate (E23) CEG and erythrocytes, ac. phos. × 501; 46. Undescribed skate (E23) NG, PAS × 971; 47. Undescribed skate (E23) CEG, PAS × 828; 48. *Callorhinchus milii* (H1) FG, post-orbital tissue, alk. phos. × 756; 49. *Chimaera phantasma* (H2) FG, AS-D × 829; 50. *Chimaera phantasma* (H2) FG, alk. phos. × 779; 51. *Chimaera phantasma* (H2) FG and CG, ac. phos. × 570; 52. *Hydrolagus novaezelandiae* (H3) FG, alk. phos. × 1452; 53. *Hydrolagus novaezelandiae* (H3) FG and CG, ac. phos. × 840; 54. *Harriotta raleighana* (H5) coarse granulocyte, spleen, ac. phos. × 1344; 55. *Conger verreauxi* (El4) N, alk. phos. × 928; 56. *Simenchelys parasiticus* (El7) N, ANAE × 968; 57. *Halosaurus pectoralis* (El12) erythrocytes, ANAE × 714; 58. *Roulenia* sp. (S2) E, spleen, Wright's × 750; 59. *Roulenia* sp. (S2) E, spleen, perox. × 2070; 60. *Roulenia* sp. (S2) N and E, PAS × 773; 61. *Talismania* sp. (S3) L, alk. phos. × 1764; 62. *Talismania* sp. (S3) E, PAS × 893; 63. *Alepocephalus* sp. (S4) N, alk. phos. × 746; 64. *Alepocephalus* sp. (S4) E, alk. phos. × 905; 65. *Persparsia kopua* (S6) E, PAS × 2295; 66. *Salmo trutta* (S11) M, β-gluc. × 1612; 67. *Salmo trutta* (S11) macrophages, spleen, β-gluc. × 639; 68. *Salmo gairdnerii* (S12) E, peritoneum, ANAE × 673; 69. *Salmo gairdnerii* (S12) E, peritoneum, AS-D × 688; 70. *Oncorhynchus tshawytscha* (S13) N, perox. + KCN × 1310; 71. *Oncorhynchus tshawytscha* (S13) L, spleen, ac. phos. × 903; 72. *Oncorhynchus tshawytscha* (S13) N, alk. phos. × 1120; 73. *Maurolicus muelleri* (St4) N, perox. × 887; 74. *Maurolicus muelleri* (St4) nucleated erythrocyte, perox. + KCN × 874; 75. *Maurolicus muelleri* (St4) fragmenting erythrocyte, perox. + KCN × 877; 76. *Maurolicus muelleri* (St4) erythrocyte, perox. + KCN × 941; 77. *Chauliodus sloani* (St6) erythrocytes and T, Wright's × 639; 78. *Chauliodus sloani* (St6) multinucleate cells, PAS × 382; 79. *Chauliodus sloani* (St6) E, alk. phos. × 1172.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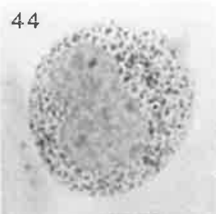
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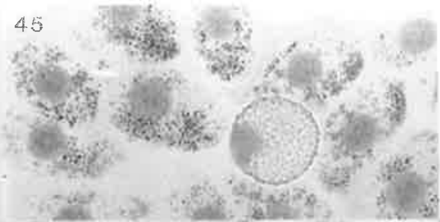
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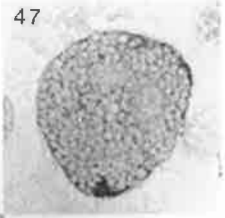
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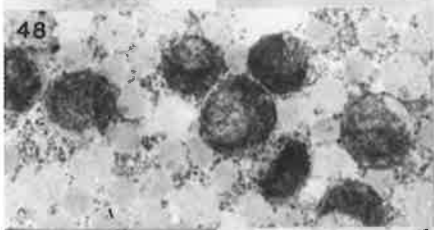
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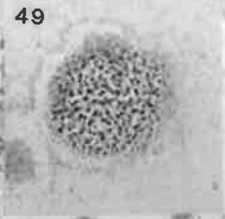
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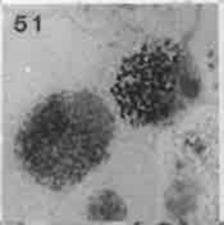
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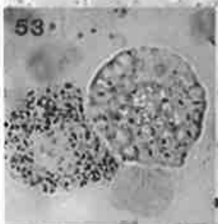
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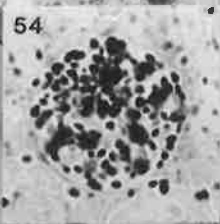
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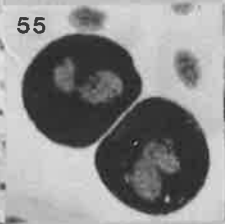
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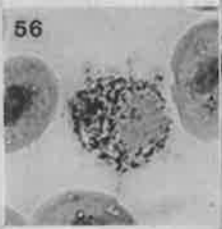
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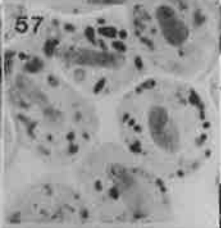
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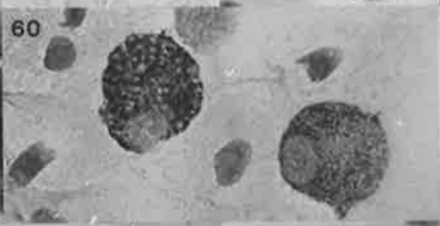
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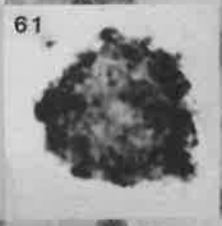
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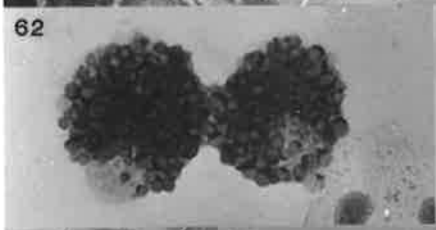
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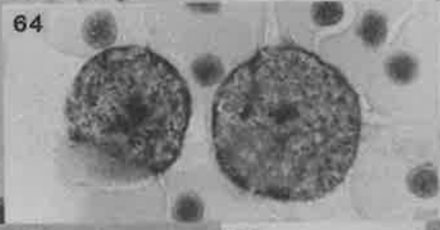
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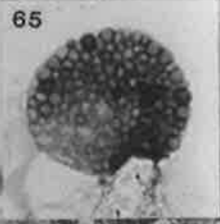
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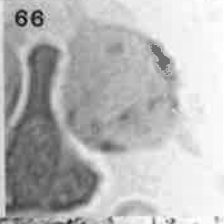
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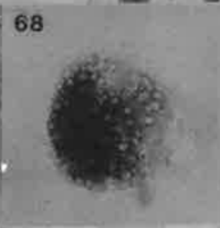
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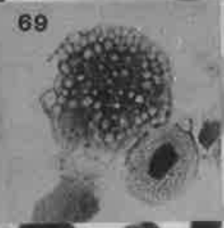
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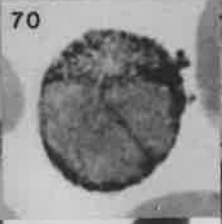
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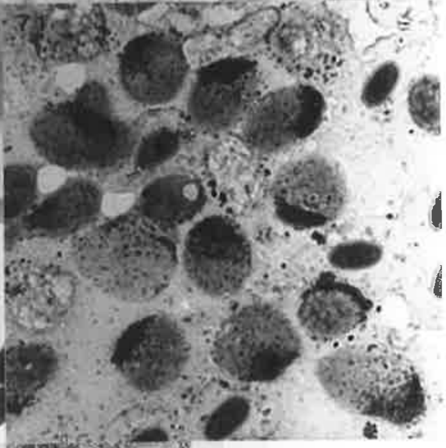
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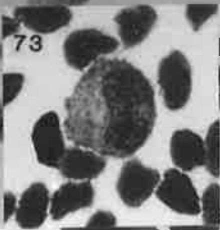
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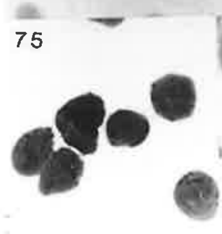
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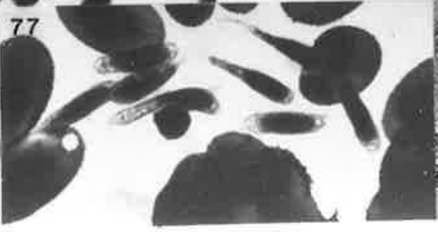
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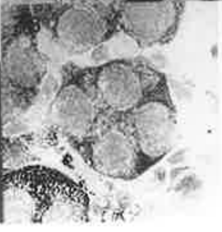
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Figs. 80–109:

80. *Malacosteus niger* (St9) N and E, perox. + KCN × 1382; 81. *Idiacanthus niger* (St10) E and M, perox. × 576; 82. *Idiacanthus niger* (St10) N and E, ac. phos. × 815; 83. *Paralepis atlantica prionosa* (Sc2) N, ac. phos. × 968; 84. *Paralepis atlantica prionosa* (Sc2) E, ac. phos. × 987; 85. *Paralepis atlantica* prionosa (Sc2) N, ANAE × 913; 86. *Paralepis atlantica prionosa* (Sc2) N and E, ANBE × 934; 87. *Macruronus novaezelandiae* (P5) N and M, alk. phos. × 837; 88. *Trachyrincus longirostris* (P7) L, alk. phos. × 997; 89. *Genypterus blacodes* (P9) N and E, alk. phos. × 1049; 90. *Brotulataenia crassa* (P10) E, AS-D × 852; 91. *Diplocrepis puniceus* (P12) M, ANBE × 913; 92. *Hoplostethus atlanticus* (A4) E, ac. phos. × 1152; 93. *Hoplostethus atlanticus* (A4) N, ANAE × 788; 94. *Hoplostethus atlanticus* (A4) N, AS-D × 706; 95. *Hoplostethus mediterraneus* (A5) N, alk. phos. × 1158; 96. *Hoplostethus mediterraneus* (A5) M and L, ac. phos. × 642; 97. *Hoplostethus mediterraneus* (A5) T, ac. phos. × 1159; 98. *Chelidonichthys kumu* (A16) L, ac. phos. × 806; 99. *Chelidonichthys kumu* (A16) L, anterior kidney, alk. phos. × 974; 100. *Congiopodus leucopaecilus* (A19) E, alk. phos. × 799; 101. *Zanclistius elevatus* (A33) E, alk. phos. × 1129; 102. *Zanclistius elevatus* (A33) E, AS-D × 1331; 103. *Pentaceros decacanthus* (A34) E, AS-D × 1344; 104. *Pseudolabrus celidotus* (A40) E, alk. phos. × 1261; 105. *Parapercis colias* (A42) M, perox. × 1452; 106. *Parapercis colias* (A42) N, PAS × 1645; 107. *Seriolella brama* (A57) N, Wright's, note refractile inclusion, × 1327; 108. *Centrolophus niger* (A60) N, ANAE × 902; 109. *Colistium nudipinnis* (A64) L, β-gluc. × 1210.


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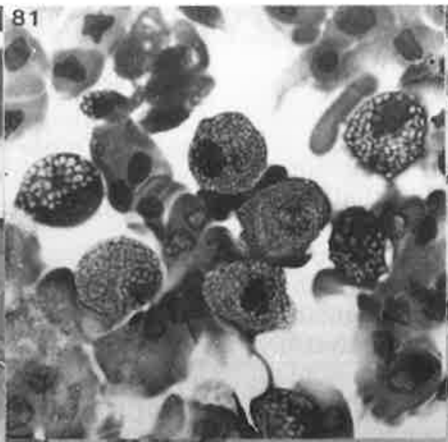
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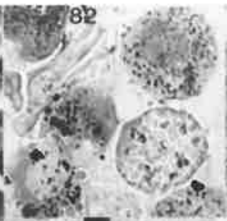
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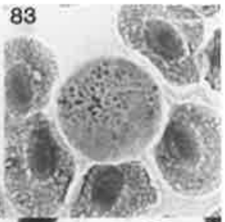
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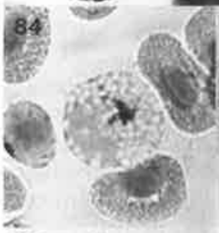
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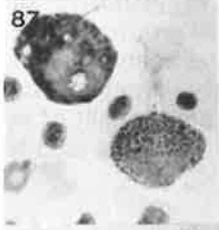
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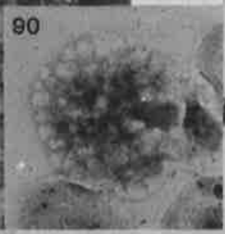
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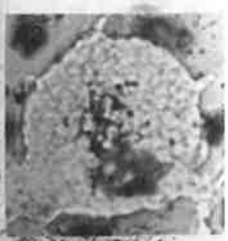


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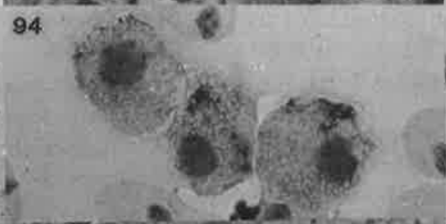
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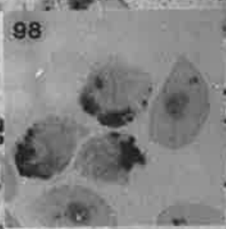
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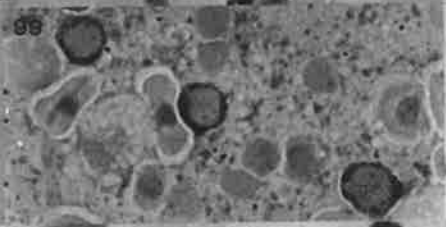
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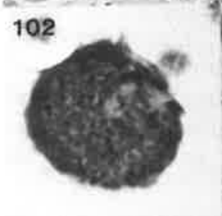
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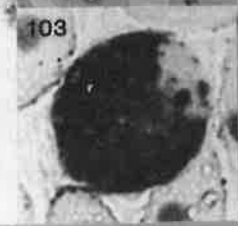
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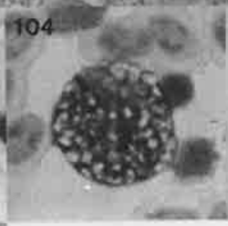
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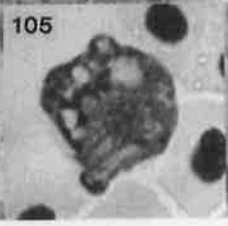
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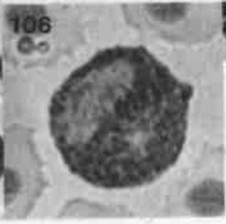
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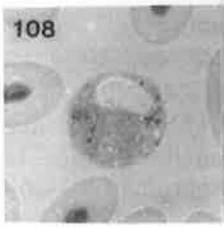
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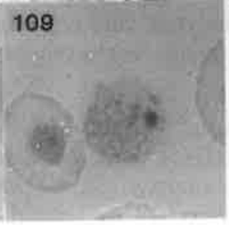
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OBSERVATIONS

Blood composition

The blood of primitive fish such as agnathans and rhinochimaerids contained cells that could only be classified as granulocytes or lymphocytes under the light microscope, though coarse eosinophilic granulocytes have been ultrastructurally demonstrated in agnathans (Rowley and Page 1985a). Conversely, elasmobranch blood often appeared to contain several types of granulocytes that were usually impossible to classify by use of the nomenclature of higher vertebrate granulocytes, though the coarse eosinophilic granulocytes were morphologically similar to the eosinophils of other vertebrate groups. Higher groups had leucocytes that were easily identifiable with those described in mammalian haematology.

Teleost neutrophils with polymorphonuclear features were most often seen in salmoniforms (29% of species from that group) and stomiiforms (30%), less often in scopelomorphs (17%) and paracanthopterygians (17%), rarely in acanthopterygians (3%), and not in elopomorphs. Neutrophils were usually neutrophilic or weakly basophilic (75.2%), though they were occasionally basophilic (5.1%) or eosinophilic (9.5%); whereas eosinophils were usually eosinophilic (50.0%), had colourless granules (46.1%), of which 14% were in basophilic cytoplasm, or were basophilic (3.6%). Neutrophils were more abundant than eosinophils in the blood of eels and scopelomorphs, but the opposite applied in 1 clupeomorph (33%), 3 salmoniforms (21%) (all of which were alepocephalids (S3–5)), 3 stomiiforms (30%), 1 paracanthopterygian (8%), and 13 acanthopterygians (19%) (mainly gurnards (A16–18), boarfish (A33, 34), and tunas (A54–56)). Neutrophils of centrolophids (A57–61) had a clear, refractile, crescent-shaped area devoid of granules containing enzymes.

Stomiiforms had very thin blood (less than 500 erythrocytes per blood film) and white livers and spleens. The blood of alepocephalids (S2–5) was also thin. Erythrocytes were so scarce in the gulper eel (E11) that they were less abundant than neutrophils. Fish with thin blood had large erythrocytes (*see* below). Conversely, *Maurolicus muelleri* (St4) had thick, burgundy-coloured blood in which intact erythrocytes were uncommon; the red cells having fragmented to produce many, small, haemoglobin-containing sacs (*see* Figs. 73–76).

Cell size

Generally, erythrocytes increased in length as depth at which the fish were taken increased (Fig. 110). This was less apparent in paracanthopterygians (P) than in other groups. Paracanthopterygian cells were also smaller than those of other groups, whereas elasmobranch cells were larger. Within the Acanthopterygii, pleuronectiforms had small blood cells.

Trends in cell types between taxa

The incidence of enzymes in cell types in different taxa is given in Table 2. Interrelationships of taxa (as proposed by Nelson (1984)) are shown in Fig. 111. In general, cartilaginous fish are regarded as primitive or resembling primitive forms. Teleost groups such as the elopomorphs, clupeiforms, and salmoniforms are regarded as basal or early groups, and perciforms and pleuronectiforms are thought of as developed or recent groups. The following trends were apparent:

Fine granulocytes, neutrophilic granulocytes, and neutrophils

1. There was an increase in perox. from a rare and weak occurrence in elasmobranchs and holocephalans to a consistent presence in teleosts other than elopomorphs and clupeomorphs.
2. There was a lack of alk. phos. in elasmobranchs, its occurrence varied in elopomorphs, and it was common in callorhynchids and chimaerids (H2–4) and in higher teleosts.
3. There was a high ANAE incidence (Table 2) and intensity (Table 3) in elasmobranchs and elopomorphs, and a high incidence of AS-D in chimaeriforms, elasmobranchs, elopomorphs, and scopelomorphs.
4. In all fish there was a consistent presence and high content of PAS substance in fine granulocytes, neutrophilic granulocytes, or neutrophils.

Coarse granulocytes, fine eosinophilic granulocytes, coarse eosinophilic granulocytes, eosinophils

5. Peroxidase was seen in only one holocephalan; was occasionally present in elasmobranchs, elopomorphs, and stomiiforms; occurred weakly in acanthopterygians (Table 4), except snapper (A30); but was not seen in salmoniforms or scopelomorphs.
6. There was no ANAE in paracanthopterygians, AS-D in salmoniforms, or esterase in stomiiforms and the one scopelomorph (Sc2) in which esterases were examined.
7. These cells usually contained some PAS substance, but staining was strong only in coarse granules of elopomorphs,

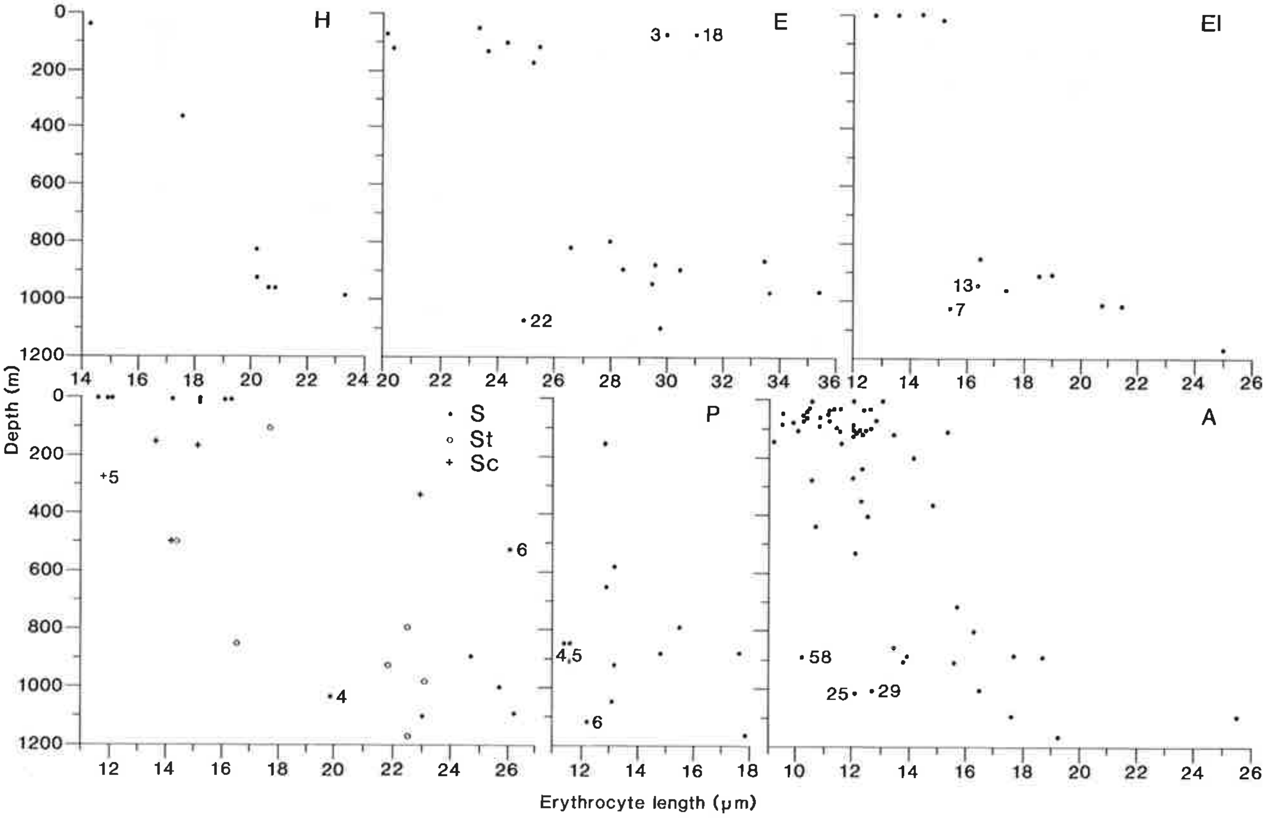

Fig. 110: Mean erythrocyte length of species in this study in relation to the mean depth at which they were caught. (Group abbreviations are as in the text, and numbers given for atypical results are the species numbers.)

salmoniforms, stomiiforms, snapper (A30), and tunas (A53–56).

Monocytes

8. Monocyte perox. was always weak, but was seen in elopomorphs, salmoniforms, and two acanthopterygians (A42, 47).
9. Monocyte ANAE was rare in all groups except elopomorphs and acanthopterygians.
10. Monocytes of all groups stained only faintly for diffuse PAS substance.

Lymphocytes

11. Lymphocyte alk. phos. was seen mainly in squalids E9, 12, 15, and 18 and in some paracanthopterygians (P1, 4, 7) (Tables 5 and 6).
12. A high incidence of lymphocyte AS-D was seen only in elopomorphs.
13. Lymphocytes of elasmobranchs often stained moderately for PAS, whereas lymphocytes in other groups contained weak PAS substance (holocephalans and elopomorphs) or no PAS substance (salmoniforms, stomiiforms, scopelomorphs, paracanthopterygians, and acanthopterygians).

Thrombocytes

14. Only squalid E12 thrombocytes contained alk. phos.
15. Thrombocyte ac. phos. was rarely seen in salmoniforms and not seen in stomiiforms.
16. Only elopomorphs had a high incidence of thrombocyte ANAE.
17. Elasmobranch E3, 7, 9, 13–18, and 23 thrombocytes were often moderately to strongly PAS, elopomorph thrombocytes contained weak diffuse reaction, but higher teleost groups were negative for PAS, except some tunas (A53, 56).

In general, perox. was most widespread in leucocytes of elopomorphs and salmoniforms; alk. phos. was seen mainly in lymphocytes and thrombocytes of elasmobranchs, but in neutrophils of teleosts; ac. phos. was ubiquitous in different taxa and different leucocytes, but was often weak in salmoniforms; and high incidences of esterases were seen in elasmobranchs and elopomorphs.

Trends within major taxa

Elasmobranchs

1. Peroxidase was most common in CEG, particularly in rays, but not as common in NG and was not seen in FEG, and it was never moderate to strong.
2. All granulocytes lacked alk. phos., except in CEG of prickly deepsea skates (E22), and β-gluc., except in NG of Baxter's lantern dogfish (E17).

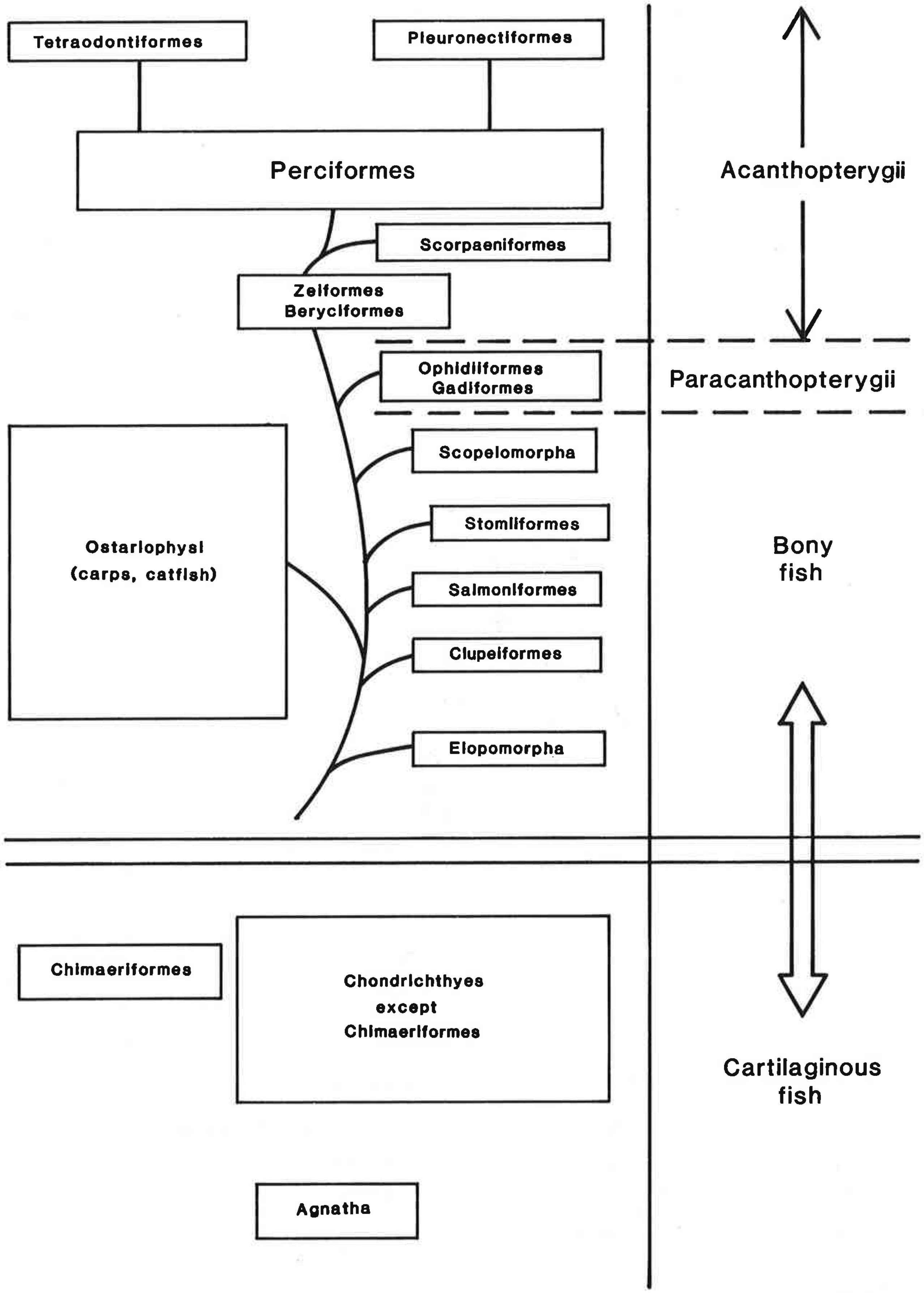

Fig. 111: The general interrelationships between major taxonomic groups (as proposed by Nelson (1984)) examined here.

TABLE 2: Incidence (%) of enzymes in relation to major taxa* and leucocyte type

	Elasmobranchii		Holocephali	Elopomorpha	Clupeiformes	Salmoniformes	Stomiiformes	Scopelomorpha	Paracanthopterygii	Acanthopterygii
Neutrophils		NG	FG							
Perox.		18	0	46	33	100	100	100	100	100
Alk. phos.		0	67	10	N/D	46	67	33	55	38
Ac. phos.		100	60	100	N/D	79	88	100	89	92
β-gluc.		7	0	0	0	13	0	17	0	0
ANAE		100	40	78	N/D	31	22	40	25	53
AS-D		77	60	67	N/D	25	22	60	8	26
PAS		100	100	100	N/D	100	100	100	100	100
Eosinophils	FEG	CEG								
Incidence in blood	71	50	67	38	100	36	50	17	45	65
Perox.	6	55	17	60	0	0	100	0†	0	13
Alk. phos.	0	10	0	0	N/D	25	0	N/D	0	17
Ac. phos.	67	90	75	50	N/D	80	75	100†	75	57
β-gluc.	0	0	0	0	0	0	0	0‡	0	0
ANAE	91	80	50	33	N/D	50	0	0‡	0	38
AS-D	40	50	33	33	N/D	0	0	0‡	25	17
PAS	100	100	100	100	N/D	100	100	100‡	100†	66
Monocytes										
Perox.		0	N/S	31	0	38	0	0	0	3
Alk. phos.		0	N/S	10	N/D	0	0	0	9	10
Ac. phos.		37	N/S	80	N/D	62	87	100	82	92
β-gluc.		0	N/S	0	0	13	0	17	0	0
ANAE		5	N/S	38	N/D	8	0	20	22	34
AS-D		12	N/S	29	N/D	17	11	20	33	19
PAS		25	N/S	75	N/D	36	57	40	45	42
Lymphocytes										
Perox.		0	0	0	0	0	0	0	0	0
Alk. phos.		25	17	10	N/D	9	0	0	27	10
Ac. phos.		79	40	100	N/D	71	50	80	73	94
β-gluc.		0	0	0	0	13	0	0	0	3
ANAE		20	20	44	N/D	15	0	0	13	32
AS-D		29	0	63	N/D	8	0	40	22	11
PAS		88	25	43	N/D	0	0	0	0	4
Thrombocytes										
Perox.		0	N/S	0	0	0	0	0	0	0
Alk. phos.		10	N/S	0	N/D	0	0	0	0	0
Ac. phos.		58	N/S	63	N/D	23	0	80	64	60
β-gluc.		0	N/S	0	0	25	0	0	0	0
ANAE		15	N/S	50	N/D	9	0	0	0	11
AS-D		29	N/S	0	N/D	0	0	20	0	8
PAS		86	N/S	43	N/D	0	0	0	0	4

* Agnatha were not included because the tests were carried out on only two species.
† Based on two fish.
‡ Based on one fish.

TABLE 3: Mean (and standard deviation) of intensity of staining* of enzymes for the major taxa†

	Perox.	Ac. phos.	ANAE	AS-D	PAS
NG, neutrophils					
Elasmobranchs	0.1 (0.2)	2.8 (0.4)	2.7 (0.5)	1.8 (1.2)	2.4 (0.5)
Holocephalans	0.0	1.4 (1.4)	0.8 (1.0)	1.2 (1.2)	2.2 (0.4)
Elopomorphs	1.2 (1.4)	2.4 (0.6)	1.9 (1.1)	1.6 (1.2)	3.0 (0.0)
Salmoniforms	2.6 (0.5)	1.0 (0.8)	0.6 (1.0)	0.4 (0.8)	2.1 (0.9)
Stomiiforms	2.3 (0.5)	1.9 (0.9)	0.7 (1.2)	0.3 (0.6)	1.9 (0.7)
Scopelomorphs	2.5 (0.5)	1.8 (0.7)	0.5 (0.8)	1.2 (1.0)	2.7 (0.5)
Paracanthopterygians	2.4 (0.5)	1.3 (0.8)	0.6 (1.0)	0.2 (0.6)	2.1 (0.7)
Acanthopterygians	2.4 (0.5)	1.9 (1.0)	1.0 (1.1)	0.6 (1.1)	2.4 (0.6)
FEG, CEG, eosinophils					
Elasmobranchs FEG	0.0 (0.1)	1.8 (1.4)	1.9 (0.9)	1.0 (1.3)	2.1 (0.6)
Elasmobranchs CEG	0.4 (0.4)	2.0 (1.2)	1.4 (1.0)	1.0 (1.1)	1.9 (0.6)
Holocephalans	0.0	1.3 (1.2)	1.0 (1.2)	0.3 (0.5)	1.0 (1.6)
Elopomorphs	1.6 (1.4)	1.0 (1.2)	1.0 (1.4)	1.0 (1.4)	2.2 (1.2)
Salmoniforms	0.0	0.4 (0.2)	0.9 (1.2)	0.0	2.6 (0.5)
Stomiiforms	2.0 (0.6)	0.9 (0.7)	0.0	0.0	2.3 (0.4)
Scopelomorphs	0.0	1.0	0.0	0.0	1.0
Paracanthopterygians	0.0	1.1 (0.9)	0.0	0.3 (0.4)	0.5
Acanthopterygians	0.1 (0.2)	1.0 (0.9)	0.8 (1.2)	0.5 (1.1)	1.2 (1.2)

* 0.0, negative; 0.5, weak and inconsistent; 1.0, weak; 2.0, moderate; 3.0, strong.
† Agnatha were not included because the tests were carried out on only two species.

TABLE 4: Mean (and standard deviation) of intensity of staining* of acanthopterygians

	Neutrophilis	Eosinophils	Monocytes	Lymphocytes	Thrombocytes
Perox.					
Beryciformes	2.3 (0.5)	0.0	0.0	0.0	0.0
Zeiformes	2.2 (0.7)	0.5	0.0	0.0	0.0
Scorpaeniformes	2.3 (0.4)	0.2 (0.4)	0.0	0.0	0.0
Perciformes	2.5 (0.5)	0.0 (0.1)	0.1 (0.3)	0.0	0.0
Pleuronectiformes	1.8 (0.4)	0.0	0.0	0.0	0.0
Tetraodontiformes	3.0 (0.0)	0.0	0.0	0.0	0.0
Alk. phos.					
Beryciformes	1.2 (1.3)	0.0	0.0	0.3 (0.7)	0.0
Zeiformes	0.5 (0.9)	0.0	0.5 (0.9)	0.8 (1.3)	0.0
Scorpaeniformes	0.6 (0.7)	1.3 (0.9)	0.0	0.8 (1.0)	0.0
Perciformes	0.9 (1.2)	0.3 (0.9)	0.1 (0.4)	0.0	0.0
Pleuronectiformes	0.0	0.0	0.0	0.0	0.0
Tetraodontiformes	2.5 (0.5)	1.0 (1.0)	1.0 (1.0)	0.0	0.0
Ac. phos.					
Beryciformes	1.8 (0.6)	1.4 (0.8)	2.3 (0.5)	2.2 (1.1)	2.0 (1.0)
Zeiformes	2.2 (0.4)	0.5	1.6 (0.5)	1.7 (0.9)	0.9 (0.9)
Scorpaeniformes	1.3 (1.4)	2.0 (0.0)	1.3 (1.1)	1.3 (1.4)	0.4 (0.8)
Perciformes	2.1 (1.0)	0.7 (0.9)	1.8 (1.0)	2.1 (0.9)	1.2 (1.1)
Pleuronectiformes	2.0 (0.0)	2.0	2.3 (0.4)	2.0 (0.7)	0.8 (0.8)
Tetraodontiformes	0.5 (0.0)	1.0 (1.0)	2.5 (0.5)	2.0 (0.0)	0.0
ANAE					
Beryciformes	1.2 (1.2)	0.0	0.4 (0.8)	0.4 (0.8)	0.0
Zeiformes	0.8 (0.8)	0.0	0.1 (0.2)	0.4 (0.4)	0.0
Scorpaeniformes	1.2 (1.2)	1.0 (1.0)	0.8 (1.0)	0.5 (0.8)	0.4 (0.8)
Perciformes	1.0 (1.2)	0.6 (1.0)	0.4 (0.7)	0.5 (0.8)	0.2 (0.5)
Pleuronectiformes	1.0 (1.4)	1.0 (1.4)	0.7 (0.9)	0.0	0.0
Tetraodontiformes	1.0 (0.0)	3.0 (0.0)	1.0 (1.0)	0.0	0.0
AS-D					
Beryciformes	1.4 (1.2)	0.0	0.4 (0.8)	0.1 (0.2)	0.1 (0.2)
Zeiformes	0.5 (0.9)	0.0	0.0	0.1 (0.2)	0.0
Scorpaeniformes	0.0	0.0	0.8 (1.3)	0.5 (0.9)	0.1 (0.2)
Perciformes	0.6 (1.1)	0.5 (1.1)	0.3 (0.7)	0.0	0.0
Pleuronectiformes	0.0	0.0	0.0	0.0	0.0
Tetraodontiformes	1.5 (1.5)	2.5 (0.5)	2.5 (0.5)	1.5 (1.5)	0.5 (0.5)
PAS					
Beryciformes	2.8 (1.4)	1.8 (1.1)	0.2 (0.2)	0.0	0.0
Zeiformes	2.0 (0.0)	0.0	0.3 (0.3)	0.3 (0.3)	0.0
Scorpaeniformes	2.0 (0.6)	0.7 (1.9)	0.1 (0.2)	0.0	0.0
Perciformes	2.4 (0.6)	1.2 (1.3)	0.3 (0.4)	0.0	0.1 (0.4)
Pleuronectiformes	2.3 (0.5)	1.3 (0.5)	0.3 (0.2)	0.0	0.0
Tetraodontiformes	3.0 (0.0)	1.5 (1.5)	0.3 (0.3)	0.0	0.0

* 0.0, negative; 0.5, weak and inconsistent; 1.0, weak; 2.0, moderate; 3.0, strong.

TABLE 5: Mean intensity* of alk. phos. and PAS in selected leucocytes

	NG, neutrophil alk. phos.		FEG, CEG, eosinophil alk. phos.		Lymphocyte alk. phos.		Lymphocyte PAS	
Elasmobranchs	0.0		0.1	(0.4)	0.5	(1.0)	1.8	(1.0)
Chimaeriform holocephalans (H1–4)	2.0	(1.4)	0.3	(0.4)	0.3	(0.7)	0.3	(0.4)
Elopomorphs	0.3	(0.9)	0.0		0.1	(0.2)	0.5	(0.7)
Salmoniforms	1.3	(1.4)	0.5	(0.9)	0.3	(0.9)	0.0	
Stomiiforms	1.5	(1.1)	0.0		0.0		0.0	
Paracanthopterygians	1.2	(1.3)	0.0		0.5	(1.0)	0.0	
Acanthopterygians	0.9	(1.2)	0.3	(0.8)	0.2	(0.6)	0.0	(0.1)

* 0.0, negative; 0.5, weak and inconsistent; 1.0, weak; 2.0, moderate; 3.0, strong.

3. Fine eosinophilic granulocyte ac. phos. occurred in the five species of squalid with FEG (E10, 11, 16, 17, 18), in three out of five sharks (E4, 5, 8, but not 3, 7), but in neither of the two rays (E21, 24).
4. Coarse eosinophilic granulocyte ANAE occurred in all three squalids with CEG (E9, 12, 15) and all four rays with CEG (E21–24), but in only one of three sharks with CEG (E7, but not 5, 8).

TABLE 6: Differential incidence (%) of enzymes in selected leucocytes of elasmobranchs

	Sharks (Hexanchiformes and Lamniformes)	Dogfish (Squaliformes)	Rays (Rajiformes)
FEG			
Ac. phos.	60	100	0
AS-D	75	25	0
CEG			
Perox.	0	50	100
ANAE	33	100	100
NG			
Perox.	0	30	0
AS-D	–*	90	33
Lymphocyte Alk. phos.	0	50	0
Lymphocyte and thrombocyte AS-D	75	0	50
Thrombocyte Ac. phos.	80	70	0

* Too few.

5. In FEG and NG, AS-D was common in sharks, as it was in NG of squalids, but it was scarce in FEG of squalids and NG of rays and was not seen in FEG of rays.
6. Although FEG, CEG, and NG had similar enzyme profiles, NG stained consistently more strongly for ac. phos., ANAE, and PAS.
7. All elasmobranch monocytes lacked perox., alk. phos., β-gluc., and ANAE, and only two (E4, 8) contained AS-D.
8. All elasmobranch lymphocytes and thrombocytes lacked perox. and β-gluc., and the lymphocytes and thrombocytes of sharks E2, 3, 4, 5, and 7 and rays E19 and 21–24 lacked alk. phos., whereas squalid lymphocytes and thrombocytes were variable.
9. All squalids lacked lymphocyte and thrombocyte AS-D.
10. Shark and squalid thrombocytes were variable for ac. phos., whereas those of all rays tested (E21–24) were negative.
11. Lymphocytes of all elasmobranchs except four (E4, 8, 14, 21) stained moderately to strongly for PAS substance.
12. Coarse eosinophilic granulocytes were common, particularly in rays.

Holocephalans

13. The peripheral blood leucocytes of all holocephalans, except pale ghost sharks (H4), lacked perox.
14. In FG, alk. phos. was strong in elephant fish (H1) and chimaerids (H2–4), but absent from rhinochimaerids (H5, 6).

Elopomorphs

15. Peroxidase neutrophils were seen in anguillid (El1–3), congrid (El4, 5), and basketwork (El6) eels only.
16. All eels, except conger eels (El4), lacked neutrophil alk. phos., though it has also been reported from European eels (El1) (Fey 1966b).
17. Neutrophil esterases were common (El1–4, 7, 11–13) and often strong (El1–4).
18. Monocytes and lymphocytes of all eel species, except one (El9), lacked alk. phos.
19. In those species in which eosinophils were seen (El2, 4–8), they stained moderately to strongly for PAS, except El5.

Salmoniforms

20. Only salmonids (S11–14) and one alepocephalid (S4) had neutrophil alk. phos.
21. Deepsea smelt (S1), common smelt (S7), and inanga (S10) neutrophils lacked ac. phos., and it was very weak in several species (S2, 8, 9, 11–13).
22. Neutrophils lacked esterases, except for those in two alepocephalids (S4, 5), ANAE in *Persparsia kopua* (S6), and very weak inconsistent ANAE in a few brown trout (S11).
23. In brown trout (S11), 7 of 78 had β-gluc. in all leucocytes, but leucocytes of the other 71 were negative for this enzyme.
24. Eosinophils were not seen in peripheral blood of common smelt (S7), galaxiids (S8–10), and salmonids, but were common in two alepocephalids (S3, 5) and *Persparsia kopua* (S6).
25. Monocytes of two galaxiids (S9, 10) and some salmonids (S11–13) showed perox.
26. Bathylagid and alepocephalid (S1–5) monocytes lacked ac. phos., whereas those of common smelt (S7), galaxiids, and salmonids were positive.
27. All monocytes lacked esterases, though there was weak activity in two salmonids (S12, 13).
28. All lymphocytes lacked alk. phos., except in *Talismania* sp. (S3), where they were strongly positive.
29. Retropinids, galaxiids, and salmonids (S7–14), but only one alepocephalid (S3), showed lymphocyte ac. phos.
30. Only S11 and 13 showed lymphocyte esterase.

Stomiiforms

31. Only lightfish (St1) neutrophils lacked ac. phos.; it was moderate (St3, 6, 7, 9) or strong (St5, 10) in other species.

32. Neutrophils and eosinophils lacked esterases, though there was strong ANAE in viperfish (St6) and scaly dragonfish (St7) neutrophils.
33. Monocytes of all species except lightfish (St1) lacked AS-D.

Paracanthopterygians

34. Lymphocyte alk. phos. was seen in three species (P1, 4, 7).

Acanthopterygians

35. Neutrophil perox. was ubiquitous, but weakest in pleuronectiforms (A62–66) and strong in both tetraodontiforms (A67, 68).
36. Pleuronectiforms lacked alk. phos. in one zeiformes (A9) and two scorpaeniforms (A15, 16) it was strongest in lymphocytes, and it was strong in tetraodontiform neutrophils.
37. Leucocyte ac. phos. was high in pleuronectiforms and some scombrids (A53, 54, 56). Acid phosphatase was high in monocytes and lymphocytes of beryciforms (A1–7), perciforms (A22–68), and tetraodontiforms, in eosinophils of snapper (A30), in neutrophils of perciforms, and it was low in neutrophils of scorpaeniforms (except A16) and tetraodontiforms.
38. Leucocyte ANAE was generally low in all groups, but high in orange roughy (A4) and boarfish (A33, 34); and ANAE was high in skipjack tuna neutrophils (A56) and tetraodontiform eosinophils.
39. Pleuronectiforms lacked AS-D, whereas it was highest in tetraodontiforms. Beryciform and most scombrid (A53, 54, 56) neutrophils contained moderate AS-D, but neutrophils of other groups contained only weak AS-D, and it was not seen in scorpaeniform neutrophils.
40. Eosinophils of beryciforms, pleuronectiforms, and tetraodontiforms stained weakly to moderately for PAS, but scombrid eosinophils stained moderately to strongly.

In general, the differential occurrence of enzymes is in accordance with major taxonomic subdivisions in elasmobranchs, holocephalans, and salmoniforms, but this was less clear in elopomorphs and acanthopterygians, and too few stomiiforms, scopelomorphs, and paracanthopterygians were examined to draw conclusions. Non-squalid sharks, squalids, and rays differed in occurrence and strength of several enzymes. Rhinochimaerid holocephalans (H5, 6) lacked FG alk. phos., whereas it occurred strongly in callorhynchid (H1) and chimaerid (H2–4) FG. In salmoniforms, argentinoids (S1–6) differed from salmonoids (S7–14) in the presence of eosinophils (except in S1), the lack of monocyte perox. and ac. phos., and the lack of lymphocyte ac. phos. (except S3). In acanthopterygians, the two most advanced groups, pleuronectiforms and tetraodontiforms, showed distinct, but dissimilar, enzyme patterns.

Distribution of enzyme activity in leucocyte granules

Under the light microscope, FEG, NG, and neutrophils only contain small granules, and thus subpopulations cannot be distinguished. Monocytes invariably contained a few granules of unequal size, but staining patterns were not related to granule size.

Eosinophils have fairly uniform coarse granules, but staining was variable within and between the granules. Eosinophil perox. was confined to the coarse granules in some eels (El2–4, 6), but in some stomiiforms (St1, 5, 8–10) reactivity was between and over the surface of coarse granules. In some eels (El1, 2, 5) and holocephalans (H2, 5, 6) ac. phos. occurred in the coarse granules, whereas in all other groups (E8, 12, 15, 21, 22, 24; C1; S3–5; St1, 5, 6, 10; Sc4; P2, 9, 10; A2, 4, 6, 8, 16, 30) it was around and between the coarse granules. Similarly, ANAE activity occurred in the coarse granules of a holocephalan (H6) and a pentacerotid (A33), but between the coarse granules in several elasmobranchs (E12, 21, 22); and AS-D occurred in the coarse granules of H2, H6, and A33 eosinophils, but around the coarse granules of E22, E23, S12, and P10 eosinophils. However, alk. phos. was seen only between coarse granules (E22; H2; S4; A19, 30, 40, 64). Periodic Acid-Schiff reaction most often occurred between coarse granules (E5, 7, 8, 12, 21, 22; S4, 5, 6; Sc2; A2, 6, 62, 65–68), but also occurred in peripheral (H4), or all, coarse granules (St9; A19, 30, 31, 34, 53, 54), in and over coarse granules (S1–3), in and between coarse granules (A55), and under the cell membrane (E22).

Enzyme inhibition

Cyanide inhibited neutrophil perox. in all but 1 (St9) of 65 species tested, but inhibited eosinophil perox. in only 1 (A30) of 9 species tested (E21; El2; St1, 5, 8–10; A8). Neutrophil perox. was totally inhibited by sodium azide in barracudina (Sc2) and by aminotriazole in *Symbolophorus* (Sc6); whereas azide usually only caused partial inhibition (S7, 11; St8–10; Sc6; P12), and aminotriazole rarely caused any inhibition (Sc2; A12). Similarly, eosinophil perox. was not inhibited by azide (St5, 8–10) or aminotriazole (St5, 9).

Urea-resistant alk. phos. occurred in elasmobranchs (E12, 15, 18), pale ghost shark (H4), and some teleosts (S4; P5, 7, 9; A7), but alk. phos. was urea inhibited in other teleosts (S11; P4; A47).

The ac. phos. of two elasmobranchs (E9, 17) and lymphocyte ac. phos. of two out of three acanthopterygians (A16, 30, but not 68) were tartrate resistant, leucocytes of deepwater dogfish (E14) showed partial inhibition, the neutrophils of eels (El2, 3, 13) showed tartrate inhibition of some granules, but tartrate-resistant ac. phos. in other granules; however, usually the ac. phos. of leucocytes was tartrate inhibited (Ag1; E7; H3; C1; S6, 7, 11, 12; St10; Sc2, 6; P12; A8, 12, 68). Thymus and spleen granulocytes of H5 contained tartrate-inhibited ac. phos., but ac. phos. of H3 spleen phagocytic cells was tartrate resistant.

Elasmobranch E4, 7, 13, and 17, eel El2, 3, and 13, and orange roughy (A4) ANAE were not fluoride inhibited; lamprey (Ag1) ANAE showed partial inhibition; and that of other species (Sc2; A12, 16, 30) showed total inhibition. For AS-D, there was differential fluoride inhibition of different leucocyte types (E4, 17).

DISCUSSION

The data presented here are based on observations from more species than in previously published studies, but include only about 0.75% of extant fish species and do not represent the full diversity of leucocyte enzyme patterns in fish. Furthermore, the observations on erythrocyte size and abundance in deepwater fish and the unusual erythrocytes in *Maurolicus muelleri* suggest some fish species, or larger taxa, have blood very unlike that usually observed in higher coastal teleosts and vertebrates in general. Despite this, the observations reported here show patterns in relation to fish phylogeny and habitat and raise questions on the functions of some enzymes, such as alkaline phosphatase and esterases, and the bactericidal mechanisms in fish lacking neutrophil peroxidase.

Phylogeny

Published studies have been mainly on agnathans (Table 7) or on readily available freshwater species such as salmonids (Table 8) and carps (Table 9); few studies have been on more than one species (Fey 1966a, 1966b, 1966c, Mitsui 1965, Johansson 1973), and the latter two only concerned peroxidase. Unfortunately, observations by Fey (1966a, 1966b, 1966c) must be treated with caution because the results are often at variance with those from other studies, and consistent observations are made on basophils (Fey 1966b), a cell type rarely seen by other workers. The study of Kelényi and Németh (1969) is not dealt with because of doubts over cell identification (Ellis 1977).

Invertebrate leucocytes and their enzymes have been reviewed by Anderson (1981) and Fletcher and Cooper-Willis (1982), and Anderson's review showed they had a similar hydrolytic enzyme content to mammalian neutrophil band forms. Morphological similarities between invertebrate phagocytes and mammalian macrophages have been reported by Kaplan and Bertheussen (1977). Of the enzymes dealt with here, both phosphatases, β-glucuronidase, N-acetyl-β-glucosaminidase, and non-specific esterases (such as ANAE) have all been reported from invertebrates, and serum lysozyme-like activity is widely distributed in many invertebrate groups (Cheng and Rodrick 1975, Anderson 1981). However, an invertebrate equivalent of mammalian myeloperoxidase appears to occur only in a few molluscs (Sminia, van der Knaap, and Boerrigter-Barendsen 1982, Granath and Yoshino 1983) and in 1% of insect haemocytes (Anderson 1977), and the mechanism by which invertebrates kill pathogens is unknown (Fletcher and Cooper-Willis 1982).

Results on agnathans (except those by Fey (1966b)) consistently show a lack of granulocyte peroxidase and the presence of alkaline phosphatase, and the β-glucuronidase reported by Page and Rowley (1983) was seen here in hagfish (Ag2).

Few studies have been made on elasmobranch leucocytes, but they report similar results to those given here. *Squalus acanthias* and *Raja* spp. may contain peroxidase (Johannson 1973, Mainwaring and Rowley 1985) or may not (Mitsui 1965), torpedoes lack peroxidase (Grimaldi *et al.* 1983), epigonal granulocytes of nurse sharks are peroxidase positive (Fänge and Mattisson 1981), and staining is negative in the dogfish *Scyliorhinus canicula* (Mainwaring and Rowley 1985) or weak and variable (Fey 1966b, 1966c). *Scyliorhinus canicula* granulocytes also contain weak alkaline phosphatase, weak acid phosphatase (except for moderate heterophil levels and strong eosinophil levels), and esterases which are strong in heterophils, moderate in eosinophils and thrombocytes, and weak in monocytes and lymphocytes (Fey 1966a, 1966b, 1966c, Mainwaring and Rowley 1985). In the Holocephali, *Chimaera monstrosa* has moderate granulocyte peroxidase (Johannson 1973), but *Hydrolagus colliei* granulocytes lack peroxidase (Mitsui 1965).

The few studies on teleosts deal mainly with freshwater species. The only two clupeiforms studied, *Clupea pallasii* and *Etrumeus micropus*, lack neutrophil and eosinophil peroxidase (Mitsui 1965). Salmoniforms have been more extensively studied. Neutrophil peroxidase is ubiquitous in salmonids and resembles mammalian myeloperoxidase (Kanner and Kinsella 1983), but the presence of monocyte peroxidase is uncertain; Yuki (1957) reported peroxidase in both cell types

TABLE 7: The results of studies published on agnathans compared with the results of this study*

	Perox.						Alk. phos.						Ac. phos.						
	N	M	E	B†	L	T	N	M	E	B	L	T	N	M	E	B	L	T	
Lampetra fluviatilis	−						+						weak +						Kelényi and Larsen (1976)
Lampetra fluviatilis	−	−			−	−							+	+			−/+‡	+	Page and Rowley (1983)
Lampetra planeri	±	±	weak +	−	−	−	+	+	+	+	−	−	−	±	−	weak +	−	−	Fey (1966a, 1966b, 1966c) and Fey and Kuntze (1970)
Lampetra spp.			−												+				Rowley and Page (1985a)
Myxine glutinosa	−	−			−	−													Johansson (1973)
Geotria australis	−				−		+				−		+				−		This study
Eptatretus cirrhatus	−				−		+				−		+				+		This study

* +, positive; −, negative; ±, presence equivocal.

† Basophils.

‡ Variable.

TABLE 8: The results of studies published on salmoniforms compared with the results of this study

	Perox.						Alk. phos.						Ac. phos.						
	N	M	E	B	L	T	N	M	E	B	L	T	N	M	E	B	L	T	
Salmo gairdnerii	+	−	+		−	−													Bielek (1981)
	+			−		−	+			weak +		−	weak +			weak +		weak +	Fey (1966a, 1966b, 1966c)
									+						+				Ezeasor and Stokoe (1980)
	+																		Finn and Nielson (1971), Haider (1968)
	+	+																	Yuki (1957)
						−						−						−	Kreutzmann (1977) *in* Kreutzmann (1978)
	+	(+)			−	−	+	−			−	−	+	+			+	−	This study
Salmo trutta	+					−													Blaxhall and Daisley (1973)
	+																		Catton (1951)
	strong +	(+)			−	−	weak +	−			−	−	weak +	+			+	−	This study
Salmo salar	+																		Conroy (1972)
Oncorhynchus kisutch	+	−																	Lester and Budd (1979)
O. masou	+	+?																	Yuki (1957)
O. keta	+	+?																	Yuki (1957)
O. tshawytscha	+	+			+	−	+	−			−	−	+	+			+	+	This study
Salvelinus leucomanis	+	+?																	Yuki (1957)
Argentina silus	strong +	+			−	−													Johansson (1973)

TABLE 9: The results of studies published on cyprinids

	Perox.						Alk. phos.						Ac. phos.						
	N	M	E	B	L	T	N	M	E	B	L	T	N	M	E	B	L	T	
Carassius auratus	+		−	−		−	+		weak +	+		−	+		weak +	weak +		+	Fey (1966a, 1966b, 1966c)
	+	−			−														Mori (1981)
	+	+?																	Yuki (1957)
	+	−			−														Mori (1981)
Carassius carassius	+		−	−			weak +		weak +	+			+		weak +	weak +			Fey (1966a, 1966b, 1966c)
	+		+	−			+		+	−									Garavini and Martelli (1981)
Cyprinus carpio	+	−	+	weak +	−	−													Bielek (1981)
	+			−			−			−			+			+			Fey (1966a, 1966b, 1966c)
			+						+						+				Varo (1970)
Tinca tinca	+	−	+	+	−	−													Bielek (1981)
	+																		Haider (1968)
Rutilus rutilus	+																		Catton (1951)
	+		−	−			+		+	+		weak +	weak +		weak +	+		−	Fey (1966a, 1966b, 1966c)
	+																		Haider (1968)
Leuciscus idus	+																		Haider (1968)
Leuciscus delineatus	+																		Haider (1968)

of *Salmo gairdnerii*, but in regard to 18 other species studied, including *Oncorhynchus masou*, *O. keta*, and *Salvelinus leucomanis*, stated that "pertinent results were obtained." It is not clear if these included monocyte peroxidase, but Johannson (1973) did report monocyte peroxidase in *Argentina silus*. Bielek (1981) reported eosinophil peroxidase only at pH 9.0, not pH 7.6, which suggested a catalase (Breton-Gorius and Guichard 1978) which is generically closer to myeloperoxidase as found in mammalian neutrophils than mammalian eosinophil peroxidase (Bolscher, Plat, and Wever 1984). *Salmo gairdnerii* mononuclear phagocytes are more phagocytic than neutrophils (Griffin 1983) and contain higher levels of acid phosphatase, which increase further on phagocytosis (Braun-Nesje, Kaplan, and Seljelid 1982). *Salmo gairdnerii* monocytes and basophils contain moderate esterase, but neutrophils, lymphocytes, and thrombocytes are weak in esterase (Fey 1966a, 1966b, 1966c).

Among the Ostariophysi, the freshwater carps (Cyprinidae) have been most extensively studied. Eosinophil peroxidase is better established than in salmoniforms; otherwise, enzyme patterns are similar. Fey's (1966a, 1966b, 1966c) studies of esterase in *Carassius auratus*, *C. carassius*, *Cyprinus carpio*, and *Rutilus rutilus* show that lymphocytes are weak; monocytes, eosinophils, and thrombocytes (except in *R. rutilus*) weak to moderate; neutrophils moderate; and *R. rutilus* thrombocytes strong in esterase. A few studies have been carried out on North American freshwater catfish (Ictaluridae) (Lester and Daniels 1976, Cannon *et al.* 1980, Garavini, Martelli, and Borelli 1981), but they are insufficient to allow the determination of overall patterns.

The few studies on higher fish have been mainly on peroxidase (Yuki 1957, Mitsui 1965, Johannson 1973) and have shown consistent peroxidase in neutrophils only, except for weak monocyte peroxidase in *Scomber scombrus* reported by Johannson (1973). Elarifi (1982) reported peroxidase and acid phosphatase from neutrophils in whiting (*Merlangius merlangus*), but not from lymphocytes; however, tests on neutrophil alkaline phosphatase and for all three enzymes in monocytes and thrombocytes were inconclusive. Plaice (*Pleuronectes platessa*) neutrophils stain erratically for peroxidase, lymphocytes and thrombocytes lack peroxidase, and monocytes stain inconclusively; all cells except lymphocytes contain acid phosphatase, but alkaline phosphatase occurs only very weakly, in neutrophils (Ellis 1976). The neutrophils and peritoneal macrophages of plaice also secrete lysozyme (Murray and Fletcher 1976).

To put fish leucocyte enzymes into perspective they must also be considered in relation to amphibians. There have been few studies on amphibian leucocyte enzymes (Mitsui 1965, Fey 1966a, 1966b, 1966c, Rogovin, Fomina, and Piruzyan 1978, Cannon and Cannon 1979). Nine species (Fey 1966a, 1966b, 1966c) showed ++→++++ peroxidase, −→++ phosphatases, and +→+++ esterase in neutrophils and no peroxidase, −→++ phosphatases (except +++ alkaline phosphatase in *Triturus alpestris* thrombocytes), and +→++ esterases in lymphocytes and thrombocytes. Monocytes of all species contained +→++ peroxidase, −→+ alkaline phosphatase, −→++ acid phosphatase, and +→++ esterase. Eosinophils of five species lacked peroxidase, but four species (all anurans) were positive (*Rana temporaria*, ++; *Rana esculenta*, +++; *Bufo bufo*, +++; and *Bombina bombina*, +++) and also contained −→++ alkaline phosphatase, −→+ acid phosphatase, and +→++ esterase. In *Bufo alvarius* peroxidase is strong in neutrophils, in the coarse granules of eosinophils, and is "abundant" in monocytes (Cannon and Cannon 1979). Lymphocytes are strongly positive or negative for acid phosphatase, which suggests two subpopulations, and in *Bufo bufo* ANAE can be used to differentiate T from non-T lymphocytes (Garavini, Rossini, and Mantovani 1982). Only *Bufo alvarius* heterophils contain alkaline phosphatase, +→++; acid phosphatase occurs in the fine granules, but also rarely in the coarse granules of eosinophils; and all leucocytes lack β-glucuronidase (Cannon and Cannon 1979). Enzyme patterns, particularly monocyte peroxidase, show similarities between amphibians, ostariophysids, and salmonids.

In summary, the following trends in leucocyte enzymes have been observed from invertebrates to amphibians:

Peroxidase

In fish peroxidase occurs in extravascular eosinophils, is very weak in blood eosinophils of a few chondrichthyans and basal teleosts, but is common in neutrophils of teleosts. Similarly, it is common in amphibian neutrophils, but is consistently present in eosinophils only in anurans. Monocyte peroxidase occurs in elopomorphs and, especially, salmoniforms and amphibians.

Alkaline phosphatase

Alkaline phosphatase occurs in insect haemocytes, agnathan and holocephalan fine granulocytes, and teleost and amphibian neutrophils, but not elasmobranch neutrophils. However, it occurs in the lymphocytes of dogfish.

Acid phosphatase

Acid phosphatase is ubiquitous in all groups.

β-glucuronidase

It is present in invertebrates and agnathans, is rare, but occurs intermittently, in other fish, and is absent from amphibians. However, it may be a common enzyme of splenic macrophages in fish.

ANAE

It occurs in invertebrates, fish, and amphibians, but is particularly intense in elasmobranch granulocytes and elopomorph neutrophils.

AS-D

It occurs in association with neutrophils, particularly in lower fish groups.

PAS

It is ubiquitous in fine granulocytes or neutrophils and in eosinophils, but is restricted to the lymphocytes of lower groups, especially elasmobranchs.

Caution must be exercised when defining patterns, because few species were examined, relationships between basal groups were ill-defined, and convergent evolution of enzymes may have occurred in very different groups. However, agnathans, holocephalans, clupeiforms, salmoniforms, stomiiforms, and higher groups show similarities in neutrophil alkaline phosphatase, the gradual development of neutrophil peroxidase, and moderate granulocyte esterases. Conversely, elasmobranchs and elopomorphs resemble each other and differ from other groups in their lack of neutrophil alkaline phosphatase and their high granulocyte esterases, including fluoride-resistant ANAE.

However, blood from elasmobranchs and elopomorphs is very different in neutrophilic granulocyte and neutrophil nuclear morphology, cell size, granulocyte tinctorial diversity, enzyme-granule distribution in eosinophils, and isozymes as suggested by different inhibition patterns. In some features, elasmobranchs more closely resemble stomiiforms (polymorphonuclear neutrophilic granulocytes and neutrophils, cell size, coarse eosinophilic granulocyte and eosinophil peroxidase) or alepocephalid salmoniforms (appearance and tinctorial properties of granulocytes and cell size).

Cell size in fish is known to increase in relation to depth (Ebeling, Atkin, and Setzer 1971), but the significance of this is not understood. As cell size is related to nuclear DNA content (Pedersen 1971, Szarski 1976), geneticists have tried to explain this in terms of degree of (undefined) "specialisation"; DNA content and cell size decreasing as fish became specialised (Hinegardner and Rosen 1972). However, this argument is unconvincing because cell size increases with depth in all groups, major groups such as elasmobranchs and paracanthopterygians have consistently large or small cells respectively, lungfish (which are very specialised) have very large cells, and deepwater fish (with their bioluminescence and specialised anatomy for exploiting a scarce food resource) appear more specialised than most shallow-water species. Large cell size in deepwater species, and haemoglobin packets in *Maurolicus*, probably reflect simple physiological requirements, for sluggish and fast movement respectively, superimposed on the cell size of the fish group concerned. This does not account for the high DNA content, much of which appears to be unused, but nor do the theories by geneticists such as Ebeling *et al.* (1971) and Hinegardner and Rosen (1972).

Inhibition studies carried out here suggest several isozymes of an enzyme type may exist and be expressed in different groups, as in mammals (Yam, Li, and Lam 1971, Oertel and Kastner 1984). The cyanide-inhibited neutrophil peroxidase and normally cyanide-resistant eosinophil peroxidase seen here show the same inhibition patterns as human peroxidases (Yam, Li, and Crosby 1971) and may have similar activities (Bolscher, Plat, and Wever 1984), but cyanide-resistant neutrophil peroxidase has also been reported from fish (Cannon *et al.* 1980, Morris and Albright 1981). Isozymes are also indicated in acid phosphatase and ANAE and alkaline phosphatase, the latter being urea inhibited in some teleosts (S11; P4; A47), as in humans (Grozdea, Vergnes, and Martin 1983), but urea resistant in elasmobranchs (E12, 15, 18), holocephalans (H4), and some teleosts (S4; P5, 7, 9; A7).

Enzyme function

The initial development of weak eosinophil peroxidase and its replacement or supplementation by neutrophil peroxidase can be compared with studies on mammals. Eosinophils are less effective than neutrophils in bactericidal activity (Baehner and Johnston 1971, Mickenberg, Root, and Wolff 1972), possibly because they are less phagocytic (Cline 1972, Bujak and Root 1974), though the results of one study (De Chatelet *et al.* 1978) showed no difference in phagocytosis. From their study and the foregoing studies, Jong, Henderson, and Klebanoff (1980) concluded that "intracellular microbicidal activity of eosinophils is not as crucial to the host defense as is the comparable activity in neutrophils. Considering the very weak coarse eosinophilic granulocyte peroxidase of elasmobranchs, the development of neutrophil peroxidase in teleosts would bring into effect more efficient host bactericidal defense mechanisms.

A review of oxidant production by phagocytes in microbicidal activity (Babior 1984) puts neutrophil myeloperoxidase into perspective as only one of several strategies for killing pathogens, albeit an important one. Furthermore, in humans with neutrophil myeloperoxidase deficiency, infections are rarely serious (Kitahara *et al.* 1981, Parry *et al.* 1981), whereas chronic granulomatous disease caused by a defect in 0_2^-, which leads to the formation of oxidase, causes serious recurrent infections (Klebanoff and Clark 1978, Babior 1984).

The effective bactericidal mechanisms in invertebrates (Anderson 1981) and lower fish, despite the widespread lack of peroxidase, suggest the myeloperoxidase bactericidal mechanisms of mammals (Karnovsky *et al.* 1981) are not of critical importance in invertebrates and fish. It is likely that non-specific factors (Ingram 1980, Fletcher 1981, Rijkers 1982), such as lysozyme in invertebrates (Chain and Anderson 1983) and fish (Fänge, Lundblad, and Lind 1976, Fletcher and White 1976, Murray and Fletcher 1976), properdin (Noguchi, Hanafusa, and Ohnishi 1980), complement (Jensen and Festa 1980), C-reactive protein (White, Fletcher, and Pepys 1983), and interferon (Beasley and Sigel 1967) in fish, play an important part in defence against pathogens (Fletcher 1978).

In mammals, lysozyme plays an important role in lysis of some pathogenic bacteria and fungi (Goldstein 1983), and in association with extracts of other leucocytes (Neeman, Lahav, and Ginsburg 1974), lysolecithin (Lahav *et al.* 1979) or complement (Schreiber *et al.* 1979), may effectively be involved in the killing and degradation of bacterial and fungal pathogens. Lysozyme, complement, and properdin become elevated in infections (Jakoniuk, Borowski, and Jabloniska-Strynkowska 1980), and other systems, independant of the myeloperoxidase-H_2O_2-halide system, may also be involved (Biggar, Buron, and Holmes 1976).

An additional mechanism, well established and possibly unique to fish, involves the melanin in melano-macrophage centres of kidney, spleen, and liver haematopoietic tissue (Roberts 1975b, Ellis 1981, Agius 1985, Rowley and Page 1985b). These centres are often closely associated with lymphoid tissue (Roberts 1975b, Agius 1981a), are associated with haemoglobin catabolism (Roberts 1975b, Ferguson 1976) in teleosts (Agius 1981b), if not elasmobranchs (Agius 1983), and sequestration of phagocytised pathogens (Roberts 1975b), which concentrates pathogen antigens near lymphocyte concentrations (Roberts 1975b, Agius 1981a, Ellis 1980, Lamers 1986). The killing of pathogens may be effected by H_2O_2 generation from melanin and its precursors (Roberts 1975b), in a similar manner to the free radical and H_2O_2 systems used by mammalian neutrophils (Klebanoff and Clark 1978, Babior 1984). If such a melanin-based bactericidal mechanism is efficient, the widespread and complex malanomacrophage centres in fish may be the primary bactericidal system, especially in fish lacking neutrophil peroxidase.

Elasmobranchs and elopomorphs are similar not only in their lack of neutrophilic granulocyte and neutrophil peroxidase, but also in their abundance of neutrophilic granulocyte and neutrophil esterases. The production and secretion of ANAE is particularly prolific in elasmobranch neutrophilic granulocytes, and this suggests this esterase is an ecto-enzyme acting in the plasma rather than an enzyme with an intracellular function. There may be functional similarity between peroxidase and esterases, as suggested by boarfish (A33, 34) (the only acanthopterygians with circulating peroxidase negative as well as positive neutrophils and high esterase levels) and also by skipjack tuna (A56), with few peroxidase positive neutrophils, but strong neutrophil ANAE. However, there are many exceptions: agnathans and holocephalans lack peroxidase, but have only moderate esterases, mainly AS-D; some acanthopterygians, such as orange roughy (A4), have high levels of both enzymes, as have the advanced tetraodontiformes (A67, 68) and humans (Hayhoe and Quaglino 1980). However, exceptions in agnathans and holocephalans may reflect the primitive status of these fish. Unfortunately, studies on mammals have not determined esterase function, though it may have a proteolytic chymotrypsin-like function (Li, Lam, and Yam 1973), and a study by Ferluga, Asherson, and Becker (1972) implicates esterases in disease processes. However, as with alkaline phosphatase (Miller, Yang, and Liepman 1984), the link with disease processes is tenuous, which suggests it is not strong.

Neutrophil alkaline phosphatase, the ubiquity of acid phosphatase, the high neutrophil AS-D, and, to a lesser extent, ANAE and the polymorphonuclear neutrophils are all long-standing primitive features which suggest that in some respects there has been little change in the basic features of leucocytes from early fish through to humans. However, leucocytes of higher teleosts seldom include polymorphonuclear neutrophils and show enzyme differences and isozyme diversity, as suggested by inhibitors, between and within groups, which suggest that not only may higher fish have developed away from the main line of evolution through the tetrapods to humans, but also that within higher teleosts leucocyte function may differ between and within groups.

ACKNOWLEDGMENTS

We would like to thank Commander M. Ingram of Marine Farm, Somerset, United Kingdom, B. Morgan of Kaikoura Fisheries, J. van Berkel of the University of Canterbury, L. Hicks of Fisheries Management Division, and R. Ashworth, E. Cudby, J. Hadfield, B. Jones, T. Murray, J. Richardson, and D. Shirer of Fisheries Research Division, Ministry of Agriculture and Fisheries for helping obtain fish and blood samples. We also greatly appreciate the help of P. Castle of Victoria University of Wellington and J. Fenaughty, P. Grimes, P. McMillan, L. Paul, D. Robertson, and D. Tracey of Fisheries Research Division for identifying fish species and for discussions on taxonomy.

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