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Comparative hematology of wild Anguilliformes (*Muraena helena*, L. 1758, *Conger conger*, L. 1758 and *Anguilla anguilla* L. 1758)

Domagoj Đikić[1,*], Duje Lisičić[1], Sanja Matić-Skoko[2], Pero Tutman[2], Daria Skaramuca[1], Zdenko Franić[3] and Boško Skaramuca[4]

[1] Department of Animal Physiology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10000 Zagreb, Croatia

[2] Institute of Oceanography and Fisheries, Šetalište I. Meštrovića 63, HR 21000 Split, Croatia

[3] Institute for Medical Research and Occupational Health, Ksaverska cesta 2, HR-10000 Zagreb, Croatia

[4] Department of Aquaculture, University of Dubrovnik, Ćira Carića 4, HR-2000 Dubrovnik, Croatia

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Abstract

The objective of this study was to compare circulating blood cell counts and morphology of three eel species: *Muraena helena* (moray), *Conger conger* (European conger) and *Anguilla anguilla* (European common eel). Moray and conger were collected from the Adriatic Sea at the Elaphite Islands near Dubrovnik, Croatia; common eels were collected in the Neretva River, Croatia. Hematological comparison was conducted using Natt-Harrick's and May-Grünwald Giemsa staining methods. The hematocrit of moray and conger were similar, while common eel had higher values by 60%. Manual cell count showed that common eel had the highest erythrocyte count. Conger had a higher erythrocyte count than moray, with a higher percentage of proerythrocytes and senescent erythrocytes compared to moray and common eel. The leukocyte count was similar in common eel and moray and slightly lower in conger. The thrombocyte count was highest in conger and lowest in moray. In all three species, the neutrophil (heterophil) nuclei appeared as either circular or bi-lobed. Moray had the highest neutrophil (heterophil) percentage and a subtype with intensively basophilic cytoplasm appearing in a similar percentage as the normal type. In common eel, neutrophils (heterophils) were the only detected granulocytes. Basophils were detected in conger eels. Eosinophils were not detected in any of the sampled fish. The size of all cell types in moray was slightly larger than in other two species. In conclusion, our findings reveal major differences in the cell count and diversity in cell subtypes between three kin species of eels.

*) Corresponding author; e-mail: magistar_djikic1@yahoo.com

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Keywords
Anguilliformes; conger; common eel; granulocytes; moray; heterophils

Introduction

Hematology is important in fish biology and ecology as it may mirror circannual changes and provide a comparative reference for captivity-reared species in aquaculture (Larsson et al., 1976). Identification of different piscine blood cells can help to indicate physiological health status, assess the conditions that cause stress to fish (disease, parasite infections, mishandling, bioaccumulation and biomagnification of pollutants), and support explanations of phylogenetic relationships amongst different fish taxa (Kakuta and Nakai, 1992; Anderson and Zeeman, 1995; Sasal et al., 1997; van Ginneken et al., 2005; Bartoli and Gibson, 2007; Clauss et al., 2008).

The basic hematological patterns in vertebrates can also be recognized in fish, though with some prominent differences. For example, the major hematopoietic organ in vertebrates is bone marrow, while in fish the major organ is the head kidney (Reavil and Roberts, 2007) with the spleen, epigonal organ and Leydig organ additionally participating in granulopoiesis (Ainsworth, 1992). Erythrocytes (red blood cells, RBC) in fish are spherical, enucleated and the most numerous blood cells, and are similar to those in other vertebrates with the exception of mammals. Hematocrit (the percentage of the volume of whole blood made up of RBC) in teleosts ranges between 20-40%, which is less than the mammalian average, e.g., normal hematocrit is 40-45% in humans or as high as 65% in some diving marine mammals (Starck and Schuster, 2012). In fish, the mean values of hematocrit and red blood cell counts increase in the following order: rays, sharks, and teleosts. A higher proportion of juvenile erythrocyte stages can be found in fish blood than in other vertebrates. Such cells are usually smaller and differently stained than mature RBC, and their proportions in circulating blood (RBC differential) may reflect the physiological status of the fish or an adaptive response to environmental conditions. In myxinoid fish, dipnoans and some elasmobranchs, the average size of mature RBC is larger than the vertebrate average and amongst the largest vertebrate cells after amphibians (e.g. *Amphiuma* spp., RBC size $\sim$65 μm). Generally, a smaller average RBC size is recorded in active teleost fish. The nuclear size of RBC is correlated to DNA content and can be indirectly used as an estimate of ploidy of the species (Filho et al., 1992; Hrubec and Smith, 2000; Kapoor and Khanna, 2004; Campbell and Ellis, 2007; Davis et al., 2009; Arican et al., 2010; Dove et al., 2010).

Different leukocyte (white blood cell, WBC) cell types and their structural heterogeneity between fish species imply that each fish species must be analyzed separately for its distinctive WBC traits. A number of papers give a classification of fish WBC, and the difficulties concerning exact classification and nomenclature continue today (Ellis, 1977; Hyder et al., 1983; Parish et al., 1986; Thuvander et al., 1987; Ainsworth, 1992; Hine, 1992; Hrubec and Smith, 2000; Kapoor and Khanna, 2004; Thrall, 2004; Thrall et al., 2004; Campbell and Ellis, 2007; Dove

et al., 2010). These authors all agree that fish leukocytes are divided into granulocytes (neutrophils, eosinophils and baosphils) and agranulocytes (lymphocytes and monocytes) and their proportions vary from species to species. The majority of classifications claim that heterophil, neutrophil and polymorphonuclear leukocytes are constituents of the innate immune system and as the first line of defence they designate the same cell with known subtypes and synonyms differently assigned by various authors (granulocytes – Type I, II, III, Type 1, heterophil, L2, G1, 2, 3, etc.). Although both neutrophils and heterophils are found in the same species of elasmobranchs or among teleosts in some cyprinids, only one cell type is usually present in the majority of teleosts (Ainsworth, 1992; Kapoor and Khanna, 2004; Dove et al., 2010). Some authors suggest that neutrophils and heterophils are not the same cell types (Hrubec, 2000). Confusion also arises from comparative hematology since both heterophils and neutrophils are found concurrently in reptilian and avian species or even in some rodents (rabbits and guinea pigs). Hence, some hematologists expect to find both cell types in fish. Azurophils, present in avian and reptilian species, have not been described in fish (Campbell and Ellis, 2007). Neutrophils are the most numerous granulocytes, while the occurrence of two other granulocytes (eosinophils and basophils) in teleost blood is rare, but it was confirmed that their lack is not caused by failure to preserve the granules through staining (Tavares-Dias, 2006). Numerous subtypes are also described for eosinophils (granulocytes – Type III, Type 2, Gl, G2, G3, & G4) since they occur more often than basophil. Basophil descriptions are rare and some authors even dispute their existence and function in fish (Ainsworth, 1992; Kapoor and Khanna, 2004). When eosinophils or basophils are detected, they are similar in appearance as in other vertebrate phyla with the exception of eosinophils of iguanas and some birds whose granules are pale blue (Campbell and Ellis, 2007). The absence of eosinophils and basophils can also be associated with the time of the year in which the blood of certain species is examined (Guijarro et al., 2003).

Agranulocytes are more easily distinguished and classified than granulocytes and they are similar in appearance as in other vertebrate phyla (Hrubec and Smith, 2000; Kapoor and Khanna, 2004). Thrombocytes are the second most abundant blood cells after RBC, but are the least studied blood cells in fish. The expression of coagulation factors and canalicular systems indicate their role in clotting. Granulated cytoplasm and phagocytic abilities of thrombocytes, which are not recorded in other vertebrates, have been noted in certain fish, especially elasmobranchs. Enucleated, elongated (oval, spindle, cone) and round fish thrombocytes resemble amphibian, reptilian and avian thrombocytes, though they have a different structure and origin than mammalian platelets (Kapoor and Khanna, 2004; Dove et al., 2010).

Surprisingly, despite abundant descriptions of fish blood cells, there are no published data dealing with the hematology of species such as *Muraena helena* (moray) and *Conger conger* (conger) as well as other species in these genera. In a previous study, a specific neutrophil (heterophil) was found in *Muraena helena* (Đikić et al., 2010, 2011). Therefore, the objectives of this study were to compare whether this

cell type also exists in other *Anguilliform* fishes and to gather information regarding the general hematology and physiology of eels (Fishbase, 2012). Therefore, this study aimed to fill the gaps in the basic knowledge on the morphology and quantitative description of blood cells of moray and conger and compare them to common eel (*Anguilla anguilla*), whose hematological profile and general biology have been studied in more detail already (Sbaihi et al., 2001; van Ginneken and Maes, 2005). The results are expected to contribute to the ongoing discussion on comparative fish hematology and will exemplify the diversity of blood cells in these three eel species.

Materials and methods

Animals and environmental conditions

Each fish species was collected on a separate sampling trip. All analyzed fish were collected in a single catch during each sampling trip at the same time of day to ensure that fish are analyzed under approximately the same environmental conditions to the greatest extent possible and to assure uniformity of the within-group sample. Morays were collected in summer (August) in the Adriatic Sea at the Elaphite Islands near Dubrovnik, Croatia. Conger eels were collected in summer (July) in the Adriatic Sea, on the south-eastern part of Mljet Island, Croatia. Environmental conditions at both locations were similar, with depths of 5-10 m, and a sea temperature of 22.5 ± 0.6°C (four measurements at various depths at which the fish were captured). Both morays and congers were caught using 200 m of long line hooks. Due to the nocturnal habits of these species, the hooks were set at 3:00 a.m. and collected two hours later. All fish appeared healthy and very agile (active-aggressive). Common eels were collected in Neretva River (full freshwater) in the town of Momići near Metković, Croatia, by nets that were set at 3:00 a.m. and collected two hours later. However, common eels were caught in the early fall (first week of October), when the water temperature was 13.3 ± 0.2°C, which corresponds with the usual water temperature and physical conditions of the Neretva River in that season (Riđanović et al., 2010). Fish of all three species were individually sedated with MS222 (Sigma) in oxygenated sea water in a 100 l plastic barrel (MS222 dose = 250 mg l^{-1}) for 15 minutes. Once sedated, morphometric parameters (BL = body length, BM = body mass) were then measured. The body mass index (BMI) was calculated from BM and BL ($BMI = BM/BL^2$).

Blood analysis

Each blood sample was collected from the heart with a 10 ml syringe with anticoagulant heparin (Sigma) and processed immediately to cell analysis (i.e., in the ship laboratory). After blood collection, all fish were sacrificed by instant decapitation. Otoliths were removed for age determination as described by Matić-Skoko et al. (2010). Detailed examinations by the veterinarian on board (Dr. A. Gavrilović,

Dept. of Aquaculture, University of Dubrovnik) established the absence of any external parasites or other pathological changes. No internal blood parasites or histopathological changes were present after inspection under the microscope.

Hematocrit was assessed immediately after blood collection by centrifugation of heparinised micro-hematocrit capillaries with the sample of blood at 115 g ($g = 118 \times 10^{-7} \times r \times n^2$; $n = 1400$ rev min^{-1}, $r = 5$ cm) for 5 minutes at room temperature in a micro-centrifuge (Microfuge) immediately upon sampling. Hematocrit was determined by the micro-hematocrit reader scale provided with the centrifuge.

Erythrocyte, leukocyte and thrombocyte counts were made from the heparin-anticoagulated blood samples using Natt and Herrick's staining method as described by Campbell and Murru (1990). Stained samples were diluted to 1 : 200 immediately after sampling and counted under a light microscope in the ship laboratory after cells became visible (approximately 10-15 minutes after blood collection) on a Bürker-Turk haemocytometer. For each fish, two counts (duplicate) were carried out and counted on the upper and lower grid. Erythrocytes, leukocytes and thrombocytes were counted separately (three counts per grid).

Blood smears were made immediately after sampling and air dried. Smears (in duplicate) were stained with the May-Grünwald Giemsa staining method (MGG, Sigma Merck) and examined at 1000× magnification for WBC and RBC differential cell counts. Size was measured on 100 cells of each type (Axiovision 4.8.2.0; Carl-Zeiss Microimaging GmbH, Germany). The WBC classification in this paper followed the recommended classification of Ainsworth (1992), Thrall (2004) and Kapoor (2004), though other sources were also taken into consideration (Ellis, 1977; Hine, 1992; Hrubec and Smith, 2000; Campbell and Ellis, 2007).

Statistical analysis

The STATISTICA 9.1 software package (Statistica software, Tulsa, USA) was used to determine descriptive statistics, data analysis and correlation analysis. Statistical analysis of the log transformed data of cell numbers and arcsine transformed data of percentages was performed to establish the differences between data. The statistical differences were compared by ANOVA and Duncan post hoc test. The level of statistical significance was set to $P < 0.05$.

Results

Morphometric data and age of analyzed fish

Measured and calculated morphometric parameters and age showed inter- and intraspecific variations between the analyzed fish (table 1). Table 1 shows the age analysis, presented as an individual number of fish in a particular age group and showing the interspecies discrepancy in uniformity between groups. Correlations between length and body mass and their statistical significance indicated that all

Table 1.
Morphometric values of wild moray eel (*Muraena helena*), European conger eel (*Conger conger*) and European common eel (*Anguilla anguilla*).

Species (N)	Parameter	Mean ± SD	Min	Max	Median
Muraena helena (18)	BMI	0.23 ± 0.06^{a}	0.18	0.38	0.21
	body weight (g)	1296.33 ± 804.45^{a}	751.00	3322.00	1043.00
	body length (cm)	72.37 ± 13.41^{a}	60.20	93.20	64.70
	ventral girth (cm)	14.53 ± 2.87^{a}	11.50	19.50	14.00
	caudal girth (cm)	11.38 ± 2.19^{a}	8.50	15.00	11.00
Conger conger (17)	BMI	0.14 ± 0.06^{b}	0.06	0.26	0.12
	body weight (g)	672.14 ± 441.96^{b}	240.00	1860.00	560.00
	body length (cm)	68.70 ± 8.70^{b}	54.5	85.00	70.00
	ventral girth (cm)	13.83 ± 2.75^{b}	10.00	17.50	14.00
	caudal girth (cm)	10.98 ± 1.91^{b}	8.50	14.00	11.00
Anguilla anguilla (17)	BMI	0.10 ± 0.04^{c}	0.04	0.20	0.10
	body weight (g)	348.56 ± 261.20^{c}	68.30	1102.00	335.70
	body length (cm)	55.40 ± 12.16^{c}	36.0	74.0	60.0
	ventral girth (cm)	9.51 ± 2.88^{c}	5.00	15.70	13.00
	caudal girth (cm)	7.83 ± 2.38^{c}	4.70	13.00	8.00

N of animals of particular age

Species	3 years	4 years	5 years	6 years	7 years	8 years	9 years	10 years	11 years
Muraena helena			5	6	2		2	3	
Conger conger	1	5	6	3				2	
Anguilla anguilla					3	3	5	5	1

Abbreviations and symbols: BMI, body-mass index; Max, maximum; Min, minimum; N, number of individuals per group; SD, standard deviation; $^{a, b, c}$, the values marked with different superscript letters are significantly different ($P < 0.05$) from the same parameter in the other fish species.

morphometric parameters had relatively high correlation within each species, although there was significant interspecies variation (supplementary table S1).

Hematocrit

The mean hematocrit value (table 2) was higher in common eel than in moray and conger, which showed similar values, although there was a slight difference in the range and median values between these two species. Hematocrit was significantly ($P < 0.05$) correlated with biometric indices in moray but not in conger and common eel (table S1).

Haemocytometer counts: RBC, WBC, thrombocytes

Moray and conger erythrocyte (RBC) values (table 2) were significantly different ($P < 0.05$), while common eel had values more than 60% higher and thus had

Table 2.
Hematological values in wild moray eel (*Muraena helena*), European conger eel (*Conger conger*) and European common eel (*Anguilla anguilla*).

Species (N)	Parameter	Mean ± SD	Min	Max	Median
Muraena	hematocrit (%)	23.22 ± 3.13^{a}	20.00	26.00	23.00
helena	RBC count ($\times 10^{12}$/l)	0.401 ± 1.60^{a}	0.214	0.749	0.431
(18)	WBC count ($\times 10^{10}$/l)	2.021 ± 0.70^{a}	0.901	2.790	1.934
	thrombocyte count ($\times 10^{10}$/l)	2.629 ± 1.20^{a}	1.384	4.710	2.190
Conger	hematocrit (%)	21.92 ± 3.80^{a}	18.00	28.00	20.50
conger	RBC count ($\times 10^{12}$/l)	0.752 ± 2.99^{b}	0.271	1.139	0.893
(17)	WBC count ($\times 10^{10}$/l)	1.377 ± 0.507^{a}	0.610	2.072	1.249
	thrombocyte count ($\times 10^{10}$/l)	6.350 ± 3.60^{b}	2.083	14.661	5.430
Anguilla	hematocrit (%)	37.76 ± 4.62^{b}	25.00	44.00	37.50
anguilla	RBC count ($\times 10^{12}$/l)	1.605 ± 0.73^{c}	0.840	4.090	1.410
(17)	WBC count ($\times 10^{10}$/l)	2.210 ± 1.39^{a}	0.059	5.730	2.063
	thrombocyte count ($\times 10^{10}$/l)	5.130 ± 2.26^{c}	2.030	10.301	4.502

Abbreviations and symbols: Max, maximum; Min, minimum; N, number of individuals per group; RBC, red blood cells; SD, standard deviation; WBC, white blood cells; $^{a, b, c}$, the values marked with different superscript letters are significantly different ($P < 0.05$) from the same parameter in the other fish species, whereas the values bearing the same superscript letter are not significantly different from the same parameter in other fish species.

significantly higher ($P < 0.05$) RBC counts than both other species. RBC count showed a significantly low ($P < 0.05$) correlation with biometric indices (table S1). Leukocyte (WBC) mean values (table 2) were similar in common eel and moray and slightly lower in conger and were not correlated with the biometrical indices of the fish (table S1). Thrombocyte mean values (table 2) were highest in conger and lowest in moray although the overall interspecific range did not exceed 4-6% of the overall cell count and was not correlated with the biometric indices (table S1).

Blood cell morphology

RBC morphology (supplementary fig. S1) was similar in all three species. Mature erythrocytes were elliptical cells with a central nucleus generally following the shape of the cell with highly condensed heterochromatin. Two types of juvenile erythrocytes were present: polychromatophilic erythrocytes and basophilic erythroblasts with generally basophilic and granulated heterochromatin and a smaller cell:nucleus ratio (fig. S1A, B, E, F, I). Senescent erythrocytes were also found. In all three species, the maturation stages of RBC were similar. The array of RBC maturation was characterized by early events such as decondensation of heterochromatin (fig. S1D, G, H), accompanied by swelling and enlargement of the cell until the entire cell is irregularly shaped until degradation in the later phases (fig. S1J, K, L).

Leukocytes (WBC) (fig. 1) were diverse in types within each of the three analyzed eel species. Neutrophil (heterophil) nuclei appeared as either circular or bi-lobed in all three species (fig. 1A, B, C, F, H, K, L). Moray was the only species that had a subtype with an intensively basophilic cytoplasm (IBG, also with circular and bi-lobed nuclei) appearing in a similar percentage as the normal type (fig. 1B). Most importantly, this type of cell was found in all analyzed morays regardless of weight/length or age. Interestingly, conger and common eel had only the standard type of neutrophil (heterophil), which was similar in appearance in all three species. However, in common eel some neutrophils (heterophils) had slightly bluish cytoplasm (fig. 1L), though not as intense as in moray and therefore it could not be stated with certainty that there are two different subtypes of neutrophil in common eel. Basophils were detected only in conger (fig. 1G). Morays and common eels did not have this granulocyte type characterized with a number of large distinctive purple granules. Eosinophils were not detected in any of the sampled fish. Monocytes (fig. 1D, I, N) in all three species had darker basophilic violet blue nuclei with clearly distinguished granular formation of eu- and heterochromatin, and the blue cytoplasm was darker than those of lymphocytes. Lymphocytes (fig. 1E, J, O) were round, often small cells with a large round nucleus that stained a dense deep red/violet colour; their nucleus occupied most of the cell and heterochromatin was compact and homogeneous. The cytoplasm was either a dark blue ring, a paucity of basophilic-staining cytoplasm.

Thrombocytes (supplementary fig. S2) in all three species appeared in four forms, oval, round, elongated (cone) and spindle, separately or in clusters.

RBC differential, WBC differential and cell size

The erythrocyte (RBC) differential (table 3) showed that conger had a higher percentage of proerythrocytes and senescent erythrocytes than moray and a slightly lower average percentage of mature erythrocytes than moray or common eel.

Leukocytes (WBC) were diverse in number (table 4) within each of the three analyzed eel species, with neutrophils (heterophils) as the most numerous granulocytes in all three analyzed species. Conger and common eel had a similar percentage of neutrophils (heterophils), which was lower than in moray. The difference between moray and conger was not statistically significant though there was a significant difference between the moray and common eel neutrophil (heterophil) counts ($P < 0.05$; table 4). However, in moray the total granulocyte percentage was generally higher due to the high percentage of IBG cells and the lack of these cells in the other two fish species (table 4). Basophils were not detected in moray or common eel. In conger, these granulocytes were found in very low percentages as approximately one cell in 100 analyzed leukocytes, though only in 11.11% of sampled congers (2 specimens; table 4). Lymphocyte percentages were lowest in moray and highest in conger, though the percentages were similar (table 4). Monocytes, accounting for approximately 4.59-13.87% of the total WBC count, were the least commonly present leukocytes. Common eel had a significantly higher ($P < 0.05$)

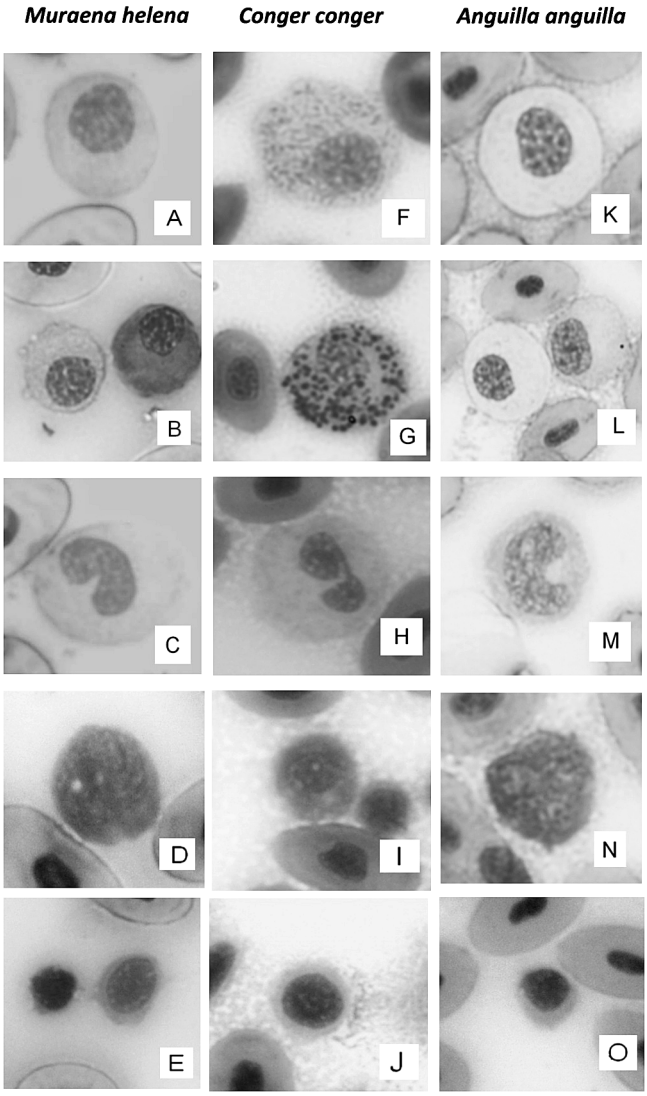

Figure 1. Leukocytes of moray (*Muraena helena*), conger (*Conger conger*) and European common eel (*Anguilla anguilla*) stained by May Grunwald Giemsa stain. (A) *Muraena helena* neutrophil (heterophil). (B) *Muraena helena* normal neutrophil (heterophil) (left cell) and neutrophil (heterophil) with intensively basophilic cytoplasm (IBG cell, right cell). (C) *Muraena helena* neutrophil (heterophil) with bi-lobed nucleus. (D) *Muraena helena* monocyte. (E) *Muraena helena* lymphocyte (right cell) compared to round thrombocyte (left cell). (F) *Conger conger* neutrophil (heterophil). (G) *Conger conger* basophile. (H) *Conger conger* neutrophil with bi-lobed nucleus. (I) *Conger conger* monocyte. (J) *Conger conger* lymphocyte. (K) *Anguilla anguilla* neutrophil (heterophil). (L) *Anguilla anguilla* normal neutrophil (heterophil) (left cell) and neutrophil (heterophil) with slightly basophilic cytoplasm (right cell). (M) *Anguilla anguilla* neutrophil (heterophil) with bi-lobed nucleus. (N) *Anguilla anguilla* monocyte. (O) *Anguilla anguilla* lymphocyte. Magnification: immersion, 1000×.

Table 3.
Percentages of differential erythrocyte (RBC) counts in wild moray eel (*Muraena helena*), European conger eel (*Conger conger*) and European common eel (*Anguilla anguilla*).

Species (N)	RBC (%)	Mature RBC	Basophilic erythroblasts	Polychromatophilic RBC (proerythrocytes)	Senescent RBC
Muraena helena (18)	mean	97.78[a]	0.19[a]	0.90[a]	0.31[a]
	SD	1.35	0.17	0.65	0.40
	min	95.50	0.00	0.09	0.00
	max	98.90	0.49	1.86	1.14
	$(n/N) \times 100$	100	55.56	100	100
Conger conger (17)	mean	88.52[b]	0.19[a]	3.88[b]	7.44[b]
	SD	4.80	0.09	2.07	4.36
	min	81.06	0.00	0.95	2.56
	max	93.65	0.32	8.21	17.52
	$(n/N) \times 100$	100	55.56	100	100
Anguilla anguilla (17)	mean	98.58[a]	0.07[b]	0.13[a]	0.54[c]
	SD	0.64	0.11	0.06	0.71
	min	97.31	0.00	0.00	0.00
	max	99.60	0.38	0.27	2.60
	$(n/N) \times 100$	100	47.05	100	76.47

Abbreviations and symbols: max, maximum; min, minimum; n, number of fishes per group in which particular cell type was found; N, number of individuals per group; RBC, red blood cells; SD, standard deviation; [a, b, c], the values marked with different superscript letters are significantly different ($P < 0.05$) from the same parameter in the other fish species, whereas the values bearing the same superscript letter are not significantly different from the same parameter in other fish species.

percentage of circulating monocytes than the other two species (table 4). All cell types were larger in moray than in the two other species and the comparison of cell and nucleus size of each cell type is shown in supplementary table S2.

Discussion

Descriptions of blood cells in moray are scarce and the literature primarily describes the general biology of moray eels (Pichiri et al., 1995; Ronchetti et al., 1995; Pichiri et al., 2000; Mehta and Wainwright, 2007). The same is true for conger (Lupo and Chieffi, 1963; Toews et al., 1983; Cerra et al., 1992; Jardas, 1996; Santos and Gibson, 2002; Matić-Skoko et al., 2010, 2011). For both species, descriptions of haemoglobin properties and composition are available (Pellegrini et al., 1995, 2003) though the basic hematology was not described.

Moray and conger hematocrit values and the RBC count fit in the hematological frame of (semi) sedentary species as explained by Filho et al. (1992). Moray RBC count values correspond with the range of values reported in the similar species *Ghymnothorax funebris* (Francis-Floyd et al., 1991), however no similar comparison was possible for the conger eel due to the lack of reference data for this species.

Table 4.
Differential leukocyte (WBC) counts in wild caught moray eel (*Muraena helena*), European conger eel (*Conger conger*) and European common eel (*Anguilla anguilla*).

Species (N)	WBC	Granulocytes (%)							Agranulocytes (%)	
		Neutrophil (Heterophil)	Neutrophil (Heterophil)-bi-lobed	Neutrophil (Heterophil)-total	IBG Neutrophil (Heterophil)	IBG Neutrophil (Heterophil)-bi-lobed	IBG Neutrophil (Heterophil)-total	Basophil	Lymphocytes	Monocytes
Muraena helena (18)	mean	45.03[a]	4.10[a]	49.13[a]	30.00	1.23	31.23	nf	15.05[a]	4.59[a]
	SD	13.77	4.42	11.11	11.98	1.19	17.58	nf	8.91	2.88
	min	25.00	0.00	31.34	0.00	0.00	8.50	nf	4.50	0.50
	max	66.00	12.92	66.50	52.07	3.33	57.60	nf	23.90	8.50
	(n/N) × 100	100	77.78	100	100	77.78	100	nf	100	100
Conger conger (17)	mean	40.29[a]	2.19[b]	41.07[a]	nf	nf	nf	0.16	50.03[b]	7.83[a]
	SD	10.02	2	7.51	nf	nf	nf	0.37	7.91	4.00
	min	28.4	0	31.93	nf	nf	nf	0	33.9	0.93
	max	58.02	7.14	58.88	nf	nf	nf	0.97	60.55	14.56
	(n/N) × 100	100	50	100	nf	nf	nf	11.11	100	100
Anguilla anguilla (17)	mean	36.71[b]	1.90[c]	38.61[b]	nf	nf	nf	nf	47.52[c]	13.87[a]
	SD	11.69	3.15	10.17	nf	nf	nf	nf	10.27	7.27
	min	11.74	0	18.31	nf	nf	nf	nf	25.60	6.00
	max	58.45	12.40	58.94	nf	nf	nf	nf	59.00	32.51
	(n/N) × 100	100	82.35	100	nf	nf	nf	nf	100	100

Abbreviations and symbols: max, maximum; min, minimum; n, number of fishes per group in which particular cell type was found; N, number of individuals per group; nf, not found; SD, standard deviation; WBC, white blood cells; [a, b, c], the values marked with different superscript letters are significantly different ($P < 0.05$) from the same parameter in the other fish species, whereas the values bearing the same superscript letter are not significantly different from the same parameter in other fish species.

Higher percentages of young and senescent erythrocytes recorded in conger may be a reflection of the faster cell life cycle (shorter cell circulation half-life, higher production, maturation and clearance ratio). Based on age and morphometric parameters, conger had higher biomorphological indices per year of age compared to other two species which indicates a higher overall metabolism and annual body weight/length gain of this species. The opposite was observed for common eel, with the lowest annual weight/length gain (higher average age and lower weight and shorter body length). This is a reflection of its slower metabolism (slower annual biomass gain) than in moray or conger, due to the seasonal changes in the freshwater environment. Presumably, the higher erythrocyte creation/destruction ratio (higher percentage of old and young circulating erythrocytes) is a reflection of the faster metabolism and growth ratio of conger.

The neutrophil (heterophil) subtype with an intensively basophilic cytoplasm, resembling reptilian or avian azurophils, was found in moray and was difficult to classify as any known fish granulocyte type (Campbell and Ellis, 2007). Interestingly, these cells were not present in conger or common eel (Orecka-Grabida, 1986; Kusuda and Ikeda, 1987; van Ginniken et al., 2005; Şahan et al., 2007; Ponsen et al., 2009). Occasionally, there are granulocyte cells that do not fit into any of the subclasses and do not resemble any known granulocyte type (Ranzani-Paiva et al., 2003; Shigdar et al., 2009). Specialized granulocyte subtypes are also common in many shark species (Hyder et al., 1983; Ainsworth, 1992). Until further analysis, those distinctive cells were classified as intensively basophilic (IBG) granulocytes of moray eel.

Such species-specific differences are common among fish (Ainsworth, 1992; Hine, 1992; Suzuki and Iida, 1992; Erickson et al., 1992; Anderson and Zeeman, 1995; van Ginneken et al., 2005; Reavill and Roberts, 2007; Clauss et al., 2008). Higher percentages of granulocytes (neutrophils + IBG) accompanied by a lower percentage of lymphocytes in moray than in the other two eel species indicate that innate immunity might play a major role in defence, while in the other two species, lymphocytes are major carrier of defence. Another possibility is that in moray, lymphocytes are adapted to physiologically compensate for the lower numbers in circulating blood. Monocytes were in concordance with the literature (Thrall et al., 2004; Şahan et al., 2007).

The presence of basophils only in conger and the absence of eosinophils in the analyzed eels is not unusual (Ellis, 1977; Cannon et al., 1980; Hendrick et al., 1986). In the genus *Anguilla*, some species are recorded to have eosinophils while others lack this cell type (Orecka-Grabida, 1986; Kusuda and Ikeda, 1987; van Ginniken et al., 2005; Ponsen et al., 2009). Further sampling during other seasons or experimental exposure to pathogens or parasites might allow for the discovery of the existence of eosinophils and the basophils in all three fish species analyzed here. Regarding size, blood cells in moray were larger than in the other two analyzed species. These results confirm that slightly larger blood cells are a general characteristic of moray eels as previously proposed by Francis-Floyd et al. (1991).

Blood cell count values did not change with the body size of individuals of all three species. Therefore, it appears that the hematological values are fairly constant during the particular developmental stage or age in these three species (in this case 3-11 years), regardless of the individual growth of the fish and this likely reflects the environmental and seasonal conditions. Similar conclusions appear in literature with the most comparable conclusions in the related genus *Anguilla* (Johansson et al., 1974). Correlation analysis supports this presumption as it is shown that RBC, WBC and thrombocyte values showed significantly low correlations with biometric parameters. Thrombocytes were the second most abundant after RBC. Often not all of four types appear together in the same or between kin species, as for example among *Sparidae* (Campbel and Murru, 1990; Pastoret et al., 1998; Pavlidis et al., 2007), though the present study indicates that all three eel species have four thrombocyte types.

In conclusion, reference intervals for domestic animals and in human medicine are usually based on a much larger sample size (100-120 individuals). For exotic animal species, this is not practical though less than 20 individuals is not considered representative of the population and therefore it is important not to overstate the significance of conclusions, especially since random field sampling resulted in an uneven sample (age, growth, etc.) within and between groups. Nevertheless, the sample size for this study (17-18 eels per species) is a good starting point for describing cell morphology and giving a preliminary estimate for complete blood count data in further studies. This paper demonstrates the cellular specifics and their occurrence among kin eel species and proposes guidelines for future work.

Supplementary material

See tables S1 and S2 and figures S1 and S2 as supplementary material in the online edition of this journal, which can be accessed via http://www.brill.com/ab.

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