DIFFERENTIAL BLOOD CELL COUNTS OF ATLANTIC HERRING, *CLUPEA HARENGUS HARENGUS*

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ABSTRACT

In differential blood counts of 200 herring, *Clupea harengus harengus*, the percentages of white cell types and immature erythrocytes in the blood were found to be different from those previously reported in the literature. Herring were sampled from February 1969 through July 1969 from the Boothbay Harbor, Maine, and Deer Island, New Brunswick, Canada, areas.

Immature erythrocytes in wild herring ranged from 6 to 38% with an average of 21; in captive herring they ranged from 2 to 22% with an average of 11. Thrombocytes were the most common type of leukocyte, followed in decreasing order by lymphocytes, neutrophils, basophils, and eosinophils in both captive and wild herring.

The percentage of each white cell type varied greatly between individual herring, but except in one instance, the mean percentage of each white cell type varied little between samples. One group of wild herring was unusual in that neutrophils averaged 21% of the leukocytes in contrast with an average of 4% neutrophils for three other wild samples and an average of 6.8% neutrophils for four captive samples.

The percentage of immature red cells varied widely between individual herring, but the mean percentage varied little between wild and captive herring within their respective categories.

The blood of herring has been studied at the Boothbay Harbor Laboratory to find physiological indicators of environmental stress that may help us determine causes of fluctuations in success of year classes. In previous published studies Naumov (1959) reported changes in the differential white cell counts of Atlantic herring, *Clupea harengus harengus*, from the Barents Sea and from the Greenland Sea in relation to the sexual cycle; Boyar (1962) reported no differences in the occurrence of blood cell types of Atlantic herring, *Clupea harengus harengus*, from the Gulf of Maine that related to length, or that offered any promise as an aging method. The differential white cell counts of herring I examined were different from those previously reported by Boyar and by Naumov. I shall report the results of my studies and discuss possible reasons for those differences.

MATERIALS AND METHODS

My analysis is based upon blood samples collected from wild herring obtained from commercial fishermen's catches and captive herring held in a tank at the Boothbay Harbor Laboratory. These data are shown in Tables 1 and 2.

All herring sampled from Boothbay Harbor were immature. Those from Deer Island were nonspawning mature fish and those from Eastport were 95% nonspawning mature fish and 5% immature fish. Captive herring were held in circular tanks having a capacity of 1,325 gallons. These tanks were supplied with unheated seawater which was pumped directly from the ocean. The water temperature was recorded at the site of capture in each instance.

Captive herring were fed a diet of canned cat food and commercial fish pellets. This diet was supplemented with natural food which entered the tanks through the water system. This natural food supply varied with the season; barnacle larvae were available in spring, and mollusk larvae were available in summer.

Slides were made from blood obtained by

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TABLE 1.—Wild herring (obtained from commercial fishermen's catches.)

Collection date (1969)	Source	No. of fish	Range	
			Length (cm)	Weight (g)
13 Mar.	Boothbay Harbor, Me.	34	11.6-19	9.4- 46.4
10 June	Eastport, Me.	32	14.5-30.4	18.0-214.5
8 July	Boothbay Harbor, Me.	45	12.5-18.2	12.7- 38.6
16 July	Deer Island, N.B., Can.	30	20 -24.6	56.5-119.3

TABLE 2.—Captive herring (held in a tank at the Boothbay Harbor Laboratory prior to collection of blood samples.)

Collection date (1969)	Months held captive	No. of fish	Range	
			Length (cm)	Weight (g)
25 Feb.	6	16	14 -17.2	14.2-29
24 Mar.	7	17	13.2-17.4	10.6-29.4
21 Apr.	8	18	14.3-17.7	14.9-33.1
23 June	10	8	13.1-18	14.1-34.7

direct heart puncture with a heparinized capillary tube. A small drop of blood from the capillary was placed on a microscope slide and the smear made. Slides were air-dried and stained by either the Wright's or Wright-Giemsa staining method. Distilled water was used as a diluent for the Wright's and Giemsa stains. The air-dried film was covered with Wright's stain for 1½ min, then an equal quantity of distilled water added and the film further stained for 3-4 min; then, either the diluted stain was flooded off with water and the slide dried, or the slide counterstained with diluted Giemsa for 20 min. Staining times varied slightly with different batches of Wright's stain. Each herring was measured for total length, weighed, sexed, marked, examined for gross parasitism, and frozen for reference in case abnormal blood properties were found. Cells were examined under oil immersion at 800 and 1,250 powers.

Differential counts were made of both red and white cells on an area of the slide where the cells were evenly distributed. A leukocyte differential count was made of a random selection of 100 leukocytes and listed under the following categories: lymphocytes, thrombocytes, neutrophils, eosinophils, or basophils. The range and mean percentage of each type of leukocyte present was calculated for each sample. An erythrocytic differential count was made of a random selection of 100 red cells and divided into mature or immature cells based upon their general morphology and staining properties. I classify herring erythrocytes in the peripheral blood according to the stage of development as erythroblasts, early polychromatics, mid-polychromatics, late polychromatics or mature cells. These terms were used by Lucas and Jamroz (1961) in describing the developmental stages of avian erythrocytes. The developmental stages for herring erythrocytes are described in Table 3. There are variations in individual herring in the size and shape between and within cell stages, and the amount of polychromasia present is the best indicator as to the stage to which the cell belongs. The primary morphological criteria I used in this paper were those of Boyar (1962). Additional references were consulted for a more definitive classification of cell types, to clarify the distinction of basophils from basophilic-staining immature erythrocytes, and to resolve confusion regarding the existence of monocytes in herring blood. These additional references were Catton (1951), Davidsohn and Nelson (1969), Jakowska (1956), Lucas and Jamroz (1961), Saunders (1968b), and Watson, Shechmeister, and Jackson (1963).

TABLE 3.—Erthrocytes in the peripheral blood of Atlantic herring, *Clupea harengus harengus*, according to the stage of development.

Stage	Description
Erythroblast	Round cell, similar in size to the early polychromatic. Has a dark blue staining cytoplasm with lightly stained spaces. The round purple-red staining nucleus is larger than that of the early polychromatic and takes up most of the cell. Erythroblasts are scarce in normal samples.
Early polychromatic	The smallest immature red cell that is normally seen in any quantity. Has a light blue to gray staining cytoplasm and appears round. The nucleus takes up most of the cell.
Mid-polychromatic	Round to slightly oval cell with a gray to light gray-orange staining cytoplasm. Cell is larger than the early polychromatic.
Late polychromatic	Slightly oval, has a larger cytoplasm and a smaller nucleus than the mid-polychromatic. The cytoplasm appears light orange-yellow.
Mature erythrocyte	Oval, has a slightly larger cytoplasm and a slightly smaller nucleus than the late polychromatic. The cytoplasm appears orange-yellow to reddish. Late polychromatic and mature erythrocytes have essentially the same appearance with Wright's and Wright-Giemsa stains.

RESULTS

Differential red and white cell counts are listed in Table 4. Blood cell measurements of all cells included in the differential counts are listed in Table 5.

The mean percentage of each white cell type varied greatly between individual herring, but except in one instance, the mean percentage for most white cell types varied little between samples. Thrombocytes were the most common leukocyte, followed in decreasing order by lymphocytes, neutrophils, basophils, and eosinophils in both wild and captive herring. One group of wild herring sampled on 8 July from Boothbay Harbor was unusual in that neutrophils averaged 21% of the leukocytes in contrast to an average of 4% neutrophils for three other wild samples and an average of 6.8% for captive herring. The herring from the 8 July sample were confined in the pocket of a stop seine overnight before being bled, in contrast to other wild herring that were bled immediately after the pocket was made in a purse seine (13 March) or in weirs (10 June and 16 July). The relative neutrophilia in the 8 July sample may be a result of the combination of being held overnight at relatively high water temperatures and crowded conditions. Temperature alone was evidently not the cause of relative neutrophilia in the July wild sample, for captive herring taken from nearly the same water temperature did not show relative neutrophilia.

Basophils and eosinophils were scarce in most herring examined, the greatest average (2% basophils) occurred in a wild sample from Eastport, Maine. Though one captive herring had 16% basophils, one wild herring from Eastport had 32% basophils, and another wild herring from Boothbay Harbor had 5% eosinophils and 17% basophils, no differences were found in those specimens in their red cell morphology or

TABLE 4.—Differential leukocyte and erythrocyte counts of wild and captive herring, *Clupea harengus harengus*, with the mean percentage and range (in parentheses) of each cell type.

Sample source and date	Water temp. (°C)	Number of herring	Type of leukocyte					Immature erythrocytes
			Thrombocytes	Lymphocytes	Neutrophils	Basophils	Eosinophils	
Wild:								
13 Mar. 1969 Boothbay Harbor, Maine	2.0	34	56.7 (33-75)	39.2 (23-58)	4.0 (0-13)	0.1 (0- 3)	0.0	19.1 (6-38)
10 June 1969 Eastport, Maine	7.7	32	53.3 (33-68)	40.8 (21-61)	3.8 (0-10)	2.0 (0-32)	0.1 (0-1)	21.2 (9-35)
8 July 1969 Boothbay Harbor, Maine	13.8	45	48.5 (21-79)	29.1 (9-62)	21.3 (8-47)	1.0 (0-17)	0.1 (0-5)	22.6 (12-33)
16 July 1969 Deer Island, N.B., Can.	9.8	30	62.7 (42-75)	31.8 (16-55)	4.5 (0-14)	0.9 (0-12)	0.1 (0-2)	19.0 (10-35)
All wild		141	54.6 (21-79)	34.7 (9-62)	9.6 (0-47)	1.0 (0-32)	0.1 (0-5)	20.7 (6-38)
Captive:								
25 Feb. 1969	1.3	16	80.2 (64-95)	13.0 (2-33)	6.6 (0-19)	0.1 (0- 1)	0.1 (0-2)	7.9 (2-19)
24 Mar. 1969	3.3	17	69.2 (29-89)	22.3 (9-51)	8.4 (0-20)	0.1 (0- 1)	0.0	11.2 (2-21)
21 Apr. 1969	4.9	18	67.8 (42-89)	26.3 (5-52)	4.6 (1-13)	1.1 (0-16)	0.2 (0-3)	12.3 (2-22)
23 June 1969	15.2	8	63.1 (35-76)	28.0 (18-48)	8.6 (1-17)	0.3 (0- 2)	0.0	13.2 (7-20)
All captives		59	70.9 (29-95)	21.8 (2-52)	6.8 (0-20)	0.4 (0-16)	0.1 (0-3)	10.9 (2-22)

TABLE 5.—Blood cell measurements in microns of wild Atlantic herring, *Clupea harengus harengus*, with the average size, standard deviation, and size range (in parentheses).

Cell type	Number of herring	Total number of cells measured	Cytosome		Nucleus	
			Length	Width	Length	Width
Erythrocytes:						
Mature RBC	8	200	10.7 ± 0.9 (9.0-14.0)	7.8 ± 0.6 (6.2-9.7)	4.6 ± 0.5 (3.3-6.1)	2.9 ± 0.2 (2.2-3.9)
Late polychromatic	6	150	10.4 ± 0.8 (9.0-12.3)	7.6 ± 0.6 (6.2-9.4)	4.8 ± 0.4 (4.2-6.2)	2.9 ± 0.2 (2.3-4.1)
Mid-polychromatic	6	150	9.5 ± 0.7 (7.8-10.9)	7.1 ± 0.7 (5.0-9.0)	5.0 ± 0.5 (4.4-6.2)	3.0 ± 0.2 (2.3-4.4)
Early polychromatic	6	150	8.2 ± 0.7 (6.2-10.6)	7.1 ± 0.7 (4.7-9.2)	4.9 ± 0.5 (3.7-6.2)	3.4 ± 0.4 (3.0-4.8)
Erythroblast	1	10	8.7 ± 0.9 (7.0-9.4)	7.9 ± 0.4 (7.5-8.6)	6.2 ± 0.2 (5.5-6.2)	5.8 ± 0.6 (4.7-6.2)
Leukocytes:						
Thrombocyte	3	100	8.9 ± 0.9 (7.0-10.9)	4.4 ± 0.5 (3.1-6.1)	7.2 ± 0.6 (5.9-9.0)	3.5 ± 0.5 (2.8-4.8)
Lymphocyte	2	100	7.7 ± 0.9 (5.0-10.1)	7.4 ± 0.8 (5.5-9.4)	6.4 ± 0.6 (4.7-8.6)	6.1 ± 0.6 (4.7-7.8)
Neutrophil	8	100	11.3 ± 1.7 (7.6-17.2)	10.9 ± 1.5 (6.2-15.6)	4.9 ± 0.9 (2.2-6.2)	7.3 ± 1.2 (3.6-10.9)
Basophil	6	100	12.4 ± 1.6 (9.4-17.2)	12.0 ± 1.2 (8.6-14.0)	5.1 ± 1.2 (3.1-8.6)	6.6 ± 1.4 (3.1-9.4)
Eosinophil	1	25	14.1 ± 1.7 (10.1-17.2)	14.3 ± 1.4 (12.5-17.2)	4.6 ± 0.9 (3.1-6.2)	7.5 ± 1.0 (6.2-9.4)

gross parasitism to distinguish them from other herring in their respective samples.

The mean percentage of immature erythrocytes varied widely between individual herring, but the mean percentage of immature erythrocytes varied little between samples of wild and captive herring within their respective categories. Wild herring had a greater percentage of immature erythrocytes at each sampling than did the herring held captive. Immature erythrocytes in wild herring ranged from 6 to 38% with an average of 21; in captive herring they ranged from 2 to 22% with an average of 11. As noted for other fish species (Catton, 1951; Hesser, 1960), films prepared from herring blood were characterized by the presence of more cellular debris and disintegrating cells than is usually found in films prepared from mammalian bloods. Erythroblasts, hemoblasts, and cells showing mitotic division were scarce in the peripheral blood of herring examined in this study.

DISCUSSION

The high variability in differential counts between individual herring is not unusual; such variability was noted in a number of vertebrate species (Altman and Dittmer, 1961:127-128). Lucas and Jamroz (1961) discussed the individual variability in blood values and the problems associated with this characteristic in avian species. Rooney, Roberts, and Dexter (1972), in differential counts of immature Atlantic salmon, found the most individual variability in thrombocytes, followed in decreasing order by lymphocytes and neutrophils.

Though I agree with Boyar (1962) in the general description of cell types in herring, my data differ markedly in the percentage of immature red cells in herring blood, in the percentage of white cell types present, and in the relative size of neutrophils in comparison to other white cell types. I found an average of 21% immature erythrocytes in 141 wild herring ranging in

length from 116 to 304 mm in my study, whereas Boyar reported immature erythrocytes and white cells combined constituted less than 3% of the total blood cells in the 85 wild herring ranging in length from 75 to 300 mm in his study.

Boyar (1962) reported neutrophils were much larger than other white cell types, with an average area twice that of the second largest cells, the eosinophils. However, in the herring I examined, the average area of neutrophils was larger than thrombocytes and lymphocytes, but smaller than basophils and eosinophils. The neutrophils in Boyar's line drawings of blood cell types have the appearance of cells that have been mechanically distorted. This flattening and spreading out would cause an increase in cell dimensions.

The average cell measurements in my studies are larger than those reported by Boyar, except in the case of neutrophils. Naumov (1959) reported ranges but not mean dimensions of leukocytes. My size range for neutrophils was similar to Naumov's, while the maximum sizes of individual lymphocytes and eosinophils were larger than reported by Naumov.

The total 8 July sample consisted of 76 herring. Five contained intracytoplasmic inclusions associated with erythrocytic degeneration (Sherburne, 1973). One herring in the total March wild sample, two herring in the total June captive sample, and two herring in the total 16 July wild sample contained erythrocytic inclusions. Though their white cell differentials did not differ markedly from other herring in their respective samples, the ten herring with inclusions are not included in this report.

Basophils were scarce and were not found in the leukocyte counts of 157 of the 200 herring in my study. Only 7 herring in my study had more than 5% basophils in their blood differentials. In contrast, Boyar (1962) reported basophils were the most abundant leukocyte in herring from brit 75 mm in length to sexually mature herring up to 300 mm. He reported basophils averaged 31% of the less than 3% cells other than mature erythrocytes (47% of the leukocytes) present in herring ranging in length from 101 to 200 mm, the predominant sizes of herring included in my study. Boyar also reported that basophils averaged from 18% to 56% of the leukocyte cell types in other size ranges of herring included in my study.

I have used the same staining method (Giemsa) used by Boyar and found no differences in the relative numbers of basophils compared with those I found using Wright's or Wright-Giemsa stains. Romer (1949) reported basophils are seldom found in fishes. Naumov (1959) found no basophils in sexually mature Murmansk (Atlantic) herring, *Clupea harengus harengus*, collected in 1938-39 from the Barents Sea and 1947 from the Greenland Sea. Saunders (1968a) found no basophils in blood smears of 49 species of teleost fishes from the Red Sea, and Saunders (1966) found basophils in the circulating blood of only 5 species out of 116 species of marine teleosts examined off Puerto Rico. The differences between Boyar's findings and my own may reflect environmental variations between the time of his study and my own. A more likely explanation is that Boyar confused certain basophilic staining immature erythrocytes with basophils. Small basophils are similar in size to basophilic staining immature erythrocytes, but in my slides these cell types were readily distinguished.

Thrombocytes were the most common leukocyte in my study; they constituted an average of 70% of the white cells in captive herring and 54% in wild herring. Boyar (1962) reported thrombocytes (spindle cells) made up 0 to 7% of the less than 3% cells other than mature erythrocytes present in his study. Saunders (1966, 1968a) reported thrombocytes were the most common leukocyte in the circulating blood of 116 species of marine teleosts from Puerto Rico and 49 species of teleost fishes from the Red Sea.

Boyar (1962) reported round, granular ghost cells averaged from 17 to 50% of the less than 3% cells other than mature erythrocytes present in his study. I also found round, anucleated cells occasionally in apparently normal herring, but most cells appeared finely vacuolated. Several blood smears from herring with intracytoplasmic inclusions associated with erythrocytic degeneration (Sherburne, 1973) had up to 50% round, anucleated cells present in the blood films. The anucleated cells appeared pale red with Wright's stain, were similar in size, and ranged from 9.4 x 9.4μ to 10.9 x 10.9μ. Some

of the cells appeared to show diffusion of nuclear material into the cytoplasm.

Naumov (1959) reported that the blood of sexually mature Murmansk (Atlantic) herring, *Clupea harengus harengus*, contained—in decreasing order of relative abundance—lymphocytes, monocytes, polymorphonuclear leukocytes, and eosinophils. Naumov, like many other authors, did not include thrombocytes in his differential counts.

In contrast to Naumov's report, neither Boyar (1962) nor I found monocytes in the Atlantic herring we examined. Jakowska (1956) discussed the confusion regarding the term monocyte, and because of the difficulty of recognizing monocytes with certainty, advised caution in including them as a separate category of teleost blood cell types. Catton (1951), Gardner and Yevich (1969), Hesser (1960), Romer (1949), Saunders (1966, 1968a) and Watson et al. (1963) did not report monocytes in teleost fish species they examined. Romer (1949) stated that monocytes, sometimes regarded as a separate category of white cells, appear to be merely large lymphocytes. I believe Naumov (1959) may have confused monocytes with large lymphocytes or neutrophils for the following reasons: Naumov reported lymphocytes in Atlantic herring ranged from 3.5 to 5.6μ, while monocytes ranged from 8.0 to 10.8μ. The lymphocytes in the herring I examined ranged from small (5μ) to large (10μ). Thus, perhaps Naumov's monocytes were actually large lymphocytes; however, he stated that during the process of regeneration of the gonads after spawning, there is an increasing quantity of monocytes and polymorphonuclear leukocytes in the blood evidently acting as phagocytes. Watson et al. (1963) stated that the neutrophil is a possible cause for some of the confusion regarding the existence of a monocyte within teleost blood, especially a neutrophil with a kidney-shaped nucleus. As the photographs in the translation of Naumov's report available to me are not clear and his monocytes and polymorphonuclear leukocytes have nearly the same range of dimensions (8.0 to 10.8μ and 8.5 to 14.4μ, respectively) perhaps his phagocytic monocytes were neutrophils.

Differential blood counts of herring fry 55-65 mm in total length, sampled in June and July 1971 from Boothbay Harbor, and mature herring 290-320 mm in total length, sampled 28 October 1972, northeast of Nantucket Shoals, confirm the findings of this study in that thrombocytes are the most common type of leukocyte in herring blood followed in decreasing order by lymphocytes, neutrophils, basophils and eosinophils.

This report provides a more accurate description of characteristic blood cell differentials found in Atlantic herring and provides a baseline for a systematic study of leukocyte differentials in physiologically stressed herring.

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