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BLOOD PROPERTIES OF PRESPAWNING AND POSTSPAWNING ANADROMOUS ALEWIVES (*Alosa pseudoharengus*)

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ABSTRACT

As part of a general investigation of the potential value of fish blood characteristics to the solution of population and migration problems, a study of modifications induced by environmental and physiological variables has been made. Six blood properties of prespawning and postspawning anadromous alewives (*Alosa pseudoharengus*) were compared. Changes that could be attributed to fresh-water migration and reproduction were found in only two of these properties; viz., significant reductions in average serum proteins and chlorides of postspawners. No important differences in average sedimentation rate, erythrocyte fragility, hemoglobin content, or serum electrophoretic pattern were found when fish entering fresh water in May were compared with seaward migrants 1 to 2 months later. Serum electrophoretic patterns were generally similar to those of other clupeoids, with fractions having mobilities comparable with human albumin and human alpha- and beta-globulins, but with little representation in the area of gamma-globulins. Great individual variations in hemoglobin content, total serum proteins, serum chloride, and sedimentation rate were found in alewives both before and after spawning.

BLOOD PROPERTIES OF PRESPAWNING AND POSTSPAWNING ANADROMOUS ALEWIVES (*ALOSA PSEUDOHARENGUS*)

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Dramatic changes in certain of the blood characteristics of humans and other higher vertebrates often occur under such physiological stresses as pregnancy or acute disease. It might be anticipated that lower vertebrates, with less precise control of their internal environment, would exhibit equally profound blood changes as the external medium or physiological conditions vary. As an adjunct to serological studies of fishes being carried on at the Bureau of Commercial Fisheries Biological Laboratory, Boothbay Harbor, Maine, it was considered important to assess the extent of environmental and physiological influences on blood properties, particularly those which might be related to serological reactions.

Erythrocyte antigens, which promise to be of great value in fish population and migration studies, have been examined most extensively in higher vertebrate groups (summarized by Dujarric de la Rivière and Eyquem, 1953; Mourant, 1954), where they have been found to be genetically determined and unmodified by environmental variations. Some evidence for genetic determination of fish erythrocyte antigens has been offered by Hildemann (1956). Serum components, which may also provide information of value to population studies, are in some cases subject to modification by other than genetic factors. For example, antibody production in fishes has been shown to vary with external temperature (Bisset, 1948) and protein fractions of fish serum to vary in amount in disease (Sindermann and Mairs, 1958). Because of possible influence of nongenetic factors on serological properties, a study of environmental and physiological effects on blood characteristics seemed advisable.

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As part of such a study, this paper is concerned with the manner in which the combined stresses of migration from the sea to fresh water and of spawning are reflected in several blood characteristics of the alewife (*Alosa pseudoharengus*). The nature and extent of serum changes in prespawning and postspawning fish have received particular attention in this investigation, although observations on cellular blood components have been included.

MATERIALS AND METHODS

COLLECTION OF BLOOD SAMPLES

Prespawning and postspawning alewives were sampled in 1958 and 1959 from two separate Maine spawning runs—Damariscotta Mills and West Boothbay Harbor. Fish were first sampled in May, as they were about to enter fresh water, and again in late June and July, as they were about to re-enter the sea. In addition to the field samples, prespawning alewives taken from both runs were held without food in live cars and seawater tanks for 2 months before blood samples were taken, to determine the effect of starvation on electrophoretic characteristics of the serum.

The fish were bled by cardiac puncture, using a glass-needle technique developed in this laboratory (Perkins, 1957). Blood was collected in screw-top vials as individual samples. Half the samples were collected in vials containing 0.2 milliliter of 6-percent sodium citrate solution, and half were collected in vials without citrate. Determinations of hemoglobin content, sedimentation rate, and erythrocyte fragility were made immediately with the citrated samples. Sera from uncitrated blood samples were decanted after expressing from the clots overnight at 4° C. Individual serum samples were kept frozen at −20° C. until determinations of chloride content, total

serum protein content, and electrophoretic pattern were made. The sex and stage of gonad development of each fish were recorded when the blood sample was taken.

DETERMINATIONS OF BLOOD PROPERTIES

Hemoglobin Content

Determination of the hemoglobin content of individual citrated blood samples was made with the cyanmethemoglobin method, using reagent and standard supplied by Hycel Hormone Chemistry Laboratory, Houston, Tex. With a Sahli pipette, .02 ml. blood was added to 5 ml. cyanmethemoglobin reagent and thoroughly mixed. Contents were transferred after 15 minutes to a cuvette and read colorimetrically with a Photovolt Lumetron colorimeter at a wavelength of 530 millimicrons. The colorimeter reading was transferred to a standard hemoglobin curve and hemoglobin concentration obtained in grams per 100 ml.

Sedimentation Rate

The rate of settling of erythrocytes was determined with a standard Westergren blood sedimentation apparatus. Individual samples of citrated blood was drawn into Westergren pipettes to the 100-millimeter mark and placed vertically in a Westergren rack. The number of millimeters that the erythrocytes had dropped at the end of 1, 2, and 3 hours—the actual sedimentation—was recorded and multiplied by 2 to make results comparable with standard tests that use the entire 200-mm. pipette length. Results are expressed in terms of the standard 200-mm. length.

Erythrocyte Fragility

Sodium chloride solutions ranging from 0.3 to 1.5 percent in 0.1-percent increasing steps were used to test erythrocyte fragility. For each sample, .05 ml. of a 50-percent cell suspension was added to 1 ml. of each saline dilution and readings of "no hemolysis," "partial hemolysis," or "complete hemolysis" were recorded for each tube at the end of 1 hour's incubation at 4° C.

Total Serum Proteins

The biuret method of Kingsley (1942) was used to determine total serum proteins. This involves precipitation with acetone-alcohol followed by addition of biuret reagent. Readings were made with a Photovolt Lumetron colorimeter at a wave length of 530 millimicrons and were plotted against a standard curve prepared with dilutions of clinical chemistry control serum, supplied by Hyland Laboratories, Los Angeles, Calif., to obtain total protein values.

Serum Chlorides

Determinations of serum chlorides were made by the standard method outlined in the manual for Photovolt Lumetron colorimeter. This involves treatment of serum with tungstic acid, silver iodate, phosphoric acid, and potassium iodide. Readings were made with the Lumetron colorimeter at a wave length of 420 millimicrons and plotted against a standard curve prepared from known chloride concentrations to obtain serum chloride values.

Serum Electrophoresis

Electrophoretic examination of serum was made with a Spinco paper electrophoresis system. Samples were run for 6 hours at 15 milliamperes, with veronal buffer of pH 8.6 and ionic strength of .05. Pooled human serum was used as a standard with each series. Filter-paper strips were dyed with bromphenol blue and analysed with a Spinco Analytrol densitometer.

COMPARISON OF BLOOD PROPERTIES

Tests of serum and cellular components of the blood of prespawning and postspawning alewives provided the data presented in table 1. Results from the two Maine spawning runs have been combined, since no consistent differences between them were noted.

HEMOGLOBIN

Variations in hemoglobin content of fish blood have been examined in a number of marine and fresh-water species by several investigators. Black (1955) found that the hemoglobin level of largemouth bass increased after forced exercise, but that of five other fish species did not. Pavlov and Krolik (1936) found that hemoglobin increased with the ripening of the sex products, while Naumov (1956) noted that it increased to the time of spawning and then dropped to a very low level. Gelineo (1957) found that hemoglobin values for several species of marine fish were some-

TABLE 1.—*Comparison of six blood properties of prespawning and postspawning alewives*

[Range in parentheses]

Blood property	Method of determination	Prespawning		Postspawning	
		Number of fish tested	Average	Number of fish tested	Average
Hemoglobin	Cyanmethemoglobin, Lumetron colorimeter	79	9.5 g./100 ml. (4.5–12.5)	50	9.4 g./100 ml. (4.0–13.0)
Sedimentation rate	Westergren apparatus	70	4.9 mm. (2.0–9.6)	51	4.7 mm. (1.0–12.0)
Erythrocyte fragility	Saline dilutions: 0.3 to 1.5 percent	60	0.6 percent (0.5–0.7)	60	0.6 percent. (0.5–0.7)
Total serum proteins	Biuret method of Kingsley (1942), Lumetron colorimeter.	40	5.9 g./100 ml. (3.9–8.6)	43	5.3 g./100 ml. (2.7–6.9)
Serum chloride	Lumetron colorimeter	30	430 mg./100 ml. (355–458)	28	395 mg./100 ml. (302–440)
Serum electrophoresis	Spinco model-R paper electrophoresis system	121	(1)	65	(1)

[1] See fig. 4.

what higher during the period of sexual activity than at other times. Findings in the present study indicated that average hemoglobin content of alewives entering fresh water to spawn is not different from that of the spent fish returning to the sea after spawning. Prespawners had an average hemoglobin value of 9.5 g. per 100 ml. (range, 4.5–12.5), while postspawners had an average of 9.4 g. per 100 ml. (range 4.0–13.0).

SEDIMENTATION RATES

The settling rate of erythrocytes has wide clinical use as an indicator of certain physiological changes. It is higher in human females than males and is greater during pregnancy and in disease. In fishes, Schumacher, Hamilton, and Longtin (1956) found that furunculosis caused a marked increase in the sedimentation rates of brook trout, while Kalashnikov (1939) found that the sedimentation rate increased as the gonads matured.

The present study indicated great individual differences in sedimentation rates of both prespawning and postspawning alewives (range, 1.0 to 12.0 mm. at 3 hours for 121 fish). However, no important changes have been disclosed by comparison of average sedimentation rates of fish entering fresh water to spawn with those of spent members of the same populations leaving fresh water 2 months later. Average sedimentation rates for prespawners were 1.2 mm. at 1 hour, 3.3 mm. at 2 hours and 4.9 mm. at 3 hours; for postspawners, 1.1 mm. at 1 hour, 3.2 mm. at 2 hours, and 4.7 mm. at 3 hours (fig. 1). Ripe females exhibited higher average sedimentation rates than did ripe males (5.4 mm. compared with 4.3 mm. in 3-hour readings), but this difference disappeared in postspawners.

ERYTHROCYTE FRAGILITY

Another indication of physiological distress is the lowered ability of red blood cells to withstand decreasing osmotic pressure of the surrounding medium. Fragility of human erythrocytes increases in certain diseases. Examination of alewife blood disclosed no changes in cell fragility due to the spawning migration. One-hour tests showed that complete lysis occurred consistently at between 0.5- and 0.7-percent saline in both prespawning and postspawning fish.

TOTAL SERUM PROTEINS

The serum proteins of animals have a variety of chemical and physical functions, including their important role in osmotic regulation. Among the invertebrates, wide individual variations (2.2 g. to 10.2 g. per 100 ml.) in total serum proteins of the lobster (*Homarus americanus*) and even greater variations (1.16 g. to 13.75 g. per 100 ml.) in the crab (*Cancer magister*) were noted by Leone (1953). In fishes, average total proteins of 5.6 g. per 100 ml. for adult salmon (*Salmo salar*), and 4.9 g. per 100 ml. for *Conger vulgaris* were reported by Drilhon, Fine, and Daoulas (1958). Keys (1933) found that the total serum proteins of eels dropped from 8.4 g. per 100 ml. in sea water to 6.8 g. per 100 ml. in fresh water.

The present study of prespawning and postspawning alewives has disclosed marked individual variations in total serum proteins—from 3.9 to 8.6 g. per 100 ml. in prespawners, and from 2.7 to 6.9 g. per 100 ml. in postspawners (fig. 2).

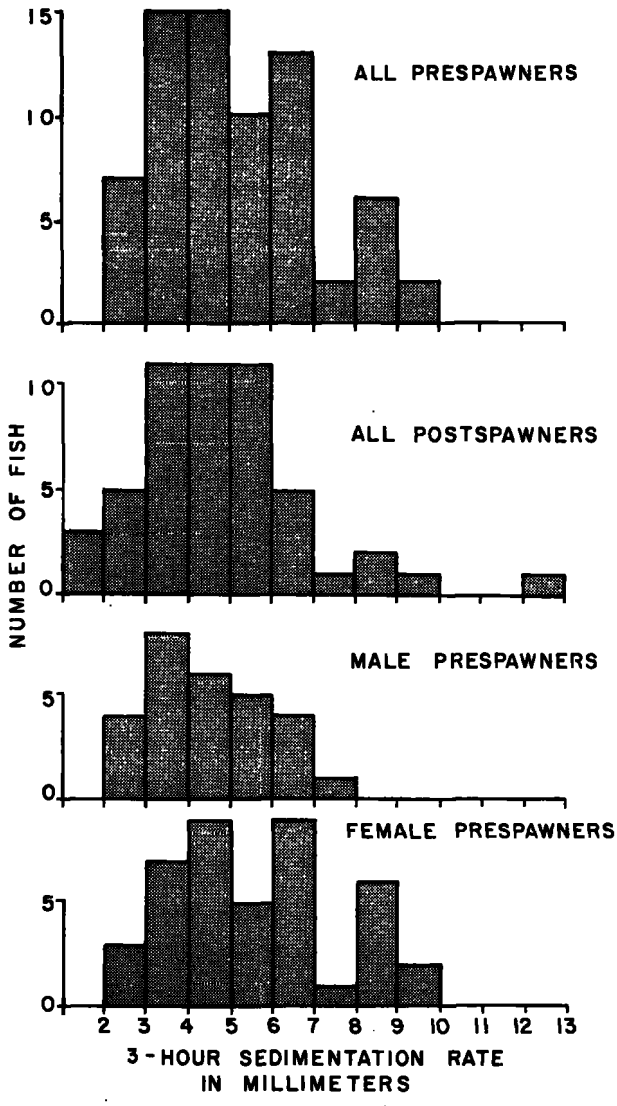

FIGURE 1.—Three-hour sedimentation rates for prespawning and postspawning alewives.

The average for prespawners was 5.9 g. per 100 ml. while that for postspawners was 5.3 g. per 100 ml.—a reduction in fresh water significant at the 5-percent level with the student T-test used for small samples, as well as with the rank sum test.

SERUM CHLORIDES

Concentrations of various ions in animal body fluids, particularly in invertebrates, may vary with environmental and physiological conditions (Prosser, 1950). The closed circulatory system of vertebrates probably effects greater ionic stability than is true for invertebrates. Numerous studies of teleost ionic regulation in varying external salinities have been made (reviewed by Fontaine and Koch, 1950, and Black, 1957). Keys (1933) found that serum chlorides of eels (*Anguilla anguilla*) were lower in fresh water than in the sea (480 milligrams per 100 ml. as opposed to 580 mg. per 100 ml.). Bond, Cary, and Hutchinson (1932) and McFarland and Munz (1958) found that in hagfish (*Polistotrema stouti*) the concentration of blood chloride varied in a linear manner with that of the surrounding medium. Harris (1959) noted a drop in blood chloride from 804 mg. to 683 mg. per 100 ml. when *Fundulus heteroclitus* were transferred from salt water to fresh.

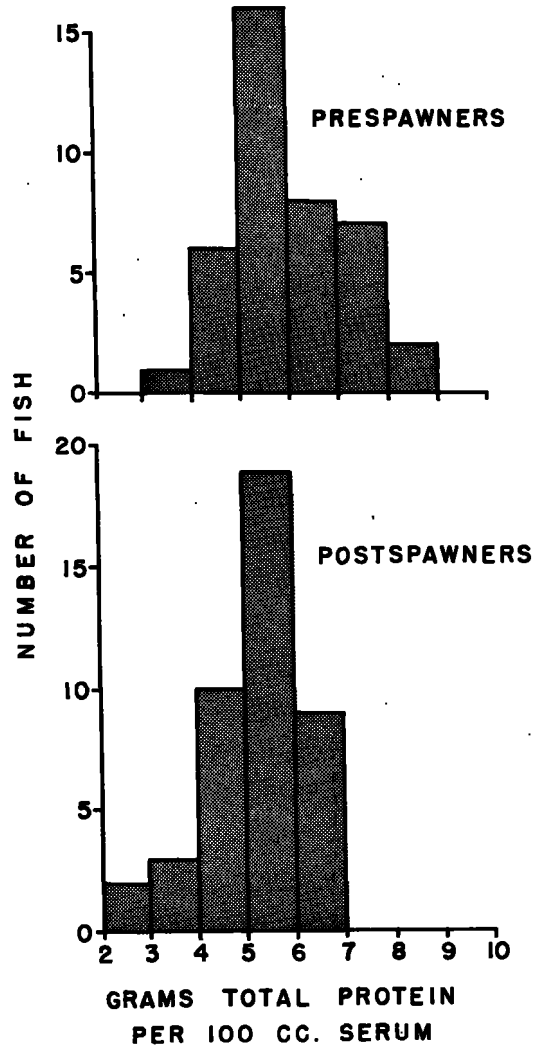

FIGURE 2.—Variation in total serum proteins in alewives before and after spawning.

The present study of serum chlorides in alewives has provided an average value of 430 mg. per 100 ml. for fish from the sea, taken just before the fresh-water migration. A decrease to an average of 395 mg. per 100 ml. was found for fish taken in fresh water just before their seaward migration (fig. 3). Within the limitations of a small sample size (30 prespawners, 28 postspawners), the rank sum test showed this decrease to be significant. As with serum proteins, individual variations in serum chlorides were marked (355 mg. to 458 mg. per 100 ml. in prespawners; 302 mg. to 440 mg. per 100 ml. in postspawners).

Comparison of these data with those of Keys (1933), which were drawn from nonspawning samples, suggests that the observed chloride reductions in alewife sera were probably associated with lower environmental salinity rather than with spawning activities. The average serum chloride of alewives in fresh water is quite similar to that of carp (401 mg. per 100 ml.) reported by Field, Elvehjem, and Juday (1943), but is somewhat lower than that of brown trout (424 mg. per 100 ml.) reported by Phillips and Brockway (1958).

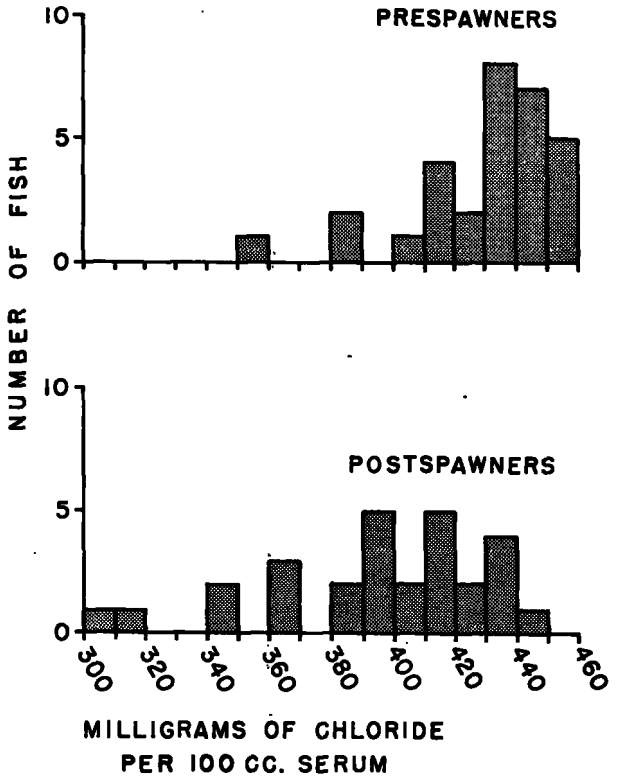

FIGURE 3.—Serum chlorides of prespawning and postspawning alewives.

SERUM ELECTROPHORETIC PATTERNS

Electrophoretic studies of fish sera have disclosed patterns which appear to be species specific (Deutsch and Goodloe, 1945; Moore, 1945). However, Drilhon et al. (1956) noted quantitative changes in albumin and beta-globulins when starved trout were compared with well-fed fish. Sindermann and Mairs (1958) found that acute fungus disease caused a drastic reduction in the albumin fraction of sea-herring blood serum. Drilhon, Fine, and Daoulas (1958) reported that neither fresh-water migration nor stage of maturity had an effect on electrophoretic patterns in trout and salmon.

The present study of prespawning and postspawning alewives has disclosed no major changes in serum patterns attributable to reproduction or to fresh-water migration. Serum patterns of alewives resembled those of other clupeoids and teleosts in general (Woods and Engle, 1957; Sindermann and Mairs, 1958) in that fractions with mobilities similar to human albumin, alpha-globulins, and beta-globulins, were consistently present. A characteristic electrophoretic serum pattern for alewives is graphed in figure 4. Fraction I, represented by the lead anodal peak, had a mobility slightly less than human albumin; fraction II occurred as a peak with mobility similar to human alpha-2 globulin; and fraction III migrated variably in the vicinity of human beta-globulin, in

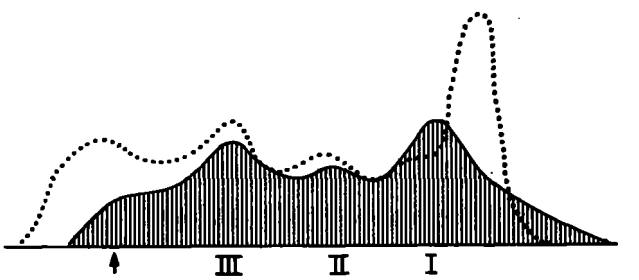

FIGURE 4—Electrophoretic pattern of alewife serum (shaded curve) compared with pattern of normal human serum. Samples were applied at a point indicated by the arrow, and the anode is to the right.

some cases occurring in two separate peaks. Little representation was found in the zone of human gamma-globulin. Fraction I slumped drastically in serum from alewives held experimentally in sea water under starvation conditions for 2 months; and is in general agreement with Keys' (1933) finding of lower total serum protein values for starved eels.

SUMMARY AND CONCLUSIONS

Comparison of six blood properties of prespawning and postspawning alewives (*Alosa pseudoharengus*) from two runs disclosed modifications in only two characteristics that could be attributed to fresh-water migration or spawning. Total serum proteins of postspawners averaged 5.3 g. per 100 ml., while prespawners averaged 5.9 g. Similarly, serum chlorides were reduced in postspawners to 395 mg. per 100 ml. from a previous average of 430 mg. Hemoglobin values, erythrocyte fragility, sedimentation rates, and electrophoretic patterns were unchanged. Hemoglobin values for alewives averaged 9.5 g. per 100 ml., with a range of 4.0 to 13.0. Complete lysis of alewife erythrocytes occurred, with little variation, between 0.5- and 0.7-percent saline in 1-hour tests. Sedimentation rates averaged 1.2 mm. for 1 hour, 3.3 mm. for 2 hours, and 4.8 mm. for 3 hours. In electrophoretic studies of serum proteins, fractions with mobilities similar to human albumin and human alpha- and beta-globulins, were found. Of these, only the beta fraction was variable, and no consistent changes resulting from fresh-water migration or spawning were observed.

The data indicated little change in most of the blood properties studied, when prespawning and postspawning alewives were compared—suggesting relative stability of the characters during this time of physiological stress. It should be noted, however, that a period of at least 1 month separated the tests of the two groups, and that no examinations were made of fish actually spawning. Recovery from any short-term effects of reproduction could have been accomplished before alewives were sampled again as seaward migrants.

Marked individual variations in such properties as sedimentation rate, hemoglobin values, serum chloride concentration, and total serum protein concentration are consistent with findings for other lower vertebrate species (Hunn, 1959, presents a list of pertinent references.) No clear association of individual variations with gonad condition, sex, or environmental salinity has been made, although the average sedimentation rate for ripe females was somewhat higher than that for ripe males. Average total serum proteins and chlorides were significantly higher in prespawners caught in salt water than in postspawners taken in fresh water—probably a reflection of environmental salinity.

Further studies are planned to assess the influences of environmental and physiological factors on the blood characteristics of fishes. In many ways, the alewife is the animal of choice for this work. Serological studies may be made with the same blood samples that are tested for other blood properties. Furthermore, offspring of small, isolated alewife populations may be examined before their seaward migration and compared with spawning adults. Also, spawning runs widely separated geographically—from the Middle Atlantic States to the Gulf of Saint Lawrence—may be compared. Finally, since landlocked populations of this species occur in North America, they may be compared with anadromous stocks.

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