CHARACTERISTICS OF THE BLOOD OF ADULT PINK SALMON AT THREE STAGES OF MATURITY

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ABSTRACT

Selected characteristics of the blood of adult pink salmon (*Oncorhynchus gorbuscha*) were studied in fish at three stages of maturity—migrating fish approaching the general area of spawning streams but still in the open ocean, fish in the immediate vicinity of the spawning stream but in the estuary, and fish in the spawning stream. Although some hematological characteristics changed little, blood proteins, glucose, and cholesterol decreased progressively, and lipid phosphorus increased.

The blood chemistry of salmon of the genus Oncorhynchus is especially interesting because of physiological changes that occur during the spawning migration from sea water to estuarine waters of reduced salinity and then into fresh water. This change in the environment is concurrent with the final stages of maturation.

Some information is already available on changes in blood characteristics at this time of the life cycle. Lysaya (1951) found several physiological changes in the blood with advancing sexual maturity in the Asiatic pink salmon (*O. gorbuscha*) and chum salmon (*O. keta*). The erythrocyte count, the hemoglobin concentration, and the blood glucose, chloride, and calcium levels fell; and the erythrocyte sedimentation rate and the blood urea and nonprotein nitrogen concentrations increased. Biologists of the Fisheries Research Board of Canada found that adult sockeye salmon (*O. nerka*) on their spawning migration up the Fraser River lost 11 to 30 percent of their body weight and had decreasing blood cholesterol (Idler and Tsuyuki, 1958); liver glycogen decreased, except for a terminal increase (Chang and Idler, 1960); and concentrations of adrenal corticosteroid hormones increased (Idler, Ronald, and Schmidt, 1959). Chinook salmon (*O. tshawytscha*) during their spawning migration up the Sacramento River and its tributaries in California showed: increased activity of the pituitary with terminal degeneration; hypertrophy of the islets of Langerhans; hyperplasia of the adrenal cortices (a rise in concentration of 17-hydroxycorticosteroids ended with degeneration of the adrenal glands); and the deterioration of the stomach, liver, spleen, thymus, kidneys, thyroid, and cardiovascular system (Robertson and Wexler, 1960, 1962; Robertson, Krupp, Favour, Hane, and Thomas, 1961; Robertson, Wexler, and Miller, 1961; and Robertson, Krupp, Thomas, Favour, Hane, and Wexler, 1961).

In 1963, under the sponsorship of the Bureau of Commercial Fisheries, I had the opportunity to study the hematology and blood chemistry of adult pink salmon in three stages of maturity in Alaska: (1) maturing fish in salt water migrating toward the spawning areas; (2) nearly mature fish milling in the estuary of a small creek; and (3) mature fish spawning in a fresh-water stream. This paper reports the results of these studies.

COLLECTION OF SAMPLES

Pink salmon in the three stages of maturity were taken from three stocks on different dates. Those migrating toward the spawning grounds (termed "migrating"), were taken from the open ocean near the community of Elfin Cove, southeastern Alaska. They were captured on August 5, about a month before

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they would have spawned; only males were sampled. Salmon milling at the mouth of a creek (called "prespawning") were taken August 9, about 2 weeks before the start of movement into fresh water, from a bay at the mouth of a stream at Little Port Walter on the southern end of Baranof Island, southeastern Alaska; equal numbers of males and females were sampled. Salmon spawning in the stream (termed "spawning") were taken from Olsen Creek, which empties into Olsen Bay on Port Gravina, Prince William Sound. They were taken on July 19 (males only) and September 2 (males and females). The Olsen Creek fish, which made up more than half of all the pink salmon sampled, were sampled on two dates because they arrive in two distinct runs. The early run typically lasts from mid-July to mid-August and the late one from late August to middle or late September. These populations may be genetically distinct.

One sample of blood was taken from each specimen while the fish was held on its back, in a wooden trough. A no. 18-½ needle on a syringe was inserted into the dorsal aorta above the roof of the pharynx, in the region of the second gill arch, and 12 ml. of blood were withdrawn. About 0.2 g. (a pinch) of potassium oxalate, an anticoagulant chosen because it is dry and hence does not cause dilution, was placed in the syringe before the sample was taken. The blood was transferred to a capped vial that also contained a pinch of the oxalate and was placed in an iced, insulated chest and transported by plane to the Bureau of Commercial Fisheries Biological Laboratory at Auke Bay. About 24 hours elapsed between collection and analysis of blood.

HEMATOLOGY

Certain hematological characteristics were determined. Specific gravity was measured by standard methods; packed cell volume was estimated after the samples were centrifuged in Wintrobe tubes at 2,700 r.p.m. for 15 minutes; erythrocytes were counted by standard techniques (Wintrobe, 1933) with 0.85 percent saline as a diluent; and hemoglobin was determined with Hycel[1] cyanomethemoglobin reagents. Mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were calculated by the formulas of Wintrobe (1933). A current general reference for work of this type is that of Hesser (1960).

The results of the analyses (table 1) are discussed in comparison with the results of other workers. Because most of the characteristics did not vary among the three stages

[1] Trade name referred to in this publication does not imply endorsement of commercial product.

TABLE 1.—*Certain hematological characteristics of adult pink salmon in three stages of maturity from three areas of Alaska in 1963*

[Numbers in parentheses are numbers of samples analyzed]

Stages of maturity	Mean specific gravity	Mean packed cell volume	Mean erythrocytes	Mean hemoglobin	Mean corpuscular volume [1]	Mean corpuscular hemoglobin [2]	Mean corpuscular hemoglobin concentration [3]
		Percent	*Number/ mm.³×10⁶*	*Grams/100 ml.*	*μ³*	*Micro micrograms*	*Percent*
Migrating (Elfin Cove)							
Males	1.061(16)	42(15)	0.98(15)	11.3(15)	439(15)	118(15)	27(15)
Prespawning (Little Port Walter)							
Males and females	1.057(15)	38(15)	.97(15)	10.7(15)	396(15)	114(15)	28(15)
Spawning (Olsen Creek)							
Males	1.059(31)	38(29)	1.01(29)	11.1(29)	403(29)	117(29)	30(29)
Females	1.058(8)	32(8)	.94(8)	10.5(8)	354(8)	114(8)	32(8)
Combined [4]							
Mean	1.059(62)	39(59)	.97(59)	11.0(59)	410(59)	116(59)	28(59)
Standard deviation	.004	4.5	.17	.9	69	22	3
Range	1.050–1.065	32–52	0.53–1.35	8.7–13	270–641	84–196	21–38

[1] $MCV = \frac{\text{Volume of red blood cells in 1,000 ml. blood}}{\text{Red blood cell count in million/mm.}^3}$

[2] $MCH = \frac{\text{Hemoglobin in grams per 1,000 ml. blood}}{\text{Red blood cell count in million/mm.}^3}$

[3] $MCHC = \frac{\text{Hemoglobin in grams percent} \times 100}{\text{Packed cell volume}}$

[4] Female pink salmon from Olsen Bay not included.

of maturity, only the mean from each stage and the grand average, range, and standard deviation for the three stages combined are given in table 1. Statistical comparisons were made by the one-way analysis of variance, or F-test (Li, 1957). No distinction is made here between early- and late-run salmon at Olsen Creek.

The specific gravities, erythrocyte counts, and hemoglobin concentrations fall within the ranges of those listed by Wintrobe (1933) for oceanic bony fishes, although the mean corpuscular volume and mean corpuscular hemoglobin were high and are comparable with the more primitive fishes.

In the comparison of my present findings on hematology of pink salmon with those of other workers, several points are of interest. In California, Robertson, Krupp, Favour, Hane, and Thomas (1961) found for chinook salmon that erythrocyte counts, hemoglobin levels, and packed-cell volumes increased during the migration and decreased during the spawning stage (to levels similar to those in animals in the open sea). My findings agree with those of Robertson and his associates in that packed-cell volumes (fig. 1) were higher in migrating males than in spawning males. The packed-cell volumes in combined male and female samples were also higher in prespawning populations than in spawning populations. Within the spawning population, the males had greater packed-cell volumes than the females. A significant increase (at the 2.5-percent level) in mean corpuscular hemoglobin concentration between the prespawning and the spawning stages was concurrent with the small decrease in packed-cell volume.

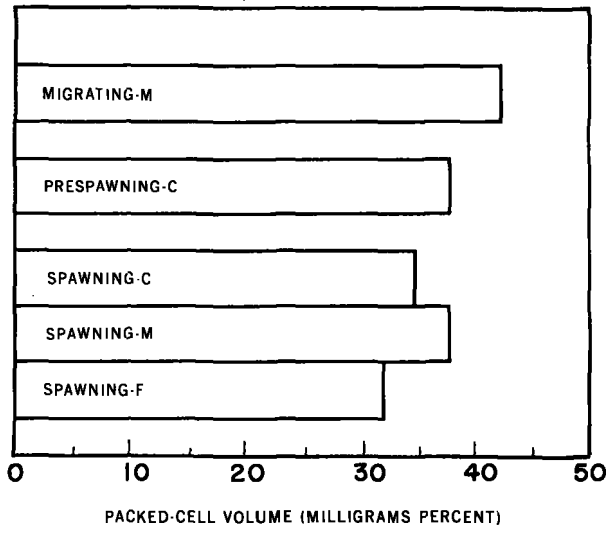

FIGURE 1.—Packed-cell volume of blood of adult pink salmon in three stages of maturity. (C, sexes combined; F, females; M, males.)

In his studies of pink salmon in Asia, Lysaya (1951) found that erythrocyte counts and hemoglobin levels fell noticeably between the time fish entered the estuary and the time they arrived on the spawning grounds. Such a trend is clearly evidenced by the decrease in packed-cell volume in my study, although it is not noticeable in the erythrocyte and hemoglobin values. The absence of a difference in hemoglobin concentrations between prespawning and spawning stages in my work, was also reported by Sinderman and Mairs (1961) for the alewife, *Alosa pseudoharengus*, a fish that returns to the sea after spawning in fresh water.

Benditt, Morrison, and Irving (1941) found that in Atlantic salmon (*Salmo salar*) affinity of hemoglobin for oxygen was greater while fish were in the spawning stage in fresh water than in the prespawning or migrating stage in salt water. This last phenomenon would compensate those changes mentioned above that would tend to decrease the oxygen-carrying efficiency of the blood. Perhaps an understanding of these points will be possible when larger numbers of fish are analyzed at all stages of migration.

BLOOD CHEMISTRY

The concentrations of several components of blood (table 2) were determined by the techniques given in Fister (1950). As with the hematology and corpuscular indices, the measured values of some of the characteristics of blood did not vary significantly among the three stages of maturity; only the mean from each stage and the mean, range, and standard deviation for the three stages combined are given in table 2.

TABLE 2.—*Average values in blood chemistry of adult pink salmon at three stages of maturity from three areas of Alaska in 1963*

[Numbers in parentheses are numbers of samples analyzed]

Stage of maturity	Albumin [1]	Globulin [1]	Glucose [2]	Cholesterol [1]	Lipid phosphorus [2]	Uric acid [2]	Urea [2]	Creatinine [2]
	Grams percent	*Grams percent*	*Milligrams percent*	*Milligrams percent*	*Milligrams percent*	*Milligrams percent*	*Milligrams percent*	*Milligrams percent*
Migrating (Elfin Cove)								
Males	1.5(11)	0.4(12)	101(16)	835(10)		2.2(16)	4.1(15)	1.0(16)
Prespawning (Little Port Walter)								
Males	1.3(8)	.7(8)				1.4(8)	5.1(15)	
Females	.7(8)	1.0(8)				2.1(8)		
Combined			68(16)	656(14)	5.8(14)			
Spawning (Olsen Creek)								
Males	1.8(26)	.6(23)					7.4(23)	1.2(16)
Early run			78(16)	494(16)	15.9(14)	.7(16)		
Late run			43(11)	580(14)	11.3(16)	1.5(16)		
Females								
Late run	.6(5)	.4(5)	41(8)	542(7)	17.5(8)	1.7(5)	6.7(6)	
Combined [3]								
Mean	1.5(53)	.7(51)	75(59)	621(54)	11.0(44)	1.8(64)	5.8(53)	1.1(32)
Standard deviation	.6	.4	30	136	3.3	.5	5.2	.5
Range	.5-3.4	.2-1.2	23-167	364-1,220	2.5-20.5	.3-2.9	.5-28	.3-2.9

[1] Plasma.

[2] Whole blood.

[3] Female pink salmon from Olsen Bay not included in combined values.

ALBUMIN AND GLOBULIN

Albumin and globulin are discussed together because both are blood proteins. Comparisons are made between males and females (table 2) in the three spawning stages (figs. 2 and 3).

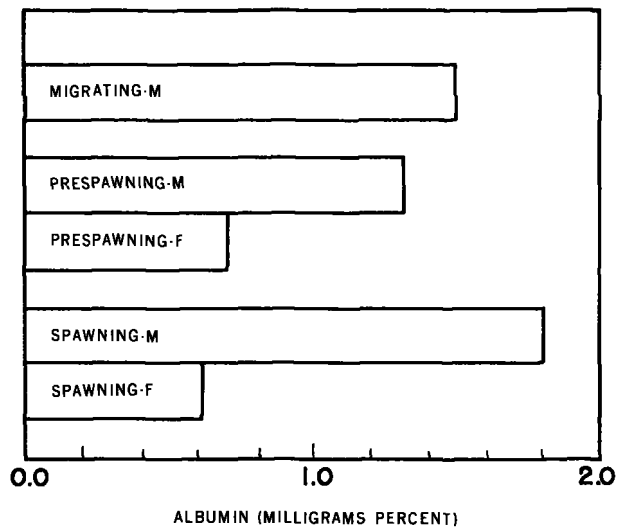

FIGURE 2.—Albumin of blood of adult pink salmon in three stages of maturity. (F, females; M, males.)

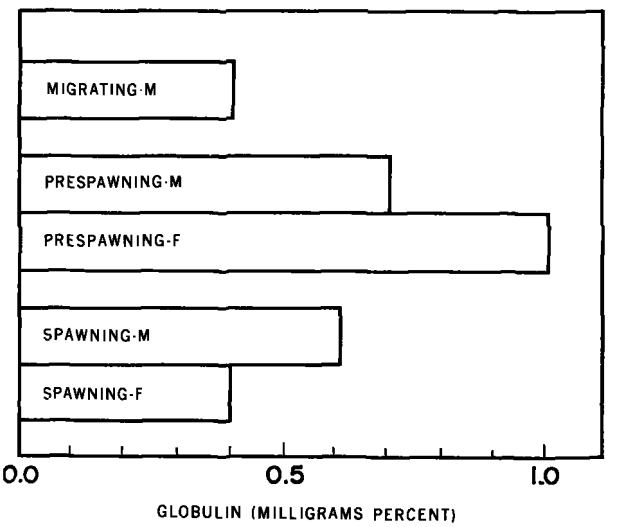

FIGURE 3.—Globulin of blood of adult pink salmon in three stages of maturity. (F, females; M, males.)

Although the range in values was large, the average concentrations of components in males showed little change from the migrating through the prespawning and spawning stages. The albumin-globulin ratio was greater than 1:1—the ratio considered normal for mammals and most fishes (Shell, 1961). The albumin values for prespawning females were about half of those for prespawning males, whereas the globulin values for prespawning females averaged higher than those for males (table 2). The albumin–globulin ratio was 0.7:1 for prespawning females. The globulin was greatly reduced in spawning females, and the albumin-globulin ratio (1.5:1) was more nearly

like the ratio for spawning males (3:1). The results of my analysis of albumin and globulin are consistent with those of Robertson, Krupp, Favour, Hane, and Thomas (1961), who found that the normal ratio of albumin to globulin of 1:2 in chinook salmon living in the sea was reversed in both sexes during migration but tended to revert to the original during spawning.

The greater reduction of albumin and globulin in females than in males by spawning time probably indicates a greater depletion of body protein in egg formation. Shell (1961), who surveyed the nutritive, osmotic, and other functions of blood proteins in fish, found a cyclic reversal of the albumin-globulin ratio in smallmouth bass, *Micropterus dolomieui,* and in his review of the literature stated that "Results of determinations of the A:G ratio in fish are confusing."

GLUCOSE

My discussion of glucose levels includes comparisons between pink salmon in the migrating and the prespawning stages and between

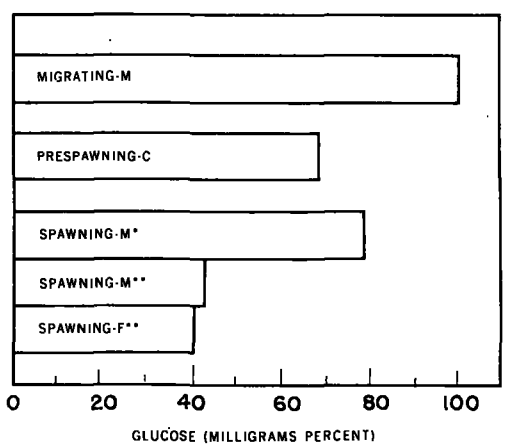

FIGURE 4.—Glucose of blood of adult pink salmon in three stages of maturity. (C, sexes combined; F, females; M, males; single asterisk, early run; double asterisk, late run.)

pink salmon in early and late runs (fig. 4 and table 2). The drop in glucose between the migrating and prespawning fish is significant at the 1-percent level, and the decrease from early to late spawners is significant at the 2.5-percent level. The different levels of glucose in spawning salmon may be attributed to the fact that the salmon of the early run at Olsen Creek were not completely ready to spawn, whereas the fish of the late run were actually spawning. The findings are in accord with those of Lysaya (1951).

It is commonly assumed that carbohydrate metabolism in fish is inefficient. Robertson and Wexler (1960), however, found an increase in the number and size of islets of Langerhans in chinook salmon during the spawning migration. Robertson, Krupp, Favour, Hane, and Thomas (1961) found an increase in blood glucose while fish were migrating from the open sea, followed by a tendency toward a decrease during spawning. They suggested that rising levels of blood glucose are due to gluconeogenesis that results from the action of increasing adrenal corticoids on muscle and fat deposits and a simultaneous increase in insulin production to utilize the product.

This viewpoint is somewhat corroborated by studies on the Fraser River in which sockeye salmon have shown an 11- to 30-percent loss of body flesh (Idler and Tsuyuki, 1958) accompanying increased production of adrenal corticosteroid hormones (Idler et al., 1959). Chang and Idler (1960) observed that liver glycogen gradually decreased during migration in fresh water but increased at spawning. These changing glycogen levels were attributed to changing hormone balances.

CHOLESTEROL

Cholesterol levels are compared among the three stages of maturity (fig 5). A downward trend in cholesterol levels from the migrating to the spawning stage was consistent, i.e. significantly lower (at the 1-percent level) in the prespawning than the migrating fish and in the spawning than the prespawning fish. Robertson, Krupp, Favour, Hane, and Thomas (1961) and Idler and Tsuyuki (1958) observed this same consistent downward trend in chinook salmon. Although I found this downward trend in cholesterol levels from the migrating to the spawning stage, levels in pink salmon within the spawning group were higher in the late run than in the early run.

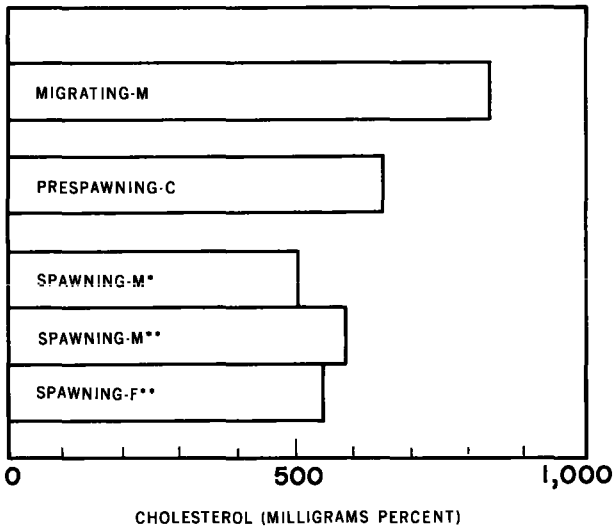

FIGURE 5.—Cholesterol of blood of adult pink salmon in three stages of maturity. (C, sexes combined; F, females; M, males; single asterisk, early run; double asterisk, late run.)

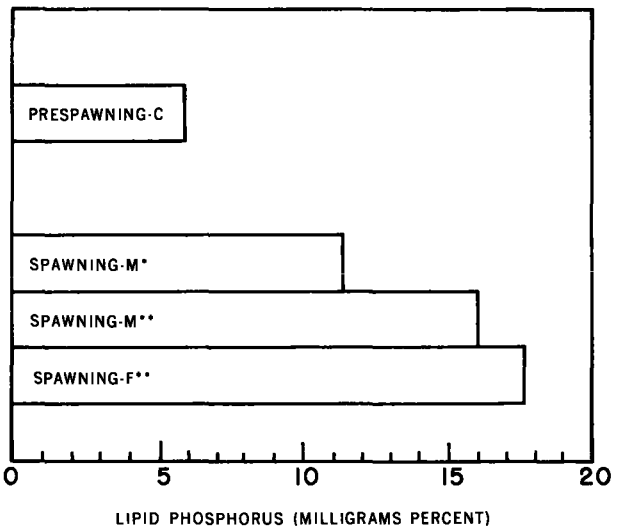

FIGURE 6.—Lipid phosphorus of blood of adult pink salmon in two stages of maturity. (C, sexes combined; F, females; M, males; single asterisk, early run; double asterisk, late run.)

The function of cholesterol in the metabolism of fishes (reviewed by Shell, 1961) remains obscure. In my study, however, cholesterol showed an inverse correlation with lipid phosphorus (significant at the 1-percent level).

LIPID PHOSPHORUS

Concentrations of lipid phosphorus in samples from the prespawning and spawning stages and the early and late spawning runs are compared (fig. 6). No data are available from the migrating group. The increase in lipid phosphorus levels from the prespawning to the spawning stage was significant at the 1-percent level. The values for males in the spawning stage in the early part of the run (fig. 6) were also significantly higher than in the late run (at the 1-percent level.) The high values for lipid phosphorus in the females sampled in the late run may be due to a terminal increase in 17-hydroxycorticosteroids in females as values at that time are dropping in males (Hane and Robertson, 1959). Although Shell (1961) found a direct correlation between lipid phosphorus and the blood proteins (albumin and globulin), no such correlation is evident in my data. Comparisons between individual animals, however, indicated a positive correlation (1-percent level of significance) with glucose. The results suggest a mechanism whereby the concentration of lipid phosphorus increases as cholesterol and glucose decrease.

URIC ACID

The values for uric acid are discussed for males and females in the three stages of maturity. The decline in uric acid concentration in the blood of males from the migrating to the prespawning stage is significant at the 1-percent level (fig. 7). The further drop in uric acid from the prespawning to the early part of the spawning stage (in the early run only) is also significant at the 1-percent level. No such drop is apparent, however, in the comparison of the males of the prespawning and the late part of the spawning stage (fig. 7). Within the prespawning stage, uric acid values were higher in the females than in the males (significant at the 5-percent level). Uric acid concentrations in females from the late spawning stage average only slightly higher than those in the males (table 2). If uric acid is accepted

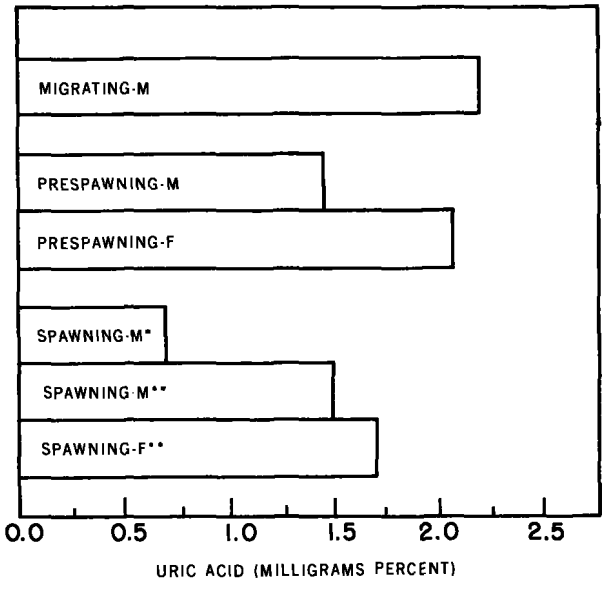

FIGURE 7.—Uric acid of blood of adult pink salmon in three stages of maturity. (F, females; M, males; single asterisk, early run; double asterisk, late run.)

as an end product of purine metabolism, a decline in purine metabolism at spawning is indicated, and females maintain a higher level longer than males.

UREA

The variations in the concentration of urea among individual specimens from an area were so great that no trend is apparent for this blood component, which is the end product of nitrogen metabolism. Lysaya (1951) noted increasing concentrations as spawning approached and attributed death of pink and chum salmon to urea poisoning.

CREATININE

No data are available on the concentrations of creatinine in blood samples from fish in the prespawning stage, and no trend is indicated by the values for the other groups. Creatinine is sometimes considered an end product of tissue catabolism, which is a dominant process in the fish sampled here. The values I found, however (table 2), are similar to those determined for smallmouth bass by Shell (1961) and for carp (*Cyprinus carpio*) and brook trout (*Salvelinus fontinalis*) by Field, Elvehjem, and Juday (1943) for fish in which catabolism was not high.

SUMMARY AND CONCLUSIONS

Blood samples were taken from adult pink salmon collected at three stages of maturation during their migration to the spawning grounds—in the ocean actively migrating, milling in the estuary of a spawning stream, and in fresh water on the spawning grounds.

Basic hematological characteristics, including specific gravity, packed-cell volume, erythrocytes, hemoglobin, corpuscular volume, corpuscular hemoglobin, and corpuscular hemoglobin concentration, were determined. Statistical analyses indicated no significant difference among groups of fish.

The concentrations of several components of blood indicate that several changes accompany migration and maturation. As pink salmon mature, utilization of protein reserves (evidenced by lowered albumin and globulin levels in females) may result from rapid building of egg tissue. Glucose levels declined, especially in females. Cholesterol concentrations also declined, although lipid phosphorus rose in both sexes; the increase was especially noticeable in females. Lipid phosphorus may play an increasingly important part in energy transfer as salmon mature.

The pink salmon from the spawning stream were from two distinct components of the run —the early and the late. The late spawners had significantly higher concentrations of cholesterol and uric acid, but lower levels of glucose and lipid phosphorus. I do not know if these differences are due to intrinsic genetic factors or are induced by extrinsic environmental factors.

I could see no trend in urea or creatinine concentrations at the three stages of maturity.

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