PACKED BLOOD CELL VOLUME, HEMOGLOBIN, AND SERUM PROTEINS OF GOLDEN SHINER, *NOTEMIGONUS CRYSOLEUCAS*, WITH REFERENCE TO INFECTION BY THE MICROSPORIDIAN PARASITE *PLEISTOPHORA OVARIAE*

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CHAPTER I

INTRODUCTION

The blood is closely related to all physiological activities within the body. It plays an important role in homeostasis as the medium of exchange for nutrients, minerals, gases and metabolites between the internal and external environment. Wells (1956, in Snieszko 1960, p. 1) stated

> . . . Since a change or lack of change in the blood picture is a fundamental characteristic of practically every physiologic or pathologic state, hematologic findings are among the most valuable and most generally useful of all laboratory diagnostic aids.

Many workers have shared this thought and expressed the importance of establishing normal hematological values in fishes to understand the effects physiological activities, diseases and the environment have on the physiological conditions (Watson et al. 1956; Weinreb 1958; Hesser 1960; Snieszko 1960; Larsen & Snieszko 1961; Summerfelt 1967).

The use of blood to evaluate the health condition of man has proved reliable. However, fish hematology in health and disease is still in a fragmented and developing state (Mulcahy 1975). Most of the work done has been concentrated on the salmon and trout (Benditt 1941; Katz 1950; Weinreb 1958; Snieszko 1961; Mulcahy 1967; Barnhart 1969; Houston et al. 1972; Conroy 1972). Most of the diseases studied have been of viral and bacterial origin (Watson et al. 1956; Clem & Sigel 1963; Hunn 1964; Klontz et al. 1965; Mulcahy 1969; Anderson & Conroy

1970; Carbery 1970; Cardwell & Smith 1971). Hematological studies of fish infected by parasites are needed due to lack of information in this area.

The golden shiner, Notemigonus crysoleucas, is an extensively cultured bait minnow in the United States. It was estimated in 1969 that 84% of the total 26,567 acres were devoted to the culture of the minnow (Summerfelt & Warner 1970a). A microsporidian parasite Pleistophora ovariae has been found to infect the ovaries of the fish (Summerfelt 1964a). Although no external disease symptom is observed, the parasite causes great economic losses by interfering with normal egg development, thus causing reduction in fecundity. The parasite was found to be widely spread in 23 out of 24 farm sources representing twelve states, and the overall incidence of infection was 46% (Summerfelt & Warner 1970a).

The present research is designed to evaluate the variations in the packed blood cell volume (PCV) or also known as hematocrit, hemoglobin concentration and percentage of serum proteins of the golden shiner, Notemigonus crysoleucas with special reference to the parasitic infection of the ovaries by the microsporidian parasite Pleistophora ovariae. Knowledge of the host response in terms of hematological changes will contribute to a better understanding of the pathological nature of the parasitic infection. No previous study on the hematological changes of the golden shiner with respect to infection by the microsporidian parasite has been found.

The techniques used in the study of blood from small fish need special skill and attention due to the small volume of blood obtainable from each fish and the relatively short coagulation time of the blood.

Snieszko (1960) has emphasized the use of microhematocrit introduced by Wintrobe (1934) as an important tool in fisheries research and management. According to Wells (1956, in Snieszko 1960, p. 1)

> ... The hematocrit reading, or the percentage of packed cells in the peripheral blood is one of the most important of all clinical constants. Because of its simplicity and high degree of reproducibility, the procedure is most useful as a routine for detection of anemia.

Strumia et al. (1954; p. 1016) stated

> ... Detection of the hematocrit reading, the erythrocyte count and the hemoglobin concentration are all used in evaluating the erythrocyte content of blood. The hematocrit determination is the most accurate of these methods inasmuch as it is not subjected to the rather large errors inherent in pipetting and diluting blood according to the other methods.

The determination of hematocrit values and hemoglobin concentrations together makes possible the estimation of the mean corpuscular hemoglobin concentration (MCHC) which is a very constant blood parameter.

The application of electrophoresis to biological and medical study has been exphasized by Luetscher (1947). Analyses of the composition of the proteins in the serum reflect the physiological and chemical changes in the body. Its application in the field of parasitic diseases has also been exphasized by Stauber (1954).

In the present study, sex, age, condition factor (C.F.), degree of gonadal development, spawning period and the effect of the parasite were considered with the intention that useful information concerning the physiology of the fish and the pathological effects of the parasitic infection could be gained. To minimize bias the various factors that may affect the physiology of the fish were categorized and considered separately.

CHAPTER II

LITERATURE REVIEW

Normal conditions for fish growth should be regarded as those under which the fish are actively feeding, growing well and when environmental stresses such as those due to oxygen deficiency, abnormal temperature, overcrowding and pollution are not presented as potential problems. Above all, an essential criterion is that no sign of disease exists. A vast amount of literature on fish hematology has been published(Booke 1964; Blaxhall 1972; Hawkins & Thomas 1972).

The use of standard methods for routine study of fish hematology has been proposed by Hesser (1960), Klontz & Smith (1968). The heart, the dorsal aorta and the caudal peduncle are the sites for bleeding. Field et al. (1943) obtained blood by puncturing the heart of carp as many as eight times produced mortality only in about 5% of the fish. The use of heparin at a concentration of 0.4 - 0.6 mg/ml blood has been considered the most satisfactory anticoagulant (Hesser 1960). Other anticoagulants used included sodium oxalate (Field et al. 1943), sodium citrate (Catton 1951) and ethylenediaminetetra-acetate (EDTA) (Mulcahy 1970). For trout blood, 4-5 mg EDTA per ml blood has been shown to be satisfactory to prevent coagulation whereas less than that amount has been shown to produce higher hematocrit values (Blaxhall 1973). For mammalian blood, 1-2 mg EDTA per ml is considered optimal. Higher amount than 2 mg/ml of blood cause osmotic shrinkage of the red blood

of the red blood cells and give falsely low hematocrit values (personal communication, Glenn B.L., 1976, Department of Veterinary Pathology, Oklahoma State University, Stillwater).

Hematocrit

Hematocrit values require a relatively simple procedure and yet are highly reproducible and consistent. A small volume of blood from 30-40 ul is sufficient to produce reliable results. Benditt, Morrison and Irwing (1941) were the first to use microhematocrit in the Atlantic salmon, Salmo solar. Salmon in brackish water were found to have a hematocrit value of 39% compared to 25% in freshwater. Species variations have been observed. The value for brook trout, Salvelinus fontinalis was 45 - 50%; brown trout, Salmo trutta was 39 - 44% and rainbow trout, Salmo gairdneri was 45 - 53% (Snieszko 1960). The mean value, however, given by Field et al. (1943) for the brook trout was 27.2% with a range of 22.2 - 35.8%. Quite a large range of variation can exist under different environmental or laboratory conditions. In twenty one pike, Esox lucius, the hematocrit values ranged from 20 - 43.5% with a mean of 32% (Mulcahy 1970). Austen et al. (1973) found very similar mean hematocrit values for wild catfish, Ictaluris punctatus, and pond cultured catfish with values of 34.2% and 34% respectively. Seasonal variations were also found with a mean of 34.2% in April, 30.9% in August and 25.0% in October. The mean hematocrit value for adult carp Cyprinus carpio was found to be 31.3% with a range of 21 - 40% (Field et al. 1943). Data on small fish species are rare. Summerfelt, Lewis and Ulrich (1967) observed a decrease in the mean hematocrit values of goldfish, Carassius auratus

between June and August. Sex difference was observed with an average of 29.4% in the female and 32.5% in the male during the same period. The fact that the males have higher hematocrit values than females have also been reported by Snieszko (1960) on trout and by Mulcahy (1970) on pike. Sano (1960) observed that the hematocrit values decreased markedly with gonad development in both sexes of rainbow trout, males being always higher than females. A change in hematocrit vlaues during the spawning period in the sockeye sazlmon, Oncorhynchus nerks has been demonstrated by Ho and Vanstone (1961). Estrogen in the form of estradiol monobenzoate was injected into the maturing fish to hasten gonadal development. Hematocrit values were significantly lower in the experimental fish compared to the control which received only the hormone suspending medium.

Hematocrit values of four 100 ul aliquot samples of blood drawn successively from bluegill sunfish declined 16% from the first to the fourth tube (Jones & Pearson, Unpublished). However, the difference between the first two tubes was not great.

A rise in hematocrit value due to insufficient concentration of EDTA was considered a source of error in fish blood examination (Blaxhall 1973). The possession of a nucleus in the red cells of fish makes it possible for some metabolism to take place outside the body of the fish. Soivio and Nyholm (1973) observed that blood samples of rainbow trout kept under hypoxic conditions have a higher hematocrit value than those kept under well oxygenated conditions. The hematocrit values of the blood samples incubated under pure oxygen reached 10% below the untreated mean value of 41.6±2.9% in two hours. During the anaerobic incubation the hematocrit values increased about 30% higher

than the mean in two hours. A rise in the blood carbon dioxide tension apparently caused the blood cells to swell. It was recommended therefore that the blood be centrifuged immediately after collection to avoid the building up of carbon dioxide tension.

Hemoglobin

The determination of hemoglobin concentration is one of the simplest yet most useful parameters in hematology. It is widely used as a routine method to detect anemia as well as other signs of disease. The consistency of the results obtained makes comparison of results of different workers possible. The method employed needs special attention since different methods produce slightly different results. The cyanmethemoglobin method was approved by a 1958 panel of the National Research Council as the method of choice for calibration of colorimetric hemoglobinometers. Wintrobe (1974) supported the idea that the cyanmethemoglobin method was gaining more universal acceptance due to the availability of a stable, accurate standard. The reagent is stable and the results are consistent (Larsen & Snieszko 1961). An error of 1.9% was noted by Wintrobe (1974) using the cyanmethemoglobin method as compared to a possible 10% error using the oxyhemoglobin or acid hematin method.

From a chemical point of view, the cyanmethemoglobin method measures the total hemoglobin content excluding sulphaemoglobin. The oxyhemoglobin method does not measure methemoglobin and carboxyhemoglobin since these compounds are not converted into oxyhemoglobin (Miale 1967). The oxyhemoglobin method is thus concerned with the amount of hemoglobin involved in respiration and has a smaller value than that obtained

by the cyanmethemoglobin method. However, in pathologic or diseased conditions that involve the formation of abnormal hemoglobin compounds. oxyhemoglobin values will be lowered. The difference between the values obtained from the two methods gives the amount of abnormal hemoglobin. The cyanmethemoglobin method requires the use of Drabkin's solution which is an alkaline solution of potassium cyanide KCN and potassium ferricyanide $K_3Fe(CN)_6$. Ferricyanide converts hemoglobin to methemoglobin which then combines with cyanide to form cyanmethemoglobin. These two reactions are rapid and stoichimetric.

Larsen and Snieszko (1961) compared four methods of hemoglobin determination in trout blood. A modified total iron method suggested by Hainline (Seligson 1958) was found more satisfactory than the original Wong Method (Wong 1928). The acid hematin method was considered unsatisfactory for fish blood due to suspension of the red cell nucleus in the solution. Results of cyanmethemoglobin and oxyhemoglobin methods were close to that of the total iron method. However, the cyanmethemoglobin method gave a slightly lower value than that of the oxyhemoglobin method. A correction for the former method was suggested for fish (Larsen & Snieszko 1961). The hemoglobin concentration in g/100 ml of blood using the respective cyanmethemoglobin and oxyhemoglobin methods for trout and catfish were : rainbow trout, Salmo gairdneri 9.6 and 10.5; brown trout, S. trutta 8.8 and 9.3; brook trout, Salvelinus fontinalis 9.8 and 10.9; catfish, Ictalurus sp.8.8 and 9.8 (Larsen & Snieszko 1961; Larsen 1964). The cyanmethemoglobin method was also considered the method of choice by Larsen (1964).

Austen (1973) observed difference in mean cyanmethemoglobin concentration between wild and pond catfish, Ictalurus punctatus with

value of 9.02 and 8.40 g/100 ml of blood respectively. A large range of variation existed in the wild fish. Seasonal variations were significant. Using the cyanmethemoglobin method, Mulcahy (1970) obtained values of 5.6 to 15 g/ 100 ml blood for the pike , Esox lucius and a mean of 8.8 g/100 ml. Differences between sexes were not significant. The mean oxyhemoglobin concentration for male and female goldfish, Carassius auratus was found to be highly significant (Summerfelt, Lewis & Ulrich 1967). The corresponding values were 7.8 and 8.9 g/100 ml of blood. The mean for males and females combined was 8.3 g/100 ml of blood.

Black (1955) studied the change of hemoglobin concentration in six species of freshwater fishes following forced exercise for 15 minutes using the acid hematin method. Only in the largemouth bass, Micropterus salmoides was a significant difference observed before and after exercise. The values were 8.1 ± 0.43 and 9.9 ± 0.42 g/100 ml of blood respectively.

The close relationship between hematocrit values and hemoglobin concentrations makes correlation between the two meaningful. Summerfelt, Lewis and Ulrich (1967) found that correlation of oxyhemoglobin and hematocrit in goldfish was highly significant. Regression equations for the two sexes were derived. With one variable known, the other variable could be predicted. Houston and DeWilde (1972) used multivariant regression equations to correlate hematocrit, hemoglobin using the acid hematin method, erythrocyte count and body weight of carp and brook trout. In the case of carp, the hematocrit values, erythrocyte counts and hemoglobin concentrations all showed significant correlation with each other ($P>0.01$), but not with body weight ($P<0.05$). In the case of the brook trout, significant correlations existed between weight and

both erythrocyte count ($P>0.01$) and hemoglobin concentration ($P>0.05$). The fact that the hematocrit values were not significantly correlated with these variables led to the speculation that still other variables such as sex, reproductive status and general health conditions might have interfered with the correlationship and thus should also be considered. Different fish stocks, diet and age were found to be the most important sources of variation for hematocrit, hemoglobin and sedimentation rate in the rainbow trout (Barnhart 1969).

Serum Proteins

The protein components in the blood serum of animals are responsible for supplying nutrient materials, hormones, ions fats and various enzymes for the body metabolic processes. A dynamic yet homeostatic balance is maintained. Some of the protein components represent the most important line of body defense against foreign proteins. The delicate chemical balance between the serum and the internal system of the body forms the basis for understanding the physiology of the body during normal and diseased conditions. Different factors can stimulate changes in the serum proteins to a varying degree. It is therefore important to know the factors that cause the changes and to identify the changes.

The study of blood proteins is made possible through the process of electrophoresis which separates molecules under the influence of an electric field. Some of the factors that influence the movement of particles in a electric field include the ionic concentration, voltage, distance from the electrode, size and shape of the molecules, solubility, degree of adsorption and temperature (Zweig & Whitaker 1967).

The complexity and variation of serum protein fractions in various animal species including fishes have been shown (Deutsch & Goodloe 1945; Deutsch & McShan 1949). The serum proteins in percentage composition and their mobilities were given. Serum patterns of individuals of the same species varied only slightly but showed obvious difference between related species. At least six protein fractions were observed in the fish studied. The alpha-globulin fractions in the lower animals were comparatively much lower than that in the mammals. Comparative studies on the serum proteins of five Indian freshwater fish species have reviewed five clearly separated components identified to be albumin, alpha-1-globulin, alpha-2-globulin, beta-globulin and gamma-globulin (Chandrasekhar 1959). Only the alpha-2-globulin fraction showed no significant difference among the species on analysis of variance. Interspecies variations were observed. The total serum proteins in the fish were found to be significantly lower than that of the mammals. Wide variability in the serum electrophoretic patterns within the sea herring, Clupea harengus was reported by Mairs and Sindermann (1960). The degree of variability was well expressed by Thurston (1967, p.2183) who recorded " No two patterns of different serum samples could be considered identical and few were sufficiently alike that some kind or degree of difference could not be readily perceived."

Irisawa and Irisawa (1954) compared the serum protein patterns of the skate, Raja kenojei and the shark, Heterodontus japonicus with that of humans and suggested that the fastest component of elasmobranchii serum differed not only in mobility but also in chemical nature from that of man and other higher animals.

Summerfelt (1966) observed six major protein fractions in the

golden shiner, Notemigonus crysoleucas. The relative mobilities and percentage composition of the serum proteins and lipoprotein fractions were observed. Protein fraction 1 and 2 were identified to be albumin. Lipoprotein fraction 4 was observed to be related to sexual maturity and reached its peak value during the spawning period (Summerfelt 1964b).

Sex difference and maturity affect the electrophoretic patterns. Although the mobilities of the protein components were very similar, the percentage composition of the albumin fractions were quite different in the golden shiner, with 38.4% in the female and 31.2% in the male (Summerfelt 1966). The protein content in the blood serum of trout was observed to increase with age until the adult stage was reached (Koroleva 1963). In the pre-spawning phase of lamprey, Petromyzon marinus, the total protein concentration as represented by the total area under the curve was 18 cm^2 for the male and only 8 cm^2 for the female (Thomas & McCrimmon 1964). Percentage-wise, protein fraction 2 identified to be albumin represented 52% of the total in the immature males and only 26% in immature females. In the mature females, fraction 2 only averaged about 7% of the total. In the rainbow trout, Thurston (1967) found significant differences in fractions 6 and 9 which were related to maturity but not to sex. However, the mean percentages of total protein under fraction 2 were higher in males than in females both at the immature and mature stages.

Electrophoretic patterns at different stages during the life cycle of coho salmon showed no sex differences were apparent prior to gonadal development (Vanstone & Ho 1961). With the onset of maturity in the female, an additional sixth protein fraction of lipoprotein in nature

was observed but disappeared at about the spawning period. The amount of serum protein diminished during the spawning migration in the Pacific salmon, Oncorhynchus tshawytscha (Robertson et al. 1961). The albumin : globulin ratio was 1:2 in the sea but was reversed during the migration and was about 1:1 during spawning.

Hormone was shown to affect the blood proteins in the sockeye salmon (Ho & Vanstone 1961). The blood proteins of male salmon after injection with estrogen approached that of sexually maturing females whereas in maturing females the condition resembled a more advanced stage of egg production. The total protein content in g/100 ml serum was higher after the hormone injection. Steroid hormones in general have a marked influence on many metabolic processes and one would expect changes in plasma proteins, as well as other substances, to occur at times when steroid hormone levels are changing drastically (personal communication, Glenn, B.L., 1976, Department of Veterinary Pathology, Oklahoma State University, Stillwater).

Certain environmental factors have been shown to affect the serum proteins of fish. Meisner and Hickman Jr. (1962) found that water temperature affects the amount of proteins in the serum of rainbow trout. The albumin: globulin ratio was higher at $8^{o}C$ than at $16^{o}C$ due to an increase in the amount of albumin and a decrease in the beta and alpha-1 globulin at the lower temperature. In the mackerel and carp, the total protein contents were lower in winter than in summer (Saito 1957). However, Koroleva (1963) using the rainbow trout, showed that the rate of feeding and the nature of the food affects the protein content of the blood significantly. He accounted for the low serum protein level in winter as due to inadequate feeding. The oxygen content of the

water was also shown to affect the serum protein fractions of bluegills and largemouth bass but did not change that of the yellow bullhead (Bouck & Ball 1965). Thurston (1967) did not observe significant change of protein fractions in the rainbow trout with dissolved oxygen as low as 1.3 ppm.

Hematology in Fish Diseases

Marsh (1906) studied the hemoglobin and cell count in healthy and diseased trout. Hemoglobin was measured by the Dare hemoglobinometer. A significant drop in hemglobin was recorded in cases of true neoplasms of a malignant nature located in the region of the gills. The same study reported that the most destructive epidemic of protozoan and bacterial infection in trout was not attended by any marked anemia. Furunculosis in rainbow trout due to infection by the bacterium Aeromonas salmonicida caused necrosis and abnormal fluid production in the kidney and spleen, the primary and secondary sites of hematopoiesis (Klontz, Yasutake & Ross 1966). Clinical pathologic changes were observed. After 40 hours postinoculation, there was a noticeable diminution of lymphocytes, neutrophilic granulocytes, macrophages and fibroblast at the site of injection. Degenerative changes also occurred in the anterior kidney and spleen. After 72 hours postinoculation, the kidney, spleen, and site of injection were devoid of granulocytes and macrophages and marked liquefaction necrosis observed. The micro-hematocrit values, hemoglobin concentrations (cyanmethemoglobin) and erythrocyte count did not vary significantly from those of the control. Young (1949), observed a decrease in hematocrit value in the opaleye, Girella negricans, infected with a tail rot disease. Loss of appetite

and general degradation of physical condition were also noted as causes for the decrease. In one study of an unidentified gill disease in the Atlantic salmon, Salmo solar, the hematocrit values increased tremendously to 62.2 ± 10.75% compared to the control of 37.2 ± 2.17% (Johansson 1968).

Study on the effect of vibrio disease on four species of marine fish showed that hemoglobin concentration (determined by acid hematin method), hematocrit values and erythrocyte count all dropped below the normal value (Anderson & Conroy 1970). Considered together with the pathology and aetiology of the condition, these three hematological parameters enhance the possibility of diagnosis and control of the vibrio disease which occurs as epizootics in wild and captive populations of marine fish and migratory species such as eels and salmonids. Cardwell and Smith (1971) studied the hematological manifestations of vibriosis upon juvenile chinook salmon. Student's t-tests of differences between hematocrit values, hemoglobin concentration (cyanmethemoglobin), erythrocyte count, mean corpuscular volume and mean corpuscular hemoglobin of healthy control and diseased chinook salmon were significantly different. The decrease in hematocrit value, hemoglobin concentration and erythrocyte count suggested that the bacterial disease was either deteriorative to the erythropoietic system or was causing accelerated destruction of red blood cells.

The hematology of the sockeye salmon, Onchorhynchus nerka infected with a viral disease was studied by Watson, Guenther and Royce (1956). Microscopic examination of blood smear showed that the abnormality in blood cells was reflected by abnormally low hematocrit values. Four days after experimental infection with the virus, the hematocrit value

rose to an average of 53% as compared to 47% for the control. The increase was speculated to be attributed to a shock incurred from the infection that resulted in a loss of plasma and thus raised the hematocrit values. Lowest hematocrit values occurred 8 to 9 days after infection. However, the highest rate of mortality occurred on the 6th day, within the period of rapid change of hematocrit values.

Mulcahy (1975) documented the blood changes associated with lymphoma in the northern pike and Ulcerative Dermal Necrosis (UDN) in Atlantic salmon. Spontaneous lymphoma is a malignant neoplasm appearing initially as soft tumors in the jaws or externally on the trunk or tail, and invades the viscera at the later stage. It is thought to be caused by a virus. The total blood picture was affected. The mean hematocrit value dropped from 32% in the healthy to 24% in the diseased pike. Cyanmethemoglobin concentration was lowered from 8.8 to 6.4 g/100 ml of blood. The red cell count dropped from 1,893,000 to 1,251,000 cell/mm^3. UDN is caused by an unknown filtrable agent. Ulceration of the epidermal layer of the skin is very often superinfected with fungal infection. Hematocrit values and hemoglobin concentrations for the infected fish were not given. However, changes in the blood parameters were observed during different stages in the life history of the fish. High red cell count, hematocrit and hemoglobin values were typical of adults in salt water. These values decreased when the adults returned to freshwater to spawn.

Disease due to dietary insufficiency of certain essential vitamins are common in fish. Folic acid was shown to be essential to prevent anemia in the rainbow trout (McLaren et al. 1947; Phillips Jr. 1963). Apart from a significant reduction in erythrocyte count, hematocrit

value and hemoglobin concentration, Smith (1968) observed poikilocytic erythrocytes, erythrocytes with segmented nuclei and other abnormalities in the peripheral blood.

Deutsch and McShan (1949) has indicated that serum protein concentration in different animals fluctuated in diseased conditions. Stauber (1954) suggested that the application of electrophoretic techniques in the study of parasitic diseases has definite advantages over other non-specific tests in identifying certain abnormal protein fractions, especially in certain parasitic infections in which only low antibody titer was present and that the production of sensitive antigens for testing was difficult. Most of the studies in fish are concerned with some of the most common and acute bacterial and viral diseases. Phillips, Jr. (1958) observed a lowering of albumin and an increase in globulin fraction in brown trout with an abundance of diplobacillus organisms associated with a kidney disease. Obvious changes in the electrophorogram between the normal brook trout and those with a kidney disease due to corynebacteria infection were observed (Hunn 1964). Significant differences were observed between the hematocrit values and the total serum protein concentration. The albumin fractions was most drastically affected. Serum protein changes in the Atlantic salmon with UDN have been studied by Mulcahy (1967; 1969; 1975). The serum proteins of UDN infected salmon were observed to show a characteristic trend of change as the disease progressed. Again, a significant drop of total serum protein was observed. A specific protein pattern associated with the earlier stages of the disease was recognised and thus contributed to the diagnosis of the disease outbreak.

CHAPTER III

MATERIALS AND METHODS

Infected stock of golden shiner, Notemigonus crysoleucas, were obtained on June 28, 1975 from the Oklahoma Cooperative Fishery Unit outdoor pond in Stillwater. These fish were offspring of females heavily infected with Pleistophora ovariae. Microscopic examination of the wet mount of a portion of the ovary to detect the presence of spores in the oocytes of the females was used to determine whether or not the fish were infected with Pleistophora ovariae. Some of the females from this source were not infected. Golden shiners were designated as Stillwater females(uninfected),Stillwater females(infected) and Stillwater males. Total length and weight measurements showed that the fish belonged to the one and two age groups. The mean weight, mean total length as well as mean ovary weight at different ages for the healthy and parasitized golden shiner have been reported by Summerflet and Warner (1970a). The fish were placed in indoor tanks in the laboratory. A photoperiod of 12 - 14 hours per day was maintained. The temperature of the water was controlled at 20 - 21 ^{o}C throughout the experimental period. The water was circulated continuously by a jet of fresh dechlorinated water. Commercial powder feed was given each day. Excess feed and feces were removed before new feed was given.

Golden shiner from an uninfected source in Fortyce, Arkansas were kept in separate tanks in the laboratory. An outbreak of bacterial

disease occurred in this group of fish. However, most of the fish recovered after treatment successively with brine, 2ppm potassium permanganate solution and antibiotic. They were treated under the same conditions as the Stillwater fish mentioned above.

Fish sampled between June and August of 1975 were used in the study. Fish were not fed one day before sampling and were captured by net randomly and transferred to a glass aquarium. The water was kept well aerated to avoid stress due to lack of oxygen. Excess water on the fish was dried by paper towel to minimise the possibility of mixing with the blood before blood samples for PCV and hemoglobin concentrations were collected by severing the ventral aorta of the fish with a commercial heparinized capillary tube of 1.2 - 1.4 mm inner diameter. The first of 20 ul of blood was collected in a pre-marked capillary tube for hemoglobin determination using the cyanmethemoglobin method. The 20ul of blood was immediately transferred to 4 ml of Drabkin's reagent and then shaken with a test tube shaker as recommended by Blaxhall and Daisley (1973). Vigorous shaking is important to reduce to a minimum any gel-like clot that may form to interfere with the results. Transmittance was read on a Spectronic 20 at a wavelength of 540 nm after 10 minutes to allow full conversion of hemoglobin to cyanmethemoglobin. The reading was converted into concentration in g/100 ml of fish blood by using a standard curve prepared with a Hycel cyanmethemoglobin certified standard.

Approximately 40-50 ul of blood was collected in the second heparinized capillary tube, sealed with "Critoseal" and centrifuged immediately for 6 minutes in a micro-capillary centrifuge (IEC model MB).

Centrifugation for 5 minutes at 10,500 revolutions per minute was recommended by Blaxhall and Daisley (1973) for fish blood. The volume of packed cells was read with an IEC micro-capillary reader.

After the PCV was read, the protein of the capillary tube containing the serum was sealed and stored in a freezer at -18°C until subjected to electrophoresis. The cellulose acetate electrophoresis method was used. The Gelman Deluxe electrophoresis Chamber with full length platinum electodes was employed. A Ortec model 4100 Pulsed Constant Power Supply providing polarization potential was attached to the electrophoresis apparatus. The constant power supply which delivers unipolar pulses regulated to constant average power, provides precise control of undesired heating effects in the electrophoresis cells and also improves the resolution of the system (Ortec Incorporated 1968). High resolution Tris-barbital buffer with a pH of 8.8 and ionic strength of 0.05 was used. New buffer solution was prepared after 4-6 runs or after one week as recommended by the maufacturer (Gelman Instrument Co. 1970). Cellulose acetate strips, Gelman Sepraphore III, 1 "X6", were soaked in the buffer for five minutes and blotted dry by absorbent paper. Approximately 1 ul of serum was applied onto the pre-moist cellulose acetate strip using a serum applicator(Gelman). Six samples were used during each run. The sample were placed on the cathode side of the chamber and were run for 35-40 minutes at 25-26 milliampere. The chamber was cooled by running water. The strips were stained for 10 minutes without agitation in Ponceau S stain with a concentration of 500mg of stain in 100 ml 5% trichloroacetic acid (Gelman Instrument Co. 1970). Decloration of background stain was achieved by agitating the strips successively in three baths of 5% acetic acid. The strips were then

allowed to air-dry for 5 minutes and then allowed to dry slowly between blotting papers to obtain a smooth surface. The strips were cleared in 13% acetic acid in methanol for 60 seconds, stripped off of excess clearing solution on a glass plate and air dried. Scanning was done on a Beckman Analytol with microzone scanning attachment model R-102. Percentage protein concentration represented as area under the curve was measured by integrator. Relative mobility of each protein fraction was measured relative to the fastest moving fraction or the one closest to the anode.

For each sample made, the date, weight, total length and sex of the fish were recorded. The condition factor (C.F.) for each fish was determined (Bennett 1970). The degree of gonadal development was arbitrarily categorized into poorly developed, well developed and very well developed stages.

Statistical comparisons of sample means among two different groups with unpaired observations and equal variance were made by using Student's t-test (Steel & Torrie 1960). The null hypothesis is that there is no difference between the population means for the two groups compared; the alternative hypothesis being there is a difference between the two population means. The sample t value was computed and compared with the tabulated t value. The significance level was chosen to be 0.05. When the claculated t value was larger than the tabulated t value at this level, the null hypothesis was rejected. When the calculated t value was smaller than the tabulated t value, there was not sufficient evidence to reject the null hypothesis.

CHAPTER IV

RESULTS

Differences Between Normal and Pleistophora ovariae Infected Fish

To determine the differences in the blood characteristics between the normal and the infected fish, 11 uninfected and 17 infected females of comparable sizes from the Stillwater stock were examined. The mean total length and weight of the infected fish were larger than that of the uninfected. The corresponding values were 111.1 mm and 12.9 g in the infected fish compared to 108.1 mm and 11.9 g in the uninfected (Table I). The ranges of variation was greater in the infected fish.

The difference in the PCV between the normal (30.5%) and infected fish (27.1%) was significant at the P=0.05 level. The difference in hemoglobin concentration between the two groups was not significant. The mean hemoglobin concentration for the uninfected females was 8.03 g/100 ml of blood whereas in the infected females it was 7.95 g/100 ml of blood.

The distribution of PCV and hemoglobin concentrations in the two groups are shown in Figure 1. The PCV and hemoglobin concentrations were obviously lower in the Pleistophora ovariae infected golden shiner below PCV of 29%. Above this value, this generalization was not necessarily true. Since the regression equations for the two groups of fish were subjected to changes by a few abnormally low or high values, their

face values could not be taken too rigidly. The present regression lines for the two groups of fish served to show the tendency of change of these two important hematological characteristics with respect tot normal and parasitized conditions.

An interesting observation related to Mean Corpuscular Hemoglobin Concentration (MCHC) was that the parasitized females in general had a higher MCHC value than the uninfected fish although Student's t-test showed that the difference was not significant (Table I).

Six major protein fractions were observed. Normal and Pleistophora ovariae infected fish sampled before July 16 were compared. Typical electrophoresis densitometer profile for the uninfected fish is shown in Figure 2. Individual variations were observed. A characteristic reduction in the relative mobility of all the protein fractions was observed in the infected fish (Table II). The percent concentrations of protein fraction 1 in the two groups were similar (Table III). The mean value for protein fractions 1 was 34.48% in the normal uninfected fish and 34.77% in the infected fish. Student's t-test showed that the difference was not significant. A rather large decrease in percent total protein was observed in fraction 4 of the infected fish as shown by the average values and the ranges. A decrease was also observed in fraction 5 of the infected fish. Fraction 6 of the infected fish, on the other hand, was slightly increased. The ratio of albumin to globulin fraction was 0.56 for the uninfected females and 0.54 for the infected females. Additional fractions assigned as fraction 2' and 3' were observed in some fish in the two groups. Fraction 2' was likely to be a small fraction of fraction 1. The presence of these two additional fractions was not considered to be specific to the infection.

Changes Attributed to Maturity

The degree of maturity of the golden shiner was categorized arbitrarily by observation of the gross morphology of the gonads. Upon dissection of the fish, the gonads were categorized as : 1, poorly developed; 2, well developed; 3, very well developed. It was observed that during the experimental period, a gradual change of the degree of gonadal development occurred. An increasing number of Stillwater fish with stage 2 and 3 gonadal development was recorded towards mid July. The Arkansas fish which were kept in the indoor tanks for a relatively longer period of time became progressively more mature towards the later part of July and in August (Table IV). Spawning took place in the middle to the later part of July in the Stillwater fish. A small number of larval fish were later observed. The PCV and hemoglobin concengrations changed during the spawning period and the periods preceeding it. In the Stillwater fish, the mean PCV and hemoglobin concentration reached the lowest point of 22% and 7.1 g/100 ml blood in mid July (Figure 3). Data for this group represented a composite sample of both the normal uninfected fish and the Pleistophora ovariae infected fish.

An unidentified eye disease was found in some of the Arkansas fish towards the latter part of July and in August. Most of the affected fish were males. As a result, data for the male fish from Arkansas were not used for analysis. The females in this group reached full gonadal development relatively later. The corresponding low values in PCV and hemoglobin concentration occurred towards the middle to the latter part of August following a long and gradual period of decline (Figure 4).

The PCV and hemoglobin concentrations were also grouped under three time periods from late June to July 6, July 7 to July 24, and July 25 to August. In the Stillwater fish, the lowest mean values were observed in the July 7 to July 24 period (Table V). Student's t-test revealed significant differences in the PCV between the uninfected females of this time period with that of the June to July 6 period, and between the uninfected females of the June to July 6 period and the Pleistophora ovariae infected females of the July 7 to July 24 period. Significant differences in the PCV were also found between the Stillwater male golden shiner in the July 7 to July 24 period and that of the other two periods. for the Arkansas female golden shiner, low mean PCV and hemoglobin concentrations were found in the July 25 to August period. However, a t-test did not show significant differences in the PCV.

The PCV and hemoglobin concentrations were also considered with respect to the relative stages of gonadal development. The two blood parameters showed that there was no difference between fish with poorly-developed gonads and those with well to very well developed gonads (Table VI). This observation was considered important. The changes in PCV and hemoglobin concentrations during the spawning period cannot be explained purely by a change in the degree of gonadal development.

Relationship between Age and Blood Parameters

To study the relationship between the age, PCV and hemoglobin concentrations of the golden shiner, the fish were aged according to sizes. Fish with total length of 94 mm and below were grouped as age

1, whereas fish of 95 mm and above were grouped as age 2 large fish. The average weight of the two age groups were about 6 g for the small fish and 12 g for the large fish. Of all the Pleistophora ovariae infected fish, 17 belonged to the age 2 group and only 4 to the age 1 group. The PCV between the two groups of fish were very similar (Table VII). The hemoglobin concentration of the smaller fish was slightly lower than that of the larger fish. In the case of the Stillwater male golden shiner, the age 1 group had slightly higher PCV and hemoglobin concentration than the age 2 group (Table VII). The average PCV was 29.8% for the age 1 fish compared to 28.9% for the age 2 group. The mean hemoglobin concentration was 8.07 g/100 ml blood for the age 1 fish and 8.02 g/100 ml blood for the age 2 fish. Student's t-tests showed that the differences were not significant. A similar trend of difference was observed in the Arkansas female golden shiner. Again, the younger fish have higher PCV and hemoglobin values. The differences between these two groups were greater than those of the other fish groups mentioned. The average PCV was 29.0% for the younger fish compared to 26.2% for the older fish. The corresponding hemoglobin concentrations were 8.50 and 7.54 g/100 ml blood. However, Student's t-test showed that the differences in PCV and hemoglobin concentrations with respect to age in these two groups of fish were not significant.

Relationship Between Condition Factor and Blood Parameters

A further consideration was made relating the condition factor (C.F.) of the golden shiner and the blood parameters. The C.F. is independent of the age. Instead, both the weight and total length are

involved. Individual fish in each group were arranged in order of decreasing C.F. No clear-cut correlation could be observed between the C.F. and the two blood parameters considered. Fish having large C.F. values could have relatively low PCV and vice versa. However, the possibility that C.F. might have an effect on the blood parameters could not be ruled out. A higher than average C.F. in each group was accompanied by a higher PCV (Table VIII). The average hemoglobin concentration remained very much the same between groups. An exception was observed in the Pleistophora ovariae infected females. This group of fish was characterized by a relatively high average C.F., but a relatively low PCV and hemoglobin value. The MCHC for each group of fish was also given (Table VIII). However, the reason for their differences was not understood. PCV was a more sensitive blood parameter than the hemoglobin concentration in measuring individual variations in the fish studied.

Differences Between The Sexes

Considerations were given to the PCV and hemoglobin concentrations between the sexes of the Stillwater golden shiners. The overall data collected during the period from June to August excluding the consideration of age, degree of gonadal development and specific time period showed that the averages of the two blood parameters were slightly lower in the males than in the female (Table VIII). Lower average PCV were also observed in the males during the two time periods from June to July 6 and from July 7 to July 24. For the mean hemoglobinconcentrations, instead of the expected lower value for the male, the average value was 8.43 g/100 ml of blood in the males and 8.14 g/100 ml of blood in the

females (Table V). However, Student's t-tests showed that the differences in each case considered more not significant. An analysis was also made of the PCV and hemoglobin concentrations in the two sexes with equivalent stages of gonadal development. Fish with well developed gonads were considered. This helped to avoid bias in analysis which might be due to other factors. The average PCV was 29.4% in the males and 30.5% in the females. The average hemoglobin concentration was 8.02 g/100 ml blood in the males and 8.12 g/100 ml blood in the females (Table VI). Student's t-tests showed that the differences in each case were not significant.

The electrophoresis serum proteins in 8 uninfected females and 13 males from Stillwater were analysized. All the fish had "well" to "very well" developed gonads. The relative mobility of fraction 5 and 6 in the two groups were similar (Table IX). Progressively larger differences were observed between corresponding protein fractions towards the anode. Since the serum samples were applied towards the cathode, the fast moving protein fractions were towards the anode. Protein fraction 1 to 4 were slightly were sluggish in the males than in the females. The average relative mobility of protein fraction 2 was 82.8 in the males and 84.3 in the females, relative to 100 for fraction 1 (Table IX). The percent concentration of protein fraction 4 was quite different between the two sexes, with a value of 13% in the males and 18.2% in the females. The average percent protein concentration of protein fraction 1 was higher in the males than in the females. A high average of 40.14% was observed in the males and 34.48% in the females (Table X). Student's t-test showed that the difference were significant at the 0.05 level. The average albumin to globulin ratio was 0.67 in

the males and 0.56 in the females in this study. The high percent protein concentration might have been one of the major reasons accounting for the slower relative mobility. Changes in the serum profile due to changes in the protein concentration of individual fractions have been proposed by Thurston (1967).

CHAPTER V

DISCUSSION

The present study evaluated variations in the PCV, hemoglobin concentration, and in certain cases electrophoresis serum protein patterns, in relation to (I) infection by the microsporidian parasite Pleistophora ovariae, (II) maturity, (III) age, (IV) condition and (V) sex. The extent to which the blood of the fish was being affected by these factors was considered. Normal values of blood parameters obtained under a defined condition helped to evaluate such effects. Normal value may be defined as that mean value or range of values that is commonly shared by the majority of physically healthy fish under a defined set of conditions that offer the least stree to the fish. Klontz and Smith (1968), Wedmeyer and Chatterton (1971) indicated that there was a rather large range of variations of blood parameters within which the fish could still be considered normal. Comparison of data subjected to influence by different physiological factors thus becomes meaningful and helps to evaluate the response of the fish to such factors.

The average PCV of Golden shiners infected by the parasite Pleistophora ovariae was 27.1% as compared to 30.5% in normal female. The difference was significant at the 0.05 level. The average hemoglobin concentration was lower than that of the normal control but the difference between the means was not significant.The hemoglobin value

is a less sensitive parameter than the PCV to detect changes since its numerical value is approximately 4 times smaller than that of the PCV in the case of fish. In the present study, the average hemoglobin concentration and PCV for the uninfected Stillwater female golden shiner was 8.03 g/100ml and 30.5% respectively (Table I). Data indicated that there was a tendency of decrease in PCV and hemoglobin concentration with respect to the parasitic infection. Regression lines for the uninfected and infected fish also indicated that the infected fish generally had a low PCV and hemoglobin concentration. Summerfelt (1967) established two regression equations for the male and female goldfish and indicated the usefulness of the equations in predicting hemoglobin values.

Serum protein fractions separated by electrophoresis on cellulose acetate strips showed that the percent relative mobilities of all the six fractions of the infected fish were slower than those of uninfected fish. Percent concentrations of protein fraction 1 were very similar and no significant difference was found between the uninfected and infected fish. Thurston (1967) considered that changes in the serum profile might be attributed to an increase or a decrease in the concentration of the proteins under consideration and also to some changes in the structure of the proteins. Redmond (1948) showed that the electrophoretic mobilities of red blood cells in pigeon containing malaria parasite, Plasmodium relictum, was slower in the infected birds than the uninfected birds. The reduction in the surface charge of the red cell was thought to alter the permeability of the red cell membrane and indirectly affecting the respiration and metabolism of the parasite. Brown (1933) documented that the reduction in the cell charge

was related to the serum of the infected birds. Mukkur and Bradley (1969) observed reduction in PCV, total serum proteins, albumin, alpha-1, beta and gamma globulins in chicken infected with sporozoan parasite Eimeria tenella. The extent of reduction was found to be dependent on the number of oocysts used for inoculation and the amount of hemorrhagic lesions produced. In the golden shiners, infection by Pleistophora ovariae was not accompanied by any hemorrhagic lesions.

Body weight and total length of infected females were usually larger than those of uninfected fish. Similar observations were made by Summerfelt and Warner (1970a). Abnormal production of sex hormones due to the infection was suggested by the same authors (1970b) as a possible explanation for the size difference. The ovary weight of the golden shiner infected with Pleistophora ovariae was found to be approximately 50% less than the uninfected fish (Summerfelt & Warner 1970a).

In the present study, the degree of gonadal development was arbitrarily classified. No significant difference in PCV or hemoglobin concentration was found between fish with poorly developed gonads and well developed gonads. Changes in PCV and hemoglobin concentrations cannot be explained purely on the basis of gross morphological differences in the gonads. The fact that significantly low PCV and decrease in hemoglobin concentrations coincided with the spawning period suggested that drastic changes in blood values took place during spawning might have been associated with certain specific physiological changes. It is important, therefore, to note the exact time of spawning and to sample the fish during this period and immediately before and after it. Summerfelt and Warner (1970a) reported that golden shiner spawn from April through August, depending on latitude. Warner (1972)

observed spawning in late April and May in Payne County, Oklahoma. Austen et al. (1973) attributed lowering of PCV in August and October to seasonal variation. Low PCV associated with well developed gonads was reported by Sano (1960) and Ho and Vanstone (1961).

The present study shows that younger golden shiners have slightly higher PCV and hemoglobin concentration than the older fish except for the Pleistophora ovariae infected females. The difference in each case was not significant. The observation was with fish of age 1 and age 2 only. Blood values in older golden shiner have not been observed. The C.F. in normal uninfected fish was not found to affect the PCV and hemoglobin concentration. No definite relationship was observed between the condition of individual fish and the blood picture. Golden shiner infected with Pleistophora ovariae were characterized by having relatively high C.F. and low PCV and hemoglobin concentrations.

The PCV and hemoglobin concentration were not significantly different between the sexes. Averages values showed that the PCV and hemoglobin concentration of the male were slightly lower than that of the female. The comparison was based on comparable conditons of gonadal development, age and time. Only in one period, June to July 6, was the average hemoglobin value of the male higher than that of the female. However, the PCV and hemoglobin concentration were observed to have wider ranges of values in the males. Such observation has also been reported for the pike, Esox lucius (Mulcahy 1970). No significant effect on blood characteristics due to sex was observed in the rainbow trout by Barnhart (1969). He attributed the result to the immaturity of the fish. Other workers including Sano (1960), Snieszko (1960), Summerfelt (1967) and Mulcahy (1970) have reported higher blood values

in male fish.

Electrophoretic study of serum proteins showed that the percent of total protein in fraction 1 was significantly higher in males (40.14%) than in the females (34.48%). The values agreed with those of Summerfelt (1966) who reported 38.4% in the males and 31.2% in the females. That males have higher percent albumin fractions has also been observed by Thomas (1964) and Thurston (1967).

It is believed that the present study provides an initial basis for understanding some of the various factors that may affect the blood picture of the golden shiner. Considerable individual variation has been observed. Since the blood picture is affected by many factors, it is valuable to consider the various factors separately. In order to obtain a more representative set of data, a larger sample size would be necessary. It would be helpful if more samples could be obtained over a short period of time to eliminate changes that arise as functions of time. It is also felt that the length of time of laboratory holding should be kept to a minimum, in order to avoid drastic deviations from the natural conditions.

CHAPTER VI

SUMMARY

Results are reported on some factors that may affect the PCV, hemoglobin concentration and serum proteins of the golden shiner, Notemigonus crysoleucas. The factors considered are infection of the ovaries of golden shiner by the microsporidian parasite Pleistophora ovariae, maturity, age, condition and sex.

Lower PCV was observed in golden shiner infected with the parasite. The difference was significant at the $P = 0.05$ level. The difference in hemoglobin concentration between infected and uninfected fish was not significant. The relative mobilities of the serum protein fractions were slower for all fractions in the infected fish. Difference in percent total protein of the albumin fraction was not significant between the infected and uninfected fish. Low blood values coincided with the spawning period. Such changes of blood values could not be detected purely by gross morphological differences in the gonadal development. Age 1 golden shiner had slightly higher blood values than the age 2 group. Condition factors and blood values were not found to correlate with each other. No significant differences in PCV and hemoglobin concentration were observed between the sexes. However, percent total protein of protein fraction 1 (albumin) was significantly higher in the males than in the females.

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APPENDIX

TABLE I

MEAN VALUES AND RANGES OF TOTAL LENGTH, WEIGHT, PCV, HEMOGLOBIN CONCENTRATION AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC) OF THE NORMAL UNINFECTED AND *PLEISTOPHORA OVARIAE* INFECTED FEMALES FROM STILLWATER. NUMBER OF FISH IN PARENTHESIS

	Total Length (mm)	Weight (g)	PCV (%)	Hemoglobin (g/100ml)	MCHC (%)
Uninfected Females (11)					
Mean	108.1	11.9	30.5	8.03	27.10
Range	95 - 126	7.2 - 19.3	25 - 40	7.23 - 9.20	21.62 - 34.48
Infected Females (17)					
Mean	111.1	12.9	27.1	7.95	29.00
Range	96 - 139	8.0 - 25.5	22 - 35	6.38 - 9.75	25.44 - 32.82

TABLE II

MEAN VALUES AND RANGES OF RELATIVE MOBILITY OF SERUM PROTEIN FRACTIONS IN THE UNINFECTED AND *PLEISTOPHORA OVARIAE* INFECTED FEMALE GOLDEN SHINER FROM STILLWATER. NUMBER OF FISH IN PARENTHESES

	Fraction Number					
	1	2	3	4	5	6
Uninfected Females (8)						
Mean	100	84.3	76.2	70.9	65.4	58.6
Range		81.8-86.7	71.6-79.3	65.1-77.1	60.3-72.5	51.1-66.5
Infected Females (14)						
Mean	100	81.0	73.7	68.5	63.4	56.3
Range		74.5-83.7	67.7-77.8	59.2-73.9	55.6-70.1	47.1-64.6

TABLE III

MEAN VALUES AND RANGES OF PERCENT TOTAL PROTEIN OF SERUM PROTEIN FRACTIONS IN THE UNINFECTED AND PLEISTOPHORA OVARIAE INFECTED FEMALE GOLDEN SHINER FROM STILLWATER. NUMBER OF FISH IN PARENTHESES

	Fraction Number						
	1	2	3	4	5	6	A/G
Uninfected Females (8)							
Mean	34.48	9.4	22.7	18.2	7.8	3.2	0.56
Range	28.2-48.1	7.2-12.8	10.4-34.7	11.2-33.7	4.8-12.0	0.9-6.7	0.39-0.92
Infected Females (14)							
Mean	34.77	10.3	23.9	13.8	6.9	3.7	0.54
Range	25.8-45.7	4.6-13.8	12.0-34.0	5.7-22.0	2.0-12.9	1.0-9.5	0.35-0.84

TABLE IV

NUMBER AND PERCENTAGE OF GOLDEN SHINER OUT OF THE TOTAL NUMBER SAMPLED DURING THREE TIME PERIODS WITH STAGE 2 AND 3 GONADAL DEVELOPMENT *

	June - July 6		July 7 - July 24		July 25 - Aug.	
Stillwater Females	6/6	100%	4/4	100%	1/8	12.5% **
Stillwater Males	13/16	81.3%	14/14	100%	3/9	33.3%
Arkansas Females	1/8	12.5%	10/14	71.4%	7/8	87.5%
Arkansas Males	7/19	36.8%	6/8	75%	10/12	83.3%

* Stages of Gonadal Development
1 = Poorly Developed
2 = Well Developed
3 = Very Well Developed

** Observation Made From Infected Fish

TABLE V

MEAN VALUES AND RANGES OF PCV AND HEMOGLOBIN CONCENTRATION FOR DIFFERENT GROUPS OF GOLDEN SHINER SAMPLED DURING THREE TIME PERIODS. NUMBER OF FISH IN PARENTHESES

	PCV (%)			Hemoglobin (g/100ml)		
	June-Jul 6	Jul 7-Jul 24	Jul 25-Aug	June-Jul 6	Jul 7-Jul 24	Jul 25-Aug
Stillwater Females (uninfected)	(6) 33 28-40	(5) 27.6 25-31	-	(5) 8.14 7.23-9.20	(5) 8.09 7.35-8.62	-
Stillwater Females (infected)	-	(14) 26 22-30	(7) 29.4 23-35	-	(10) 7.69 6.38-8.71	(7) 8.28 7.20-9.72
Stillwater Males	(16) 31 24-42	(14) 26.4 22-32	(8) 31.5 28-34	(12) 8.42 7.03-9.8	(13) 7.59 6.22-9.45	(8) 8.23 7.33-8.94
Arkansas Females	(7) 27.1 23-35	(12) 27.8 20-35	(4) 23.8 19-30	(9) 8.05 6.95-9.25	(12) 8.04 6.42-10.40	(4) 7.10 6.68-7.51

TABLE VI

MEAN VALUES AND RANGES OF PCV AND HEMOGLOBIN CONCENTRATION FOR DIFFERENT FISH GROUPS WITH DIFFERENT STAGES OF GONADAL DEVELOPMENT.* NUMBER OF FISH IN PARENTHESES

	PCV (%)		Hemoglobin (g/100ml)	
	Gonadal Development		Gonadal Development	
	1	2-3	1	2-3
Stillwater Females (uninfected)	-	(11) 25-40 30.5	-	(10) 7.23-9.20 8.12
Stillwater Males	(9) 27-34 30.2	(28) 22-42 29.4	(8) 7.03-8.94 8.13	(25) 6.22-9.80 8.02
Arkansas Females	(7) 23-35 27.1	(14) 20-35 27.1	(9) 6.95-9.25 8.05	(14) 6.42-10.40 7.76

* Gonadal Development
1 = Poorly Developed
2 = Well Developed
3 = Very Well Developed

TABLE VII

MEAN VALUES AND RANGES OF PCV AND HEMOGLOBIN CONCENTRATION FOR DIFFERENT FISH GROUPS CLASSIFIED ACCORDING TO AGE. NUMBER OF FISH IN PARENTHESES

	PCV (%)		Hemoglobin (g/100ml)	
	Age 1	Age 2	Age 1	Age 2
Stillwater Females (infected)	(4) 27.3 25-29	(17) 27.1 22-35	(3) 7.71 7.38-8.08	(15) 7.95 6.38-9.75
Stillwater Females (uninfected)	-	(11) 30.5 25-40	-	(10) 8.12 7.23-9.20
Stillwater Males	(21) 29.8 22-42	(19) 28.9 24-35	(16) 8.07 6.22-9.78	(17) 8.02 6.95-9.80
Arkansas Females	(7) 29.0 20-37	(14) 26.2 20-35	(8) 8.50 7.20-10.40	(15) 7.54 4.80-9.30

TABLE VIII

MEAN VALUES AND RANGES OF CONDITION FACTOR (CF), PCV, HEMOGLOBIN CONCENTRATION AND MCHC IN DIFFERENT GROUPS OF FISH STUDIED. NUMBER OF FISH IN PARENTHESES

	C.F.	PCV (%)	Hemoglobin (g/100ml)	MCHC (%)
Stillwater Females (Uninfected)	(11) 322.55 287.11-385.28	(11) 30.55 25-40	(10) 8.12 7.23-9.20	(10) 27.10 21.62-34.48
Stillwater Females (Infected)	(19) 328.50 289.68-377.85	(19) 27.21 22-35	(16) 7.97 6.38-9.75	(16) 29.00 25.44-33.31
Stillwater Males	(39) 318.14 270.60-368.74	(38) 29.42 22-42	(33) 8.05 6.22-9.80	(33) 28.01 21.77-39.38
Arkansas Females	(23) 292.96 222.28-336.59	(19) 28.06 22-37	(21) 8.10 6.42-10.40	(17) 29.47 22.5-37.65

TABLE IX

MEAN VALUES AND RANGES OF RELATIVE MOBILITY OF SERUM PROTEIN FRACTIONS IN THE FEMALE AND MALE GOLDEN SHINER. NUMBER OF FISH IN PARENTHESES

	Fraction Number					
	1	2	3	4	5	6
Stillwater Females (Uninfected) (8)	100	84.3 81.8-86.7	76.2 71.6-79.3	70.9 65.1-77.1	65.4 60.3-72.5	58.6 51.1-66.5
Stillwater Males (13)	100	82.8 80.8-85.4	74.9 72.0-79.3	69.5 62.7-75.0	65.8 58.2-71.8	58.6 50.5-66.9

TABLE X

MEAN VALUES AND RANGES OF PERCENT TOTAL PROTEIN OF SERUM PROTEIN FRACTIONS IN THE FEMALE AND MALE GOLDEN SHINER. NUMBER OF FISH IN PARENTHESES

	Fraction number						
	1	2	3	4	5	6	A/G
Stillwater Females (Uninfected) (8)	34.48 28.2-48.1	9.4 7.2-12.8	22.7 10.4-34.7	18.2 11.2-33.7	7.8 4.8-12.0	3.2 0.9-6.7	0.56 0.39-0.92
Stillwater Males (13)	40.14 30.2-48.9	10.3 5.2-14.7	21.7 14.6-33.9	13.0 7.3-19.4	7.4 3.7-19.9	3.2 1.2-4.5	0.70 0.43-0.96

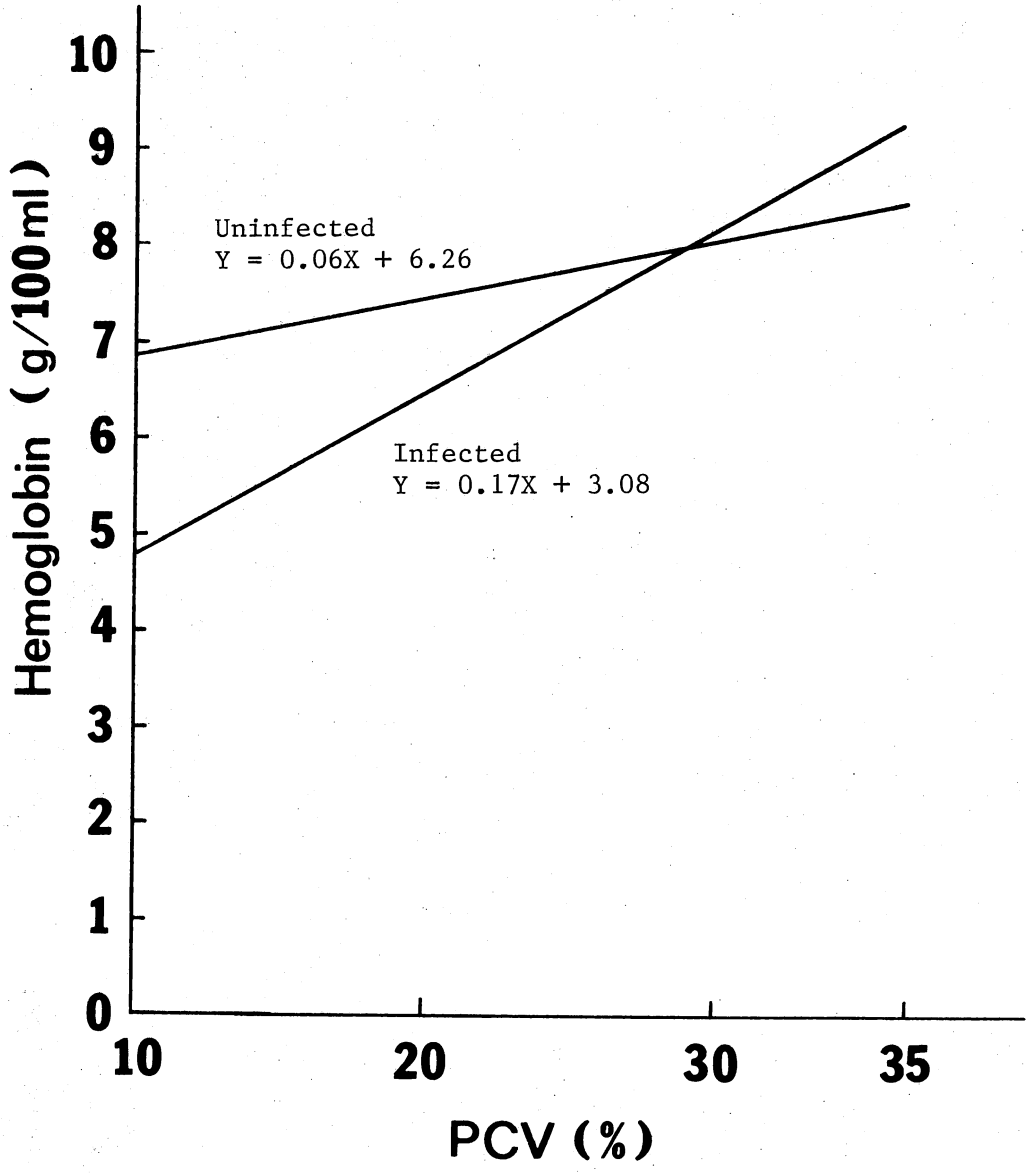

Figure 1. Regression Lines for Uninfected and Pleistophora ovariae Infected Female Golden Shiner, Notemigonus crysoleucas from Stillwater

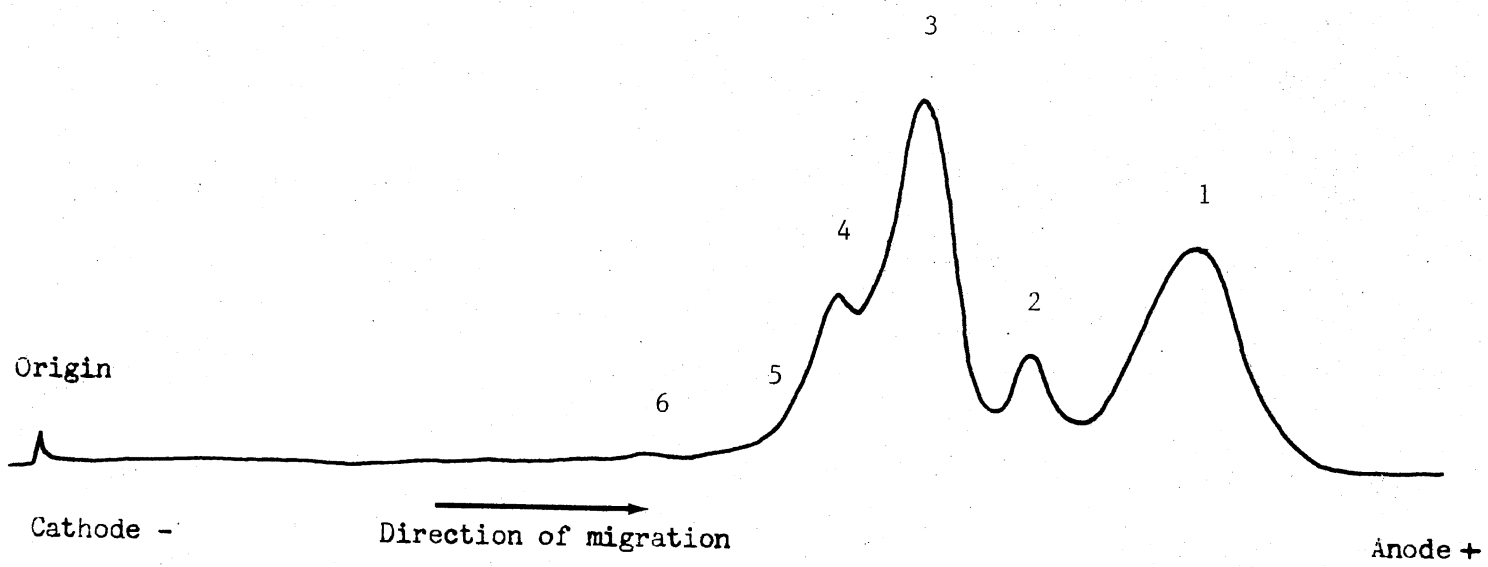

Figure 2. Typical Electrophoresis Densitometer Profile of Female Golden Shiner, Notemigonus crysoleucas

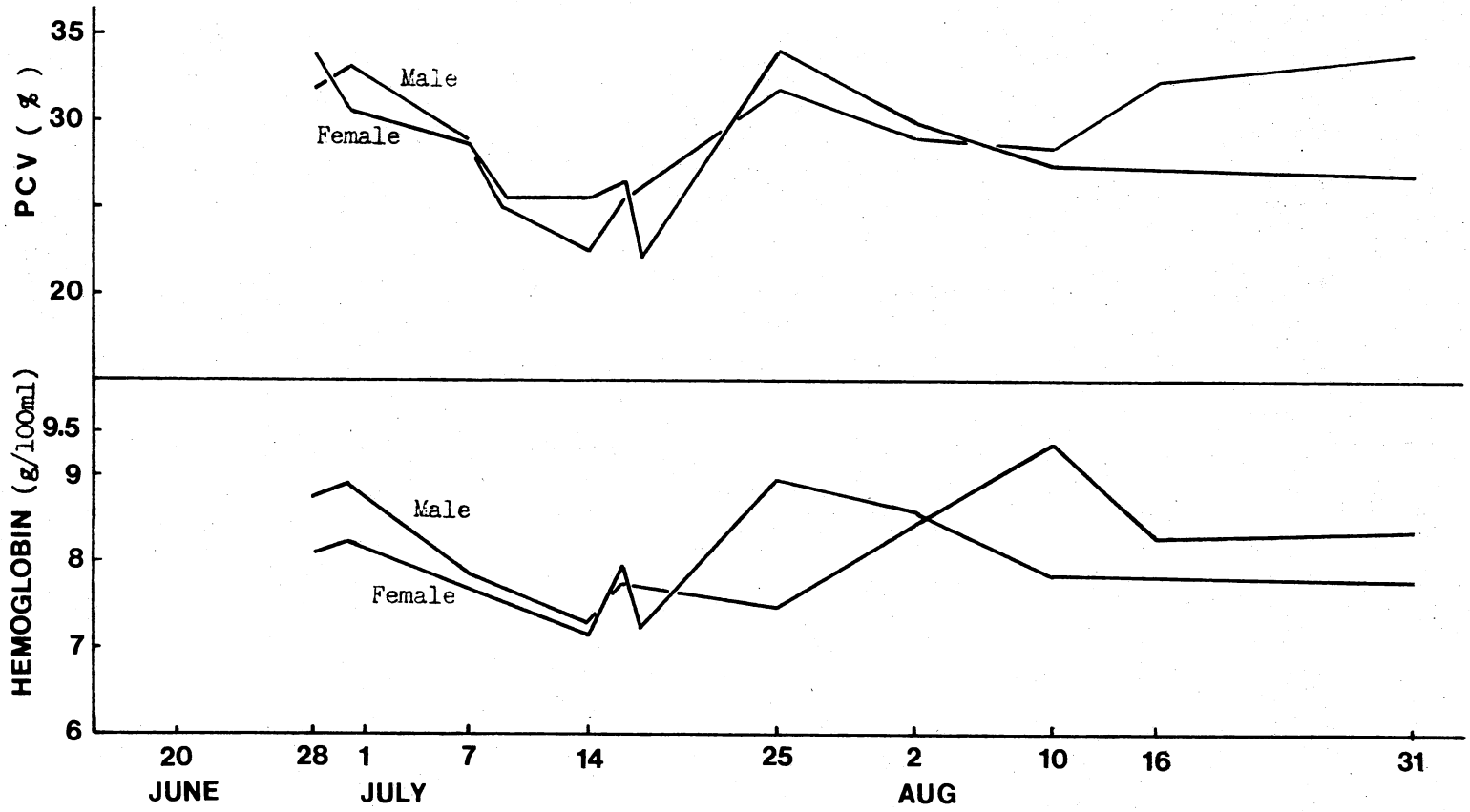

Figure 3. Changes in PCV and Hemoglobin Concentrations with Time in Golden Shiner from Stillwater

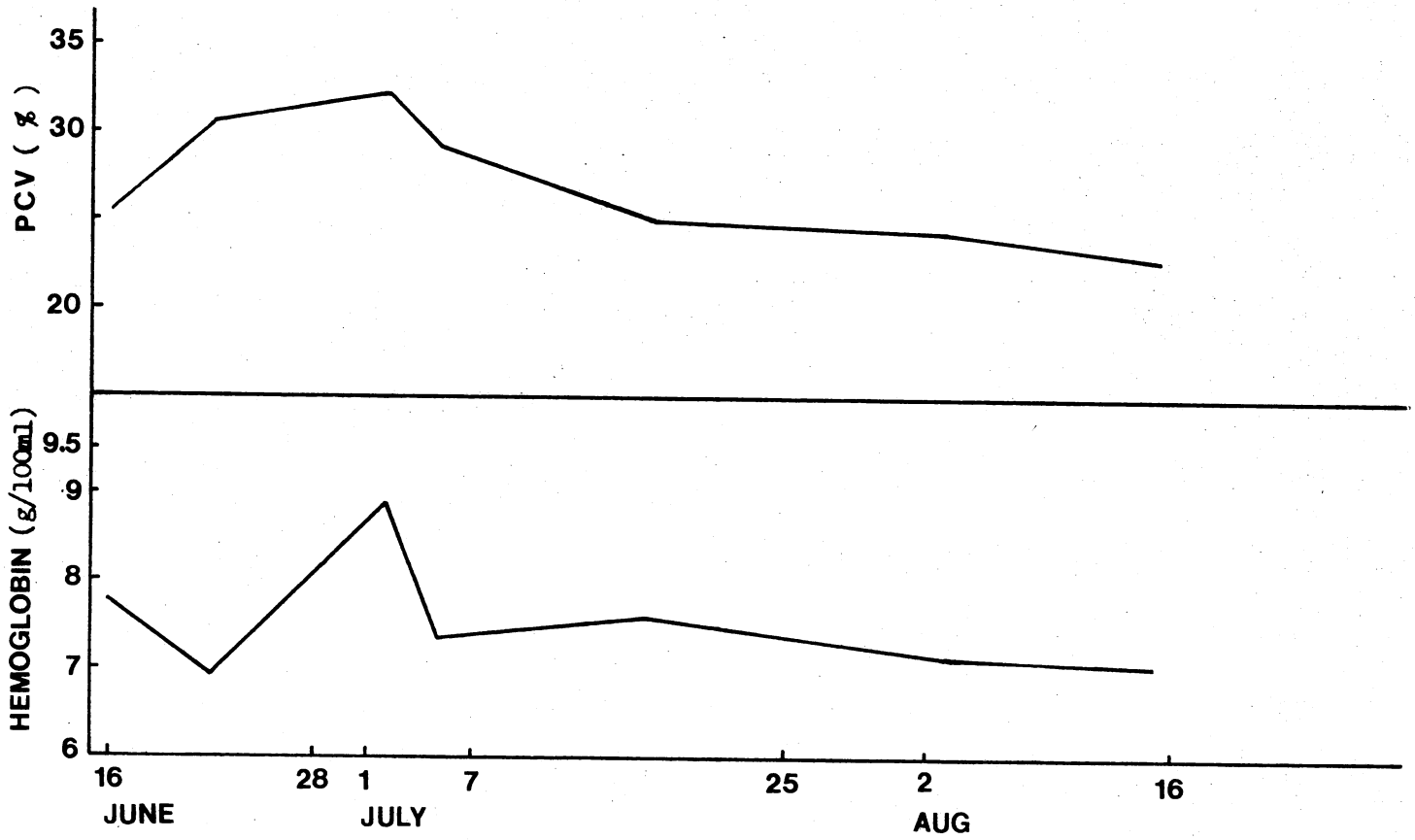

Figure 4. Changes in PCV and Hemoglobin Concentrations with Time in the Female Golden Shiner from Arkansas

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