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GROWTH, MORTALITY, AND HEMATOLOGY OF CUTTHROAT TROUT EXPERIMENTALLY INFECTED WITH THE BLOOD FLUKE *Sanguinicola klamathensis**

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Abtsract: Studies were made of the growth, mortality, and blood changes of cutthroat trout (*Salmo clarki*) experimentally infected with the blood fluke, *Sanguinicola klamathensis* Wales 1958. Five hundred non-infected cutthroat trout fingerlings and 500 exposed to a population of 6000 *Fluminicola fusca* snails with a 6% prevalence of infection with the blood fluke *S. klamathensis* were maintained for several months. Following 3 months exposure to the blood fluke infection, the experimental group had 80% mortality. Packed cell volumes and oxyhemoglobin levels were reduced significantly in the experimental fish as compared to the controls. Control fish continued to grow logarithmically in total weight, while the experimental fish declined in total weight due to parasitism and mortality. There were significant differences between the two groups for the average weight per fish and the average length per fish during the period of mortality.

INTRODUCTION

Only three species of blood flukes are known to occur in salmonids in the world. *Sanguinicola davisi* was described by Wales[14] and *S. klamathensis* was described by Wales[14] and Evans and Heckmann,[9] while Meade and Pratt[11] described *Cardicola alseae*. The descriptions of *S. klamathensis* were primarily of the morphology and life history.

There is little information concerning the effects of the blood fluke, *S. klamathensis* upon its salmonid hosts. This study was undertaken to provide observations useful as a reference to scientists and fishery agencies.

MATERIALS AND METHODS

One thousand non-infected cutthroat trout were used in the experiments. Five hundred cutthroat trout each were placed into two separate, standard hatchery tanks. No snails were included with one group of trout and 6000 snails of the species *Fluminicola fusca* with a 6% prevalence of infection with *S. klamathensis* were included with the other group. A screen barrier was used to separate the fish from the snails. The barrier still permitted passage of cercariae through the screen. The water source for the hatchery is from subterranean springs, and the temperature was constant at 15C. The water flow rate was maintained at 85 l/min. Algae-coated rocks were placed with the snails, and a 20 watt, fluorescent lamp was installed above both tanks to accelerate algal growth. Further details can be obtained by reference to an earlier article on *S. klamathensis* by the author.[8]

Samples for histopathology and hematology were taken on 21 December ,1972 and 4 January, 1973. Tissue samples were also taken on 25 April, 1973, but blood samples were not taken on this date.

* This research was part of the author's recent Ph.D. dissertation.

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Two samples of 10 and 15 fish, respectively, were taken from each group for blood studies. Each fish was weighed, measured, and killed by transecting the brain. The tail of each fish was severed at the caudal peduncle, and the first drop of blood was blotted with an absorbent paper towel. The blood sample for hemoglobin determination was taken directly from the caudal artery using a 20 μl glass microcapillary pipette. This aliquot was prepared for oxyhemoglobin determination using the method of Collier.[7] The cuvettes were covered with aluminum foil and read 10-15 min after preparation in a spectrophotometer. Two blood samples for packed cell volumes (PCV) were also taken from the caudal artery of each fish. Blood was collected in capillary tubes (75 mm long, 0.5-0.6 mm ID), sealed with clay, and centrifuged for 3 min. at approximately 12,500 rpm. Packed cell volumes were determined on a micro-hematocrit reader, and averages of the two samples from each fish were recorded.

Feed projections, weighing, and counting were done every 2 weeks for the first 2 months of the study. For the last 5 months these data were calculated on a monthly basis.

Fish were weighed in a 19 l bucket containing approximately 7.5 l of water. Crowding screens were used to confine the populations wherein a hand-net was utilized to transfer the fish to the bucket. After weighing, the fish were counted and placed back into the tanks. Estimates of growth were calculated by consulting standard measurement tables.[4] Food calculations and projections were made by the technique used by the Hagerman National Fish Hatchery.[6] Dead fish were removed and counted daily.

Samples of tissue were obtained from both groups of trout for examination for viral and bacterial microorganisms. Scrapings with a platinum wire loop of the posterior kidney from ten experimental and ten control fish were streaked onto trypticase soy agar slants and microscope slides. Tests were run according to a flow chart[5] to identify the microorganisms present.

Samples of tissue from the spleen and kidney were removed from both groups of fish, ground, and inoculated onto RTG-2 tissue culture tubes. These tubes were incubated for a 2-week period. Positive and negative control tubes were set up with each sample tube.

The analysis-of-variance was used to analyze the effects of the presence or absence of the blood fluke upon oxyhemoglobin levels, PCV, and length and weight per fish on two sampling dates.

In addition, a two sample T-test to detect the differences between two proportions was used to analyze the differences in mortality rates of the control and experimental groups.[12]

RESULTS AND DISCUSSION

Few reports are available in reference to PCV of diseased fishes. There is more data on oxyhemoglobin levels. Papers by Blaxhall,[2] Blaxhall and Daisley,[3] and the bibliography by Hawkins and Maudesley-Thomas[10] are good sources to consult for hematolological techniques.

Since kidney damage was apparent it was suspected that hematopoiesis would be reduced in infected fish. Therefore, PCV and oxyhemoglobin levels were selected in an attempt to show significant changes between the blood constituents of infected and non-infected salmonids.

Growth Parameters

Figure 1 shows that control fish continued to grow logarithmically while the experimental fish declined in total weight due to parasitism and high mortality. There were no significant differences in average lengths and weights per fish between the control and experimental groups for the 7-month period. The F ratio for the average length per fish was 1.63 while the F ratio for the average weight per fish was 3.58. There were significant differences between the two groups for the average weight per fish ($F=11.1$) (Fig. 1) and average length per fish ($F=11.5$) during the period of high mortality, however.

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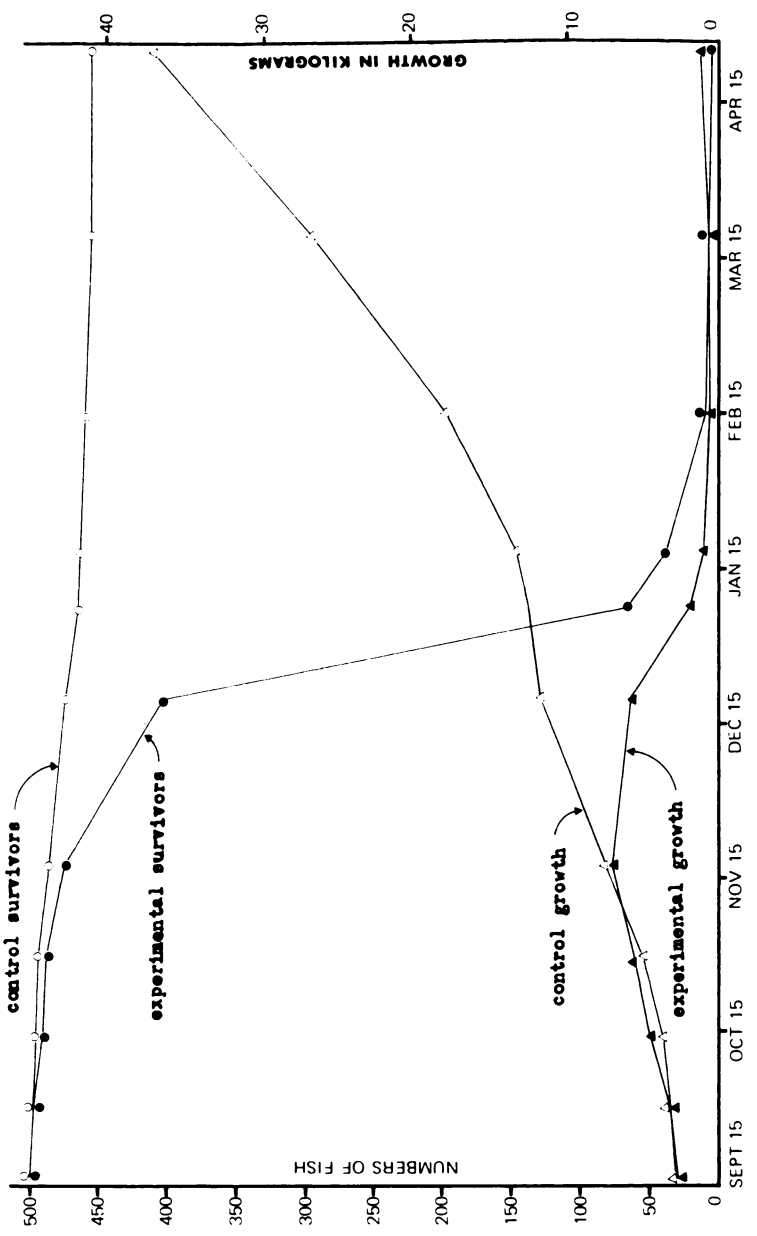


FIGURE 1. Survivors, mortality, and weight patterns of non-infected cutthroat trout (**Salmo clarki**) and cutthroats experimentally infected with **Sanguinicola klamathensis**.

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Mortality

The greatest mortality of the experimental fish occurred between days 96 and 110 (21 December, 1972 and 4 January, 1973), the interim between major samples (Fig. 1). The experimental infection, therefore, took about 3 months to reach a peak. Death rate among controls was similar to expected mortality for this hatchery.

A two-sample T-test was performed for four separate dates. The T-value for the mortality on day 64 was 2.23. This value increased to 7.70 on day 96 and 39.35 on day 110. The latter was the largest increase observed, statistically indicating that the greatest mortality took place between day 96 and 110. The next calculated T-value was 56.35 which occurred in the period between days 110 and 123.

Blood Parameters

Tables 1 and 2 indicate significant differences between the PCV means of the two groups. Control fish PCV averaged 40% while the experimental PCV averaged 16%. Statistical analyses of the PCV showed significant differences between the samples of day 96 and day 110 with an F value of 5.6 which is well above 4.08 or significant at the 0.05 level. Day-group interaction analysis yielded an F value of 8.8 which is highly significant at the 0.01 level. The oxyhemoglobin levels which were inversely proportional to the PCV were statistically significant.

In a study related to the blood fluke, Smitherman[13] pointed out that the PCV decreased in bluegills (*Lepomis macrochirus*) infected with trematode larvae (*Posthodiplostomum minimum*). According to Watson et al,[15] in a study of healthy and virus-diseased sockeye salmon (*Onchorhynchus nerka*), PCV values fell to 16% by the 8th day after exposures to the virus compared to 47% in the controls. Anderson and Conroy[1] pointed out that fish suffering from a *Vibrio* infection showed marked anemia characterized by lowered red cell counts, PCV, and hemoglobin concentrations. The blood was watery and lacked coloration in cutthroat trout experimentally infected with *S. klamathensis*. Blood loss due to hemorrhage or a decrease in erythropoiesis reduces PCV and oxyhemoglobin values. Hemorrhage is probably due to mass exodus of miracidia through the gill epithelium. Migration of flukes through the hematopoietic tissue of the kidney and encystment of eggs and miracidia in the same organ contribute to reduced erythropoiesis and possibly renal failure.

Survey of Microorganisms

There were no effects in the tissue cultures, indicating the apparent absence of cytopathic fish viruses. Bacterial organisms were not found in Gram-stained kidney smears, but cultured samples contained bacteria. Three different bacterial organisms were observed: *Pseudomonas* sp., *Aeromonas* sp., and an enteric bacterium, none of which were seen in the original kidney smears. All were gram-negative, and none are considered pathogenic to hatchery fishes.[5]

Host-Parasite Relationship

The survival of salmonid hosts infected with *S. klamathensis* appears to be dependent upon the degree of infection and their inherent resistance. Transmission and propagation of the blood fluke infection within hatchery production fishes was found to be directly dependent upon either wild or escaped *Salmo gairdneri* or *S. clarki* within the water supplies. Fish examined from the hatchery water supplies and raceways were found to be infected with *S. klamathensis*.

Observations of the behavior of infected fish during the peak infection period revealed "flashing" by the fish and scraping of their bodies against the tank bottom and sides. Also, mass surfacing, gasping for air, and crowding near the water inflow were observed. Those fish which continuously orientated at the surface were lethargic and usually died a few minutes thereafter. Additional observations indicated diminished or discontinued feeding during the peak infection

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period. The alimentary tracts of infected fish were either empty or partially filled.

Wales[14] noted that eggs and miracidia of *S. davisi* were either absent or degenerate in infected adult trout which had been moved to snail-free water previous to examination. This is probably due to the eggs maturing to miracidia and the latter escaping from the host permitting the fish to recover from the infection. The potential for egg production is less for *S. davisi* than *S. klamathensis*.[14] Anemic changes in experimental fish were prominent during this project (Tables 1 and 2). It appears that the change in normal blood parameters occurs when miracidia break out through the gill epithelium causing multiple petechiae resulting in blood loss and probably death.

The potential for *S. klamathensis* for reaching epizootic proportions among its salmonid hosts in hatcheries or in nature should be evident. The need for more extensive research in the future on this trematode and closely related species is great among cultured fishes and natural populations.

TABLE 1. Blood parameters and physical characteristics of 10 control and 10 experimental cutthroat trout examined on day 96 (Percent transmittance in parentheses).

	Oxyhemoglobin g/100 ml*	%T	PCV**	Total Length (mm)	Body Weight (g)
			Control		
Means	10.4	(44.3)	40	148	32.2
			Experimental		
Means	3.8	(71.1)	16	131	19.9

* Hemoglobin values determined with Bausch and Lomb spectrophotometer model 20.

** Packed cell volumes determined by microhematocrit method.

TABLE 2. Blood parameters and physical characteristics of 15 control and 15 experimental cutthroat trout examined on day 110. (Percent transmittance in parentheses).

	Oxyhemoglobin g/100 ml*	%T	PCV**	Total Length (mm)	Body Weight (g)
			Control		
Means	9.9	(46.2)	38	186	59.7
			Experimental		
Means	7.2	(57.9)	27	169	43.7

* Hemoglobin values determined with Bausch and Lomb spectrophotometer model 20.

** Packed cell volumes determined by microhematocrit method.

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