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Relationship of season, thermal loading and red-sore disease with various haematological parameters in *Micropterus salmoides*

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One hundred and fifty largemouth bass (*Micropterus salmoides*) were caught during the mid-winter and mid-summer months in Par Pond, an 1120 ha cooling reservoir located on Savannah River Plant near Aiken, SC. Equal numbers of fish were taken from ambient and thermally altered (> 10° C ambient) areas of the lake. The total length and weight of each bass was noted, along with the presence or absence of lesions associated with red-sore disease (caused by the bacterium, *Aeromonas hydrophila* and the ciliate, *Epistylis* sp.). Blood was drawn, by heart puncture, from each bass and the following haematological parameters measured: haematocrit, haemoglobin, iron, total iron-binding capacity, thyroxine, sodium and potassium. Only the total iron-binding capacity was significantly higher in bass with red-sore disease; none of the other blood parameters were significantly different in infected and uninfected bass. Bass from thermally altered locations had consistently higher haematocrits during both summer and winter. Bass from thermal and ambient locations were not different with respect to iron, iron-binding capacity, sodium or potassium levels. However, bass taken during summer months had higher levels of circulating iron than individuals in winter months. Summer bass had higher thyroxine levels than bass in winter. These results are discussed in terms of their relationship to red-sore disease, season and the impact of thermal effluent.

I. INTRODUCTION

Red-sore disease is a common malady of fish in the southeastern United States. Recently, Miller & Chapman (1976) reported that several fish kills in North Carolina reservoirs could be attributed to epizootics of red-sore disease. The aetiological agent is *Aeromonas hydrophila*, a motile, gram negative bacterium. Secondary infection by *Epistylis* sp., a colonial peritrich, is common but unnecessary for production of a typical red-sore lesion (Hazen, unpubl. obs.). Esch *et al.* (1976) demonstrated that incidence among largemouth bass *Micropterus salmoides* Lacépède, was correlated with body condition (*K*-factor) of the bass. They showed that fish with low *K*-factors are more likely to be infected, and that body condition in all fish was related (directly or indirectly) to temperature. With this in mind, haematological assessment of fish fitness was made using a number of parameters. By determining correlations between blood parameters and fish variables (sex, season, habitat, infection), effects of thermally altered water on *M. salmoides* were measured.

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II. MATERIALS AND METHODS

The study area was Par Pond, located on the Savannah River Plant, Aiken, South Carolina. Par Pond is an 1120 ha, artificial impoundment with a mean depth of 6·2 m, a shoreline length of 53 km and a volume replacement time of six months (Lewis, 1974; Bennett & Gibbons, 1972). Heated water is discharged into the reservoir from a single nuclear production reactor (Fig. 1). Heat distribution patterns and water quality parameters in the reservoir have been reported elsewhere (Lewis, 1974; Tilly, 1973).

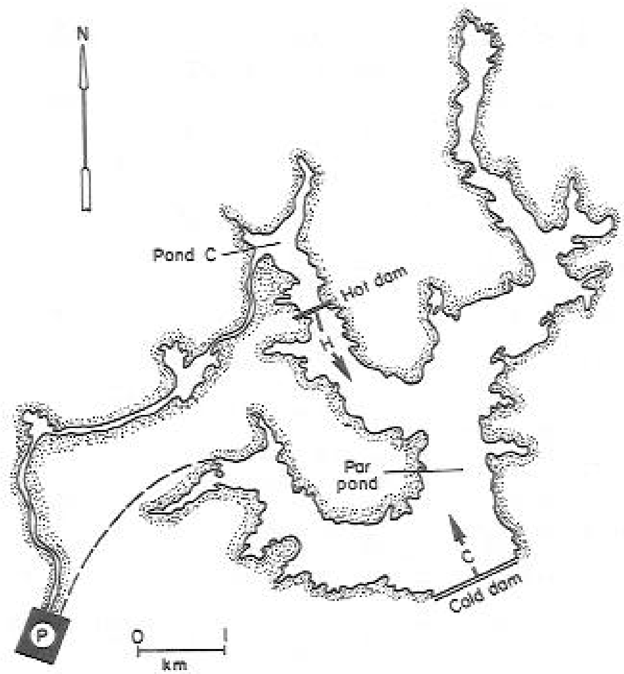

FIG. 1. Map showing the Par Pond system near Aiken, SC. The source of thermal effluent is a nuclear production reactor (P). Temperature of water in the thermally-altered location (H) averages 10° C higher than water in the ambient location (C).

Bass were collected from two parts of the reservoir, HD and CD (Fig. 1). Site HD was considered thermal all the year round ($\triangle T \geq 10°$ C), while CD was always ambient in temperature. Movement of bass between these two areas was shown to be minimal by Quinn, *et al.* (in press). During July, 1975, 50 *Micropterus salmoides* were collected from each site. In January, 1976, 25 more *M. salmoides* were collected from the two sites. Collection was by a combination of angling and electrofishing, but mostly the latter.

All fish were bled within one hour of capture by cardiac puncture with heparinized, 10 ml draw vacutainers (made by B-D Rutherford, New Jersey, U.S.A.). Haematocrits were determined using the microhaematocrit method. The cyanmethaemoglobin method was used to determine haemoglobin content. Both haematocrit and haemoglobin determinations were made immediately after collection, as described by Blaxhall (1972). The whole blood that remained was centrifuged and the serum removed and frozen for later analysis. Serum iron and total iron-binding capacity were measured by automated analysis using 2, 4, 6-tripyridl–*s*–triazine (TPTZ) as a colour reagent. Unsaturated iron-binding capacity (UIBC) was determined by adding excess iron to serum binding sites. Sodium and potassium were measured by flame emission spectroscopy with an internal lithium standard. Thyroxine (T4) was estimated by a competitive protein binding assay modification of Murphy & Pattee (1967).

III. RESULTS

While there are distinct trends, the data shown in Table I indicate that the body conditions of infected largemouth bass are not significantly different from uninfected bass, in either ambient or thermal locations. However, these data do not accurately reflect the situation since all of these bass were captured in summer. When each season is considered separately, it has been shown that infected bass in fall, spring, and winter have significantly lower body conditions than uninfected individuals in both ambient and thermal locations (Esch *et al.*, 1976). If infection is disregarded as a variable, then it can be seen (Table I) that bass in thermal locations have significantly lower body conditions than those in ambient locations. Further, this relationship persists for bass taken in summer or winter.

There is not a significant relationship between haematocrits of infected and non-infected bass taken from ambient or thermal locations during the summer months (Table II). However, the haematocrits of both infected and non-infected bass were found to be significantly higher in bass from thermal locations as compared to those from ambient areas. Moreover, when infected and non-infected summer bass are pooled and compared with their winter counterparts, it was found that the relationship

TABLE I. Comparison of body condition (*K*-factor) for infected and non-infected bass by location and for all bass (disregarding infection) by season and location

Location	Red-Sore Disease (summer only) Infected	vs.	Non-infected	Season (disregarding infection) Summer	vs.	Winter
Ambient	1·22	n.s.	1·27	1·27	$P=0{\cdot}0004$	1·41
vs.	n.s.		n.s.	$P=0{\cdot}04$		$P=0{\cdot}03$
Thermal	1·07	n.s.	1·15	1·14	$P=0{\cdot}001$	1·32

TABLE II. Comparison of haematocrits for infected and non-infected bass by location and for all bass (disregarding infection) by season and location

Location	Red-Sore Disease (summer only) Infected	vs.	Non-infected	Season (disregarding infection) Summer	vs.	Winter
Ambient	24·9	n.s.	27·1	26·7	$P=0{\cdot}003$	35·3
vs.	$P=0{\cdot}0019$		$P=0{\cdot}0253$	$P=0{\cdot}0017$		$P=0{\cdot}0188$
Thermal	37·0	n.s.	32·2	33·0	$P=0{\cdot}0042$	40·1

TABLE III. Comparison of haemoglobin for infected and non-infected bass by location and for all bass (disregrading infection) by season and location

Location	Red-Sore Disease (summer only) Infected	vs.	Non-infected	Season (disregarding infection) Summer	vs.	Winter
Ambient	6·6	n.s.	7·2	7·1	$P=0{\cdot}0795$	8·1
vs.	n.s.		n.s.	$P=0{\cdot}0188$		n.s.
Thermal	6·4	n.s.	5·9	6·0	$P=0{\cdot}001$	8·2

persisted with the highest mean values being recorded in winter bass from thermal locations.

Haemoglobin levels in infected and non-infected bass are not significantly different at either thermal or ambient locations (Table III). When winter bass from thermal and ambient locations are compared in terms of haemoglobin, there is not a significant difference. However, it is apparent that thermal loading in summer does play a significant role in depressing haemoglobin levels in bass since the lowest haemoglobins were observed in summer bass taken in thermal locations.

Infected bass in thermal locations during the summer months had higher iron levels than non-infected bass during the same months (Table IV). Significant differences were not noted between infected or non-infected bass from thermal and ambient locations. However, iron concentrations do vary with season. Thus, summer bass had higher levels of circulating iron in both ambient and thermal locations. Lowest iron concentrations were noted in winter bass from ambient locations.

The pattern for total iron-binding capacity (TIBC) was approximately the same as for circulating iron except that significant differences were not observed when levels for infected bass were compared to those for non-infected individuals (Table V). The lowest TIBC values were observed in bass taken from ambient locations during the winter months.

Concentrations of thyroxine (T_4) were not significantly different when infected and non-infected bass were compared (Table VI). Nor were T_4 levels significantly different for bass from ambient and thermal locations during winter or summer. However, significantly lower T_4 values were noted in winter as compared to summer bass.

Sodium concentrations were not affected by infection or thermal loading during summer months (Table VII); nor do sodium levels significantly vary from summer to winter in either ambient or thermal locations. However, sodium levels appear to be significantly elevated in bass from thermal areas taken in winter months as compared

TABLE IV. Comparison of iron for infected and non-infected bass by location and for all bass (disregarding infection) by season and location

Location	Red-Sore Disease (summer only) Infected	vs.	Non-infected	Season (disregarding infection) Summer	vs.	Winter
Ambient	150·8	n.s.	143·7	145·0	$P=0·0001$	72·3
vs.	n.s.		n.s.	n.s.		$P=0·0194$
Thermal	161·3	$P=0·0093$	133·0	137·9	$P=0·0001$	92·6

TABLE V. Comparison of total iron-binding capacity (TIBC) for infected and non-infected bass and for all bass (disregarding infection) by season and location

Location	Red-Sore Disease (summer only) Infected	vs.	Non-infected	Season (disregarding infection) Summer	vs.	Winter
Ambient	634·6	n.s.	576·0	585·3	$P=0·0001$	347·5
vs.	n.s.		n.s.	n.s.		$P=0·0001$
Thermal	605·6	n.s.	586·2	590·1	$P=0·0005$	492·7

with individuals taken in ambient locations during the same season. Exactly the same kind of relationships can be seen for potassium levels as for sodium in bass taken during the two seasons in ambient and thermal locations (Table VIII).

TABLE VI. Comparison of thyroxine (T_4) for infected and non-infected bass and for all bass (disregarding infection) by season and location

	Red-Sore Disease (summer only)			Season (disregarding infection)		
Location	Infected	vs.	Non-infected	Summer	vs.	Winter
Ambient	1·50	n.s.	1·58	1·58	$P=0{\cdot}0054$	0·88
vs.	n.s.		n.s.	n.s.		n.s.
Thermal	1·96	n.s.	1·37	1·51	$P=0{\cdot}0007$	0·68

TABLE VII. Comparison of sodium for infected and non-infected bass by location and for all bass (disregarding infection) by season and location

	Red-Sore Disease (summer only)			Season (disregarding infection)		
Location	Infection	vs.	Non-infected	Summer	vs.	Winter
Ambient	163·3	n.s.	157·2	158·2	n.s.	157·9
vs.	n.s.		n.s.	$P=0{\cdot}0958$		$P=0{\cdot}0297$
Thermal	166·1	n.s.	161·2	162·0	n.s.	163·2

TABLE VIII. Comparison of potassium for infected and non-infected bass by location and for all bass (disregarding infection) by season and location

	Red-Sore Disease (summer only)			Season (disregarding infection)		
Location	Infected	vs.	Non-infected	Summer	vs.	Winter
Ambient	4·6 meq l^{-1}	n.s.	4·0 meq l^{-1}	4·1 meq l^{-1}	n.s.	5·0 meq l^{-1}
vs.	n.s.		n.s.	n.s.		n.s.
Thermal	3·0 meq l^{-1}	n.s.	3·8 meq l^{-1}	3·7 meq l^{-1}	n.s.	4·0 meq l^{-1}

IV. DISCUSSION

According to Amlacher (1961), erythrocyte counts and haemoglobin levels are suggestive of severe anaemia in carp infected with *Aeromonas liquefaciens* (this species has been synonomized with *A. hydrophila*; see Buchanan and Gibbons, 1974). The data generated in the present study do not suggest anaemia in bass infected with red-sore disease. Indeed, the only blood parameter which could be identified as being characteristically associated with *A. hydrophila* infections in bass was circulating iron. While the bass in this study were not examined histologically, others have been and, in those cases, there were internal lesions, characteristic of infection with *A. hydrophila* (=*A. liquefaciens*) as described by Amlacher (1961). *A. hydrophila* was also cultured from external lesions of infected bass. Studies are continuing in an effort to further analyze the apparent discrepancies between our findings and those of Amlacher (1961).

The seasonal variability in body condition and blood parameters of all bass reveal both expected and unexpected findings. The body conditions of bass in summer and winter confirm an earlier report by Esch *et al.* (1976). Thus, during summer, bass in both ambient and thermal locations are known to have lower body conditions than those in winter and, furthermore, bass in thermal locations have lower body conditions than those from ambient areas during all seasons except summer. The data for thyroxine levels are consistent with the observations for body condition even though significant differences were not observed when bass from ambient and thermal locations are compared within a given season. An explanation for the seasonal and locational differences in body condition has been suggested by Gibbons *et al.* (in prep.). They argue that elevated temperatures, such as those recorded in summer in all locations and in thermal locations during fall, winter and spring stimulate metabolism of the bass, resulting in a reduction in body fat and a general decline in body conditions. This, coupled with a reduction in the density of prey and/or an increase in refuge sites such as dense growth of *Myriophyllum* which occurs in Par Pond in summer, would exacerbate the effects of temperature on metabolism. Long-term studies on more than 10 000 largemouth bass in Par Pond tend to support this hypothesis.

Causes for elevation or depression of the various other blood parameters, as measured seasonally, can be associated with a number of factors and, in general, agree with the finding reported for blood of other freshwater teleosts (Snieszko, 1969; Field, Elvehjem & Juday, 1943; Summerfeldt, Lewis & Ulrich, 1967).

Haematocrits of largemouth bass were higher in winter than in summer and higher in thermal locations during both seasons. According to Blaxhall (1972) changes in haematocrit may be attributed to several variables including collecting technique, season and disease. As previously noted, *A. hydrophila* infection does not appear to be a contributing factor in variations in haematocrit levels in Par Pond bass nor does sex since significant differences were not observed between males and females. As noted by Mulcahy (1970), microhaematocrit values in healthy adult pike, *Esox lucius* are variable, ranging from 20·0 to 43·5 with a mean of 32·0. The same kind of variability was observed in the present study. Thus, haematocrits from bass in thermal locations had a coefficient of variation (cv.) of 28·4% in summer (range = 10–50) and 24·4% in winter (range = 14–56): the cv. of haematocrits of bass from ambient locations in summer was 37·5% (range = 7–47) and 24·3% in winter (range = 17–51). Speculation relating to these differences and the high degree of variability centre upon the possibility that bass in thermal locations had a decreased intravascular volume due to some abnormality in oxygen-haemoglobin saturation and dissociation such as might occur in the presence of an abnormal haemoglobin. However, such speculation must be considered cautiously until such time as further studies can be completed.

Haemoglobin levels in bass blood followed the same seasonal pattern as for haematocrit, except that significant differences in ambient and thermal bass were not noted in winter, nor were significant differences noted between ambient bass in summer and winter. This latter observation may not be indicative of the real situation since the p-values for the *f*-test closely approach the 5% level of confidence, suggesting additional sampling could provide a more valid perspective. It should also be noted that haemoglobin values for Par Pond bass are within limits reported by other investigators for other teleost hosts (McCarthy *et al.*, 1973; Houston & deWilde, 1972; Soivio & Oikari, 1976).

Iron and TIBC are of interest because they are directly related to each other and because they are indirectly related to haematocrit and haemoglobin. It is worth noting that the iron and TIBC levels follow the same patterns in thermal and ambient locations during summer and winter. Thus, the lowest levels are noted in ambient areas in winter and the highest in summer in both ambient and thermal locations. There is, therefore, a clear relationship between temperature, iron and TIBC. It seems reasonable to speculate that iron levels are lower in winter because additional portions of the circulating iron stores have been removed from the blood to be incorporated into the increased haemoglobin production already noted to have occurred during the winter months. TIBC is a reflection of transferrin levels in blood. It is possible that depression of transferrin levels in winter coincides with an overall decline in general protein production by the bass during the time of year when activity was diminished due to lower temperature. This would probably occur during the period of the year when bass are foraging less extensively, resulting in less nutrient input and, hence, less protein anabolism. This line of reasoning would fit well with the generalized reduction in levels of circulating T_4.

The sodium and potassium levels observed in the present study are, generally, consistent between locations and seasons. This finding was expected and compares favourably with reports of other investigators (Palacios *et al.*, 1972; Holmes & Donaldson, 1969). Umminger (1969, 1970) has presented evidence of a decrease in levels of sodium in response to reduced temperatures; our own observations in this regard certainly do not indicate such changes from winter to summer. However, we can see a difference between locations within a single season. We cannot provide a suitable explanation for such an observation.

In general, it is clear from the present studies that infection with *Aeromonas hydrophila* does not elicit significant haematological changes in largemouth bass in the Par Pond system. The impact of seasonal effects and thermal loading have, however, been clearly demonstrated. These changes are undoubtedly related, primarily, to temperature. However, it should also be noted that seasonal variability in feeding behaviour and availability of prey species may play a role in producing results such as these. Our data do not suggest that sexual differences are significant for the parameters measured in this study. The only exception to this rests with TIBC levels which are highest among male bass from ambient locations. This observation, however, should be treated with caution since it represents but a single parameter among the entire series and could thus be spurious.

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