## INTERNAL TEMPERATURE OF FISH

AS A FUNCTION OF ENVIRONMENTAL TEMPERATURE

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A Thesis

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### INTRODUCTION

Classically animals have been separated into poikilotherms, whose body temperature closely follows ambient temperature, and homeotherms, whose body temperature is regulated within narrow limits. As more body temperature measurements are made, more and more animals have been placed in an intermediate category, the heterotherms or partial regulators (Prosser, 1973).

Fish are considered poikilothermic, but tuna and some species of sharks have been shown to have a significantly higher body temperature than the water in which they live (Carey and Teal, 1966). These fish are able to maintain a higher temperature because they possess a rete mirabile or arterialvenous heat exchange system which conserves body Since it seemed reasonable to ask if some heat. freshwater fishes also maintain body temperature significantly above environmental temperature, the present detailed study was undertaken. Body temperatures were measured in three selected Kansas fishes, the black bullhead (Ictalurus melas), the carp (Cyprinus carpio), and the largemouth bass (Micropterus salmoides).

Carey and Teal (1966), who performed elaborate studies on the tuna, used expensive devices to obtain their results. One of the purposes of this study was to design and build a simple, inexpensive thermistor apparatus that would give precise data on the internal temperature of fish.

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### METHODS AND MATERIALS

The internal temperature of fish was recorded by a servo recorder (Heathkit Model EU-20-29), a modified Wheatstone bridge, and a thermistor matched to the bridge.

After much experimentation with different resistors, potentiometers, and thermistors, a combination of components that allowed internal temperatures to be measured with great sensitivity was discovered. The final model allowed measurement of internal temperatures of fish between 0 C and 30 C, with an accuracy of  $\pm$  .05 C. A schematic diagram of the bridge circuit is given in Fig. 1 and the physical arrangement of the circuit is shown in Fig. 2. With the exception of the recorder, parts cost about five dollars and can be obtained from many sources.

The thermistor (Fenwal Electronics glass probe), was fastened to the ends of the wires that lead from the resistor bridge as indicated in Fig. 3. Dow-Corning Silicone Rubber Aquarium Sealer was used to make the connection waterproof. The amount of sealant on the junction was kept to a minimum to allow insertion of the thermistor through a sharpened cannula.



Figure 1. Schematic diagram of bridge circuit.



Figure 2. Physical arrangement of modified Wheatstone bridge.









Figure 4. Temperature regulated aquarium with confining box.

The thermistors cost about three dollars apiece, and several should be obtained since they are easily broken.

A step by step account of the measurements of the internal temperature of a fish follows. The fish was placed in the desired starting water temperature at least two hours before experimentation began. This gave the fish time to equilibrate with the new environmental temperature. The fish was confined by a 25 cm x 40 cm x 20 cm plastic box within the aguarium (Fig. 4). The confining box had numerous 3 cm x 5 cm holes to allow circulation of water. If the fish were allowed too much swimming area, the thermistor might be pulled from its body. "Hypno" tranquilizer (Jungle Laboratories, Orlando, Florida) was used when the fish was overactive. Since it seemed probable that internal temperature would vary slightly, five different points in the body were chosen for the insertion of the thermistor. The sequence of thermistor sites and depth of the sites are shown in Figs. 5 and 6. The insertions were made with the help of a trocar (Fig. 7). Both the cannula and the stylet of the trocar were inserted into the body of the fish. The stylet was then removed and replaced by the thermistor, then the

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Figure 5. Sequence of thermistor sites.



Figure 6. Cross section of fish with thermistor in place. Shaded areas show where a rete mirabile, if one exists, would be expected.



Figure 7. Trocar for intramuscular insertion of thermistor.

cannula was pulled back onto the wires, leaving the thermistor imbedded in body tissue. The recordings at these sites were made in terms of differences between body temperature and water temperature and were made by placing the thermistor first in the circulating water of the aquarium (Instant Ocean), then inserting the thermistor into a site in the fish and noting the deflection of the recorder. A calibration curve (Fig. 8) was made by comparing readings from the servo recorder with known temperature readings from a thermometer graduated to 0.01 degree C. This allowed the conversion of data obtained from the thermistor into degrees Celsius.

In many of the experiments to be reported, internal temperatures were followed after the animals were suddenly placed in water 10 C higher or lower than the temperature with which they had equilibrated. Because of the high sensitivity of the recording system, the pen position had to be reset to zero two or more times during the usual warming or cooling experiment. In constructing the warming or cooling curves of Fig. 9 through 14, an average value of 0.25 inch deflection/0.1 degree C was used. As can be seen from the calibration curve of Fig. 8, this introduces an error, but it is a small one, probably no larger than the reading error if the recording



Figure 8. Calibration curve: Deflections produced on recorder by 0.1 degree C temperature changes at different base temperatures.

system had been set up for 10 C for full-scale deflection.

In order to see if any of the three species have, to some degree, a rete mirabile system, colored latex (General Biological, Inc.) was injected into their veins and arteries. The latex is fluid in neutral or alkaline solutions and hardens when exposed to acidic solutions. A plastic syringe was used to inject approximately 30 cc of buffered formalin into the sinus venosus and dorsal aorta in the caudal peduncle of fish 25 to 35 cm. in length. The buffered formalin was used to flush the veins and arteries of blood. Blue latex was then injected into the sinus venosus and red latex into the dorsal aorta. A cotton swab was used to place 25 percent HCl to stop leakage of latex from injection site. After the needle was pulled from the injection site, more HCl was placed over the hole in order to plug the opening. The fish was then placed in weak acetic acid to harden the latex throughout the animal.

#### RESULTS

Data on the difference between ambient and body temperature  $(T_{a-b})$  were collected from three individuals from each of the three species studied. Temperatures were recorded from five standard sites (Fig. 5) in each individual. Depth of insertion of the thermistor in sites 2, 3, and 4 in the three species was approximately 1.5 cm. A single environmental temperature (20 C) was used. The results are shown in Table 1. The largest  $T_{a-b}$  values were recorded from the hearts, 0.50 C for the largemouth bass, 0.35 C for the black bullhead, and 0.40 C for the carp. The rectal temperatures in the three species were found to have the smallest  $T_{a-b}$ .

An incidental but possibly significant observation was made on the carp. As the probe was partially removed from insertion sites 3 and 4, a temperature increase of approximately 0.05 C was noted at a depth of about 1 cm. No such increase was noted in the other two species.

The data on the internal temperatures of the three species were subjected to the student T-test p=.05 level of significance. The test showed that there was not a significant difference between any two of the three species. Table I. Average  $T_{a-b}$  of three largemouth bass, carp, and black bullheads, from five standard sites in each individual. Environmental temperature was held at 20 C  $\pm$  0.15 C throughout all experiments. In any one day the variation was less than  $\pm$  0.02 C.

Average T <sub>a-b</sub> at 5 sites for three species								
	large- mouth bass	carp	black bullhead					
Site 1 Rectal	.05	.05	.05					
Site 2 Peduncle	.20	.10	.20					
Site 3 Mid lateral	.40	.30	.35					
Dorsal Site 4 anterior muscle	.40	.30	.35					
Site 5 Heart	.50	•110	•35					
Average weight (grams)	420	390	382					

Cooling and warming rate data were collected for the three species. Fish of nearly the same weight were selected. Kleiber (1961) pointed out that smaller spheres cool off more readily than larger spheres, so smaller poikilotherms would be expected to cool more rapidly than larger poikilotherms, and if different sized fish are used, a correction factor would have to be used. The internal temperature was followed when a fish which had been kept at 20 C was suddenly placed in water at 10 C. The fish was then kept at 10 C for at least an hour after it had equilibrated, then recordings were made when it was returned to the 20 C water. The number 4 thermistor site (in muscle near the dorsal aorta) was used throughout the heating-cooling experiment. Results of the cooling experiments are shown in Figs. 9, 10 and 11. Results of the heating experiments are shown in Figs. 12, 13 and 14.

The largemouth bass was found to take 60 minutes to cool from 20 C to 10 C, but it only took 37 minutes to rewarm to 20 C. These results suggest an active role on the part of the fish to conserve heat during the cooling process and/or to increase heat absorption during the warming process. These curves could be explained by the constriction of superficial blood vessels during cooling, or by dilation of superficial







Figure 10. Cooling data for two black bullheads. Fish were moved from water at 20 C to water at 10 C at 0 minutes. To show that the cooling process is not just the reverse of the werming process the average warming curve for the same species has been drawn with a deshed line. Ordinate for cooling experiment at left. Ordinate for warming experiment at right.



Figure 11. Cooling data for two carp. Fish were moved from water at 20 C to water at 10 C at 0 minutes. To show that the cooling process is not just the reverse of the warming process the average warming curve for the same species has been drawn with a dashed line. Ordinate for cooling experiment at left. Ordinate for warming experiment at right.



Figure 12. Warming data for two largemouth bass. Fish were moved from water at 10 C to water 20 C at 0 minutes. To show that the warming process is not just the reverse of the cooling process the average cooling curve for the same species has been drawn with a dashed line. Ordinate for warming experiment on left. Ordinate for cooling experiment on right.



Figure 13. Warming data for two black bullheads. Fish were moved from water at 10 C to water at 20 C at 0 minutes. To show that the warming process is not just the reverse of the cooling process the average cooling curve for the same species has been drawn with a dashed line. Ordinate for warming experiment on left. Ordinate for cooling experiment on right.



Figure 14. Warming data for two carp. Fish were moved from water at 10 C to water at 20 C at 0 minutes. To show that the warming process is not just the reverse of the cooling process the average cooling curve for the same species has been drawn with a dashed line. Ordinate for warming experiment on left. Ordinate for cooling experiment on right. vessels during

The fish multiplet to different temperatures to determine where or not core and ambient temperatures would consider at some point. It was found that in all there species the core temperature remained above the ambient temperature even at temperatures is a 0.5 C. Care had to be taken in lowering the fish's body temperature, as most were found to die if subjected to 15 or 20 C temperature differences.

by a rete mirabile system.

Latex injections were made in at least two specimens from each of the species studied. A rete mirabile was not detected by this means in any of the three species. If a rete is present, its vessels must be extremely small, since the injections demostrated quite small vessels.

#### DISCUSSION

It is an established fact that not all fish are cold blooded. John Davy in 1835 found that the tuna was 10 C warmer than the surrounding water (Carey 1966). The body temperatures of most fish are very near environmental temperature because of their modes of respiration. Fish must obtain their oxygen from water which has only one-fortieth of the oxygen found in an equal volume of air, but has 3,000 times the capacity of air for absorbing heat, so that extracting oxygen from water can be 120,000 times more costly in heat loss than can be expected from extracting oxygen from air.

The blood of a fish is cooled as it is pumped through the gills. It may then be expected to lose most of the metabolic heat it has accumulated. A change in metabolism due to excitement or rapid swimming would not be expected to affect the temperature significantly since a concomitant increase in blood flow through the gills would cause temperature reduction in the blood. Also, due to the fish's low caloric intake and limited supply of oxygen, the fish can not produce enough metabolic heat to raise the temperature of the blood to any significant degree. A study by Altman (1970) indicated the temperature differences recorded here can not be due to species differences in metabolic rates. Altman gave date comparing  $0_2$  uptake rates for the three species studied (Table 2). According to his data, the metabolic rate for the carp is two to three times that of the largemouth bass and black bullhead. If all other factors were equal, internal temperature of the carp should be higher than that of the other two species. Internal temperatures found in this study (Table 1) indicate just the opposite, that the carp maintains the lowest  $T_{a-b}$  of the three species.

How then can the largemouth bass maintain higher internal temperatures than their environment? At the turn of the century a rete mirabile or "wonderful net" was found in some fish. It is a system of parallel arteries and veins that forms a countercurrent heat exchange system. Venous blood warmed by metabolic processes gives up heat to newly cooled oxygenated blood from the gills. Some fish have a more advanced rete mirabile than other fish. An experiment was performed with these three species of fishes in an attempt to demonstrate the presence of a rete mirabile and its degree of complexity. The direct attempt to demonstrate a rete by latex injection was unsuccessful, but this could be explained

Table II. Oxygen uptake in three species of fish studied (data from Altman, 1970).

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Sandar	Resting metabolic rate									
Species	Temperature	Body wt.	<u>ml 02</u> g br							
Black bullhead	17	50	.051							
Carp	19	40	.177							
Largemouth bass	20	44	.071							

readily if the rete vessels were too small to be demonstrated by standard latex injection. On the other hand, the fact that the fish warmed up more rapidly than they cooled provides indirect physiological evidence either for a rete to conserve heat during cooling or for a means of shunting more blood to the surface during warming. Whether or not these fish possess a rete mirabile could possibly be settled at some future time by vessel-injection techniques using materials less viscous then the latex normally used.

### SUMMARY

Internal temperatures of the largemouth bass, black bullhead, and carp were recorded at multiple sites. It was found that among the three, the largemouth bass maintained the highest temperature above the environmental temperature. Heating and cooling curves revealed that the bass was able to gain heat at a higher rate than it lost heat, suggesting it actively conserves heat in a cold environment and absorbs heat in a warm environment. LITERATURE CITED

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