A Study Of The Temperature Selection And Upper Lethal Temperature Responses For Two Species Of Sculpin, Cottus Cognatus And Cottus Confusus, In The Flathead River Basin Of Northwestern Montana

Eric Froines
Carroll College

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A STUDY OF THE TEMPERATURE SELECTION AND UPPER LETHAL TEMPERATURE RESPONSES FOR TWO SPECIES OF SCULPIN, COTTUS COGNATUS AND COTTUS CONFUSUS, IN THE FLATHEAD RIVER BASIN OF NORTHWESTERN MONTANA

Submitted in Partial Fulfillment of the Requirements for Graduation with Honors to the Department of Biology at Carroll College, Helena, Montana

Eric John Froines
Joseph John Biby
March 23, 1982
This thesis for Honors recognition has been approved for the Department of Biology.

Dr. John Christenson, Advisor

Dr. Jean Smith

Rev. Robert Butko

March 23, 1981
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ACKNOWLEDGEMENTS

Our most sincere appreciation is extended to Dr. Jack Stanford who directed and guided us through this project. We would also like to thank our thesis director, Dr. John Christenson, and our readers, Dr. Jean Smith and Father Robert Butko for their time and suggestions.
ABSTRACT

The temperature selection and upper lethal temperature for two species of sculpin in the Flathead River Basin of northwestern Montana were investigated. Comparisons were made between Cottus cognatus and Cottus confusus concerning these thermal parameters to examine the possible relationship between altered thermal discharges below Hungry Horse Dam and reported hybridization between the two species. The data suggests that C. confusus has a higher preferred temperature and upper lethal temperature tolerance.
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INTRODUCTION AND LITERATURE REVIEW

Temperature, for stream fishes, is an important ecological factor which limits both broad geographical distributions and local occurrences within a single water-course. Most obligate riverine fishes are adapted to certain daily, seasonal, and yearly temperature regimes. Therefore, changes in the preferred thermal regime will affect these fish, and the greater the change, the greater the impact or effect (Ward and Stanford, 1979). Limits in temperature tolerance determine, in part, the relative distribution of closely allied species. For example, *Cottus biardi* lives in warmer waters of streams along the north shore of Lake Superior than does its related species *C. cognatus* (cited in Hynes, 1970). Changes in thermal regime and their corresponding effects on species distribution have been investigated in thermally altered river systems where hydroelectric development has occurred (Ward and Stanford, 1979).

The lower South Fork of the Flathead River in northwest Montana is regulated by Hungry Horse Dam. Operation of the dam has altered normal discharge and temperature regimes from the South Fork and modified conditions in the main Flathead River. The influence of discharge from Hungry Horse Dam on the main Flathead River is modified by the
combined natural flows from the North and Middle Forks, however hypolimnial water released from Hungry Horse Dam does lower summer water temperatures and elevate winter water temperatures in the main stem (Graham, 1980). These thermal modifications have resulted in a relatively constant annual thermal regime. Weekly mean temperatures in the South Fork fluctuate only slightly above or below 7 C, compared to an annual thermal variation in unregulated segments of 0-18 C (Ward and Stanford, 1979; Fig. 1).

Assessments of impact from thermal modification and discharge from Hungry Horse Dam on aquatic organisms has largely centered on game fish and aquatic invertebrates in the drainage. The thermally altered hypolimnial releases from the Dam have produced extreme ecological disturbances that have significantly reduced insect species diversity and biomass in comparison to unaltered river segments (Stanford and Hauer, 1978). In this constant thermal regime many species of insects presumably cannot complete their life cycles (Graham, 1980). Stanford and Hauer (1978), and Hauer (1980) have determined that temperature is an important environmental factor affecting the benthos in the regulated areas of the Flathead River.

Of the game fish studied, the kokanee salmon, *Onchorhynchus nerka* has been shown to be directly affected by operations at Hungry Horse Dam. Prior to impoundment of the South Fork by the reservoir, most kokanee spawning took place along Flathead lakeshore, in McDonald Creek, and
Fig. 1 Monthly temperature fluctuations of regulated and unregulated rivers in the Flathead River Basin.
in the Whitefish River (Graham, 1980). After impoundment a significant shift toward river spawners was noted (Hanzel, 1964) with a large portion of the systems kokanee spawning in the main stem Flathead River below its confluence with the South Fork. According to Graham (1980), the reason for increased river spawning has not been determined but is probably related to warmer winter water temperatures in the main stem river due to discharges from Hungry Horse Dam.

Studies of the impacts from altered temperature on other fishes in the drainage have been limited. For species of sculpin living in the Flathead River system, little is known about specific aspects of the *Cottus* life history, habitat requirements, and population dynamics (Graham, 1980). There are two species of sculpin commonly found within the drainage: *(Cottus cognatus)* the slimy sculpin and *(Cottus confusus)* the shorthead sculpin (Brown, 1971). A third species *(Cottus biardi)* the mottled sculpin, has been identified in a North Fork tributary (Graham, 1980), but this is under further investigation and has not been supported by other reports. The morphological characters of *C. confusus* and *C. cognatus* are so similar that field identification is extremely difficult and identification using morphometric and meristic characters is often inaccurate (Graham, 1980).

A recent study (Zimmerman and Wooten, 1980) examined electrophoretic variation in proteins encoded by 33 gene loci for 16 populations of *C. confusus* and six populations of *C. cognatus* from the Flathead river system. It was
determined that the two species could be separated based on the expression of alternate alleles at six gene loci. Allozymic variation indicated a narrow zone of hybridization between the two species of Cottus taken from one locality below the reservoir. On this basis it was suggested that hybridization is occurring in the disturbed habitat where hypolimnion release of water has resulted in altered thermal regimes and habitat structure.

In determining the effects of temperature upon the two species of sculpin in the drainage, both a lethal effect and a directive effect can be investigated as determined by (Fry, 1947). Under Fry's definition a lethal effect is either a high temperature or a low one sufficient to kill an organism in a finite time that would otherwise be within its normal lifespan. A directive effect occurs when temperature influences the spontaneous movement of organisms. For example, a directive effect may occur when fish are caused to aggregate in a certain specified region of a temperature gradient when exposed to a wider choice of temperatures.

In describing the responses of fish to temperature under laboratory conditions, investigators (Fry, 1947 and Brett, 1952) have established descriptions of the environmental history of a given fish. These investigators have determined that acclimation and acclimatization can affect the thermal responses of fish. An artificial stabilization of the environmental history of a fish is designated as acclimation. In measuring the effects of temperature in the laboratory, fish are often acclimated by maintaining them at a constant
temperature for several days or weeks. Depending upon the acclimation temperature a fish has been maintained at, the temperature tolerance and the preferred temperature of that fish can vary (Ferguson, 1958).

Acclimatization, on the other hand, concerns the environmental history of a fish determined by the natural conditions of the climate in which it exists prior to any laboratory test. Consequently, the influence of the natural environment from fertilization up to the period of testing must be considered (Rose, 1964). When a fish is captured in the field its acclimatization state is unknown. The fish has usually been subjected to varying temperatures (with behavior playing a role in the temperatures experienced) and has also been subjected to numerous nonthermal factors which can affect acclimatization, including photoperiod or other seasonal influences (Reynolds et al. 1977; Barans and Tubb, 1973). Investigators seeking to minimize the variability in thermal responses of fish under laboratory conditions have controlled the acclimatization factors by rearing fish under identical conditions from the egg stage (Brett, 1952).

A determination of temperature influences upon fish from a lethal effect can be investigated both at upper and lower tolerance levels. The upper and lower limits of temperature which fish can withstand define the extreme of a tolerable thermal environment. While a variety of experimental procedures have been used to determine the lethal limits (Brett, 1944; Fry, 1947), in each case an effort has been made to provide a simple index, usually expressed as
the mean or median temperature tolerance for a given sample of fish when exposed for some predetermined interval of time. Under carefully controlled conditions the upper lethal temperature is remarkably precise. Its sensitivity has permitted a comparison of species tolerance with significant differences obtained between closely related species (Brett, 1952). The lethal limits are only consistent within a species when repeated for fish from similar acclimation temperatures and comparable laboratory conditions (Brett, 1956). As the temperature of acclimation increases, the lethal temperature correspondingly increases (Fry, Hart and Walker, 1946; Brett, 1971). By working systematically with acclimation temperatures ranging from 0 C to the maximum tolerable for any given species an upper and lower boundary of thermal tolerance can be determined and graphed (Fig. 2).

The range of temperature in lethal effect determination can be divided into a zone of resistance over which it will operate to kill an organism in a determinate period of time and a zone of tolerance in which the life span of the organism is not influenced by the direct lethal effect of the temperature concerned (Fry, 1971). Temperature is not a lethal factor until the threshold between tolerance and resistance is reached. The boundary between tolerance and resistance is designated as the incipient lethal level (Brett, 1956). The period of tolerance prior to death for an individual fish is known as the resistance time (Fry, 1947).
Fig. 2 LETHAL TEMPERATURE RELATIONS FOR TWO SPECIES OF FISH.

The bullhead, *Ameiurus nebulosus*, is a highly tolerant species in contrast to the chum salmon, *Onchorhynchus keta*. The area enclosed by each trapezium is the zone of tolerance (Data from Brett, 1944 and 1952).
The lethal temperature is theoretically conceived as the temperature within which fifty per cent of the population die after indefinite exposure. The duration of the experimental test has been subject to variation with each investigator settling on an index suitable for the demands of the experimental design (Brett, 1952). The question of deciding when a fish has died from higher temperatures seems to present little trouble (Brett, 1944; Hart, 1947). A cessation of respiratory movements and muscular contraction accompanied by complete loss of response to stimuli have been regarded as decisive criteria (Brett, 1952).

Many studies have shown that fish placed in laboratory thermal gradients tend to spend most of their time within a narrow range of temperature (Fry, 1958). The response can be described quantitatively as the temperature range where the frequency of occurrence is greatest when observations are made periodically over a reasonable length of time. The term given this modal value is usually the "preferred temperature". Fry (1947) has defined the preferred temperature as "the region, in an infinite range of temperature, at which a given population will congregate with more or less precision," and the final preferendum as "a temperature around which all individuals will ultimately congregate regardless of their thermal experience before being placed in the gradient." Fry (1947) has further indicated that a final preferendum may be defined as the temperature where the preferred temperature equals the acclimation temperature.
Like the lethal temperature, the preferred temperature is subject to variation due to acclimation and acclimatization. Factors such as light, conditioned responses related to feeding routines, seasonal variation, and social behavior can interfere with the expression of the response to temperature (Ferguson, 1958). Ferguson (1958) found that subdued lighting conditions were most commonly needed for preferred temperature experiments with fish, however, full daylight was required in experiments with some fish. Barons and Tubb (1973) and Cherry et al. (1975) showed that seasonal variation can affect temperatures selected by fish species.

The most widely used method of presenting a temperature gradient to a fish is the longitudinal horizontal gradient. In this method the fish are placed in a trough in which the water changes temperature from one end to the other so that the gradient and swimming path of the fish are forced into the same plane and are parallel (Javaid and Anderson, 1967; Ogilvie and Anderson, 1965).

A second method of determining thermal preferenda has been to establish a vertical thermal gradient (Brett, 1952; Ferguson, 1958) in a tank large enough to allow the fish freedom in a horizontal path and in which temperature selection is affected by the fish swimming higher or lower. In this chamber the fish may react to depth as well as temperature (Garside and Tait, 1958).

A third method utilizes a single-choice trough rather than a gradient of choices and is designed after a horizontal
linear trough (Cherry et al., 1975).

More recently developed test apparatus allow an individual fish to serve as its own thermostat in regulating the temperature of the tank it occupies (Beitinger, Magnuson et al., 1975).
METHODS

Collection

We collected sculpin from river sites which Zimmerman and Wooten (1980) had established as having populations of a single species. Sculpin were collected during the months of June, July, August, and September of 1981 from the Flathead River Basin.

*Cottus confusus* were collected from the following areas: North Fork Flathead River at (1) mouth of Camas Creek, (2) 3 km below the mouth of Deep Creek, and (3) Blankenship Bridge at the confluence of the North & Middle Fork; Middle Fork Flathead River at (4) 0.3 km above Blankenship Bridge, and (5) 0.4 km below West Glacier.

*Cottus cognatus* were collected from the following areas: Mill Creek at (6) 0.3 km below the junction of Mill Creek and Highway 35; Swan River at (7) 13 km NW Swan Lake, (8) mouth of Coat Creek, (9) mouth of Holland Creek; and Lost Creek at (10) 4 km South Swan Lake (Fig. 3).

Sculpin were located by disturbing the substratum of riffle areas and pools. Fish were then collected with rectangular aquatic insect sampling nets. Only those sculpin between 4 and 10 cm were kept for use in the thermal experiments.

Following collection the sculpin were separated by
Fig. 3 Flathead River Basin sampling sites for C. confusus and C. cognatus.
species into 44 by 25 by 30 cm white fiberglass troughs. The bottoms of the troughs were covered with a gravel layer and rocks up to approximately 15 cm in size. A continuous stream of 8.3 C water from Yellow Bay Creek provided a continuous supply of fresh aerated water and removed wastes. The room containing the tanks was not lighted except for feeding periods. During the time the sculpin were kept at the Flathead Lake Biological Station except for testing periods, the sculpin were provided a continuous supply of insects supplemented with chopped earthworms.

**Acclimation**

The acclimation procedure was the same for each of our experiments. In each case the acclimation time was established at two weeks. At each acclimation temperature two adjacent white fiberglass tubs 75 by 65 by 60 cm were used to acclimate the two different species. The bottom of each tub was covered with a 20 cm layer of rocks. Water for the acclimation tubs was 8.3 C Yellow Bay Creek water mixed with creek water heated to 29.4 C to achieve the correct acclimation temperature. The water was divided with a T-junction and equal flows were directed to each tub. A center drain fitted with a screen-covered standing drain pipe maintained the water level in the tubs at a depth of 25 cm.

**Thermal Gradient Trough**

The thermal gradient trough used was based on horizontal gradients developed by others (Ogilvie and Anderson,
A temperature gradient was established by slowly changing the temperature of water as the water flowed through a long continuous trough. We made one significant change in the procedure. The water was cooled rather than heated as it flowed through the gradient trough. By cooling the water, gas supersaturation problems which have been associated with heating water were avoided (McCauley, 1977).

The gradient trough was a rectangular metal trough 228 by 6 by 6 cm. A screened one-half inch inlet pipe was placed flush with the inside of one end of the trough and an identical outlet pipe was placed at the opposite end. By adjusting the flow of water entering the trough and the amount of water siphoned from the outlet a constant depth of 4.5 cm was maintained. We used a water cooling system to cool the water as it flowed through the trough. The gradient trough was placed in a 244 by 25 by 30 cm fiberglass tank which had a circulating supply of 8.3 C water. This cold water surrounding the gradient trough entered the fiberglass tank adjacent to the gradient trough outlet. The cold water flowed within the fiberglass trough in a direction opposite to the flow of water in the gradient trough. A standing overflow drain adjacent to the gradient trough inlet maintained the water level in the fiberglass tank of the same height as the water in the gradient trough. Water entered the gradient trough at 20.0 C and was cooled at a uniform rate of 0.08 C per cm to 12.2 C at the outlet.
To facilitate position recording, the top edge of the gradient trough and the adjacent wall of the fiberglass tank were marked off into 15 sections each 15.24 cm long.

The lowest possible light conditions which still allowed observation were used. A single flourescent light the length of the gradient trough was positioned so that no direct light reached the trough and all areas of the trough were uniformly illuminated.

We observed the fish at a distance by the use of inclined mirrors placed directly above the gradient trough. This allowed the observer to see all areas of the trough without disturbing the fish.

Fish of each species were acclimated in groups of ten to each acclimation temperature. The acclimation temperatures used were 8.3 C, 11.1 C, 13.9 C and 16.7 C.

For each trial a maximum of five fish were placed in the gradient trough at the position corresponding to their acclimation temperature and allowed to orient for 1 hr. During the second hr the number of fish in each of the 15 positions in the gradient trough was recorded at two-min intervals.

Two control tests were run with the gradient trough to determine fish preference to positions in the trough and orientation to the directional flow of water. One control trial was run with a constant temperature of 8.3 C and no water flow within the trough. The second trial was run with the constant temperature of 8.3 C and a uniform water flow.
Single-Choice Trough

To supplement the results of the thermal gradient we used a single-choice or avoidance trough. The single-choice trough is a simple trough which employs two separate streams of differing water temperature each flowing from opposite ends toward a central drain (McCauley, 1977; Cherry et al., 1975). This trough maintained two distinct temperatures from which the fish could choose.

A 45-cm section of four-inch PVC drain pipe with end caps was cut longitudinally to form a trough 6 cm deep in the center of the trough. A screened one-half inch pipe was installed flush with the inside of each end cap for water inlets. A series of 2 mm holes was drilled in the center of the trough to provide a central drain. In the center of the trough along both edges, a 2-cm long notch was cut to function as an overflow drain. The water temperatures for the inlets were 8.3 C and 13.9 C. The two distinct temperatures were maintained in the trough to within 3 cm of either side of the central drain.

Light for observation was the lowest level possible. A single shielded 60 watt bulb was placed so that the trough was not in direct light. No shadows were produced and the whole trough was uniformly illuminated.

The same inclined mirrors used within the gradient trough trials were used to observe the fish.

Ten fish from each species were acclimated to each acclimation temperature. The acclimation temperatures used were 8.3 C, 11.1 C, and 13.9 C. For each trial a maximum of
of five fish were placed within the trough and left to orient for 30 min.

During the following 30 min the number of fish occupying both sections of the trough was recorded at one-min intervals.

Upper Lethal Determination

The method of lethal temperature determination as conducted by Brett (1952) was utilized with both species of sculpin tested. Each set of lethal baths was regulated to a constant temperature with four sets of baths at temperatures of 21.7 C, 22.8 C, 24.4 C and 25.6 C. Sculpin were acclimated for two weeks prior to the test at 8.3 C. Groups of five fish from each species were placed in the four sets of temperature baths. Records of the times to death for all fish were kept by close inspection at 5-min intervals for the first hr and at 15-min intervals for the remaining 11 hrs. A cessation of respiratory movements and a loss of response to physical stimuli were taken as criteria for death. A check was done immediately after "death" by removing the fish to a lower temperature and checking for recoveries. Fish that did not die within the 12 hr test period were assumed to be tolerant to the lethal effects of the temperature concerned.
RESULTS

Thermal Gradient Trough

The results of the control tests indicated an attraction to the ends of the trough but no orientation to the directional flow of water. At a constant temperature of 8.3°C and no flow of water in the thermal gradient, the distribution of fish was clearly bimodal. Fish were found at the ends of the trough in approximately equal numbers far more frequently than at the intermediate locations. When flowing water of a constant temperature was used, a similar distribution was obtained.

The frequency distribution of each species in the thermal gradient was determined for each acclimation temperature (Table 1). The mode temperature for C. cognatus remained constant at 12.2°C for each acclimation temperature. The mode temperature for C. confusus was 12.2°C when acclimated to 8.3°C and 13.9°C and 20°C in acclimation temperatures of 13.9°C and 16.7°C respectively.

Single-Choice Trough

The percentage distribution in the single choice trough was calculated for both species at each acclimation temperature (Table 2). Both species selected the colder end of the trough at every acclimation temperature, but a greater percentage of C. confusus selected the warmer
<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>8.3</th>
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<td>cog.</td>
<td>con.b</td>
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<td>12.2</td>
<td>30.3</td>
<td>33.6</td>
<td>49.3</td>
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<td>12.8</td>
<td>20.9</td>
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<td>13.9</td>
<td>9.0</td>
<td>3.5</td>
<td>5.3</td>
<td></td>
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<td>4.9</td>
<td>6.2</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>15.0</td>
<td>1.0</td>
<td>3.9</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>15.6</td>
<td>1.4</td>
<td>1.6</td>
<td>2.0</td>
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<td>16.1</td>
<td>3.8</td>
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<td>4.0</td>
<td>.8</td>
</tr>
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<td>2.7</td>
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<td>6.6</td>
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<td>1.6</td>
<td>2.0</td>
<td>5.4</td>
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<td>3.8</td>
<td>6.2</td>
<td>1.3</td>
<td>6.1</td>
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<td>3.5</td>
<td>5.1</td>
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<td>20.3</td>
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<td>19.4</td>
<td>4.9</td>
<td>2.7</td>
<td>2.0</td>
<td>37.8</td>
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<td>20.0</td>
<td>2.1</td>
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<td>25.7</td>
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<td>Total No. Observations</td>
<td>287</td>
<td>256</td>
<td>150</td>
<td>148</td>
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<tr>
<td>No. of fish</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mode (°C)</td>
<td>12.2</td>
<td>12.2</td>
<td>12.2</td>
<td>13.9</td>
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</tbody>
</table>

Table 1. The percentage frequency distribution in a thermal gradient of *C. cognatus* and *C. confusus* acclimated to four different temperatures.
<table>
<thead>
<tr>
<th>Trough Temperature (°C)</th>
<th>8.3</th>
<th>11.1</th>
<th>13.9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cog.</td>
<td>con.</td>
<td>cog.</td>
</tr>
<tr>
<td>8.3</td>
<td>72.6</td>
<td>72.0</td>
<td>85.8</td>
</tr>
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<td>13.9</td>
<td>27.4</td>
<td>28.0</td>
<td>14.2</td>
</tr>
<tr>
<td>Total no. Observations</td>
<td>259</td>
<td>256</td>
<td>234</td>
</tr>
<tr>
<td>No. of fish</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 2. The percentage frequency distribution in a single-choice trough of *C. cognatus*[^a] and *C. confusus*[^b] acclimated to three different temperatures.

[^a]: Reference to a footnote indicating the acclimation conditions for *C. cognatus*.
[^b]: Reference to a footnote indicating the acclimation conditions for *C. confusus*. 
temperature in each trial.

**Upper Lethal Determination**

For each species at each acclimation temperature both the percent mortality and the mean time of death were determined for each lethal bath temperature (Table 3.) Both species showed an increased tolerance at successively higher acclimation temperatures. *C. confusus* had longer resistance times at all four lethal temperatures.
<table>
<thead>
<tr>
<th>Lethal Bath Temperature (°C)</th>
<th>21.7</th>
<th>22.8</th>
<th>24.4</th>
<th>25.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Mortality</td>
<td>25.0</td>
<td>50.0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ave. Time to Death (min.)</td>
<td>255</td>
<td>277</td>
<td>41</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 3. The percent occurrence of death and average times of resistance for *C. cognatus* and *C. confusus* in lethal water baths of varying temperatures. An acclimation temperature of 8.3 °C was used for all trials.
DISCUSSION AND CONCLUSION

The results of the thermal gradient experiment are not a clear representation of the preferred temperatures. The mode temperature selected by the sculpin of both species was one of the two end positions in the trough in all but one trial. Because of this attraction to the ends of the trough we can only draw general conclusions about temperature selection by comparing which end of the trough was most favorably selected by the fish. At acclimation temperatures of 8.3 C both species selected the coldest end of the trough. At acclimation temperatures of 11.1 C, 13.9 C, and 16.7 C _C. cognatus_ continued to select the coldest position in the trough, but _C. confusus_ selected the warmest end. This suggests that _C. confusus_ has a warmer preferred temperature than _C. cognatus_ at equal acclimation temperatures and that the temperature selected increases as the acclimation temperature is raised.

The preference of the fish for the end positions of the trough could represent a tactile response. The results of the control tests showed no reaction to the directional flow of water, but suggest a strong thigmotactic response of the fish to both ends of the trough. Several gradient trough experiments have shown a similar bimodal distribution before the gradient had been established, but in each case
the attraction to the ends of the trough appeared negligible when the gradient was established (Ogilvie and Anderson, 1965; Reynolds et al., 1977). However, with the sculpin the bimodal distribution was still apparent after establishing the gradient. Apparently, the tactile response of the fish to the walls and corners of the trough was stronger than the temperature selection response at the temperatures used.

It may be that the sculpin's natural environment and defense mechanisms place a priority on tactile stimulation and cover that is not found in most other free-swimming fish. Sculpin inhabit riffle areas of streams and rivers where there is an abundance of rocks and rubble and are usually absent from pools where the bottom is sandy or clay (Bailey, 1952). In addition, sculpin are nocturnal feeders that remain hidden from predators during the day under rocks or other cover. At night they come out to feed on aquatic insects and invertebrates but rarely leave the substratum.

Sculpin normally have a restricted movement and possibly are not prepared to deal with water temperature fluctuations as most fish can. Emery (1970) showed that within a natural sieche, rapid temperature fluctuations that were easily avoidable by other fish were fatal to C. bairdi. The restricted movements of the sculpin and an inability to escape temperature fluctuations along with a constant substratum contact may suggest that the temperature selection of the sculpin is secondary to a tactile response.
A thermal gradient with an extended length and extended temperature range would possibly be more effective in determining temperature preference for the sculpin. An extended trough with temperature extremes that were completely uninhabitable could force the sculpin into the middle sections of the trough where the tactile stimuli would be equivalent for all positions.

In the design and use of the single-choice trough we hoped to avoid the suggested tactile response observed for fish in the gradient trough. The design of the single-choice trough provided only two temperatures, but the physical dimensions of the trough and the water flow were identical for both temperatures. In repeated trials using different temperatures we could have theoretically pinpointed the preferred temperature for each species, but we were limited by the range of water temperatures available for use.

The tactile response was eliminated as an influencing factor in the single-choice trough and the results were successful within the temperature range in which we were able to work. The results show that, with water temperatures of 8.3°C and 13.9°C in the trough, both species selected 8.3°C at all three acclimation temperatures, but a higher percentage of C. confusus selected the warmer water in each trial. In this experiment both species showed an increase in the percentage of fish selecting the lower temperature as the acclimation temperatures increased. This type of inverse reaction to increasing acclimation temperature has been observed before (Garside and Tait, 1958;
Brett, 1952; Zahn, 1962, cited in Javiad and Anderson, 1967; Ogilvie and Anderson, 1965) but is opposed both to the normal response recorded for most fishes and the results from our thermal gradient experiment. Although the acclimation relationship is not normal, a comparison of the two species in each trial showed *C. confusus* consistently selected the warmer temperature.

The results of the upper lethal temperature determination indicate a greater capacity to withstand higher temperatures in *C. confusus* than in *C. cognatus*. *C. confusus* showed greater tolerance to higher temperatures both in the percent of fish which showed tolerance at each temperature and in the length of resistance when the temperature proved to be lethal. These results correspond well with the results of both the thermal gradient trough and the single-choice trough in suggesting that *C. confusus* has a higher temperature tolerance and preference than that of *C. cognatus*.

When comparing the results of thermal studies to other experiments or to fish in natural environments, variations in sample populations and testing procedures must be considered. Variation in thermal histories, size and age, as well as acclimation times under laboratory conditions may have influenced the thermal responses of the fish.

Since the fish used were collected from a variety of rivers and sampling sites, a possible variation in acclimatization histories must be considered (Reynolds and Casterlin, 1979). Even within the same river system thermal histories
of individual fish can vary. The fish were collected over a 3-mo period that included a seasonal variation that may have affected thermal responses (Barans and Tubb, 1973; McCauley, 1977).

The fish collected may not be truly representative samples because different collecting methods were necessary at different collecting sites. In all areas we were limited by the depth of water in which we could effectively work. In the larger rivers this made sampling possible only along the river edge, while in smaller streams we were able to sample from the entire stream. Often we were more successful in rocky pools of the larger rivers and the small riffle areas of the smaller streams.

The age and relative size of fish may also be a factor in temperature selection and lethal tolerance (Brett, 1956; Ferguson, 1958; Barans and Tubb, 1973). We tried to limit the size of the fish used within the trials, but there was variation in both size and age that could be a possible source of variation from results received with a homogeneous age group.

Our collecting methods also created a variety of acclimation times. The 2-week acclimation period before each experiment was uniformly controlled. However, since we collected fish over several months, varying periods of maintenance in an artificial environment were required. The artificial environment in our laboratory had a characteristic temperature, food supply, oxygenation, density of fish, water chemistry and photoperiod which could affect
the temperature selected by the fish (McCauley, 1977; Ferguson, 1958; Reynolds and Casterlin, 1979). The acclimation period was severely affected by the presence of a fungus growth which occurred on fish at the upper acclimation temperatures. The fungus growth produced symptoms of sluggishness leading to the appearance of blistered skin and finally death. We did not use fish that were noticeably affected by the fungus in our trials. However, infected fish not showing symptoms of fungal infection could have been used during the trials and the results possibly affected.

In the thermal gradient acclimation period the fungal growth killed all of the *C. confusus* fish at 16.7 °C and several at 13.9 °C. Noticeable symptoms were present in some fish of both species at 16.7 °C and 13.9 °C and perhaps all the fish were affected to some degree at these temperatures. In acclimation periods for the single-choice trough, the fungus was present at both the 13.9 °C and 11.1 °C temperatures. A possible explanation for the inverse acclimation relationship in the single-choice trough trials was the presence of the fungus on acclimating fish. The sluggishness, characteristic of the infected fish would limit the exploration by the sculpin causing a higher percent of the time in the trough to be spent in the selected temperature. The fungus was most prominent on fish that were acclimating to the higher temperatures, and this corresponds with the results.
Beitinger and Fitzpatrick (1979) have suggested a relationship of thermal preferendum to the behavior of fish in a natural environment that involves three hypotheses:

1) The final temperature preferendum is species specific.

2) When other factors such as food and cover distribution are equal, fishes will tend to occur in nature at their thermal preferendum when it is available.

3) The final thermal preferendum will coincide with temperatures at which key physiological, biochemical and life history processes are optimized.

In addition, Brett (1956) has determined that fish reproduction is limited to a much more specific temperature range than most fish functions. Spawning activity, release of gametes, and rate of development of egg and embryo are each sensitive to temperature fluctuations in the environment. Our data suggests that the temperature preference of C. confusus and C. cognatus is species specific and this, in conjunction with Brett's work, supports the hypothesis of Zimmerman and Wooten on sculpin hybridization. We have shown that C. confusus selects for higher temperatures and has a greater thermal tolerance than C. cognatus when acclimated at the same temperatures. The difference in the species with respect to thermal preferences and tolerances shows that the altered temperature regimes below Hungry Horse Dam could be the cause of simultaneous reproductive activity and corresponding hybridization in the two species of Cottus.
LITERATURE CITED


