UNIVERSITY OF TORONTO
BIOLOGICAL SERIES NO. 60

Geographic Variations of Some Physiological and Morphological Characters in Certain Freshwater Fish

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Publications of the
Ontario Fisheries Research
Laboratory, No. LXXII

UNIVERSITY OF TORONTO PRESS: 1952

Printed in Canada
London: Geoffrey Cumberlege
Oxford University Press

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Abstract

Study of the geographic variation of lethal temperatures and diagnostic morphological characteristics in ten species of freshwater fish showed that the variability was relatively greater for the morphological characteristics. No evidence was found for physiological as distinct from morphological races, and many of the species studied possessed uniformity in lethal temperatures from Ontario to Tennessee or Florida. As a result, the lethal temperature findings did not confirm the accepted taxonomic status in several species with recognized subspecies over this range. In others, with geographic differences in lethal temperatures, there was agreement with the taxonomic status.

The discrepancies in the geographic variation between physiological and morphological characteristics appear to be associated with non-genetic morphological variation and with factors tending to suppress formation of geographic physiological races (wide lethal temperature limits, small geographic habitat differences within the species range and possibly low mutation rates for lethal temperatures). The lethal temperatures appear well above the thermal extremes commonly encountered in the environment and may therefore have little ecological significance.

GEOGRAPHIC VARIATIONS OF SOME PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERS IN CERTAIN FRESHWATER FISH

Introduction

The taxonomist today is attempting to carry out two tasks. The first is to classify the organic world according to phylogenetic relationships; the second is to study the process of speciation and evolution.

The most useful tool of taxonomy has been morphology. It has enabled biologists almost at a glance to classify the organic world into a series of units or species according to gross similarities and differences in structure. The species, at least in sexually reproducing animals, can be considered a natural unit.

The physiology of organisms has been considered for taxonomic purposes only in certain rare instances. It is utilized in those groups where morphological distinctions are not evident (bacteria, fungi) and also in other groups to strengthen the morphological findings. In almost every case, where it has been possible to compare the physiological characters of species differentiated morphologically, it has been found that conclusions based on physiological differences have confirmed the previous morphological findings. The most notable contributions in this respect have been the well-known surveys based on precipitin reactions (Nutall, 1904; and others), crystallography of haemoglobins (Reichert and Brown, 1909) and properties of plant starches (Reichert, 1919). Furthermore geneticists have found that there is every reason to believe that physiological characteristics are inherited in the same manner as morphological ones. Likewise, morphological differences have been considered as reflecting underlying physiological differences, and it has also been shown that structural characteristics, which seemingly have no adaptive significance, may nevertheless be linked with physiological characteristics which may have adaptive value (McEwen, 1918; Keeler and King, 1941; Heuts, 1947). Thus, although it has been recognized that physiological characteristics are perhaps the more important with respect to the survival of the species, morphological characteristics are the more convenient for taxonomic purposes and have been utilized almost wholly as synonyms of physiological ones.

This parallelism, however, often appears to break down when we consider the actual speciation process among smaller groups and populations within the species. In the first place, there is evidence that physiological differentiation may exist although there is no evident morphological differentiation, and therefore the important changes leading to the formation of a new species may be passed before ever a structural difference has been formed. This problem can be attacked by studying both the structural and physiological differentiation among populations of a species. The question has been investigated by the writer for a series of species of fish.

In the second place, reasoning from morphological attributes may not apply to physiological ones with respect to the direct action of the environment on the individual. Environmental factors, such as temperature, food, or moisture, may completely alter the phenotypic expression of a genetic factor, largely by their action during the early stages of development (Hubbs, 1918, 1926; Mottley, 1931; Martin, 1948).

Certain physiological characteristics, however, may be modified rapidly and reversibly by environmental influences during any stage of the life history of the animal. Thus, any previous differences caused by the environment can be erased by such induced changes, whereas the characteristics mentioned in the previous paragraph are fixed at an early stage in development. These physiological characteristics, such as temperature tolerance or low oxygen tolerance, can be changed by a process called acclimation or acclimatization.

The term acclimation has been used to signify definite physiological changes produced in an animal by experimental conditions in the laboratory (Hathaway, 1927), and the term acclimatization has been restricted to the broader process of adjustment to different climates within the same generation. Problems of gradual adaptation (racial acclimatization) of organisms to different climates or environments through selection over a number of generations are outside the scope of this paper.

The consequence to the taxonomist of rapid and reversible change in characteristics through acclimation is that he can compare a series of populations under the same environmental history, and attempt in this way to sort out inherent from acquired (through acclimation) geographic physiological differences without crossing or transplanting the various samples. Of course, the modification of physiological characteristics through acclimation may not be the only way in which the environment influences these characteristics. It is also possible that body size, seasonal changes, growth rates, or early life history may exert some influence. The effects of size and season apart from acclimation have been considered in subsequent sections of this paper.

The method adopted in this study has been to acclimate samples of fish to the same temperature levels in different localities. These temperature levels are the "standards" by which the populations are compared. When once acclimated, the temperatures required to kill the samples of fish are compared geographically. Lethal temperatures have been chosen as a criterion since there is a large amount of evidence that the acclimation process is rapid (Davenport and Castle, 1895; Loeb and Wasteneys, 1912; Wells, 1914; Sumner and Doudoroff, 1938; Mellanby, 1940; Brett, 1946; Mills, 1933; and others) and the determinations are quite precise.

In addition, certain morphological characters of the fish in the samples were measured to obtain an understanding of the relationship between lethal temperatures and diagnostic characteristics used for taxonomic purposes. An attempt has been made to compare both the variation in morphology of fish from the same population in relation to resistance to high temperatures and also the geographic variation in morphology in relation to lethal temperatures. The lethal temperature variation has been finally discussed in relation to morphological variation, to taxonomy of the species, and to the thermal characteristics of their environments.

The data were obtained in the years 1945–7 and are included in more complete form in a doctoral thesis filed in the library of the University of Toronto. In this thesis the following tabular data are given that are excluded in this report: Length of time used for acclimating fish, mortality in acclimation tanks, median mortality times and incipient lethal temperatures with their experimental errors for each species, analyses of variance for geographic variation in *Notemigonus crysoleucas*, physiological data for respiratory tolerance and stroke output of the heart, and comparison of distribution limits with average maximum summer temperatures and lethal temperatures.

ACKNOWLEDGEMENTS

The study was undertaken with the aid of a liberal grant furnished by the Committee for Scientific Research of the University of Toronto and of personal support from the Department of Veterans

Affairs. The work was made possible by the help of my wife, and by the scientific guidance of Dr. F. E. J. Fry who inspired the undertaking of this research. Thanks are also due to Dr. J. R. Dymond, F. P. Ide, and other members of the writer's graduate committee. The writer also wishes to express his indebtedness to Dr. T. H. Langlois of the Franz Theodore Stone Laboratory at Put-in-Bay, Ohio, to Dr. J. S. Rogers and others of the University of Florida, and to Mrs. F. A. Ault and the late Dr. E. B. Powers of Knoxville, Tennessee, for providing laboratory facilities and living quarters at these respective localities.

MATERIALS

The material chosen for this study consisted of native fish collected in the field. The equipment was transported to the different regions where the work was carried out. Throughout, every effort was made to hold the fish the minimum possible time prior to experiments, in most cases not over four or five days. It is felt that this precaution enabled the procuring of data on many species which would otherwise not have been possible owing to deterioration during long storage. However, some species were held in the laboratory for much longer periods of time.

Collections

Living material for experimental work and preserved material for morphological investigations were both collected at the same time. The fish to be preserved were placed directly in 10 per cent formalin on capture. Supplementary morphological data were obtained from the fish used in the lethal experiments.

The fish were collected by seine for the most part and to a limited extent by trap. A modified seining procedure was found which greatly reduced the mortality caused through damage to the fish in the process of capture. The seine was never removed from the water with fish in it. A pocket containing the fish was formed and they were then removed and placed in tanks by means of a dipper without ever being actually taken from the water.

Storage of fish

The fish were held in tanks at various constant temperatures for a sufficient but minimum length of time for the completion of acclimation to that particular temperature. When the storage temperatures were but slightly above environmental temperatures it was necessary to hold them for only a few days prior to lethal temperature tests. When the fish were held for longer periods feeding was necessary. Where possible the fish were fed living food such as small fish, insects, and worms. During winter experiments in Toronto they were fed largely ground beef liver with cod liver oil added. Compressed air and running water were supplied throughout the storage period.

In general the fish fed well during the course of storage. However, some species did not feed adequately in captivity. For example, gizzard shad and lake shiners rapidly become emaciated at high temperatures owing presumably to elevated food requirements that they do not satisfy. For short periods of captivity no serious errors appeared to be introduced by starvation and hence the only safeguard was to reduce the storage time as much as possible by rapid acclimation. Brett (1944) found that there was no difference during the summer months in lethal temperature of the bullhead, *Ameiurus nebulosus,* starved for various lengths of time up to forty days. However, perch appear to deteriorate after long starvation and to give erroneous lethal temperature results. These observations appear to be in agreement with findings of Krogh (1939) for *Ameiurus* and *Perca flavescens* where the former species does not lose mineral rapidly when starved but the latter does.

Mortality

It was found impossible to avoid mortality of fish in the tanks. In almost every collection some fish died during the acclimation period. Detailed records were kept throughout and lots were not used in experiments when the fish were unhealthy.

It is recognized that mortality during acclimation may be selective, since the less resistant individuals that die might also be the least resistant in experiments and results obtained without them would thus be too high. When mortality is due to disease such as *Saprolegnia,* those individuals with the visible disease usually die before their unaffected neighbours in an experiment, and the cause of the discrepancy is obvious. When there is no visible sign of disease, however, there is rarely evidence to suggest any selective effect. This viewpoint is supported by the fact that most of the mortality occurs sharply during the first few days of storage and levels off quite sharply so that fish that are "dying" are not used in experiments. The early fortuitous mortality is correlated with the treatment of the fish during capture where scales and mucous are invariably rubbed from some of them. This treatment is random. No correlation has been noted between lethal temperatures and percentage mortality when the stock was healthy at the time of the tests. Death from unknown causes was on the whole rare. It occurred, however, in *Ameiurus nebulosus* and *Perca flavescens* which were held for long periods of time during the winter at Toronto, and also in certain lots of Florida *Micropterus salmoides*. These mortalities were not necessarily associated with development of fungus but with other types of disease and led to deterioration of the stock. The fish would not take any food and they would not acclimate properly to higher temperatures. In later experiments with perch (McCracken and Starkman, 1948), where the fish were induced to feed on mealworms, no difficulties with acclimation were experienced. Erroneous results, such as those obtained above, had to be excluded when they were found incorrect following repetition of the experiments under more favourable circumstances.

Species investigated

The list below gives the species studied together with the various geographic regions where studies were carried out.

Clupeidae

Dorosoma cepedianum (Le Sueur), Gizzard shad.
  Put-in-Bay, Ottawa Co., Ohio.
  Loudon Lake (Tennessee R.), Knox Co., Tenn.

Cyprinidae

Semotilus atromaculatus atromaculatus (Mitchill), Northern Creek chub.
  Don R., York Co., Ont.
  Willow Fork, Beaver Creek, and Freeway Branch of Bull Run creek, Knox Co., Tenn.
  Hinds Creek and Braden Branch, Anderson Co., Tenn.
Rhinichthys atratulus meleagris Agassiz, Western blacknose dace.
  Don R., York Co., Ont.
R. a. obtusus Agassiz, Southern blacknose dace.
  Willow Fork and Freeway Branch, Knox Co., Tenn.
  Hinds Creek and Braden Branch, Anderson Co., Tenn.
  Roaring Fork Creek, Sevier Co., Tenn.
Notemigonus crysoleucas auratus (Rafinesque), Western golden shiner.
  Algonquin Park, Ont.
  East Harbor, Catawba Island, Ottawa Co., Ohio.

N. c. bosci (Valenciennes), Florida golden shiner.
  St. Johns R. and tributary lakes and streams, Putnam Co., Fla.
Notropis atherinoides atherinoides Rafinesque, Lake emerald shiner.
  Chippewa Creek (Welland R.), Welland Co., Ont.
  Put-in-Bay, Ottawa Co., Ohio.
N. cornutus chrysocephalus (Rafinesque), Central common shiner.
  Willow Fork and Freeway Branch, Knox Co., Tenn.
  Hinds Creek, Anderson Co., Tenn.
N. c. frontalis Agassiz, Northern common shiner.
  Don R., York Co., Ont.
Hyborhynchus notatus (Rafinesque), Bluntnose minnow.
  Etobicoke Creek, York Co., Ont.
  Put-in-Bay, Ottawa Co., Ohio.

Ameiuridae

Ictalurus lacustris lacustris (Walbaum), Northern channel catfish.
  Sandusky Bay, Ottawa Co., Ohio.
I. l. punctatus (Rafinesque), Southern channel catfish.
  St. Johns R. and tributaries, Putnam Co., Fla.
Ameiurus nebulosus nebulosus (Le Sueur), Northern brown bullhead.
  Algonquin Park, Ont.
  Rouge R., York Co., Ont.
  Sandusky Bay, Ottawa Co., Ohio.
A. n. marmoratus (Holbrook), Marbled brown bullhead.
  St. Johns R. and tributaries, Putnam Co., Fla.
A. natalis natalis (Le Sueur), Yellow bullhead.
  St. Johns R. and tributaries, Putnam Co., Fla.

Poeciliidae

Gambusia affinis affinis (Baird and Girard), Western mosquito fish.
  Loudon Lake, Knox Co., Tenn.
G. a. holbrooki Girard, Eastern mosquito fish.
  St. Johns R. and tributaries, Putnam Co., Fla.

Percidae

Perca flavescens (Mitchill), Yellow perch.
  Black creek, Simcoe Co., Ont.
  Put-in-Bay, Ottawa Co., Ohio.

Centrarchidae

Micropterus salmoides salmoides (Lacépède), Largemouth bass.
Put-in-Bay and East Harbor, Catawba Island, Ottawa Co., Ohio.
Loudon Lake, Knox Co., Tenn.
St. Johns R. and tributaries, Putnam Co., Fla.
Lepomis macrochirus purpurescens (Cope), Bluegill.
St. Johns R. and tributaries, Putnam Co., Fla.

The nomenclature of the above species is based on Hubbs and Lagler (1947) for species in the Great Lakes Region, Kuhne (1939) together with a supplementary list supplied by the T.V.A., Norris, Tennessee, for the Tennessee Region, and Carr (1936) for the Florida species. Changes in the nomenclature later than those given in these papers have also been incorporated when available.

The time and place of experiments varied. Work in Ontario was carried on at the University of Toronto during the winter of 1945–6, and in the summer of 1947. The Ohio species were studied in the summer of 1946 at the Franz Theodore Stone Laboratory at Put-in-Bay, Ohio. Species listed for Tennessee were studied during the summer of 1947 at Knoxville, and the Florida species during the winter of 1946–7 at the University of Florida Conservation Reserve, Welaka.

METHODS

The fish were acclimated to the same temperature levels at different geographical locations and the upper and lower lethal temperature relations determined at these acclimation temperatures. Temperature was controlled to within ±0.2°C. during acclimation and ±0.1°C. during the lethal temperature experiments. Oxygen was kept at a high level and CO_2 at a minimum by means of running water or aeration, so that these factors would not interfere with the results. It was the desire of the writer to study only the lethal effects of temperature. Unfortunately other factors, such as the physical and chemical properties of the water used in different places, were not controlled. Although changes in lethal temperatures of animals with different salinity levels have been noted in the literature (Davenport and Castle, 1895; Zoethout, 1898; Loeb and Wasteneys, 1912; Sumner and Doudoroff, 1938), it will be indicated later that errors due to variations in physical and chemical properties of the water probably were not serious here except, perhaps, in one or two instances. Some properties of the water in the different places are shown in Table I. In Tennessee it was not possible to use running water owing to chlorine and copper contamination; therefore raw creek water was used and kept oxygenated by aeration alone. Analyses of this water before and after holding fish for a period of time are shown in Table I.

Details of acclimation

The lethal temperature method, modified from that of Hathaway and various other authors, has been described previously in various papers by Fry *et al.* (1942, 1946, 1947). Originally it was felt that acclimation to any particular temperature could be carried out at any time regardless of season. In essence this is probably quite true, but for practical purposes it was necessary to carry out work at low acclimation temperatures in the winter and high acclimation temperatures in the summer. Errors arising from disregard of this principle will be considered under seasonal effects (p. 15) and appear to be associated with the rate of acclimation and possibly specific seasonal differences in lethal temperatures for a given acclimation temperature.

Because work in a particular locality was carried out in either summer or winter it was not feasible to acclimate fish to temperatures over their whole thermal range. Apart from the winter work in Toronto most of the acclimations were made when environmental temperatures were above 15°C. Geographic comparisons were made at two or more acclimations five centigrade degrees apart at or above 15°C.

Lethal temperature determinations were carried out only after it was reasonably assured that acclimation was complete following storage at a given temperature. The uncertainty of this state constituted the most serious error in the lethal temperature method. The only empirical assurances were the approximations to former determinations on the same species and repetition of lethal temperature determinations on batches of fish from the same storage lot after different periods of time.

Rate of acclimation to a given temperature has been studied by various authors (Loeb and Wasteneys, 1912; Sumner and Doudoroff, 1938; Doudoroff, 1942; Brett, 1944, 1946). It has been found that it proceeds much faster from a lower to a higher than from a higher to a lower temperature. Furthermore, acclimation as judged by rate of gain of heat tolerance proceeds more quickly the higher the temperature. Therefore, by utilizing the upper part of the thermal range for most of the species studied, and always acclimating the fish to temperatures above habitat temperatures, acclimation was for the most part very rapid and only three or four days' storage time was necessary. During the winter experiments in Toronto (Hart, 1947), however, acclimation appeared to be very slow and there is grave doubt that reliable results were obtained for some species.

Lethal temperature determination

The descriptions of various manifestations of death at high and low temperatures (Wells, 1914; Britton, 1923; Doudoroff, 1942) are in excellent agreement with that found for the present species. The criterion of death was a complete cessation of all movements either spontaneous or induced. Occasionally recovery occurred in certain species (particularly bullhead and channel catfish) after removal from the tanks. A few minutes additional exposure eliminated this and no appreciable error resulted.

The whole upper lethal range for each acclimation level has been studied by measuring both the time to death of samples where all the fish die, and the ultimate temperature at which just 50 per cent of the fish die. The former is termed the *resistance time,* and the latter the *incipient upper lethal* (Fry *et al.,* 1946). The resistance time is a series of determinations expressing the relation between temperature and time to death of 50 per cent of the sample. In practice, a sample of five fish was removed from the acclimation storage tank and subjected to a high temperature that killed 50 per cent of the fish in roughly ten to twenty minutes. Further samples were then removed and placed at lower and lower temperatures 0.5 centigrade degrees apart. The time to death of these samples became progressively longer until finally certain individuals of a sample were no longer killed after exposure to the elevated temperature for perhaps several days. At still lower temperatures only one or two individuals were killed, and from a series of values thus determined, the incipient upper lethal temperature was calculated.

In several species lower incipient lethals were determined also. Since lower lethal resistance times were not determined, it was not always possible to tell how long lower lethal experiments should be continued in order to place the incipient lower lethal. Although it is realized that the results might be too low, most lower lethal experiments were not continued for more than twenty-four hours. If the fish were still alive but showed signs of dying after twenty-four hours at a low temperature the experiment was continued until a final decision could be reached. It has been indicated (Doudoroff, 1942) that it may require many days to attain the incipient lower lethal, but for the purpose of comparison of different races the twenty-four-hour incipient lethal appears adequate.

A description of other characteristics of lethal temperature relationships has been given by Fry *et al.* (1942, 1946) and by Hart (1947). These concern the course of change in the temperature pattern as the acclimation temperature is changed. Other attributes considered in this study are the *ultimate incipient upper lethal* and the *thermal tolerance.* The former is the highest possible level to which the species can be acclimated, and the latter is the zone within which no lethal effects are experienced, expressed quantitatively as the area within the lethal levels over the range in which the species can be acclimated. Comparisons of thermal tolerance were utilized only for *Notemigonus crysoleucas* and *Ameiurus nebulosus,* since the data were too inadequate to permit its evaluation in other species.

A criticism often levelled against the "sudden jump" lethal temperature technique is that the fish might die from "shock" resulting from the change in temperature rather than from the normal course of death expected from the elevated temperature itself. The prevalence of this belief is indicated by the hatchery procedure of tempering prior to planting of fish in waters differing in temperature from the fish storage temperature. However, Brett (1941) showed that tempering had no effect on the lethal temperatures of speckled trout. In the present studies the only indication that "shock" had any influence on the results was obtained with *Rhinichthys atratulus* acclimated to 20°C. in Tennessee. At this particular acclimation the fish died almost instantaneously if suddenly exposed, regardless of the temperature in the lethal range to which they were subjected. By tempering for a few minutes at intermediate temperatures before placing the fish at the desired temperature, death from shock was avoided.

Calculation of results

In the past, investigators have determined resistance times for samples of fish by calculating the average survival time at a given constant temperature (Loeb and Wasteneys, 1912), the geometric mean survival at a given constant temperature (Fry *et al.,* 1946), or the temperature at which a given percentage survive a given length of time (Hathaway, 1927; Doudoroff, 1942). The method of Fry *et al.* (1946) was used in this investigation.

It was shown by the same writers that the median or 50 per cent survival time for speckled trout and mean logarithm of the time of death of individuals of a sample coincide. The mean logarithm and its experimental error has therefore been used for resistance time comparisons. Alternatively, resistance times were determined by plotting the per cent mortality as probits against the logarithm of time, and the median obtained by inspection.

Calculation of the incipient upper lethal was based on the dosage mortality method of Bliss (1935). De Beere (1945) provided a special graphic modification applicable to bioassay methods which enables one to calculate the median by inspection and the experimental error by greatly simplified procedure. This technique proved applicable for temperature work also. Most of the limits of error have been omitted from this publication but are on file in the library of the Ontario Fisheries Research Laboratory.

Measurements used in morphological comparisons

Where possible, all counts and measurements of fish that had been preserved were made directly after capture. No correction for shrinkage during preservation was made and the magnitude of this factor was not determined. The weights of the fish, however, were determined fresh and after preservation in 10 per cent formalin for three to six months followed by 65 per cent alcohol. The loss in weight varied from 5 to 17 per cent. In some instances, however, owing to shortage of material, measurements had to be made on the fish used in the experiments. Structural comparisons were restricted entirely to fish used in experiments only for *Ameiurus nebulosus*, *Micropterus salmoides* and *Notemigonus crysoleucas* from Put-in-Bay and Algonquin Park. In these species characters were chosen that would not be markedly affected by the treatment of the fish.

Below are the definitions of the morphological characters. All measurements were made with calipers and estimated to the nearest tenth millimetre.

Total length—the greatest dimension in a straight line from tip of snout to tip of dorsal lobe of caudal fin.

Standard length—the distance from tip of snout to end of hypural plate. In accordance with the accepted practice (Hubbs and Lagler, 1947), the end of the plate was determined without dissection by moving the caudal fin from side to side. It was later discovered that this was about 1.5 per cent less than the actual distance measured after dissection in the case of *Notemigonus, Rhinichthys,* and *Semotilus.* The error appears to be due to the fact that the hypural plate itself may bend when the caudal rays overlap it.

Body depth—the greatest dorso-ventral dimension exclusive of fins or fin base.

Depth of caudal peduncle—the least depth of that part.

Dorsal origin to occiput—the distance from the origin of the dorsal fin to the most anterior scaled portion of the nape.

Anal base—the greatest over-all length from the structural origin of the first ray to the end of the membrane at the base.

Head depth—the distance from the mid-line of the occiput vertically downward to the ventral surface of the head.

Head length—the distance from the tip of the snout to the most posterior bony part.

Snout length—the distance from the tip of the snout to the front margin of the orbit.

Eye diameter—the distance from the anterior to the posterior rim of the orbit made by pressing the dividers apart on either side of the eyeball.

Length of upper jaw—the tip of the snout to the most posterior point of the maxillary.

Pectoral spine of catfish—left spine length from base to tip.

Lateral line scale count—the number of scales along the lateral line ending at the posterior margin of the hypural plate. In those species where the lateral line was measured 1.5 per cent short of the true length the scale count would average one below the correct value.

Scales above lateral line—the number of scales from origin of dorsal fin to, but not including, the lateral line.

Scales below the lateral line—the number of scales from origin of anal fin to, but not including, the lateral line. The counts were made obliquely following the scale rows.

Predorsal scales—all scales intercepting the mid-line from the dorsal origin to the occiput.

Vertebrae count—the number of vertebrae exclusive of the hypural plate. No fused vertebrae were encountered. In the Cyprinidae the first four vertebrae in the Weberian apparatus were included. Counts were made on X-ray photographs.

Dorsal rays were counted in *Gambusia* as the total developed number in the dorsal fin.

Anal rays—In *Ameiurus* the count included both rudimentary and developed rays. In *Notemigonus* only developed rays were counted. The last two bases were counted as one ray.

RESULTS

Influence of size or age on lethal temperature

The literature on lethal temperatures contains frequent references to the effect of size or age. Bélehrádek's list (1935, p. 201), for species from various phyla, showed that there was a tendency for thermal resistance to decrease with size or age but no consistent rule was found. In fish, similar resistance to high temperature in specimens of differing size and age was reported by Hathaway (1927) for six and eighteen month perch, Sumner and Doudoroff (1938) for gobies, Mason (1939) for barbels and Fry *et al.* (1946) and Hart (1947) for various freshwater fish. On the other hand an increased ability of younger fish to withstand high temperatures has been reported by Day (1886) for fry relative to adults in various species, Huntsman and Sparks (1924) for American plaice, and Huntsman (1942) for Atlantic salmon and speckled trout. In most of these records size and age vary together and hence their separate effects have not been analyzed except for yearling speckled trout (Fry *et al.*, 1946) where there was no size effect.

In the present study it was also not possible to study the separate effect of weight and age except where underyearling fish were involved (*Dorosoma* and *Micropterus*). The trend of weight in relation to order of death is shown in table 2. A study of this table indicates that the mean weights of the first and last fish to die are generally similar. There is, however, evidence of a trend for *Rhinichthys atratulus, Notropis cornutus,* and *Lepomis macrochirus*. In the first two of these species the largest fish tended to die first, in *Lepomis*, however, the opposite was true.

These trends were statistically significant at the 5 per cent level for *Rhinichthys atratulus, Notropis cornutus,* and *Lepomis macrochirus* as measured by the "t" test. In *Rhinichthys* the differences were significant only for the Toronto August data. In *Notropis* significance was obtained only for Tennessee data, in *Notemigonus* only for Put-in-Bay data, and in *Lepomis* for Welaka data. The size effect noted in the table for *Lepomis* is particularly interesting because the larger fish were more resistant than small to high temperatures.

Analyses of variance of body weight in relation to order of death (combining all results obtained at different localities) were made for *Rhinichthys, Semotilus, Notropis, Ameiurus,* and *Ictalurus,* but the differences were significant only for *Notropis.*

The feasibility of correcting the lethal temperatures to a common body weight has been considered for species in which a weight difference in relation to order of death has been found. No correction has been applied, however, because the relationship was not the same in the different geographic groups or in the same group tested at different times (compare Toronto summer and winter data). This inconsistency, which perhaps tends to mask the general selective action of high temperature, was caused partly by differences in the age or size range in the geographic groups (compare northern and southern *Ameiurus, Notemigonus*) and partly by the narrowness in the size range of fish in many of the experiments. It is also evident that in some of the fish samples studied there was no size trend at all.

Effect of season on lethal temperature

It is possible that fish tested at different times of the year would not have similar lethal temperatures even when acclimated to the same temperature. In other words it may not be possible to duplicate the natural lethal temperature relationships in the laboratory when seasonal effects are disregarded.

There is little published information on the subject for fish. It is possible that there may be a seasonal cycle associated with spawning. Seasonal changes independent of acclimation temperature may possibly explain results reported by Fry *et al.* (1946) for *Salvelinus fontinalis* where the authors compared their data for autumn and winter fish with somewhat different results obtained by Brett for spring fish. A similar interpretation may be given to data on preferred temperatures in *Salvelinus* (Sullivan and Fisher, 1948). Some additional information has been obtained for species tested during the summer and winter (table 3).

Table 3 indicates that *Rhinichthys atratulus, Notropis atherinoides, Hyborhynchus notatus,* and *Perca flavescens* from Toronto were not able to withstand, during winter, temperatures as high as those tolerated by the same species from Put-in-Bay during the summer. These differences, which were first thought to be geographic, are interpreted to be seasonal in character, because comparable seasonal changes were also observed for *Rhinichthys atratulus, Semotilus* *atromaculatus,* and *Notropis cornutus* from the same locality (Toronto). It is possible that some of these seasonal changes were independent of temperature acclimation.

However, several facts suggest that seasonal changes independent of temperature acclimation were not present. First, certain species had identical lethal temperature patterns during the summer and winter. The results for *Ameiurus nebulosus,* obtained in December before the stock deteriorated, indicate substantial winter and summer agreement. Second, although most of the work was carried out in summer months it was by no means all obtained at the same season. Geographic similarities to be discussed later, therefore, suggest that seasonal factors did not complicate the results. Third, the observed seasonal differences in *Perca flavescens* from Put-in-Bay and Toronto (table 3) were not found when the Toronto fish were induced to feed in later experiments. The winter fish were found to have thermal resistance equal to that of summer fish (Fry, unpublished). Apparently feeding had affected the acclimation process. Other observations (table 3) suggest incomplete acclimation. In *Rhinichthys* the incipient upper lethals and resistance times for 20°C. were similar during summer and winter. Thus it appears that *Rhinichthys* was similar during winter and summer up to 20°C. and was incompletely acclimated to 25°C. in the winter period. A similar result was obtained for *Semotilus* (figure 11), where the winter lethal temperature pattern at 20°C. and below corresponded to the summer pattern. In *Hyborhynchus notatus* and *Notropis cornutus,* however, both 20°C. and 25°C. acclimation data showed seasonal differences.

It is concluded that the existence of a seasonal cycle independent of thermal acclimation is uncertain. Since acclimation to higher temperatures during the winter is a very slow process, any differences may be caused by incomplete acclimation during the winter. These difficulties have been reduced to a minimum in subsequent work by carrying out acclimation to high temperatures in the summer months where the thermal differential between environmental and laboratory acclimation levels was small.

Association between lethal temperature and morphological characteristics

The problem considered in this section is the possible genetic linkage between physiological and morphological aspects and the consequent selective action of environmental factors on the structure of animals. The selective action of adverse environmental conditions on the structure of English sparrows has been reported by Bumpus (1899) who examined a series of the birds after a storm in which sixty-four succumbed and seventy-two recovered. He found a whole series of morphological differences in the two groups. Crampton (1904) also showed differences in characteristics of Lepidoptera pupae during selection. Only 319 out of 1090 pupae lived through the winter. Equal numbers of dead and surviving pupae were measured and a series of differences were found to be statistically significant. Keys (1931) found selection in relation to salinity and asphyxiation for relative head length in *Fundulus.* In fish killed by asphyxiation the smaller fish died first and the first to die had shorter heads than the fish dying last. Differential selection of the sexes was also reported. Heuts (1947) demonstrated in *Gasterosteus aculeatus* that the fish with more lateral plates survived longer in sea-water than those with fewer plates when both groups had a low average plate number. Also, within the same group, fish with fewer plates had a greater ability to survive at high temperatures.

The observations cited above suggest that some of the morphological characteristics used in this study might be correlated with ability to survive at high or low temperatures. The selective action of upper lethal temperatures has been determined by comparing certain proportional measurements of the first and last fish to die in the experiments. Measurements of body parts were divided into the standard length and the differences of the means of the ratios tested for significance by the "t" test. The distributions of discontinuous variables were tested by the chi square method given by Pearl (1940, p. 329).

The analyses of the various measurements are given in table 5, and the counts in tables 7, 11, 12, 16, and 17 for *Notropis cornutus, Notemigonus crysoleucas, Ameiurus nebulosus,* and *Micropterus salmoides.* Most of these were tested at more than one locality. The correlation between order of death in lethal temperatures and the sex of fish were examined for *Rhinichthys atratulus* and *Ameiurus nebulosus* (table 6).

Unfortunately, the small number of fish used for lethal temperature work precluded the use of an adequate series of individuals for comparisons of this type, but it is felt that if there were any general correlation some trends would be evident even when small numbers were used. In this study, however, there were no significant differences in the characteristics of the first and last fish to die and no trends of any such relationships were apparent. The only approach towards significance is seen in *Ameiurus nebulosus* from Algonquin Park for the ratio $\frac{\text{standard length}}{\text{pectoral spine}}$. This ratio shows a significant correlation with sex (table 15), and the correlation resulted because more males died last than females. When other localities are considered, however, it is apparent that there is no general tendency for the high temperatures to have a greater selective action on females than on males (table 6).

In conclusion it may be stated that, with the minor exceptions noted above, no evidence has been obtained of association between lethal temperatures and either morphological characteristics or sex among the fish populations studied.

Comparison of Populations in Different Localities

Species showing geographic variations in lethal temperatures

Out of ten species within which comparisons were made only four showed any appreciable geographic differences in lethal temperatures.

Notropis cornutus. This species is split geographically into several sub-species based mainly on differences in the lateral line, predorsal scale count and pigmentation (Ortenburger and Hubbs, 1926; Hubbs, 1941; Hubbs and Lagler, 1947; Trautman, 1946). The race studied at Toronto has been identified as belonging to *Notropis cornutus frontalis* (personal communication R. M. Bailey) and the Tennessee form as *N. c. chrysocephalus.* The former subspecies has smaller scales, especially in the predorsal region, a fact that is in accordance with the interpretation that it is a cool water, slowly developing form. Since the predorsal scales are the last to form they would be laid down later in *frontalis* than in *chrysocephalus* on a longer axis and hence would be ultimately more numerous (Hubbs, 1941).

The predorsal and lateral line scales have been counted for the two groups and are given in table 7. The geographic differences were in accordance with expectations and were highly significant. Vertebrae were examined by X-ray and it was found that the southern group had fewer vertebrae than the northern. These results are in general agreement with the north-south gradient for number of vertebrae or scales in fishes (Hubbs, 1925; Kendall, 1902; Johnsen, 1936; Schnakenbeck, 1931; and others). The scales of

creek shiners from Knoxville and Toronto were examined and the former group of fish were found to have a somewhat more rapid growth rate than the latter (table 8). The growth rate of the Tennessee fish was found to be in agreement with that found by Marshall (1939) for Ohio fish which belonged to the same subspecies. Although local differences in growth rate are probably involved, it is possible that *Notropis cornutus frontalis* as a whole has a slower growth rate than *N. c. chrysocephalus.*

Certain body measurements were also made and plotted against standard length in figure 1. The lines through the points were de-

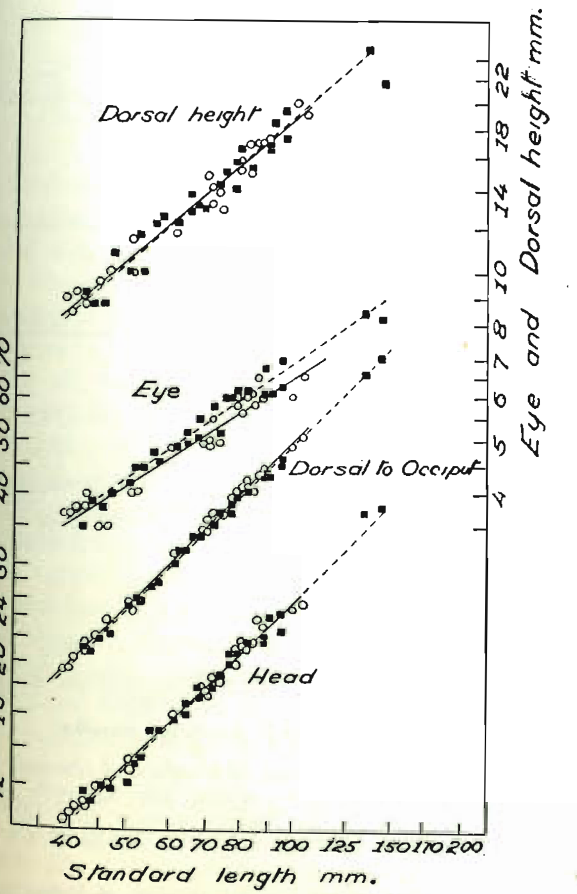


FIGURE 1. Body parts in relation to standard length for *Notropis cornutus* from Toronto, Ontario (circles) and Knoxville, Tennessee (squares). Double log. grid. Regression lines drawn from equations in table 9.

termined by least squares and the equations for these lines are shown in table 9. There do not appear to be any differences in these characters between the Knoxville and Toronto samples. Therefore the only distinct differences between the subspecies are the differences in growth rates and scale counts between the Toronto and Knoxville groups.

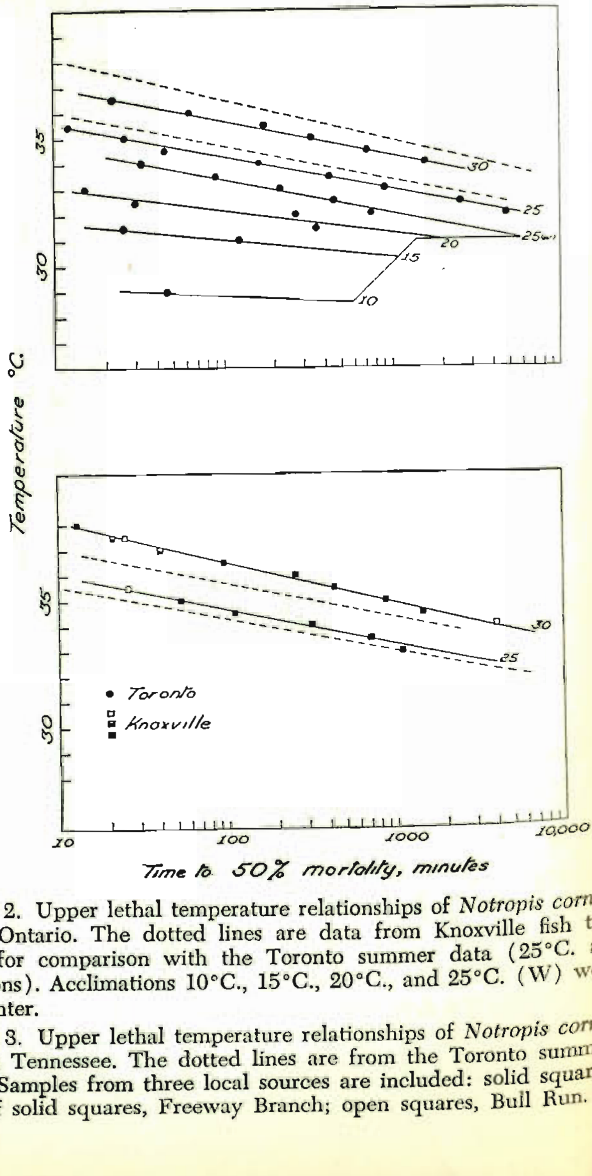


FIGURE 2. Upper lethal temperature relationships of *Notropis cornutus*, from Toronto, Ontario. The dotted lines are data from Knoxville fish taken from figure 3 for comparison with the Toronto summer data (25°C. and 30°C. acclimations). Acclimations 10°C., 15°C., 20°C., and 25°C. (W) were carried out in winter.

FIGURE 3. Upper lethal temperature relationships of *Notropis cornutus* from Knoxville, Tennessee. The dotted lines are from the Toronto summer data in figure 2. Samples from three local sources are included: solid squares, Willow Fork; half solid squares, Freeway Branch; open squares, Bull Run.

Lethal temperatures of the Toronto and Knoxville populations are shown in figures 2 and 3. The two populations can be compared at acclimation levels of 25°C. and 30°C. At both these temperatures the resistance times for the Knoxville samples were considerably greater than those of the Toronto samples at the same temperatures but the slopes of these time temperature lines appear similar. These differences cannot be related to differences in acclimation level in the two series and are possibly related to the subspecific variation. The differences are significant since there is scarcely any overlapping in individual times at the two localities. The geographic differences in lethal temperatures also cannot be explained by the effect of size because the Knoxville fish average larger than the Toronto fish in the summer and the larger fish tend to die before the smaller ones. If the resistance times were adjusted to a common weight the geographic differences would be accentuated.

Comparisons of the incipient upper lethals (table 4) at the upper levels of acclimation are difficult in this species owing to the length of time the experiments have to be carried on to reach these levels. Thus, in the Knoxville fish the incipient upper lethal for 25°C. acclimation was attained in approximately 5000 minutes. To decide this an experiment at 32°C. had to be carried out for nearly ten days (14,000 minutes) in which three out of ten fish died. In the Toronto fish, however, all the fish died at 32°C. with 4800 minutes for the 50 per cent mortality. The incipient lethal level may be much lower than this (possibly 31°C. as found for the winter results, Hart, 1947) but such long-term experiments are impracticable to carry out. Likewise, the determinations for 30°C. acclimated fish were not carried to the incipient lethal level. However, only three out of four Knoxville fish died at 34°C. with a 50 per cent mortality at 4000 minutes, in spite of the fact that the experiment was continued for 14,000 minutes. It appears, therefore, that the incipient level for 30°C. acclimated fish would approximate 33.5°C. (figure 3).

It is concluded from these results that *Notropis cornutus* shows geographic variation in both structural and physiological characteristics.

Gambusia affinis. The southern and Atlantic coast subspecies, *Gambusia affinis holbrooki*, was formally recognized as specifically distinct from the Tennessee form, *G. a. affinis*, (Hubbs, 1926a). Differences between these species in dorsal rays and gonopodial characters were described by Hubbs. Carr (1936) listed the West

Florida form, *G. a. affinis* (intergrades), as having fewer dorsal rays and smaller head and eyes than the eastern form.

Accordingly these characters were examined on *Gambusia* from Welaka and Knoxville. The fish from Knoxville were found to have a significantly lower number of dorsal rays (table 10). The Knoxville fish examined also had smaller heads but much larger eyes than the Florida form (figure 4). The eye difference was quite distinct and could be seen at a glance when the two groups of fish were placed side by side. The measurements, however, confirm records in the literature that distinct differences exist between the subspecies and show that such differences are applicable to the populations on which lethal temperature determinations were made.

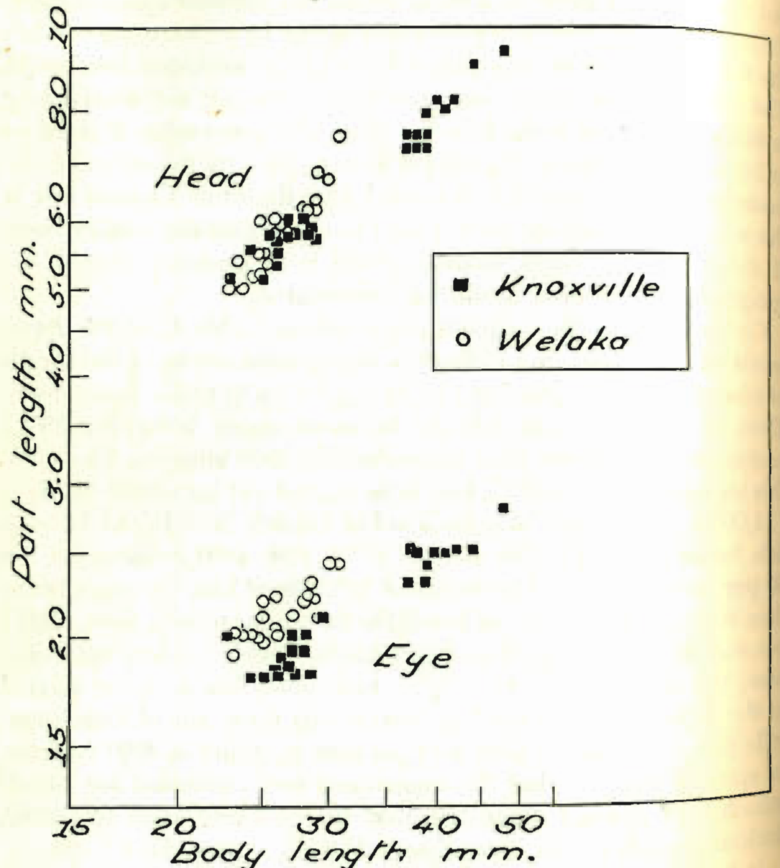


FIGURE 4. Head length and eye diameter in relation to total length for *Gambusia affinis* from Knoxville, Tennessee and Welaka, Florida. Double log. grid.

The lethal temperatures of *Gambusia* are shown in figure 5. Although not as many data have been gathered here as would have been desirable, a difference in the pattern of the lethal temperatures in the two races is indicated. Both forms were compared at common acclimation temperatures of 30°C. and 35°C. The results are similar at 30°C. but differ at 35°C., where the times to death were greater at each temperature in the Knoxville fish. In addition, two points were obtained in Knoxville at an acclimation temperature of 25°C. for fish that were found in a cool spring. Although no Welaka data were obtained for this acclimation temperature, it is evident from figure 5 that the patterns for the two geographic samples studied are dissimilar. For the lower acclimation levels, the Welaka fish may have higher lethals than the Knoxville fish. It is interesting that, in spite of the apparent differences, the two races show similarities in slopes of the 30°C. acclimation lines and in the ultimate upper incipient lethal (the horizontal boundary of the upper resistance lines).

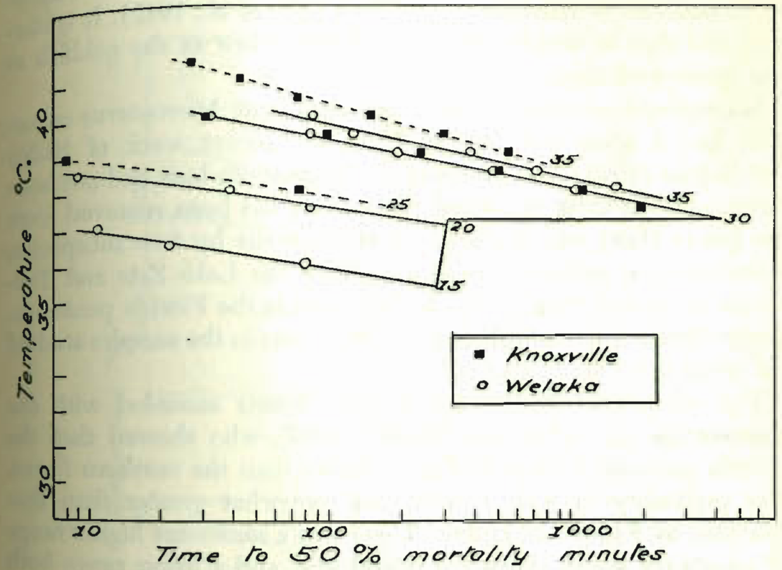


FIGURE 5. Comparison of upper lethal temperatures of *Gambusia affinis* from Welaka, Florida and Knoxville, Tennessee. The two populations may be compared at acclimation temperatures of 30°C. and 35°C.

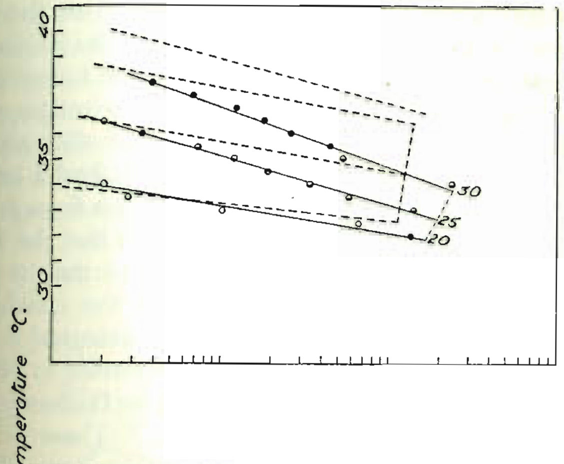
Gambusia can withstand very high temperatures in conformity with its habitat. At acclimation temperatures of 15°C. and 20°C. it has higher incipient upper lethals than any species heretofore reported. In the neighbourhood of 37°C., however, there appears to be no further increase in incipient upper lethal and hence it is not able to withstand for any great length of time temperatures up to 40°C. that can be tolerated by goldfish (Fry *et al.*, 1942). It is likewise not able to withstand temperatures as low as the goldfish at any given acclimation level.

Micropterus salmoides. The largemouth bass, *Micropterus salmoides,* has a wide distribution. Until the recent work of Bailey and Hubbs (1949) subspecies of the largemouth bass had not been recognized. In their treatment the species has been removed from the genus *Huro* and replaced in *Micropterus* by two subspecies, *Micropterus s. salmoides* occupying both the Lake Erie and Tennessee areas and *Micropterus s. floridanus* in the Florida peninsula. Comparisons of the morphological differences in the samples studied are given in tables 11 and 12.

The subspecific differences in scale counts accorded with the observations of Bailey and Hubbs (1949) who showed that the Florida peninsular form had more scales than the northern forms. The variability in scale counts was somewhat greater than that observed by Bailey and Hubbs. There was a somewhat higher range of counts for scales above the lateral line, and a lower range both below and along it. Some of these differences are probably due to differences in method of counting. The Welaka fish also had more dorsal soft rays than the Put-in-Bay fish but vertebral counts showed no significant differences.

Another interesting trend is seen when comparisons are made between local groups of fish reared under different pond conditions. The fish referred to as "normal" (tables 11 and 12) were large fish over 90 gms. The "stunted" group were fish of the same age (nine to eleven months) as the first but were under 25 gms. The trend is for the "stunted" fish to have fewer scales than the "normals"; this, however, is significant only in lateral line scales. Thus, not only do the bass exhibit local variations in structure from apparently environmental causes, but the fish which exhibited the slower growth showed characteristics tending towards those of the northern races more than did the fish exhibiting the faster growth. The differences appear to be due to crowding and food conditions. Comparable conditions have been described by Eschmeyer (1936) for perch populations.

The geographic differences in lethal temperatures found among the races of this species were very large. The data are shown in figures 6 and 7 for Welaka, Knoxville, and Put-in-Bay. In Welaka many bass were used in attempts to obtain comparable results with the Put-in-Bay fish. Although acclimation over long periods was carried out (only three to eight days was required to acclimate these



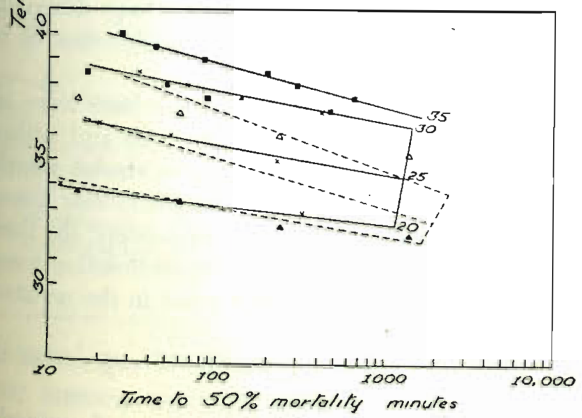


FIGURE 6. Upper lethal temperatures of *Micropterus salmoides* from Welaka, Florida, at acclimation temperatures of 20°C., 25°C., and 30°C. The dotted lines are lethal temperatures for Knoxville and Put-in-Bay samples (figure 7). The various symbols represent different samples varying considerably in their average weight.

FIGURE 7. Upper lethal temperatures of *Micropterus salmoides* from Put-in-Bay, Ohio (crosses) and Knoxville, Tennessee (squares). The dotted lines represent Welaka fish for comparison (figure 6). Data for Lake Mendota fish (Hathaway, 1927) at 22°C. and 30°C. are included (closed and open triangles).

fish) the lethal temperature pattern found was a series of converging resistance-time lines with non-parallel slopes (figure 6). The difference between the two races was least at 20°C. and became progressively greater at the higher acclimation levels. The respective patterns are superimposed as dotted lines in figures 6 and 7. The difference in the incipient upper lethals between the races was 1.8°C. at 25°C. acclimation, and 2.7°C. for 30°C. acclimated fish. The most surprising feature of the differences is that the northern race withstood higher temperatures better than the southern race.

Further data on fish of the year were obtained at Knoxville during August. These are shown in figure 7. Since the water temperatures at that time reached 28°C., the fish were tested after acclimation to 30°C. and 35°C. The bass acclimated to 30°C. had a lethal temperature distribution that was similar to that of fish from Put-in-Bay rather than to those from Welaka. This suggests that the Knoxville sample resembles the one from Put-in-Bay more than it does the Welaka sample, which is in agreement with the conclusions of Bailey and Hubbs (1949) based on their morphological findings.

Lethal temperatures of largemouth bass acclimated to 22°C. from Lake Mendota, Wisconsin, have been studied by Hathaway (1927), and the results are also illustrated in figure 7. These fish appear somewhat less resistant to high temperatures than Knoxville or Put-in-Bay fish, but the resistance pattern resembles that of bass from Put-in-Bay rather than the Welaka pattern.

Three local populations of the largemouth bass were studied at Welaka. The fish were reared in different ponds and, although they were all approximately the same age (nine to eleven months), there were marked differences in the sizes attained, due apparently to crowding and nutritional conditions. Unfortunately, the three weight groups were compared largely at different acclimation temperatures but even so there was no apparent difference in the resistance times of the three groups.

The rather surprising lethal temperature divergence of the southern largemouth bass can be interpreted in various ways. It is possible that inherent differences between the northern and southern races are involved. However, there is a large difference in the total dissolved solids and chloride between the St. Johns River and northern waters (table 1). Also, the bass were taken from ponds in which the chloride is probably considerably lower than in the river and hence acclimation to chloride changes may have taken place. The operation of such an accessory factor (Fry, 1947) may have

lowered the lethal temperatures and brought about a chance agreement with the taxonomic findings of Bailey. It is felt that inherent differences in lethal temperatures within the species may be present, but further work is required.

Lepomis macrochirus. Unfortunately adequate data on the geographic variation of lethal temperatures for this species were not obtained but the data on the Welaka bluegill, described as *Lepomis macrochirus purpurescens* (Hubbs and Allen, 1943), are compared with certain data given by Hathaway (1927) and with one isolated point for Knoxville fish. These data indicate that bluegills may also vary geographically in lethal temperature relationships.

Data for fish acclimated to 25°C. from two different drainage systems in Florida but tested in St. Johns river water showed substantial agreement (figure 8). Geneva and Santa Fe lakes drain into the Suwannee River System, whereas the other fish were from the St. Johns River. Fowler (1945) describes bluegill of the Suwannee System as a different subspecies (*Lepomis macrochirus macrochirus*) but the basis for his decision is not clear. The fish from Geneva and

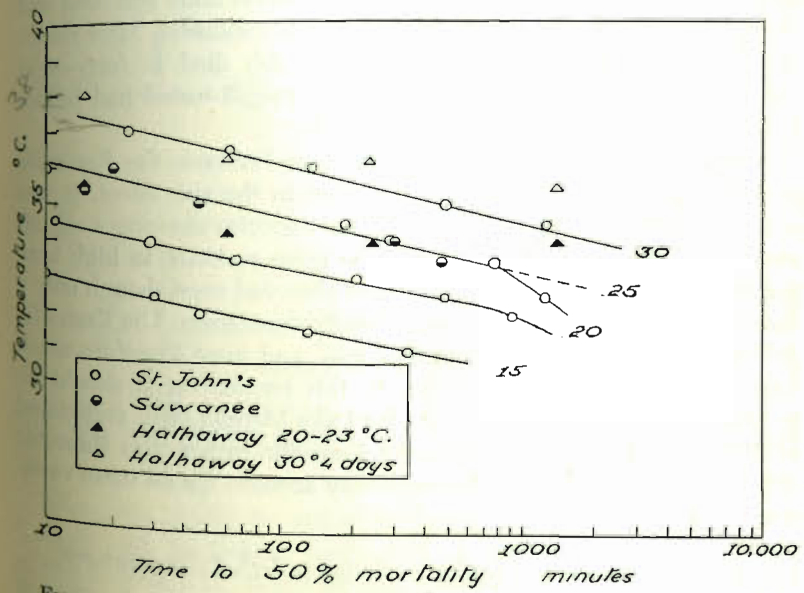


FIGURE 8. Lethal temperature relationships of *Lepomis macrochirus* from Welaka, Florida acclimated to 15°C., 20°C., 25°C., and 30°C. Fish from two local sources are compared at 25°C. Data for Lake Mendota (Hathaway, 1927) are also shown.

Santa Fe lakes, however, did possess certain colour differences among which were redder fins than the St. Johns River fish. The lethal temperature comparisons support the viewpoint that local differences in lethal temperatures are uncommon in fish.

The dotted line shows Hathaway's data for fish acclimated to 22°C.–23°C. Although the agreement is fairly good at resistance times up to about seventy minutes, there were departures for longer times to death which indicate a much higher incipient lethal (34°C.) for 22°C.–23°C. than would be expected for the Florida fish (about 32°C.). Also, fish held at 30°C. for four days were more resistant than fish acclimated to 30°C. in Florida.

In Knoxville only one sample of bluegill was tested; this was at a temperature of 36.5°C. They had been acclimated to 30°C., and according to the Welaka results, 50 per cent should have died within about sixty minutes. However, after 240 minutes none had died and the temperature was raised to 38°C. in twenty minutes. After reaching this temperature 50 per cent of the fish died in forty-eight minutes. It is evident that the Knoxville bluegill tested had higher lethal temperatures than the Welaka fish.

A possible explanation of the differences between the Knoxville and Welaka fish, however, may be found in the size effect. It was noted (p. 00) that bluegills were the only species showing a significant tendency for the larger fish to be more resistant to high temperatures. A significant co-relation was observed even though only a few large fish were present in the Welaka population. The Knoxville fish tested ranged from 15.8 to 33.7 gms. and were therefore much larger than the Welaka average. In this connection it should be pointed out that Hathaway's data for Lake Mendota fish acclimated to 30°C. conform with the data for Knoxville rather than those for Welaka fish. Size or age differences may account for all these variations.

Species not showing geographic variation in lethal temperatures

Dorosoma cepedianum. The gizzard shad has a wide distribution from the lower Great Lakes through the Mississippi drainage basin westward to Florida and up the Atlantic coast drainage to New Brunswick. It is recognized as being a plastic species by Hubbs and Whitlock (1929) in their description of diverse forms of young from different waters. The differences were considered to result from direct action of the environment. No subspecies have been recognized, perhaps because of this plasticity. Likewise no evidence of physiological differences within the species have been found.

Underyearlings of this species were compared at acclimation temperatures of 25°C., 30°C., and 35°C. at both Put-in-Bay and Knoxville. The results are compared in figure 9. Unfortunately small specimens were not available at Welaka, although large fish were plentiful. Young gizzard shad have never been reported by any collectors in that area.

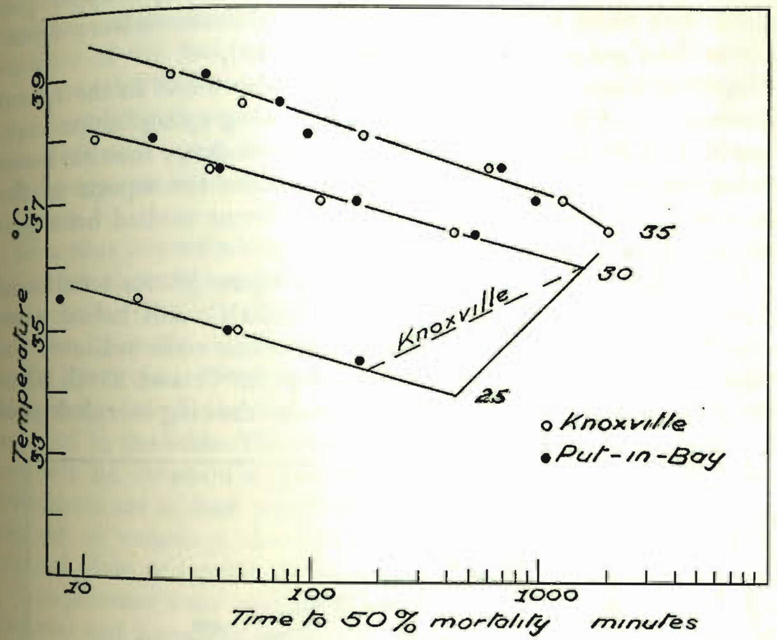


FIGURE 9. Upper lethal temperatures of *Dorosoma cepedianum* from Put-in-Bay, Ohio, and Knoxville, Tennessee, for fish acclimated to 25°C., 30°C., and 35°C.

Dorosoma is able to withstand rather high temperatures in conformity with its habitat preference of shallow open water. Comparisons of data for Knoxville and Welaka samples show agreement within the range of experimental error. The greatest difference was found at 25°C. where Knoxville fish had an incipient upper lethal 0.6 centigrade degrees higher than the Put-in-Bay form (table 4). This difference is not interpreted as signifying any real difference between the races, owing to possible differences in acclimation level and to difficulties such as were encountered at Knoxville in maintaining some of the fish in a healthy state during long experiments. More consistent results were obtained when the water was changed daily.

Rhinichthys atratulus. This species has four recognized subspecies (Hubbs, 1936). The form examined at Toronto is within the distribution range of *Rhinichthys atratulus meleagris* and the Tennessee form is within the range of *R. a. obtusus.*

Measurements made on the two groups revealed the following trends: *Rhinichthys* from Knoxville had deeper bodies, greater caudal peduncle depth, narrower heads, and longer upper jaw length than *Rhinichthys* from Toronto. The differences were significant at the 5 per cent level or above (table 15).

Significant geographic differences were also found in the lateral line scale count with the Knoxville form having a distribution overlapping that of the Toronto form but with a tendency towards fewer scales (table 13). These observations confirm the reports in the literature that the Knoxville and Toronto forms studied belong to two distinct subspecies.

The lethal temperatures are given in figure 10. As mentioned earlier, the winter and summer data at 20°C. and below were similar. Furthermore, no significant differences were indicated for Knoxville and Toronto fish acclimated to 20°C. and 25°C. Thus *Rhinichthys* is a good example of a species showing morphological differentiation into well-defined subspecies without showing comparable variation in the physiology of lethal temperatures.

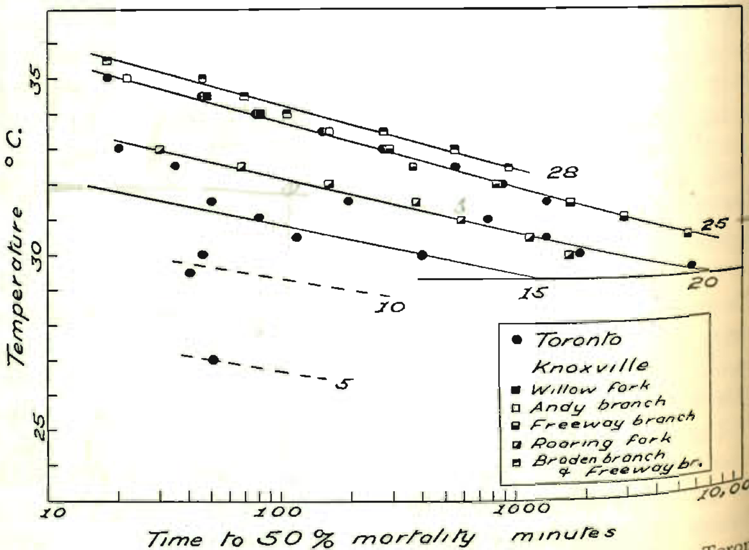


FIGURE 10. Upper lethal temperatures of *Rhinichthys atratulus* from Toronto, Ontario and Knoxville, Tennessee. Acclimation temperatures are 5°C., 10°C., 15°C., 20°C., 25°C., and 30°C. Knoxville fish from various local sources as indicated.

The lethal temperature pattern of *Rhinichthys atratulus* is an unusual one. There appears to be no further increase in the incipient upper lethal at acclimation temperatures above approximately 13°C. Also, the change in position of the resistance lines with increase in the acclimation temperature is less above than below this acclimation temperature. *Rhinichthys atratulus* is a species for which determination of the incipient upper lethals at the higher acclimation levels was difficult and impracticable because death occurred only after very long exposures. With the exception of *Salvelinus,* which has an ultimate upper incipient lethal of 25.3°C., the ultimate upper lethal temperature of *R. atratulus* (29.3°C.) is the lowest of any yet reported.

Semotilus atromaculatus. Both the Knoxville and Toronto forms are within the range of *Semotilus atromaculatus atromaculatus.* The same characteristics studied by Greeley (1930) were used to compare the Knoxville and Toronto samples. Predorsal scale counts and lateral line scale counts are shown in table 14. No geographic differences were found. Greeley's data for fish from Six Mile Creek are included in the table. The results for predorsal scales are confirmatory but he obtained a greater count for lateral line scales. The differences are at least partly due to a systematic difference in the method of counting, since Greeley did not use the end of the hypural plate as a point for terminating his scale counts.

Comparisons were also made of the head lengths of fish from Toronto and Knoxville (table 15). In these samples the males had a mean head length slightly longer than that of the females in accordance with Greeley's findings, but the differences were not significant. When both sexes were lumped together, the Knoxville sample had somewhat shorter heads than the Toronto sample. The differences were significant ($P = .02$).

The growth rates determined by scale examination (table 8) for Toronto and Knoxville samples were similar to those reported by Greeley for the northern subspecies and there is little evidence of any marked difference between the two geographic groups.

The results obtained confirm Greeley's findings that the species is fairly uniform morphologically over most of its range, although local variations are encountered. The characteristics of the Knoxville and Toronto groups on which lethal temperature determinations were made are thus similar.

The lethal temperatures were determined at Knoxville and at Toronto for acclimation temperatures of 25°C. and 30°C. during the summer, and at Toronto for lower acclimation temperatures during the winter. The results indicate a common pattern with no geographic differences. The lethal temperature comparisons confirm the taxonomic status of the species whose populations belong to the same subspecies in both areas studied (figure 11).

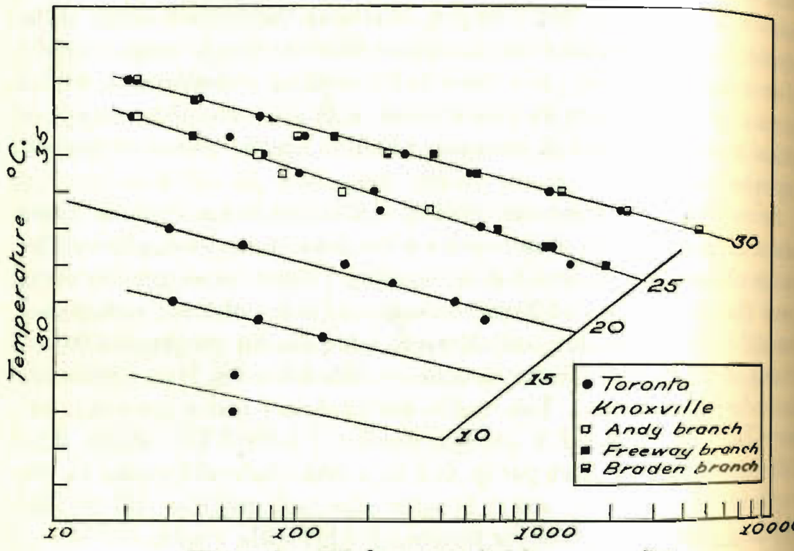


FIGURE 11. Upper lethal temperature relationships of *Semotilus atromaculatus* from Toronto, Ontario and Knoxville, Tennessee. Toronto fish acclimated to 10°C., 15°C., 20°C., 25°C., and 30°C. Knoxville samples from various local sources as indicated.

The lethal temperature results obtained with *Semotilus* subjected to the higher acclimation temperatures are uncertain in long experiments, owing to the difficulties experienced in maintaining the fish in good condition. The ultimate incipient lethal was therefore not precisely determined but probably does not greatly exceed 33°C. However, this seems to permit the distribution range of the species to extend considerably south of Tennessee. It would be interesting to determine whether any difference exists between this subspecies and the more southern *Semotilus atromaculatus thoreauianus*.

Notemigonus crysoleucas. The geographic variation of every morphological character examined in this species was considerable.

This variation has been observed by Hubbs (1918) who demonstrated a cline in anal rays. This cline was noted as an exception to the general rule associating more meristic parts with northern and fewer with southern races. The work was continued by Schultz (1927) who correlated the increased number of anal rays in southern localities directly with the temperature of the water during development. Geographic areas for equal numbers of anal rays were related to air temperature isotherms for May which gave an indication of the temperature of the water during larval development. In spite of this evidence for environmental control, the nomenclature of the species has remained polytypic even though the characters by which the subspecies are distinguished fluctuate markedly from environmental causes. The Florida form studied by the writer is within the range of *Notemigonus crysoleucas bosci*, whereas both the Put-in-Bay and Algonquin Park forms are within the range of *N. c. crysoleucas*.

Determinations of the lateral line scales, anal rays, and vertebrae are shown in table 16 for golden shiners from the above mentioned localities. The variation in anal rays confirms the previous reports. The Welaka fish had fewer scales but more anal rays than those at Put-in-Bay. The differences between Put-in-Bay and Algonquin Park fish in this respect were not significant.

The variations observed in vertebral count were remarkable. A cline was found with fewer vertebrae in the northern localities. The greatest difference occurred between Algonquin Park and Put-in-Bay; the differences between Put-in-Bay and Welaka were not significant. The vertebral counts in this series thus had a tendency to follow the same trend as the anal rays but the spheres of variation in these two characters occurred in two different geographic ranges. The vertebral counts from Put-in-Bay and Welaka fish were similar and probably confirm findings of Gosline (1948). The anal rays, however, have their greatest variation within this range. Between Put-in-Bay and Algonquin Park the differences in anal rays are not significant but the differences in vertebral count are significant.

Geographic variations have also been found in the lengths of head and snout and eye diameter. The equations for the co-relations of these parts to the standard length are shown in table 9, and the results plotted in figure 12. When figure 12 is examined it will be observed that the data for head length and eye diameter fall into three distinct groups: the Put-in-Bay and Welaka fish with a standard length above 65 mm., the same group below this length, and the Algonquin Park fish. The latter group had distinctly larger heads and eyes than either the Put-in-Bay or Florida group over the length range at which they could be compared. The regression line equations for data above 65 mm. standard length have been averaged in figure 12 for the Put-in-Bay and Florida fish on the basis of their apparent similarity. There is a significant change in the slope of the lines for head and eye data for fish below 65 mm. from these two localities. There is also a significant difference in the

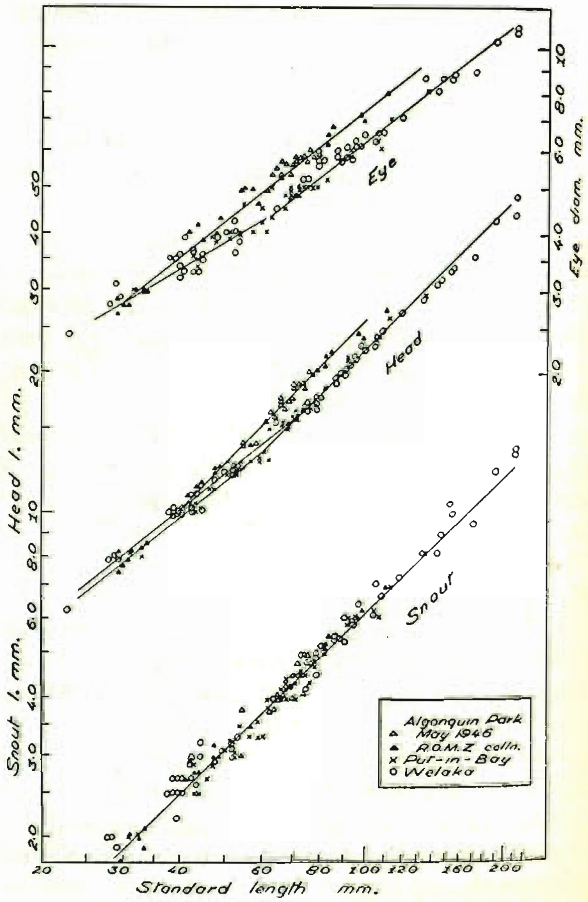


FIGURE 12. Head length, snout and eye diameter in relation to standard length for *Notemigonus crysoleucas* from three localities. Change of slope is indicated at approximately 65 mm. in the case of head and eye for fish from Put-in-Bay, Ohio and Welaka, Florida. Regression lines are drawn from formulae in table 9 as explained in the text.

position but not in slope between the Algonquin Park and Put-in-Bay groups of data for head lengths over the same range. The same conclusion does not hold for eye diameter where the two groups appear to coincide. For this reason the slopes of the lines for head lengths of Put-in-Bay and Welaka groups of fish below 65 mm. have been averaged but plotted at their different positions in figure 12, whereas a common average line has been drawn for eye diameter for the same groups of fish. The snout length was similar in all three localities and the three regression lines have been averaged in figure 12. The head and eye proportions, like the vertebral counts show their greatest differences between Put-in-Bay and Algonquin Park.

The picture for geographic variation of the head and eye in the golden shiner resembles that reported elsewhere (Doan, 1939; Wilder, 1944; Martin, 1949), in which differences in body proportions result from differences in the length of the fish at which growth inflection occurs. The mechanics of environmental control of these variations have been discussed by Martin (1948), who showed that the great majority of geographic differences within a given growth stanza are such that the position, but not the slope, of the lines varies. In general, growth inflection occurs during larval development and at the onset of maturity, and any environmental factors that alter the rate of differentiation actually alter the size at which growth inflection occurs during these stages. In the Put-in-Bay and Florida groups of golden shiners growth inflection for the head and eye occurs around 65 mm. In the Algonquin Park fish inflection probably occurs around 45 mm., but data are lacking for this size range.

The growth rates of the golden shiners from the different localities show a gradient from north to south (table 8). Again, the local nature of growth rate characterization was shown by Cooper (1936); golden shiners from southern Michigan grew faster than those in the northern part of the state. The difference was correlated with the length of the growing season in the two areas. In Algonquin Park (Lake Opeongo and Costello Creek), the shiners showed the slowest growth, with fish in their second year averaging about 70 mm. The fish from this area attained a greater age but a smaller size than those from the other areas. Shiners from Put-in-Bay had faster growth rates than those from Algonquin Park, fish in their second year averaging about 100 mm. This growth is comparable to that for northern Michigan fish given by Cooper. The Put-in-Bay fish also attained a larger size than the Algonquin Park fish but not so great an age. The shiners from Welaka showed the most rapid growth, fish in their second year averaging about 150 mm. These shiners also attained the greatest length but the maximum age attained was less than that of fish from any of the other areas. At the outset it was thought that age determination would be difficult in these southern fish but in most cases well defined annuli were found. Annuli close to the edge of the scales were found during the period from January to March which coincided with low water temperatures.

In contrast to the morphological variation, there was an absence of geographic physiological variation. Lethal temperatures of shiners from three localities have been determined at acclimation temperatures from 10°C. to 30°C. It was not necessary to study this whole acclimation range at each locality because data from each area conformed so well to one common pattern. This was found in spite of the fact that the Florida sample studied corresponds to subspecies *Notemigonus crysoleucas bosci*, and the Put-in-Bay and Algonquin Park samples of *N. c. auratus*. The data shown in figures 13 and 14 illustrate the fact that there are no geographic differences apparent at any one acclimation temperature. A common difference of approximately 1.6 centigrade degrees exists between the parallel resistance-time lines for each five-degree change in acclimation temperature.

In order to test the possibility of significant differences between localities, an analysis of covariance was carried out for the 20°C. and 25°C. resistance-time lines. By this test neither the slopes nor the position of the lines differed significantly for the three localities. It can thus be said that no significant geographic differences with respect to lethal temperature are evident in *Notemigonus*.

The upper and lower incipient lethal limits in relation to the acclimation temperature are shown in figure 14 and given in table 4. No geographic differences are evident and the thermal zone within which the species can live (thermal tolerance) has been bounded by a line common to all localities studied. The ultimate incipient upper lethal was not determined, but it is estimated to be approximately 35°C. This temperature would give the species a fair margin of safety above the high water temperatures of marshy areas. If 35°C. is the highest point to which the species can be acclimated, then the thermal tolerance zone as defined (area within the curve) is 940 centigrade square units. Thus, in comparison with stream species

(Hart, 1947), this species is relatively eurythermal in conformity with the thermal conditions of its habitat.

In summary, the geographic variation of structural characteristics in the golden shiner can be interpreted causally from environmental factors modifying the rate of development during various growth periods (Hubbs, 1926). It is recognized, however, that differences in the rate of development of the various races may possibly have a genetic basis. According to these data, the differences between

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HIGH LETHAL TEMPERATURES OF GOLDEN SHINERS (*NOTEOMIGONUS CRYSOLEUCAS*).—The highest temperatures that most North American fish can tolerate range from about 25.0° C to about 36.0° C (Clark, 1969). The high lethal temperatures for the golden shiner (*Notemigonus crysoleucas*) range from 30.5° C for fish acclimated at 15.0° C to 34.7° C for fish acclimated at 30.0° C (Carlander, 1969).

This study indicates the high lethal temperature for *N. crysoleucas* to be near 40.0° C for fish acclimated at 22.0° C for 56 days.

Three *N. crysoleucas*, ranging in size from 7.0 to 8.0 cm and in weight from 2.2 to 3.5 g, were acclimated at 22.0° C for 56 days in an aerated 2.5 gal tank. On the 57th day the tank was placed in a Lab-Line water bath with inside dimensions of 91.4 × 45.7 × 22.9 cm. The water level in the water bath was maintained at 15.3 cm and in the tank within 2.0 cm of the top. The fish were fed a commercially prepared food three times weekly during the acclimation and testing periods. The temperature was raised approximately 0.75° C per day. On the 24th day two fish died at 39.5° C and one at 40.0° C.

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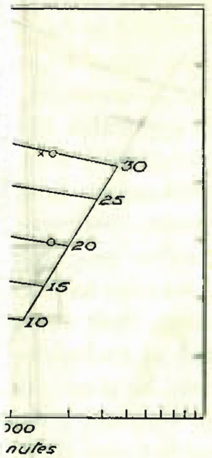

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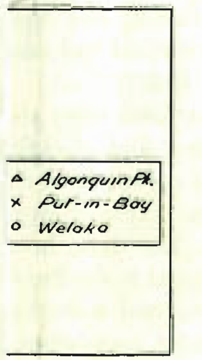

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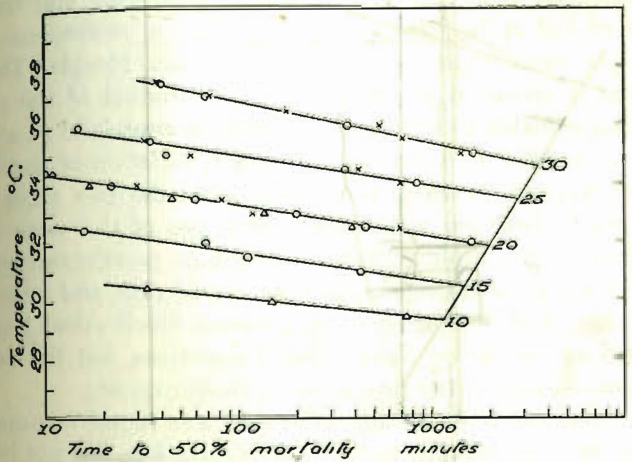

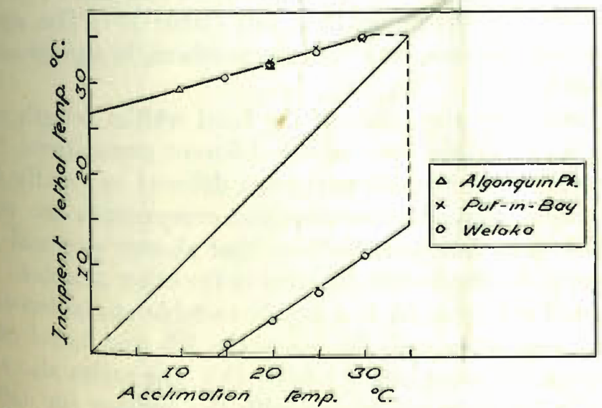

FIGURE 13. Upper lethal time-temperature relationships of *Notemigonus crysoleucas* from Algonquin Park, Ontario (acclimated to 10°C. and 20°C.), Put-in-Bay, Ohio (acclimated to 20°C., 25°C., and 30°C.) and Welaka (acclimated to 15°C., 20°C., 25°C., and 30°C.).

FIGURE 14. Relation between incipient upper and lower lethal temperatures and acclimation temperature for *Notemigonus crysoleucas* from the same localities as in figure 13.

Algonquin Park and Put-in-Bay groups within the range of the same subspecies are just as great as those between Put-in-Bay and Welaka fish belonging to different subspecies. The question of inherent versus induced differences will have to await further experimentation but the geographic similarity in lethal temperatures suggests that we are dealing with a uniform group.

Ameiurus nebulosus. This species is recognized as consisting of two subspecies, the northern, *Ameiurus nebulosus nebulosus*, specimens of which were obtained from Algonquin Park and from the vicinity of Put-in-Bay, and the southern, *A. n. marmoratus*, from which specimens were obtained from Welaka, Florida. The only published information on the geographic variation of the species, which is applicable to catfishes in general, is contained in a review by Hubbs (1940) who observed a parallel variation of the fish for certain characters between species, between the two sexes of the same species, between young and old stages of the same species and between geographic races. In southern populations younger fish and females have longer spines, more anal rays, and more terete bodies than their complements. A common biochemical basis was suggested as underlying these parallel variations, but the common factor may be simply the rate of larval development.

In my material a significant difference was found in number of anal rays between the Put-in-Bay and Welaka fish, but not between the Put-in-Bay and Algonquin Park fish (table 17). The southern samples studied had more rays than the northern, in agreement with Hubbs (1940).

Comparisons were also made of the head widths, length of pectoral spine, and anal fin base of the different populations (figure 15). The catfish from the different areas differed so greatly in size that their ranges showed no overlap and comparisons are scarcely possible. The data, however, indicate that shorter pectoral spines were present in the Put-in-Bay fish than in the other samples.

The pectoral spine length is a highly variable characteristic and a significant correlation with the sex of the fish was found only for the group from Algonquin Park (table 15). The males also tended to have wider heads in accordance with the findings for catfish in general, but the differences were significant only for Welaka fish. Little geographic variation was observed in proportionate measurements for head widths (table 15) but the data are not comparable because the southern fish examined were so much larger than the northern.

Geographic colour variation of these fish has been long recognized. The northern populations from areas in Algonquin Park and Toronto were very dark, with no pattern. The Put-in-Bay fish were also dark but a mottled pattern was often evident especially in the larger fish. The Welaka fish were light, with a strikingly variegated pattern.

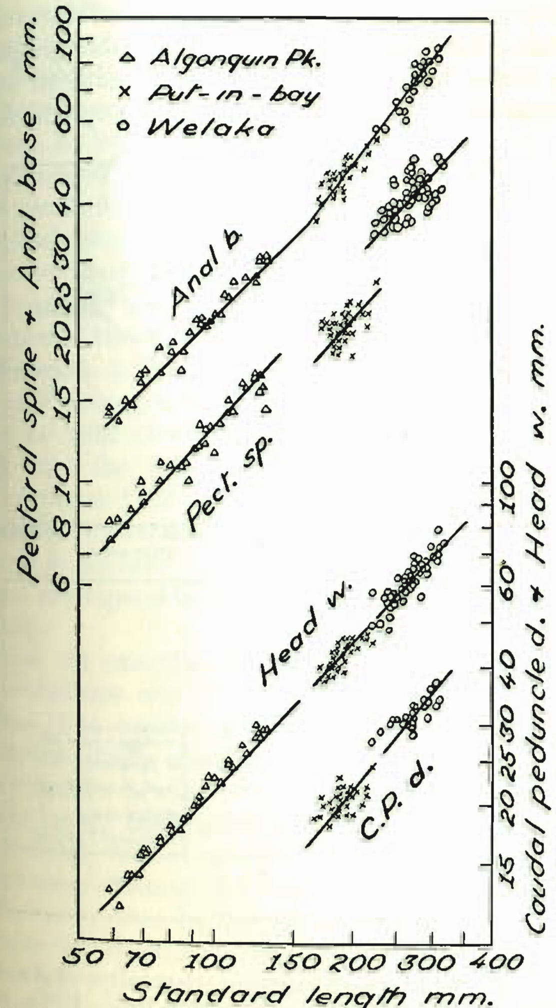

FIGURE 15. Body parts of *Ameiurus nebulosus* in relation to standard length in samples from Algonquin Park, Ontario, Put-in-Bay, Ohio, and Welaka, Florida. Double log. grid.

Lethal temperatures were determined on samples of fish from Algonquin Park to Florida at acclimation temperatures indicated in figures 16 and 17. Fish were acclimated to temperatures from 5°C.

to 34°C. and the data from all localities suggested a common pattern. No long experiments were carried out in Florida since the large fish broke the thermo-regulation apparatus and electrocuted themselves during such experiments.

The lethal temperatures of the brown bullhead were similar to those of the golden shiner. The two species inhabit the same general areas. Similarly, this bullhead shows no appreciable geographic differences in upper lethal temperatures from north to south. The incipient lethal changes approximately two Centigrade degrees for a change of five Centigrade degrees in the acclimation temperature. The ultimate incipient lethal temperature is slightly below 35°C. as determined for Put-in-Bay fish. While it cannot be said that the lethal temperatures of the Florida subspecies conform precisely to those of the northern fish, because only short experiments were performed, the results suggest that they do.

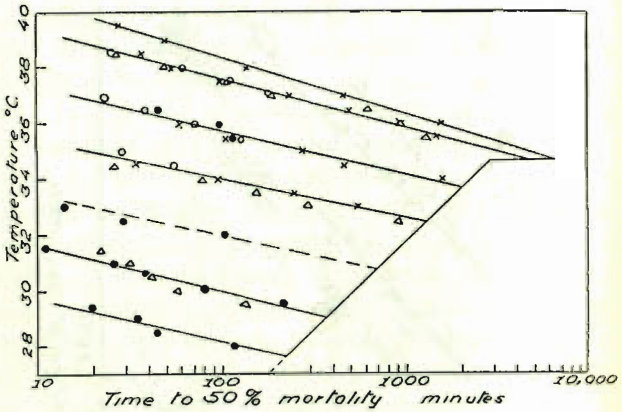

FIGURE 16. Upper lethal time-temperature relationships of *Ameiurus nebulosus* from Algonquin Park, Ontario (acclimated to 20°C., 25°C., and 30°C.), Toronto, Ontario (acclimated to 5°C., 10°C., 15°C., and 25°C.), Put-in-Bay, Ohio (acclimated to 20°C., 25°C., 30°C., and 34°C.), and Welaka, Florida (acclimated to 20°C., 25°C., and 30°C.).

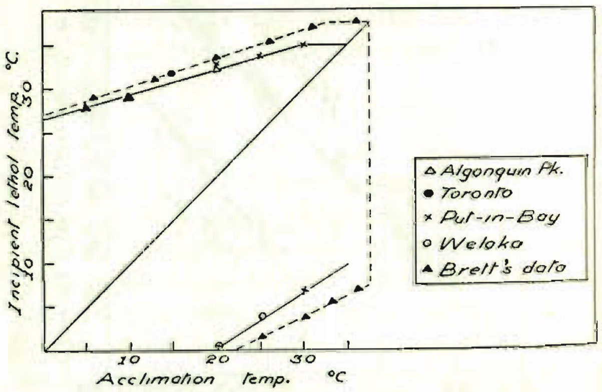

FIGURE 17. Relation between incipient upper and lower lethal temperatures for *Ameiurus nebulosus* from the same localities as in figure 16. Brett's (1944) data for Algonquin Park fish are also shown.

Data for the brown bullhead have been obtained by Brett (1944) who determined the incipient upper lethal temperatures for the whole acclimation range but did not extend his experiments beyond twelve hours. As indicated here, twelve hours is insufficient to determine the incipient lethal level at acclimation temperatures above 15°C. The results obtained by Brett should therefore be higher than those observed here by an amount consistent with the resistance time of twelve hours (720 minutes). However, the observed differences (figure 17) are greater than those expected from differences in the duration of the experiment alone.

The differences in lower lethals of catfish determined by Brett and by myself were greater than those observed for upper lethals, and cannot be satisfactorily explained by differences in method. It is possible that the Algonquin Park race is more eurythermal (thermal tolerance 1162 Centigrade square units) than the more Southern races (tolerance 1029 units). If such a temperature race exists, which is doubtful, the break between the races is northern and does not correspond in distribution to the subspecies recognized taxonomically.

Apart from the exception noted above, geographic variation in *Ameiurus nebulosus* was thus mainly morphological rather than physiological. The various samples studied were morphologically but not physiologically distinct.

Ameiurus natalis. In view of the general similarity of geographic races of the brown bullhead it was felt desirable to obtain some results on a closely related species, *Ameiurus natalis*. No geographic comparisons were obtained; the data were all obtained from Welaka fish. Brown and yellow bullheads have somewhat similar habitat preferences in Welaka and it is difficult to catch one species without taking the other.

In spite of the similarities in the habitat of the two species, the lethal temperatures of yellow bullheads are much higher than those of the browns at comparable acclimation temperatures. The 20°C. acclimated fish were similar to brown bullheads acclimated to 25°C., and fish acclimated to 30°C. were more resistant than the brown bullheads at their highest acclimation. No two species have yet been found with identical lethal temperature patterns.

Ictalurus lacustris. The two subspecies of the channel catfish, *Ictalurus lacustris lacustris* and *I. l. punctatus* were studied respectively at Put-in-Bay, Ohio, and Welaka, Florida. The southern form apparently intergrades with the northern one in Ohio and according to Trautman (1946), with "intergrades probably comprising a large proportion of population."

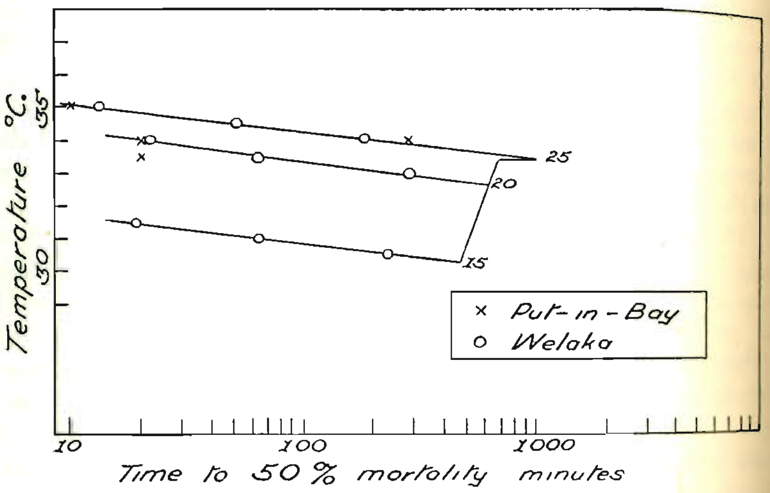

FIGURE 18. Upper lethal temperature relationships for *Ictalurus lacustris* from Put-in-Bay (acclimated to 20°C. and 25°C.) and Welaka (acclimated to 15°C., 20°C., and 25°C.).

Lethal temperature determinations on this catfish are by no means satisfactory owing to the small amount of data obtained. However, at acclimation temperatures of 20°C. and 25°C. resistance time and incipient lethal data were similar (figure 18) for fish from both Welaka and Put-in-Bay. There is a suggestion, however, that lower lethals may differ geographically, as Welaka catfish have a lower lethal of 4.7°C. while Put-in-Bay fish have one of 2.5°C. when acclimated to 20°C. The ultimate upper lethal for this species appears to be around 33.5°C. as suggested by the relatively small differences in lethal temperatures for fish acclimated to 25°C. and 30°C. If the latter assumption is correct *Ictalurus* has a lower ultimate incipient lethal than the other catfish studied.

DISCUSSION

Comparison of physiological and morphological variation

The morphological variation has been summarized by relating the number of characters and the number of species showing geographic variation to the total number studied. Each structural feature studied is treated as a single character. Of the characters compared geographically, about two-thirds showed significant differences between two or more localities. All seven species showed significant geographic differences in one or more characters.

It is possible to compare the physiological variation in the same way but unfortunately this involves a somewhat arbitrary definition of a physiological character. The lethal temperature pattern of a species can be considered as a whole series of characters, but here it will be considered as two—the upper and lower lethal series.

When the lethal temperature characters are enumerated as described above, only four out of thirteen studied geographically or about 31 per cent showed differences (upper lethals in *Notropis cornutus, Gambusia affinis,* and *Micropterus salmoides,* and lower lethals in *Ictalurus lacustris*). This arbitrary but convenient method of comparing the geographic variation of morphological and physiological characteristics shows over twice the variability in the former as in the latter. This can be seen when compared on the basis of total numbers of characters or total number of species showing differences.

There are apparently few published reports with which these data may be compared. Heuts (1945) found that populations of *Gasterosteus aculeatus* from different localities in Europe showed gradations in the temperature–salinity range within which osmotic equilibrium was maintained, but these gradations were associated with well-defined morphological differences in lateral plates. Physiological differences in survival of eggs and larvae in relation to salinity were also described (Heuts, 1947). The only other data on physiological comparison of races of fish are those of Black and Black (1946) in which marine and landlocked races of the Atlantic salmon possessed blood that showed no physiological differences. Morphological differences in the races have been observed (Wilder, 1948) but they are not thought to be inherited (Huntsman, 1947).

In the Amphibia, results obtained by Moore (1942) were similar in certain respects to those obtained on fish by the writer with respect to geographic variation. Five species of frogs were compared with respect to breeding period, water temperature at breeding, embryonic temperature tolerance, rate of development, Q 10 for development, egg diameter, and specific distribution limits. The species fell into a series with the following order for all of these factors: *Rana sylvatica, Rana pipiens, Rana palustris, Rana clamitans,* and *Rana catesbiana. Rana sylvatica,* the most northern species, spawned at the lowest temperature, had the lowest embryonic temperature tolerance range, developed at the slowest rate for any given temperature and had the smallest eggs. Geographic races of three of these species were studied. No differences were found in *Rana pipiens* from Vermont, New Jersey, and Quebec in this respect, but frogs from Florida developed faster at high temperatures and more slowly at low temperatures than Vermont frogs. This physiological difference was accompanied by slight morphological differences in egg diameters, markings, and shape of head. The Florida form studied was probably *Rana sphenocephalus* (Carr, 1940). In this study temperature was an important factor limiting distribution through its effects on the developing embryo, and physiological differences when found were associated with well-defined morphological differences and with the species or incipient species level.

The presence among species belonging to other phyla, of populations which show inherited physiological differences when studied under laboratory conditions and which show no evident structural differences, thus has no parallel in the fish species studied. Defining such populations as physiological races it may be said that no valid examples have been reported for fishes and few for vertebrates. The sex races in European frogs described by Witschi (1942) appear to be authentic physiological races. In other groups, particularly invertebrates, physiological races have been described. They are usually associated with isolation of the groups in question either by geographic or biotic factors. Nevertheless, we are often uncertain whether the differences described are inherited or are the result of direct action of temperature on the animals concerned. The geographic differences in developmental rates described by Runnström (1929, 1936) and Fox (1938) for *Paracentrotus lividus* and other marine invertebrates, may thus be due to seasonal changes in the organisms as was suggested by Horstadius (1925). Similarly the geographic physiological differences described by Fox *et al.* (1936-9) for marine invertebrates and possibly those described by Krumbiegel (1932, 1936) for *Carabus* may be confounded by non-genetic variation. The work on *Drosophila* by Timofeef-Ressovsky (Dobzhansky, 1941) appears to involve authentic physiological races in which geographic differences in viability at different temperatures were found.

Finally, numerous physiological races have been described for the lower forms in which structural differences are not evident. These are found mainly among the bacteria (White, 1931) and fungi, but there are many others, e.g. the mating types in *Paramoecium* (Sonneborn, 1938), races with different temperature optimum for division in *paramoecium* (Gause *et al.*, 1942), spontaneous occurrence of thermal races of *Daphnia* in the laboratory (Banta, 1921, 1929), and the classical experiments of Dallinger (1887) in which the thermal environment of flagellates was raised from a normal of about 15°C. to about 60°C. over a period of years.

Another class of variation, commonly described in the literature for insects and lower groups, is the occurrence of differences in behaviour patterns of closely related forms that are often indistinguishable morphologically. Reviews on this subject are given by Goodey (1931), Thorpe (1930, 1931), White (1931), and Smith (1941). Authentic examples of this type of variation in fish are difficult to find. There are numerous records of biological variation in fish populations: the races of herring described by Schnackenbeck (1931) with different spawning periods; behaviour differences in Kokanee and sea-going Sockeye salmon (Ricker, 1938); racial differences in *Zoarces* described by Schmidt (1921); populations among certain species that show differences in growth rates or in differentiation from the normal types living in the same or neighbouring area (Kennedy, 1944), Dence (1937), Greene (1929), Greeley and Bishop (1932); all are illustrations of this type of variation. However, in fish, the behaviour differences are sometimes thought to be associated with the direct action of the environment (White and Huntsman, 1938) and hence not hereditary. Furthermore, there are few valid examples of races showing inherited biological differences that have no demonstrated morphological differences.

The populations of fish studied by the writer showed morphological variability similar to that frequently described in the literature and present the usual difficulties for those who wish to know whether the differences are inherited or acquired. In contrast, racial differences in lethal temperatures were not prominent and these findings do not lend support to the assumption that physiological races are common in fish as one might infer from a study of lower forms.

It is thought that the observed differences in the degree of geographic variability of the lethal temperatures on the one hand and of the morphological characters on the other may be partly caused by differences in the mode of action of the environment on the two classes of characters. Physiological characters of the type studied can be modified rapidly and reversibly by acclimation at any stage in the life history. Now since there is little evidence of geographic variation in lethal temperatures within several species, and since the populations studied must have had widely differing early environmental histories during the larval stages, early environmental history and rates of larval development cannot have had much influence on the lethal temperatures for these species.

It is well known, on the other hand, that morphological characters cannot be rapidly and reversibly changed at any stage, but are markedly affected by the early environmental influence on the rate of larval development (Schmidt, 1921; Hubbs, 1926b; Gabriel, 1944; Martin, 1949). Thus, the control of physiological characters through acclimation has its counterpart in the control of morphological characters by early environmental influence. In consequence, only if the fish are reared under conditions in which the pertinent factors are controlled throughout development can we expect to eliminate the environmental influence on morphology to an extent comparable with that obtained by acclimation in the case of physiological characters. It is therefore not possible to decide from this study whether the inherited lethal temperature characteristics show more or less variation than the inherited morphological characteristics, because it is not known to what extent the latter were modified by direct action of the environment.

The environment thus has an entirely different action on the morphological and physiological characteristics studied. This is further evidence that they are independent. This independence has also been demonstrated by tests in which morphological characteristics of fish from the same population showed no correlation with resistance to high temperatures. Hence, although there is a general parallelism between physiological and morphological differentiation, it would be incorrect to assume that there is a close association of physiological with morphological variation. The geographic variations of physiological and morphological characteristics of fish studied by the writer appear to be entirely independent.

Interpretation of observed geographic variations of lethal temperatures in relation to taxonomy of species

It is difficult to escape the conclusion that the lethal temperature patterns of species are inherited, when they are constant throughout an area that constitutes a large proportion of the range of those species. This conclusion applies to *Notemigonus crysoleucas, Dorosoma cepedianum, Rhinichthys atratulus, Semotilus atromaculatus, Ameiurus nebulosus,* and *Ictalurus lacustris.* These species showed little or no geographic variation (there may be differences in lower lethals in the catfishes) and the lethal temperature patterns appear reliable for taxonomic purposes. However, in only two of these species, *Dorosoma cepedianum* and *Semotilus atromaculatus,* did the lethal temperature findings confirm the taxonomic status. In all the others the populations studied are recognized as belonging to different subspecies. Although it is realized that a whole spectrum of physiological characteristics must be studied before one can compare them with the range of structural characteristics observable at a glance, these findings suggest that the taxonomic status of species such as *Notemigonus crysoleucas* might be entirely revised if further experiments under controlled conditions were carried out.

On the other hand, interpretation of geographic differences in lethal temperatures in relation to the taxonomic status of the species is difficult, because it cannot be assumed that all the observed racial differences are inherited. The following influences may modify the lethal temperatures:

(1) Thermal acclimation. The effect of acclimation is so large that it may completely mask racial and specific differences. No conclusions should be drawn concerning inherited physiological differences if acclimation has been ignored. It is thought that geographic differences caused by acclimation effects are small or absent in this study, but it may account for some of the differences in lower lethals observed between my results for *Ameiurus nebulosus* and those of Brett (1944) for the same species.

(2) Seasonal effects. Data have been presented to show that seasonal differences in lethal temperatures may be present even though precautions were taken to acclimate the fish. Seasonal effects may give pseudo-geographic variation. However, all the species that showed geographic variation were compared in the summer months and so it is thought that seasonal effects do not complicate the picture.

(3) Age and size effects. It has been indicated that age or size of the fish influenced the lethal temperature results for certain species. Geographic differences between Knoxville and Welaka bluegills were cited as possible examples of this phenomenon. The possibility of a correlation between body weight and resistance to high temperatures was examined for *Notropis cornutus* and *Micropterus salmoides,* both of which showed geographic variation. In the latter species no size effect was found, and it is concluded that the observed geographic differences were not due to size differences. In *Notropis cornutus* the larger fish tended to die first. Since the Knoxville fish averaged larger than the Toronto (summer) fish and had higher lethal temperatures, the geographic differences in body size do not account for the lethal temperature differences between the races. In *Gambusia affinis* there were more large fish in the Knoxville sample and it is possible that the observed geographic variation in lethal temperatures may be due to size effects.

(4) Accessory factors. Accessory factors are environmental influences that impose a burden on the organisms in addition to the thermal stress and modify the lethal temperature pattern. The condition of the water in which the fish were acclimated may modify the lethal temperature characteristics. Chemical analyses of the water in the different areas (table I) have shown that only minor differences were apparent between Toronto tap water (Dom. Dept. of Public Health data) Lake Erie water (Clark, 1924; Wright and Tidd, 1933) and Bearden Creek water at Knoxville (T.V.A. Stream Sanitation Laboratory analyses in 1947). However, the St. Johns River water (Pierce, 1947) had a much higher chloride and total dissolved solid content than the other waters, and it is possible that these differences might have modified the lethal temperature results for *Gambusia affinis* and *Micropterus salmoides.* However, it should be noted that they did not apparently modify the results for *Ameiurus, Ictalurus,* and *Notemigonus.*

Micropterus salmoides from Welaka were obtained largely from hatchery ponds having a lower salt content than river water, but these fish were acclimated in the river water. *Gambusia affinis* were obtained from hatchery ponds and springs in the area and were also acclimated in river water with relatively high chloride content. Loeb and Wasteneys (1912) showed that salinity modified the lethal effect of temperature in *Fundulus.* The osmotic load operates as an accessory factor. It is therefore possible that the differences between Knoxville and Welaka water may have caused some of the observed geographic differences in lethal temperatures for these two species. Although the lethal temperature findings are in agreement with the taxonomic status of the species, this agreement may be apparent rather than real.

In several of the species discussed above (*Micropterus salmoides, Gambusia affinis,* and *Ameiurus nebulosus*) the interpretation of observed geographic differences has been rendered difficult owing to the operation of certain uncontrolled factors in the experiments. In one species, *Notropis cornutus,* the observed differences in lethal temperatures are not subject to any of the objections cited above and in this species the agreement between the lethal temperature results and taxonomic status appears to be real.

Bearing in mind the above reservations, it is concluded that lethal temperature results confirm the taxonomic status in five species (*Dorosoma cepedianum, Semotilus atromaculatus, Micropterus salmoides, Notropis cornutus, Gambusia affinis*) and contradict it in four species (*Ameiurus nebulosus, Ictalurus lacustris, Notemigonus crysoleucas, Rhinichthys atratulus*). In nearly 50 per cent of the species studied, the physiological findings were not in agreement with the current taxonomic status of those species. In all these species geographic differences in lethal temperatures were small or absent, although the fish belonged to different subspecies in the northern and southern localities.

On the whole it is not surprising that the physiological findings do not confirm the taxonomic status in some of the species. The disagreement may be caused partly by non-genetic variation in diagnostic morphological characters in certain of the species (e.g. *Notemigonus*) as discussed previously. Another important factor is that the physiological characteristics are adaptive, but as will be shown later, there is little evidence of geographic differences in the thermal environments of the various subspecies and hence there may not have been sufficient selective pressure for evolution of physiological differences. The consequences of these considerations are that the physiological characters studied may not be as sensitive as the morphological ones for taxonomic purposes.

Interpretation of geographic physiological variation in relation to thermal environment

The adaptive character of physiological properties of animals has long been recognized. Most physiological properties are dependent on temperature and we therefore expect to find physiological differences in animals living in different thermal environments. Similarly we expect to find adaptive physiological variation among geographic races or closely related species from northern and southern localities.

The literature gives a number of records of geographic physiological variations. In the field of developmental physiology, references have already been cited (Runnström, 1929, 1936; Fox, 1938) in which races differing in temperature range for development were described for *Mytilus edulis, Paracentrotus lividus, Ciona intestinalis,* and *Ascidia mentella.* Reference has also been made to geographic variation in rates of development by Moore (1942) for various species and races of frogs, and Brown (1929, a & b) described differences in rates of development as well as in lethal temperatures for Cladocera that were correlated with their distribution ranges.

Geographic variation of physiological characters in species or races has been described for Protozoa (Gause, 1942), Coelenterata (Mayer, 1914), Insecta (Timofeef-Ressovsky, 1933, 1935; Krumbiegel, 1932), and marine invertebrates (Fox *et al.,* 1936–9). In fish, geographic physiological differences in races of *Gasterosteus* have been described by Heuts (1945, 1947) and differences in lethal temperatures for fish species correlated with their distribution range have been observed by Huntsman and Sparks (1924), Battle (1926), and Storey (1937). The observed physiological differences described above have been correlated with the thermal characteristics of the environments of the various species.

In the species studied by the writer, a considerable variation of lethal temperatures was observed. In order to compare the species, they have been listed (table 18) in order of decreasing incipient upper lethals (20°C. acclimation temperature), and data on lower lethals, oxygen consumption, and habitats are also given. It can be noted that increasing upper lethals are correlated with a succession from deep lakes and cold streams, to warm streams, to marshy streams, and finally to marsh conditions. The correlation of lower lethals with habitat was poor, but, with the notable exception in *Ameiurus nebulosus,* the succession is associated with decreasing metabolic rates. Similar gradations of physiological characteristics associated with ecological succession of species have also been observed by Shelford (1911), Wells (1914), Wright (1918), Fox *et al.* (1933, 1935), Ide (1935), Clausen (1936), Whitney (1939), and Fry (1939).

Although there is a considerable overlap in range, the species listed in table 18 also illustrate the phenomenon of physiological gradations in relation to geographic range. The species with low upper lethal levels that are found in cool environments are members of the northern fauna, whereas the marsh inhabitants are members of the southern fauna. The lethal temperature characteristics of the species, in a rough way, are correlated with their geographic distribution.

In contrast, the lethal temperature characteristics of the geographic races and subspecies studied showed a very poor correlation with their geographic distribution. Correlation with habitat conditions was observed only for upper lethals of *Notropis cornutus* and possibly for lower lethals of the catfishes. *Notropis cornutus frontalis* inhabits stream areas further towards the source than *N.c. crysocephalus* (Gerking, 1945; Trautman, 1946) and the former subspecies had lower lethal temperatures than the latter. In *Micropterus salmoides* the subspecies differences in lethal temperatures can hardly be regarded as adaptive since the Florida form had lower tolerance to high temperatures than the Tennessee form. Geographic differences in *Gambusia* were too small to permit interpretation of their adaptive significance. Racial physiological differences were absent for the remaining species.

The interpretation of the paucity of racial physiological variation among these fish species in contrast with its abundance in other groups discussed above may be aided by consideration of some of the factors responsible for geographic variation.

Methodological factors

1. Non-genetic variation. Geographic pseudo-races may result from the methods utilized in making the study, i.e. from variations in environmental conditions prior to or during the study.
2. Differences in the physiological age of animals from the various populations.

Factors affecting speciation

1. Differences in the rate of mutation. Differences in mutability may account for most of the reported variations among populations. Basically, speciation is the phenomenon we are attempting to assess but the interpretation is often difficult because of the interferences discussed above. Generally speaking, the rate of mutation of insects and other invertebrates, which display extensive geographic physiological variation, may be faster than that of the fish species studied by the writer.

2. Phenotypic plasticity. Plasticity may indirectly influence the rate of speciation. A population of organisms exposed to any given environmental extremes in nature should theoretically be less subject to the action of natural selection, if the individuals of the population are able to adjust physiologically within wide limits. Eurythermal species in a given thermal environment should be less exposed to selection than stenothermal species in the same environment. In support of this concept, Gause (1947) showed that genotypic specialization tended to vary inversely with phenotypic plasticity. Resistant populations changed less during acclimation than populations with low initial resistance. Although this relationship has not been found to apply to the species studied by the writer, several of the species studied possess rather wide thermal tolerance limits (bullhead catfish 1029 units, golden shiner 940 units) and this may circumvent the necessity of geographic race formation with respect to lethal temperatures.

3. Influence of the environment. The environment influences speciation through natural selection and perhaps by other means. If the mutation rate is relatively low, and the habitat conditions of a species do not differ geographically at comparable seasonal periods, then it should presumably require a long time for racial differences to be built up among the populations. Many insects which possess rapid speciation rates are restricted to microhabitats and micro climates in which the populations are isolated. In the present work, although the populations studied were isolated by geographic barriers, there is no evidence that the climatic factors of their habitats differ sufficiently to cause distinct physiological differences in lethal temperatures among the populations.

As indicated earlier, if there were geographic differences in lethal temperatures among the populations we should expect to find geographic differences in water temperatures. It is well known that the average water temperature increases from north to south but lethal temperatures would probably be correlated with temperature extremes rather than averages. Observations suggest, however, that the temperature extremes inhabited by a given species do not vary geographically to a great extent. The only obvious geographic temperature difference noted was that the St. Johns river, habitat of the catfish, bass, and golden shiners, does not freeze in the winter, unlike waters in the north. Maximum water temperatures in the St. Johns river, however, do not usually exceed 30°C. (Pierce, 1947), and similar temperatures have been recorded in marshy areas near Put-in-Bay.

Stream temperatures in the Tennessee area recorded by the writer did not exceed 27°C. and Ide (personal communication) reported maximum temperatures approaching 28°C. in the Humber river, Ontario, in 1948. For Illinois streams, where certain of the species studied by the writer also can be found, water temperatures exceeding 30°C. were reported (Thompson and Hunt, 1930). The average maximum summer air temperatures in the localities from which the fish were taken show very small differences (Brooks and Connors: climate maps of North America).

There apparently is only one report dealing with death from high temperature in freshwater fish (Huntsman, 1946). Reports of death from cold are more common (Verrill, 1901; Storey, 1937; Gunter, 1941), but there are no records of mortality due to high or low temperatures among the species studied from the various localities at which tests were carried out. It would therefore appear that the thermal extremes encountered by these species do not exert much selection pressure on them.

In conclusion, the paucity of geographic variation in lethal temperatures is interpreted to mean that there is no well-marked gradation in temperature extremes over the range studied. This perhaps has reduced selection for temperature resistance, and, coupled with high phenotypic plasticity (through thermal acclimation) and relatively low rate of mutation, has discouraged the building up of geographic physiological races. Furthermore, it would appear that the lethal temperatures of the species studied are well above the thermal extremes usually encountered in their environments, and that these physiological characteristics have little ecological significance over the geographic range considered.

SUMMARY

1. Lethal temperatures have been compared in fourteen species at common temperatures of acclimation. Comparison of samples from different locations over their geographic range was undertaken in ten of these species. Samples were obtained from Ontario, Ohio, Tennessee, and Florida waters.

2. Morphological characters were also studied in seven of these species. No correlation was found between the lethal temperature and morphological characteristics in the same population.

3. Lethal temperature comparisons in winter and summer on the same species showed that some species were less resistant in winter than in summer at the same acclimation temperatures. The differences may have resulted from incomplete acclimation to higher temperatures during the winter season.

4. Size or age was shown to have a significant effect on upper lethal temperature relationships in three species. In *Notropis cornutus* and *Rhinichthys atratulus* large fish died before smaller ones; in *Lepomis macrochirus* the smaller fish died before the larger. The size or age effect was usually small and not consistent in the various localities, but in *Lepomis machrochirus* the effect of size differences on lethal temperatures was relatively large.

5. Geographic differences in upper lethal temperatures were found only in three species (*Notropis cornutus* from Knoxville and Toronto, *Gambusia affinis* from Welaka and Knoxville, and *Micropterus salmoides* from Welaka and Knoxville or Put-in-Bay). These populations were also taxonomically distinct, so that physiological and morphological findings were confirmatory. However, the geographic physiological differences were complicated by accessory factors in certain of these species.

6. In two species, *Dorosoma cepedianum* and *Semotilus atromaculatus,* no geographic differences in upper lethal temperatures were found. These findings are in agreement with the taxonomic status of these species in which subspecies are not recognized over the range studied.

7. No geographic differences were found in upper lethal temperatures of *Ameiurus nebulosus* from Algonquin Park to Welaka, or of *Ictalurus lacustris* from Put-in-Bay to Welaka. Although the data are insufficient there are indications of geographic differences in lower lethal temperatures.

8. No geographic differences were found in the upper or lower lethal temperatures of *Notemigonus crysoleucas* from Algonquin Park to Welaka, nor in upper lethals of *Rhinichthys atratulus* from Knoxville and Toronto. Significant geographic differences in morphology were found for both of these species. The former was noted as being highly variable. The lethal temperature findings therefore did not conform with the accepted taxonomic status of these species which have recognized subspecies.

9. The physiological characteristics on the whole showed a greater geographic constancy than the morphological ones. The greater apparent constancy of physiological characteristics was in part caused by control of previous environmental history through acclimation.

10. The upper lethal temperatures were correlated with habitat. Eurythermal species were found in southern localities and in ponds or base level streams; more stenothermal species were found in northern localities in cooler water. However, southern races within the species were not more resistant to high temperatures than northern races, and similarly summer habitat temperature extremes of these races may not be greater in southern than in northern localities. There was no correlation between lethal temperatures and geographic distribution range of races within the species studied.

11. These results are interpreted as meaning that the geographic variation in temperature extremes encountered by the races studied has not been sufficient to impose selection for temperature resistance. This explanation appears plausible in view of the plasticity (thermal acclimation) of the races and because large geographic differences in maximum air or water temperatures apparently do not exist over the range considered. Furthermore, it appears that the lethal temperatures of these species are well above the thermal extremes usually encountered in their environments, and that these physiological characteristics have little ecological significance.

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TABLE 1

Analyses of water comparable to that used for acclimating fish. Bearden Creek water analyzed by T.V.A. Stream Sanitation *Laboratory in 1947. Differences* between raw water (conditioned) from tank in which fish have been kept approximately three *weeks are shown. Toronto* tap water analysis obtained from Public Health Department at Toronto. St. Johns River water *according to Pierce (1947).* Lake Erie water (1) from Clark (1924) for central and eastern end and (2) according to Wright and *Tidd (1933)* for western end of lake.

Item p.p.m.	Toronto tap water	Lake Erie	Tennessee—Bearden Creek		Florida St. Johns R. (Dec.—May)
			Raw	Conditioned	
pH	7.6	7.7-8.4(2)	8.15	7.90	7.5-8.1
Alkalinity:					
Phenolphthalein	0.0		3.8	0.0	0.0
Methyl orange	93.5	85-103(2)	136.2	137.0	56-89.5
Bicarbonate as $CaCO_3$		114.0(1)	138.0	136.0	
Carbonate as $CaCO_3$	0.0		2.18	1.21	
Organic N	0.065	0.151(2)	0.976	1.46	.166-.360
Free ammonia N	0.005	0.013(2)	0.172	0.672	0.0-.002
Nitrite N	0.001	0.005(2)			0.0-.002
Nitrite + Nitrite N	0.101	0.105(2)	1.10	6.48	0.0-.402
Total dissolved solids	161.7	133.0 (1)	193.0	208.0	355-900
Total fixed solids			104.0	138.0	
Total volatile solids			89.0	70.0	
Suspended total solids			24.0	7.0	
Suspended fixed solids			10.0	1.0	
Suspended volatile solids			14.0	6.0	
Chloride	9.8	7.2 (1)	3.5	3.5	89-370
Calcium, Ca	34.3	34.1 (1)	41.7	44.4	
Magnesium, Mg	5.7	8.3 (1)	11.3	13.4	
Sulphate, SO_4	18.6	19.8 (1)	3.29	9.77	

TABLE 2

Mean weight of fish and order of death. Numbers in brackets are the variance of the mean. For *Micropterus* from Put-in-Bay missing data give variable numbers of fish in each class. *Micropterus* from Welaka are segregated into three weight groups.

Species	Locality w. (winter) s. (summer)	*Mean weight and variance of mean* 1st Death	2nd Death	3rd Death	4th Death	5th Death	No. in each class
Rhinichthys atratulus	Toronto w.	2.25 (.027)	2.21 (.032)	2.05 (.054)	1.88 (.066)	2.38 (.062)	18
	Toronto s.	2.70 (.076)	2.41 (.079)	2.00 (.137)	1.41 (.066)	1.47 (.077)	9
	Knoxville s.	2.10 (.129)	1.81 (.074)	1.85 (.058)	1.78 (.062)	1.75 (.074)	22
Semotilus atromaculatus	Toronto w.	6.73 (2.586)	5.40 (.700)	3.90 (.431)	7.80 (4.52)	7.28 (2.69)	21
	Toronto s.	4.27 (1.82)	9.46 (34.4)	4.35 (1.72)	2.41 (.320)	2.22 (.555)	10
	Knoxville s.	4.74 (1.35)	4.53 (.761)	4.73 (2.42)	2.73 (.322)	2.09 (.183)	18
Notropis cornutus	Toronto w.	7.23 (.884)	6.12 (.496)	8.11 (.866)	7.66 (1.21)	5.73 (.582)	27
	Toronto s.	3.42 (.785)	4.10 (1.01)	1.96 (.126)	2.39 (.144)	2.04 (.184)	13
	Knoxville s.	7.26 (2.85)	4.58 (.957)	4.61 (1.11)	3.68 (.256)	3.93 (.508)	22
Notemigonus crysoleucas	Algonquin	5.01 (.177)	7.03 (1.07)	5.87 (.295)	6.46 (.262)	6.37 (.441)	10
	Put-in-Bay	10.11 (5.54)	4.28 (1.42)	4.35 (.333)	3.62 (.534)	3.52 (.397)	14
	Welaka	34.2 (107)	39.6 (161)	49.1 (143)	39.1 (148)	46.8 (172)	12
Ameiurus nebulosus	Algonquin	19.1 (7.87)	17.0 (4.95)	17.1 6.40	22.2 (5.74	21.6 (8.23)	19
	Toronto	22.4 (5.01)	23.6 (9.06)	21.2 (11.1)	22.5 (20.4)	29.0 (26.0)	21
	Put-in-Bay	125.4 (42.5)	147.6 (35.6)	132.5 (35.3)	134.1 (34.2)	141.8 (50.0)	17
	Welaka	422.6 (648)	443.5 (731)	466.8 (1962)	477.4 (2003)		12
Ictalurus lacustris	Welaka	88.3 (103.6)	88.4 (51.9)	103.2 (99.6)	119.9 (436.5)	122.6 (406.2)	7
Lepomis macrochirus	Welaka	5.81 (2.63)	8.32 (9.58)	6.98 (4.11)	8.42 (5.91)	14.2 (11.4)	18

TABLE 2—*Continued*

Mean weight of fish and order of death. Numbers in brackets are the variance of the mean. For *Micropterus* from Put-in-Bay missing data give variable numbers of fish in each class. *Micropterus* from Welaka are segregated into three weight groups.

Species	Locality w. (winter) s. (summer)	*Mean weight and variance of mean* 1st Death	2nd Death	3rd Death	4th Death	5th Death	No. in each class
Micropterus salmoides	Put-in-Bay	53.0 (197)	42.6 (139)	57.9 (642)	51.2 (171)	64.1 (423)	5-8
	Knoxville	0.90 (.071)	1.30 (.078)	0.89 (.043)	0.85 (.008)	1.15 (.049)	6
	Welaka:						
	Large fish	152 (215)	158 (126)	159 (264)	146	176 (58.6)	3-6
	Medium "	39.0 (12.1)	28.4 (3.60)	32.3 (4.89)	35.2 (14.2)	30.5 (6.65)	8-10
	Small "	6.3 (0.58)	8.6 (8.73)	7.6 (0.51)	6.65 (0.66)	6.8 (0.18)	7-8
	Average	60.5	58.8	54.2	40.3	56.7	21

TABLE 3

Comparison of lethal temperature relationships during the summer and winter periods. Toronto (T) and Algonquin Park (AP) winter data were obtained during 1945 and 1946, Toronto summer data in 1947, Put-in-Bay (PIB) data summer 1946, and Knoxville (Knox) data summer 1947.

Species	Accl. Temp. °C.	Locality and seasons of testing	Time to 50 per cent mortality in minutes at test temperature °C.																			Incipient Lethal °C.	
			29.5	30.0	30.5	31.0	31.5	32.0	32.5	33.0	33.5	34.0	34.5	35.0	35.5	36.0	36.5	37.0	37.5	38.0	38.5	Upper	Lower
Rhinichthys atratulus	20	T-winter	5700	1850	1335	750	190		35	20	3											29.3	
		Knox-summer		1700	1100	592	367	157	60	30												29.5	
	25	T-winter		4000	2000	1600	550	265	90	43	18	14											
		T-summer					1340	770	545	270	150	78	46	18									
		Knox-summer			5500	2930	1700	810	358	280	158	47	22										
Semotilus atromaculatus	25	T-winter				900	500	280	180	130	88	45	16	15								30.3	
		T-summer						1335	1130	580	220	209	102	73	53	21						31.5	
Notropis cornutus	25	T-winter						750	450	220	90	32											
		T-summer						4800	2570	910	320	165	45	26	12								
Notropis atherinoides	25	T-winter					3800	1500	700	350	245	35										30.7	8.0
		PIB-summer											515	210	49	15						30.7	9.3
Hyborhynchus notatus	20	T-winter							440	400	120	16										31.7	4.2
		PIB-summer									180	65										32.7	7.0
	25	T-winter										550	42	21								33.3	7.5
		PIB-summer											620	320	265	50						34.0	8.5
Ameiurus nebulosus	20	AP-winter									127	80	35									32.5	
		PIB-summer								570	251	115	30									32.7	
	25	T-winter										1900			120	90	45						
		PIB-summer										1600	480	282	105	60						33.7	
	30	AP-winter													1400	1000	600	190	108	49	30		
		PIB-summer													1500	950	500	238	98	54	37		
Perca flavescens	25	T-winter		2500		1050	550	300	160	33												29.7	3.7
		PIB-summer							790	205	87	60	15									32.3	8.7

TABLE 4

Incipient lethal temperatures. In this table are gathered the data on incipient lethal temperatures collected during the investigation. The upper value in each pair is an upper incipient lethal temperature, the lower, a lower incipient lethal. For the definition of the incipient lethal temperature see page 10. Data given in table 3 have not been included here.

Species	Locality	Acclimation Temperature °C.						
		5	10	15	20	25	30	35
Dorosoma cepedianum	Put-in-Bay					34.0 10.8	36.0 14.5	36.5 20.0
	Knoxville					34.6	35.8	
Semotilus atromaculatus	Toronto		27.3 —	29.3 —	30.3 0.7	31.5 —		
	Knoxville					31.6		
Rhinichthys atratulus	Toronto	26.5 —	28.8 —	29.6 —	29.3 2.2			
	Knoxville				29.3			
Notemigonus crysoleucas	Algonquin Park		29.3 —		31.8 —			
	Put-in-Bay				32.1 —	33.7 7.5		
	Welaka			30.5 1.5	31.9 4.0	33.2 7.0	34.7 11.2	
Notropis cornutus	Toronto	26.7 —	28.6 —	30.3 0.0				
	Knoxville					32.3	33.5?	
Ictalurus lacustris	Put-in-Bay				32.7 4.7	33.5		
	Welaka			30.3 0.0	32.8 2.5	33.5 6.0		
Ameiurus nebulosus	Algonquin Park		29.0 —		32.3 —			
	Toronto		27.7 —	29.0 —	31.7 —			
Ameiurus nebulosus	Put-in-Bay				32.7 0.5	33.7 —	34.7 6.8	
	Welaka				— 0.5	— 4.0		

TABLE 4 (*Continued*)

Species	Locality	Acclimation Temperature °C. 5	10	15	20	25	30	35
Gambusia affinis	Knoxville						37.3	
							—	
	Welaka			35.4	37.3			37.3
				1.5	5.5			14.5
Micropterus salmoides	Put-in-Bay				32.5	34.5	36.4	
					5.5	—	11.8	
	Knoxville						36.4	
							—	
	Welaka				31.8	32.7	33.7	
					5.2	7.0	10.5	
Lepomis macrochirus	Welaka			30.7	31.5	—	33.8?	
				2.5	5.0	7.5	11.0?	

TABLE 5

Proportionate measurements of first and last fish to die from high temperatures. Standard error given in parentheses.

Species	Locality	Ratio of standard length to:	Mean ratio First	Last	No. of tests
Notropis cornutus	Knoxville	Dorsal origin to occiput	2.03 (.022)	2.06 (.014)	10
		Head length	3.92 (.034)	4.01 (.062)	10
		Head depth	5.68 (.068)	5.74 (.109)	10
		Dorsal height	5.16 (.107)	5.32 (.065)	10
Notemigonus crysoleucas	Algonquin Park	Head length	3.89 (.029)	3.86 (.067)	10
		Eye diameter	12.4 (.213)	12.4 (.173)	10
Ameiurus nebulosus	Welaka	Head width	4.43 (.096)	4.37 (.085)	11
		Pectoral spine	6.49 (1.68)	6.37 (.254)	11
		Anal base	3.69 (.066)	3.74 (.053)	11
	Algonquin Park	Head width	4.30 (.192)	4.24 (.042)	17
		Pectoral spine	7.51 (.114)	7.82 (.138)	17
		Anal base	4.30 (.061)	4.24 (.042)	17
Micropterus salmoides	Welaka	Head length	2.94 (.035)	2.92 (.036)	10
	Put-in-Bay	" "	2.92 (.040)	2.86 (.046)	8
	Both localities	" "	2.93 (.022)	2.89 (.029)	18
Micropterus salmoides	Welaka	Upper jaw	2.12 (.138)	2.08 (.034)	10
	Put-in-Bay	" "	2.03 (.032)	2.05 (.041)	8
	Both localities	" "	2.08 (.027)	2.07 (.026)	18
Notropis cornutus	Knoxville	Eye diameter	3.25 (.074)	3.17 (.080)	10
Notemigonus crysoleucas	Algonquin Park	Snout	4.26 (.077)	4.35 (.066)	10

TABLE 6

Number of fish of each sex dying first and last when exposed to lethal high temperature.

Species	Locality	Number of Fish First Death ♂	♀	Last Death ♂	♀
Ameiurus nebulosus	Welaka	7	4	6	5
	Put-in-Bay	12	6	8	10
	Algonquin	9	8	12	5
	All localities	28	18	26	20
Rhinichthys atratulus	Knoxville and Toronto	8	10	9	9
Perca flavescens	Put-in-Bay	4	4	5	3
Notemigonus crysoleucas	Algonquin	8	2	6	4
All Species	Total	48	34	46	36

TABLE 7

Distribution of scale counts and vertebrae for *Notropis cornutus* from Toronto, Ontario, and Knoxville, Tennessee. First and last deaths apply to lethal temperature tests.

Locality	Deaths	Vertebrae 37	38	39	40	x^2	P
Toronto		1	11	17	5	9.88	<.05
Knoxville		3	20	11			

Locality	Deaths	Lateral line scales 36	37	38	39	40	41	x^2	P
Toronto				1	14	13	4	47.43	<.001
Knoxville		2	5	25	8				
Toronto	First				4	2			
	Last				4	1	1		
Knoxville	First		5	4	1				
	Last			9	1				

Locality	Deaths	Predorsal scales 16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	x^2	P
Toronto								1		1	3	5	5	8	3	1	5	372.00	<.001
Knoxville		1	6	5	6	10	4	3	3	1	1								
Toronto	First							1				1	1	2			1		
	Last										2	1	2	1				8.30	>.30
Knoxville	First		3		1	2	1	1	2										
	Last			3	1	3	1	1		1									

TABLE 8

Growth rates for Notropis cornutus, Semotilus atromaculatus, and Notemigonus crysoleucas. Plus sign after age symbol indicates considerable growth beyond *annulus. No plus sign after age symbol indicates annulus at, or very close to, edge of scale.* Standard length given for each annulus.

				Annuli											
Species	*Locality*	*Date*		0+	I	I+	II	II+	III	III+	IV	IV+	V	V+	VII+
Notropis cornutus	Knoxville	May 20 to July 15, 1947.	Average length			54.2	76.5	81.9	90.0	109.8		145			
			Range			46.0 – 67.0		76.0 – 93.0	82.0 95.0	95.0 – 135.0					
			No. of fish			16	1	10	4	4		1			
	Toronto	August 25, 1947.	Average length			46.2		69.0		82.5		104			
			Range			39.0 – 60.0		61.0 – 73.8		70.0 – 90.0					
			No. of fish			11		10		11		1			
Semotilus atromaculatus	Knoxville	May 20 to July 15, 1947.	Average length			58.1		77.5	92.2	96.1		106			
			Range			47.0 – 73.0		61.5 – 95.0							
			No. of fish			8		12	2	2		1			
	Toronto	August 25, 1947.	Average length			59.0		68.7	75.7	140		155			
			Range			45.0 – 60.0		63.0 – 75.5							
			No. of fish			13		7	2	2		2			
Notemigonus crysoleucas	Welaka	December-March, 1946-7.	Average length	68.0	89.0	99.2	123	152	157	181	185	215			
			Range	29.0 – 23.0	77.5 – 94.0	91.0 – 120	100 – 135	143 – 177	137 – 182	170 – 210					
			No. of fish	16	5	10	4	14	4	5	2	1			
	Put-in-Bay	June 12 to Sept. 3 1946.	Average length	45.8		71.5			104		113.5		138		
			Range	32.0 – 57.0		61.5 – 93.0									
			No. of fish	23		23			3		2		1		
	Algonquin Park	July 9-Sept. 9, 1941.	Average length	43.7		53.4	65.5	70.4	71.5	79.6		92.5		97.0	111
			Range	38.0 – 47.5		45.0 – 58.3		62.7 – 78.2		66.0 – 86.0					
		May, 1946.	No. of fish	5		6	2	14	1	5		2		1	1

TABLE 9

Relation between standard length and body parts for *Notemigonus crysoleucas* and *Notropis cornutus*. (D.O. dorsal fin origin).

Species	Locality	Body part in relation to standard length	Standard length range	Formula x (log standard length) y (log body part)
Notemigonus crysoleucas	Welaka	Head length	Over 65.0	$y=0.934x-0.521$
			Under 65.0	$y=0.786x-0.248$
		Eye diameter	over 65.0	$y=0.667x-0.531$
			under 65.0	$y=0.524x-0.304$
		Snout	over 65.0	$y=1.000x-1.210$
			under 65.0	$y=0.890x-1.017$
	Put-in-Bay	Head length	over 65.0	$y=0.902x-0.466$
			under 65.0	$y=0.839x-0.358$
		Eye diameter	over 65.0	$y=0.659x-0.532$
			under 65.0	$y=0.707x-0.615$
		Snout	over 65.0	$y=0.919x-1.070$
			under 65.0	$y=0.886x-1.030$
	Algonquin	Head length	all lengths	$y=1.023x-0.629$
		Eye diameter	all lengths	$y=0.782x-0.699$
		Snout	all lengths	$y=1.010x-1.240$
Notropis cornutus	Knoxville	Head length	38-150 mm.	$y=1.010x-0.608$
		Eye diameter	38-150 mm.	$y=0.686x-0.536$
		D.O. to occiput	38-150 mm.	$y=0.999x-0.309$
		Dorsal height	38-150 mm.	$y=0.875x-0.474$
	Toronto	Head length	38-104 mm.	$y=0.960x-0.513$
		Eye diameter	38-104 mm.	$y=0.659x-0.503$
		D.O. to occiput	38-104 mm.	$y=1.000x-0.300$
		Dorsal height	38-104 mm.	$y=0.846x-0.419$

TABLE 10

Distribution of dorsal rays for *Gambusia affinis* from Welaka, Florida and Knoxville, Tennessee.

Locality	6	7	8	x^2	P
Welaka		5	14		
Knoxville	8	19		29.7	$>.001$

TABLE 11

Distribution of scale counts and dorsal soft rays for *Micropterus salmoides* from Put-in-Bay, Ohio, and Welaka, Florida. First and last deaths apply to lethal temperature tests.

Locality		7	8	9	10	11	12	13	14	15	16	17	x^2	P
		Scales above lateral line												
Put-in-Bay		16	26	20	2								23.35	$<.001$
Welaka		2	26	14	14	1								
Put-in-Bay	First	3	4	4	1								4.07	$>.30$
	Last	1	7	1	1									
Welaka	First	1	5	3	1								1.32	$>.80$
	Last		5	3	2									
	Normal		7	6	8	1							10.24	$<.20$
	Stunted	2	9	6	1									
		Dorsal soft rays												
Put-in-Bay							19	42	3				10.02	$<.02$
Welaka							4	40	7					
Put-in-Bay	First						4	7						
	Last						4	6	1				2.69	$>.30$
Welaka	First						1	9						
	Last						2	6	2				5.93	$>.10$
		Scales below lateral line												
Put-in-Bay							1	3	27	14	17	2	19.47	$<.01$
Welaka									5	20	18	5		
Put-in-Bay	First							2	7		2		1.30	$<.90$
	Last							2	5		2	1		
Welaka	First								1	7	2		2.49	$>.70$
	Last								1	4	4	1		
Welaka	Normal								2	6	11	3	8.29	$>.10$
	Stunted								3	11	4			

TABLE 12

Distribution of lateral line scales and vertebrae for *Micropterus salmoides* from Put-in-Bay, Ohio, Knoxville, Tennessee, and Welaka, Florida. First and last deaths apply to lethal temperature tests.

Locality		Lateral line scales																			x²	P
		57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	77		
Put-in-Bay		2	3	4	5	9	17	15	10	3	2	2	1									
Knoxville			1	2	2	4	3	2	1												6.57	>.80
Welaka									1	2	2	5	10	12	8	6	2	1	3	1		
Put-in-Bay	First	1		2	1		4	2													5.90	>.50
	Last			1	1	1	3	2	1	1												
Welaka	First											2	3	3				1	1		13.20	>.20
	Last										2		2	1	2	2	1			1		
Welaka	Normal											1	2	5	7	4	1	1	2	1		
	Stunted								1	2	2	2	6	4	1						22.56	>.05
		Vertebrae																				
		30	31	32																		
Put-in-Bay		3	49	7																		
Welaka		2	27	1																	1.80	>.50
Welaka	Normal	1	18	3																		
	Stunted	2	31	4																		

TABLE 13

Distribution of lateral line scales for *Rhinichthys atratulus* from Toronto, Ontario and Knoxville, Tenn.

Locality	47	50	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	73	x²	P
Knoxville	1			1	1	1		2	2	4	4	7	6	6	7	5	2	1	29.05	<.05
Toronto	1	1	2		3	3	4	5	6	5	6	3	2	1	2	1	1			

TABLE 14

Distribution of lateral line scales and predorsal scales for *Semotilus atromaculatus* from Toronto, Ontario and Knoxville, Tennessee. Results compared with data obtained by Greeley (1930) for Six Mile Creek, N.Y.

Locality	Lateral line scales															x²	P
	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67		
Knoxville	1	3	2	7	4	4	1	1									
Toronto	1	6	3	5	3	3	4	2								2.48	>.90
Six Mile Creek			1		3	4	1	5	10	3	2	3	2		1	37.0	<.001
	Predorsal scales																
	32	33	34	35	36	37	38	39	40	41	42	43	44	45			
Knoxville		1	1	2	7	3	3		3	2	1			1			
Toronto	2	2	3	2	3	4	4	2		2	1	2				11.4	>.50
Six Mile Creek	3	2	7	3	5	5	3	6	1							11.3	>.50

TABLE 15

Proportionate measurements of *Rhinichthys atratulus*, *Semotilus atromaculatus* and *Ameiurus nebulosus* from various localities. Standard error given in parentheses.

Species	Locality	Ratio of standard length to:	Mean, both sexes	No. of tests
Rhinichthys atratulus	Knoxville	Head length	3.69 (.054)	16
	Toronto	" "	3.74 (.028)	20
	Knoxville	Caudal peduncle	8.09 (.188)	16
	Toronto	" "	8.39 (.076)	20
	Knoxville	Head depth	6.29 (.076)	16
	Toronto	" "	6.13 (.053)	20
	Knoxville	Head width	6.28 (.093)	16
	Toronto	" "	5.98 (.063)	20
	Knoxville	Body depth	4.46 (.070)	16
	Toronto	" "	4.71 (.042)	20
Semotilus atromaculatus	Knoxville	Head length	3.58 (.024)	25
	Toronto	" "	3.50 (.023)	28

		Ratio of head length to:		
Rhinichthys atratulus	Knoxville	Upper jaw	3.37 (.058)	16
	Toronto	" "	3.76 (.053)	20

		Ratio of standard length to:	Mean ♂	Mean ♀	No. of tests ♂	No. of tests ♀
Semotilus atromaculatus	Knoxville Toronto combined	Head length	3.51 (.028)	3.57 (.023)	18	24
Ameiurus nebulosus	Welaka	Head width	4.32 (.044)	4.50 (.057)	26	23
		Pectoral spine	6.44 (.116)	6.44 (.115)	26	22
	Put-in-Bay	Head width	4.37 (.028)	4.48 (.045)	27	23
		Pectoral spine	8.76 (.120)	8.49 (.138)	26	22
	Algonquin	Head width	4.47 (.033)	4.48 (.072)	22	12
		Pectoral spine	7.83 (.039)	7.36 (.088)	22	12

TABLE 16

Distribution of anal rays, lateral line scales and vertebrae counts for Notemigonus crysoleucas from Algonquin Park, Ontario, Put-in-Bay, Ohio, and Welaka, Florida. First and last deaths apply to lethal temperature tests.

Locality	Deaths	Anal Rays 11	12	13	14	15	16	17	18	χ^2	P
Algonquin			23	18	1					7.58	>.10
Put-in-Bay		3	16	28	5						
Welaka					3	6	20	8	3		
Algonquin	First		7	3						.80	>.50
	Last		5	5							
Put-in-Bay	First	2	3	7	1					3.05	>.50
	Last		6	6	1						
Welaka	First				1		4	1			
	Last					1	2	3			

Locality	Deaths	Vertebrae 37	38	39	χ^2	P
Algonquin		18	19	1	21.90	<.001
Put-in-Bay		4	10	14	6.24	=.10
Welaka			11	25		

Locality	Deaths	Lateral Line Scales 40	41	42	43	44	45	46	47	48	49	50	51	52	53	χ^2	P
Algonquin		1		1	1	3	1	3	9	5	4	1	1		1		
Put-in-Bay				1	1	2	7	6	5	10	10	5	3	1		12.24	>.50
Welaka		1	2	2	5	8	15	9	1	1						33.36	<.01
Algonquin	First	1		1	1	1		2	2		1				1		
	Last					1	1	1	4		1	1	1				
Welaka	First	1	1			1	2	1									
	Last			1		2	1		1	1							

TABLE 17

Distribution of anal rays of *Ameiurus nebulosus* from Algonquin Park, Ontario, Put-in-Bay, Ohio, and Welaka, Florida. First and last deaths apply to lethal temperature tests.

Locality	Deaths	19	20	21	22	23	24	25	26	x^2	P
Algonquin		2	8	16	8						
Put-in-Bay		3	19	20	4					6.32	>.10
Welaka			2	5	16	15	8	1	1	57.97	<.001
Algonquin	First	2	3	8	4						
	Last		5	8	4					2.47	>.50
Welaka	First				5	4	2			5.60	>.30
	Last			1	4	4		1	1		

TABLE 18

Relation between physiological characteristics and habitat. (1) Fry, Brett, and Clausen (1942); (2) Fry, Hart, and Walker (1946); (3) Hart (1947); (4) Fry and Hart (1948); (5) Clausen (1936); (6) Graham (1948); (7) Fry (1947); (8) Wiebe and Fuller (1933); (9) Gibson (unpublished).

Species	Resting O_2 consumption 20°C. mg/kilo/hr.	Incipient upper lethal 20°C.	Lower lethal 20°C.	Habitat
Cristivomer namaycush		23.5(9)		Deep lakes
Salvelinus fontinalis	140(6)	25.3(2)	>0(2)	Cool streams, deep lakes
Rhinichthys atratulus		29.3(3)	2.2(3)	Warm and cool streams, riffles
Semotilus atromaculatus		30.3(3)	0.7	Warm and cool streams, pools
Notropis c. frontalis	131(5)	31.0(3)	3.7(3)	Warm and cool streams, pools
Notropis c. chrysocephalus		31.5 (estimate)		As above but warmer streams
Notemigonus crysoleucas	97(5)	32.0	4.0	Slow streams with vegetation marsh
Ictalurus lacustris		32.7	4.7	Large rivers
Hyborhynchus notatus		32.7	7.0	Streams, mostly at base level; shallow lakes
Micropterus salmoides	65(5) 36-53(6)	32.5	5.5	Marsh
Ameiurus melas	73(5)			Marsh; shallow zone of lakes
Ameiurus nebulosus	270(7)	32.7	0.5	Marsh; shallow zone of lakes
Carassius auratus	61(4)	34.8(1)	2.5(1)	Very shallow water
Gambusia affinis holbrooki		37.3	5.5	Very shallow water