The Physiological Ecology of Larval Muskellunge and Norlunge:
Temperature Tolerance and Growth Rates Under Hatchery Conditions.

THESIS

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requirements for the degree of Master of Arts at the
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By

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ABSTRACT OF THESIS

The Physiological Ecology of Larval Muskellunge and Norlunge: Temperature Tolerance and Growth Rates Under Hatchery Conditions.

Critical Thermal Maxima (CTM) and growth rates of larval muskellunge (Esox masquinongy) and norlunge (E. masquinongy ♀ x E. lucius ♂) were determined under hatchery conditions. Two groups of fish were maintained at ambient water temperatures with natural photoperiod. Weekly water samples were collected to monitor the troughs. Parameters measured were dissolved oxygen, pH, and ammonia which were within normal limits.

Norlunge had higher temperature tolerance and developed faster than muskellunge. CTM of larval norlunge (30.9° to 36.0°C, $\bar{X}$ =34.0°C) were significantly different ($P<0.01$) from those of muskellunge (29.9° to 35.6°C, $\bar{X}$ =32.8°C).

Both species exhibited a sharp decrease in CTM during swim-up, followed by a slow recovery period indicated by a general increase in CTM values. Both species steadily increased in length during development. Weight remained stable through swim-up, decreased with absorption of the yolk sac and then increased as the fry began to feed.

The results obtained in this study indicates that both age and past thermal history had a significant effect on the temperature tolerance of muskellunge and norlunge fry.

John David Bonin

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Date

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INTRODUCTION

The muskellunge (Esox masquinongy) is a member of the family Esocidae. It is one of the largest pikes reaching a length of 140 cm. and a weight of over 27 kg. It ranges from the headwaters of the Mississippi River system in the northern United States and Canada, south to Tennessee and north to Quebec. A voracious carnivore, it eats a wide variety of prey including fish, frogs, waterfowl, and small mammals (Hourston 1952). Like all pikes it prefers clear water, sandy bottoms, and considerable aquatic vegetation (Trautman 1957).

Natural hybridization sometimes occurs between the muskellunge and the northern pike (Esox lucius), since in some waters they utilize the same spawning areas and have similar feeding habits (Oehmeke 1951). Because the F₁ hybrid is sterile, grows rapidly, and is an effective predator, it can be important in the management of lakes and streams where it is used to control fish communities which have become overpopulated. Artificial propagation of this hybrid has been possible for many years (Black and Williamson 1947).

The muskellunge is abundant in Chautauqua Lake, New York where it is an ecological dominant and is the basis for one of the State's most important sport fisheries (Heacox 1942, Mooradian and Shephard 1973). The muskellunge is the only Esocid present in the lake. A state fish hatchery at Pendergast Point propagates muskellunge for stocking in Chautauqua and smaller nearby lakes to offset intense fishing pressure. Some norlunge are also produced at the hatchery and used in management programs in other New York lakes.

The history of muskellunge propagation at the Chautauqua

Lake fish hatchery has been briefly reviewed by Hassan and Spotila (1976). Additional information indicates that the muskellunge catch in Chautauqua Lake has been dropping since 1905, and that natural reproduction has been insufficient to maintain the fishery. Whipple (1906) in the report of the State Fish Culturist noted that muskellunge raised on lake water grew faster than those raised on well water. This was attributed to the higher temperatures ($+7^{\circ}$ to 8°C) of the lake water. It was also noted in 1958 and 1962 that erratic temperature fluctuations occurring during development caused excessive mortality (New York Department of Environmental Conservation 1958 and 1962). The hatchery has experienced complete fry mortality in seven out of the last ten years (Colesante 1974). Death of the fry occurred at the swim-up stage of development and has been attributed by N.Y.D.E.C. personnel to a bacteria pathogen in the water. Swim-up refers to that stage of development at which a functional upper jaw appears, the yolk sac is totally absorbed, and fry begin actively swimming and feeding. Recent experiments by N.Y.D.E.C. personnel (summer of 1975) proved inconclusive because of the lack of a control. I believe that a closer look at the environmental factors affecting the fry may provide information that would prevent their deaths. Hassan and Spotila (1976) showed that temperature was important in the development of muskellunge fry, and may have been a contributing factor in the mortalities of fry at the Chautauqua Lake fish hatchery.

Temperature has been shown to be an important factor in the hatching success and survival of many species of fish larvae (Allblough and Manz 1964, Colby and Brooke 1970, Combs 1965,

Edsall 1970, Hubbs 1966, Kelley 1968, and McCormick, Jones, and Syrett 1971). Thermal requirements of fish in early stages of development often are more exacting than in the adult stage (Brett 1956, Banner and Van Arman 1973, Hokanson et al. 1973, and Hokanson, McCormick, and Jones 1973). Wisconsin fisheries personnel have reported that temperature was a major factor in periodic die offs of muskellunge fry at their Woodruff and Spooner hatcheries. Walker (1968) stated that abnormally low temperatures produced weak fry and high mortalities. Eggs and larval stages were extremely sensitive to physical shock induced by excessive drops or rises in temperature. Oehmeke (1969) reported that high mortalities resulted from weak fry and differential hatching that were due to high and low water temperatures.

Adult muskellunge can tolerate temperatures as high as 32°C although 24°C appears to be optimum (Scott 1964, Ferguson 1958). Galat and Eipper (1969) found that 13°C was an optimum temperature for incubation of muskellunge eggs. Eggs kept at this temperature hatched in 12 days and swim-up fry were in excellent condition.

Hassan and Spotila (1976) studied the effects of rearing temperatures on the development of muskellunge. They found that muskellunge reared at 15°C exhibited a decrease in temperature tolerance during swim-up that was followed by a slow recovery period, indicated by an increase in tolerance. At a higher temperature (25°C) development was rapid and a sensitive period was not noted, while at a lower temperature (7°C) development was abnormal and high mortalities occurred. Fry appeared to be at least as tolerant of high temperatures as were the larger individuals tested by Scott (1964).

Historically temperature tolerance has been determined by using one of two methods. The first is the lethal temperature method (L.T. 50) developed by Fry and his colleagues at the University of Toronto (Fry 1967), and the second is the critical thermal maximum method introduced by Cowles and Bogert (1944).

The procedure for the lethal temperature test has been standardized by Brett (1952) and Edsall and Colby (1970). Mortality data from this test are usually analyzed by the method of Fry et al. (1946) to determine the incipient upper lethal temperature (I.U.L.T.). The I.U.L.T., as defined by Edsall and Colby (1970), "was that temperature falling midway between the test temperature that killed 50% or more of a test group in 10,000 minutes and a second test temperature that was 0.5 or 1.0°C closer to the acclimation temperature and killed less than 50% of a second test group in 10,000 minutes". In this method the variable is time since temperature is held constant.

Cowles and Bogert (1944) defined the CTM as "the thermal point at which locomotor activity becomes disorganized and the animal loses its ability to escape from conditions which will promptly lead to its death". Lowe and Vance (1955) and Hutchison (1961) modified this definition to include a standard methodology for tests of CTM, this entailed rapid controlled heating (1°C/min.) of the test animal from its acclimation temperature to the end-point defined above. The CTM is two dimensional, involving a rise in body temperature over a period of time. Critical thermal maximum is an indicator of the thermal tolerance of a fish, and is a measure of its ability to acclimate to changes in temperature.

If done correctly the CTM test can be a valuable tool in the study of thermal ecology. The validity and usefulness of this test have been proven by Hutchison and his students (Hutchison 1961, Hutchison and Kosh 1964, Hutchison et al. 1973, Hutchison and Rowlan 1975, and Mahoney and Hutchison 1969) as well as by Heatwole et al. 1965, Dunlop 1968, and Spotila 1972. Hassan and Spotila (1976) have demonstrated its utility in the study of temperature tolerance of newly hatched fish.

Improper methods (incorrect heating rate and/or endpoint) raise a question concerning the results of CTM tests as reported by several authors (Starkey et al. 1972, Lashbrook and Livezey 1970, Cox 1974, and Campbell and Davies 1975). While these authors have indeed measured the temperature tolerance of their test organisms, they have not ascertained the organisms' CTM.

The purpose of this study was to determine the effect of age and thermal history on the temperature tolerance and growth rates of larval muskellunge (E. masquinongy) and norlunge (E. masquinongy ♀ x E. lucius ♂) raised under hatchery conditions. Temperature tolerance was determined by the critical thermal maximum (CTM) method.

METHODS AND MATERIALS

This study was conducted at the Chautauqua Lake Muskellunge Hatchery on Pendergast Point in Chautauqua county, New York. The Chautauqua Lake hatchery is owned and operated by the state of New York and is one of the largest muskellunge hatcheries in the northeastern United States.

Muskellunge and norlunge fry were raised in outside troughs that measured 310 x 61 x 46 cm. with a flow rate of 6 liters/min. Water was supplied from the lake at natural temperatures until lake temperatures reached 20°C. Well water was then mixed with the lake water to maintain water temperatures below 20°C. Troughs were partly covered with wood to reduce heating due to solar radiation and to prevent predation by birds. One end of each trough was left uncovered to provide a natural photoperiod. Fry were maintained by hatchery personnel.

On May 25, 1975 approximately 10,000 day old muskellunge and norlunge fry were placed in each of 2 troughs, on wire screens just under the water surface. After a few days those fry still on the screens were assumed to be dead and were discarded. After swim-up, muskellunge and norlunge fry were fed brine shrimp and zooplankton ad lib. Beginning on day 20, muskellunge were fed live minnows. Norlunge beginning on day 13, were raised on a commercial fish pellet dispensed by automatic feeders at 15 minute intervals. Experimentation continued until fry reached fingerling size (61-63 cm.) at which time they were stocked.

Water quality was monitored throughout the study to determine dissolved oxygen, pH, and ammonia (see Appendix A for procedures). A laboratory was maintained at the Chautauqua Lake hatchery and

all water quality measurements were made at this facility. Dissolved oxygen (DO) was determined by the modified winkler method and was measured at least once a week. The DO's up to day 13 had to be discarded because of improper reagents. Hydrogen ion concentration (pH) was also measured once a week, using a Coleman pH meter standardized with 6.8 buffer. Ammonia was determined weekly using the phenate method. All water quality tests were determined at 1400 EDT. Temperature was recorded every test date at 0900 with a standardized mercury thermometer graduated to 0.1°C .

Critical thermal maxima (CTM) were determined between 0930 and 1400 EDT, according to the method described by Hutchison (1961). The test apparatus used was a 1-liter beaker with a 1.28×61 cm. Briskeat silicone heating tape wound around the beaker and connected to a variable transformer. The transformer was used to control the heating rate. Two fry were placed into the beaker with 300 ml. of water, at ambient temperature ($\pm 1^{\circ}\text{C}$) and heated at a rate of $1.0 \pm 0.2^{\circ}\text{C}/\text{min}$. until they reached their CTM. The onset of spasms was used as the endpoint for the post swim-up fry (see results for discussion of endpoint).

Aeration was supplied through an airstone to mix the water so that even heating could be maintained and in order to keep the water saturated with oxygen. Water temperature was measured with a standardized mercury thermometer graduated to 0.1°C .

Ten fry of each species were collected once a week to determine growth rates. Fry were preserved in 10% formalin and total length was measured using a Helios dial caliper accurate to ± 0.005 mm. Wet weight was determined using an Ainsworth Type SCN analytical balance accurate to ± 0.00005 g. Fish were

blotted dry with a paper towel to remove excess moisture and then weighed singly.

A one-way analysis of variance (ANOVA) was performed on the individual values for the two species to determine overall statistical significance between the species. Differences in CTM through time of each species were analyzed using a one-way ANOVA and graphically by the method of Hutchison (1961). Calculated F values for ANOVA were considered significant at the 5% level of probability ($P < 0.05$) and highly significant at the 1% level ($P < 0.01$).

RESULTS

Water Quality

Water quality in the rearing troughs was normal during the course of this experiment. Dissolved oxygen ranged from a high of 8.8 mg/l on day 13 to 6.0 mg/l on day 49 (Fig. 1). Through the experiment dissolved oxygen decreased as temperature increased and as the amount of oxygen in the lake also began to decline. Ammonia remained well below 0.5 $\mu\text{g/l}$ and pH was stable. Temperature ranged from 16.0°C on day 1 to 22.5°C on day 37. On day 37 water was analyzed through the night to check for diurnal fluctuations in ammonia, oxygen, and temperature. Ammonia was below 0.5 $\mu\text{g/l}$ throughout the night. Dissolved oxygen was 8.0 mg/l at 1600 and dropped to a low of 5.9 mg/l at 2100, increasing to 7.8 mg/l at 0830 (Fig. 2). Temperature decreased from 22.5°C at 0900 to 20.4°C at 2100, and 19.9°C at 0800 on day 38, then increasing to 20.7°C at 1100.

Critical Thermal Maxima

It was difficult to ascertain the endpoint for the pre swim-up fry. There seemed to be no clear cut point which could be used. Dorsal lateral bending observed by Hassan and Spotila (1976) was not noted. The only activity observed in these fry was slight jerking movements. Movement would occur when fry were stimulated with a glass rod. The endpoint used was the temperature at which the fry would no longer respond to the stimulus of a glass rod.

After the fry swam-up the endpoint used was the onset of spasms. When these fry were placed into the test beaker they

Fig. 1 Water quality of fish troughs at the Chautauqua Lake fish hatchery during the course of this experiment.

Fig. 2 · Dinurnal fluctuations in the temperature and dissolved oxygen of experimental troughs on days 37 and 38.

swam continuously. As the temperature increased, activity increased with fry occasionally losing equilibrium. At this stage they would always recover and resume swimming. Prior to reaching their CTM the fry would slow down and begin to gasp at the surface. The CTM was noted as the temperature at which spasms began. This was immediately followed by complete loss of equilibrium and cessation of opercular movement. Spasms were rapid, beginning at the head and moving posteriorly. Within this general pattern, variations of the endpoint did occur. Some fry exhibited only a slight shudder of the head region, while others became rigid and lost equilibrium without any noticeable spasms. At this point if the fry were removed from the test beaker and placed in colder water, they would completely recover within 30 minutes. If the fry were allowed to remain in the test beaker after the CTM was reached, no activity would occur for a few moments, then the fry would go into violent spasms. These fry would never recover, even when placed in colder water. This endpoint was a lethal temperature and should not be confused with the CTM which did not involve death.

There was a highly significant difference ($P < 0.01$) in the average CTM of norlunge fry (34.0°C) and muskellunge fry (32.8°C) in this experiment (Table 1). There was a highly significant difference ($P < 0.01$) in the CTM of muskellunge fry with time during the course of the experiment (Table 2). CTM ranged from 29.9°C on day 4 to 35.6°C on day 37 (Fig. 3). Muskellunge fry on day 2 had a CTM of 31.7°C , which significantly decreased on day 4 to 29.9°C . Swim-up of muskellunge began on day 4 and was complete on day 6. After swim-up was complete CTM's began to

Table 1: One-way ANOVA for the average CTM of muskellunge and norlunge reared at the Chautauqua Lake fish hatchery.

Source	df ¹	SS ²	MS ³	F ⁴
Species	1	119.23	119.23	40.08**
Individuals	370	1100.62	3.97	

**P<0.01

1. degrees of freedom
2. sum of squares
3. mean square
4. F value

Table 2: One-way Anova for the effect of time on the CTM of muskellunge reared under hatchery conditions.

Source	df	SS	MS	F
Test days	18	638.31	35.46	131.14**
Individuals	167	45.16	0.27	

**P<0.01

Table 3: One-way ANOVA for the effect of time on the CTM of norlunge reared under hatchery conditions.

Source	df	SS	MS	F
Test days	18	384.18	21.34	108.34**
Individuals	167	32.89	0.19	

**P<0.01

Fig. 3. Critical thermal maxima of muskellunge reared for 55 days at Chautauqua Lake fish hatchery. Vertical lines bounded by horizontal lines are ranges; center horizontal lines are means; black rectangles represent $\bar{X} \pm 2.262$ SE, black rectangles on day 2 represent $\bar{X} \pm 2.571$ SE; sample size is ten unless otherwise specified. If the black rectangles of any two given samples do not overlap, they are considered to be statistically significantly different at the 0.05 level or beyond. After the method of Hutchison (1961).

increase, a drop in water temperature (T_w) from 19.2°C on day 5 to 16.5°C on day 6 had no apparent effect on the CTM measured on days 6-10. From day 10 to day 16 CTM remained stable as did water temperature (Fig. 1). The CTM on day 19 was not significantly different from that on days 13, 16, and 25, despite a drop in T_w from 18.7° to 16.6°C during this period. However the CTM on day 25 was significantly different from those on days 2-16. The increase in CTM beginning on day 25 was correlated with increasing water temperatures (Fig. 1 and 3). The CTM was 32.8°C ($T_w=17.8^{\circ}\text{C}$) on day 25 and increased to a high of 35.6°C on day 37 ($T_w=22.5^{\circ}\text{C}$). When water temperatures decreased to 18.7°C on day 43 the CTM correspondingly decreased to 34.6°C and then stabilized.

There was a highly significant difference ($P<0.01$) in the CTM of norlunge fry with time in this study (Table 3). Values ranged from 30.9°C on day 4 to 36.0°C on day 40. Norlunge on day 2 had a CTM of 32.5°C which significantly decreased on day 4 to 30.9°C (Fig. 4). Swim-up of the norlunge fry began on day 3 and was complete on day 4. After swim-up, norlunge like muskellunge fry, showed an increase in CTM with values increasing to 31.9°C on day 6 and 33.4°C on day 10. The drop in T_w on day 6 had no apparent effect on CTM. There was little change in CTM from days 10 to 19 despite a drop in T_w from 18.7°C on day 13 to 16.6°C on day 19. On day 31 CTM began to rise in association with a general increase in T_w . When T_w decreased from 22.5°C on day 37 to 18.7°C on day 43 norlunge CTM remained stable. Critical thermal maxima finally decreased to 35.2°C on day 53 after 10 days of low water temperatures.

Fig. 4 Critical thermal maxima of norlunge reared for 55 days at Chautauqua Lake fish hatchery. Vertical lines bounded by horizontal lines are ranges; center horizontal lines are means; black rectangles represent $\bar{X} \pm 2.262$ SE, black rectangles on day 2 represent $\bar{X} \pm 2.571$ SE; sample size is ten unless otherwise specified. If the black rectangles of any two given samples do not overlap, they are considered to be statistically significantly different at the 0.05 level or beyond. After the method of Hutchison (1961).

Growth Rates

Muskellunge upon hatching averaged 9.95 mm. in length and weighed 0.0108 g. Muskellunge fry steadily increased in length through development. Growth was slow from swim-up on day 6 until day 25, when fry began to eat live minnows. Length then increased rapidly to 61.45 mm. on day 53 (Fig. 5). The pattern of weight gain appeared similar to that for length. Weight decreased when the yolk sac was completely absorbed (day 7) and rapidly increased starting on day 20. On day 53 fry averaged 1.2000 g. (Fig. 6).

Norlunge averaged 10.27 mm. and 0.0130 g. upon hatching. They increased in length through swim-up and showed a slow down in growth from day 10 to day 16, similar to that observed in muskellunge. After this time norlunge increased in length to 58.69 mm. on day 43. From day 43 to day 53 length only increased 4.89 mm. to 63.58 mm. (Fig. 5). Weight of larval norlunge continued to increase rapidly throughout development to 1.4907 g. on day 53 (Fig. 6).

Fig. 5 Growth in length of muskellunge and norlunge reared under hatchery conditions. Each dot represents the mean of ten fry for each day sampled.

Fig. 6 Growth in weight of muskellunge and norlunge reared under hatchery conditions. Each dot represents the mean of ten fry for each day sampled.

DISCUSSION

Critical Thermal Maximum

Throughout the course of this study norlunge fry had a significantly higher temperature tolerance than muskellunge fry. These results indicates that norlunge are better able to adjust physiologically to fluctuating environmental temperatures. Scott (1964) reported that the lethal temperatures of juvenile muskellunge acclimated to 25°C was 32.5°C (resistance time of 400 minutes) as compared to a lethal temperature of 32.5°C for northern pike and 33°C for their F₁ hybrids. He stated that this increase in thermal tolerance was an indication of hybrid vigor. The results of this study appear to support his findings.

Both age and past thermal history had a significant effect on the temperature tolerance of muskellunge and norlunge fry (Figs. 3 and 4). On day 13 and day 43, T_w was the same but CTM were different. This was due to differences in the past thermal regime and in the developmental stage of the fry on these days. These results are consistent with the findings of other studies on fish (Allen and Strawn 1971, Banner and VanArman 1973, and Hassan and Spotila 1976), salamanders (Hutchison 1961, Spotila 1972), and frogs (Brattstrom 1968, Mahoney and Hutchison 1969).

Both species exhibited a marked decrease in CTM during swim-up. This indicates that swim-up was a critical period in the development of these fish. It is during this period that environmental factors such as temperature, toxic substances, disease, etc. would have their greatest effect. Hassan and Spotila (1976) reported the same effect for muskellunge fry raised in the laboratory. Fry reared at 15°C showed a sharp drop in thermal

tolerance during swim-up. Water temperature at the hatchery ranged from 16.0°C on day 1 to 19.2°C on day 5 and dropped to 16.5°C on day 6. Temperature below 20°C appears to be detrimental to fry during swim-up. Hassan and Spotila (1976) found that fry raised at 25°C developed rapidly and did not show a reduction in thermal tolerance during swim-up. Similar effects have been reported for bluegill sunfish in the process of swimming up (Banner and VanArman 1973) and for northern pike (Franklin and Smith 1963).

After swim-up the temperature tolerance of muskellunge fry gradually increased (Fig. 3). From swim-up until day 25, CTM increased regardless of water temperature. After day 25 CTM changed in response to changes in T_w . The CTM of norlunge fry followed the same pattern but was higher than those of muskellunge fry. After day 31, CTM increased with increasing T_w (Fig. 4). These results suggest that initially (up to 25 days after hatching for the muskellunge) developmental effects are the dominant factors in determining temperature tolerance of these fry. This period may be longer for norlunge fry since their CTM did not drop for several days after a drop in T_w that began on day 37 (Fig. 4). This early period in development is a time of rapid change at the cell, tissue, and organ levels. It appears to be a refractory period during which moderate changes in environmental temperatures have little or no measurable effect on CTM. The mechanisms involved in this phenomenon are unknown.

Growth Rates

Changes in growth rates through time were related to changes in development, diet, and environmental conditions. Growth was slow in the sac fry stage. Both species increased slightly in length prior to swim-up. A weight reduction was observed in muskellunge fry when they absorbed their yolk sac. This was not noted in norlunge because of their rapid rate of development and early swim-up (day 3).

Beginning on day 16, muskellunge grew rapidly (Figs. 5 and 6). This was due to changes in diet and temperature. On day 16, T_w began to rise steadily and on day 20 fry began to eat live minnows instead of zooplankton and brine shrimp. By the end of the experiment muskellunge fry were similar in length and weight to the norlunge.

On day 13 norlunge began to receive dry feed rather than live minnows as food. Many fish could not adjust to this change in diet. Many fish died and cannibalism was common. After several days the remaining fish became adapted to this diet. These fry steadily increased in length throughout the experiment. They gained weight rapidly until day 25. By this time all of the norlunge fry were eating dry food. Their rate of weight gain was reduced during the remainder of the experiment.

RECOMMENDATIONS

The results of this study suggest that temperature is an important factor in the development of muskellunge and norlunge. Temperature control is vital to the successful propagation of these fry, especially during swim-up. Maintenance of high temperatures (20°-25°C) during development would decrease the length of the critical swim-up period and would cause an increase in the survival of these fry. Additional research at the hatchery is needed to determine the optimum temperature needed for development.

Norlunge fry are hardier than muskellunge fry. They have a higher temperature tolerance, develop more rapidly, are less sensitive to low temperatures during swim-up and grow more rapidly on live food. Muskellunge eating live minnows grow more rapidly than norlunge eating dry food. Dry food can be used to grow norlunge but it is probably not as effective as live minnows. Its use should be restricted to those instances when minnows are unavailable.

Caution should be applied in the application of these findings. Although norlunge fry are easier to raise and hardier than muskellunge fry, large scale propagation of this hybrid could cause an ecological disaster. This hybrid does not reproduce, it probably can outcompete both northern pike and muskellunge. Introduction of this hybrid into lakes or rivers that now support either of its parents, or other members of the family Esocidae, could result in the disappearance of these fish from those waters.

APPENDIX A

Dissolved oxygen - modified winkler method

Apparatus

1. Sample bottles - 300 ml. $\pm$ 3 ml. capacity BOD bottles
2. Pipets

Reagents

1. Manganous sulfate solution: Dissolve 364 g. of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in distilled water and dilute to 1 liter.
2. Alkaline iodide solution: Dissolve 700 g. of potassium hydroxide (KI) in distilled water and dilute to 1 liter. To this solution add 10 g. of sodium azide (NaN_3) dissolved in 40 ml. of distilled water.
3. Sulfuric acid, concentrated
4. Starch solution
5. Sodium thiosulfate, stock solution, 0.75 N: Dissolve 186.15 g. $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 ml. chlorform.
6. Sodium thiosulfate standard titrant, 0.0375 N: Prepare by diluting 50 ml. of stock solution to 1 liter.
7. Potassium bi-iodate standard, 0.375 N: Dissolve 4.873 g. potassium bi-iodate, previously dried 2 hours at 103°C , in 1 liter of distilled water. Dilute 250 ml. to 1 liter for 0.0375 N.

Standardization of 0.0375 N sodium thiosulfate

Dissolve 2 g. KI in 100 ml. distilled water, add 10 ml. of 10% H_2SO_4 followed by 20 ml. standard potassium bi-iodate. Place in dark for 5 minutes, dilute to 300 ml., and titrate with the standard sodium thiosulfate titrant to a pale straw color. Add 1-2 ml. starch solution and continue the titration drop by drop until the blue color disappears.

Procedure

To the sample collected in the BOD incubation bottle, add 2 ml. of the manganous sulfate solution followed by 2 ml. of the alkali-iodide-azide reagent, well below the surface of the liquid, stopper with care to exclude air bubbles, and mix well. When settling has produced at least 100 ml. of clear supernate, carefully remove the stopper and immediately add 2 ml. of conc. H_2SO_4 by allowing the acid to run down the neck of the bottle, re-stopper and mix by gentle inversion until the iodine is uniformly distributed throughout the bottle. Complete the analysis

within 45 minutes. Transfer the entire bottle contents by inversion into a 500 ml. wide mouth Erlenmeyer flask and titrate with 0.0375 N thiosulfate solution to a pale straw color. Add 1-2 ml. of starch and continue to titrate to the first disappearance of the blue color.

Calculation

Each ml. of 0.0375 N sodium thiosulfate titrant is equivalent to 1 mg/l DO when the entire bottle contents are titrated.

Precision and Accuracy

Exact data are unavailable on the precision accuracy of this technique; however, reproducibility is approximately 0.2 mg/l of DO at the 7.5 mg/l level due to equipment tolerance and uncompensated displacement errors.

Ammonia - phenate method

Apparatus

1. Spectrophotometer
2. Magnetic stirrer

Reagents

1. Ammonia free water
2. Hypochlorous acid reagent: To 40 ml. of distilled water add 10 ml. of a 5% commercial bleach. Adjust pH to 6.5-7.0 with HCl. Prepare weekly.
3. Manganous sulfate solution, 0.003 M: dissolve 50 mg. $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in 100 ml. distilled water.
4. Phenate reagent: dissolve 2.5 g. sodium hydroxide, NaOH, and 10 g. phenol, in 100 ml. of ammonia free water. Prepare weekly.
5. Stock ammonium solution: dissolve 381.9 mg. anhydrous ammonium chloride NH_4Cl dried at 100°C in ammonia free water and dilute to 1 liter. 1 ml. = 122 μg NH_3 .
6. Standard ammonium solution: dissolve 5 ml. stock solution to 1 liter with ammonia free water. 1 ml. = 0.610 μg NH_3 .

Procedure

To a 10 ml. sample in a 50 ml. beaker add 1 drop manganous sulfate solution. Place on a magnetic stirrer and add 0.5 ml. hypochlorous solution, followed immediately by the addition, a

drop at a time of 0.6 ml. phenate reagent. Measure the color after zeroing the photometer on the blank at 630 mμ.

Calculation

Beer's law governs. Calculate the ammonia concentration as follows:

$$\mu\text{g/l Ammonia } N = \frac{A \times B}{C \times S}$$

Where A=absorbance of sample, B=μg ammonia N in standard taken, C=absorbance of standard, and S=ml. unknown water sample used.

LITERATURE CITED

- Allbaugh, C.A., and J.V. Manz. 1964. Preliminary Study of the Effects of Temperature Fluctuations on Developing Walleye Eggs and Fry. *Prog. Fish-Cult.* 26(4):175-180.
- Allen, K.O., and K. Strawn. 1971. Rate of Acclimation of Juvenile Channel Catfish, Ictalurus punctatus, to High Temperatures. *Trans. Amer. Fish. Soc.* 100(4):665-671.
- Banner, A., and J.A. VanArman. 1973. Thermal Effects on Eggs, Larvae and Juveniles of Bluegill Sunfish. Report, Ecological Research Series, EPA-R3-73-041. 111pp.
- Black, J.D. and L.O. Williamson. 1947. Artificial Hybrids Between Muskellunge and Northern Pike. *Wisc. Acad. Sci., Arts and Letters.* 1947:299-314.
- Brattstrom, B.H. 1968. Thermal Acclimation in Anuran Amphibians as a Function of Latitude and Altitude. *Comp. Biochem. Physiol.* 24:93-111.
- Brett, J.R. 1952. Temperature Tolerance in Young Pacific Salmon, genus Oncorhynchus. *J. Fish. Res. Bd. Can.* 9(6):265-323.
- _____. 1956. Some Principles in the Thermal Requirements of Fishes. *The Quarterly Review of Biology.* 31(2):75-87.
- Campbell, C.M. and P.S. Davies. 1975. Thermal Acclimation in the Teleosts, Blennius pholis (L). *Comp. Biochem. Physiol.* 52:147-151.
- Colby, P.J. and L.T. Brooke. 1970 Survival and Development of Lake Herring (Coregonus artedii) Eggs at Various Incubation Temperatures. In Biology of Coregonid Fishes. C.C. Lindsey and C.S. Woods (eds.). pp. 417-428. University of Manitoba Press, Winnipeg, Canada.
- Colesante, R. 1974. Summary of 1974 Muskellunge Fry Studies in New York State. In Sixth Interstate Muskellunge Workshop. Morehead, Kentucky. Sept. 25, 1974. pp.45-47.
- Combs, B.B. 1965. Effect of Temperature on the Development of Salmon Eggs. *Prog. Fish-Cult.* 27:134-137.
- Cowles, R.B. and C.M. Bogert. 1944. A Preliminary Study of the Thermal Requirements of Desert Reptiles. *Bull. Amer. Mus. Natur. Hist.* 83:261-296.
- Cox, D. 1974. Effects of Three Heating Rates on the Critical Thermal Maximum of Bluegill. In Thermal Ecology. Gibbons, J. W. and R.R. Shairtz (eds.). AEC Symposium Series (Conf.-730505).
- Dunlap, D.G. 1968. Critical Thermal Maximum as a Function of Temperature of Acclimation in Two Species of Hylid Frogs. *Physiol. Zool.* 41:432-439.

- Edsall, T.A. 1970. The Effect of Temperature on the Rate of Development and Survival of Alewife Eggs and Larvae. Trans. Amer. Fish. Soc. 99(2):376-380.
- _____ and P. Colby. 1970. Temperature Tolerance of Young-of-the-year Cisco Coregonus artedii. Trans. Amer. Fish. Soc. 99(3):526-531.
- Ferguson, R.G. 1958. The Preferred Temperature of Fish and Their Midsummer Distribution in Temperate Lakes and Streams. J. Fish. Res. Bd. Can. 15(4):607-624.
- Franklin, D.R. and L.L. Smith, Jr. 1963. Early Life History of Northern Pike, Esox lucius L., with Special Reference to Factors Influencing Numerical Strength of Year Classes. Trans. Amer. Fish. Soc. 92(2):91-110.
- Fry, F.E.J. 1967. Responses of Vertebrate Poikilotherms to Temperature. In Thermobiology. Rose, A.H. (ed.). Academic Press, New York. pp.375-409.
- _____, J.S. Hart and K.F. Walker. 1946. Lethal Temperature Relations for a Sample of Young Speckled Trout (Salvelinus fontinalis). University of Toronto. Stud. Biol. Ser. No. 54. Public Ont. Fish. Res. Lab. 66:5-35.
- Galant, D.L. and A.W. Eipper. 1969. Preliminary Investigations on the Effects of Incubation Temperature on the Survival of Muskellunge (Esox masquinongy) Embryos. Report. New York Co-op. Fish. Unit, Cornell University. 18p.
- Hassan, K.C. and J.R. Spotila. 1976. The Effect of Acclimation on the Temperature Tolerance of Young Muskellunge Fry. In Thermal Ecology. AEC Symposium Series (Conf.-750425). In Press.
- Heacox, C. 1942. The Chautauqua Lake Muskellunge: Research and Management Applied to a Sport Fishery. Trans. North Amer. Wildl. Conf. 11:419-425.
- Heatwole, H., N. Mercado, and E. Ortiz. 1965. Comparisons of Critical Thermal Maxima of Two Species of Puerto Rican Frogs of the Genus Eleutherodactylus. Physiol. Zool. 38:1-8.
- Hokanson, K.E.F., J.H. McCormick, and B.R. Jones. 1973. Temperature Requirements for Embryos and Larvae of the Northern Pike, Esox lucius (Linnaeus). Trans. Amer. Fish. Soc. 102(1):89-100.
- _____, _____, _____, and J.H. Tucker. 1973. Thermal Requirements for Maturation, Spawning and Embryo Survival of the Brook Trout, Salvelinus fontinalis. J. Fish. Res. Bd. Can. 30:975-984.
- Hourston, A.S. 1952. The Food and Growth of the Maskinonge (Esox masquinongy Mitchill) in Canadian Waters. J. Fish. Res. Bd. Can. 8(5):347-368.

Hubbs, C. 1966. Fertilization, Initiation of Cleavage, and Developmental Temperature Tolerance of the Cottid Fish, Clinocottus analis. Copeia. 1966:29-42.

Hutchison, V.H. 1961. Critical Thermal Maxima in Salamanders. Physiol. Zool. 34:92-105.

_____, and R.J. Kosh. 1964. The Effect of Photoperiod on the Critical Thermal Maxima of Painted Turtles (Chrysemys picta). Herpetologica. 20:233-238.

_____, G. Engbretson, and D. Turney. 1973. Thermal Acclimation and Tolerance in the Hellbender, Cryptobranchus alleganiensis. Copeia. 1973:805-807.

_____, and S.D. Rowlan. 1975. Thermal Acclimation and Tolerance in the Mudpuppy, Necturus maculosus. J. of Herpetology. 9(4):367-368.

Kelley, J.W. 1968. Effects of Incubation Temperature on Survival of Largemouth Bass Eggs. Prog. Fish-Cult. 30:159-163.

Lashbrook, M.K., R.L. Livezey. 1970. Effects of Photoperiod on Heat Tolerance in Sceloporus occidentalis occidentalis. Physiol. Zool. 43(1):38-46.

Lowe, C.H. Jr., and V.J. Vance. 1955. Acclimation of the Critical Thermal Maximum of the Reptile Urosaurus ornatus. Science. 122:73-74.

Mahoney, J.J., and V.H. Hutchison. 1969. Photoperiod Acclimation and 24-Hour Variations in the Critical Thermal Maxima of a Tropical and Temperate Frog. Oecologia. 2:143-161.

McCormick, J.H., B.R. Jones, and R.F. Syrett. 1971. Temperature Requirements for Growth and Survival of Larval Ciscos (Coregonus artedii). J. Fish. Res. Bd. Can. 28:924-927.

Mooradian, S.R., and W.F. Shephard. 1973. Management of Muskellunge in Chautauqua Lake. N.Y. Fish. Game. J. 20(2):152-157.

New York State Department of Environmental Conservation. 1958. Fish Propagation. In New York State Conservation Departments 48th Annual Report. 109(1959):104-111.

_____. 1962. Fish Propagation. In New York State Conservation Departments 52th Annual Report. 106(1963):103-104.

Oehmeke, A.A. 1951. Muskellunge Yearling Culture and Its Application to Lake Management. Prog. Fish-Cult. 2:63-70.

_____. 1969. Muskellunge Management in Wisconsin. Wisc. Dept. Natur. Res., Div. Fish, Game and Enforcement, Bureau of Fish Management, Report No. 19. 18p. (mimeo).

- Scott, D.P. 1964. Thermal Resistance of Pike (Esox lucius L.), Muskellunge (E. masquinongy Mitchill), and Their F1 Hybrid. J. Fish. Res. Bd. Can. 21:1043-1049.
- Spotila, J.R. 1972. Role of Temperature and Water in the Ecology of Lungless Salamanders. Ecological Monographs. 43:85-125.
- Starkey, E.E., J.A. Kagmierski, M.R. Fletcher, and O.E. Maughan, 1972. The Effect of Photoperiod on Thermal Resistance of Speckled Dace. Trans. Amer. Fish. Soc. 101:715-718.
- Trautman, M.B. 1957. The Fishes of Ohio. Ohio State University Press, Ohio. pp.215-220.
- Walker, K.W. 1968. Temperature Control in Northern Pike and Muskellunge Egg Hatching. North Central Warmwater Fish Culture Workshop. Ames, Iowa. Feb. 15. 10p.
- Whipple, J.S. 1906. Report of the State Fish Culturist for the Year 1906. In State of New York Forest, Fish, and Game Commission. Annual Reports 1904-1905-1906. pp.181-182.