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# PRELIMINARY STUDIES ON THE TOLERANCE OF AQUATIC INSECTS TO HEATED WATERS[1]

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ABSTRACT

The mature larvae of twelve species of aquatic insects (Trichoptera, Diptera, Ephemeroptera, Plecoptera, Odonata) were tested to determine their relative sensitivity to heated waters in the laboratory. The temperature at which 50% died after 96 hours ($TL_m^{96}$) was recorded as the lethal temperature. This ranged from 21°C. for winter stoneflies to 33°C. for dragonflies.

## INTRODUCTION

Artificial heating of surface waters in the United States is increasing at a rapid rate, especially from steam-electric power generating facilities. The effects the increased temperatures may have on aquatic life are diverse and the changes that occur are complicated. Knowledge concerning the effects of thermal pollution on aquatic organisms must be available in order to determine if the warmed waters are harmful or beneficial to the specific stream so affected. The requirements of the biota in the stream must be known before one can say that changes in stream temperatures are affecting the biota.

This paper summarizes the results of acute, short term 96-hour tests ($TL_m^{96}$) used in screening 12 aquatic insect species to determine their relative sensitivity to heated water. The 96-hour $TL_m$ (Standard Methods, 1960) was used as the measure of effect in these tests. This work will lay a foundation for further studies dealing with the effects of temperature on factors such as feeding rates, molting, adult emergence, reproduction, and long term survival.

Literature concerning the effects of heated waters on aquatic insects contains a limited amount of information even though a wide variety of aquatic insects under various laboratory and field conditions have been studied. Several reviews have adequately summarized the short term data. Altmann and Dittmer (1966) issued a list of many marine and freshwater organisms which included some data on aquatic insects. An excellent review of temperature effects on aquatic insects was included in the third Seminar on Biological Problems in Water Pollution, U. S. Public Health Service (Tarzwell, 1965). Coutant (1962) made a field study on the effects of a heated water effluent on the macroinvertebrate fauna of the Delaware River. He found drastic reductions in the volume and numbers of organisms in affected areas of the stream.

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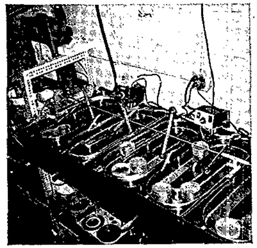

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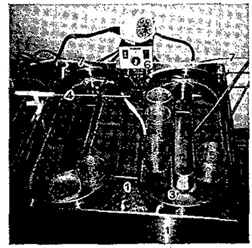

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Figs. 1, 2. Test Chambers

LABELLED EQUIPMENT

1 water bath for temp. control
2 drain from water bath
3 circulating artificial stream
4 paddle for circulating water
5 cage holding test specimens
6 water pump-heater control
7 constant flow water source
8 stream support rods

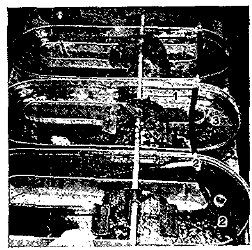

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Fig. 3. Holding-Acclimating Chambers

1 circulating artificial stream
2 fresh water source
3 drain
4 paddle for circulating water

Laboratory studies have been conducted by several workers, many of whom failed to consider the acclimation temperature; few used methods or reported data so that their results could be compared with those of other workers.

## METHODS AND MATERIALS

All tests were conducted in oval stainless steel tanks immersed in a stainless steel water bath for precise temperature control (Figs. 1, 2). The tanks are similar to those used by Surber and Thatcher (1963). Six test chambers were used, each with two oval tanks ("streams") for running duplicate tests; five chambers for temperature testing and one for a control, with the control maintained at the initial acclimation temperature. The oval tanks were used as artificial streams where various water flows could be maintained, either with the stream of water from the pump (Fig. 4) or with additional paddle wheels (Fig. 3). The oval

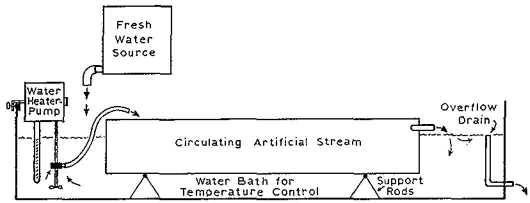


Fig. 4. Diagram of test chamber shown in Fig. 2.

tanks are 26.5 inches (67.5 cm) long, 11 inches (28 cm) wide, and 4 inches (10 cm) deep. Each water bath (2 baths used for convenience) is 76.5 inches (194 cm) long, 28.5 inches (72.5 cm) wide, and 10 inches (25.5 cm) deep, and is divided into 3 chambers each 25 inches (63.5 cm) wide. Each chamber is separated by a ½ inch (1.3 cm) insulating double partition.

A diagram of one test chamber is shown in Fig. 4. Fresh water was continually added to the system (6 water baths, 12 test tanks) at a rate of 1 liter per minute to remove waste products, etc. The Porta-temp* (Precision Inst. Co.) combination water heater-pump-temperature controller maintained the water bath at a constant temperature, continually mixed the water bath and pumped the water into the oval test tank. The water drained from the oval "stream" back into the water bath and was recirculated for heating. An overflow drain is located in the water bath to maintain water level and discharge displaced water. Stainless steel supporting rods hold the oval tanks above the bottom of the water bath so that they are completely surrounded by water thereby giving more uniform temperature control.

The test organisms were collected from local trout streams near the National Water Quality Laboratory, Duluth, Minnesota, and were placed in acclimation tanks (Fig. 3) for one week. This insured that healthy specimens could be chosen from material collected and that a definite acclimation temperature could be maintained prior to testing. Natural substrate and flow were maintained in the holding "streams". All test specimens in this study were mature larvae and nymphs obtained about three weeks before emergence of the adults.

Testing began with an initial survey series of temperatures usually

* Mention of commercial products does not constitute endorsement by the Federal Water Pollution Control Administration or the U. S. Department of the Interior.

TABLE I. Temperatures at which 50% of the test species died after 96 hours exposure ($TL_m^{96}$) when acclimated at 10°C. for one week.

| SPECIES TESTED | $TL_m^{96}$ (°Celcius) |
|---|---|
| *Taeniopteryx maura* (winter stonefly) | 21 ° |
| *Ephemerella subvaria* (mayfly) | 21.5° |
| *Isogenus frontalis* (stonefly) | 22.5° |
| *Allocapnia granulata* (winter stonefly) | 23 ° |
| *Stenonema tripunctatum* (mayfly) | 25.5° |
| *Brachycentrus americanus* (caddisfly) | 29 ° |
| *Pteronarcys dorsata* (stonefly) | 29.5° |
| *Acroneuria lycorias* (stonefly) | 30 ° |
| *Paragnetina media* (stonefly) | 30.5° |
| *Atherix variegata* (true fly) | 32 ° |
| *Boyeria vinosa* (dragonfly) | 32.5° |
| *Ophiogomphus rupinsulensis* (dragonfly) | 33 ° |

ranging from 20° to 35°C. in order to find the approximate lethal temperature. A final test was conducted using 5 temperatures 1°C. apart, such as 28°, 29°, 30°, 31°, and 32°C., with 10°C. as control. The tests were run again at least once for additional replicate data. The test tanks were all initially set at the acclimation temperature of 10°C.; the specimens were placed in the test tanks and the temperature gradually raised to the appropriate test temperature (2°–4°C. per hour). This eliminated some of the "shock" of immediate transfer from one temperature to another and also reduced the acclimation by the specimens (to higher temperatures) which accompanies a slow rise of test temperatures.

The water used for all phases of the testing and holding operations was obtained from Lake Superior via the Duluth city water supply. It was dechlorinated with carbon filters just prior to use. The chemical properties of the water were quite stable. The dissolved oxygen level was maintained between 90 and 100% of saturation. The pH varied from 7.2 to 7.5; total alkalinity remained near 40 ppm; $CO_2$ was fairly constant at 3–5 ppm; with total hardness from 35–40 ppm (alkalinity, $CO_2$ and hardness expressed as ppm of $CaCO_3$). Water temperature was maintained near ± .2°C. during final testing.

Earlier tests had shown that the aquatic insects could live for several weeks without food, so they were not fed during the 4-day tests. Many survivors were held for several weeks without food after testing and no mortality occurred.

If deaths occurred in either of the duplicated controls the entire test was discarded. In addition, all chamber temperatures were monitored with 7-day recording thermographs so that a continuous record was obtained. If the temperature deviated by more than .5°C. from the desired test temperature the test was also terminated.

The temperature at which 50% of the organisms died was obtained by using a modification of the straight line graphical interpolation method as outlined in Standard Methods (1960).

All tests were duplicated so as to be amenable to statistical analysis. Duplicate (and additional replicate) test streams were recorded as separate samples of the given population and were compared statistically by means of the group comparison or Student's 't' test. The differences between the two samples were not found to be significant for the conditions of the test.

## RESULTS

The mature larvae and nymphs of 12 species of aquatic insects were tested to determine their tolerance to high water temperatures. A decided difference in susceptibility was apparent (Table I) between the different species. The winter stoneflies (*Taeniopteryx maura* and *Allocapnia granulata*) died at 21 and 23°C., respectively, and did not withstand large temperature increases. *Ephemerella subvaria,* a small, widely distributed, spring emerging mayfly, was also very sensitive to temperature increases, exhibiting a 96-hour $TL_m$ of only 21.5°C. The stonefly *Isogenus frontalis* was also quite sensitive, 50% dying at 22.5°C. The active mayfly *Stenonema tripunctatum* was somewhat more tolerant with a $TL_m$ value of 25.5°C. The remaining species can be loosely classified as more heat tolerant. The caddisfly *Brachycentrus americanus,* extremely abundant in certain locations, exhibited 50% mortality at 29°C. Three common large stoneflies, *Pteronarcys dorsata, Acroneuria lycorias,* and *Paragnetina media,* had 96-hour $TL_m$ values of 29.5, 30, and 30.5°C., respectively. *Atherix variegata,* a rhagionid fly, was very tolerant with a $TL_m$ of 32°C., though not as much so as two species of dragonfly, *Boyeria vinosa* ($TL_m = 32.5$°C.) and *Ophiogomphus rupinsulensis* ($TL_m = 33$°C.). They survived temperature increases of 22–23°C., proving the most tolerant to heat of all the insect species tested.

## DISCUSSIONS

Seasonal and ecological conditions should be considered when interpreting the above results. The nymphs of winter stoneflies live in streams where the water temperature seldom gets above 5°C. The adults emerge in late winter, lay their eggs, and these species pass through the warmer summer months as eggs or prenymphal forms. Activity and growth are not resumed until the stream temperatures are again lowered the next fall. *Ephemerella subvaria* also emerges in late spring before stream temperatures reach higher summer levels. Adult emergence occurred weeks earlier in the laboratory (abruptly terminating several tests) when water temperatures were raised but not brought to lethal levels. An artificial increase in stream temperatures during the winter would very likely cause these species to develop more rapidly, emerge earlier, and be killed by cold air temperatures, effectively eliminating the species.

The stonefly, *Isogenus frontalis,* is rather restricted in habitat requirements as reflected by its intolerance to increased water temperature. *Stenonema tripunctatum* is a mayfly restricted to rapid flowing, non-silted, rocky streams and may be adapted to the lower temperatures usually associated with this type of stream. *Atherix variegata,* the snipefly or clusterfly, is a widespread, temperature-tolerant creature. Incomplete tests with higher acclimation temperatures indicate that, with proper conditioning, it could survive even higher temperatures than those tested.

The three large stoneflies, *Pteronarcys dorsata, Acroneuria lycorias,* and *Paragnetina media* require three years to complete their life cycle and are therefore subjected to the warmer waters of late summer which many aquatic insects avoid by emergence in the spring. This factor is reflected in their comparative tolerance to higher temperatures.

The two dragonflies, *Boyeria vinosa* and *Ophiogomphus rupinsulensis* live in the slower, warmer reaches of streams and reflect tolerance to a variety of extreme conditions.


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