# Effects of Thermal Shocks on Drifting Aquatic Insects: A Laboratory Simulation

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Effects of temperature shocks from thermal plumes on drifting *Isonychia* (Ephemeroptera: Baetidae) and *Hydropsyche* (Trichoptera: Hydropsychidae) larvae were examined in a laboratory simulation. Groups of both insects were collected at various seasons and acclimated in the laboratory at temperatures comparable to those in the field. Groups were exposed to thermal shocks of varying duration and observed for periods of 10 days afterward. Consistent statistically significant differences in mortality between treatment and control groups were not evident until shock temperatures neared the respective upper lethal limits for the two insects. While consistent treatment related differences in molting frequency in *Isonychia* were not obtained, changes in the patterns of molting were observed. Behavioral tests with *Isonychia* indicated no discernable treatment effects on rheotaxis, phototaxis, and substrate orientation. No discernable effects of treatment on susceptibility to predation of *Isonychia* by *Cottus carolinae* were observed.

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Les auteurs ont étudié les effets de chocs thermiques causés par des couches superficielles chaudes simulées en laboratoire sur des larves dérivantes d'Isonychia (Ephemeroptera: Baetidae) et d'Hydropsyche (Tricoptera: Hydropsychiae). Des groupes des deux espèces d'insectes ont été recueillis à diverses saisons et acclimatés au laboratoire à des températures comparables à celles qui prévalaient sur le terrain. Les groupes ont été exposés à des chocs thermiques de durée variable et ensuite observés pendant des périodes de 10 jours. Il n'y a pas de différences uniformes et statistiquement significatives dans les mortalités des groupes traités et des groupes témoins tant que les températures de choc ne se rapprochent pas des limites létales supérieures des deux insectes. Bien qu'il n'y ait pas de différences uniformes liées au traitement dans la fréquence des mues d'Isonychia, on observe des changements dans les modalités de la mue. Des expériences de comportement avec Isonychia ne révèlent aucun effet discernable résultant du traitement sur le rhéotactisme, le phototactisme et l'orientation sur le substrat. On ne détecte aucun effet du traitement sur la susceptibilité d'Isonychia à la prédation par Cottus carolinae.

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Interest in the evaluation of the effects of power production on aquatic ecosystems has heightened in recent years. A major area of interest has been the chronic effects of heated discharges from power plants on aquatic insect communities (Coutant 1962, 1967; Durrett and Pearson 1975; Gibbons et al. 1975; Langford and Deffern 1975). Related studies on individual aquatic insect species have included sublethal effects of chronic thermal

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Printed in Canada (J4548) Imprimé au Canada (J4548) stress on feeding rates (Nebeker 1971a), emergence (Nebeker 1971b; Gaufin and Hern 1971), and drift dynamics (Wojtalik and Waters 1970). Lethal effects of continuous exposure to elevated temperatures have been examined (e.g. Nebeker and Lemke 1968; Gaufin and Hern 1971), as has the effect of acclimation temperature on lethality (Martin and Gentry 1974).

One area that apparently has received no attention is the effects of instantaneous thermal shocks as might be experienced by riverine aquatic insects drifting through a thermal plume. Such shocks might result in outright mortality or in less drastic sublethal effects of the sort occurring under chronic exposure to thermal stress.

This paper presents results of a laboratory study designed to assess the effects of short-term thermal shocks on certain aspects of the survival and behavior of drifting stream insects by simulating the thermal regime encountered while drifting through a thermal plume. The important features of this study are the short durations of the shocks (from 1 to 40 min) and the use of lethal and sublethal criteria in evaluating their effects.

# **Materials and Methods**

COLLECTION AND PRESHOCK HOLDING OF EXPERIMENTAL ANIMALS

The two insects selected for testing, Isonychia nr. sadleri (Ephemerotera: Baetidae) and Hydropsyche spp. (Trichoptera: Hydropsychidae), were used because they are important components of invertebrate drift in New River (E. F. Benfield unpublished data), the large stream from which test animals were collected. Isonychia and Hydropsyche were collected with nets and acclimated in the laboratory for 5-7 days prior to testing. All animals were maintained without feeding in 19-\ellectrian plastic containers with aeration, under approximately natural photoperiods, and within 1°C of stream temperatures at the time of collection. Preliminary data demonstrated that both insects could be held longer than 3 wk under these conditions without significant mortality.

City tap water drawn from New River was dechlorinated with charcoal filters and used for holding and experimentation. Sodium thiosulfate was used to eliminate any residual amounts of chlorine remaining after charcoal treatment. Water hardness averaged  $54.2 \pm 4.8 \text{ mg}/\ell$  as CaCO<sub>3</sub> and pH averaged  $7.8 \pm 0.15$ ; measurements were made irregularly throughout the testing period.

# POSTSHOCK HOLDING FACILITIES

Postshock holding facilities consisted of a series of fiberglass screen cages  $(13 \times 7 \times 9 \text{ cm})$  with plexiglass frames. Two such cages were supported inside a single plastic box  $(20 \times 14 \times 10 \text{ cm})$  and 20 boxes were supported by an epoxy-coated wooden frame. The frame was immersed in a 400- $\ell$  Min-O-Cool® holding tank, which maintained the desired holding temperature. Immersible pumps provided a current through the 40 cages. The tank was fitted with a wooden cover, and Westinghouse Coolwhite® fluorescent bulbs were used to produce an illumination of approximately 1076 lx at cage-top level.

# Administration of Thermal Shocks

The shock delivery system for all tests was a constant temperature, circulating water bath fitted with an overflow, and a reservoir of water at the acclimation temperature of the particular test. Shocking was carried out by immersing larvae contained in

the fiberglass cages into preheated water baths, exposing them for appropriate times, and then gradually returning the bath temperature to ambient over a period of 15-20 min by introducing cool water from the reservoir. This instantaneous increase, maintenance, and gradual reduction of temperature simulated the thermal regime an organism would encounter while drifting completely through a thermal plume.

EXPERIMENTAL PROTOCOL FOR MORTALITY TESTS WITH Hydropsyche AND Isonychia

For test purposes each Min-O-Cool tank was considered as a unit. Ten or more animals were placed in each cage 24 h prior to testing for acclimation to cage conditions. Because cannibalism occurred among Hydropsyche during preliminary testing, each animal was isolated in a cylindrical fiberglass screen container, and the containers were placed in the cages. After shocking, the cages were returned randomly to avoid possible effects from microdifferences in the holding tank. Generally, 10 cages of animals were used as controls and the remaining 30 as experimentals. Therefore a matrix of three shock temperatures and two shock durations would include a minimum of 50 experimental animals (5 cages) at each temperature-duration combination and 100 control animals (10 cages). Postshock observations for mortality in both insects lasted 10 days. Molting frequency and emergence patterns were observed in Isonychia during the 10-day period. Each cage was checked at 2-h intervals the 1st day and at 24-h intervals thereafter. Dead animals were measured (body length) to the nearest millimetre. At termination of a test, survivors were killed with hot water and similarly measured for comparison. Long-term experiments were conducted under essentially the same protocol as the 10-day test except they were terminated at 122 and 53 days for Hydropsyche and Isonychia, respectively.

Preliminary tests demonstrated that a total shock temperature (ambient plus increment) of 36°C killed all *Isonychia* exposed 10 min or longer, and 35°C was arbitrarily chosen as the maximum shock temperature. Analogous temperatures for *Hydropsyche* were 39 and 38°C, respectively. A shock temperature-duration matrix was established for each insect, initially with temperature increments of 3 deg C and shock durations of 1, 5, and 10 min. The matrix was continuously modified as previous tests were evaluated.

Mortality in treatment and control groups for each test were compared with three different data arrangements. First, results for each combination of increment temperature and shock duration were evaluated at 1, 4, 7, and 10 days after shock. Second, to reduce overall variation and better evaluate the effects of shock duration, all responses for days after shock were combined and differences were compared for temperature–duration combinations. Third, all days after shock and shock durations were combined and analyses performed for increment temperatures alone. Two-sided Student's t-tests were used to test for sig-

nificant differences between treatment and controls at the 95% level ( $\alpha = 0.05$ ).

EXPERIMENTAL PROTOCOL FOR BEHAVIORAL TESTS WITH Isonychia

Rheotaxis, phototaxis and substrate orientation -Paired plexiglass troughs  $(56 \times 5 \times 4 \text{ cm})$  were used to simulate a flowing stream for the experiments. To provide suitable substrate for nymphs, the trough bottoms were covered with removable plexiglass plates coated with either black or white aquarium grit. The plates allowed the color and pattern of the trough bottoms to be changed. A small, moveable wooden cover extending over both troughs was used to selectively darken portions of the bottom during tests. Current was created by immersible pumps, and a shunt diverting water from the troughs allowed control of water velocity. Light intensity was varied by changing the distance between the troughs and light source; black plastic was used to prevent light from entering the bottom, sides, and ends of the troughs.

In testing, shocked *Isonychia* nymphs were placed in the middle of either trough and a similar number of nonshocked nymphs in the other. Observations on the positioning and general behavior of the nymphs were made at regular intervals, although animals were not observed continuously as individuals. The test was then repeated, with trough positions of test and control animals reversed. To maximize possible responses, nymphs were shocked at 33 °C for 30 min for all tests. This often induced a 1–3-min torpor in the animals, and only individuals recovering from the torpor were used in testing. Individuals were never reused in subsequent experiments.

Susceptibility to predation — Predator susceptibility tests with Isonychia were performed in a 120 × 23 × 12-cm artificial stream. Coarse aquarium gravel was placed on the bottom to a depth of 1 cm, and stones ranging in size from 3–12 cm diam were added to provide a substrate similar to that at the collection sites of the mayflies. Water depth was maintained at 8 cm and continuous current produced by an immersible pump. For all predation tests photoperiod was 12:12 and light intensity approximately 540 lx at the surface of the stream. Ambient temperatures were 14°C, and nymphs were shocked at 33°C for 30 min to maximize effects.

The banded sculpin, Cottus carolinae, was chosen as the predator because of its abundance at the Isonychia collection sites and its known insectivory. Cottus, like many bottom dwellers, is an opportunistic feeder and preliminary tests demonstrated that it readily fed upon Isonychia. A test was begun by introducing 50 nymphs of assorted sizes into the stream. Two hours later three fish that had been held at ambient water and temperature conditions for at least 5 days without feeding were added. After 24 h, the number of Isonychia remaining was recorded. The fish were removed, measured, and immediately tested for satiation with Isonychia; this apparently never occurred. To avoid errors resulting from

familiarization with the artificial stream and test conditions the fish were not reused.

#### Results

# MORTALITY AND RELATED OBSERVATIONS

Mortality due to short-term thermal shocks was evaluated for *Hydropsyche* and *Isonychia*. Because of the larger number of instars and correspondingly shorter stadia it was also feasible to examine molting frequency, emergence, and egg production in *Isonychia*.

Hydropsyche — Six tests, encompassing acclimation temperatures from 7 to 24°C, were performed with Hydropsyche. About 17% of the mortality comparisons (35 of 210) made for individual days after shock were significant of which approximately half were at temperatures of 36°C or higher (Table 1). When all days after shocks were combined (Table 2), 9 of 33 comparisons (ca 27%) were significant. With all days and shock durations combined (Table 3), 7 of 19 comparisons (ca 37%) differed at the 5% level. In most tests, more shocked animals died than controls, indicating a consistent effect of thermal increases. The increasingly higher percentages of significant values obtained by combining the data could be due to the successive lowering of overall variability. It appeared that the overall shock temperature was more important than either acclimation temperature or shock duration, since the majority of differences occurred at temperatures within 3 or 4 deg C of lethal levels. No effects were observed below a shock temperature of 28°C.

A long-term mortality test with *Hydropsyche* was begun in November and lasted 122 days, during which time about 90% of the larvae died. Sixteen of 102 comparisons were significant (ca 16%) and deaths occurred primarily from 13 to 31 days after the shock, and all at 30-min exposures. Mortality beyond 10 days suggests the possibility of lingering effects of the thermal shocks; however, significant differences in treatment and control deaths did not occur after day 31.

Body length analyses indicated no consistent differences between the average size of shocked and control animals dying during the short- and long-term tests. Larvae dying during the test did not differ in size from those killed at termination, nor were there correlations between the average size of larvae dying and the number of days after treatment.

Isonychia — Eleven mortality tests similar to

Table 1. Shock temperature–duration matrix for *Hydropsyche*. Significant differences ( $\alpha = 0.05$ ) in mortality between treatments and controls at 1, 4, 7, and 10 days after shock are indicated.

	Acclimation temp (°C)	Increment temp (°C)	Duration ( <i>min</i> )	Significant difference in mortality	
				Days after shock	Duration (min)
Mar.	7	11	1, 5, 10		
	7	14	1, 5, 10		
	7	17	1, 5, 10		
	7 7	20	1, 5, 10		
June	21	11	10		
	21	14	10		
	21	17	10	1, 4	10
Aug.	24	6	10		
	24	10	10		
	24	14	10	4	10
Sept.	20	10	30	1	30
	20	14	30	1	30
	20	18	30	1, 4, 7, 10	30
Oct.	16	12	10, 30	7	10
	16	16	10, 30	1	10
	16	20	10, 30	1, 4, 7, 10	10, 30
Nov. <sup>a</sup>	16	15	10, 30		,
	16	17	10, 30		
	16	19	10, 30	4, 7, 10	30

<sup>&</sup>lt;sup>a</sup>Long-term test: significant values were at days 13, 16, 19, 22, 25, 28, 31 for the 15°C increment at 30-min duration, and at days 13, 16, 19, 22, 25, and 28 for the 19°C increment at 30-min duration.

Table 2. Shock temperature–duration matrix for *Hydro-psyche*. Significant difference ( $\alpha = 0.05$ ) in mortality between treatments and controls when days after shock are combined are indicated.

	Acclimation temp (°C)	Increment temp (°C)	Significant difference in mortality at specific durations (min)
Mar.	7	11	
1,141,	7	14	
		17	
	7 7	20	
June	21	11	
	21	14	10
	21	17	10
Aug.	24	6	
·	24	10	
	24	14	
Sept.	20	10	30
•	20	14	
	20	18	30
Oct.	16	12	10
	16	16	
	16	20	30
Nov.	16	15	30
	16	17	
	16	18	30

TABLE 3. Shock temperature-duration matrix for Hydropsyche. Significant difference ( $\alpha=0.05$ ) in mortality between treatments and controls when days after shock and shock durations are combined are indicated.

	Acclimation temp (°C)	Significant differences at specific increments ( ${}^{\circ}C$ )
Mar.	7	
June	21	17
Aug.	24	14
Sept.	20	10, 18
Oct.	16	20
Nov.	16	15, 19

those for *Hydropsyche* were conducted with the mayfly *Isonychia*, encompassing acclimation temperatures of 4–24°C. Of the 302 mortality comparisons at 1, 4, 7, and 10 days postshock, only 13 (ca 4%) were significant, and over half of these were at shock temperatures of 33°C and above (Table 4). With data from all days after shock combined 4 of 80 (5%) comparisons were significant. Combining all days and shock durations resulted in only one difference in 36 comparisons. Most comparisons indicated more shocked than control *Isonychia* dying, as was

Table 4. Shock temperature-duration matrix for *Isonychia*. Significant differences ( $\alpha = 0.05$ ) in molting (italicized numerals) and mortality between treatment and controls at 1, 4, 7, and 10 days after shock are indicated.

	Acclimation temp (°C)	Increment temp (°C)	Duration of shock (min)	Significant differences in mortality and molting	
				Days after	Duration
Mar.	4.0	11	1, 5, 10		
	4.0	14	1, 5, 10		
	4.0	17	1, 5, 10		
	4.0	20	1, 5, 10		
Apr.	9.5	11	5, 10		
	9.5	14	5, 10		
	9.5	17	5, 10	10	5
	9.5	20	5, 10		
May	13.5	11	1, 5, 10		
	13.5	14	1, 5, 10		
	13.5	17	1, 5, 10		
	13.5	20	1, 5, 10	4	5
May	15.0	11	1, 5, 10	1; 1	1; 10
•	15.0	14	1, 5, 10	1; 1	1; 10
	15.0	17	1, 5, 10	1	$I^{'}$
	15.0	20	1, 5, 10	1; 1, 4	5; 10
June	17.0	14	10		•
	17.0	17	10	4	10
July	24.0	5	5, 10	1	10
•	24.0	8	5, 10		
	24.0	11	5, 10		
Aug.	24.0	5	5, 10	1, 4; 1	5; 10
·	24.0	8	5, 10	, ,	,
	24.0	11	5, 10		
Sept.	18.0	8	10, 40		
	18.0	11	10, 40	4	10
	18.0	14	10, 40		
Sept.	20	8	20, 40		
	20	11	20, 40		
	20	14	20, 40	1	20
Nov.ª	10	17	10, 30		
	10	20	10, 30	4	10
	10	23	10, 30	1, 4, 7	30

<sup>a</sup>Long-term tests: additional significant mortality occurred at day 26 for the 23°C increment at 30-min duration. A significant difference in molting occurred at day 26 for the 17°C increment at 30-min duration.

found for the *Hydropsyche*; below a shock temperature of 26.5°C no significant mortality was observed.

Only 2% of the molting data were significantly different for days after shock (Table 4) and no differences appeared in either of the two data combinations. One result of note, however, was the pattern of molting. At shock temperatures of 26–29°C, a significantly higher portion of shocked animals molted than controls, but temperatures above 29°C reduced molting significantly. Although the data are few, the fact that moltinfluencing temperatures were generally lower than those affecting survival suggests a greater sensitivity of the molting cycle to thermal shocks.

The set of long-term mortality tests with *Isonychia* lasted 53 days by which time all nymphs had died. Five of 72 comparisons (ca 7%) for mortality were significant at individual days after shock, but only one was significant for molting differences. Significant lingering mortality was not indicated in the long-term test.

Nymphs dying during the test did not differ significantly in size from those killed afterwards, nor were there consistent differences between the average sizes of shocked and control animals dying during the test.

Isonychia emerged from the holding cages sporadically throughout the tests, but in only one test, begun in September, did enough mayflies

emerge to be analyzed statistically. At a shock temperature of 34°C (increment of 14°C) emergence was reduced by an average of 86% when compared with the other two increment temperatures, 8 and 11°C. This was significant at the 1% level when compared with controls. Emergence at the 8 and 11°C increments did not differ from control groups, nor did the difference in shock durations (20 and 40 min) have an effect.

Mayflies that emerged were held during transformation (if it occurred) to the imago. Females' abdomens were detached and softened in cold KOH, and eggs removed and counted. Successful transformations did not differ statistically between shocked and control groups, nor did numbers of eggs per female.

### BEHAVIORAL STUDIES

Behavioral studies were initiated because mortality—molting studies alone might not reflect the total effects of the thermal shocks. Subtle behavioral aberrations produced by sublethal thermal increases could influence survival by modifying orientation, feeding, shelter seeking, and avoidance of predators. Because *Isonychia* is free living and active when compared with *Hydropsyche* and is known to exhibit phototaxis and rheotaxis, it was used as the experimental animal for behavioral studies.

Rheotaxis, phototaxis and substrate orientation — With currents of 17-18 cm/s, Isonychia began changing positions 10-15 min after being placed in the troughs. After 30-60 min, most had congregated at the trough ends, primarily at the outflow. Under conditions of continuous light these aggregates were stable for several hours. Eventually individuals that had initially moved to the intake (upstream) end began moving downstream, but it was not clear whether they were weakened or merely shifting position. After 16-24 h essentially all the Isonychia, shocked and control groups, were at the outflow end. Changing light intensity from 376 to 2152 lx had no noticeable effect on this position pattern, nor did changing the color patterns of the black and white substrate tiles.

When current velocity was reduced to 2 cm/s, no aggregations were formed after 16 h under continuous light in either shocked or control groups. Instead, positioning was apparently random throughout the troughs. Shocked *Isonychia* required up to 1 min to face into the current at these low velocities, whereas controls generally took only a few seconds. In faster currents both groups normally oriented too quickly for any differences to be seen.

Covering portions of the troughs to exclude light showed that Isonychia was photonegative, tending to remain under the covers during periods of light. This negative phototaxis was strongly influenced by current velocity. At a current of 2 cm/s the nymphs distributed themselves at random, just as they had done in the absence of the cover. With an increase in velocity to 16-18 cm/s, however, Isonychia tended to remain beneath the covers, regardless of where the covers were placed relative to the inflow or outflow of the troughs. Repositioning the covers resulted in a corresponding shift in positions of the nymphs after several hours. At the fastest current tested, 29 cm/s, both controls and shocked mayflies were at the downstream end of the troughs after 24 h. Apparently current took precedence over photic responses at these higher velocities.

These results suggest that Isonychia responds primarily to current velocity, with phototactic responses being secondary, particularly at high water velocities. The pattern or color of the substrate had no apparent influence on positioning. Isonychia is a filter feeder, depending on the current to provide a food supply, and this type of orientation is not surprising. Clemens (1917) came to essentially the same conclusions in studies of Isonychia behavior. Throughout these experiments no differences were noted between control and shocked animals other than the slower orientation into the current seen in the latter at low velocities. Qualitative observations of feeding actions and general mobility of the nymphs revealed no distinguishable effects due to the thermal shocks.

Susceptibility to predation — The second phase of behavioral studies concerned the influence of thermal shocks on the ability of *Isonychia* to avoid predators. Should thermal shocks decrease reaction time of nymphs, or markedly disrupt their orientation, shocked individuals might be more susceptible to predators.

Predation tests using shocked nymphs were alternated with those using control groups. Eleven such tests were performed, five of these with control groups. The average number of non-shocked nymphs eaten per fish per day was  $7.5 \pm 0.4$  (mean  $\pm$  sp) and the corresponding number of shocked nymphs was  $7.2 \pm 0.3$ . These were not significantly different. Data from preliminary tests, which had been carried out at  $9^{\circ}$ C, were compared with the  $14^{\circ}$ C data; no differences in the numbers of nymphs eaten at the two temperatures were indicated. All fish used were approximately the same size, averaging  $60 \pm 8$  mm in body length.

# Discussion

Analysis of our findings suggests that neither acclimation temperature nor the magnitude of thermal shocks was consequential in precipitating lethality until combinations of the two approached the upper lethal temperature of the respective organisms. With Hydropsyche, about half the significant t values for mortality were at total temperature exposures of 36-38°C, and none occurred at <28°C exposures. No mortality occurred in *Isonychia* at temperature exposures <26.5°C and half the significant differences were at 33.5-35°C. Similar conclusions can be drawn from ongoing research on the effects of acute thermal shock (12 min) to the cladoceran, Daphnia pulex, in that no significant changes in oxygen consumption or reproduction are observed until shock temperatures are within 1-2 deg. C of the upper lethal temperature (S. R. Sherberger unpublished data).

The major portion of significant differences observed suggest that Hydropsyche may be more susceptible than Isonychia to thermal shocks. Isonychia may have been unaffected by the treatments, at least with respect to mortality, because the portion of significant t tests was close to that expected (5%) when testing for treatment effects at the 95% level.

The behavioral work was a natural follow-up to the mortality studies. Predation is a significant factor in many insect populations and its effects could be enhanced if the animals were behaving abnormally by, for example, failing to be negatively phototactic. Behavioral aberrations that might expose shocked nymphs to increased predator pressure were investigated first and were followed by experimental predation studies.

No significant effects of thermal shocks on rheotaxis and phototaxis in *Isonychia* were observed, and one must therefore conclude that if nymphs survive the thermal shock they suffer no detectable aberrations of the kinds of behavior monitored. Although our behavioral analyses did not generally extend longer than 24 h after shock, it seems unlikely that changes would appear later, as might be expected with a persistent toxicant.

Predation studies indicated that shocked Isonychia were not more susceptible than control groups. Of possible importance is that only individuals that appeared fully recovered from the torpor induced by heat shock were used. In natural situations Isonychia would undoubtedly be more susceptible to predation while in that state, being carried passively by the current, unable to swim or avoid attack. Coutant (1973) found that a 30-min holding period after treatment more than tripled the exposure time re-

quired to obtain increased predation rates by adult rainbow trout (Salmo gairdneri) on thermally shocked juvenile conspecifics.

Extrapolating these findings to a natural system, one would not expect either Hydropsyche or Isonychia to suffer distinct effects while drifting through a thermal plume when ambient river temperatures and thermal increments added together were below 27-29°C, other than possible changes in molting frequency in Isonychia. Mortality would probably be particularly noticeable in Isonychia at the 31-33°C range, as might changes in emergence patterns during appropriate times of the year. At 36°C and above, significant mortality would occur in both animals. These projections are tentative because they do not include possible effects of chlorine, which is often present in power plant cooling water discharges; synergistic action could lower thermal tolerance estimates for a variety of aquatic insects as was demonstrated by Gregg (1974).

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