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Research and Development

Effects of Thermal Discharges on Aquatic Insects in the Tennessee Valley

Interagency Energy/Environment R&D Program Report

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EFFECTS OF THERMAL DISCHARGE ON AQUATIC INSECTS IN THE TENNESSEE VALLEY

by

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ABSTRACT

The primary objectives of this research project are (1) to determine the thermal tolerances of selected aquatic insects and (2) to investigate growth and emergence of selected aquatic insects in the vicinity of Tennessee Valley Authority electric generating plants. The information may help establish thermal effluent limits to protect the aquatic ecosystem.

The burrowing mayfly Hexagenia bilineata (Say) and the midge Coelotanypus sp. were chosen for study because they are abundant and they occur in areas affected by thermal discharges from Tennessee Valley Authority electric generating plants.

The thermal plume in the vicinity of Johnsonville Steam Plant (TRM 98 to 101), Humphreys County, Tennessee, reached the river bottom only during the fall and winter (1976-1977). Therefore, benthic insects were subjected to above-ambient temperatures during the coldest part of the year. In early spring, H. bilineata nymphs collected from the area influenced by the thermal plume were larger on the average than those collected from the ambient station. However, growth at the ambient station accelerated during late spring, and adult emergence occurred almost simultaneously at both stations.

Tolerance of the immature stages of both study species to thermal shock was great; abrupt changes in temperature of 20°C (at low acclimation temperature of 10 and 15°C) resulted in low percentages of mortality although sample numbers were low.

Another stage in the life cycle that is subjected to ΔTs resulting from thermal plumes is the egg. The optimum range of constant temperatures for development of H. bilineata eggs is from 31 to 34°C; the upper limit for egg development is near 37°C. Eggs subjected to a brief (5 to 15 min) shock of 10°C at the time of oviposition yielded a mean percentage of hatching comparable to that of the control treatment. However, a shock of 15°C resulted in a greatly reduced mean percentage of hatching.

No difference in fecundity of adult females was found between the ambient and thermal plume stations at Johnsonville Steam Plant. Adult males emerging from the heated discharge channel were significantly larger on the average than males from the ambient station.

A drift study conducted at TVA's John Sevier Steam Plant using damselfly nymphs (Enallagma spp.) and mayfly nymphs (Stenonema spp.) in a thermal plume resulted in little or no mortality at ΔTs that normally result from the heated water.

This report was submitted by the Tennessee Valley Authority, Division of Environmental Planning, in partial fulfillment of Energy Accomplishment Plan 80-BDR under terms of Interagency Agreement D8-E721-DR with the Environmental Protection Agency. Work was completed in October 1977.

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LIST OF ABBREVIATIONS AND SYMBOLS

ΔT	--	change in temperature above ambient
°C	--	degrees Celsius
df	--	degrees of freedom
DO	--	dissolved oxygen
h	--	hour
HRM	--	Holston River mile
km	--	kilometer
m	--	meter
min	--	minute
ml	--	milliliter
mm	--	millimeter
n	--	number of individuals in a sample
P	--	probability
r	--	correlation coefficient
s	--	standard deviation
TRM	--	Tennessee River mile
TVA	--	Tennessee Valley Authority
$\bar{x}$	--	mean value

ACKNOWLEDGMENTS

The cooperation of several people in TVA's Water Quality and Ecology Branch, Division of Environmental Planning, is gratefully acknowledged: Mr. Barry O. Bell, who assisted in collecting the field and laboratory data; Ms. Sylvia A. Murray, who helped in the experimental design of temperature effects on mayfly eggs, helped with the analyses, and critically reviewed the manuscript; and Mr. Thomas W. Toole, who calculated statistical parameters for the fecundity data and generated the regression equation for egg development. We especially thank Dr. Richard D. Urban for his initial planning of the thermal effects research on aquatic insects and for advice and criticism throughout the study. Mr. James R. Marsh, Laboratory Branch, is also acknowledged for his fine work in the design and fabrication of the emergence traps and flow-through egg containers.

SECTION 1

INTRODUCTION

The continued increase in demand for electric power increases the amount of heated water discharged from fossil-fueled electric generating plants, which in turn further alters the temperature regimes of aquatic environments. How much can temperatures be altered before changes occur in the biology of aquatic plants and animals? What are the limits on thermal discharge that will not jeopardize a balanced indigenous flora and fauna?

The theory that diversity in an ecosystem ensures stability is generally supported by results of ecological research. Insects are a major component of diversity in aquatic ecosystems, and their importance in the food web and energy flow is well documented. Yet relatively little is known about the thermal tolerances of aquatic insects, especially species in the southeastern United States.

In this study, the thermal tolerances of selected species of aquatic insects in the Tennessee River Valley were investigated. Emphasis was placed on those stages of the life cycle that are subject to thermal discharge from Tennessee Valley Authority (TVA) electric-generating plants. These stages include the eggs, the immatures (stationary or drifting), and the stage of emergence to the adult insect.

Several questions were asked about what actually happens in a field situation:

1. What stages of the selected species' life cycle are influenced by thermal discharge?

2. What magnitudes of change in temperature above ambient (ΔT) do these stages experience?

3. What are the thermal tolerances of these stages?

4. Does entrainment within a thermal plume affect survival?

5. How do elevated temperatures affect growth rate, emergence time, and fecundity?

The species chosen for study were the burrowing mayfly, *Hexagenia bilineata* (Say) (Ephemeroptera: Ephemeridae) and the midge, *Coelotanypus* sp. (Diptera: Chironomidae), both of which are important food items for fish. Both species are sufficiently abundant to facilitate experimentation, and both occur in areas subject to thermal discharges. Also, mortality of these species due to laboratory conditioning is low. Damselflies of the genera *Enallagma* and *Ischnura* and mayflies of the genus *Stenonema* were also studied.

SECTION 2

CONCLUSIONS

Results from this study support other reports that insects inhabiting large rivers and reservoirs are quite tolerant of thermal shock. Eggs of the burrowing mayfly Hexagenia bilineata developed and hatched (mean hatching percentage was 63%) after a 15-min exposure to 40°C (ambient = 30°C, ΔT = 10°C) immediately after oviposition. However, a 5- to 15-min exposure to 45°C (ΔT = 15°C) resulted in a low mean percentage of hatching (13%). A constant temperature of 37°C limited egg development. Nymphs of H. bilineata and larvae of the midge Coelotanypus sp. withstood thermal shocks as high as 20°C in the laboratory at low acclimation temperatures (5 to 15°C).

Although acute effects from moderate thermal shocks in the laboratory were minimal, effects on several aspects of the life cycle of H. bilineata were found. Nymphal growth was greater in areas where the thermal plume extends to the bottom during the winter and early spring than in ambient areas. However, development in ambient areas accelerated in late spring so that adults emerged at about the same date as those in thermal areas. Size and fecundity of female H. bilineata emerging from thermal plume areas and from ambient areas did not differ significantly, although males from thermal plume areas may be significantly larger. These differences in the mayfly's biology found to date were not expected to adversely affect the species in the study area. Damselfly and mayfly nymphs (Enallagma and Stenonema) were held in the thermal plume at John Sevier Steam Plant, resulting in no significant mortality, compared with controls.

SECTION 3

RECOMMENDATIONS

The effect of various acclimation temperatures on the thermal tolerance of aquatic insects should be investigated further; results may be useful for predicting seasonal effects. Tolerance to cold shock should be determined. Organisms that survive experimental heat and cold shocks should be observed afterward for latent mortality and chronic effects. The experiment on the effect of thermal shock on *H. bilineata* eggs should be repeated with better control. The study of the effects of thermal discharges at Johnsonville Steam Plant on the time of emergence, size, and fecundity of *H. bilineata* should be continued for another year.

Establishing temperature limits to protect aquatic insects will require study of species from other orders because tolerances no doubt differ. From the information gathered in this study, it appears that thermal plumes extending to the river bottom that are below 37°C in summer would fully protect the most sensitive stages of *H. bilineata*.

SECTION 4

EXPERIMENTAL PROCEDURES

Workers use two approaches to study the effects of temperature increase. In the field, qualitative and quantitative changes in the fauna are monitored, and results are usually reported as changes in population structure and diversity indexes. In the laboratory, various stages in the life cycle are subjected to different temperature regimes to determine thermal tolerances. Rarely have these approaches been combined. This study used both approaches. The field studies were aimed at discovering (1) which species and which stages of the life cycles of those species are influenced by a thermal plume and (2) whether the plume affects growth, time of emergence, and fecundity. Laboratory experiments were devised to investigate implications from field data under controlled conditions.

FIELD STUDIES

Field studies were conducted at TVA's Johnsonville Steam Plant and vicinity (TRM 98 to 101), on Kentucky Lake in Humphreys County, Tennessee, and at TVA's John Sevier Steam Plant on the Holston River (HRM 106.5 to 106) in Hawkins County, Tennessee.

Stages of Life Cycle Subjected to Thermal Plume

To determine whether the benthic, immature stages of *H. bilineata* and *Coelotanypus* sp. are subjected to the thermal plume from Johnsonville Steam Plant, monthly vertical temperature profiles were determined with a calibrated thermistor. Temperatures were measured at 1-m intervals at five stations (Figure 1) between October 1976 and October 1977. Bottom temperatures indicate whether the thermal plume reached the benthic habitat and reveal the magnitude of ΔTs experienced by the benthos.

Mating swarms of *H. bilineata* were observed in the vicinity of Johnsonville Steam Plant on June 5, 1977, around dusk. Mated females were observed to determine whether oviposition takes place in the discharge channel, which would expose eggs to above-ambient temperatures.

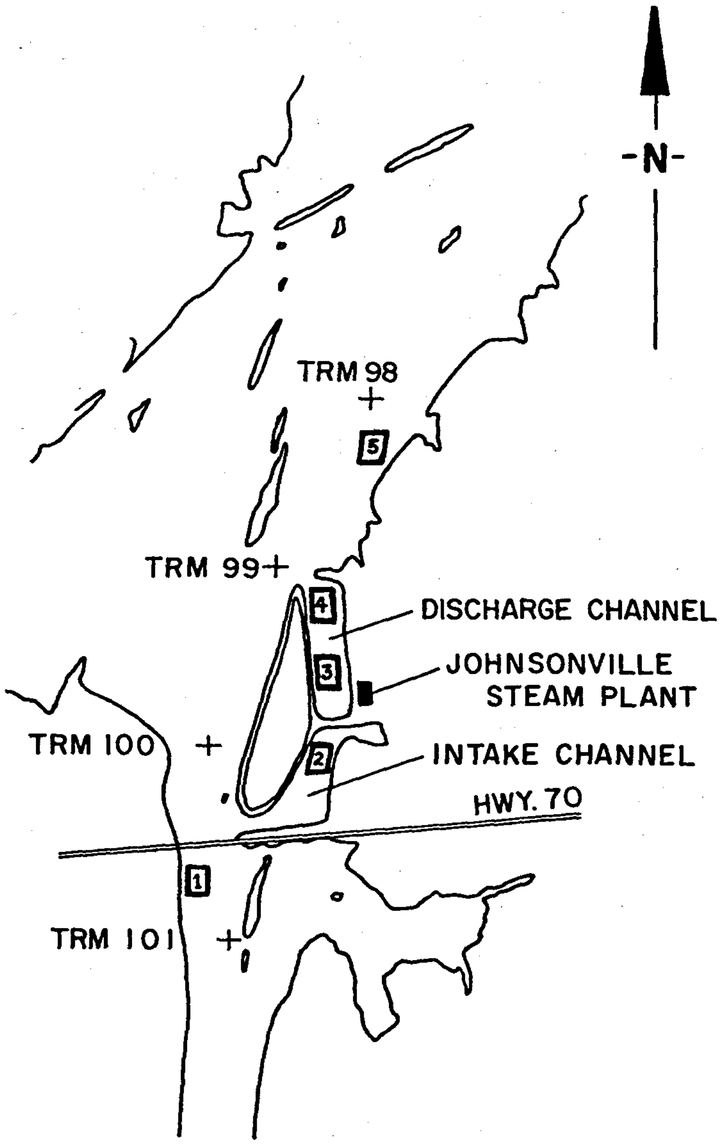

Figure 1. Outline map of Kentucky Lake in vicinity of Johnsonville Steam Plant showing locations of the five sampling stations.

Determination of Growth, Time of Emergence, and Fecundity

To compare growth rates of H. bilineata nymphs in ambient temperatures with those in thermal plume temperatures, five samples were taken with a Ponar dredge at stations 1 and 3 (TRM 100.6 and TRM 99.5 respectively) at 3-week intervals from March 31, 1977, to August 24, 1977. The sediments were field-washed on a 48-mesh sieve, and the mayfly nymphs were preserved in an 80% ethanol solution.

Nymphal head capsule widths and wing pad lengths were measured with an ocular micrometer on a binocular microscope. Because individual instars cannot be exactly determined for H. bilineata,[1] size categories of 0.2 mm were established for head capsule width. Data were transformed to percentages of each size category, and histograms were constructed. Mean head width and mean wing pad length were calculated for each station and sampling date.

Time of emergence of H. bilineata at stations 1 and 3 was determined by trapping emerging subimagoes with 1-m^2 floating traps (Figure 2). Three traps were anchored at each station from June 1 to June 6, 1977; trapped adults were collected and preserved in the mornings.

Fore wing and abdomen lengths were measured with a millimeter rule. Egg count for each female was estimated by stirring the eggs in 10 ml of distilled water and pipetting a 1-ml subsample for counting. A linear correlation of size and fecundity was drawn for each station, and the two stations were compared. Student's t-test was used to compare the mean lengths of wing and abdomen and mean egg counts between the two stations.

Entrainment

A drift study was conducted at John Sevier Steam Plant on July 20, 1976, to determine the effect of the thermal plume on survival. This site was chosen because of the distance the thermal plume extends down the Holston River (over 8 km) and the usually high ΔTs (6 to 10°C) that occur. These two factors create conditions for a maximum-exposure field test.

The experimental insects, damselfly nymphs of the genus Enallagma and mayfly nymphs of the genus Stenonema, were collected from the cooling-water intake channel. Ten insects were placed in each Plexiglas box attached to a flotation apparatus (Figure 3). One flotation apparatus was anchored in the thermal plume, and another was anchored in the ambient temperature area near the right bank. After 6 h of exposure, the insects were removed, and the dead organisms were counted.

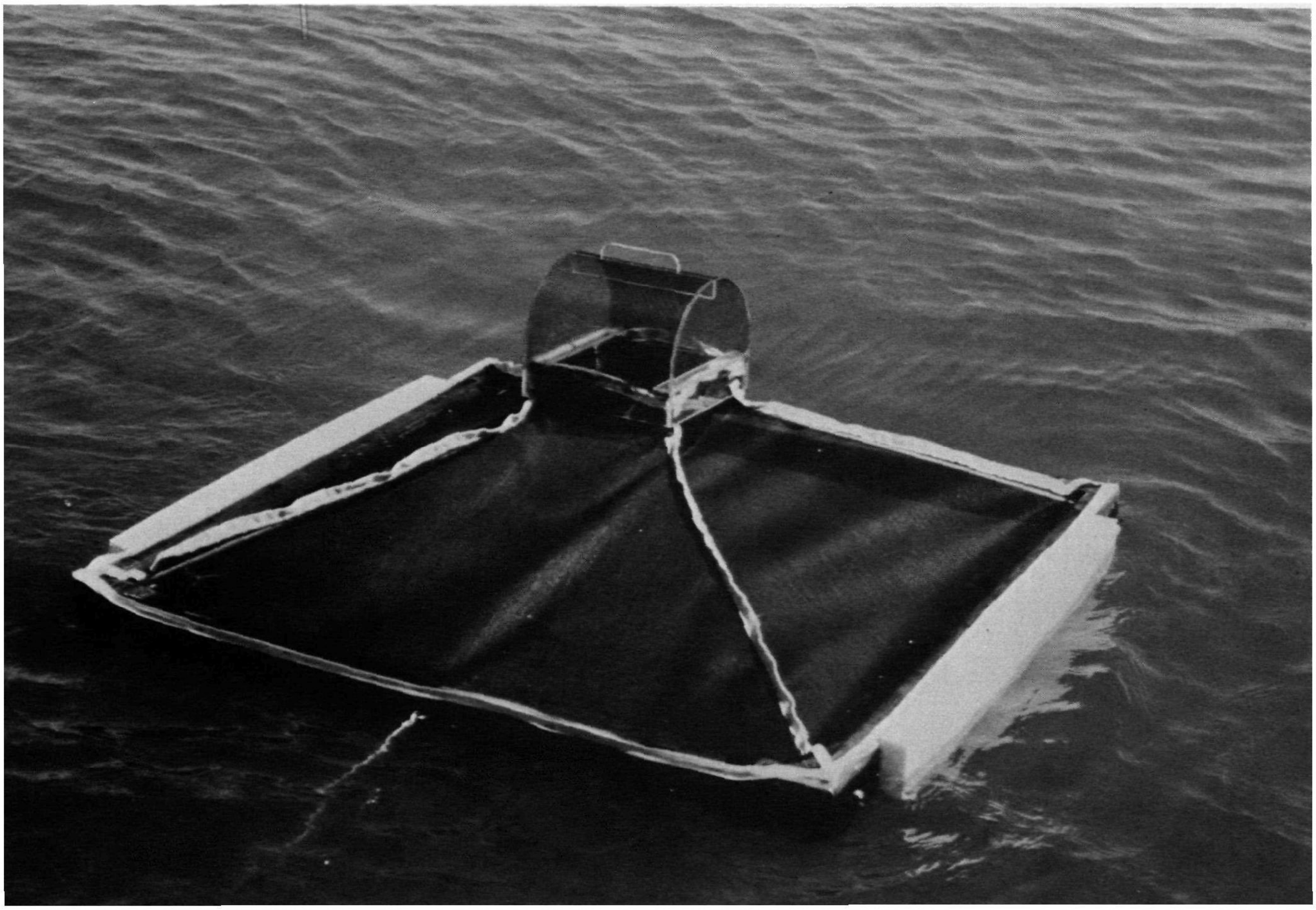

Figure 2. Emergence trap used to collect emerging mayflies.

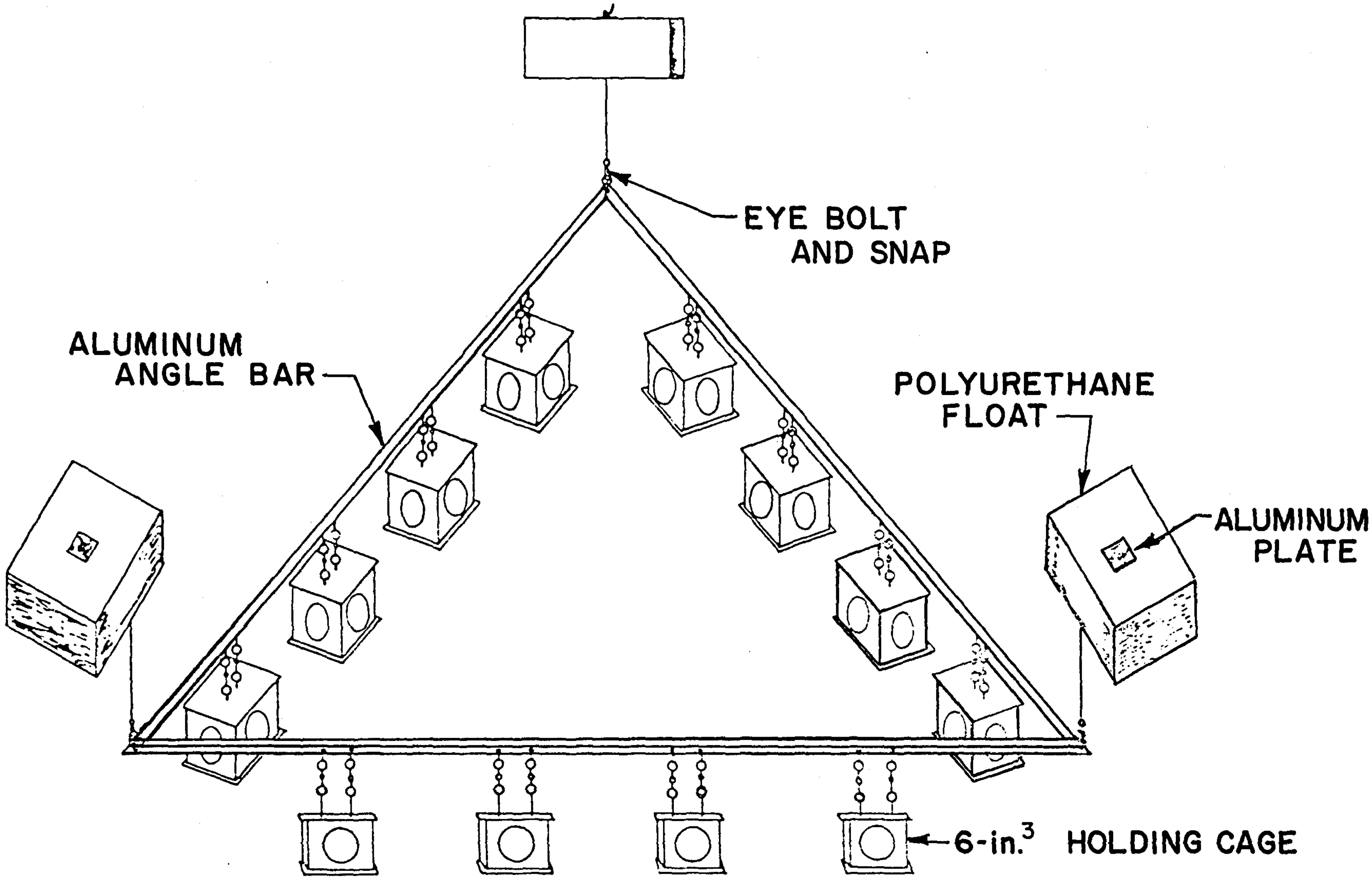

Figure 3. Flotation device used to hold aquatic insects for drift study.

LABORATORY STUDIES

Testing Immature Stages for Thermal Tolerance

Larvae of Coelotanypus sp. were collected from Shoal Creek (near TRM 264.5) in Lauderdale County, Alabama, on November 18, 1976. The larvae were acclimated to 15°C for three days in the laboratory. After the acclimation period, larvae were shocked at three different temperatures--25, 35, and 40°C--which exposed them to ΔTs of 10, 20, and 25°C, respectively, above the control temperature of 15°C. Ten larvae were used for each treatment. Mortality was checked at 15-min intervals for 2.5 h. Death was assumed when larvae did not respond when squeezed gently with a pair of forceps. At the end of the test the larvae were held at 25°C for 8 h to check for latent mortality.

Nymphs of H. bilineata were collected from impounded areas of Second Creek (near TRM 275) near Highway 72 West, Lauderdale County, Alabama, on November 22, 1976; substrate temperature was 10°C at time of collection. The nymphs were acclimated in aerated aquariums in substrate from their natural habitat at 10°C for 8 days. The nymphs were then subjected to ΔTs of 10, 20, and 30°C; a control group was held at a constant temperature of 10°C. The treatment consisted of ten nymphs per replicate and three replicates per treatment. The number of dead were counted at 15-min intervals for 3.5 h. Dissolved oxygen was determined with an oxygen meter (YSI model 54).

Thermal Tolerance of H. bilineata Eggs

Two experiments were conducted: (1) Eggs were cultured at different constant temperatures to determine the upper limit and the optimum range for development; and (2) eggs were exposed during oviposition to several ΔTs for various durations to simulate deposition of eggs in a thermal plume before they sink to an ambient temperature.

Experiment (1). Constant Temperature and Development--

About 300 mated females were collected under lights between 9 and 10 p.m. along Brush Creek (near TRM 264.5) in Lauderdale County, Alabama, on July 8, 1977. The females were placed in 27°C water, where they released their eggs. The eggs were transported to the laboratory, where 1-ml aliquots (700 to 2000 eggs) were placed in Plexiglas flow-through chambers (Figure 4). Twenty chambers were placed in each of eight aquariums that were half-filled with 27°C water. Each aquarium was placed in a refrigerator-size incubator set at one of eight temperatures--19, 22, 25, 28, 31, 34, 37, and 40°C. The aquariums were gently aerated, and the temperatures were allowed to equilibrate overnight. By the next day the eggs had adhered to the bottom of the chambers. The aquariums were then filled, and the water was circulated with Dynaflow-II motor filters.

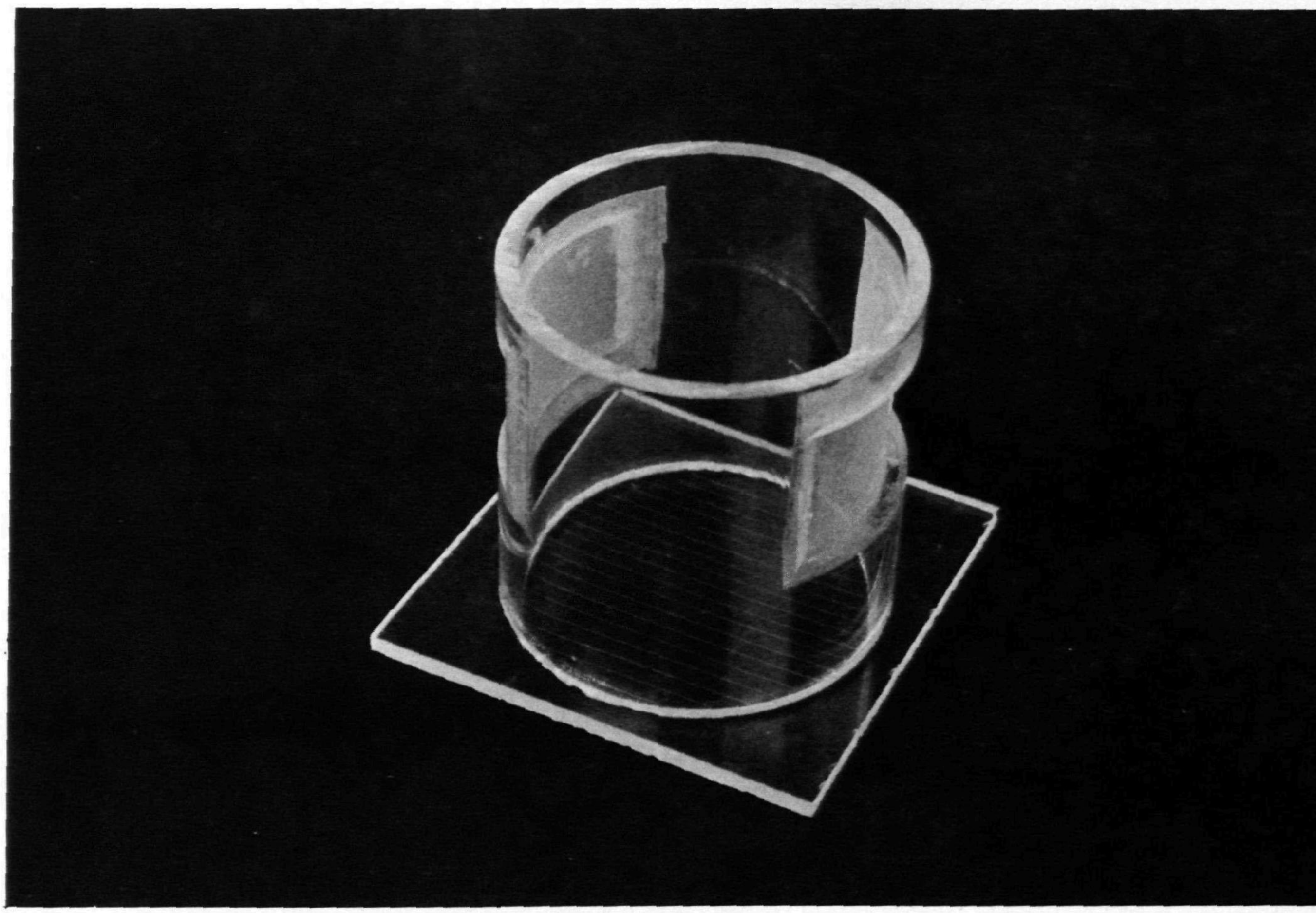

Figure 4. Plexiglas flow-through container for culturing H. bilineata eggs.

When hatching began, five chambers were removed each day for four days, and the number of eggs hatched and the number not hatched were recorded. The data were transformed to percentage hatched and analyzed by a 2-factor analysis of variance.

Experiment (2). Egg Tolerance to Brief Thermal Shock--

About 200 females were collected around 9 p.m. at Shoal Creek (near TRM 264.5) in Lauderdale County, Alabama. About 50 individuals were simultaneously placed in each of four large glass dishes containing water of four different temperatures--30, 35, 40, and 45°C. These temperatures subjected the females to ΔTs of 0 (control), 5, 10, and 15°C. After 3 min these females were removed; 5 min after oviposition some of the eggs from each dish were transferred to separate, labelled containers of 30°C water. The same was done after 10 and 15 min of exposure, yielding 12 treatments of varying temperature and duration. The eggs that had been transferred were then transported to the laboratory and placed in flow-through containers as described in experiment (1), except that all incubators were set to maintain a temperature of 30°C.

When hatching began, three containers (replicates) per treatment were removed each day for four days (total 144 samples). Data were collected, transformed to percentage hatched, and analyzed by a 3-factor analysis of variance.

SECTION 5

RESULTS AND DISCUSSION

TEMPERATURE PROFILES AT JOHNSONVILLE STEAM PLANT AND IMPLICATIONS FOR AQUATIC INSECTS

Surface temperature data from October 1976 to October 1977 indicate that the thermal plume extended from 1 to 2 km downstream from the plant. Surface temperatures at station 5 ranged from 0 to 3.0°C above ambient throughout the year. These results agree with a mathematical model of the plume.[2]

The vertical temperature profiles revealed that the depth to which the thermal plume extended was variable (Figures 5 through 7), depending on time of year. Throughout late fall and winter at station 3 and throughout winter at station 4, the thermal plume extended to the river bottom (Figure 5). During this 5-month period, the substrate, which is inhabited by benthic insects, was about 4 to 6°C above ambient (Figure 8). The greatest ΔT measured was 6.5°C, which occurred in February 1977. Beginning in April 1977, the depth of the thermal plume diminished, and bottom temperatures in the discharge channel were at or very near ambient throughout the spring, summer, and early fall (Figures 6 and 7).

These temperature data indicate that the organisms inhabiting the substrate of the discharge channel were living at temperatures 4 to 6.5°C above ambient throughout the coldest part of the seasons. The average bottom temperature at station 3 from November 17 to March 31 was 15.2°C, compared with 10.6°C at station 1 (average based on five samples). Growth of *H. bilineata* nymphs becomes negligible at 10°C, according to a field study,[3] and at 14°C, according to a laboratory study.[4] Temperature of the substrate at station 3 was never below 10°C on any of the sampling dates, whereas at station 1 it was well below 10°C on December 22, 1976, and February 16, 1977.

GROWTH AND EMERGENCE TIME OF *H. BILINEATA*

If growth of *H. bilineata* nymphs becomes negligible as temperatures approach a low of 10°C, nymphs inhabiting heated areas, where temperatures stay above 10°C (Figure 8), should be larger than those in ambient areas by the end of winter. Therefore, nymphs in heated discharge areas should mature faster, and it is plausible that they would emerge sooner as adults.

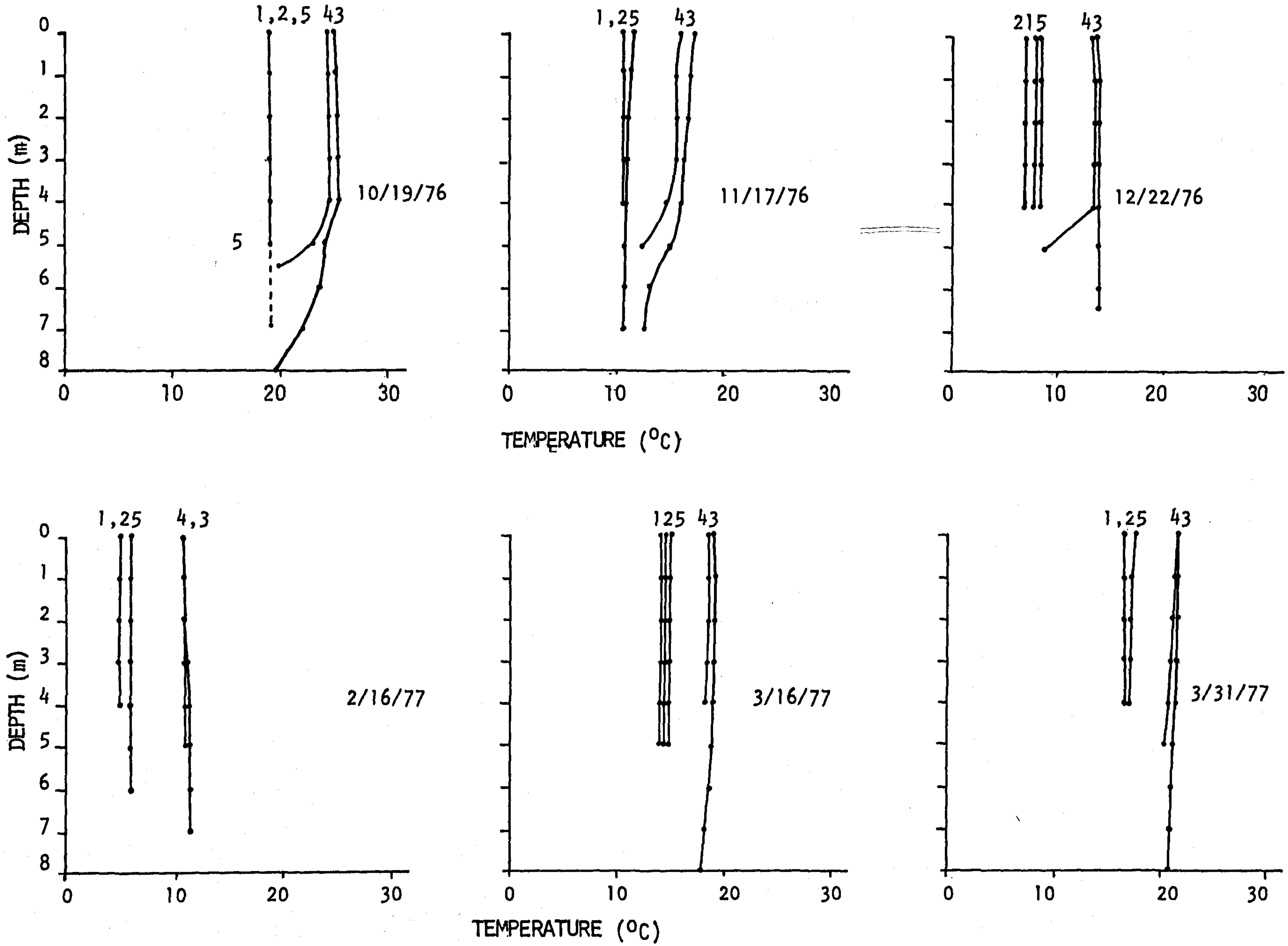

Figure 5. Vertical temperature profiles at five stations in vicinity of Johnsonville Steam Plant, Kentucky Lake, from October 1976 to March 1977.

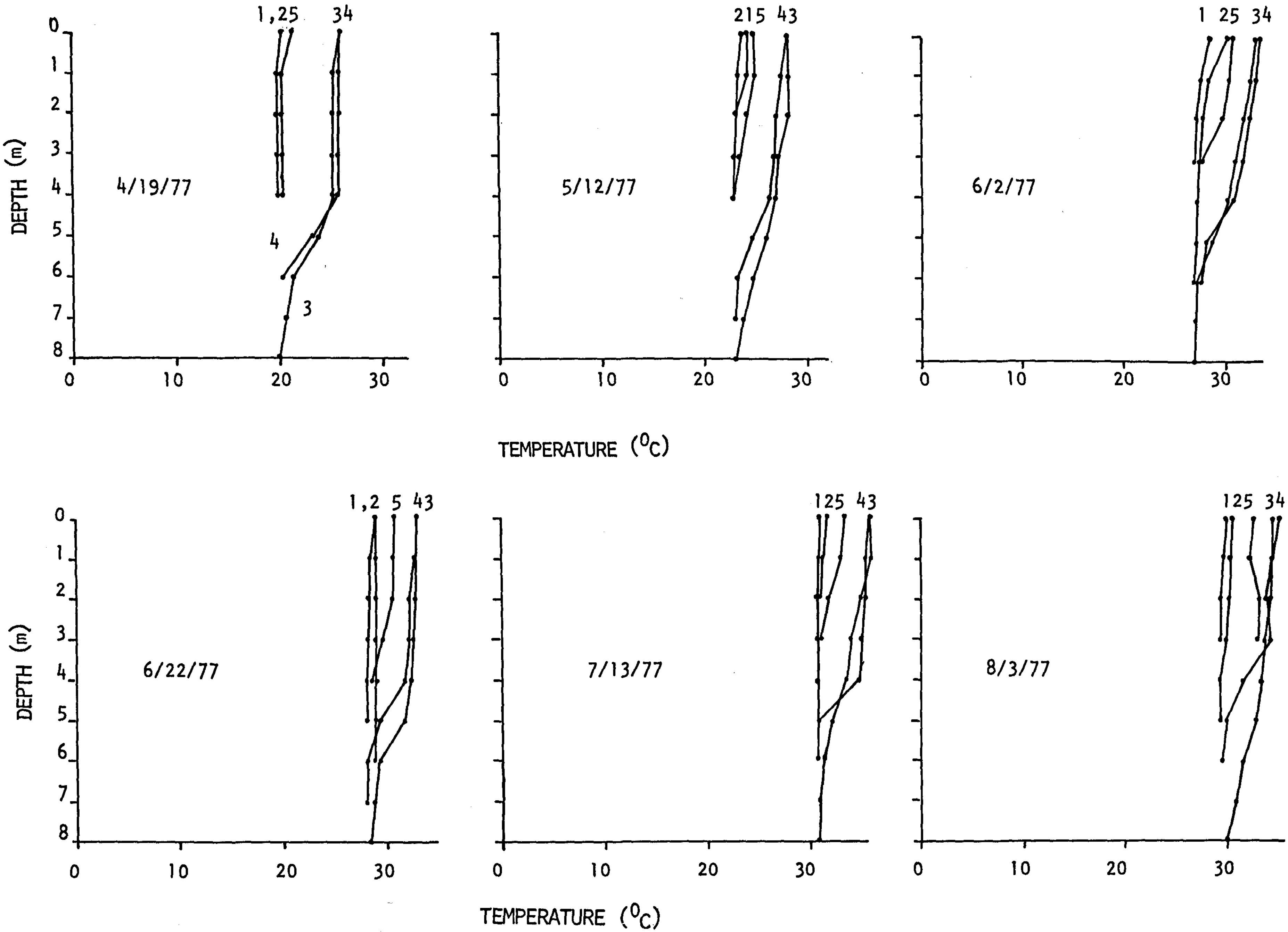

Figure 6. Vertical temperature profiles at five stations in vicinity of Johnsonville Steam Plant, Kentucky Lake, from April to August 1977.

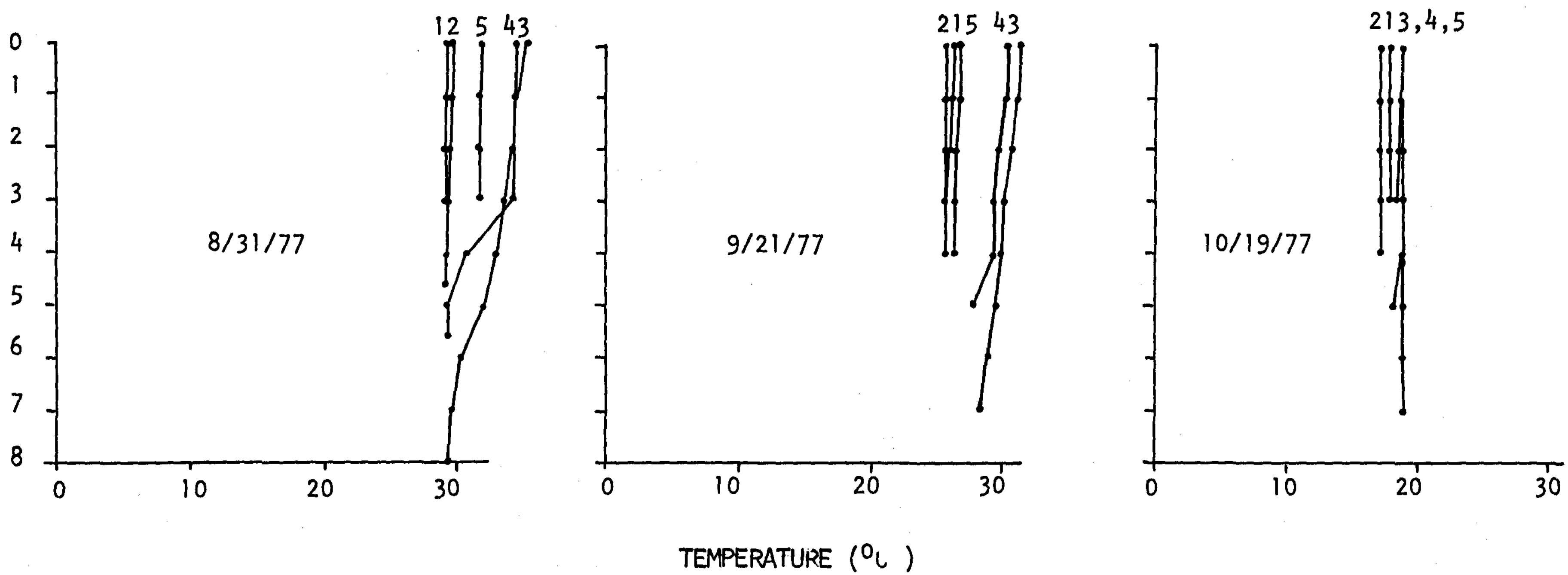

Figure 7. Vertical temperature profiles at five stations in vicinity of Johnsonville Steam Plant, Kentucky Lake, from August to October 1977.

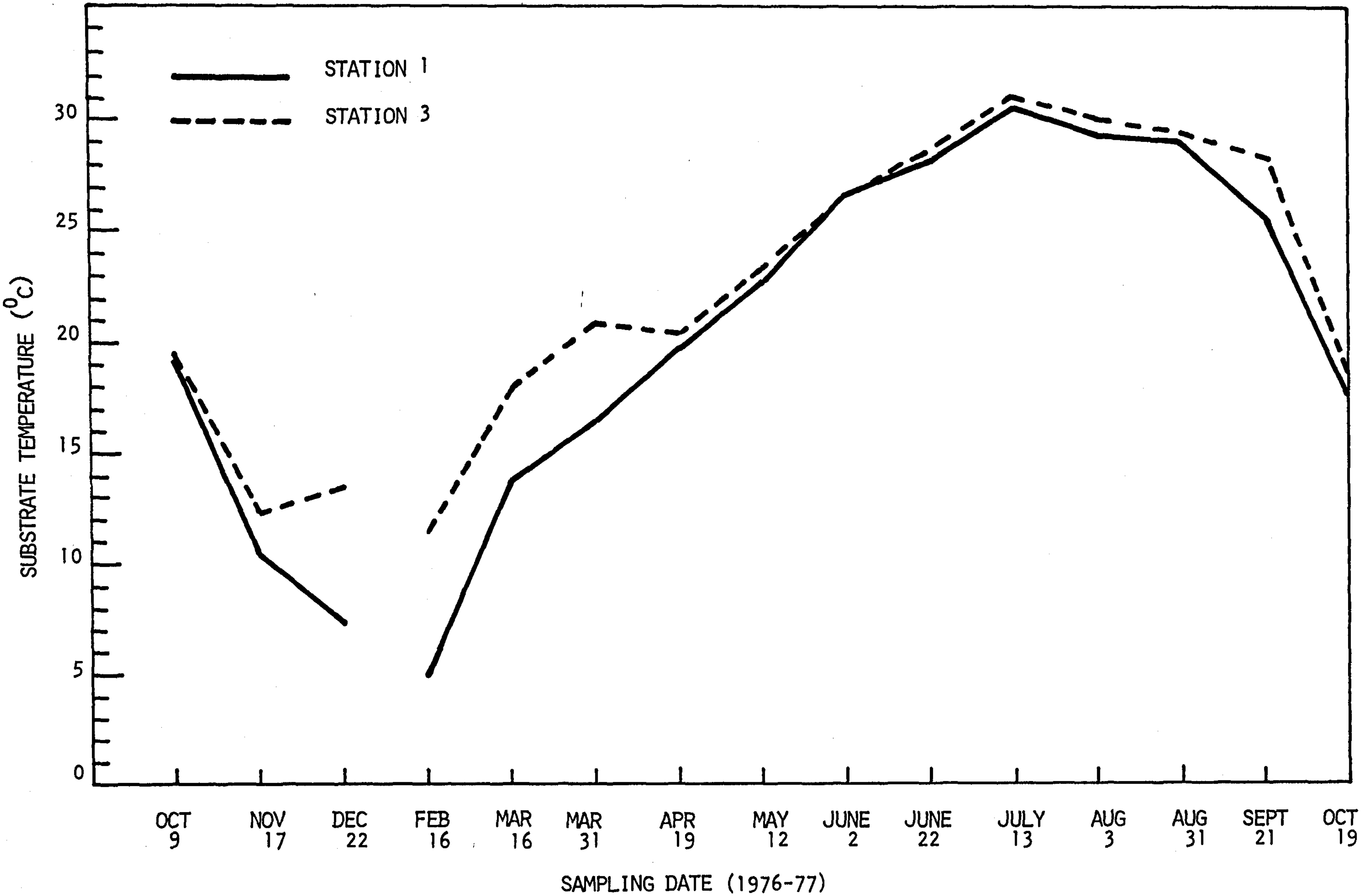

Figure 8. Substrate temperatures at two stations near Johnsonville Steam Plant, Kentucky Lake, from October 19, 1976, to September 21, 1977. No data were obtained in January 1977.

Measurements of head capsules and wing pads of H. bilineata nymphs near Johnsonville Steam Plant show that mean size was greater at station 3 (in the heated discharge channel) than at station 1 from March 31 to June 2 (Figure 9), with the exception of mean head width on June 2. Frequency histograms of nymphs in size classes of 0.2-mm head width showed higher percentages of large nymphs at station 3 from March 31 to June 2 (Figures 10 through 13). After June 2, mean head width was slightly greater at station 1 (Figure 9). The percentage of nymphs with head widths greater than 2.8 mm on June 22 was higher at station 1 (Figure 14); on July 13 and August 3, these percentages were again higher at station 3 (Figures 15 and 16). Mean wing pad length was greater at station 3 on all sampling dates except June 22 (Figure 9). The total number of nymphs collected (n) on successive sampling dates decreased, probably because of drift and increased predation by fish.

The differences in percentages of nymphs in different size classes (based on 0.5-mm intervals of head width) and the changes in these percentages through time are shown in Figure 17. A greater percentage of nymphs were in the larger size classes (2.0 to 3.5 mm) at station 3 from March 31 to at least June 2. Nymphs at station 1 first reached the 3.0- to 3.5-mm size class sometime between May 12 and June 2. However, on June 2, nearly equal percentages of nymphs were in the 3.0- to 3.5-mm class at the two stations.

These data on nymphal head size through time indicate that, although the nymphs in the heated discharge channel were larger on the average than those at the unheated station throughout most of the spring, nymphal growth at station 1 accelerated, yielding a mean size comparable to that at station 3 by June 2.

Emergence to the subimago (adult) stage occurs shortly after the wing pads become thickened and black. No emergence occurred in the vicinity of Johnsonville Steam Plant before the first week of June 1977. On June 2, 1977, 14 of 57 nymphs (19.7%) collected at station 3, as compared with 1 of 27 (3.7%) at station 1, had thick, black wing pads (Figure 18). A chi-square test showed the difference to be significant ($P < 0.05$) although the number of observations was low. The differences in the percentages for June 22 and July 13 are not significant.

Emergence to the adult occurred about two or three days earlier at station 3. On June 2, one adult was seen at station 3 in the discharge channel; no adults were seen at station 1. On the night of June 4, a large number of emerging mayflies were collected in emergence traps at station 3. No mayflies were trapped at station 1, although subimagoes were present along the nearby bank on the morning of June 5. Subimagoes were also present 2 to 6 km downstream from the steam plant. Another large emergence at station 3 occurred on the night of June 5; an emergence also occurred at station 1, but again no adults were found in the emergence traps. Therefore, subimagoes were later netted at station 1 for size and fecundity comparisons with those trapped at station 3.

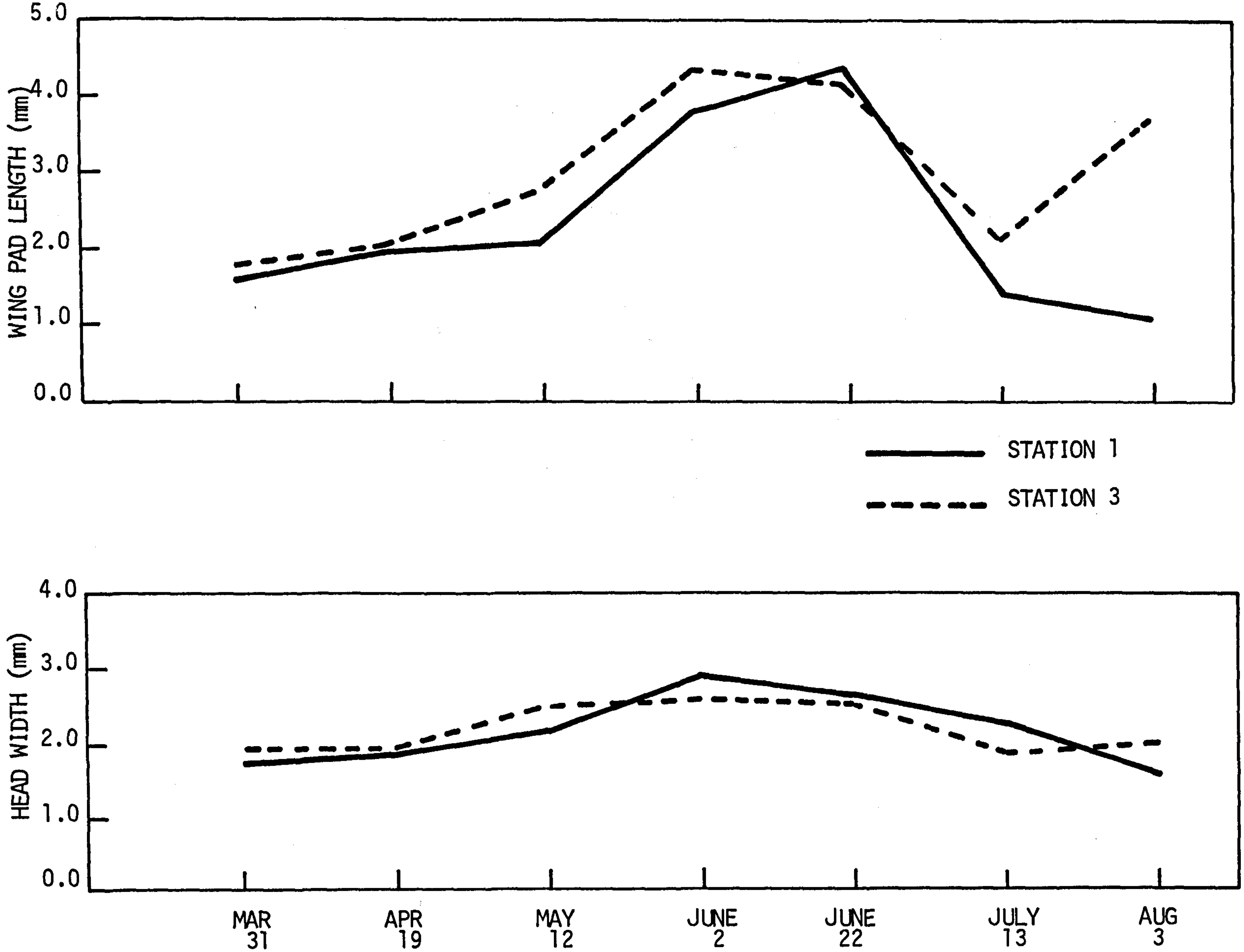

Figure 9. Average head width and fore wing pad length of *H. bilineata* nymphs collected at stations 1 and 3 near Johnsonville Steam Plant at 3-week intervals from March 31 to August 3, 1977.

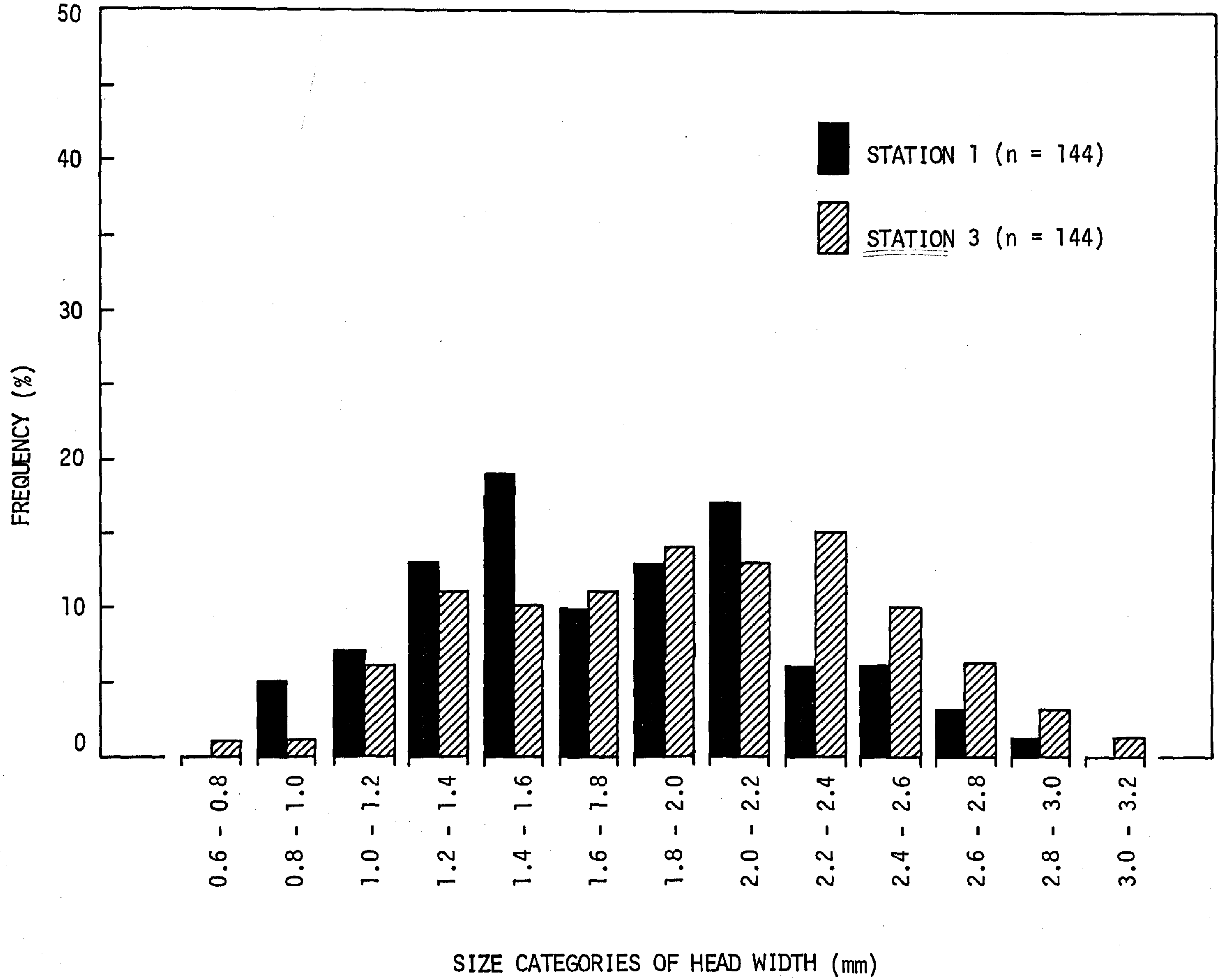

Figure 10. Comparison of frequency (%) of *H. bilineata* nymphs in various size classes (based on head width) at stations 1 and 3 near Johnsonville Steam Plant on March 31, 1977.

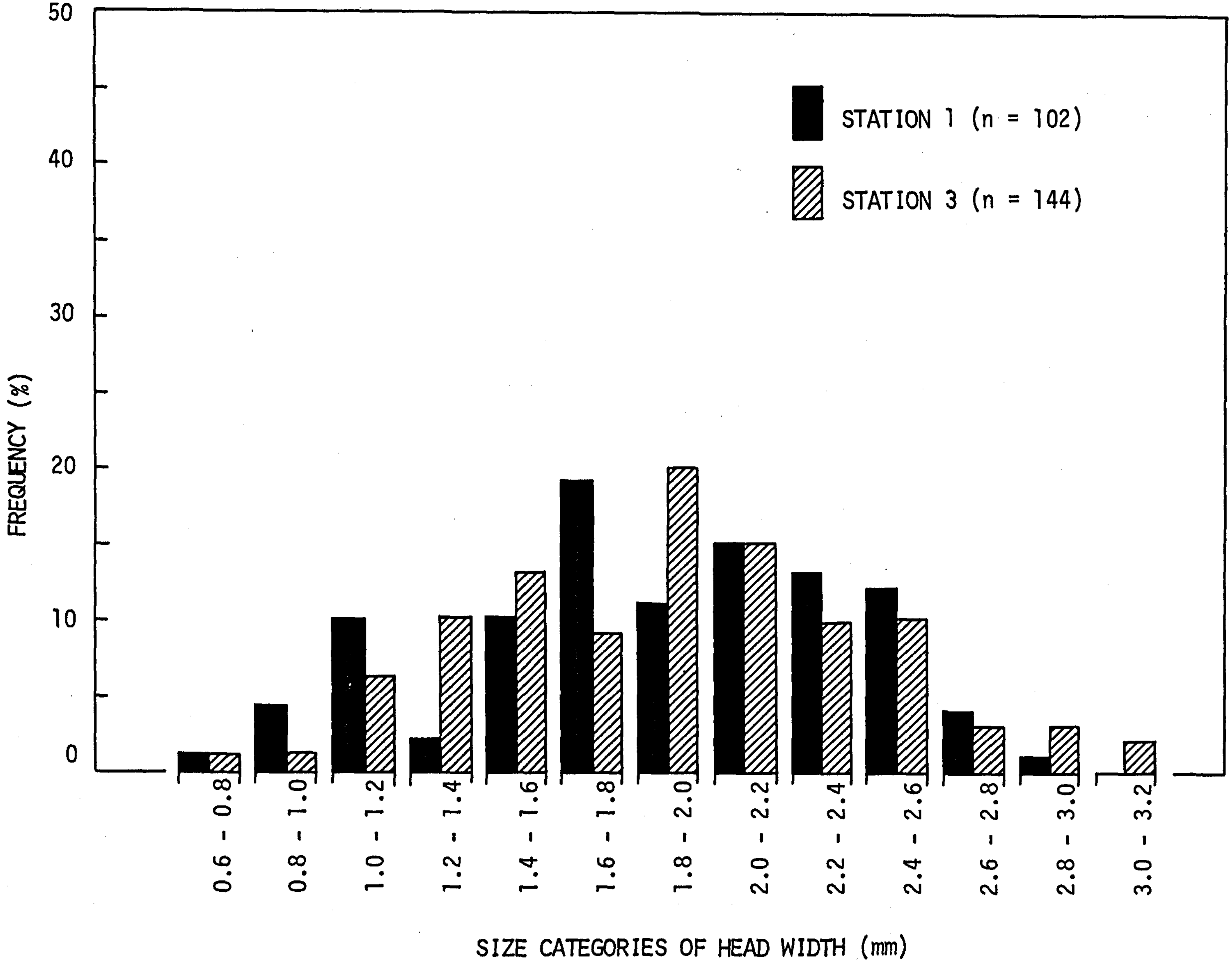

Figure 11. Comparison of frequency (%) of H. bilineata nymphs in various size classes (based on head width) at stations 1 and 3 near Johnsonville Steam Plant on April 19, 1977.

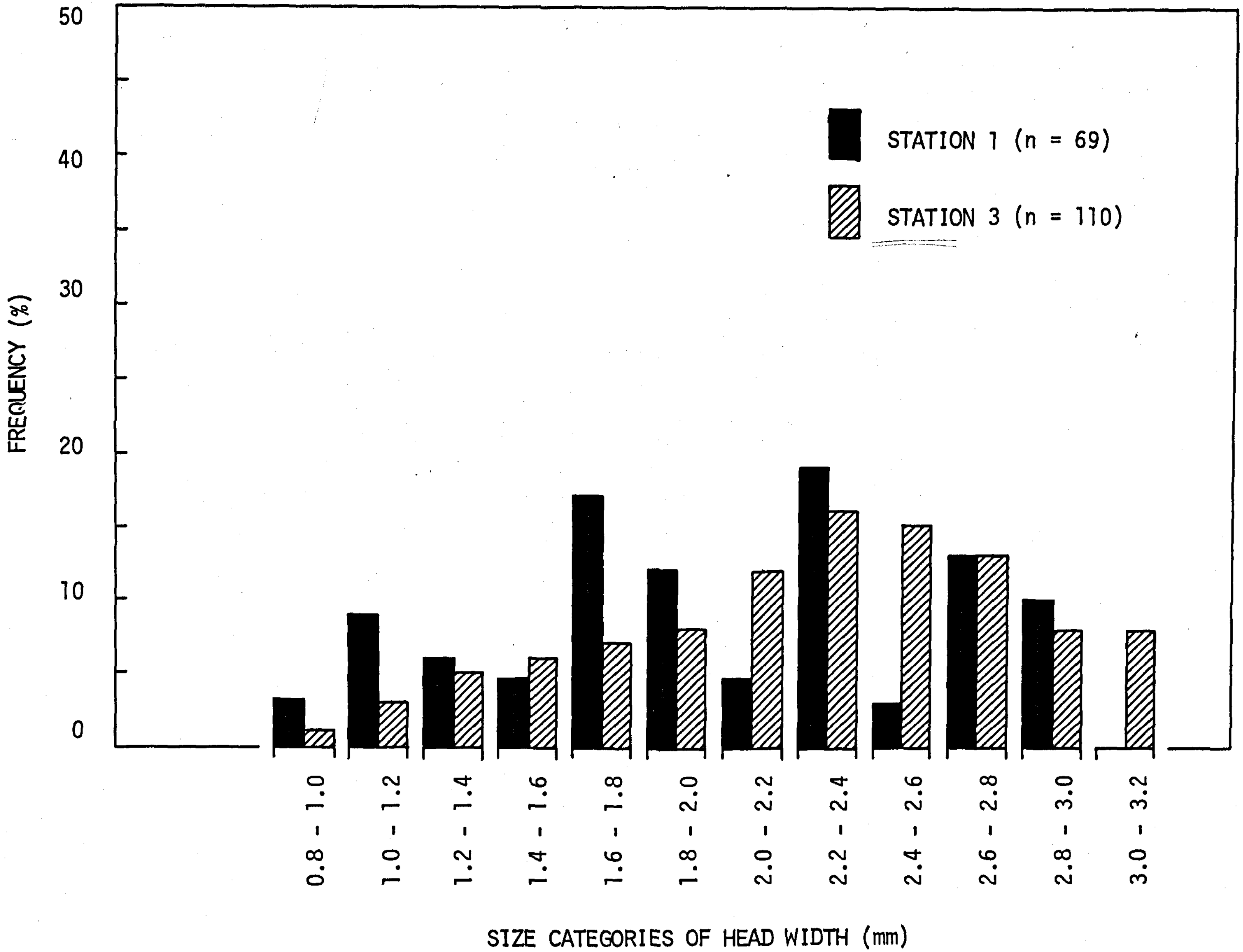

Figure 12. Comparison of frequency (%) of H. bilineata nymphs in various size classes (based on head width) at stations 1 and 3 near Johnsonville Steam Plant on May 12, 1977.

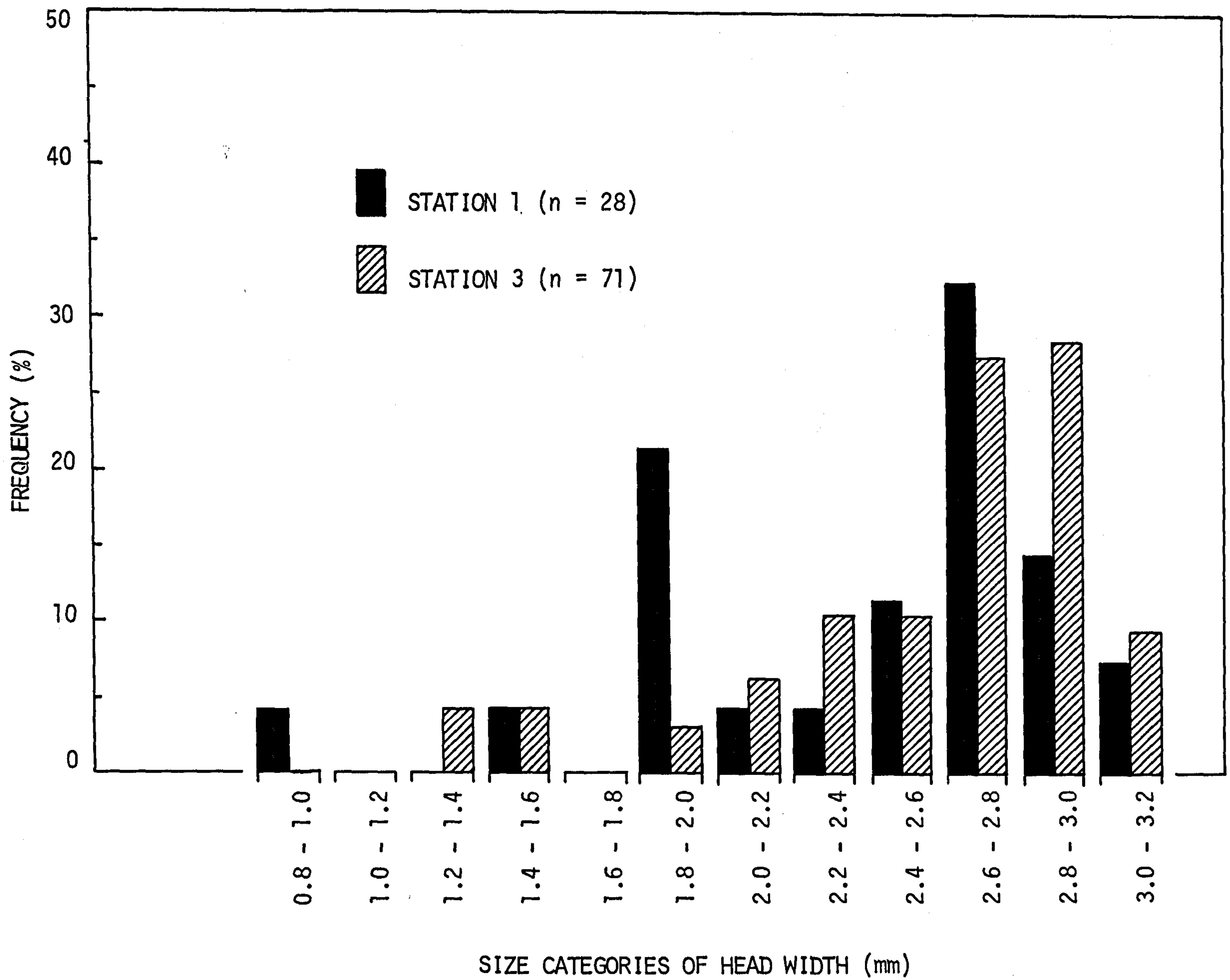

Figure 13. Comparison of frequency (%) of H. bilineata nymphs in various size classes (based on head width) at stations 1 and 3 near Johnsonville Steam Plant on June 2, 1977.

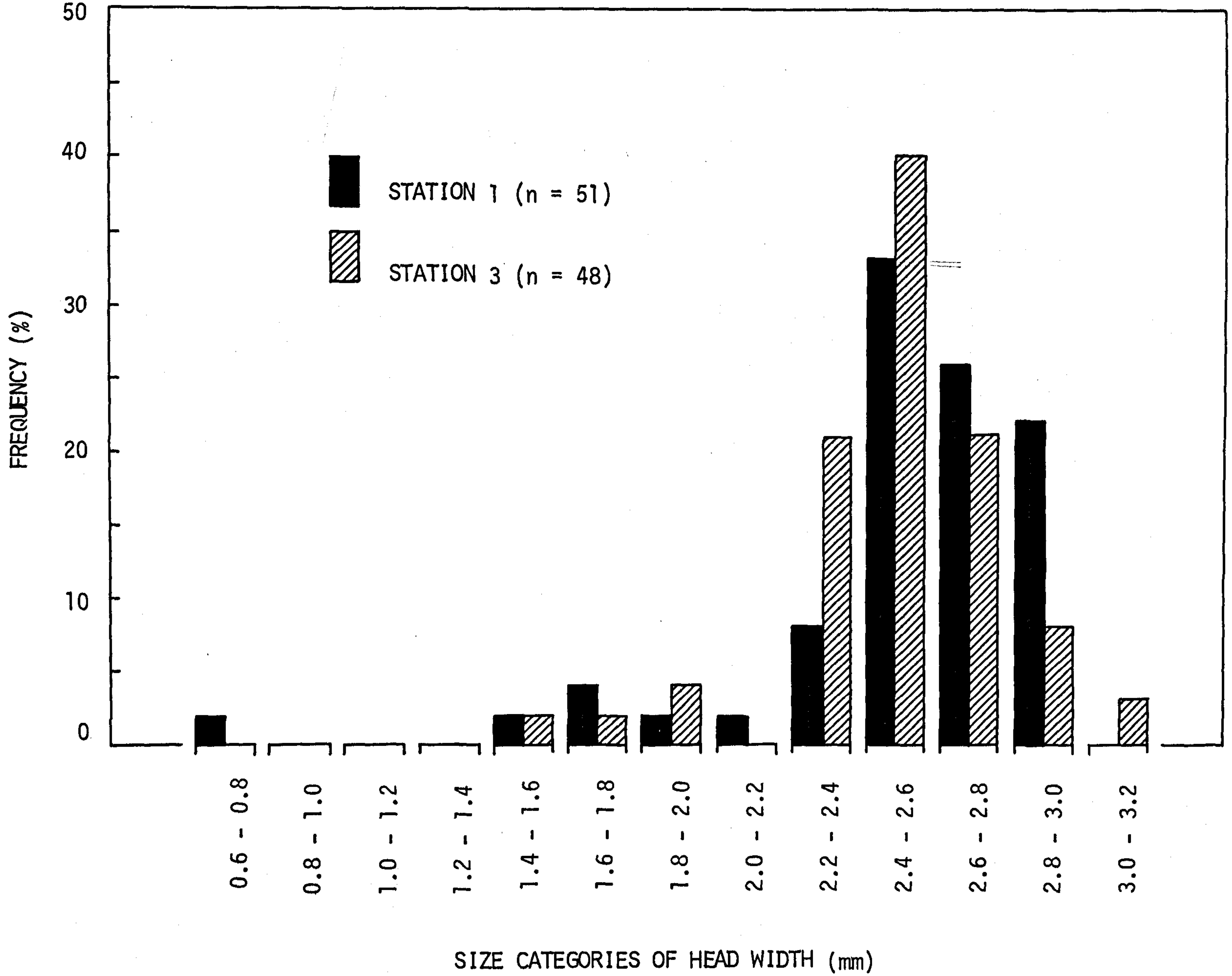

Figure 14. Comparison of frequency (%) of H. bilineata nymphs in various size classes (based on head width) at stations 1 and 3 near Johnsonville Steam Plant on June 22, 1977.

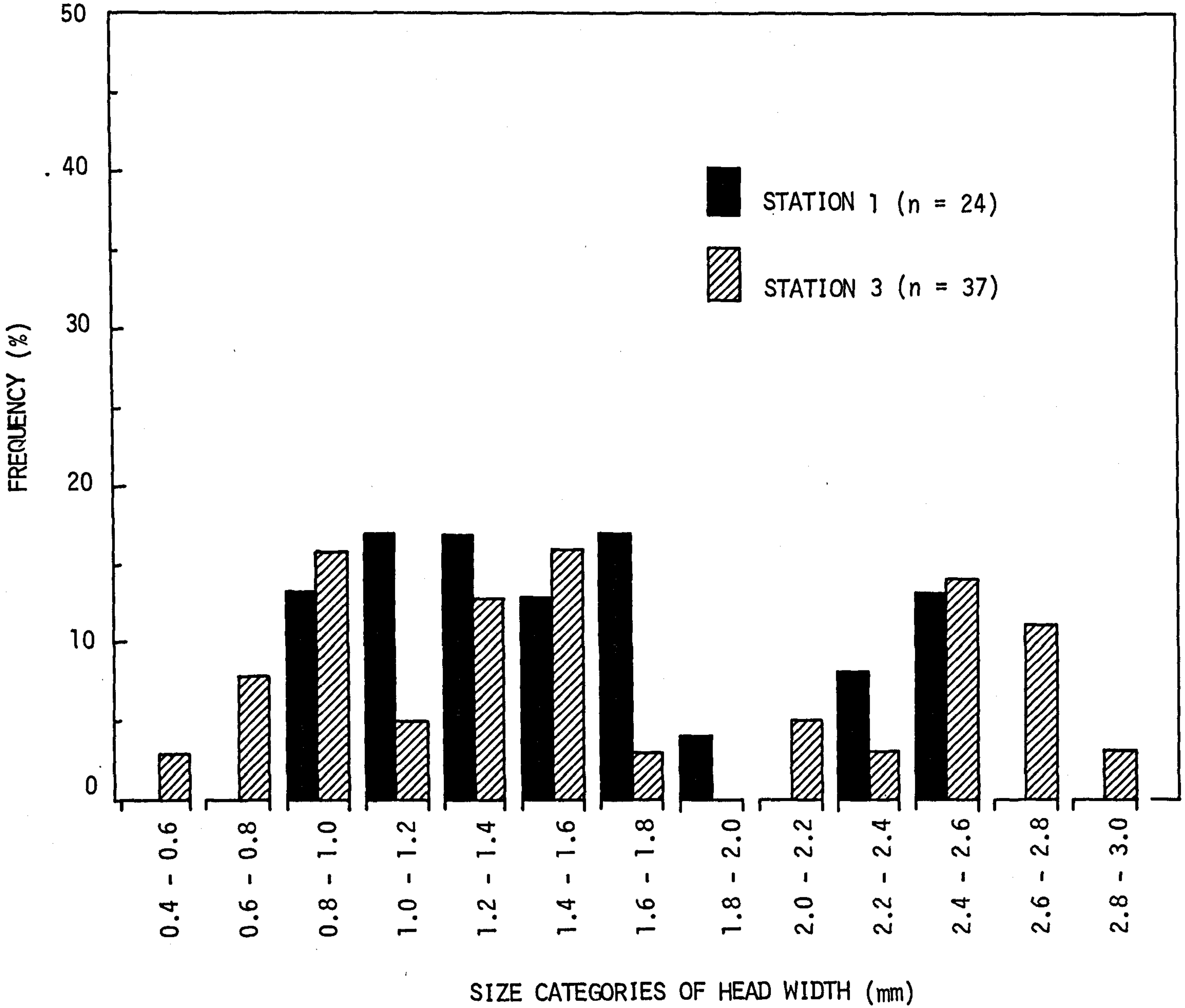

Figure 15. Comparison of frequency (%) of H. bilineata nymphs in various size classes (based on head width) at stations 1 and 3 near Johnsonville Steam Plant on July 13, 1977.

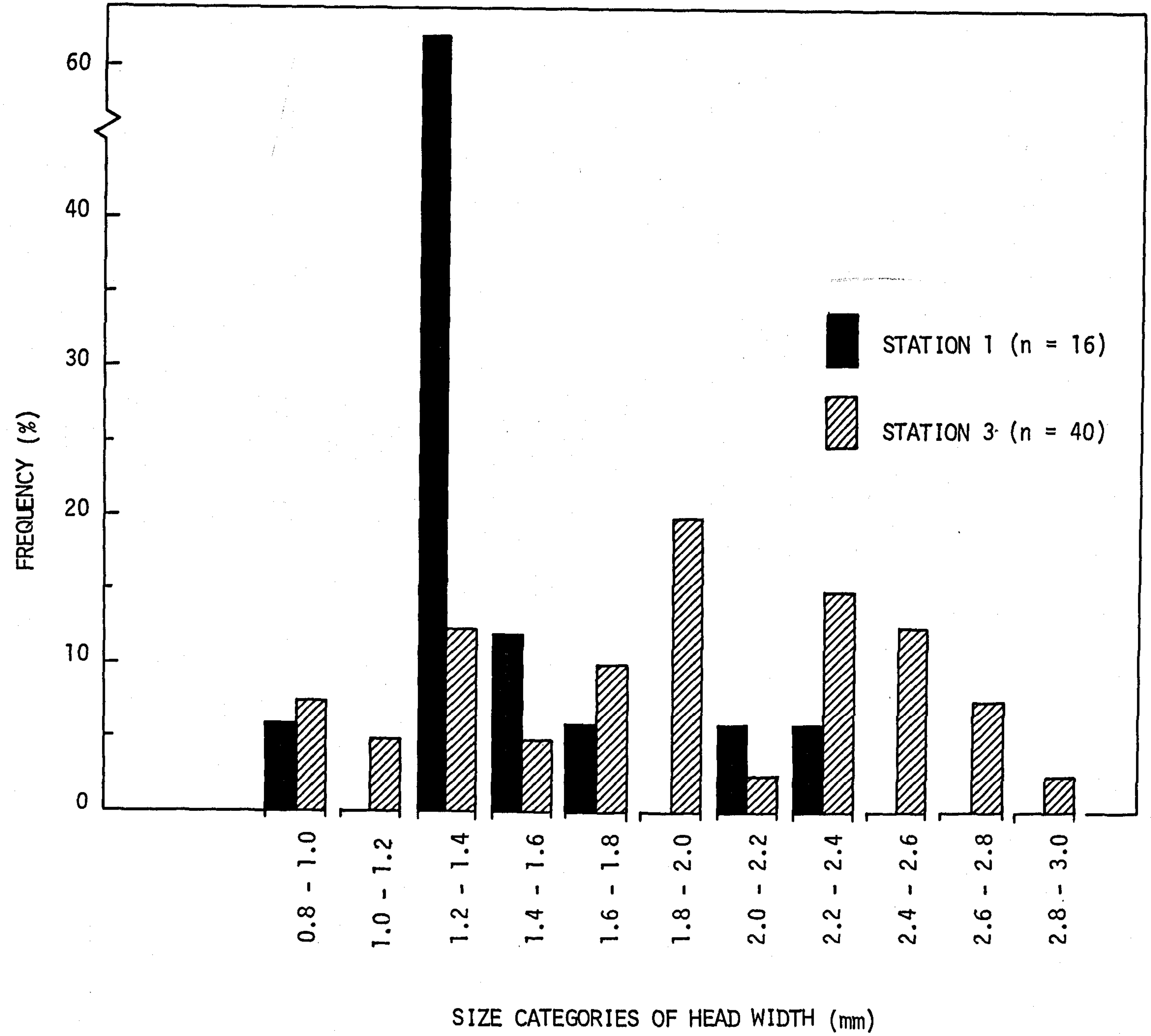

Figure 16. Comparison of frequency (%) of H. bilineata nymphs in various size classes (based on head width) at stations 1 and 3 near Johnsonville Steam Plant on August 3, 1977.

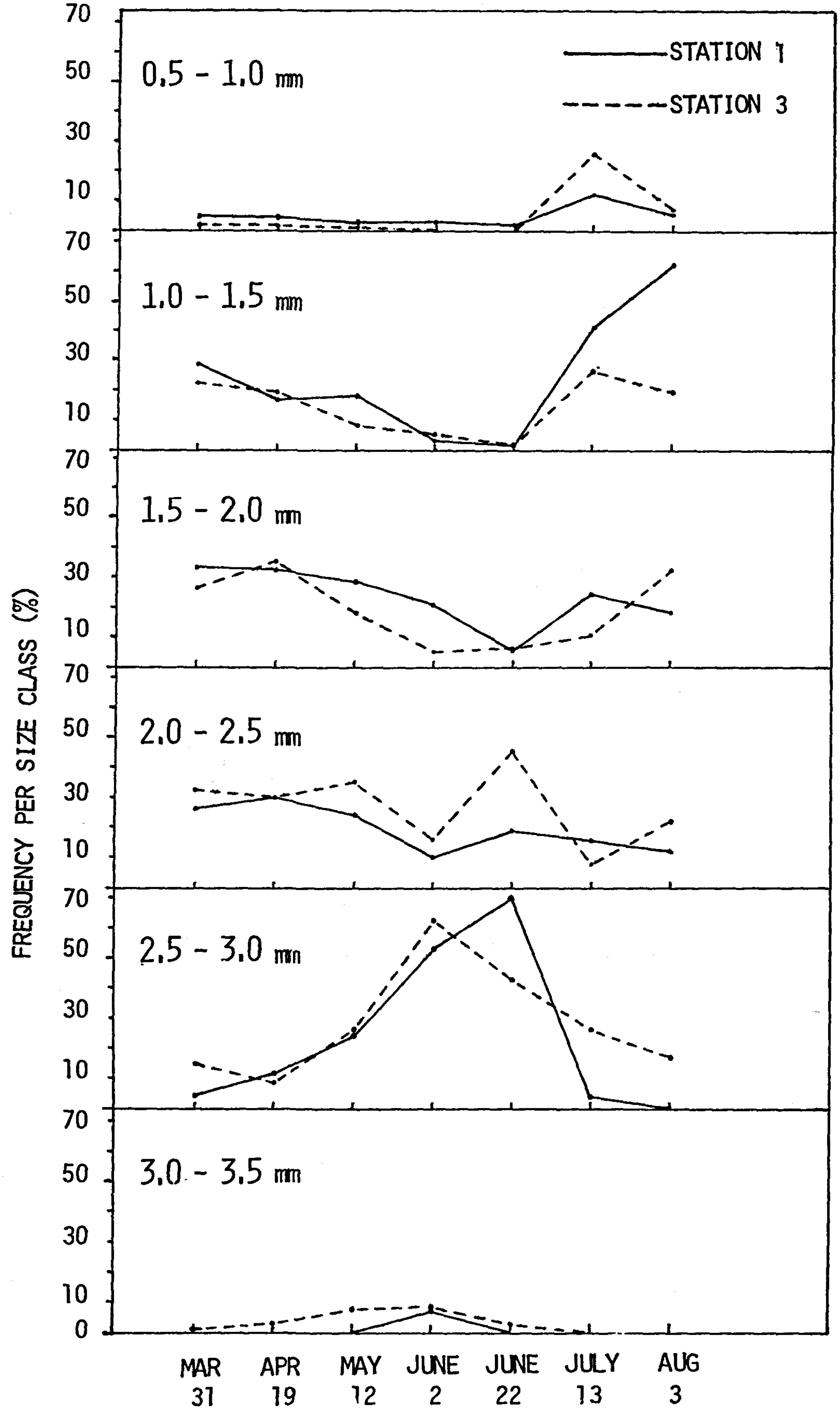

Figure 17. Comparison of frequency (%) of *H. bilineata* nymphs in six size classes of head width (interval = 0.5 mm) from March to August 1977 at stations 1 and 3 near Johnsonville Steam Plant.

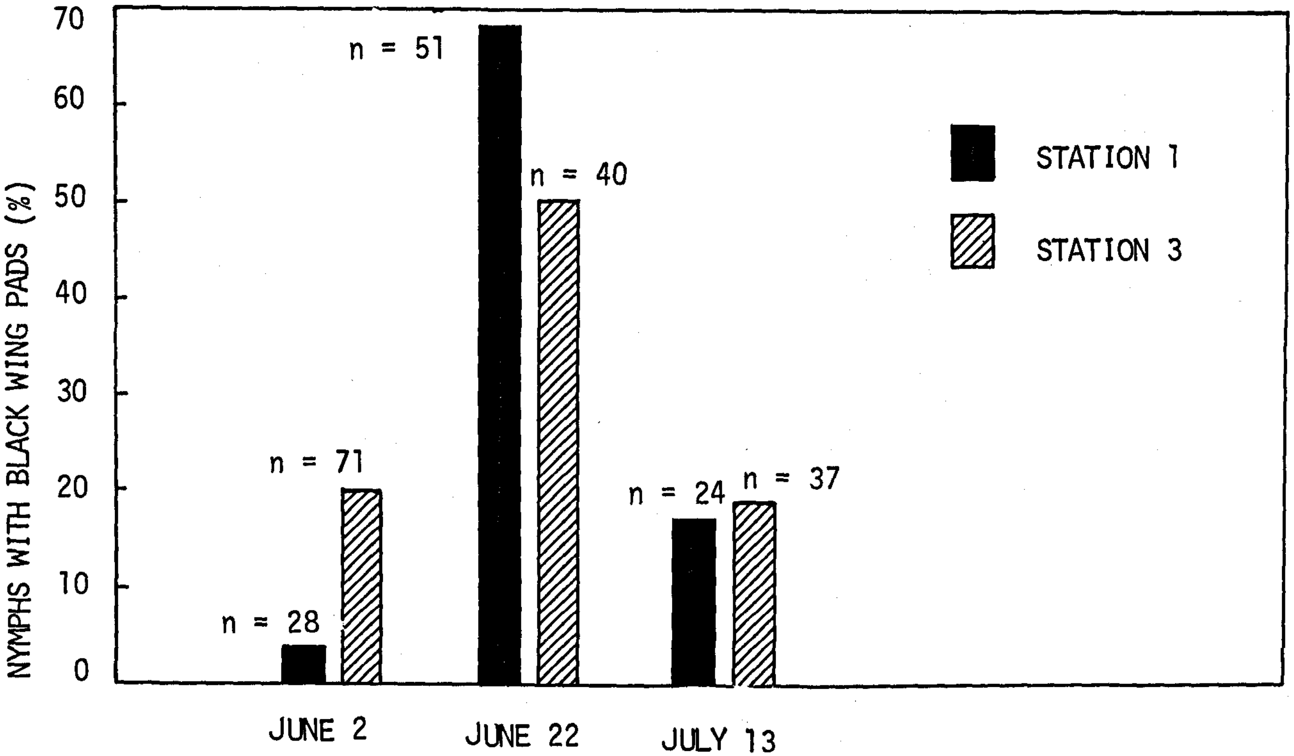

Figure 18. Percentage of H. bilineata nymphs having black wing pads at stations 1 and 3 on three sampling dates, 1977.

That elevated water temperatures can induce early emergence of aquatic insects, by as much as six months, has been established.[5,6] In contrast to these reports, a dragonfly has been shown to develop in the laboratory at a rate similar to that in the field, despite higher temperatures during the winter.[7] A field study showed that Ephemeroptera, Trichoptera, and Megaloptera below a power station in England do not emerge earlier than those in ambient areas.[8] Adult H. bilineata live only two or three days, in which time they must mate and lay eggs. Therefore, synchronization of emergence to the adult stage is critical for propagation of the next generation. H. bilineata near Johnsonville Steam Plant completed development sooner in the spring of 1977 in areas receiving thermal effluent, but evidently did not emerge appreciably earlier in these areas than in ambient areas. Some combination of extrinsic factors, rather than just temperature, must control the timing and hence synchronization of emergence. Although daylength may be an important cue, the exact mechanism is unknown.[9]

SIZE AND FECUNDITY COMPARISONS OF H. BILINEATA

The mean values for H. bilineata wing length, abdomen length, and egg count are given in Table 1. The number of eggs per female was positively correlated with abdomen length. The correlation coefficient r for females at station 1 was 0.58, slightly higher than that found at station 3 (0.52); both values are significant (P = 0.01). However, the variation in egg count for a particular abdomen length was very large (Figure 19).

Results of t-tests on mean size and fecundity between stations 1 and 3 are given in Table 2. Males at station 1 were significantly smaller on the average than those at station 3. Females differed in average abdomen length, but not in average wing length or number of eggs. Unfortunately, the specimens from station 1 were collected on a later date (June 25, 1977) than those from station 3 (June 4 and 5, 1977). Possibly, a seasonal decrease in size, a phenomenon known to occur in Hexagenia spp.,[9] is responsible for the differences in male size. This study will be repeated to ensure collection of individuals from the two stations on the same date.

THERMAL TOLERANCE OF IMMATURE AQUATIC INSECTS

H. bilineata Nymphs

Results of the thermal shock experiment are given in Table 3. All nymphs survived the control and the 10°C ΔT, and 29 of 30 survived the 20°C ΔT after 4 h of exposure. Mortality was high at the 30°C ΔT; 23 nymphs died within the first 15 min of exposure. Dissolved oxygen (DO) was probably not a factor in mortality, because nymphs can survive at DO levels less than 6.5 ppm at lower temperatures.

TABLE 1. MEAN VALUES ($\bar{x}$), STANDARD DEVIATIONS (s), AND NUMBER OF OBSERVATIONS (n) FOR MALE AND FEMALE *H. BILINEATA* WING LENGTH, ABDOMEN LENGTH, AND EGG COUNT AT STATIONS 1 AND 3 NEAR JOHNSONVILLE STEAM PLANT

	Wing length (mm)		Abdomen length (mm)		
	Male	Female	Male	Female	Egg count
	Station 1 (6/25/77)				
$\bar{x}$	14.07	17.63	9.93	12.71	3779.79
s	1.143	0.992	1.201	1.369	1058.24
n	29	24	29	24	24
	Station 3 (6/4/77-6/5/77)				
$\bar{x}$	14.95	17.73	11.41	13.82	3548.61
s	1.36	1.73	1.28	1.76	1623.17
n	44	108	44	108	108

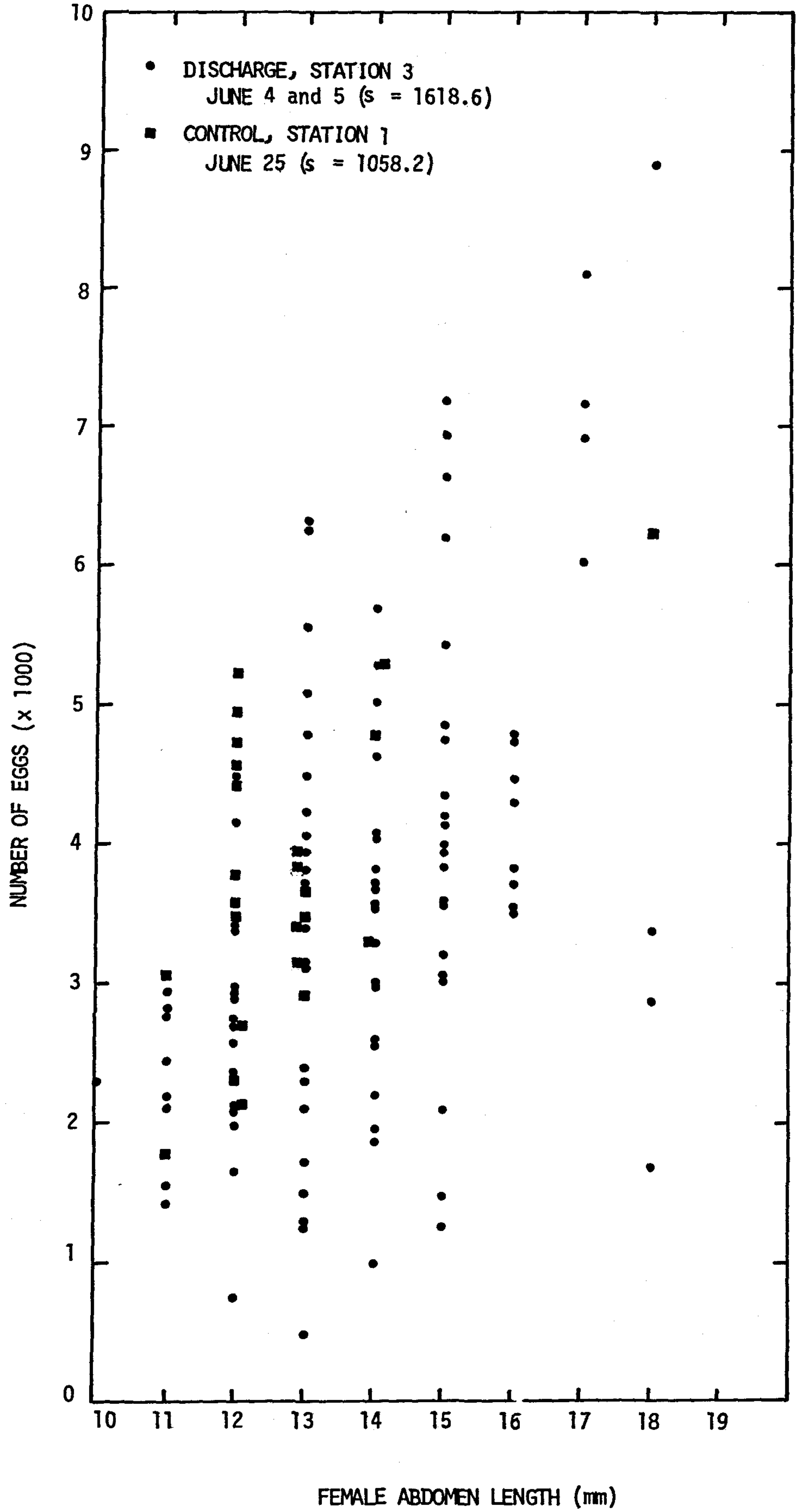

Figure 19. Scatter diagram of relationship between abdomen length and number of eggs in H. bilineata females.

TABLE 2. RESULTS OF t-TESTS COMPARING MEAN SIZE AND FECUNDITY OF *H. BILINEATA* SUBIMAGOES AT STATIONS 1 AND 3 NEAR JOHNSONVILLE STEAM PLANT

Character	df	Value of t	P
Male wing length	71	2.88**	<0.01
Male abdomen length	71	4.95**	<0.01
Female wing length	130	1.62	0.11
Female abdomen length	130	2.89**	<0.01
Egg count	130	0.07	>0.95

TABLE 3. NUMBER OF *H. BILINEATA* NYMPHS SURVIVING FOUR EXPERIMENTAL THERMAL SHOCKS

Thermal shock temperature[a] (°C)	Dissolved oxygen (ppm)	Replicate number	Number of survivors of exposure duration of							
			15 min	30 min	45 min	60 min	90 min	120 min	180 min	240 min
10 (ΔT = 0)	9.2	1	10	10	10	10	10	10	10	10
		2	10	10	10	10	10	10	10	10
		3	10	10	10	10	10	10	10	10
20 (ΔT = 10)	8.6	1	10	10	10	10	10	10	10	10
		2	10	10	10	10	10	10	10	10
		3	10	10	10	10	10	10	10	10
30 (ΔT = 20)	7.4	1	10	10	10	10	10	10	10	10
		2	9	9	9	9	9	9	9	9
		3	10	10	10	10	10	10	10	10
40 (ΔT = 30)	6.5	1	1	0						
		2	2	0						
		3	4	3[b]	1	1				

[a]Acclimation temperature was 10°C.

[b]Removed and slowly brought to 25°C.

The nymphs exposed to 20 and 30°C ΔTs immediately ceased moving; 3 min later the nymphs exposed to 20°C ΔT showed gill and leg movements, but no movement was seen in the nymphs exposed to 30°C ΔT. At all three higher ΔTs, air bubbles appeared on the gills of many nymphs. At the 15-min check, most of the nymphs exposed to 30°C ΔT were floating; those alive showed very little gill movement. The three nymphs still living after 30 min were slowly brought to 25°C; two died within 15 min, but one survived.

Although the number of nymphs available for the tests was low, results indicate that *H. bilineata* can tolerate relatively high thermal shocks (at least 20°C) for short periods when acclimated to a low temperature. Determination of the effects of various acclimation temperatures on thermal tolerance would be helpful for establishing seasonal tolerances.

Coelotanypus sp. Larvae

Results of the thermal shock experiment on *Coelotanypus* sp. larvae are given in Table 4. Survival after 2.5 h was high (90 to 100%) at all four temperatures. After the larvae were slowly brought to 25°C, some mortality was observed in larvae exposed to shock; the highest mortality (40%) occurred in the 25°C ΔT treatment. The effects of acclimation temperature on survival of these larvae should also be determined.

EFFECTS OF ENTRAINMENT WITHIN THERMAL PLUME ON AQUATIC INSECTS

Results of the field study at John Sevier Steam Plant on July 20, 1976, are presented in Table 5. The difference in temperature between the thermal plume station and the ambient station was 6 to 7°C throughout the 8-h test. Of the two damselfly nymphs that died, one had been partially eaten and was therefore probably killed by another damselfly. The other had drowned as it tried to emerge to the adult stage without a suitable support. The single dead mayfly may have been killed by a damselfly nymph present in the test basket.

The thermal plume from John Sevier Steam Plant sometimes extends several kilometers down the Holston River.[10] An insect drifting the length of the plume could be exposed to the heated water for many hours. Although the test was limited, the data indicate that damselfly nymphs of the genus *Enallagma* and mayfly nymphs of the genus *Stenonema*, common inhabitants of the Holston River, would probably experience little or no mortality due to the heated water. A simulated laboratory study[11] showed that mayflies of the genus *Isonychia* and caddisflies of the genus *Hydropsyche* were not adversely affected until shock temperatures neared the upper lethal limits.

TABLE 4. NUMBER OF *COELOTANYPUS* SP. LARVAE SURVIVING FOUR EXPERIMENTAL THERMAL SHOCKS

Thermal shock	Number of survivors of exposure duration of										
temperature[a] (°C)	15 min	30 min	45 min	60 min	75 min	90 min	120 min	150 min[b]	210 min	10 h	48 h
15 (ΔT = 0)	10	10	10	10	10	10	10	10	10	10	10
25 (ΔT = 10)	10	10	10	10	9	9	9	9	9	9	8
35 (ΔT = 20)	10	10	10	10	10	10	10	10	10	9	8
40 (ΔT = 25)	10	10	9	9	9	9	9	9	8	7	6

[a]Acclimation temperature was 15°C.

[b]Temperature in each treatment was brought slowly to 25°C.

TABLE 5. NUMBER OF DAMSELFLY AND MAYFLY NYMPHS SURVIVING AFTER 8 HOURS OF EXPOSURE IN THE THERMAL PLUME AND AT AMBIENT STATION NEAR JOHN SEVIER STEAM PLANT, JULY 20, 1976

Insect	Number of survivors per replicate[a]					
	Thermal plume station				Ambient station	
	1	2	3	4	1	2
Damselflies	8	10	10	10	10	10
Mayflies	10	10			9	

[a]10 nymphs per replicate.

EGG DEVELOPMENT AND THERMAL TOLERANCE

The results of egg hatching at seven constant temperatures are summarized in Table 6. No eggs hatched at 40°C, and although eggs hatched at 37°C, the percentage was low (21%) and the development time was comparatively long (minimum of 12 days). The shortest development time was 8 days, both at 31 and 34°C. These data agree with Fremling's report[4] of 8 days for development at 32°C and indicate a range of optimal temperatures for development. The relationship between temperature and incubation time is shown in Figure 20. The regression equation is $Y = 139.44 - 8.437t + 0.1349t^2$, where Y is development time in days and t is temperature. The observed values lie close to and twice coincide (at 25 and 37°C) with the predicted values.

The average cumulative percentages of hatching (at the end of the 4-day sampling period) at 31 and 34°C are significantly different at the 1% level, as determined by Duncan's multiple range test (Table 7). The abrupt drop in average cumulative percentage of hatching from 34 to 37°C indicates that 34°C is the peak temperature for speed of development. (See Davidson[12] for discussion on speed of development.)

Embryos never developed in eggs cultured at 40°C. After 5 days at this constant temperature, the cytoplasm was concentrated near the center of the oval eggs and the ends were translucent. After 2 weeks at 40°C, most of the eggs were almost entirely transparent, containing only small scattered pieces of opaque material. At 37°C, most of the eggs that did not hatch after 2 weeks were opaque, but very few embryos could be seen. Some eggs had the cytoplasm centered, with the ends translucent. Dissolved oxygen was maintained at the saturation level for each temperature and therefore was not a limiting factor.

Because 34°C is close to the upper temperature limit for development of H. bilineata eggs, 30°C was chosen as the culture temperature to follow the thermal shocks. Results of the thermal shocks simulating females laying eggs in a thermal plume are given in Table 8. Dissolved oxygen values were above 8.0 ppm. The hatching percentages were highly variable, partly because of the variable number of eggs placed in the containers. Some containers had fewer than 100 eggs, whereas others contained over 2000. These discrepancies resulted from the total number of eggs the females laid in each treatment. Especially notable was the comparatively low number of eggs laid in the 45°C water.

The average percentages of hatching in the control treatments (ΔT = 0°C) were low compared with the percentages reported for the constant temperatures 28 and 31°C. Reasons for this discrepancy are not clear; it is doubtful that the physical transfer of the eggs caused the lower percentage of hatching. Possibly the later date of collection (August 10 compared with July 8) was an important factor.

The 3-factor analysis of variance is summarized in Table 9. The three main effects--shock temperature, shock duration, and hatching day (day after oviposition)--were partitioned by one-degree-of-freedom tests for significant responses. All interactions were found to be significant.

TABLE 6. MEAN CUMULATIVE PERCENTAGE OF HATCHING THROUGH 4 DAYS OF *H. BILINEATA* EGGS CULTURED AT 8 CONSTANT TEMPERATURES[a]

Day after oviposition	Mean cumulative hatch (%) at constant temperature of							
	40°C	37°C	34°C	31°C	28°C	25°C	22°C	19°C
1-7								
8			74.8	62.5				
9			93.1	85.9				
10			94.7	87.8	26.1			
11			95.4	89.1	76.1			
12		9.5			87.6			
13		14.4			88.1	34.8		
14		19.8				81.6		
15		21.0				85.1		
16						88.0		
17							12.7	
18							40.7	
19							66.7	
20							81.6	
21-28								
29								2.5
30								12.0
31								24.1
32								35.8

[a]Each value is the mean of five replications.

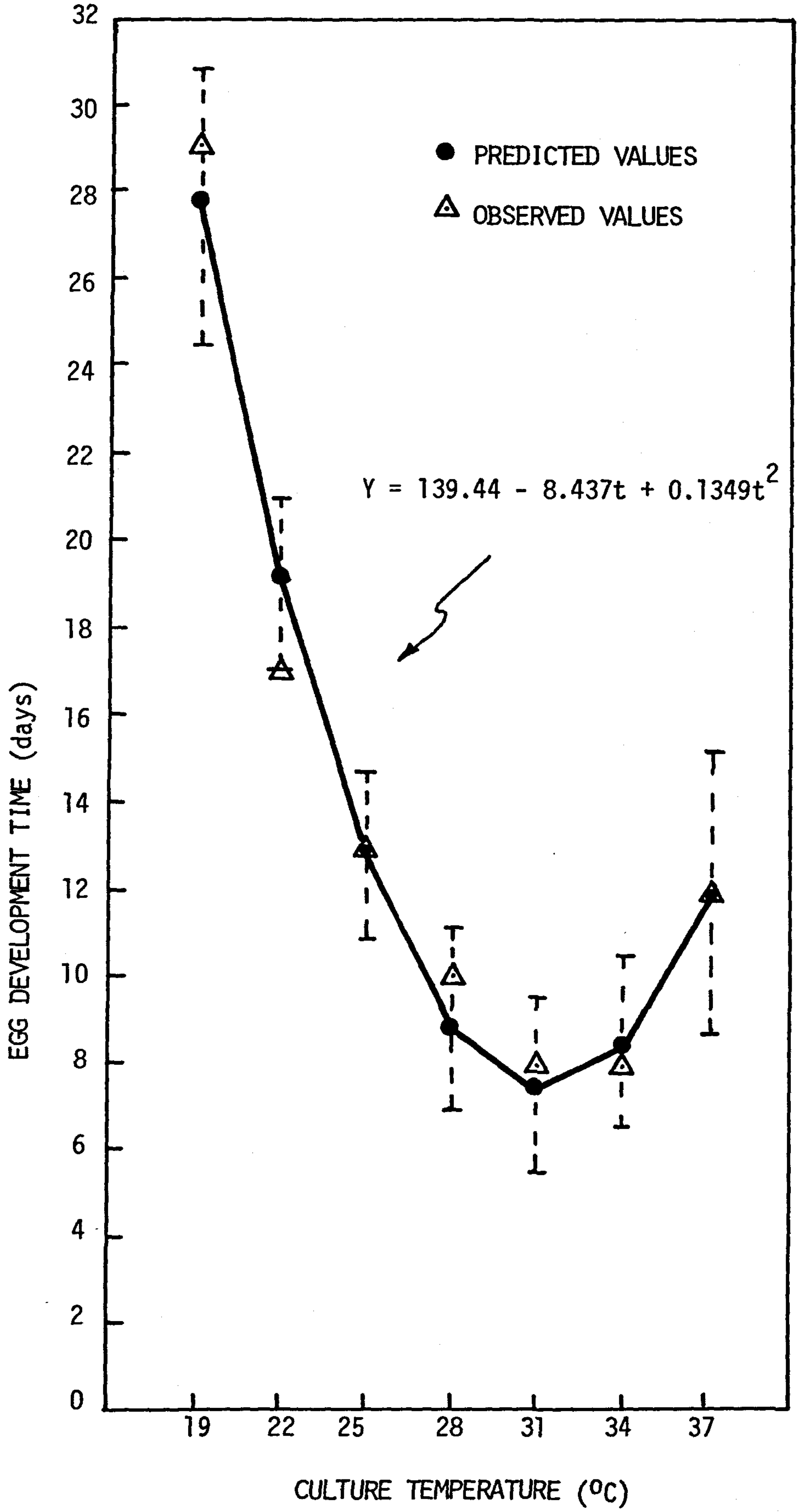

Figure 20. Predicted and observed development times (in days) for *H. bilineata* eggs cultured at seven nearly constant (±1°C) temperatures.

TABLE 7. SUMMARY OF HATCHING RESULTS AT CONSTANT TEMPERATURE AND TESTS FOR SIGNIFICANCE OF DIFFERENCE BETWEEN MEANS AS DETERMINED BY DUNCAN'S MULTIPLE RANGE TEST

Constant temperature (°C)	Mean cumulative hatch[a] (%)
19	35.8 a
22	81.6 b
25	88.0 c
28	88.1 c
31	89.1 c
34	95.4 d
37	21.0 e

[a]Means followed by different letters are significantly different at the 1% level.

TABLE 8. PERCENTAGES OF HATCHING OF H. BILINEATA EGGS EXPOSED TO THERMAL SHOCKS (ΔTs OF 5, 10, AND 15°C) FOR THREE DURATIONS (5, 10, AND 15 MIN) IMMEDIATELY AFTER OVIPOSITION

Pulse temperature (°C)	Pulse duration (min)	Date hatched (1977)	Hatching percentage (%) by replicate number 1	2	3	Total (%)	Average (%)
30 ($\Delta T = 0$)	5	8/18	35.79	27.91	13.16	76.86	25.62
		8/19	60.44	90.91	42.80	194.15	64.72
		8/20	37.09	44.95	39.47	121.51	40.50
		8/21	43.75	49.49	44.09	137.33	45.78
	10	8/18	44.32	85.71	27.50	157.53	52.51
		8/19	13.04	22.62	53.42	89.07	29.69
		8/20	58.00	51.43	43.33	152.76	50.92
		8/21	45.05	21.26	64.00	130.32	43.44
	15	8/18	14.20	8.13	16.44	38.77	12.92
		8/19	45.29	27.03	18.93	91.25	30.42
		8/20	34.29	75.14	75.82	185.25	61.75
		8/21	47.22	34.62	41.77	123.61	41.20
35 ($\Delta T = 5$)	5	8/18	16.98	28.07	20.83	65.88	21.96
		8/19	53.62	56.18	59.26	169.06	56.35
		8/20	71.43	90.11	91.49	253.03	84.34
		8/21	83.23	79.79	92.04	255.06	85.02
	10	8/18	29.69	7.84	28.27	65.80	21.93
		8/19	62.37	62.50	78.72	203.59	67.86
		8/20	51.49	30.69	38.89	121.07	40.36
		8/21	88.17	79.02	87.93	255.12	85.04
	15	8/18	15.38	5.71	2.80	23.89	7.96
		8/19	78.32	79.31	79.80	237.43	79.14
		8/20	89.91	87.57	89.87	267.35	89.19
		8/21	82.79	90.25	88.65	261.69	87.23
40 ($\Delta T = 10$)	5	8/18	7.81	11.70	13.59	33.10	11.03
		8/19	43.52	41.09	42.63	127.24	42.41
		8/20	29.23	35.68	46.13	111.04	37.01
		8/21	62.92	63.32	58.73	184.97	61.66
	10	8/18	10.96	8.46	6.88	26.30	8.77
		8/19	52.26	52.36	35.39	140.01	46.67
		8/20	69.92	36.87	64.35	170.53	56.84
		8/21	59.84	71.54	54.13	185.51	61.84

TABLE 8 (continued)

Pulse temperature (°C)	Pulse duration (min)	Date hatched (1977)	Hatching percentage (%) by replicate number 1	2	3	Total (%)	Average (%)
	15	8/18	12.32	9.76	11.53	33.61	11.20
		8/19	42.03	35.96	43.00	120.99	40.33
		8/20	60.53	64.01	67.05	191.59	63.86
		8/21	73.52	69.72	54.72	197.96	65.99
45 (ΔT = 15)	5	8/18	0.00	0.00	0.86	0.86	0.29
		8/19	22.84	11.54	12.16	46.54	15.51
		8/20	0.00	6.90	27.27	34.17	11.39
		8/21	63.48	14.05	11.54	89.07	29.69
	10	8/18	2.91	1.36	0.41	4.68	1.56
		8/19	0.00	0.00	0.00	0.00	0.00
		8/20	1.01	7.69	1.79	10.49	3.50
		8/21	3.25	9.35	2.97	15.57	5.19
	15	8/18	3.54	3.13	33.33	40.00	13.33
		8/19	3.39	2.53	3.43	9.35	3.12
		8/20	12.39	9.18	14.39	35.96	11.99
		8/21	5.35	3.67	3.40	12.42	4.14

TABLE 9. ANALYSIS OF VARIANCE OF 3-FACTOR THERMAL SHOCK EXPERIMENT ON *H. BILINEATA* EGGS

Source of variation	df	Mean square	F
Replications	2	11.51	
Shock temp., T (t = 4)	(3)	(17,046.26)	
Linear response	1	25,134.29	333.47**
Quadratic response	1	25,183.05	334.11**
Cubic response	1	821.46	10.90**
Shock duration, D (d = 3)	(2)	(175.79)	
Linear response	1	8.61	0.114
Quadratic response	1	342.97	4.55**
Hatching day, H (h = 4)	(3)	(8,869.24)	
Linear response	1	23,008.16	305.26**
Quadratic response	1	3,094.33	41.05**
Cubic response	1	505.25	6.70**
TD (t-1)(d-1)	6	363.32	4.82**
TH (t-1)(h-1)	9	1,538.56	20.41**
DH (d-1)(h-1)	6	549.84	7.30**
TDH (t-1)(d-1)(h-1)	18	479.18	6.36**
Error	96	75.373	

The mean percentages of hatching due to the three main effects are shown in Figure 21. The average percentage of hatching after a shock of 15°C was much lower than that after lesser shocks (Figure 21A). Shock duration appeared to have no effect on hatching response when averaged over all treatments (Figure 21B). Cumulative percentage of hatching increased through time as expected (Figure 21C).

Partitioning the main effect of shock temperature showed that there was a highly significant linear response of hatching to shock temperature, but also that the response was curvilinear (Table 9). These results indicate that the increase in hatching at the 5°C ΔT, as shown in Figure 21A, is significant. The main effect of shock duration showed a significant quadratic response when partitioned. The reason for the lower hatching response to the 10-min treatments is unknown. The main effect of hatching day showed significant linear, quadratic, and cubic responses. Hatching increased sharply from day 1 to day 2, but then tended to reach a plateau (Figure 21C).

The interaction between shock temperature and shock duration is illustrated in Figure 22. Hatching success responded in different patterns at each shock duration, which showed the complex relationship between these two variables. Increased shock duration for the 15°C ΔT treatment decreased the hatching success, although the response is curvilinear. The interaction between shock temperature and hatching day revealed a lower hatching success at 15°C ΔT for each hatching day (Figure 23). At the 0, 5, and 10°C ΔTs, the percentage of hatching increased appreciably through the 4-day observation period, but at the 15°C ΔT the percentage increase was much less. The response of hatching to the interaction between shock duration and hatching day was highly variable (Figure 24). Shock duration apparently affects the percentage of hatching differently on each day that eggs hatch.

The significant 3-way interaction (Table 9) indicates that the relationship between shock temperature, shock duration, and day after oviposition, as they affect egg development, is complex. The percentages obtained in the experiment were quite variable, and trends are difficult to define. Shock durations of 5 to 15 min did not appear to affect hatching success significantly unless the temperature change was at least 15°C (ambient was 30°C); in this case, longer shock duration lowered the hatching responses, although there was a slight cumulative increase in the response through the 4-day observation period.

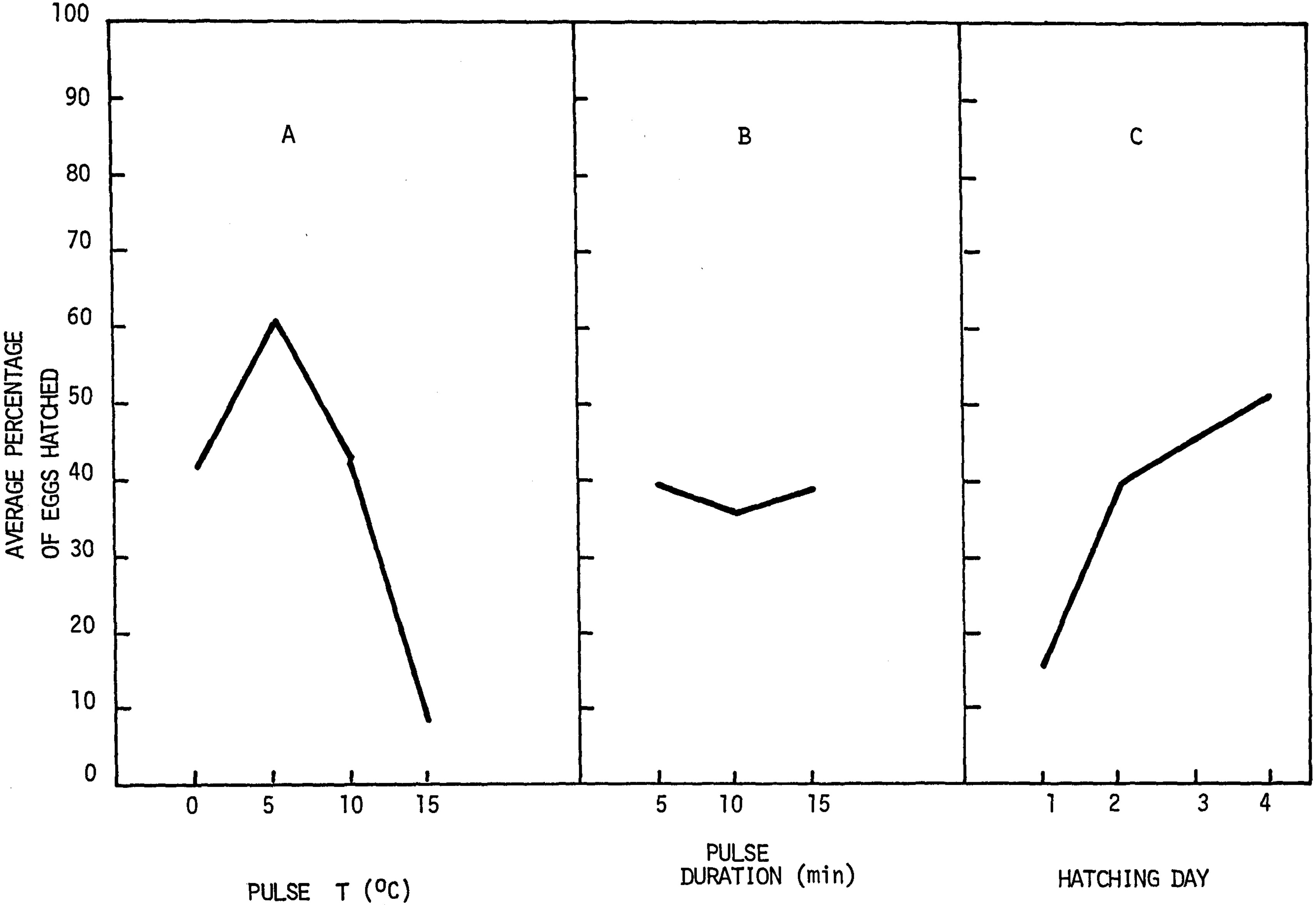

Figure 21. Average percentage of hatching of H. bilineata eggs due to main effects of shock temperature (A), shock duration (B), and day after oviposition (C).

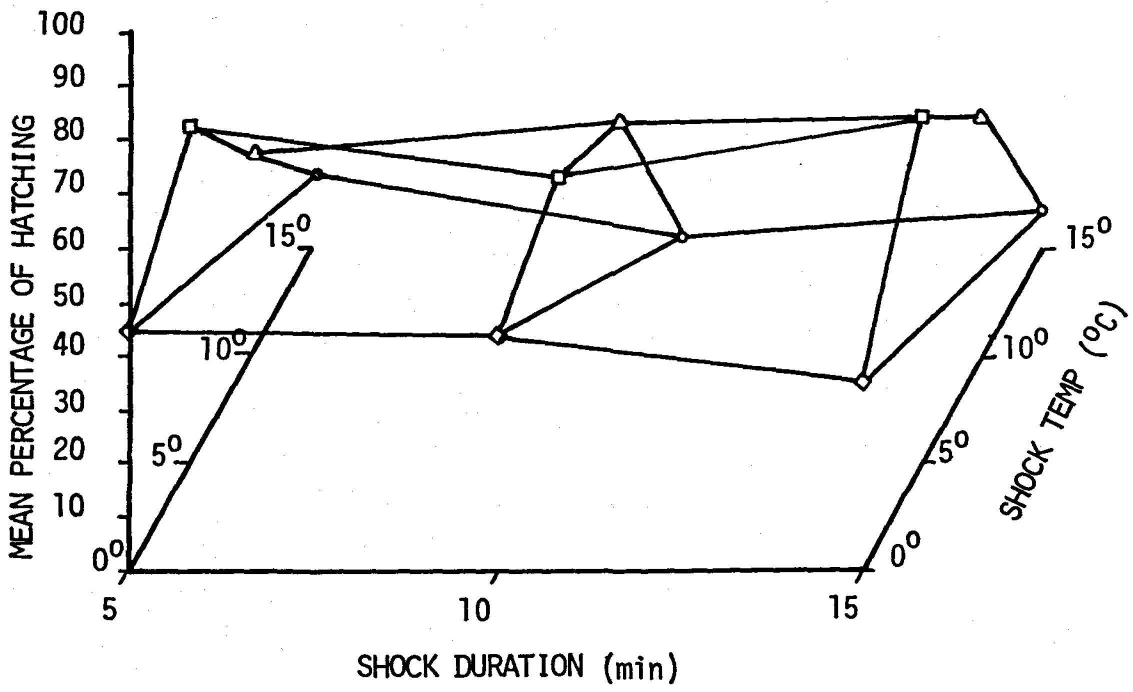

Figure 22. Surface response curves for shock temperature and shock duration (interaction) effects on mean percentage of hatching of H. bilineata eggs over a 4-day hatching period.

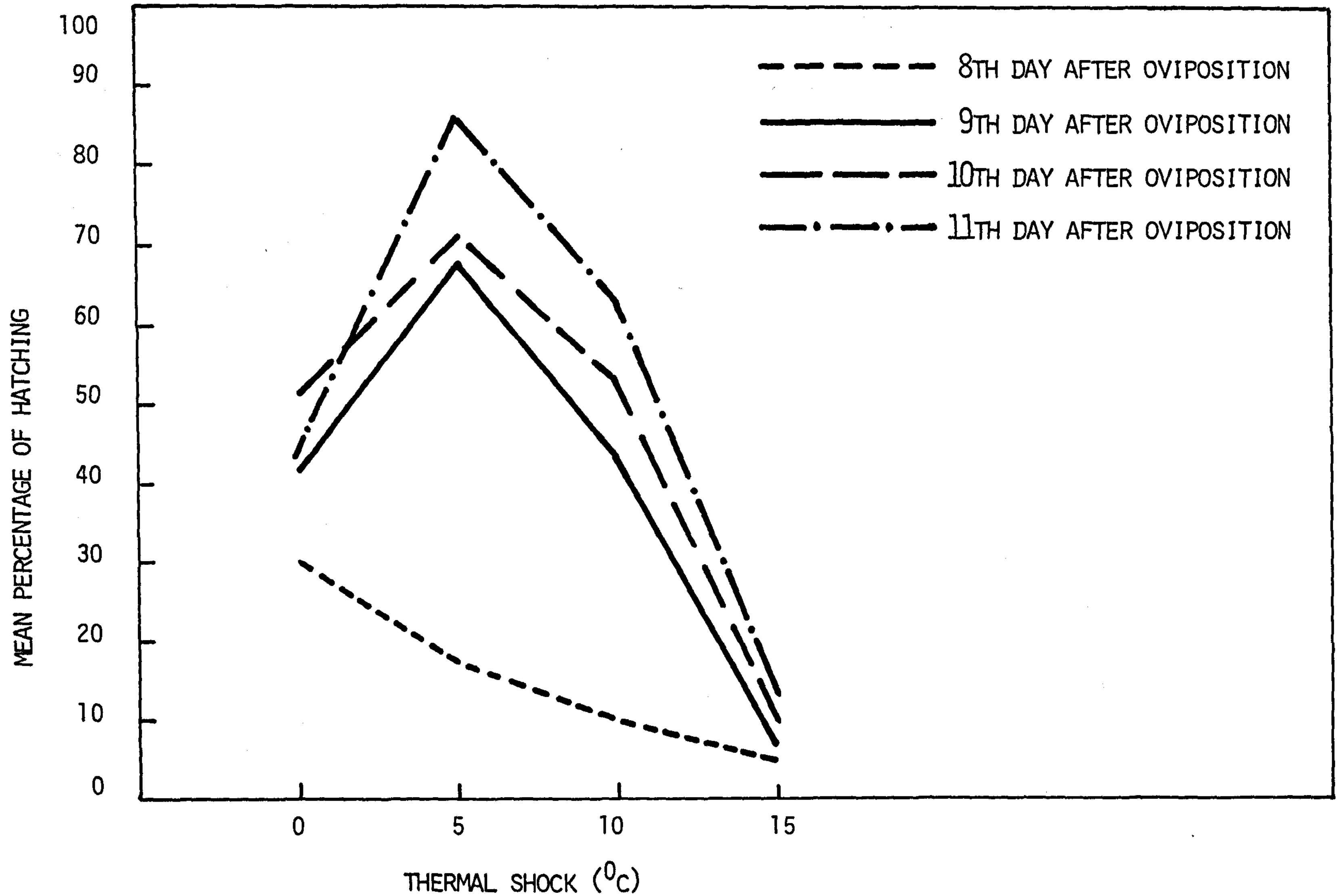

Figure 23. Effect of shock temperature on mean cumulative percentage of hatching of H. bilineata eggs exposed to thermal shock immediately after oviposition.

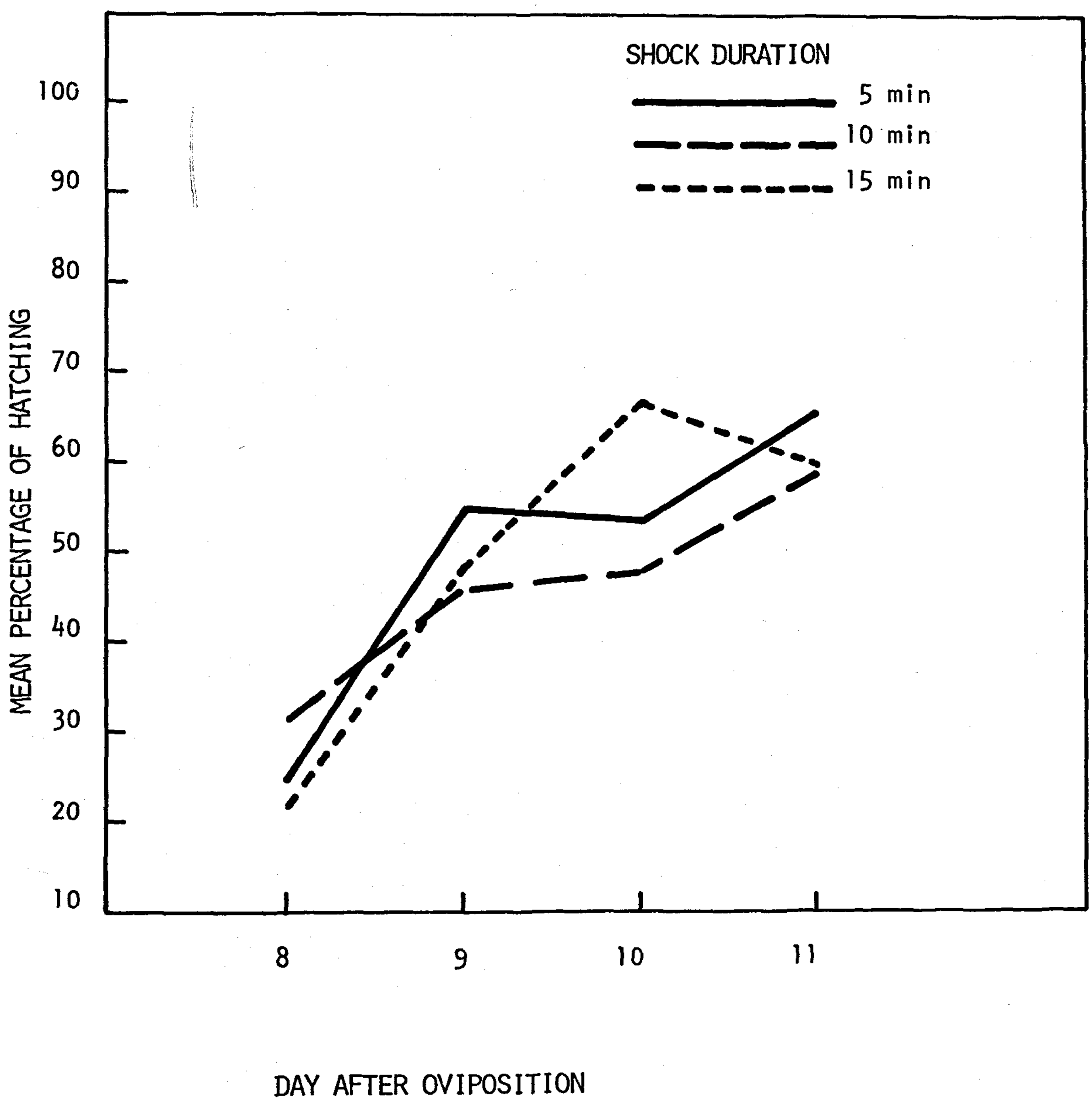

Figure 24. Interaction of shock duration and hatching day on the mean percentage of hatching of H. bilineata eggs exposed to thermal shock immediately after oviposition.

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GLOSSARY

ambient: Surrounding environmental condition.

entrainment: Transport by the flow of a liquid.

fecundity: Ability to produce offspring; reproductive potential.

instar: A stage in the life cycle of an insect between molts.

subimago: Immature adult stage of mayflies, duller and darker than adult to which it molts.

thermal plume: Warm water discharged from once-through cooling by electric generating plants; boundary is 2°C above ambient isotherm.

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/7-78-128	2.
3. RECIPIENT'S ACCESSION NO.	
4. TITLE AND SUBTITLE: EFFECTS OF THERMAL DISCHARGE ON AQUATIC INSECTS IN THE TENNESSEE VALLEY	5. REPORT DATE: July 1978
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15. SUPPLEMENTARY NOTES

This project is part of the EPA-planned and coordinated Federal Interagency Energy/Environment R&D Program.

16. ABSTRACT

The Tennessee Valley Authority (TVA) conducted studies to (1) determine the thermal tolerances of selected aquatic insects and (2) investigate growth and emergence of those insects in the vicinity of TVA electric generating plants. Results of the study will be used to help establish thermal effluent limits to protect the aquatic ecosystem.

Tolerance of the immature stages of Hexagenia bilineata (Say) and Coelotanypus sp. to thermal shocks (ΔTs) of up to 20°C was found to be great. However, eggs subjected to a shock of 15°C resulted in a greatly reduced mean percentage of hatching. No difference in fecundity of adult females was found between ambient and thermal plume stations. Adult males from the heated discharge channel were significantly larger on the average than adult males from the ambient station.

A drift study of Enallagma spp. and Stenonema spp. in a thermal plume showed little or no mortality at ΔTs that normally result from the heated water.

17. (Circle One or More) KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Ecology, Environments, Earth Atmosphere, Environmental Engineering, Geography, Hydrology, Limnology, Biochemistry, Earth Hydrosphere, Combustion, Refining, Energy Conversion, Physical Chemistry, Materials Handling, Inorganic Chemistry, Organic Chemistry, Chemical Engineering	Control Technology: Energy Extraction, Coal Cleaning, Flue Gas Cleaning, Direct Combustion, Synthetic Fuels, Nuclear, Thermal, Improved Efficiency, Advanced Systems; Processes & Effects: Transport Processes, Ecological Effects, Charac., Meas. & Monit., Health Effects, Integrated Assessment; Energy Cycle: Extraction, Processing, Conversion, Utilization; Fuel: Coal, Oil/Gas, Oil Shale, Nuclear, Geothermal, Solar, Waste as Fuel, Hydroelectric, Multi-fuel (5 or more)	6F 8A 8F 8H 10A 10B 7B 7C 13B
Other:	Other:	

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