EFFECTS OF INCREASED WATER TEMPERATURE ON *DAPHNIA PULEX*

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ABSTRACT

Techniques were developed to study the effects of increased water temperature on certain zooplankters; specific studies were conducted on *Daphnia pulex*, an abundant and important zooplankter of the lower Columbia River. Study methods simulated prolonged exposure to constant high temperatures in thermal discharges and short exposures to increased temperatures in condensers of cooling systems. Effects were evaluated on the basis of survival and reproduction for periods ranging from 34 to 90 days. The time to death of 50% of the *D. pulex*, both mature and young, was less than 24 h at temperatures above 27°C. Temperatures of 27°C and below required an exposure of at least 192 h to cause 50% mortality. The young females were more tolerant of temperature increases than older females. The greatest reproduction by older females was at the control temperature (15°C), whereas reproduction by the young females was low at lower temperatures. No reproduction occurred above 27°C.

Two groups of *D. pulex* (one from the Seattle, Wash., area and the other from the Columbia River) studied at increased temperatures for prolonged periods revealed similar patterns of survival and reproduction, but the Columbia River group appeared less tolerant of increased temperatures. A short exposure (15 min) to increased temperatures up to 30°C had little effect on survival and reproduction.

It was concluded that temperatures should not exceed 26° or 27°C for prolonged periods or 30°C for more than 15 min to protect *D. pulex* populations in the river.

The lower reaches of the Columbia River (below Portland, Oreg.) support extensive and valuable commercial and sport fisheries as well as other types of recreational activities. This section of the river is also becoming increasingly industrialized. Associated with the industrialization is 1) the extensive use of river water for cooling purposes and 2) the discharge of heated cooling water back into the river. This increasing use of the river for industrial cooling has created concern that the aquatic biota is endangered by thermal pollution. North and Adams (1969) have described thermal conditions at outfalls and in condenser cooling systems of some California plants. They pointed out that increases of +10°F (5.6°C) above normal are considered significant biologically at all seasons of the year. Coutant (1970) presented a diagram of the hypothetical time-course of acute thermal shock to any organism entrained in condenser cooling water systems that indicates they could be exposed to the maximum increase (10.8°C) for at least 9 min in diffuser systems and to substantial increases from 12 to 20 min in the discharge canal system. He also noted the average temperature rise reported is about 10.8°C but may be as great as 16°C.

I studied the effect of increased water temperatures on one of the abundant cladocerans of the area, *Daphnia pulex*. It has been found to be important in the diet of valuable stocks of juvenile chinook salmon, *Oncorhynchus tshawytscha*, in certain seasons of the year (Craddock et al.[2]). Cladocerans may be thermally affected by a thermal nuclear power plant where, along with other zooplankton, they may be entrained with intake cooling water and pass through the condenser cooling system encountering sudden and sizable temperature increases. Increased cooling water use by industrial and power plants may increase the temperature of certain areas of the river (bays and eddies) for extended periods and also affect zooplankton.

The specific objectives of the study were: 1) to develop techniques for laboratory study of thermal effects on zooplankton and 2) to assess the

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effect of both prolonged and short exposure to increased temperatures on survival and reproduction of *D. pulex*.

METHODS AND MATERIALS

Two stocks of *D. pulex* were cultured at two acclimation temperatures and subjected to three types of tests to determine their thermal tolerance. One stock was obtained from the Columbia River and the other from a small pond north of Seattle, Wash. They were cultured separately and will be referred to as the Columbia group and the Seattle group. Stock cultures were maintained in 5-liter battery jars of Lake Washington water filtered through No. 25 Swiss silk bolting cloth to remove zooplankton and phytoplankton, but not bacteria. Taub and Dollar (1968) felt that bacteria were important to the nutrition of *Daphnia*, especially in relation to reproduction. Stock cultures were reared and acclimated at either 15° or 20°C in a controlled temperature incubator. Continuous fluorescent lighting (45-50 foot candles, cool white) provided similar lighting in the incubator and in the laboratory and was consistent for all animals, test and control. Algae, *Chlorella* and *Chlamydomonas*, were cultured using medium No. 63 developed by Taub and Dollar (1968) and fed to *D. pulex*. Water in the test vessels was changed weekly, and the animals were fed three times a week.

The test temperatures were maintained by using primary and secondary water baths and immersion heaters activated by temperature controllers (Figure 1). The primary bath was a Plexiglas[3] tank 150 × 30 × 23 cm supplied with flowing water at 10° to 15°C. The secondary baths consisted of six or seven 5-liter battery jars, 23 × 14 × 17 cm, placed in the primary bath. The temperature in each of these secondary baths was raised progressively from the water inlet end to the outlet end of the primary tank. Temperatures in the secondary baths could be maintained from 10° to 36°C ± 0.5°. Air continually bubbling into each secondary bath eliminated stratification. Experimental subjects were held in 50-ml jars of filtered lake water suspended in the secondary baths and equilibrated to the test temperature in those baths.

Parthenogenetically produced animals of the same age, either young females (less than 24 h old) or mature females (approximately 1 wk old), were selected from the stock cultures and held in 10-ml vials for a day before the start of the experiment to check for handling mortality. At the start of an experiment, the bulk of the water in the vials was canted off, and the appropriate number of test animals was poured directly into the 50-ml test chamber at the test temperature. The control groups were treated identically with the others, except that they were held at acclimation temperatures. A large bore pipette was used when individual animals were handled.

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

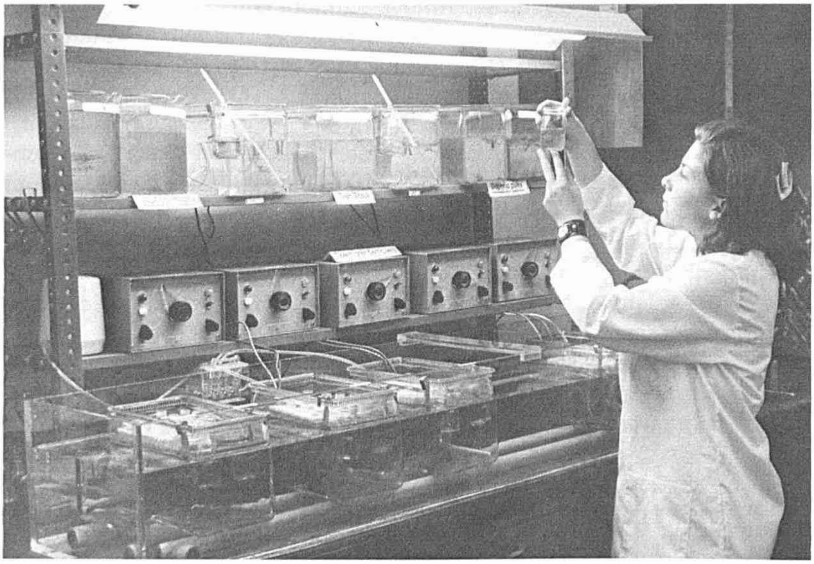

FIGURE 1.—Experimental equipment used to study temperature effects on zooplankton showing primary and secondary water baths, test vessels, and temperature controllers.

Three experiments were conducted to simulate thermal conditions that *D. pulex* might encounter. Two experiments studied the effect of increased temperatures that might be encountered in the area of a heated plant outfall, whereas the third simulated the thermal conditions small organisms could encounter in the condenser cooling system of a thermal power plant.

The first experiment compared the effect of prolonged exposure (50-52 days) to constant temperatures of 15° (control), 18°, 21°, 24°, 27°, 30°, and 33°C. Test organisms were both mature females and young females (at the start of the tests) of the Seattle group acclimated at 15°C. There were 18 mature females per test temperature, 6 per test jar, and 10 young females were tested per test temperature and test jar. Ten *Daphnia* per 50-ml jar were well below the number that would cause harmful metabolic waste buildup or oxygen depletion (Pratt 1943); 10 animals has long been accepted as a standard for bioassays, Doudoroff (1951), American Public Health Association (1971), and Sprague (1973).

The second experiment compared the effect of prolonged exposure (34 days) to temperatures of 20° (control), 23°, 26°, 29°, and 32°C on mature females of the two groups (Seattle and Columbia) acclimated at 20°C. There were 10 animals per test temperature and test jar.

The third experiment subjected mature females of the Seattle group acclimated to 15°C to a short exposure (15 min) to temperatures of 15° (control), 19°, 21°, 24°, 27°, 30°, 33°, and 36°C. Test organisms were then returned to acclimation temperature where they were held and observed for 90 days. Twelve animals were tested at each temperature.

Test animals were examined frequently to determine the effect of increased temperatures, usually hourly during the first 8 h of a test. The next day or two, they were examined two or three times a day and subsequently once each week day. During each observation, the mortalities were noted and removed, and newly born *Daphnia* were counted and removed. The animals were assumed to be dead when they lay on the bottom and there was no detectable movement of the antennae, thoracic legs, or the post abdomen.

Temperature effects were evaluated on the basis of survival and reproduction by animals tested at the various temperatures. In this study, my evaluation criterion was the time at a particular temperature until 50% mortality; therefore, I use the term TD_{50} (time to death of 50% of the test animals at a particular temperature).

RESULTS

Experiments Relating to Discharges of Heated Water

Seattle *Daphnia* Acclimated to Water of 15°C

Death occurred rapidly for both mature and young *D. pulex* at 33°C. Some animals in both groups lost equilibrium within the first hour, TD_{50} occurred before the third hour, and none survived the fourth hour of exposure (Table 1). Mature and young *D. pulex* subjected to temperatures above 27°C reached TD_{50} in less than 24 h. Temperatures of 27°C and below required an exposure of at least 192 h (8 days) to cause 50% mortality. The younger females did not succumb to moderately high temperatures (18°, 21°, and 24°C) as quickly as the older females. Temperatures of 21°, 24°, and 27°C caused TD_{50} among the older females after an average of 238 h, whereas the younger females did not reach TD_{50} until an average of 768 h.

TABLE 1.—Mortality of *Daphnia pulex* introduced as mature and young females and maintained at temperatures of 15° to 33°C (Seattle race, acclimated at 15°C).

	Mature females		Young females	
Test temp (°C)	Hours to 50% mortality[1]	% mortality at end of test (50 days)	Hours to 50% mortality[1]	% mortality at end of test (52 days)
15	1,008 (42)	67	1,224 (51)	50
18	888 (37)	78	[2]1,248 (51)	40
21	259 (9)	89	1,152 (48)	60
24	192 (8)	100	648 (27)	100
27	264 (11)	100	504 (21)	100
30	19	100	21	100
33	3	100	3	100

[1]Days in parentheses.
[2]50% mortality not reached.

All animals died before producing young at 30° and 33°C; rate of reproduction was highest at 24° and 27°C before all subjects died. Total offspring produced and rate of reproduction varied for the two age-groups of females tested at 21°C or below (Table 2).

First reproduction by the mature females occurred 5 days earlier at test temperatures of 27°, 24°, and 21°C than at the control temperature (15°C). Only one peak of production occurred at 27°C before 100% mortality was reached. Reproduction at 15°C was stable with peaks occurring

TABLE 2.—Reproduction of *Daphnia pulex* introduced as mature and young females and maintained at temperatures of 15° to 33°C (acclimated at 15°C, Seattle race).

Test temp (°C)	Mature females: Total young produced[1]	Mature females: Average no. young/adult per day[2]	Young females: Total young produced[3]	Young females: Average no. young/adult per day[2]
15	1,162	2.20	33	0.09
18	652	1.05	40	0.08
21	244	1.11	286	0.74
24	466	2.61	366	2.67
27	318	3.02	629	3.25
30	0	0.00	0	0.00
33	0	0.00	0	0.00

[1]Total reproduction from 18 animals during 50 days of experiments.
[2]Average reproduction based on number of days survivors remained.
[3]Total reproduction from 10 animals for 52 days of experiments.

regularly at 6-day intervals, whereas at higher temperatures reproduction was erratic. The greatest numbers of offspring were produced by the older females at 15°C and generally decreased with increasing temperatures. The highest rates were at 27° and 24°C where the survivors reproduced rapidly before they all succumbed on the 13th and 27th day, respectively. High temperatures increased the rate of reproduction for a short period before total mortality, but the increased rate was short lived and did not match total production by animals at a more normal temperature (15°C).

Reproduction by females who were young at the start of the experiment increased with increasing temperature, contrary to the trend shown by the older females (Table 2). No reproduction occurred at 15°C until the 34th day; at 18° and 21°C, initial reproduction took place on the 3rd to 6th day but did not resume until the 44th and 22nd day, respectively. At 24° and 27°C, the first reproduction occurred on the 3rd to 6th day, stopped for 3 or 4 days, and then continued at a high rate until the death of all females on the 34th and 27th day, respectively. The low reproduction by the younger females at 15° and 18°C is not explained.

Seattle and Columbia *Daphnia* Acclimated to Water of 20°C

Both Seattle and Columbia *Daphnia* reached TD_{50} within 24 h at 32°C (Table 3); 90% mortality occurred in less than 24 h in the Columbia group and within 48 h in the Seattle group. At 29°C, both groups reached 50% mortality in 120 h. There were significant differences in the length of time to 50% mortality for each of the two groups at 20°, 23°, 26°, and 29°C (Seattle—χ^2 = 37.9,

TABLE 3.—Mortality of *Daphnia pulex* (Seattle and Columbia races) acclimated at 20°C and introduced as maturing females to temperatures of 20° to 32°C.

Test temp (°C)	Seattle race: Hours to 50% mortality[1]	Seattle race: % mortality at end of test (34 days)	Columbia race: Hours to 50% mortality[1]	Columbia race: % mortality at end of test (34 days)
20	[2]>816 (34)	40	216 (9)	70
23	648 (27)	80	456 (19)	100
26	120 (5)	100	48 (2)	100
29	120 (5)	100	120 (5)	100
32	<24 (1)	100	<24 (1)	100

[1]Days in parentheses.
[2]50% mortality not reached.

$P<0.01$, 3 df; Columbia—$\chi^2 = 18.8, P<0.01$, 3 df). The Columbia group seemed to succumb more rapidly than the Seattle group, but the more rapid demise of the Columbia *Daphnia* at 20°C (the acclimation temperature) casts doubt upon these results. However, a test of homogeneity for temperatures of 23°, 26°, and 29°C indicated significant differences between the two groups in days to 50% mortality ($\chi^2 = 22.6, P<0.01$, 2 df).

Comparatively little reproduction took place at temperatures of 26°C and above (Table 4). The greatest reproduction for the Seattle group was at 23°C and for the Columbia group at 20°C. The Columbia animals remaining after the initial unexplained mortality at 20°C outproduced the Seattle animals at the same temperature. The Seattle animals produced 62% of the total young produced by the two groups.

TABLE 4.—Reproduction of *Daphnia pulex* (Seattle and Columbia races) acclimated at 20°C and introduced as maturing females to temperatures of 20° to 32°C.

Test temp (°C)	Seattle race: Total young produced[1]	Seattle race: Average no. young/adult per day[2]	Columbia race: Total young produced[1]	Columbia race: Average no. young/adult per day[2]
20	246	1.12	299	1.82
23	424	1.68	152	1.02
26	0	0.00	3	0.67
29	90	2.14	16	0.32
32	0	0.00	0	0.00
Total	760		470	

[1]Total reproduction by 10 animals for 34 days of experiments.
[2]Average reproduction based on the number of days survivors remained.

Experiments Relating to Water Passing Through Cooling Systems

Exposure for 15 min at temperatures of 30°C or less seemed to have little or no effect upon the survival of *D. pulex* (Table 5). The only mortalities observed during the exposure period were at 36°C: within 5 min, over 50% of the animals at this temperature were dead; all but one died in 15

TABLE 5.—Mortality and reproduction of *Daphnia pulex* (Seattle race) exposed as maturing females for 15 min to various temperatures and returned to acclimation temperature of 15°C.

	Survival		Reproduction	
Shock temp (°C)	Hours to 50% mortality[1]	% mortality after 90 days	Total young produced[2]	Average no. young/adult per day of test[3]
15	1,178 (49)	92	2,051	3.64
19	1,320 (55)	83	1,340	2.30
21	1,008 (42)	92	1,365	3.57
24	1,512 (63)	75	2,640	3.50
27	1,464 (61)	67	1,716	2.26
30	1,536 (64)	92	1,832	2.48
33	792 (33)	100	363	0.78
36	0.083	92	[4]480	5.33

[1]Days in parentheses.
[2]Total reproduction for 90 days of test.
[3]Average daily reproduction per surviving adult.
[4]Produced by the one survivor of the 15-min exposure during the succeeding 90 days.

min. One hour after exposure, one animal had died at 33°C, but TD_{50} took 792 h (33 days) at 33°C and 1,008-1,536 h (42-64 days) after exposure to temperatures below 33°C. Time in days to reach TD_{50} was not statistically significant (χ^2 = 6.89, 5 df) for temperature treatments of 15° to 30°C. A temperature in excess of 30°C for the 15-min exposure was necessary to significantly increase mortality.

The rate of reproduction was not significantly changed by an exposure of 15 min to increased temperatures through 30°C (χ^2 = 0.79, 5 df). The greatest total reproduction was by those *D. pulex* tested at 24°C (Table 5) where survival was also good. Reproduction at 27° and 30°C exceeded the reproduction at 19°C, so it appears that reproduction is not materially affected by a short exposure to temperatures through 30°C that do not seriously affect survival. Reproduction by animals tested at 33° and 36°C was drastically reduced because most of the test animals died.

DISCUSSION

In zooplankton sampling of the Prescott-Kalama section of the Columbia River in 1968-69, *D. pulex* was more abundant during periods of higher water temperature (Craddock et al. see footnote 2). Numbers of *D. pulex* were low during the portion of the year when the temperature remained below 15°C (late fall, winter, and spring), but as water reached and exceeded this temperature the population increased rapidly until the peak abundance was reached at the maximum water temperature (approximately 21°C). The mean daily water temperature in August (the month of highest temperature) ranged from 19.3° to 22.8°C in 1968 and from 19.7° to 21.1°C in 1969 (Snyder and McConnell 1971). Tauson (1931) found temperatures of 16°-22°C favorable for parthenogenetic reproduction by *D. pulex,* but above or below this range production was reduced considerably. The upper limit was 30°C. Ivleva (1969) reviewed literature on the thermal range of *Daphnia* and noted that several researchers reported the optimum temperature range for development of *D. pulex* as 18°-20°C. Ivleva made the general observation that the optimal range varies with age and the young are more resistant to high temperature than the old, as was indicated by my experiments. Other researchers reviewed by Ivleva found that mass mortalities could occur in the range of 28°-32°C. Some of these researchers indicated that when *Daphnia* species are acclimated to higher or lower temperatures over a long period they become more resistant to further increases or reductions in temperature.

My experiments to determine the effect of increased temperature on *D. pulex* that were 1 wk old and 1 day old (i.e., at the start of the experiment) indicated that the younger animals adapted better to increased temperatures. Temperatures of 21°C and above seriously reduced the length of survival of the older females (21°C = TD_{50} in 259 h), whereas temperatures of 24° to 27°C or more were required to have the same effect on the younger females (24°C = TD_{50} in 648 h; 27°C = TD_{50} in 504 h). Temperatures above 27°C caused TD_{50} in a short time (less than 21 h) for both age-groups.

Although the younger females survived better at the control and lower test temperatures (15°, 18°, and 21°C), their eventual production of young was considerably less than that of the mature animals. This difference was not due solely to the 1-wk difference in age, and I do not have an adequate explanation.

My experiment comparing survival and reproduction of the Seattle and Columbia races indicated that the Columbia *Daphnia* may be less resistant to increased temperatures. The results of the tests of Seattle *D. pulex* acclimated at 15° and 20°C are not directly comparable and, although there is some indication that the higher temperature acclimation increases resistance in the midrange (23°-24°C), the effect was not apparent in the high range (26°-27°C) and no conclusion could be made.

My experiments indicate that an increase of 6°C in the area of an outfall could cause TD_{50} in about 168 h (7 days) among important segments of the reproducing population. To minimize damage to *Daphnia* populations in the Columbia River, the temperature should not be raised more than 6°C above ambient or higher than 26° or 27°C for any prolonged period.

A short exposure (15 min) to increased temperatures as might occur in a condenser cooling system did not cause a significant reduction in time to TD_{50} or in reproduction unless the temperature exceeded 30°C. There is a period from mid-July through September when the lower Columbia River temperatures may exceed 20°C. In these instances, the temperature increase in condenser cooling systems should be less than 10°C if the *Daphnia* are to survive. It must be kept in mind that temperature is only one of several factors including pressure, abrasion, and toxic chemicals that could be acting synergistically to damage zooplankton in a condenser cooling system (Marcy 1973; Becker and Thatcher[4]).

To protect *D. pulex* populations, water temperatures in condenser cooling systems should not exceed 30°C and passage through the system should take less than 15 min.

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