SHORT-TERM THERMAL RESISTANCE OF ZOEAE OF 10 SPECIES OF CRABS FROM PUGET SOUND, WASHINGTON

BENJAMIN G. PATTEN[1]

ABSTRACT

Zoeae of 10 crab species were subjected to tests that simulated thermal stress associated with steam-powered electric stations. Shortly after hatching, the unfed zoeae were subjected to conditions simulating passage through heat exchangers (held at elevated test temperatures for 20 min with an abrupt increase and decrease from ambient) or mixing with thermal plumes (held at test temperature 1 to 4 h with temperatures gradually rising and decreasing from ambient). All species used in tests were hatched from February to November and were naturally acclimated to ambient conditions of the littoral zone. Observations were made on the point in temperature that zoeae became torpid in heat exchanger tests and on the TL_{50} (maximum temperature–time that 50% or more of the subjects survived 48 h after testing).

In the heat exchanger tests, the most sensitive species, the Bering hermit crab, *Pagurus beringanus*, and the porcelain crab, *Petrolisthes eriomerus*, did not become torpid at 24°C; their torpid point and their TL_{50} were at 26°C. The economically important Dungeness crab, *Cancer magister*, did not become torpid at 28°C; its TL_{50} was at 30°C. The TL_{50} of other species ranged from 30° to 34°C.

The TL_{50} of zoeae given the thermal plume test ranged from 26° to 34°C for a 1-h exposure and 24° to 32°C for a 2- to 4-h exposure.

Thermal conditions in heat exchangers are postulated to be more critical to the survival of zoea than mixing with thermal plumes. The maximum temperature that should be permitted in heat exchangers to protect the most sensitive species studied is 24°C for the Puget Sound area.

Thermal resistance of marine organisms should be understood before seawater in a specific area is used for industrial cooling. In the State of Washington, for example, nuclear power plants are being planned for construction by municipalities and industries. These plants require large quantities of seawater to cool condensers of the steam turbine system; their waste hot water would be discharged back into the environment, along with toxic chemicals (Becker and Thatcher[2]). Organisms entrained into steam electric stations would be subjected to mechanical injury (Marcy 1973) from passage through such a system. Studies are needed to fully evaluate the impact of entrainment and the discharge of altered waste water on the associated life; temperature effects are considered here.

Some information is available on the thermal maximums and optimums of two species of Puget Sound crabs (Todd and Dehnel 1960; Reed 1969; Prentice 1971; Mayer[3,4]). These studies show the effects of long-term temperature increases but do not depict situations related to industrial use of seawater for cooling. Experiments reported here were designed to simulate the stress that zoeae would be exposed to in passing through heat exchangers of steam electric stations and in mixing with thermal plumes of the waste water released into the environment.

This study is one of a series describing the thermal resistance of selected species of planktonic organisms. The time-temperature combinations used are considered a measure of thermal resistance (Fry 1971) because they are probably beyond the environmental tolerance of the species used. This paper describes the elevated temperatures that cause immediate and imminent

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[1] Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, 2725 Montlake Boulevard East, Seattle, WA 98112.

[2] Becker, C. D., and T. O. Thatcher (compilers). 1973. Toxicity of power plant chemicals to aquatic life. Battelle Pac. Northwest Lab., Richland, Wash., WASH-1249, U.S. AEC, misc. pagination.

[3] Mayer, D. L. 1973. Thermal tolerance of *Cancer magister* eggs. *In* Q. J. Stober and E. O. Salo (editors), Ecological studies of the proposed Kicket Island nuclear power site, p. 412–419. Univ. Wash., Coll. Fish., FRI-UW-7304.

[4] Mayer, D. L. 1973. Response of Dungeness crab in a thermal gradient. *In* Q. J. Stober and E. O. Salo (editors), Ecological studies of the proposed Kicket Island nuclear power site, p. 420–429. Univ. Wash., Coll. Fish., FRI-UW-7304.

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death and stress to the zoeae of four species of anomuran and six species of brachyuran crabs acclimatized to natural ambient conditions. These crabs constitute some of the more important types in the littoral zone and include species important in sport and commercial fisheries. Testing was done at the National Marine Fisheries Service facility at Mukilteo, Wash., from May to October 1971 and in February 1972.

MATERIALS AND METHODS

Ovigerous crabs were collected from the mid-Puget Sound areas of Possession Sound, Poverty Bay, and at Alki Point. Graceful crab, *Cancer gracilis*, Dungeness crab, *C. magister*, and kelp crab, *Pugettia producta*, were collected subtidally; other species were taken on beaches during low tides. The messmate crab, *Pinnixa littoralis*, was collected inside horse clam, *Tresus capax*, that had been excavated. Most of the experimental species were ovigerous in May and June; the mud flat crab, *Hemigrapsus oregonensis*, black clawed crab, *Lophopanopeus bellus*, and porcelain crab, *Petrolisthes eriomerus*, had ovigerous individuals to August. *Pugettia producta* were ovigerous July to November.

Ovigerous crabs and pre- and posttest zoeae were held in aquaria receiving running seawater of temperatures ranging from 8.2° to 23.5°C (Table 1); salinity ranged from 24.1 to 28.3‰; and dissolved oxygen ranged from 5.6 to 9.0 ppm. Laboratory water was sometimes 3°C higher than ambient temperatures at the surface in the afternoon on sunny days in July and August because of heating of the water supply pipe. Other than this, the ambient water temperatures of the Mukilteo area were similar to that expected of central Puget Sound locations (Wennekens 1959).

TABLE 1.—Temperature of Mukilteo, Wash., laboratory seawater summarized by 10-day periods in 1972.

	Water temperature (°C)		
	Average		
Month	Low	High	Range
May	9.3	10.0	8.2–10.7
	9.9	10.4	9.1–11.1
	9.5	11.4	8.8–12.7
June	10.4	12.2	10.0–13.3
	10.4	12.7	9.7–14.3
	10.8	13.5	10.4–14.3
July	11.0	13.1	10.4–14.0
	—	—	—
	12.3	16.4	11.3–18.2
August	12.9	16.4	11.3–18.2
	16.6	20.7	13.5–23.5
	15.7	18.6	13.0–23.0
September	13.2	14.9	12.6–15.6
	12.9	15.5	12.5–16.8
	13.7	15.9	12.5–16.8

Test facilities consisted of floating holding boxes for test groups of zoeae and 5-liter battery jars for maintaining water baths of a controlled temperature. Holding boxes were 2.5 cm^3, with two screened sides having 0.110-mm apertures, attached to Styrofoam[5] for floatation. Battery jars received 3 liters of seawater immediately before testing. Temperatures were maintained within ±0.5°C of the test temperatures during experiments. Continuous aeration insured mixing and oxygenation.

Zoeae generally hatched within a week after their parents were collected, but some parents were held a month before hatching occurred. When the zoeae hatched (hatching of all ova of a parent occurred within about 12 h), 10 were counted into each of the holding boxes within 24 h of hatching and remained there, unfed, to the termination of an experiment. Zoeae used as controls were held at the temperature of laboratory water, and others were given two types of thermal tests.

To simulate passage through heat exchangers, holding boxes containing 10 zoeae were removed from water of ambient temperature and placed directly into battery jars having water of an elevated temperature ranging from 24° to 38°C by 2°C increments (Table 2). The zoeae remained at the elevated temperature for 20 min and were then placed into water of ambient temperature. Actual temperature change within the holding boxes was delayed. On the average, the increase from ambient to midway to the test level occurred in 5 s. Temperatures were within 1°C of the test level in 2 min. Decreases from test temperatures to ambient occurred in about 1½ min. Activity of zoeae was noted before, during, and after testing.

To simulate conditions encountered in thermal plumes, zoeae in holding boxes were placed in water of ambient temperature in the battery jar. The temperature of the water was then elevated to a test temperature ranging from 24° to 36°C by 2°C increments (Table 2) over a 30-min period. Specific groups of zoeae were held at specific test temperatures for durations of 1, 2, or 4 h. After this, the temperature was gradually decreased to ambient level over a 20-min period, and the

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

TABLE 2.—Percentage survival of first stage zoeae 48 h after testing of 10 species of crabs subjected to a range of temperatures at four durations (percentages are from combined data of two or more tests). Increases to and decreases from a test temperature were rapid for the 20-min test (heat exchanger test) and gradual for the longer durations of exposure (thermal plume test).

Species	Minutes held at test temperature	Date at end of test	No. of parents	Control		No. zoeae tested	Survival at different water temperatures (°C)							
				No. zoeae	Percent survival		24°	26°	28°	30°	32°	34°	36°	38°
							Percentage							
Anomuran:														
Bering hermit crab,	20	6/20	2	60	53	30	80	[1]*55*	47	0	0	—	—	—
Pagurus beringanus	60	7/28					55	*73*	37	10	0	—	—	—
	120						*50*	30	0	17	0	—	—	—
	240						*65*	37	3	0	0	—	—	—
Granular hermit crab,	20	6/25	3	90	92	30	—	87	83	*70*	0	0	—	—
Pagurus granosimanus	60	6/27					—	93	80	*77*	0	0	—	—
	120	7/2					—	90	90	*53*	0	0	—	—
	240						—	90	*83*	23	0	0	—	—
Hairy hermit crab,	20	6/5	3	80	93	10-30	—	80	80	*53*	10	7	0	—
Pagurus hirsutiusculus	60	6/13					—	100	77	*85*	37	0	0	—
	120	6/23					—	*100*	80	*80*	30	0	—	—
	240						—	*80*	83	65	7	0	0	—
Porcelain crab,	20	6/23	3	40	83	10-30	—	45	30	0	0	—	—	—
Petrolisthes eriomerus	60	6/25					—	70	*50*	0	0	—	—	—
	120	7/2					—	*100*	0	0	0	—	—	—
	240						—	*80*	0	0	0	—	—	—
Brachyuran:														
Black clawed crab,	20	6/16	3	100	89	40	100	98	90	95	*63*	13	0	—
Lophopanopeus bellus	60	6/23					100	98	98	*85*	5	3	—	—
	120	9/16					90	100	98	*75*	8	0	—	—
	240						80	98	83	*55*	0	3	—	—
Dungeness crab,	20	6/7	4	60	93	50	100	90	80	*78*	14	0	—	—
Cancer magister	60	6/9					100	94	*74*	18	6	0	—	—
	120	6/28					100	96	*90*	0	0	0	—	—
	240	2/29/72					100	94	*62*	2	0	0	—	—
Graceful crab,	20	7/16	2	60	95	40	88	90	90	*88*	23	0	—	—
Cancer gracilis	60	7/18					93	90	*90*	25	0	0	—	—
	120						90	83	*93*	3	0	0	—	—
	240						93	85	*80*	0	0	0	—	—
Kelp crab,	20	9/2	2	80	100	40	—	100	100	100	*90*	10	0	—
Pugettia producta	60	10/15					—	98	100	98	*88*	0	0	—
	120						—	98	90	*93*	13	0	0	—
	240						—	95	*95*	30	0	0	0	—
Messmate crab,	20	7/30	2	80	95	40	90	83	85	*83*	25	0	—	—
Pinnixa littoralis	60	8/4					95	98	88	*60*	3	0	—	—
	120						83	85	*95*	30	0	0	—	—
	240						93	93	*63*	13	0	0	—	—
Mud flat crab,	20	6/13	5	130	98	20-50	—	100	100	100	92	*52*	0	0
Hemigrapsus oregonensis	60	6/18					—	97	98	96	94	*54*	10	—
	120	7/2					—	100	100	100	*100*	46	0	—
	240	7/8 8/28					—	100	90	100	*98*	48	0	—

[1] Italic denotes the TL_{50}

holding boxes containing zoeae were replaced in aquaria with running seawater.

The numbers of replicate tests made at a temperature for a test varied because of numbers of ovigerous crabs available and numbers of zoeae resulting from a hatching. The offspring from at least two parent crabs of a species were used (Table 2). Some species were tested at intervals over a 2- to 3-mo period to indicate seasonal acclimation effects. One test for *C. magister* was made in 1972; all other species were tested in 1971. Percentage survival of a species of crab for a given duration and temperature is the combined survival of two to five tests made for a species (Table 2).

Observations were made on the levels of activity, point of torpor, and the TL_{50} (maximum temperature–time combination survived by 50% or more of subjects 48 h after testing) to evaluate the effects of experimental conditions. A 48-h posttest observation duration was deemed appropriate for these tests as the zoeae were not fed and could have been affected by starvation although they readily survived to 72 h.

TEMPERATURE EFFECTS

Temperature–time combinations for a type of test that was critical to the survival of the zoeae of a species were indicated by survival of the controls and by experimental conditions affecting activity and survival of the test subjects.

Zoeae used as controls had survival rates ranging from 53 to 100% (Table 2). Guidelines set in the American Public Health Association (1971) state that losses of greater than 10% of control subjects invalidate an experiment. Control zoeae of the Bering hermit crab, *Pagurus beringanus*, with a survival of 53%, *L. bellus* with a survival of 89%, and *Petrolisthes eriomerus*, with a survival of 83% fall below this standard. Although the TL_{50}'s are invalid for these species, the point of torpor is valid as it demonstrates an immediate condition the zoeae lapse into with a given temperature stress.

Activity and survival of a species of zoeae decreased with increasing temperature and duration at an elevated test temperature (Table 2). In heat exchanger tests, zoeae experienced a rapid temperature change and were initially hyperactive, probably as a result of thermal shock (Kinne 1964). With time, zoeae at a temperature 4°C below the TL_{50} appeared normal. Those at 2°C below TL_{50} had reduced activity and had difficulty maintaining themselves off the bottom. Subjects placed in water at the TL_{50} temperature and above were initially hyperactive, but in 2 to 7 min became torpid and sank to the bottom. Heat exchanger test temperatures producing torpor were 26°C for *Pagurus beringanus* and *Petrolisthes eriomerus* and 30°C for most other test species; the maximum was 32°C for *L. bellus, Pugettia producta,* and *H. oregonensis.* After the zoeae were returned to ambient conditions, those tested at the TL_{50} temperature had not become active after 20 min.

Zoeae subjected to the heat exchanger tests generally had high survival to the point of the TL_{50} (Table 2). Thereafter, mortalities were complete at 2° to 4°C higher except in the case of the hairy hermit crab, *Pagurus hirsutiusculus*, where all died at 6°C above the TL_{50}. The minimal TL_{50} was at 28° and 30°C for most other crabs; it was at 32°C for *Pugettia producta* and *L. bellus* (Table 2). The most tolerant species was *H. oregonensis* with a TL_{50} at 34°C.

Zoeae subjected to the thermal plume tests had lower TL_{50}'s than those given the heat exchanger tests (Table 2). The TL_{50} of zoeae given the 60-min test was similar to or 2°C lower than those given the 20-min heat exchanger test; TL_{50}'s were at progressively lower temperatures for the 120- and 240-min tests. Mortalities were complete at 2° to 4°C above the TL_{50}. The least tolerant species were the *Cancer* crabs (Table 2) with TL_{50}'s at 28°C for the 60- and 240-min tests. TL_{50}'s were generally at 30°C for the other crabs for the three time durations they were tested. The species with the highest tolerance was *H. oregonensis* with a TL_{50} at 34°C for the 60-min test and at 32°C for the 120- and 240-min tests.

DISCUSSION

The situation postulated to be most critical to the survival of the planktonic zoeae is their passage through heat exchangers; zoeae will be entrained into heat exchanger systems but those encountering thermal plumes will probably only be exposed to lowering temperatures (Coutant 1970) at the periphery where turbulence occurs.

The maximum temperature limit that should occur in heat exchangers is best described as the one causing no adverse effects to the least resistant species—to be consistent with the protection of all species tested. Conditions that could be overtly recognized as affecting the survival of the zoeae were the degree of stress causing torpor and the TL_{50}. While the TL_{50} directly relates to death, torpor indicates a condition that could indirectly cause death. Torpid zoeae would have their feeding interrupted and they would not be able to evade predators until they recovered. Selective predation on zoeae subjected to a stress below that causing torpor could also be a factor of survival at sublethal temperature–time combinations. In fish, for example, Coutant (1973) experimentally observed that rainbow trout, *Salmo gairdneri*, predators selectively preyed on juvenile rainbow trout and chinook salmon, *Oncorhynchus tshawytscha*, that had been exposed to shock temperature treatments of durations below that required for the prey to lose equilibrium.

The maximum temperature that had no observable effect on the species studied was 24°C, as this was the greatest stress that did not cause *Pagurus beringanus* and *Petrolisthes eriomerus* to become torpid. The maximum for other species should be no greater than 28°C for *Cancer* and up to 30° to 32°C for the most resistant species.

A properly sited steam electric station should not discharge hot waste water in quantities or at locations where thermal plumes would retain their integrity over periods of 1 to 4 h. This could be a problem if Puget Sound waters were intensively used for cooling. TL_{50}'s for the zoea subjected to the 1- to 4-h thermal plume test ranged from 28° to 32°C, except that *H. oregonensis* had a TL_{50} of 34°C for the 1-h test.

The maximum temperature increase in a steam electric station that will not cause mortality to the species studied can be estimated from the seawater temperature in Puget Sound and the maximum temperatures tolerated by zoeae. Surface temperatures of Puget Sound range from about 10°C in the spring when most zoeae hatch to 15°C or more in some locations in the summer (Wennekens 1959). Temperatures in heat exchangers can be increased 14°C in the spring and 9°C in the summer without causing direct or indirect mortalities to the least resistant species. Synergistic effects from the release of toxic chemicals and from mechanical damage may act to lower the thermal maximums tolerated.

Knowledge of the temperature tolerance of the zoeae studied provides a partial input into the assessment of the impact of a steam electric station using Puget Sound waters for cooling. Zoeae are generally a minor component of zooplankton within the depths of Puget Sound that would be subject to entrainment (Hebard 1956; Patten unpubl. data). Also, the volume of water entrained by a steam electric station would be small in comparison to that of Puget Sound. Therefore, if all entrained zoeae were destroyed in a steam electric station, the proportion lost may be of minor concern on the population level. Losses of zoeae from high temperature conditions may be more serious if a series of steam electric stations used Puget Sound waters for cooling. In this case, some conservation measures should be considered.

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