MERCENARIA MERCENARIA (MOLLUSCA: BIVALVIA): TEMPERATURE-TIME RELATIONSHIPS FOR SURVIVAL OF EMBRYOS AND LARVAE[1]

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ABSTRACT

To estimate the effects of entrainment of *Mercenaria mercenaria* embryos and larvae in the cooling-water systems of steam-electric power plants, we used a thermal gradient apparatus. Cleavage stages, trochophore larvae and straight-hinge veliger larvae were subjected to 11 different temperatures for 8 different time periods. There was a direct relationship of mortality with temperature increase and, at higher temperatures, with increase in time exposure. As the clams aged, temperature tolerance increased, with cleavage stages most sensitive to higher temperature and straight-hinge larvae least sensitive. Multiple regression analyses of percentage mortality on temperature and time produced estimating equations that allow prediction of percentage mortality under different conditions of temperature and time exposure. Entrainment of *M. mercenaria* embryos and larvae in cooling systems of power plants should be as short as possible if mortality is to be held to a minimum.

Passage of plankton through the cooling system of steam-electric power plants is a matter of concern (Coutant, 1970). Mortality caused by such entrainment (e.g. Marcy, 1971) might lead to loss of species from the vicinity of a power plant, with various ecological and economic consequences. It is estimated that increased demand for cooling water may necessitate the location of power plants in estuarine and marine environments (Tarzwell, 1972). Thermal tolerances of planktonic organisms in these environments must be determined to allow estimation of lengths of entrainment and increases in temperature that are least harmful to entrained organisms.

The hard clam, *Mercenaria mercenaria* (L.), is an abundant and commercially important bivalve found in shallow inshore waters of the east coast of North America. It is easily spawned in the laboratory and has been the subject of numerous investigations on the influence of various factors on its larval biology (see Loosanoff and Davis, 1963; Calabrese and Davis, 1970 for appropriate references). We used an aluminum thermal gradient apparatus (Thomas, Scotten, and Bradshaw, 1963) to determine thermal tolerances of hard clam embryos and larvae at different combinations of temperature and time exposure. This simulated exposure of these organisms to heat for varying time periods in power plant cooling systems. The research was undertaken in the summer of 1972 and spring of 1973 at the Eastern Shore Laboratory, Virginia Institute of Marine Science, Wachapreague, Va. Similar experiments have been made on embryos and larvae of the coot clam, *Mulinia lateralis* (Say) (Kennedy et al., 1974). Reference should be made to that paper for fuller details of experimental apparatus and techniques.

MATERIALS AND METHODS

Mercenaria mercenaria were stimulated to spawn by fluctuating water temperatures (Loosanoff and Davis, 1963) over the range of 22° to 30°C. Gametes from 3 to 32 females and 2 to 30 males were pooled in each experiment to provide genetic diversity (Calabrese and Davis, 1970). We used three developmental stages: early cleavage stages (2 h old); trochophore larvae (10-11 h); straight-hinge veliger larvae (32-50 h).

Wild stock collected as needed in the summer near Wachapreague provided the gametes. After the experiments ended in 1972, the preservative

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TABLE 1.—Percentage mortality of cleavage stages of *Mercenaria mercenaria* under different temperature-time combinations. Values in parentheses are temperatures corrected for the influence of injection water.

Time (min)	Temperature °C										
	17.5	20.2	22.7	25.1	27.6	30.0	32.5	34.9	37.4	39.7	42.4
1	0	39	14	12	12	35	0	0	3	26	85
	(18.8)	(21.5)	(23.3)				(31.6)	(34.0)	(35.8)	(37.7)	(39.4)
5	26	0	24	11	21	0	0	12	64	99	100
	(18.4)	(21.1)	(22.7)				(31.9)	(34.3)	(36.4)	(38.5)	(40.3)
10	22	0	12	0	16	0	0	78	98	100	100
	(18.3)	(21.0)						(34.1)	(36.4)	(38.5)	(40.8)
30	0	16	1	0	8	40	63	100	100	100	100
60	11	0	12	0	22	46	88	100	100	100	100
120	0	0	10	0	16	0	91	100	100	100	100
180	0	7	0	28	0	54	72	100	100	100	100
360	21	0	0	0	0	0	98	100	100	100	100

TABLE 2.—Percentage mortality of trochophore larvae of *Mercenaria mercenaria* under different temperature-time combinations. Values in parentheses are temperatures corrected for the influence of injection water.

Time (min)	Temperature °C										
	17.6	20.1	22.6	25.0	27.5	29.9	32.4	34.8	37.3	39.7	42.3
1	29	19	14	0	0	2	11	0	0	14	20
	(18.9)	(21.4)	(23.2)				(31.5)	(33.9)	(35.7)	(37.7)	(39.3)
5	25	30	19	14	0	0	0	0	0	41	93
	(18.5)	(21.0)	(22.6)				(31.8)	(34.2)	(36.3)	(38.5)	(40.2)
10	25	21	2	3	1	0	0	0	2	86	100
	(18.4)	(21.1)						(34.1)	(36.3)	(38.5)	(40.7)
30	20	13	0	17	8	1	2	10	55	100	100
60	20	0	5	7	6	0	0	0	100	100	100
120	17	0	0	0	0	0	0	63	100	100	100
180	18	1	0	0	0	0	0	100	100	100	100
360	11	8	7	10	0	3	47	100	100	100	100

TABLE 3.—Percentage mortality of straight-hinge larvae of *Mercenaria mercenaria* under different temperature-time combinations. Values in parentheses are temperatures corrected for the influence of injection water.

Time (min)	Temperature °C										
	18.3	21.0	23.5	26.1	28.5	30.9	33.4	35.8	38.2	40.7	43.1
1	3	2	3	2	2	3	4	4	3	5	4
	(19.6)	(21.6)				(30.0)	(32.5)	(34.2)	(36.4)	(38.4)	(40.1)
5	3	3	3	5	5	4	6	4	4	4	5
	(19.2)					(30.3)	(32.8)	(34.8)	(37.0)	(39.2)	(41.0)
10	3	3	3	4	3	4	3	5	5	5	7
	(19.1)							(35.0)	(37.2)	(39.5)	(41.5)
30	3	2	3	3	4	3	5	5	8	5	29
60	5	4	2	4	4	4	5	7	5	47	76
120	3	3	5	2	5	4	5	3	9	13	99
180	5	4	4	4	6	3	3	11	6	12	96
360	4	4	4	5	5	6	4	8	9	97	98

in most of the vials containing the cleavage stages and trochophores was found to have developed a precipitate that hindered microscopic analysis of the results. Consequently, in March 1973 we spawned 2-yr-old individuals of the F_2 generation produced in the hatchery in 1971 from the same local wild stock. These clams had been held in beds near the hatchery. They were conditioned for 6 wk in warm water before being spawned (Loosanoff and Davis, 1963) and provided us with replacement cleavage stages and trochophore larvae. Results of experiments using these replacements did not appear to differ from preliminary results of the 1972 experiments.

Embryos and larvae not used immediately in experiments were held at ambient temperatures in 60-liter plastic containers at a density of about 33/ml (cleavage stages, trochophores) or about 17/ml (straight-hinge) with the seawater changed daily. Development appeared normal with no high mortalities observed. Larvae not used in our experiments were successfully carried through to metamorphosis.

We used clarified, ultraviolet irradiated seawater (28-31‰) in the experiments. The cast aluminum block, bored to hold 88 test tubes (25 mm) in an 8 + 11 matrix (see Figure 1, Kennedy et al., 1974) provided a thermal gradient

that was approximately linear, varying by 2.3° to 2.7°C from one column of test tubes to the next. Each of the 11 columns in the block represented a different temperature level (Tables 1-3). Temperatures rarely varied more than ± 0.3°C either within a test tube or from one test tube to another in a column. The eight rows represented different time exposures (1, 5, 10, 30, 60, 120, 180, 360 min). There were a total of 88 different temperature-time combinations or treatments.

Twenty-six milliliters of water placed in each test tube were brought to stable temperature levels. Four milliliters of water containing the appropriate developmental stages were injected into each test tube to give a concentration of about 9 to 12 animals/ml. We inoculated the 11 test tubes in any row simultaneously, using an apparatus holding 11 syringes whose plungers were depressed together (see Figure 1, Kennedy et al., 1974).

Eighty-eight plastic beakers, each holding about 340 ml of seawater, were placed in an 8 × 11 matrix in a water bath at 25° to 26°C. When a time period in the block ended, we removed the appropriate row of 11 test tubes at once and washed the contents of each test tube into its corresponding beaker. Survivors were incubated in the beakers for 19 h (trochophores) or 23 h (cleavage stages, straight-hinge) after the experiments ended. Preliminary experiments indicated that this allowed surviving cleavage stages and trochophores to develop to the straight-hinge stage and bacteria to decompose dead individuals. It also allowed bacteria to decompose the meat of dead straight-hinge larvae.

At the end of the incubation period, the animals in each beaker were preserved in 1% buffered Formalin.[4] Numbers of straight-hinge larvae that were alive or dead at the end of an experiment were counted for each treatment. Indications of death included an empty shell or decomposing meats within a shell. For each experiment, we used 10 control test tubes held at room temperature, with the experimental animals treated to all handling described except exposure in the block. Three experiments were made on each of the three developmental stages.

Temperature of the injected water was about 23° to 25°C. This altered the temperature in the test tubes at the cold and warm ends of the block for a short period of time after injection. We made approximate corrections for these changes (Kennedy et al., 1974), and the corrected temperatures are noted in Tables 1 to 3. No corrections were made for periods longer than 10 min.

In a separate experiment, we measured oxygen levels in the test tubes over a 6-h period using straight-hinge larvae of *M. mercenaria*. Numbers of larvae were similar to numbers used in temperature-time experiments. Over the temperature range of 18° to 43°C, dissolved oxygen levels remained near saturation, with almost no change during the 6 h. We concluded that there would be no stress from low oxygen levels (Morrison, 1971) so we did not aerate during the experiments.

Multiple regression analyses of percentage mortality on temperature and time were calculated by a UNIVAC 1108 using a BMD02R stepwise regression program, version of 2 May 1966, from the Health Sciences Computing Facility, University of California at Los Angeles.

Davis and Calabrese (1964) indicated that accuracy in experiments involving sampling and handling bivalve larvae is about ± 10%. Thus, differences of less than 20% in percentage mortality from one treatment to another may not be meaningful.

RESULTS

There was a direct relationship of mortality with temperature increase and, at higher temperatures, with increase in time exposure (Tables 1-3; Figures 1-3). As the animals aged, temperature tolerance increased, with cleavage stages most sensitive to higher temperature and straight-hinge larvae least sensitive (Tables 1-3; Figures 1-3).

The general mortality patterns for the triplicated experiments at each developmental stage were scrutinized and judged to be similar so the data were combined. Over the (approximately) 20° to 26°C interval (columns 2 to 4 in the block), survival was high at each temperature level. The average number of straight-hinge larvae found alive in each of these columns and in the controls were compared, with no significant differences found ($P > 0.05$). Therefore there was no unusual

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

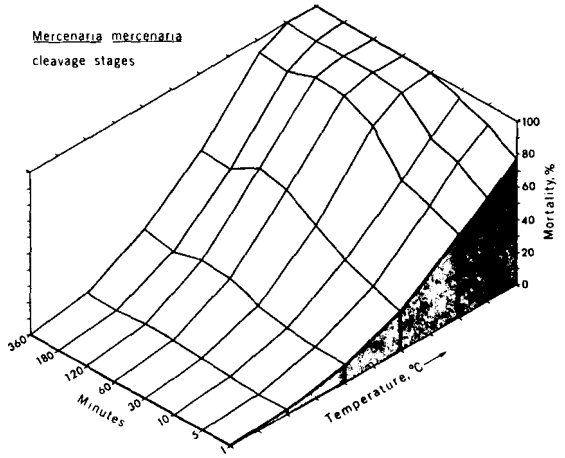

FIGURE 1.—*Mercenaria mercenaria* cleavage stages. Response surface generated from multiple regression analysis of percentage mortality on temperature and time. Refer to Table 1 for appropriate temperatures.

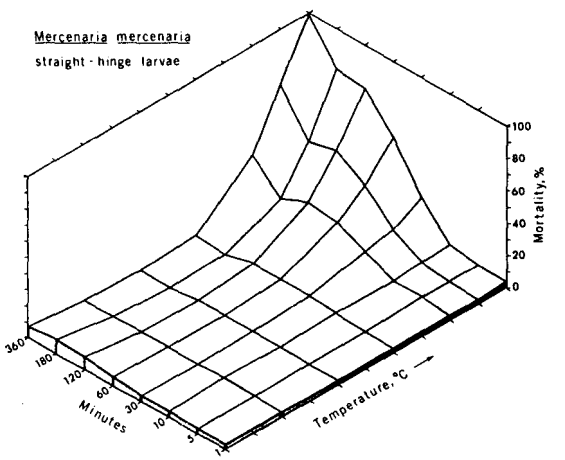

FIGURE 2.—*Mercenaria mercenaria* trochophore larvae. Response surface as in Figure 1. Refer to Table 2 for appropriate temperatures.

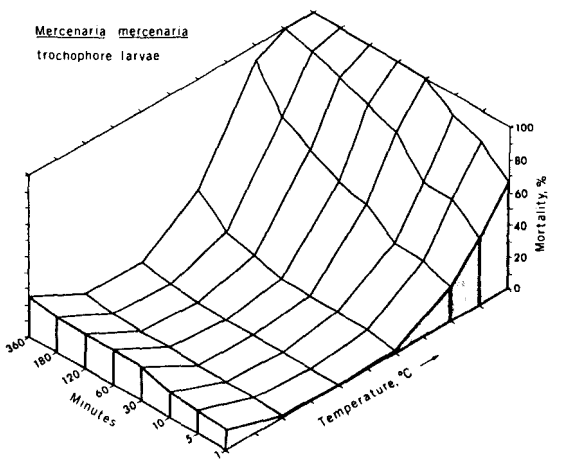

FIGURE 3.—*Mercenaria mercenaria* straight-hinge larvae. Response surface as in Figure 1. Refer to Table 3 for appropriate temperatures.

mortality associated with exposure in the block at normal temperatures (Kennedy et al., 1974). Percentage mortality data for each developmental stage were determined as for *Mulina lateralis* (Kennedy et al., 1974) and are presented in Tables 1 to 3. The stepwise multiple regression program transformed these data to arcsine square root of the percentage mortality to allow the distribution to approximate the normal. First, second, and third order terms for main effects (temperature, time) and all possible interactions were scrutinized. Only those terms (variables) with $F \geq 3.96$ ($P = 0.05$, *d.f.* 1, 80) were entered in the final equation. The program selected variables making greatest reduction in residual sum of squares until no further variables satisfied the acceptance criteria. The final empirical models appeared to be good predictive equations for all three stages (Table 4). The derived constant and variables selected for each stage are presented in Table 5, along with other statistics. The equations incorporating these constants and variables allow calculation of predicted percentage mortality for different combinations of temperature and time. The resulting estimates are in transformed form and must be converted to untransformed values (Sokal and Rohlf, 1969). Figures 1 to 3 were constructed using these equations and seven temperature levels to outline the basic pattern of the estimated response surface.

For each stage, the coefficient of determination ranged between 71% to 80% when all the variables were selected (Table 5), indicating that most of the variation in mortality can be explained by these variables (Steel and Torrie, 1960). For cleavage stages and trochophore larvae, T^3, by itself, was the best single predictor of percentage mortality. This was also true for straight-hinge larvae although T^3 was eventually eliminated by the program as new variables entered. In combination with the other variables in the final predictive equation, T^3 continued to be the most useful variable in estimating or predicting percentage mortality for trochophore larvae, as

TABLE 4.—Analysis of variance of multiple regression of percentage mortality on temperature and time for embryos and larvae of *Mercenaria mercenaria*. *** — significant at the 0.001 level; *d.f.* — degrees of freedom; *MS* — mean squares.

Source of variation	Cleavage stages		Trochophore		Straight-hinge	
	d.f.	*MS*	*d.f.*	*MS*	*d.f.*	*MS*
Regression	4	20557.780***	3	23484.304***	6	2903.295***
Residual	84	399.213	84	332.115	81	54.931

indicated by the values of the standard partial regression coefficients (Table 5). However, for cleavage stages, in the final predictive equation T^3 became relatively less important (Snedecor, 1956; Steel and Torrie, 1960).

The 88 residuals were tested for skewness (g_1) and kurtosis (g_2) (Sokal and Rohlf, 1969). Both statistics were normally distributed for cleavage stages ($g_1 = -1.02$; $g_2 = -1.78$). They were as follows for trochophore larvae: $g_1 = 2.02$*; $g_2 = 0.90$ and for straight-hinge larvae: $g_1 = -0.12$; $g_2 = 5.92$** (* $P = 0.05$; ** $P = 0.01$).

We estimated temperature levels for 10%, 50%, and 90% mortality for each period of time exposure by plotting percentage mortality from Tables 1 to 3 against log temperature on probability paper. These values (Figure 4) allow us to estimate the possible effects of temperature elevation over time on the survival of the different stages.

DISCUSSION

Early cleavage stages of molluscs appear to have a narrower range of tolerable temperatures than older stages (Pelseneer, 1901; Loosanoff, Miller, and Smith, 1951; Loosanoff and Davis, 1963; Goodwin, 1970; Kennedy et al., 1974). Results for *Mercenaria mercenaria* indicate that increased temperature tolerance occurred as early as the trochophore stage. This is in agreement with our results for *Mulinia lateralis* (Kennedy et al., 1974).

Cleavage stages of *Mercenaria mercenaria* were generally more temperature sensitive than those of *Mulinia lateralis* (Kennedy et al., 1974). Trochophore larvae of both species were generally similar in their thermal tolerances. Straight-hinge larvae of the hard clam were more temperature tolerant.

The sensitive cleavage stages of the hard clam are of primary importance in relation to the effects of entrainment and exposure to high temperature in cooling systems. If the cleavage stages are killed, obviously it does not matter that the next stages would be more temperature tolerant. The hard clam spawns during the summer throughout its geographical range (e.g. Loosanoff, 1937; Landers, 1954; Carriker, 1961; Porter, 1967; Chanley and Andrews, 1971). However, little precise information exists as to the temperature range for spawning. Carriker (1961) found that hard clams spawned between 22° and 30°C in Little Egg Harbor, N.J., with maximum frequency over the range of 24° to 26°C. This is in general agreement with the sparse

TABLE 5.—Statistics of multiple regression of percentage mortality on temperature and time. M = minutes; T = °C; b = regression coefficient; S_b = standard error of b; $100R^2$ = coefficient of determination (increases as each new variable is added); S_{rs} = standard error of the response surface (decreases as each new variable is added); b' = standard partial regression coefficient (absolute values).

Developmental stage	Constant	Variable	b	S_b	$100R^2$	S_{rs}	b'
Cleavage stages	−3.6	T^3	1.05×10^{-3}	1.40×10^{-4}	67.1	21.01	0.61
		MT^3	1.36×10^{-5}	4.90×10^{-6}	68.2	20.76	1.90
		M^2T^3	-9.24×10^{-8}	4.11×10^{-8}	69.9	20.34	4.40
		M^3T^3	1.61×10^{-10}	7.96×10^{-11}	71.3	19.98	2.74
Trochophore larvae	152.6	T^3	3.78×10^{-3}	4.40×10^{-4}	48.4	24.28	2.38
		T	−8.33	1.23	65.9	19.87	1.85
		MT^3	1.82×10^{-6}	4.40×10^{-7}	71.6	18.22	0.27
Straight-hinge larvae	8.7	MT^3	5.00×10^{-5}	5.87×10^{-6}	44.2	11.91	17.10
		MT^2	-2.48×10^{-3}	3.40×10^{-4}	61.7	9.92	21.74
		MT	3.43×10^{-2}	5.91×10^{-3}	69.7	8.88	8.20
		M^2T^3	-5.90×10^{-8}	1.10×10^{-8}	74.8	8.19	6.89
		M^3T^2	4.98×10^{-9}	1.11×10^{-9}	77.6	7.78	5.48
		M^3	-1.26×10^{-6}	4.33×10^{-7}	79.7	7.41	1.21

reports elsewhere (Nelson, 1928; Belding, 1931; Loosanoff, 1937; Porter, 1967). No temperatures have been published for spawning in Maryland or Virginia waters. We will assume conservatively that the range of 23° to 29°C would apply in these waters and that hard clam embryos and larvae would be present in the plankton under these conditions. Coutant (1970) estimated that the average temperature increase expected in cooling water carrying entrained organisms through a nuclear power plant would be 10.8°C. Therefore, entrained embryos and larvae of hard clams could be subjected to temperatures of 33.8° to 39.8°C while passing through such a facility. In spring (Figure 4), 90% of the cleavage stages could be eliminated if entrained for about 30 min. Fifty percent could be killed in about 13 min and 10% in about 6 min. For trochophores, 10% could be killed in about 25 min, perhaps longer. Straight-hinge larvae would appear to be unaffected by the temperature increase in spring. In late summer, 90% of the cleavage stages might be killed in 1 min or less, with 90% of the trochophores dying in less than 5 min. Over 180 min of exposure would be needed to kill 90% of the straight-hinge larvae.

The equations we have developed should allow predictive evaluations to be made concerning the effects of entrainment of hard clam embryos and larvae in Maryland and Virginia waters and elsewhere. Discharge canals of steam-electric power plants are usually located to avoid directing heated water over beds of commercial bivalves. It appears that it is also important to avoid taking in water that might come from the area of a bed of hard clams during spawning season. Should such water contain embryos and larvae of hard clams, long exposures in the cooling system of the plant (whether within the facility or in a discharge canal) could be lethal to the entrained

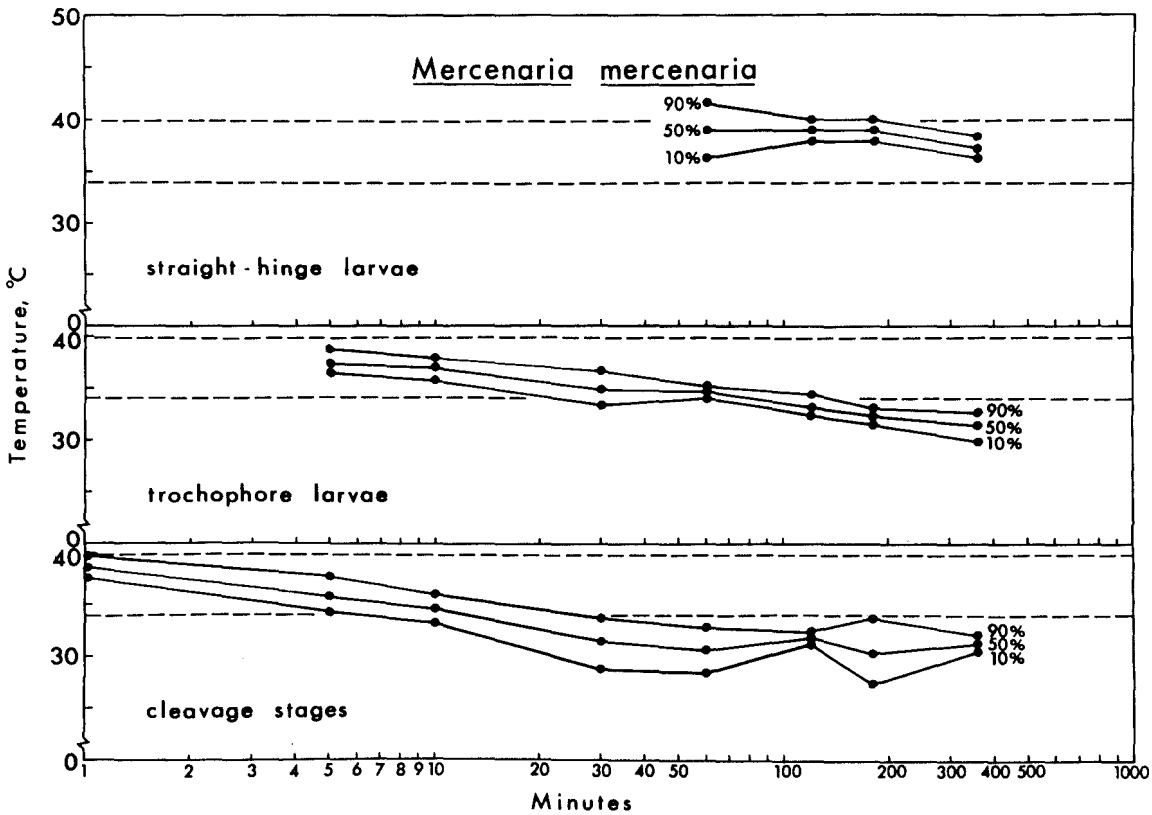

FIGURE 4.—Estimates of three percentage mortality levels for different exposure times. Percentage mortality for trochophore larvae and straight-hinge larvae was estimated to be less than 10% at 1 min and up to 30 min, respectively. For each stage, the dashed lines represent an increment of 10.8°C over estimated spawning temperature in spring (23°C - lower dashed line) and late summer (29°C - upper dashed line) in Maryland and Virginia waters.

organisms. Whether such mortality would affect the continued existence of the local hard clam resource depends upon local circumstances. Minimum destruction of the resource during use of cooling water from the vicinity of hard clam habitat requires that entrainment of embryos and larvae be as short as possible. The thermal discharge should be mixed with receiving water as quickly as possible to provide a rapid return to ambient temperature. In making evaluations it should be remembered that organisms passing through cooling systems are also subject to various stresses due to pressure changes, mechanical effects, and chlorination, in addition to temperature.

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LITERATURE CITED

BELDING, D. L.
1931. The quahaug fishery of Massachusetts. Mass. Dept. Conserv., Div. Fish Game, Mar. Fish. Serv. 2, 41 p.

CALABRESE, A., AND H. C. DAVIS.
1970. Tolerances and requirements of embryos and larvae of bivalve molluscs. Helgoländer wiss. Meeresunters. 20:553-564.

CARRIKER, M. R.
1961. Interrelation of functional morphology, behavior, and autecology in early stages of the bivalve *Mercenaria mercenaria*. J. Elisha Mitchell Sci. Soc. 77:168-241.

CHANLEY, P., AND J. D. ANDREWS.
1971. Aids for identification of bivalve larvae of Virginia. Malacologia 11:45-119.

COUTANT, C. C.
1970. Biological aspects of thermal pollution. I. Entrainment and discharge canal effects. CRC Crit. Rev. Environ. Control 1:341-381.

DAVIS, H. C., AND A. CALABRESE.
1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. U.S. Fish Wildl. Serv., Fish. Bull. 63:643-655.

GOODWIN, C. L.
1970. Some observation on laboratory spawning of the geoduck, *Panope generosa*, and the culture of its larvae. Proc. Natl. Shellfish. Assoc. 60:13-14.

KENNEDY, V. S., W. H. ROOSENBURG, H. H. ZION, AND M. CASTAGNA.
1974. Temperature-time relationships for survival of embryos and larvae of *Mulinia lateralis* (Mollusca: Bivalvia). Mar. Biol. (Berl.) 24:137-145.

LANDERS, W. S.
1954. Seasonal abundance of clam larvae in Rhode Island waters, 1950-52. U.S. Fish Wildl. Serv., Spec. Sci. Rep. Fish. 117, 29 p.

LOOSANOFF, V. L.
1937. Spawning of *Venus mercenaria* (L.). Ecology 18:506-515.

LOOSANOFF, V. L., AND H. C. DAVIS.
1963. Rearing of bivalve mollusks. Adv. Mar. Biol. 1:1-136.

LOOSANOFF, V. L., W. S. MILLER, AND P. B. SMITH.
1951. Growth and setting of larvae of *Venus mercenaria* in relation to temperature. J. Mar. Res. 10:59-81.

MARCY, B. C., JR.
1971. Survival of young fish in the discharge canal of a nuclear power plant. J. Fish. Res. Board Can. 28:1057-1060.

MORRISON, G.
1971. Dissolved oxygen requirements for embryonic and larval development of the hardshell clam, *Mercenaria mercenaria*. J. Fish. Res. Board Can. 28:379-381.

NELSON, T. C.
1928. On the distribution of critical temperatures for spawning and for ciliary activity in bivalve molluscs. Science (Wash., D.C.) 67:220-221.

PELSENEER, P.
1901. Sur le degré d'eurythermie de certaines larves marines. Bull. Acad. R. Belg. Cl. Sci., p. 279-292.

PORTER, H. J.
1967. Seasonal gonadal changes of adult clams, *Mercenaria mercenaria* (L.), in North Carolina. Proc. Natl. Shellfish. Assoc. 55:35-52.

SNEDECOR, G. W.
1956. Statistical methods applied to experiments in agriculture and biology. 5th ed. Iowa State Univ. Press, Ames, 534 p.

SOKAL, R. R., AND F. J. ROHLF.
1969. Biometry; the principles and practice of statistics in biological research. W. H. Freeman, San Franc., 776 p.

STEEL, R. G. D., AND J. H. TORRIE.
1960. Principles and procedures of statistics: with special reference to the biological sciences. McGraw-Hill, N.Y., 481 p.

TARZWELL, C. M.
1972. An argument for the open ocean siting of coastal thermal electric plants. J. Environ. Qual. 1:89-91.

THOMAS, W. H., H. L. SCOTTEN, AND J. S. BRADSHAW.
1963. Thermal gradient incubators for small aquatic organisms. Limnol. Oceanogr. 8:357-360.