Marine Environmental Research **25** (1988) 175–193

Effects of Cold Shock on Egg, Larval, and Juvenile Stages of Tropical Fishes: Potential Impacts of Ocean Thermal Energy Conversion

Yara Lamadrid-Rose* & George W. Boehlert

Southwest Fisheries Center Honolulu Laboratory,
National Marine Fisheries Service, NOAA,
2570 Dole Street, Honolulu, Hawaii 96822-2396, USA

(Received 31 July 1987; revised version received 11 November 1987; accepted 26 March 1988)

ABSTRACT

Ocean thermal energy conversion (OTEC) facilities will bring large volumes of deep, cold water to the surface, where it will be mixed with warm surface water after the utilization of the thermal differential. Mixing of these two water types will result in exposure of larval and juvenile fishes entrained in the warm water intake to lowered temperatures. A time course of cold shock from simulated entrainment was used to determine potential effects upon egg and larval mahimahi, Coryphaena hippurus, *manini,* Acanthurus triostegus, *and juvenile striped mullet,* Mugil cephalus. *Experiments were designed to test varying exposure times and temperature changes upon different early life stages of tropical fishes.*

Earlier stage eggs were more sensitive to temperature shock, with mortality increasing with increasing delta T as compared to controls; exposure time, however, did not exert a significant effect. Significant excess mortality for all stages of eggs occurred at the range of times and temperatures used in the experiments, although in some cases these exceed values proposed in current OTEC engineering plans. For mahimahi larvae, excess mortality increased with age to values higher than for latest stage eggs, but we consider this to be caused by stress associated with yolk exhaustion. A single size range of juvenile mullet was exposed to the same experimental protocol as the eggs and larvae. Although exposure time was not significant, delta T had a significant effect upon mortality, and there was a significant interaction between exposure time and delta T.

* Present address: Department of Animal Science, Prawn Aquaculture Research Program, University of Hawaii, Honolulu, HI 96822, USA.

Marine Environ. Res. 0141-1136/88/$03·50

INTRODUCTION

Studies on man-induced thermal effects on aquatic ecosystems have generally considered elevated temperatures resulting from once-through cooling systems of fossil fuel or nuclear power-plants (Van Winkle, 1977; Schubel *et al.*, 1978; Talmage & Coutant, 1979). The majority of these studies have concentrated on higher latitude marine and freshwater ecosystems (Hoss *et al.*, 1974; Burton *et al.*, 1976; Smith *et al.*, 1979; Barker *et al.*, 1981). As the utilization of marine waters for cooling increased, concerns about negative impacts upon fisheries developed (Saila, 1975). Most studies of thermal effects in marine systems have concentrated upon elevated temperatures; the proposed development of ocean thermal energy conversion (OTEC), however, raises new concerns about exposure to decreased temperatures in the effluent plume (Myers *et al.*, 1986). Hoss *et al.* (1986) studied the effects of decreased temperature on juvenile French grunt and observed no major mortality effects at temperatures expected from OTEC operations. In this study, we consider the effects of lowered temperatures upon early life history stages of tropical fishes with special attention to egg and larval stages.

Effects of thermal stress on egg and larval stages of fishes are important because ichthyoplankton is vulnerable to entrainment and highly sensitive to temperature fluctuations (Horst, 1975; Schubel, 1975; Rosenthal & Alderdice, 1976). Again, most of these studies have concentrated upon the effects of elevated temperature, primarily as it affects mortality (Smith *et al.*, 1979; Barker *et al.*, 1981). Relatively little work has been conducted upon the lower thermal tolerances of fish larvae or upon the effects of lower temperatures upon development, particularly in tropical systems. Tropical species, however, have less tolerance to cold than do temperate species (Shimada *et al.*, 1985). Even slightly lowered temperatures may have subtle but important effects upon the larval development. Lasker (1964), for example, noted normal development of larval Pacific sardine, *Sardinops caerulea*, at temperatures of 14°–21°C, but no development of a functional jaw at temperatures of 13°C and below. Larvae were unable to initiate feeding at temperatures which were only slightly lower than those observed in nature. Pacific cod (*Gadus macrocephalus*) eggs can tolerate variability in salinity and dissolved oxygen if within a temperature range of 3°–5°C, but egg viability decreased sharply, irrespective of other environmental conditions, below 3°C (Alderdice & Forrester, 1971). Lowered temperatures may also affect the order of appearance of developmental features in embryos (Hayes *et al.*, 1953) and may result in abnormal hatching. Eggs of *Bairdiella icistia* reared at 18°C failed to develop past the blastula stage despite normal embryonic development between 20° and 30°C (May, 1974).

These and other studies demonstrate that temperatures only a few degrees below ambient may cause significant mortality in fish embryos and larvae.

Temperature shocks in nature may also be characterized by sublethal or stress effects, where the probability of subsequent mortality is increased (Rosenthal & Alderdice, 1976; Mazeaud *et al.*, 1977). Sublethal stresses may decrease predator avoidance capabilities in nature (Coutant, 1973; Farr 1977). Coutant *et al.* (1974) observed increased predation in juvenile channel catfish, *Ictalurus punctatus*, and largemouth bass, *Micropterus salmoides*, exposed to varying cold thermal shocks. The incidence of predation increased with increasing magnitude of shock temperatures.

In the present study we describe the effects of lowered temperatures on egg and larval stages of two representative species of tropical marine fishes, the mahimahi, *Coryphaena hippurus*, and the manini, *Acanthurus triostegus*, and on juvenile striped mullet, *Mugil cephalus*. By considering pelagic, reef-dwelling, and nearshore species, the results of these experiments are pertinent to understanding potential thermal effects of OTEC on early life history stages of fishes.

MATERIALS AND METHODS

Experiments were conducted at the Kewalo Research Facility of the Southwest Fisheries Center Honolulu Laboratory, where the capability to naturally spawn pelagic and reef fishes provided experimental animals. Our experimental design considered an analysis of the time–temperature histories for seawater pumped through and discharged from typical OTEC operations (E. Myers, Ocean Minerals and Energy, NOAA, Washington, DC 20235, pers. comm.). This design (Fig. 1) is patterned after the engineering plans for the Ocean Thermal Corporation's (1983) planned Kahe Point facility. Warm surface water (74·54 m^3/s at 25·5°C) is drawn into the plant and immediately mixed with the warm water effluent from the existing oil-fired plant (31·85 m^3/s at 31·3°C), raising the temperature of the mixed water to 27·2°C. Flow through the heat exchangers results in a small temperature drop, and mixing of cold and warm water causes the final temperature to drop to approximately 16·7°C. The time for a change from ambient to mixed temperatures is < 1 min. The transit through the mixed effluent pipes would be approximately 8 min (Fig. 1) before release near a depth of 100 m, where a dilution period of about 1 min would bring the temperature back to within 2°C of the surface temperature.

Attempts were made to simulate representative thermal histories in the experiments, but we did not include the small temperature increase (Fig. 1) associated with the heated effluent addition before thermal shock. In our

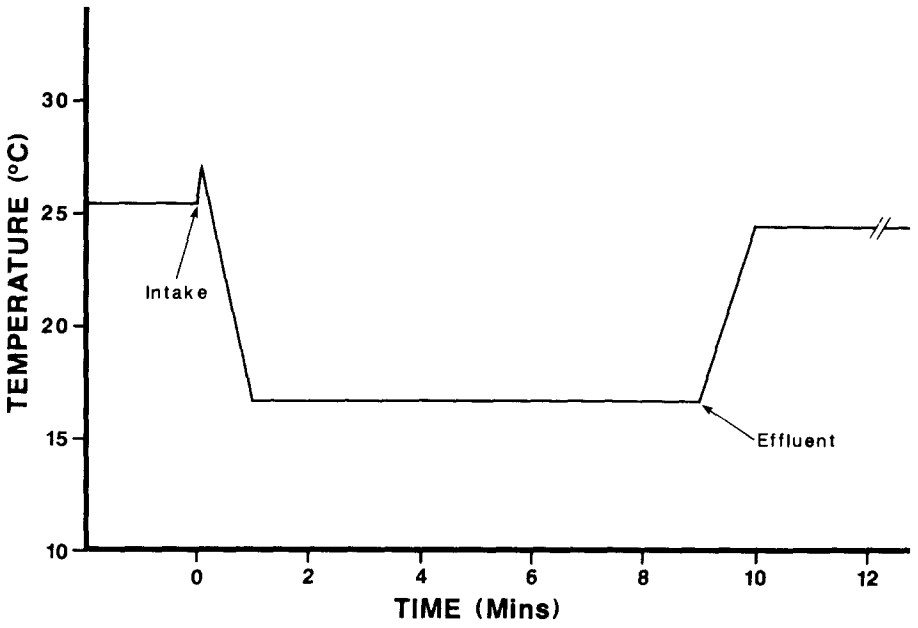

Fig. 1. Time–temperature history for a water parcel or organism entrained into the warmwater intake of the proposed Kahe Point facility (OTC, 1983). At time zero, the water enters the plant, mixes with the heated effluent from the nearby oil-fired plant, decreases in temperature as it proceeds through the heat exchangers and then mixes with the cold water. The mixed effluent takes approximately 8 min to reach the terminus of the pipe, where it gradually increases to the ambient temperature at the release depth (near 100 m).

design, the experimental variables considered are stage of egg or larval development, magnitude of thermal shock (delta T), and duration of lowered temperature exposure. Developmental stage classification for mahimahi was obtained from Palko *et al.* (1982); manini eggs were staged using Oppenheimer's (1937) classifications. We used delta T's of 5°, 10°, and 15°C and exposure times of 8, 16, and 24 min in our experiments. The values for delta T and exposure times exceeded those in Fig. 1, but were chosen to encompass a range that would include alternate plant designs.

Mahimahi brood stock was maintained in various flow-through circular tanks (5 or 7·2 m diameter) at ambient temperatures of 23·5° to 24·5°C. During the course of the experiments, the brood stock spawned every other day without artificial induction. Mahimahi eggs are spherical (1·52–1·66 mm in diameter), buoyant, and have a single oil globule (0·31–0·34 mm in diameter). At an ambient water temperature of 26°C, the eggs hatched approximately 54 h after spawning and larvae ranged from 4·3 to 5·4 mm standard length (SL).

A complete series of experiments for mahimahi was composed of three tests; each test consisted of a single delta T with samples from six

developmental stages at three exposure times including a control and replicates. The developmental stages were 0, 12 and 24 h post-fertilization eggs; larvae were at day of hatch, and day 2, and day 4 post hatch. Three exposure times (8, 16 and 24 min) and a control were used for each of three temperature shocks (delta T, 5, 10 and 15 C°) below the ambient temperature (26°C). Three replicate samples for the three exposure times and control resulted in 12 samples per developmental stage per temperature drop (a total of 72 samples per test). Fifty eggs or 20 larvae were tested per replicate. For each test, eggs and larvae used were obtained from a single spawning day. Specific controls for each spawning were necessary because egg quality may vary among spawnings (T. K. Kazama, Southwest Fisheries Center Honolulu Laboratory, National Marine Fisheries Service, NOAA, Honolulu, HI 96822-2396, pers. comm.).

Mahimahi eggs were collected within 10–20 min after spawning. The eggs were gathered in a fine-mesh dip net as they floated along the surface of the water; they were placed in a 22·8-liter (6 gallons) bucket with seawater from the parental tank. The procedure was repeated until 4000–5000 eggs were collected. The eggs were taken into the laboratory and mildly aerated. Samples were removed and checked for normal fertilization and development. They were then placed in a 100-liter rearing tank within a water bath maintained at 26°C. The 100-liter tank and the water bath contained aerated seawater filtered through a 0·22-μm Millipore filter system. A photoperiod with 18 h of light and 6 h of darkness (18L: 6D) was maintained with timers on a fluorescent light system directly above the water bath tank. Marine microalgae (*Tetraselmas* sp. and *Isochrysis* sp.) were added in small quantities to the 100-liter tank and the ambient water bath tank to maintain water quality. On day 1 post hatch, the 100-liter tank was inoculated with rotifers (*Brachionus* sp.) at a concentration of 1–2/ml. This food density was maintained until the termination of the experiments at day 5 post hatch. At 26°C yolk was absorbed by day 2 post hatch.

Eggs and larvae were placed in 1-liter plastic beakers modified for water transfer by cutting six windows symmetrically around the top of the beaker. The windows were covered with 0·35-mm Nitex mesh attached with silicone caulk. Beakers were labeled and the eggs or larvae randomly distributed among the beakers. The beakers were suspended in the ambient temperature (26°C) water bath by rings of styrofoam. A fine-pore airstone was suspended in each beaker and the seawater gently aerated. The beakers to be subjected to the thermal shock were individually transferred from the ambient-temperature, water-bath tank (holding tank) to the treatment tank (shock tank). The shock tank was maintained at the desired temperature for the test—delta T of 5 (21°C), 10 (16°C), or 15 C° (11°C, all $\pm$0·5°C)—by use of a cooling unit in the shock tank.

The transfer was made by allowing the ambient seawater in the beaker to drain to the bottom of the screened window, approximately one-fourth the height of the beaker. It was then placed in the shock tank and the colder seawater allowed to bring the water volume to 1 liter. Then, 4 liters of seawater from the shock tank were siphoned into each 1-liter test beaker through a 5-mm inside diameter (ID) hose. This produced a 1-min temperature drop from the ambient temperature (26°C) to the desired shock temperature. This procedure was repeated for each of nine test beakers per stage exposed to the thermal shock. Controls were handled identically but were placed back into the holding tank.

After the appropriate exposure time in the shock tank, each test beaker was again allowed to drain to the bottom of the screened window, returned to the holding tank, and, over a period of 1 min, flooded with ambient seawater from the water bath to bring the temperature up to 24°C. The beaker was left in the holding tank so that the water temperature could slowly return to the ambient (26°C); this required approximately 1 h. The test beakers were kept in the holding tank at ambient temperature until the termination of the test. The control beakers were handled in the same manner but with no thermal shock.

Eggs were held in the test beakers until day 1 post hatch. Mortality and development data were recorded daily. Approximately 12 h after the thermal shock occurred, eggs exposed to the shock and the control samples were checked for mortalities and incidences of abnormalities as described by Rosenthal & Alderdice (1976). Thereafter, eggs were checked every 24 h until day 1 post hatch for mortality or abnormality. On day 1 post hatch, experiments were terminated; surviving and dead eggs were counted and recorded. Observations of abnormalities in surviving eggs and larvae, as well as time delays in hatching, were also recorded. Methodology for handling larvae was similar. Dead larvae were removed 1 h after the thermal shock, and survivors kept in the test beakers for periods ranging between 16 and 24 h post shock. When the experiment was terminated, the numbers of dead and surviving larvae were recorded.

Manini brood stocks were kept in a 7·2-m diameter tank at ambient temperatures (23·1° to 23·4°C). Various species of tuna were usually also in the tank but did not interfere with the spawning activity of the manini. The brood stock spawned naturally two to three times a week until mid-October, 1984, after which spawning became less frequent (once a week at most). A few days before periods of a full moon, however, spawning activity increased. Manini eggs are spherical, 0·63–0·70 mm in diameter, buoyant, and have a single oil globule (0·15–0·18 mm in diameter). At an ambient seawater temperature of 26°C, the eggs hatched approximately 36 h after spawning and larvae ranged from 2·03 to 2·43 mm SL.

The experimental design was identical to the mahimahi procedures with minor exceptions. Three developmental stages were used for each test (two egg stages, 0 and 18 h post-fertilization, and one yolk sac larval stage, day 1 post hatch); thus, the complete series of experiments consisted of three tests, each with one delta T, three developmental stages at three exposure times with a control, and three replicates of each. Manini eggs were collected within 10–30 min after spawning. The eggs were handled and processed in the same manner as the mahimahi eggs. The experimental beakers were modified by painting the bottoms black to facilitate observations of the small, semitransparent larvae; the windows were covered with 0·25 mm Nitex mesh. The water bath, rather than individual beakers, was aerated. The only microalga used as water conditioner in these experiments was *Isochrysis* sp.

The procedure for treating the smaller eggs and larvae after the thermal shocks differed from mahimahi experiments. The eggs were held in test beakers until day of hatch; mortality data were recorded only at the termination of each experiment. Mortality and survival of eggs and larvae, as well as time delays in hatching, were also recorded at this time. Larvae were held for 24 h post shock; the numbers of survivors were recorded at the termination of experiments. At the termination of each test, all surviving eggs and larvae were labeled and preserved in 3% formalin (in seawater).

Juvenile striped mullet, *Mugil cephalus*, were collected between 8 November and 18 December at Waialae Beach Park, Honolulu, Hawaii, and kept in 2-m circular flow-through tanks in water between 25° and 26°C. They were fed a varied diet including trout and catfish chow, live brine shrimp, *Artemia* sp., and commercial flake fish food. The fish used in the experiments ranged from 25·8 to 66·5 mm SL.

The basic experimental design was similar to that used with larval stages, except that the juvenile experiments were conducted on a larger scale. A complete series of experiments consisted of three tests. Only one stage (juvenile fish) was used in each test, but exposure times and delta T were the same as in larval experiments. Two complete series of experiments were run. In the first series, three replicates were used for each exposure time and control; in the second series, only two replicates were used due to the numbers of fish available. Ten juveniles were used for each replicate.

Juveniles were randomly transferred from outside holding tanks, in groups of five fish, to 38-liter (10 gal) glass aquaria until each tank contained ten fish. A 200-W submersible heater in each tank maintained a 26°C seawater temperature; water was circulated by use of an airstone suspended in each tank. The alga, *Tetraselmas* sp., was added in small quantities to maintain water quality. The fish were allowed to acclimate in the tanks for 4 to 5 h before the exposure experiments were conducted. Fish in tanks to be

subjected to the thermal shock were transferred to a 4-liter bucket 5 min before the exposure. The buckets were modified to facilitate water exchange; each bucket had 3-mm holes uniformly cut from the top to the 1·5-liter level. Transfer to the shock tank involved gradual immersion of the bucket into the colder seawater to produce a 1-min temperature drop from the ambient temperature.

Two minutes before termination of the exposure, cold seawater was added to the holding tanks to reduce the temperature to 24°C. After the appropriate exposure time for each sample, buckets were drained to the 1·5-liter mark and gradually submerged and flooded with seawater in the holding tank to bring the test fish to 24°C in 1 min. The heaters raised the temperature to 26°C over a period of 30 min to 1 h. The control fish were handled in the same way, except that the water was maintained at the ambient temperature (26°C). The fish were kept in the holding tanks for 24 to 48 h post shock.

Data analysis consisted of three-way analysis of variance (ANOVA) with Duncan's multiple range test (Nie *et al.*, 1975) to compare treatment means. Two ANOVA's were run for each experiment. The data analyzed in the first were proportion survival, with factors delta T (three levels), stage of development (varying levels by taxa), and exposure time (four levels, where controls represented zero exposure time). In the second ANOVA, the datum analyzed was proportion excess mortality. Control mortality varied with egg quality; past experience with mahimahi shows that egg viability varies from day to day. To adjust for this variability, mean mortality values for controls were subtracted from the relevant treatment values to determine 'excess mortality'. Both values, as proportions, were arcsin transformed before running the ANOVA.

RESULTS

Coryphaena hippurus, Mahimahi

Five complete tests were conducted using six stages of mahimahi eggs and larvae with three tests at 5 C°, one test at 10 C°, and one test at 15 C°. For 0 h post-fertilization eggs, delta T of 10 and 15 C° produced 100% mortalities irrespective of exposure times (Fig. 2a), generally within 12 h of exposure to the cold shock. These eggs, shocked between the 2 and 16 cell stage, failed to develop beyond the gastrula stage and died. The control eggs, from the same age group, however, were developing normally and at the early neurula stage. Newly fertilized eggs exposed to 5 C° delta T had higher excess mortalities than later stages exposed to the same delta T and exposure time. The 12 h post-fertilization eggs were shocked at the late gastrula–early neurula stage. Again, the greatest excess mortalities were observed at delta

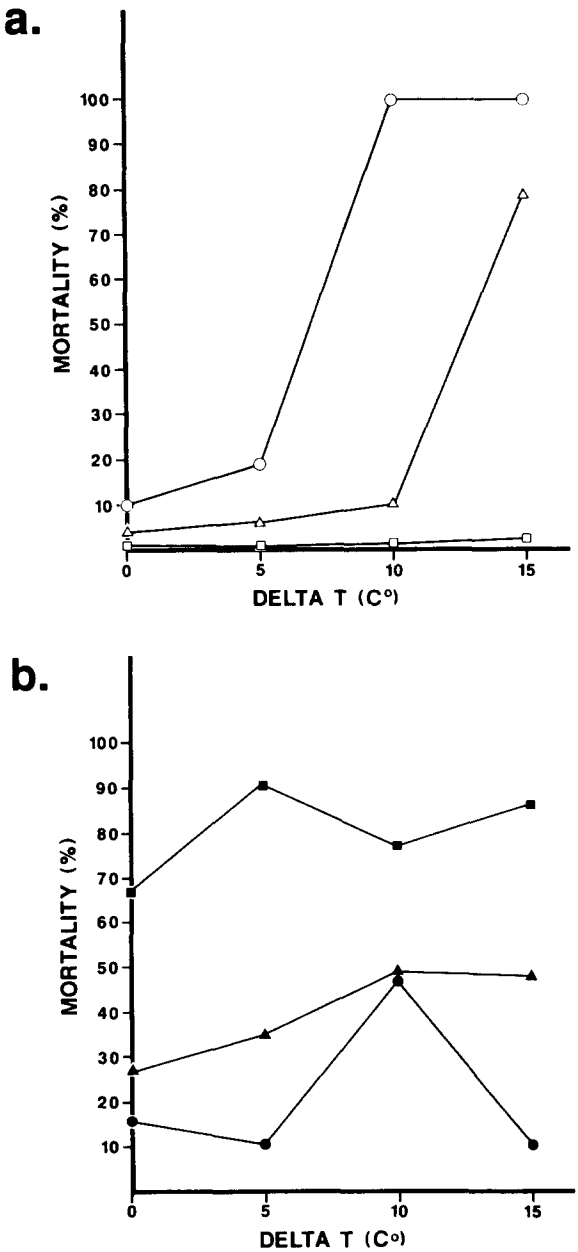

Fig. 2. Results of thermal exposure experiments on egg (a) and larval (b) stages of mahimahi, *Coryphaena hippurus*. Each data point is the mean of three replicates at each exposure time with the exception of the delta T 5 C° and the controls (delta T 0 C°); the former is based upon three experiments and the latter upon the mean of controls run each day. Actual data points were arcsin transformed before taking means and means were transformed back to percentage mean mortalities. ○ = 0 h post-fertilization; △ = 12 h post-fertilization; □ = 24 h post-fertilization. ● = day-of-hatch; ▲ = day 2; ■ = day 4.

T 15 C°, and mortality decreased at the lower delta T's (Fig. 2(a)). The 24-h post-fertilization eggs were exposed to thermal shock at the developing embryo stage; the embryo occupied approximately one-half the circumference of the egg, the optic vesicles were formed, and the yolk was pigmented. Excess mortality of the eggs exposed at this stage was significantly lower than eggs exposed at earlier stages and not significantly different from controls. Excess mortality increased slightly, however, in eggs exposed to greater delta T's.

In all the egg stages shocked and reared to hatching in the 1-liter beakers, some deaths were observed on the day of hatch. Abnormalities were also observed in these experiments and included yolk and oil globule deformities, bent body, tail and head deformities, and an amorphous embryo mass. Abnormalities in 0-h post-fertilization eggs were more common than in eggs exposed to cold thermal shock at later stages; the incidence of abnormalities in the 12-h post-fertilization eggs was greater than that in eggs shocked at 24 h post-fertilization. The 12- and 24-h stage egg data could not effectively be compared to the 0-h stage egg data due to the 100% mortalities at the higher delta T's (Fig. 2(a)). The percentage of abnormalities in the 24-h post-fertilization tests, however, was very low.

The results for the larval stages were almost inverse to that seen for the egg stages. The newly hatched larvae generally had lower excess mortalities than day 2 and day 4 post-hatch feeding larvae but neither the delta T nor exposure time significantly affects the survival of the day-of-hatch larvae (Fig. 2(b)). The day 2 and day 4 larvae have significantly higher mortality than day-of-hatch larvae, but excess mortality is not significantly different due to high mortalities in the controls (Table 1). In our experiments, all larvae showed similar reactions to the cold shock; they turned slightly dark, sank to the bottom of the beaker, and were less active. When returned to the ambient temperature water bath, they quickly resumed their normal coloration and position in the water column. The degree and speed of their response to the cold thermal shock were directly proportional to the magnitude of delta T. The greater mortalities seen in day 2 and day 4 larvae may have been a result of starvation. Although the larvae were fed before the experiments, lack of food could have resulted in sublethal stress within the 24-h post shock period; the excess mortalities (Table 1) may have been the result of the additional stress of the thermal shock.

Analyses of the mahimahi egg and larva data, including the treatment of eggs and larvae separately and combined, are presented in Table 1. Interaction effects of the factors were not considered. Generally, the ANOVA analysis on the excess mortality most clearly demonstrates the important effects. Overall, the most significant effects were from delta T and developmental stage; exposure time had no effect (the effect of exposure

TABLE 1

Thermal Exposure Experiment on Mahimahi, *Coryphaena hippurus*, Egg and Larval Stages. Three-way ANOVA and Duncan Multiple Range Test Summary. Results Shown are from Arcsin Transformation Data Analysis which have been Converted Back to Per Cent Mean Mortality and Per Cent Excess Mortality. The Results of the Three-way ANOVA and Duncan Multiple Range Test are given for the Three Experimental Variables: Delta T, Developmental Stage, and Exposure. Duncan Multiple Range Test: x = Mean; D–G = Duncan Grouping, Means with the Same Letter are not Significantly Different. Three-way ANOVA Analysis: PR > F Values, ≥0·05 = NS; <0·05 = *; ≤0·01 = **; ≤0·001 = ***.

Variable		*Mahimahi combined*						*Mahimahi eggs*						*Mahimahi larvae*					
		Mean mortality (%)			*Excess mortality (%)*			*Mean mortality (%)*			*Excess mortality (%)*			*Mean mortality (%)*			*Excess mortality (%)*		
		x	*D–G*	*ANOVA*	x	*D–G*	*ANOVA*	x	*D–G*	*ANOVA*	x	*D–G*	*ANOVA*	x	*D–G*	*ANOVA*	x	*D–G*	*ANOVA*
Delta T (C°)	5	17·82	C		13·64	B		5·42	C		7·13	C		36·06	B		19·94	A	
	10	32·07	B	***	34·42	A	***	15·22	B	***	46·63	B	***	53·66	A	*	21·60	A	NS
	15	37·58	A		37·27	A		34·76	A		57·64	A		40·48	B		14·89	A	
Developmental stage																			
Egg	0 h	34·63	B		52·47	A		34·63	A		52·47	A		—	—	—	—	—	—
	12 h	12·32	C		20·29	B		12·32	B	***	20·29	B	***	—	—	—	—	—	—
	24 h	0·24	D		0·83	C		0·24	C		0·83	C		—	—	—	—	—	—
				***															
Larvae	DOH	12·86	C		16·63	B		—	—	—	—	—	—	12·86	C		16·63	A	
	Day 2	36·68	B		17·55	B	***	—	—	—	—	—	—	36·68	B	***	17·55	A	NS
	Day 4	81·44	A		23·73	B		—	—	—	—	—	—	81·44	A		23·73	A	
Exposure	8 min	25·86	A		20·29	A		13·01	A		23·07	B		42·30	A		17·49	A	
	16 min	26·48	A	***	22·58	A	NS	14·36	A	***	26·71	A	NS	40·92	A	**	18·59	A	NS
	24 min	31·80	A		23·65	A		15·33	A		25·45	A, B		50·42	A		21·85	A	
	Control	13·31	B		—	—		3·91	B		—	—		27·72	B		—	—	

time in the analysis of percentage mean mortality is significant, but this is due to the inclusion of controls as zero exposure time; this group is significantly less in all cases but treatment means are equal). The effects of delta T were apparent for all data combined and for eggs, but not for larvae alone. For the combined analysis, the 5 C° differed from the 10 and 15 C° data, whereas for the eggs, the treatment means increased with increasing delta T. As a function of developmental stage, in the combined analysis, excess mortality for newly fertilized eggs is significantly greater than that for the group with 12-h eggs and all three larval groups; the 24-h post-fertilization eggs suffer significantly lower excess mortality than either group. For eggs alone, each group differs in the same manner as in the combined analysis, whereas the larval groups analyzed alone are not significantly different.

Acanthurus triostegus, Manini

Three complete tests, one at each delta T, were conducted using the three stages of manini eggs and larvae. The results (Fig. 3) were similar to those in

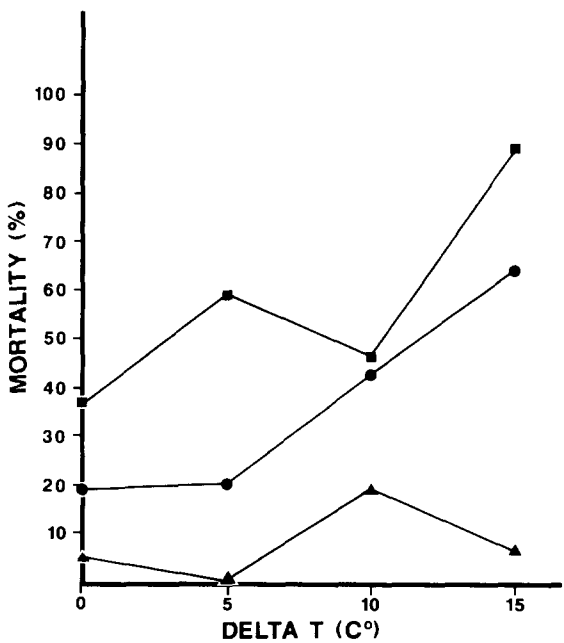

Fig. 3. Results of thermal exposure experiments on egg and larval stages of manini, *Acanthurus triostegus*; data points are means across exposure times. Actual data points were arcsin transformed before taking means and means were transformed back to percentage mean mortalities. Egg stages: ● = 0 h post-fertilization; ▲ = 18 h post-fertilization. Larval stage: ■ = day 1.

the mahimahi experiments. For newly hatched eggs, mortalities increased with increasing delta T. The later egg stage (18-h post-fertilization) had greatly decreased mortalities at all delta T's. The day 1 yolk sac larvae, including the controls, showed very high mortalities (Fig. 3); when calculated as excess mortality, no trends related to delta T's or exposure period were apparent. The manini data showed significant effects of all variables when mean per cent mortality data were included, but exposure time was not significant when considering excess mortality (Table 2). Percentage excess mortality for 5 and 10 C° delta T was not significantly different, but both differed from 15 C° delta T. The only significant effect of development stage was that 18-h post-fertilization eggs had a very low mortality compared to early stage eggs or yolk sac larvae (Table 2).

Mugil cephalus, Mullet

The juvenile mullet were used in two complete series of experiments. In the first series, larger fish (mean 50·1 mm SL) were used in three tests at each

TABLE 2

Thermal Exposure Experiment on Manini, *Acanthurus triostegus*, Egg and Larval Stages. Three-way ANOVA and Duncan Multiple Range Test Summary. Results shown are from Arcsin Transformation Data Analysis which have been Converted Back to Per Cent Mean Mortality and Per Cent Excess Mortality. The Results of the Three-way ANOVA and Duncan Multiple Range Test are given for the Three Experimental Variables: Delta T, Developmental Stage, and Exposure. Duncan Multiple Range Test: x = Mean; D–G = Duncan Grouping, Means with the Same Letters are Not Significantly Different. Three-way ANOVA Analysis: PR > F Values, ≥0·05 = NS; <0·05 = *; ≤0·01 = **; ≤0·001 = ***

Variable		*Mean mortality* (%)			*Excess mortality* (%)		
		x	D–G	*ANOVA*	x	D–G	*ANOVA*
Delta T (C°)	5	17·21	B		9·69	B	
	10	32·81	A	***	11·88	B	***
	15	33·40	A		37·39	A	
Developmental stage							
Egg	0 h	34·48	B		28·98	A	
	18 h	4·27	C	***	3·78	B	***
Larvae	Day 1	56·58	A		26·43	A	
Exposure	8 min	31·26	A		20·69	A	
	16 min	31·21	A	***	20·67	A	NS
	24 min	31·78	A		18·17	A	
	Control	16·55	B		—	—	

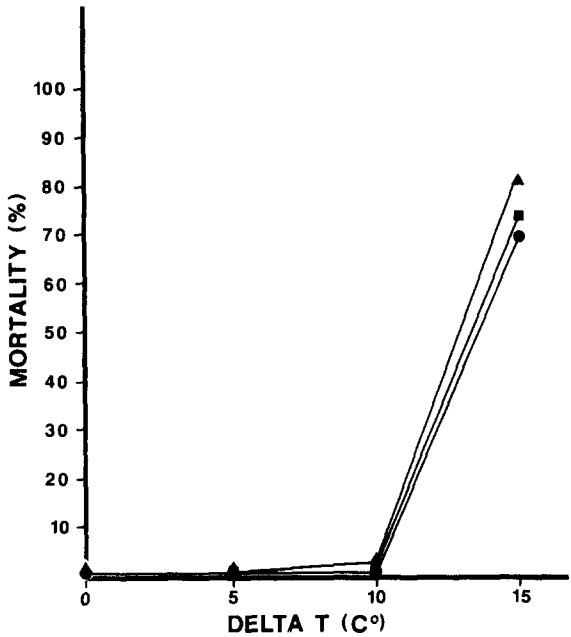

Fig. 4. Results of thermal exposure experiments on juvenile striped mullet, *Mugil cephalus*; data points are means across exposure times. Actual data points were arcsin transformed before taking means and means were transformed back to percentage mean mortalities. Each data point is the mean of five replicates. ● = Experiment I; ▲ = Experiment II; ■ = Experiment I and II combined.

delta T with three replicates. In the second series, smaller fish (mean 33·0 mm SL) were used with two replicates. The size difference between the groups of fish was significant (t-test, $P < 0{\cdot}01$), but there was no significant difference in excess mortality (t-test, $P > 0{\cdot}05$); data from both series of experiments were thus combined. The juvenile mullet showed no significant excess mortality at 5 and 10 C° delta T (Fig. 4). Behaviorally, juveniles placed into the cold shock at those temperatures typically turned dark, decreased activity, and remained near the bottom of the container. A few of the fish at 10 C° delta T swam in quick, jerking motions for a few seconds before settling like the others. At 15 C° delta T, however, the fish placed in the water swam in similar quick jerking motions with their opercula flared and lost equilibrium after a few seconds. Most of the fish that exhibited this behavior did not recover and died; 25% of the fish did recover and survived to the end of the experiment (Table 3). In general, the behavior patterns of fish exposed to this thermal shock was much like that described in Hoss *et al.* (1986). In these experiments, unlike the ones above, exposure time did have a significant effect on excess mortality within the 15 C° delta T. As the exposure time increased, excess mortality also increased (Table 3).

TABLE 3

Thermal Exposure Experiment on the Striped Mullet, *Mugil cephalus*, Juvenile Stage. Three-way ANOVA and Duncan Multiple Range Test Summary. Results shown are from Arcsin Transformation Data Analysis which have been Converted back to Per Cent Mean Mortality and Per Cent Excess Mortality. The Results of the Three-way ANOVA and Duncan Multiple Range Test are Given for the Three Experimental Variables: Delta T, Developmental Stage, and Exposure. Duncan Multiple Range Test: x = Mean; D–G = Duncan Grouping, Means with the Same Letter are Not Significantly Different. Three-way ANOVA Analysis: PR > F Values, $\geq 0{\cdot}05$ = NS; $< 0{\cdot}05$ = *; $\leq 0{\cdot}01$ = **; $\leq 0{\cdot}001$ = ***

Variable		*Mean mortality* (%)			*Excess mortality* (%)		
		x	D–G	*ANOVA*	x	D–G	*ANOVA*
Delta T (C°)	5	0·10	B		0·33	B	
	10	0·15	B	***	2·01	B	***
	15	43·92	A		79·81	A	
Exposure	8 min	9·61	A		21·65	A	
	16 min	10·47	A	***	35·97	A	NS
	24 min	12·23	A		35·36	A	
	Control	0·05	B		—	—	

DISCUSSION

Survival of fishes through early life history stages is an important aspect of population dynamics; oviparous fishes which produce large numbers of small eggs obviously suffer high mortality in early life history stages, primarily from starvation and predation (Hunter, 1976, 1981). Environmental perturbation or pollution may clearly increase mortalities during these early life history stages through either direct effects (Hunter *et al.*, 1979; Longwell & Hughes, 1981) or sublethal effects (Rosenthal & Alderdice 1976). Thermal pollution, depending upon the magnitude, may be either lethal or sublethal to aquatic organisms.

High temperatures, such as those encountered in typical nuclear or oil-fired electrical generation operations, have been shown to have potentially negative impacts upon early life history stages of marine fishes (Smith *et al.*, 1979; Talmage & Coutant, 1979; Barker *et al.*, 1981). These studies, however, have typically been conducted in temperate regions, where fishes may be more eurythermal than in tropical regions. Concern over cold shock in these regions has mainly related to mortality of animals in warm effluent plumes during periods of electrical plant shutdown in winter (Ash *et al.*, 1974; Pilati, 1976); these impacts are typically of short duration and occur locally. Fish

kills from either cold weather or intrusions of cold water have been documented in tropical regions (Bohnsack, 1983), demonstrating that tropical fishes generally have less tolerance to low temperature shock than temperate fishes. Furthermore, acclimation to low temperature is typically slower than to high temperature (Doudoroff, 1942). Early life history stages of fishes may also be adapted to the upper range of temperatures experienced by a species, as shown by laboratory (Medvick & Miller, 1979), distributional (Norris, 1963), and growth and energetics studies (Boehlert, 1982; Boehlert & Yoklavich, 1983). Thus, cold shock may exert a strong negative effect upon early life stages of tropical fishes (Shimada *et al.*, 1985).

Our results suggest that lowered temperatures likely to be encountered by a fish egg or larva in currently proposed OTEC operations (Fig. 1) may cause slightly increased mortalities. The highest excess mortalities due strictly to the thermal effects occur for the earliest developmental stages of eggs for mahimahi (Fig. 2(a) and (b); Table 1) and manini (Fig. 3; Table 2), whereas later stage eggs suffer less thermal mortality. The highest mortalities occur at delta T greater than that currently proposed for OTEC operations (Fig. 1); alternate designs, however, could conceivably produce greater temperature drops. Low hatching rates for Atlantic menhaden eggs and low survival of early larvae occur at 12°C, whereas 15 mm larvae can withstand 3·5°C (W. F. Hettler, Jr, NMFS Beaufort Laboratory, Beaufort, NC, pers. comm., 29 May 1987). Thus, eggs and newly hatched larvae may be relatively stenothermal and become more eurythermal during development. Larvae show particularly high mortality in later stages, but this occurs in the controls as well, implicating either problems with the rearing system or starvation-related effects. Nonetheless, the significant excess mortalities in the larval stages suggest that sublethal effects may be exerted upon these stages, making the probability of subsequent mortality higher (Mazeaud *et al.*, 1977). For the juvenile mullet considered in this study, significant mortality was only observed at delta T 15 C° (Fig. 4). This is considerably higher than planned temperature changes for a parcel of water entrained in the warmwater intake (Fig. 1) and thus older stages may be tolerant of the lowered temperatures likely to be encountered. Furthermore, juvenile stages will be better able to avoid either entrainment or low temperature conditions in the environment. These results agree with conclusions of Hoss *et al.* (1986).

Entrainment of fish eggs or larvae through an OTEC facility would result in several stresses, including thermal (Fig. 1), pressure and mechanical effects, exposure to biocides, and displacement from preferred habitat. Matsumoto (1984) suggested that these effects would result in 100% mortality of entrained fish eggs and larvae. Our results suggest that the thermal stresses alone will not result in such high mortality, but synergism of thermal effects with the other direct impacts (Hoss *et al.*, 1977) may result in high mortalities

before the larvae leave the system in the effluent. Koo & Johnson (1978) suggested that mortality and hatchability are not sufficiently good criteria of thermal shock in eggs, since thermal shock increased larval deformities. Such sublethal effects, including developmental anomalies, deformation, increased vulnerability to predation, and displacement from feeding areas are all possible impacts from lowered temperature not considered relative to OTEC operations.

ACKNOWLEDGEMENTS

This study was conducted at the Kewalo Research Facility of the Southwest Fisheries Center Honolulu Laboratory. We thank Thomas K. Kazama for considerable assistance with facilities and broodstock and D. E. Hoss and W. F. Hettler, Jr, for reviews of the manuscript.

REFERENCES

Alderdice, D. F. & Forrester, C. R. (1971). The effects of salinity, temperature, and dissolved oxygen on early development of the Pacific cod (*Gadus macrocephalus*). *J. Fish. Res. Board Can.*, **28**, 883–901.

Ash, G. R., Chymko, N. R. & Gallup, D. N. (1974). Fish kill due to 'cold shock' in Lake Wabamun, Alberta. *J. Fish. Res. Board Can.*, **31**, 1822–4.

Barker, S. L., Townsend, D. W. & Hacunda, J. S. (1981). Mortalities of Atlantic herring, *Clupea h. harengus*, smooth flounder, *Liopsetta putnami* and rainbow smelt, *Osmerus mordax*, larvae exposed to acute thermal shock. *Fish. Bull., U.S.*, **79**, 198–200.

Boehlert, G. W. (1982). Ontogenetic changes in growth and their relationship with temperature and habitat change. In *GUTSHOP '81: Fish Food Habits Studies.* ed. G. M. Cailliet & C. A. Simenstad, *Proceedings of the Third Pacific Workshop, December 6–9, 1981, Pacific Grove, California.* Washington Sea Grant Program, University of Washington, Seattle, WSW-WO 82-2, pp. 115–23.

Boehlert, G. W. & Yoklavich, M. M. (1983). Effects of temperature, ration, and fish size on growth of juvenile black rockfish, *Sebastes melanops*. *Environ. Biol. Fishes*, **8**, 17–28.

Bohnsack, J. A. (1983). Resiliency of reef fish communities in the Florida Keys following a January, 1977 hypothermal fish kill. *Environ. Biol. Fishes*, **9**, 41–53.

Burton, D. T., Richardson, L. B., Margrey, S. L. & Abell, P. R. (1976). Effects of low ΔT powerplant temperature on estuarine invertebrates. *J. Water Pollut. Control Fed.*, **48**, 2259–72.

Coutant, C. C. (1973). Effects of thermal shock on vulnerability of juvenile salmonids to predation. *J. Fish. Res. Board Can.*, **30**, 965–73.

Coutant, C. C., Ducharme, H. M., Jr. & Fisher, J. R. (1974). Effects of cold shock on juvenile channel catfish (*Ictalurus punctatus*) and largemouth bass (*Micropterus salmoides*) to predation. *J. Fish. Res. Board Can.*, **31**, 351–4.

Doudoroff, P. (1942). The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). *Biol. Bull.* (Woods Hole), **83**, 219–44.

Farr, J. A. (1977). Impairment of antipredator behavior in *Palaemonetes pugio* by exposure to sublethal doses of parathion. *Trans. Am. Fish. Soc.*, **106**, 287–90.

Hayes, F. R., Pelluet, D. & Gorham, E. (1953). Some effects of temperature on the embryonic development of salmon (*Salmo salar*), *Can. J. Zool.*, **31**, 42–51.

Horst, T. S. (1975). The assessment of impact due to entrainment of ichthyoplankton. In *Fisheries and Energy Production: A Symposium.* ed. S. B. Saila, Heath Publishing Company, Lexington, Massachusetts, pp. 107–18.

Hoss, D. E., Clements, L. C. & Colby, D. R. (1977). Synergistic effects of exposure to temperature and chlorine on survival of young-of-the-year estuarine fishes. In *Physiological Responses of Marine Biota to Pollutants.* Academic Press, New York, pp. 345–55.

Hoss, D. E., Hettler, W. F., Jr. & Coston, L. C. (1974). Effects of thermal shock on larval estuarine fish—ecological implications with respect to entrainment in power plant cooling system. In *Early Life History of Fish.* ed. J. H. S. Blaxter, Springer-Verlag, New York, pp. 357–71.

Hoss, D. E., Peters, D. S. & Hettler, W. F., Jr. (1986). Effects of lowered water temperature on the survival and behavior of juvenile French grunt, *Haemulon flavolineatum. Bull. Mar. Sci.*, **39**, 134–8.

Hunter, J. R. (1976). Report of a colloquium on larval fish mortality studies and their relation to fishery research, January 1975. *NOAA Tech. Rep. NMFS Circ.*, **395**.

Hunter, J. R. (1981). Feeding ecology/predation. In *Marine Fish Larvae: Morphology, Ecology and Relation to Fisheries.* ed R. Lasker, Washington Sea Grant Program, Seattle, pp. 34–77.

Hunter, J. R., Taylor, J. H. & Moser, H. G. (1979). Effect of ultraviolet irradiation on eggs and larvae of the northern anchovy, *Engraulis mordax*, and Pacific mackerel, *Scomber japonicus*, during the embryonic stage. *Photochem. Photobiol.*, **27**, 1–14.

Koo, T. S. Y. & Johnson, M. L. (1978). Larval deformity in striped bass, *Morone saxatilis* (Walbaum) and blue back herring *Alosa aestivalis* (Mitchill) due to heat shock treatment of developing eggs. *Environ. Pollut.*, **16**, 137–49.

Lasker, R. (1964). An experimental study of the effect of temperature on the incubation time, development, and growth of Pacific sardine embryos and larvae. *Copeia*, **1964**, 399–405.

Longwell, A. C. & Hughes, J. B. (1981). Cytologic, cytogenetic, and embryonic state of Atlantic mackerel eggs from surface waters of the New York Bight in relation to pollution. *Rapp. P.-V. Reun. Cons. Int. Explor. Mer*, **178**, 76–8.

Matsumoto, W. M. (1984). Potential impact of ocean thermal energy conversion (OTEC) operation on fisheries. National Marine Fisheries Service, NOAA, Honolulu, Hawaii. *Southwest Fisheries Center Administrative Report H-84-4*, 29 pp.

May, R. C. (1974). Effects of temperature and salinity on yolk utilization in *Bairdiella icistia* (Jordan and Gilbert) (Pisces: Sciaenidae). *J. Exp. Mar. Biol. Ecol.*, **16**, 213–25.

Mazeaud, M. M., Mazeaud, F. & Donaldson, E. M. (1977). Primary and secondary effects of stress in fish: Some new data with a general review. *Trans. Am. Fish. Soc.*, **106**, 201–12.

Medvick, P. A. & Miller, J. M. (1979). Behavioral thermoregulation in three Hawaiian reef fishes. *Environ. Biol. Fishes*, **4**, 23–8.

Myers, E. P., Hoss, D. E., Matsumoto, W. M., Peters, D. S., Seki, M. P., Uchida, R. N., Ditmars, J. D. & Paddock, R. A. (1986). The potential impact of ocean thermal energy conversion (OTEC) on fisheries. *NOAA Tech. Rep. NMFS*, **40**.

Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K. & Bent, D. H. (1975). *Statistical Package for the Social Sciences.* (2nd edn), McGraw Hill, New York, 675 pp.

Norris, K. S. (1963). The functions of temperature in the ecology of the percoid fish *Girella nigricans* (Ayres). *Ecol. Monogr.*, **33**, 23–62.

Ocean Thermal Corporation. (OTC) (1983). Ocean Thermal Energy Conversion (OTEC) site specific environmental assessment. Document No. L3 submitted to Department of Energy under Contract No. DE-AC01-82CE30716. Ocean Thermal Corporation, New York (Draft).

Oppenheimer, J. M. (1937). The normal stages of *Fundulus heteroclitus*. *Anat. Rec.*, **68**, 1–15.

Palko, B. J., Beardsley, G. L. & Richards, W. J. (1982). Synopsis of the biological data on dolphin-fishes, *Coryphaena hippurus* Linnaeus and *Coryphaena equiselis* Linnaeus. *NOAA Tech. Rep. NMFS Circ.*, **443**. *FAO Fish. Biol. Synop.* **130**.

Pilati, D. A. (1976). Cold shock: Biological implications and a method for approximating transient environmental temperatures in the near-field region of a thermal discharge. *Sci. Tot. Environ.*, **6**, 227–37.

Rosenthal, H. & Alderdice, D. E. (1976). Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. *J. Fish. Res. Board Can.*, **33**, 2047–65.

Saila, S. B. (ed). (1975). Fisheries and energy production. Heath Publishing Company, Lexington, Massachusetts, 300 pp.

Schubel, J. R. (1975). Some comments on the thermal effects of power plants on fish eggs and larvae. In *Fisheries and Energy Production.* ed S. B. Saila, Heath Publishing Company, Lexington, Massachusetts, pp. 31–54.

Schubel, J. R., Coutant, C. C. & Woodhead, P. M. J. (1978). Thermal effects of entrainment. In *Power Plant Entrainment: A Biological Assessment.* ed J. R. Schubel & B. C. Marcy, Academic Press, New York, pp. 19–73.

Shimada, S., Egami, N. & Shima, A. (1985). Comparison of cold, heat, and radiation sensitivities of the fish embryos among the genus Oryzias. *Comp. Bioch. Physiol.*, **82A**, 815–8.

Smith, C. F., Schubel, J. R., Greges, M. P., Itzkowitz, N., DiPiero, S. J., Longo, J. & Morgan, M. A. (1979). Thermal resistance characteristics of early life history stages of finfish from Long Island waters. *Mar. Sci. Res. Cent. Spec. Rep.* (Stony Brook), **26**, 79–9.

Talmage, S. S. & Coutant, C. C. (1979). Thermal effects. *J. Water Pollut. Control Fed.*, **51**, 1517–54.

Van Winkle, W. (Ed.). (1977). *Assessing the Effects of Powerplant Induced Mortalities of Fish Populations.* Pergamon Press, New York, 380 pp.