THERMAL SHOCK EFFECT ON EGGS OF THE SUMMER FLOUNDER

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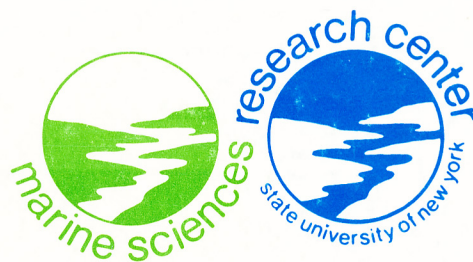
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THERMAL SHOCK EFFECT ON EGGS OF

THE SUMMER FLOUNDER

by

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August, 1979

Special Report 29

Reference 79-12

Approved for Distribution

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Abstract

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Fertilized summer flounder eggs in different embryological stages of development (cleavage, early embryo, and late embryo), were subjected to various excess temperature-exposure time combinations $\Delta T(^\circ C)$-t (min) to assess the effects of thermal shock on hatching success and sublethal effects on individual larvae that hatched from these eggs. Using the Chi-square test, significant differences were found to occur between experimental and control samples, for the cleavage experiment, between $\Delta 16$-16. For the late embryo experiment significant differences were found to occur between $\Delta 16$-16 and $\Delta 18$-2. The thermal shock region for the early embryo experiment was found to exceed the highest temperature-time combination used in this study, $\Delta 20$-16.

Viable larvae hatched from thermally shocked eggs were examined for sublethal effects in two experiments. Significant differences were found, in the late embryo experiment, occurring between experimental and control samples between $\Delta 14$-4 and $\Delta 14$-8. Another significant difference, for this experiment, was found to occur between $\Delta 16$-2 and $\Delta 16$-4. The results for the larvae hatched from eggs used in the cleavage experiment were inconclusive.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

INTRODUCTION

In estuaries and coastal areas productivity is high and a wide diversity of biological life exists. Many of our commercially important fishes use these zones for spawning or nursery areas. McHugh (1966) concluded that approximately 64%, by value, of the total commercial catch of fish and invertebrates on the Atlantic coast of the United States, consisted of estuarine-dependent species.

According to Clark and Brownell (1973), within estuaries or coastal waters, 147 operational or proposed steam electric generating plants are situated and 140 of them use once-through cooling. Once-through cooling is the most economical method for condensing exhaust steam from the turbines of a steam electric generating plant but it involves passage of large quantities of water through steam condensers (Coutant, 1970; Committee on Entrainment, 1978). The water used to cool the condensers of steam electric generating plants with once-through cooling systems was increased in temperature (ΔT) from 5.5° to 23.3 °C (Committee on Entrainment, 1978). Characteristically, water is drawn from the aquatic body and is pumped through 25 mm (1 inch) diameter tubes surrounded by exhaust steam. Heat energy in the form of high pressure steam drives the turbines and generators of a power plant to produce electricity. The excess steam in the form of "waste" heat is returned to the cooling water which is

then returned to the water body from which it was drawn (Coutant, 1970; Committee on Entrainment, 1978). Figure 1 illustrates a typical power plant. Recirculation of the discharged water is avoided by following the natural flow pattern of the water body, or by construction of engineering devices (Coutant, 1970).

The probability of an organism being entrained in the cooling system of a power plant depends upon a variety of factors including: the rate of withdrawal of cooling water compared with the rate of renewal of the water body; the mesh size of the screens covering the intake pipe, usually 9 to 13 mm; and the distribution of organisms in the environment. The movements of the tides back and forth past an intake structure maximizes entrainment with the same water mass moving to and fro and subjecting planktonic organisms to multiple hazards of entrainment (Committee on Water Quality Criteria, 1973).

Entrained organisms encounter three forms of stress in their passage through the condenser cooling system of a power plant: thermal (temperature rise across the condenser), mechanical (pressure changes, shear forces, acceleration forces, and abrasion), and chemical (chlorine gas, Cl_2) or sodium hypochlorite (NaOCl). As a result of these stresses, and the mortalities which they cause, a power plant acts like a "predator". In addition, some of the surviving organisms become debilitated and may be vulnerable to increased natural predation (Coutant, 1973;

STEAM ELECTRIC GENERATING STATION

TRAVELING SCREEN—
IMPINGEMENT, ENTRAPMENT—
(Physical)

STEAM LINE

TURBINE

ELECTRICITY

CONDENSER—
Thermal,
Chemical,
Physical

TRASH RACK—
IMPINGEMENT, ENTRAPMENT—
(Physical)

BOILER

GENERATOR

WATER

CHLORIDE

COOLING
WATER
SYSTEM

RECEIVING WATERS

INTAKE
WATERS

FUEL

HEATED
PLUME

BIOCIDE—
(Chemical)

DISCHARGE
CANAL OR PIPE—
Thermal,
Chemical,
Physical

DIFFUSER—
Thermal,
Chemical,
Physical

ENTRAINMENT—
(Meroplankton,
Zooplankton,
Phytoplankton)

PUMP—
PHYSICAL—
(Acceleration,
Pressure,
Shear,
Abrasion)

Fig. 1 - Schematic diagram of a typical power
plant showing sites of various stresses

3

4

Yokom and Edsall, 1974). Figure 1 illustrates where various stresses occur. Site studies have been made to assess the effects of entrainment, but they generally do not distinguish between the effects of the several stresses. An exception is the work done by Marcy (1973) at Connecticut Yankee. Other cases were cited by Beck and Lackie (1974), Beck and Miller (1974), and Beck and the Committee on Entrainment (1978). Marcy (1973) indicated that 80% of the mortality encountered by nine species of young fish could be attributed to mechanical damage and 20% to thermal shock and prolonged thermal exposure in the 1.8 km (1.1 mile) discharge canal. He found that chlorination, in the form of sodium hypochlorite, had no measurable effect.

The objective of our study is to determine the effects of various time-temperature combinations on hatching success of summer flounder (*Paralichthys dentatus*) eggs and sublethal effects on individual larvae that hatched from these eggs.

BIOLOGY OF THE SUMMER FLOUNDER

General Statements

Summer flounder, or fluke, *Paralichthys dentatus* (Linnaeus), is an important commercial and recreational fish. It ranges from the Gulf of Maine to the east coast of Florida (Gutherz, 1967; Bigelow and Schroeder, 1953), but is most abundant from south of Cape Cod to Cape Hatteras (Gutherz, 1967)--the Middle Atlantic Bight.

Summer flounder are described by their left-handedness, that is, both eyes are on the left side, while it lies on the bottom on its right side. It has the ability to adapt its coloration to the background on which it rests and can assume a wide range of tints and hues of brown and gray (Bigelow and Schroeder, 1953) with numerous ocellated spots (Ginsburg, 1952). The most conspicuous spots are generally located caudally (Ginsburg, 1952). The mouth is large and has relatively large teeth (Ginsburg, 1952; Lux et al, 1966). They feed on small fishes and macroinvertebrates (Poole, 1964; Smith and Daiber, 1977).

Seasonal Movements

When the inshore waters warm in late spring (April or May), summer flounder begin to migrate into relatively shallow

water of 4 to 37 m (2 to 20 fathoms) (Ginsburg, 1952). In the
New York Bight area, by June they are found in bays, estuaries,
and shallow coastal waters (Murawski, 1970), where they are
vulnerable to capture by sport fisherman, commercial traps (fyke
and pound nets), seines, and trawls (Lux et al., 1966). In fall-
winter (late September-December or January), dependent on latitude,
as the water column cools, summer flounder begin to migrate south
and east into waters of 36 to 146 (20 to 80 fathoms) (Bigelow and
Schroeder, 1953), or 183 m (100 fathoms) in depth to the edge of
the continental shelf (Ginsburg, 1952; Murawski, 1970), where they
are accessible to the commercial trawl fishery (Lux et al., 1966).
Tagging experiments conducted by Poole (1962) and Murawski (1970)
indicate that the migration pattern of the summer flounder is not
extensive and that there is a strong tendency for adults to
return to the same summer grounds in subsequent years. The
remaining fish move to the northeast. In the New York Bight, the
major spawning area (Smith, 1973), 3,423 metric tons were caught
in 1975 by commercial fishermen (McHugh, 1977; McHugh and Williams,
1976; McHugh and Ginter, 1978). The sport fishery for summer
flounder, during the same year, probably caught an amount equal
to or exceeding the commercial catch (Lux et al., 1966;
J.L. McHugh, personal communication).

Spawning

The offshore migration is associated with spawning.
Presumably both events are associated with environmental factors,
temperature and light, and physiological factors, such as secre-
tions by the endocrine glands (Woodhead, 1975; Woodhead and
Woodhead, 1965). As summer flounder leave the summer grounds,
gonadal maturation occurs in fish greater than 37 cm long, 2-3
years old (Murawski, 1964; Poole, 1966). Smith (1973) observed
a seasonal progression of spawning from north to south. North of
Chesapeake Bay, spawning occurs from September to December, the
peak in spawning activity occurs in October. South of Chesapeake
Bay, spawning peak occurs in November, spawning taking place from
November to April. For the New York Bight, the spawning period
has been confirmed by Murawski (1965) who observed that during
the first half of October there was a sharp increase in gonodal
size and running ripe females were found during late September
through early November. Smith (1973) considers the New York Bight
to be the most productive spawning ground for summer flounder.
Murawski (1964) located spawning areas in the Bight from 27 m
(15 fathoms) off Long Island to 101 m (55 fathoms) East-South-East
of Cape May, New Jersey.

Studies by Powell (1974) indicate that summer flounder are
highly fecund and females measuring 50.6 to 68.2 cm contain 1.0
to 1.7 million eggs. Spawning takes place at temperatures of 12

to 19°C (Smith, 1973). When fertilized the egg chorion meaures 0.9 - 1.1 mm (Smith and Fahay, 1970). The fertilized egg is pelagic and can withstand a wide range of temperatures from 9.1 to 22.9°C (Smith, 1973; Smith et al., 1975), while drifting passively with the prevailing surface currents. During fall, surface currents carry the eggs and larvae shoreward to the south and southwest (Bumpus and Lauzier, 1965). Most larvae are transported to the sounds of North Carolina, Chesapeake Bay, and to the bays on the eastern Virginia shore--the nursery grounds of juveniles (Poole, 1966).

Metamorphosis is completed by the time the larva has grown to 12 or 13 mm (Smith and Fahay, 1970). From this time the larva spends the remainder of its life on or close to the bottom (Lux et al., 1966).

Juvenile Populations

Local populations of juveniles have been described by Poole (1961) and Murawski (1964; 1965; 1966). Poole (1961; and personal communication) encountered young-of-the-year and juvenile summer flounder in Great South Bay. Murawski (1964; 1965; 1966) captured young-of-the-year and juveniles in New Jersey estuaries (Manasquan, Cunning, and Shark Rivers) indicating that the prevailing currents transport some larvae into New Jersey estuaries during the fall. Murawski (1964) caught young-of-the-year and

juveniles from the end of October through the end of December in New Jersey estuaries, indicating that the fry did not move off-shore but remained in the estuaries over the winter. The behavior of the young-of-the-year and juveniles was probably similar to that observed by Powell (1974); Powell and Schwartz (1977) for the fry which inhabit North Carolina estuaries as nursery grounds. Powell (1974); Powell and Schwartz (1977) concluded that both of the first two year classes were present in the estuaries from spring to mid-summer, but thereafter juveniles moved into the ocean while young-of-the-year remained in the estuaries.

EXPERIMENTAL DESIGN

The experimental design is a randomized complete block design with 7 levels of excess temperatures and 4 exposure times, with controls.

Excess Temperature, ΔT (°C):   0 8 10 12 14 16 18 20
Exposure Time, (min):   0 2 4 8 16

The 7 excess temperature levels selected span the range of temperature rises experienced across the condenser tubes of proposed and operating power plants with once-through cooling systems (Committee on Entrainment, 1978). The exposure times were chosen to span the range of residence times at high excess temperatures within typical cooling systems (Coutant, 1970), without long discharge canals.

MATERIALS AND METHODS

Fish Capture and Laboratory Handling

Summer flounder were collected from the West Passage of
Narragansett Bay, Rhode Island, between Wickford Harbor and the
Jamestown Bridge (Fig. 2) on August 24 and September 18, 1977.
Tows were made for 20-30 min, at a speed of approximately 4 knots,
using a small otter trawl. The fish were transferred first from
the trawl to a tray containing seawater where they were sorted
and then to a fiberglass tank with running seawater. They were
transported from the ship to the Environmental Research Laboratory-
Narragansett (Rhode Island), U.S. Environmental Protection Agency,
in the fiberglass tank of seawater and aerated with portable air
pumps.

The laboratory holding tank was a fiberglass cylinder
2.4 m (8 ft) in diameter and 1.2 m (4 ft) high equipped with
running seawater at ambient temperature. The fish were fed
chopped hard clams (*Mercenaria mercenaria*) every third day. Live
killifish (*Fundulus* spp.) and silversides (*Menidia* spp.) were
maintained in the same tank as live food. The fish were treated
with Furance (Nifurpirinol, Abbot), a prophylactic against vibro
disease (G.K. MacPhee, personal communication).
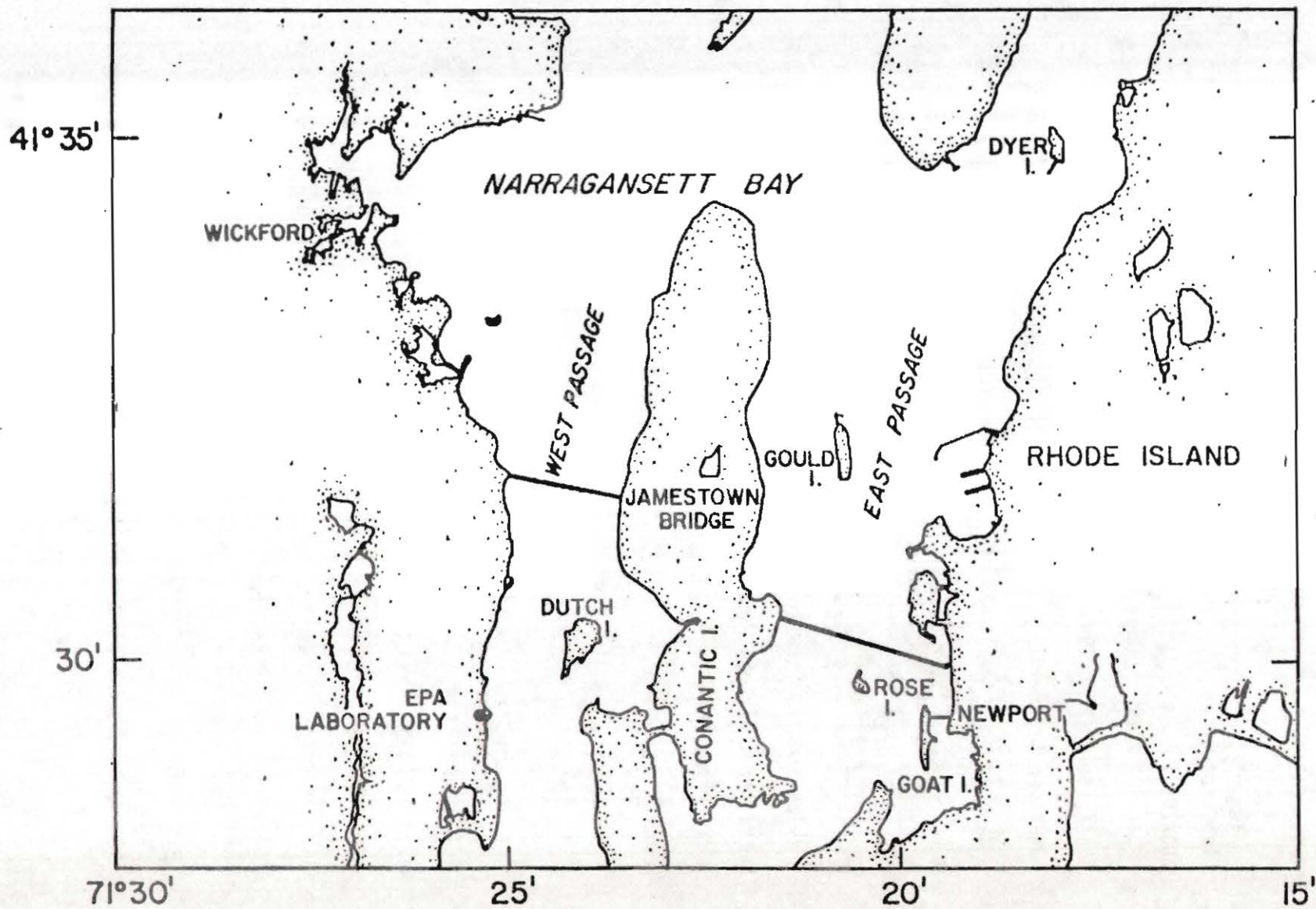
Fig. 2 - Area of fish capture

Hormone Induced Spawning and Egg Handling

The failure of summer flounder to spawn naturally when held in aquaria (Bigelow and Schroeder, 1953; Smigielski, 1975), necessitates hormonal treatment to induce spawning (Smigielski, 1975). Freeze-dried carp pituitary hormone in saline solution was injected intramuscularly into the back muscle below the dorsal fin. The dosages given to individual flounders were proportional to their weight (Smigielski, 1975). The hormonal treatment was repeated daily, until the mature eggs were released. The fish were then stripped by hand.

The eggs for these experiments were stripped from a single female and fertilized with milt from several males. The eggs and milt were placed in a plastic bowl containing seawater to ensure maximum fertilization. The bowl was allowed to float in an acclimation tank for approximately 30 min. The fertilized eggs were placed on a 550 μm mesh plastic screen and the excess milt washed off. The eggs were returned to the plastic bowl and replaced in the acclimation tank. A subsample of the fertilized eggs was removed and inspected under a dissecting microscope to determine the percentage of fertilization. The percentage of eggs fertilized varied between experiments: only 20-25% for the cleavage experiment, but 90-95% for the early and late embryo experiments. The fertilized eggs used in the late embryo experiment were placed in a hatching jar (Fig. 3) where they remained

14

AIR
INFLOW

SEAWATER
INFLOW

WATER LEVEL

AIR STONE

DRAINAGE
FAUCET
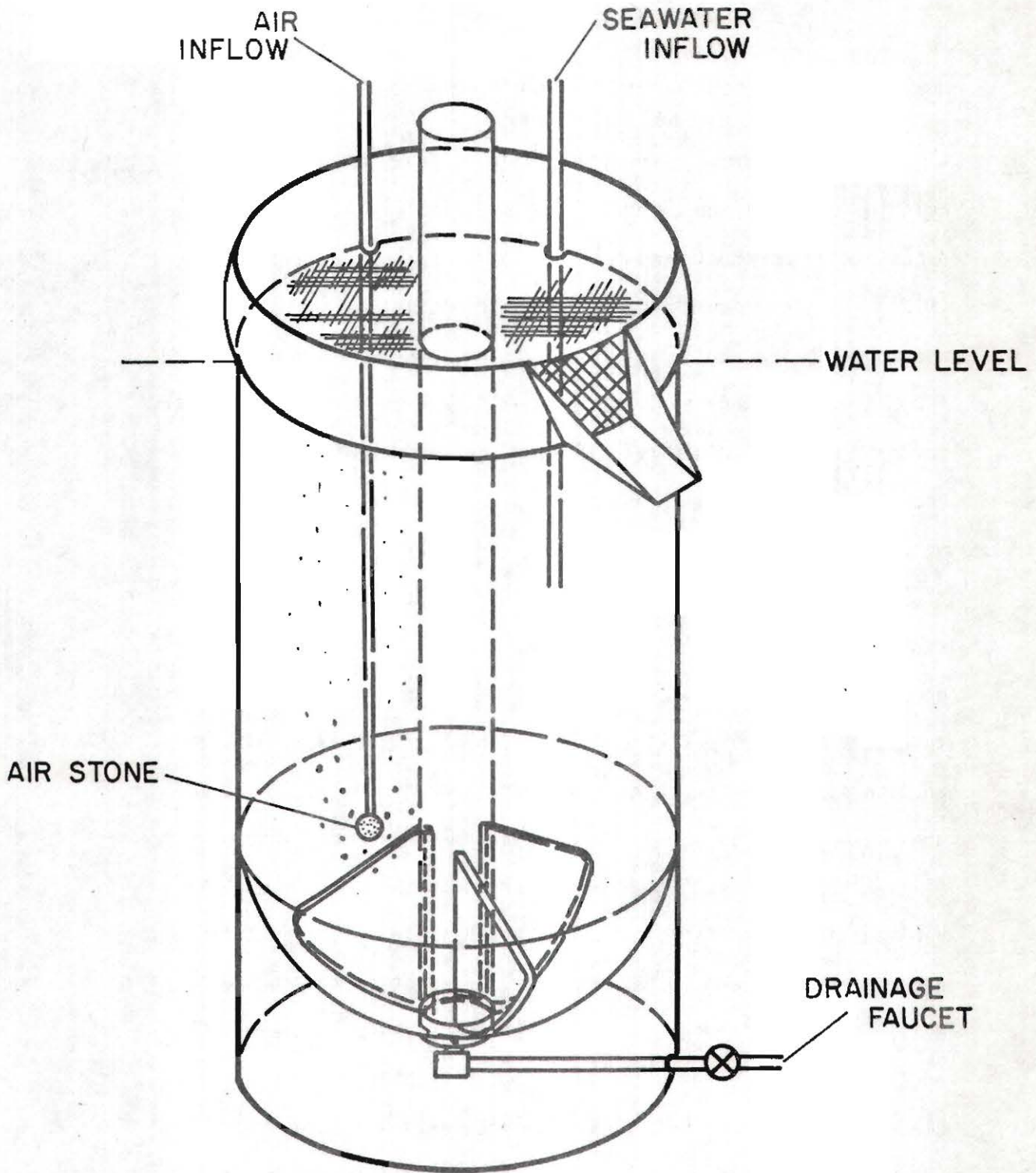
Fig. 3 - Hatching jar; clear acrylic
cylinder and PVC rim

until they were placed in hatching boxes (Fig. 4) just before the experiment was begun. The eggs for the other experiments were placed in hatching boxes a few hours after fertilization.

The salinity of the water used in the experiments was 32 ± 2 ^{o}/oo and the ambient temperature of the seawater in the acclimation tank was controlled by regulating the flow of water entering the tank. It was necessary to place some hatching boxes in different acclimation tanks. There was, therefore, some small variation in the range of ambient water temperatures among the several experiments: cleavage (L) 15.5 - 16.3 °C, cleavage (S) 14.5 - 16.0 °C (L and S are abbreviations for large and small tank), early embryo 14.5 - 15.0 °C, late embryo 14.0 - 15.0 °C.

A 60 x 15 mm glass petri dish was used to transfer the fertilized eggs to the hatching boxes. Varying numbers of fertilized eggs were placed in the hatching boxes: cleavage, 34 to 320; early embryo, 167 to 1963; late embryo, 66 to 540. Replicates were included when enough eggs were available. Each hatching box consisted of a polyvinyl chloride frame covered with monofilament bolting cloth, Nitex, 243 μm mesh opening (Fig. 4), which retained the newly hatched larvae.
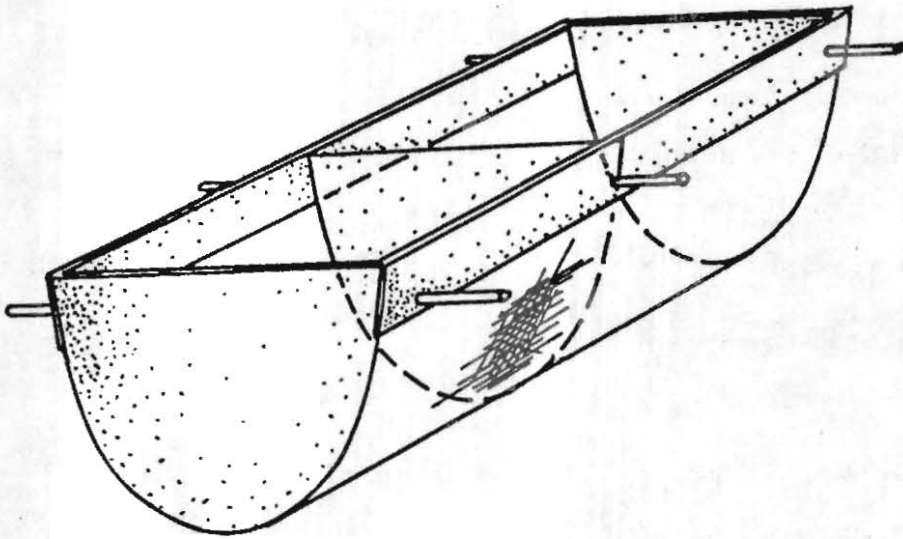
Fig. 4 - Hatching box; PVC frame covered with
monofilament bolting cloth, Nitex,
243 μm mesh opening. Strips of poly-
ethelene foam provided floatation

Experimental Procedure

Before beginning the thermal shock, a subsample was removed from a control hatching box to determine the developmental stage of the eggs. It was assumed that the eggs in the other hatching boxes were in a similar stage of development.

The two control hatching boxes experienced the same physical handling as the experimental boxes except that they were not subjected to the thermal shock. Each hatching box was floated into a plastic container by submerging the container beneath the hatching box in the acclimation tank. At the appropriate time a hatching box was transferred from the acclimation tank (base temperature) to a partially filled 5 gal (18.9 ℓ) aquarium, that was partially submerged in the well of a constant temperature bath (Blue M, model number MR-3220A-1). The aquarium was aerated and was used as the experimental chamber. At each ΔT, a different hatching box was placed in the experimental chamber for 2, 4, 8, or 16 min. The ΔT in the initial experiment was 8°C, in subsequent experiments the ΔT was increased by an increment of 2°C, until the final ΔT of 20°C was attained. An aquarium heater was used to decrease the time required to equilibrate the temperature of the aquarium to that in the constant temperature bath. Following the thermal shock, the experimental subsamples, in their hatching boxes, were quickly returned to the acclimation tank. At the time of the thermal shock the acclimation or base temperatures for

the various experiments were: cleavage experiment 16.0°C, early embryo experiment 14.5°C, late embryo experiment 14.0°C.

The temperatures of the water in the constant temperature bath and in the aquarium were monitored during the experiments using two calibrated thermometers. In all experiments, the temperatures of the bath and aquarium were essentially the same with the exception of the late embryo experiment at Δ18 during which the aquarium was 0.5°C higher than the bath for 10-12 min.

After hatching, the larvae were preserved in 10% buffered formalin for later analysis. Approximately 10 days later the 10% solution was replaced with a 5% solution.

Primary Separation of Samples

A dissecting microscope with a 10X (power) ocular was used in segregating the samples.

Eggs

The following terminology is used throughout the remainder of this paper.

Aborted eggs were defined as those eggs that stopped developing before the tests were begun. Such eggs were in the cleavage, blastula, or gastrula stage of development. For many of them it was impossible to tell which of the three stages the eggs were in since the cellular material formed an amorphous

mass (Fig. 5).

Shocked eggs were defined as those eggs that stopped developing after they were subjected to a thermal shock. They are characterized by an embryonic form in the egg (Fig. 6) or pigmentation spots in the amorphous mass, or pre-hatched larvae that were opaque.

Delayed hatch eggs were defined as those eggs that still had larvae enclosed and were transparent (Fig. 7). Those that were opaque were classified with the shocked eggs.

Larvae

Viable larvae were defined as those larvae that hatched, were normally formed, and transparent (Fig. 8 - 13).

Non-viable larvae were defined as those larvae that were imperfectly formed or were opaque (Fig. 14).

Further Separation of the Viable Larvae

A dissecting microscope with a 15X ocular was used in segregating the samples of viable larvae. This was done only for the experiments containing complete sets of temperature-time exposures, cleavage and late embryo experiments, and not for the early embryo experiment for which data are missing.

The viable larvae were further subdivided according to whether or not they showed vertebral or other deformities. The
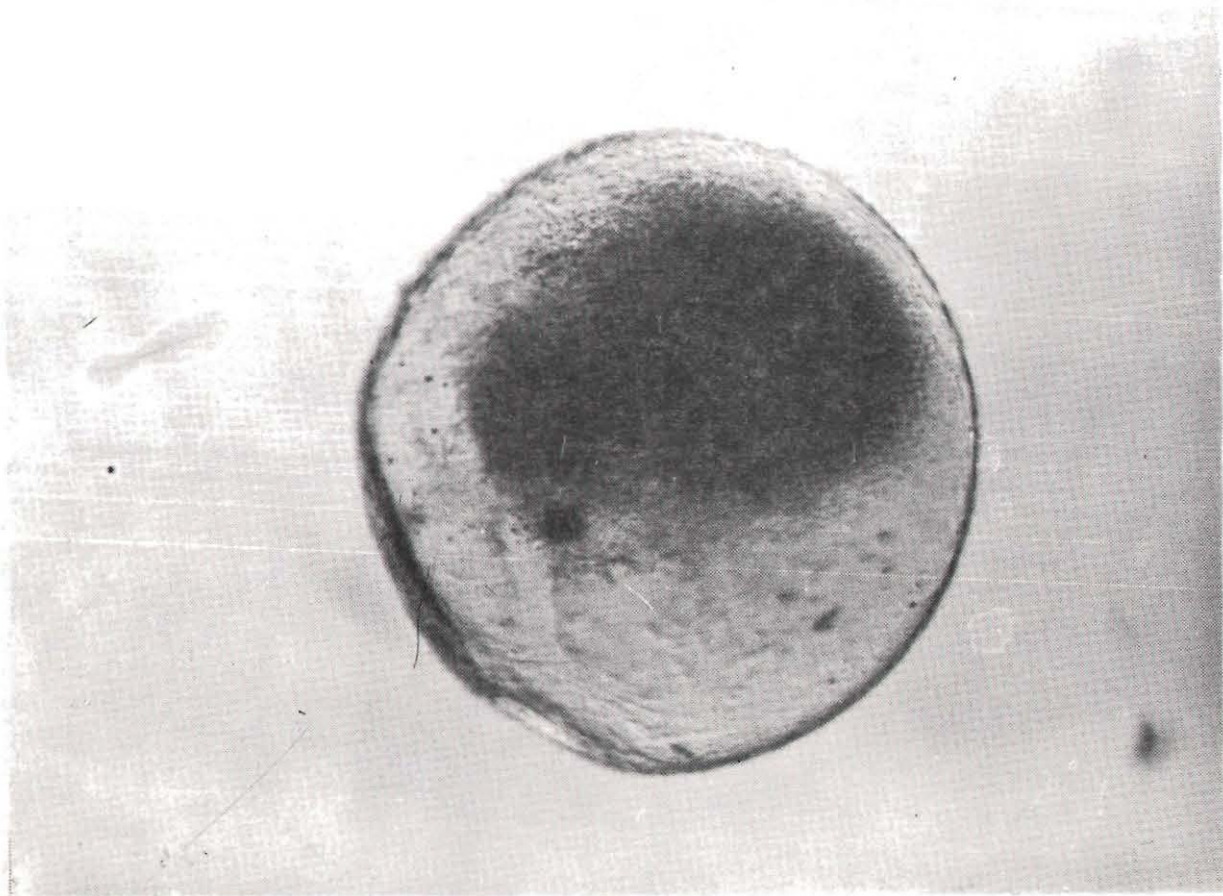
Fig. 5 - An aborted egg
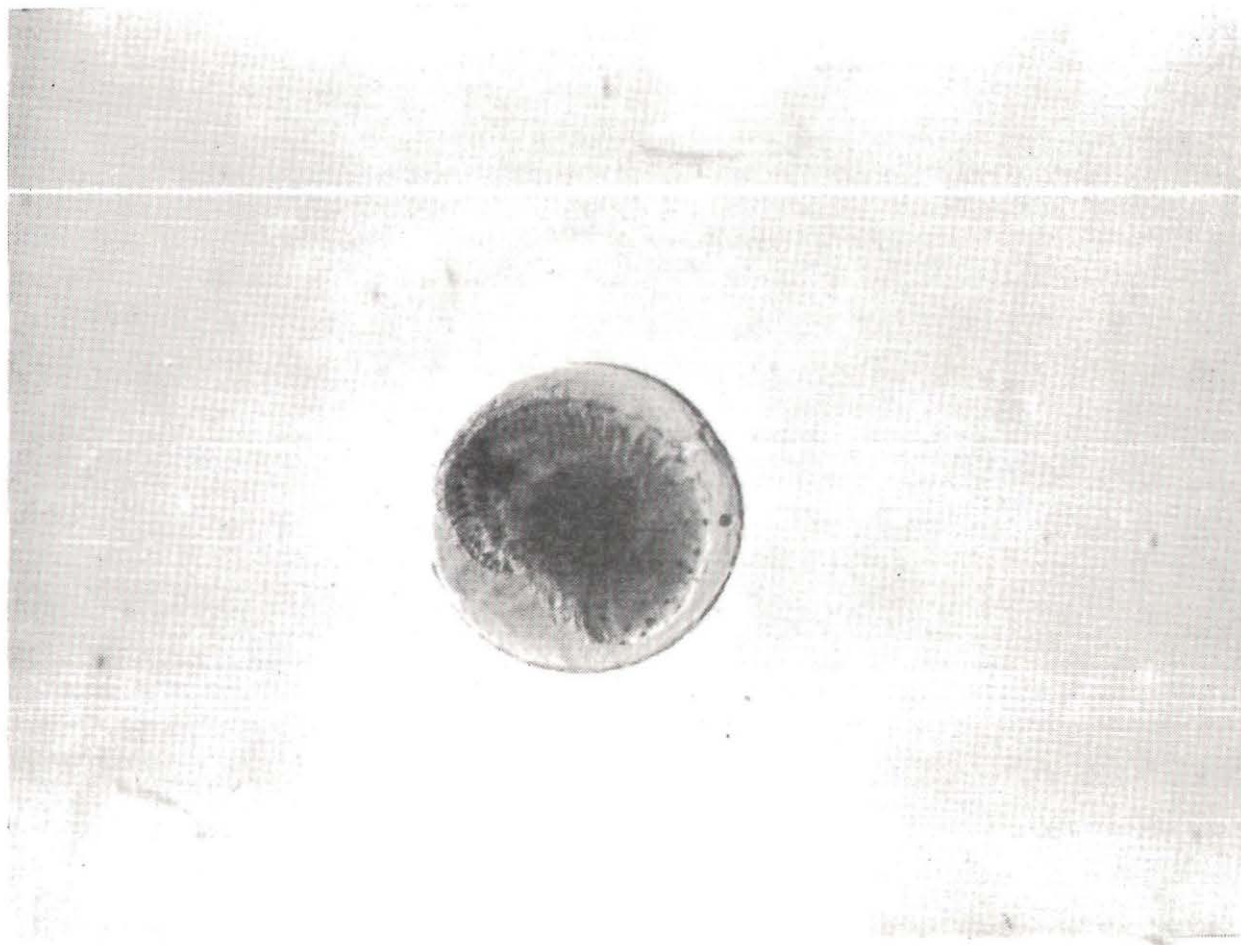
Fig. 6 - A shocked egg
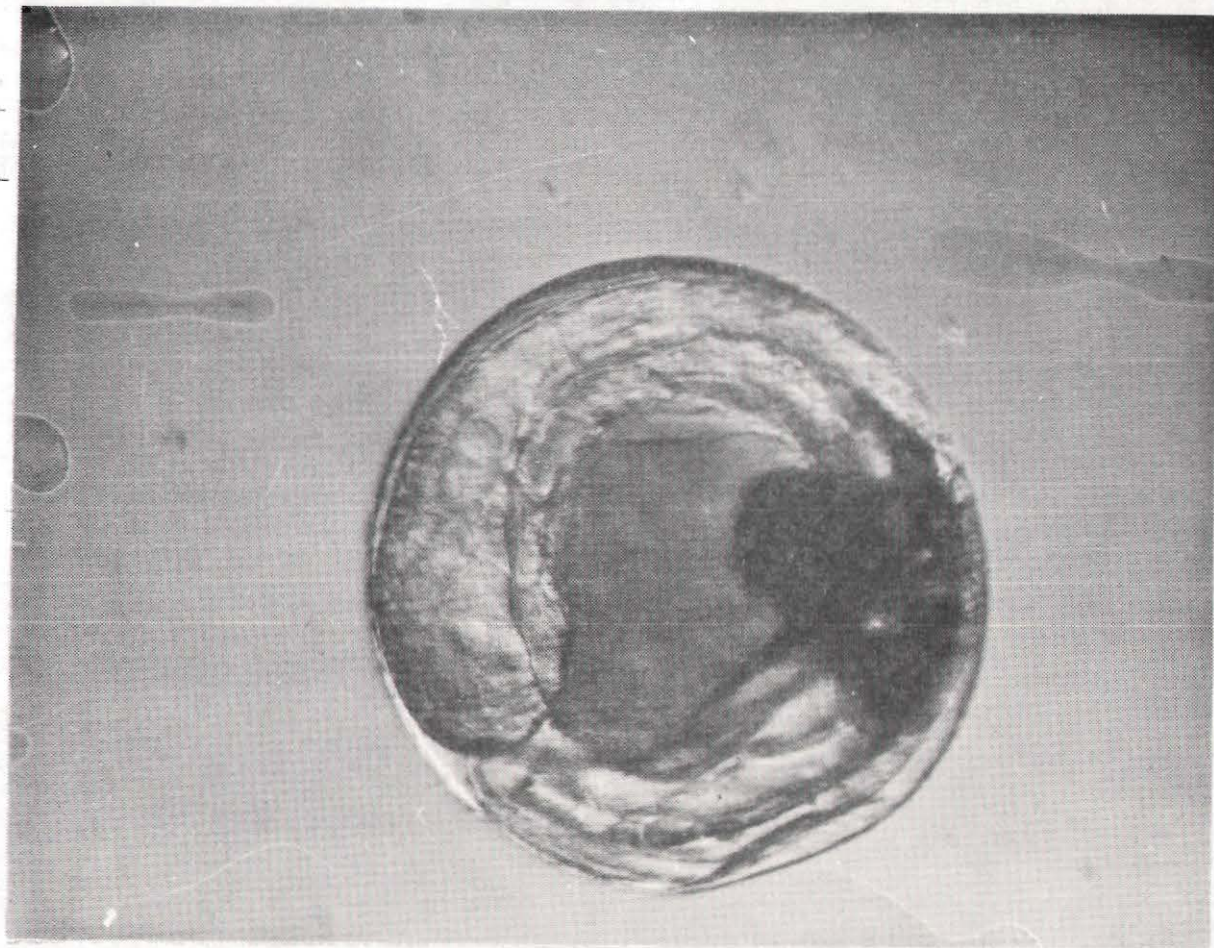
Fig. 7 - A delayed hatched egg
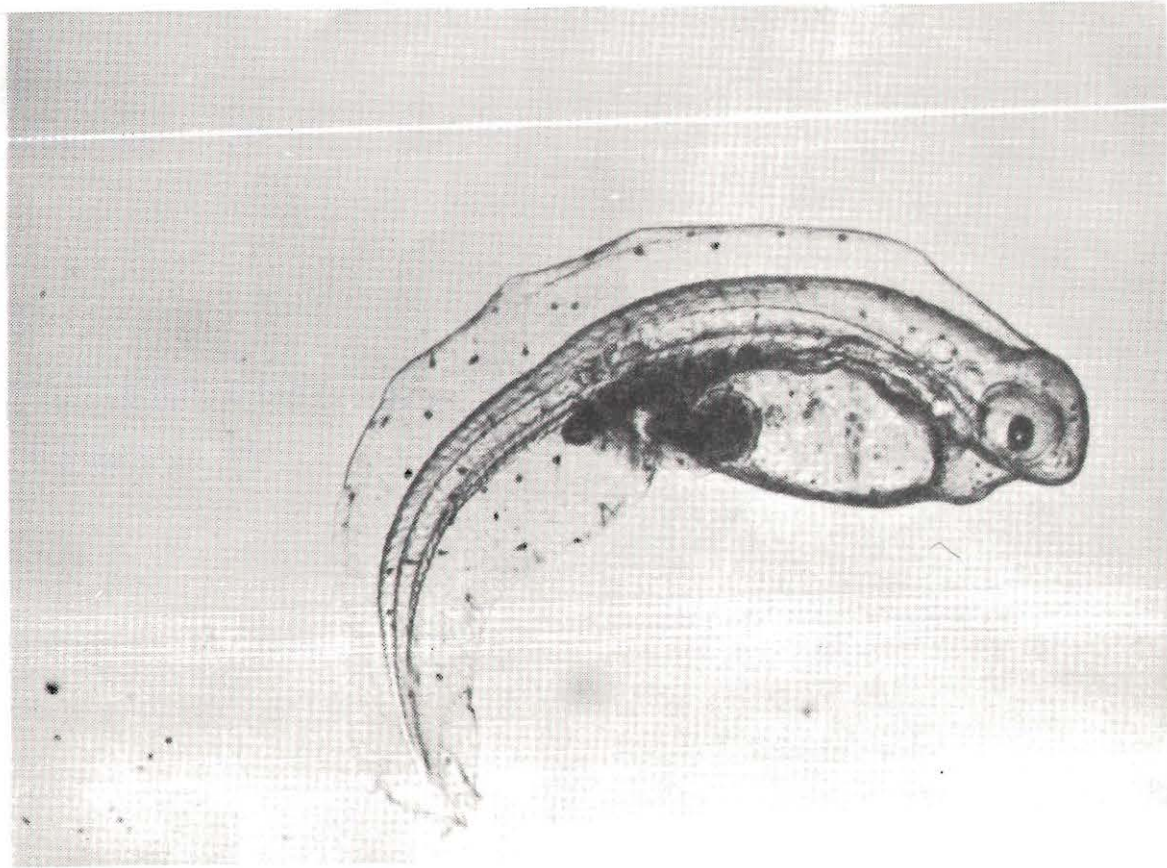
Fig. 8 - Larva exhibiting kyphosis
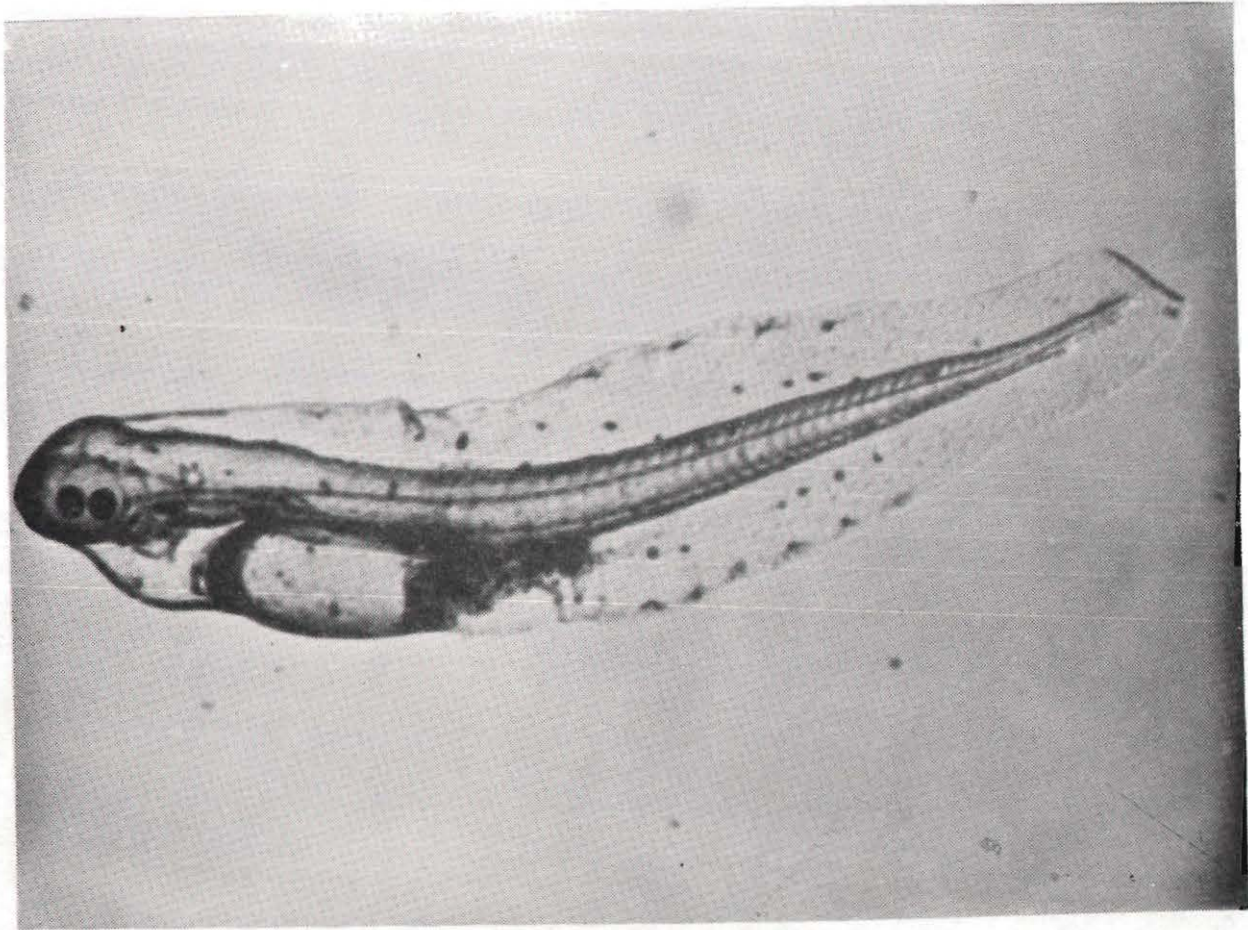
Fig. 9 - Larva exhibiting lordosis
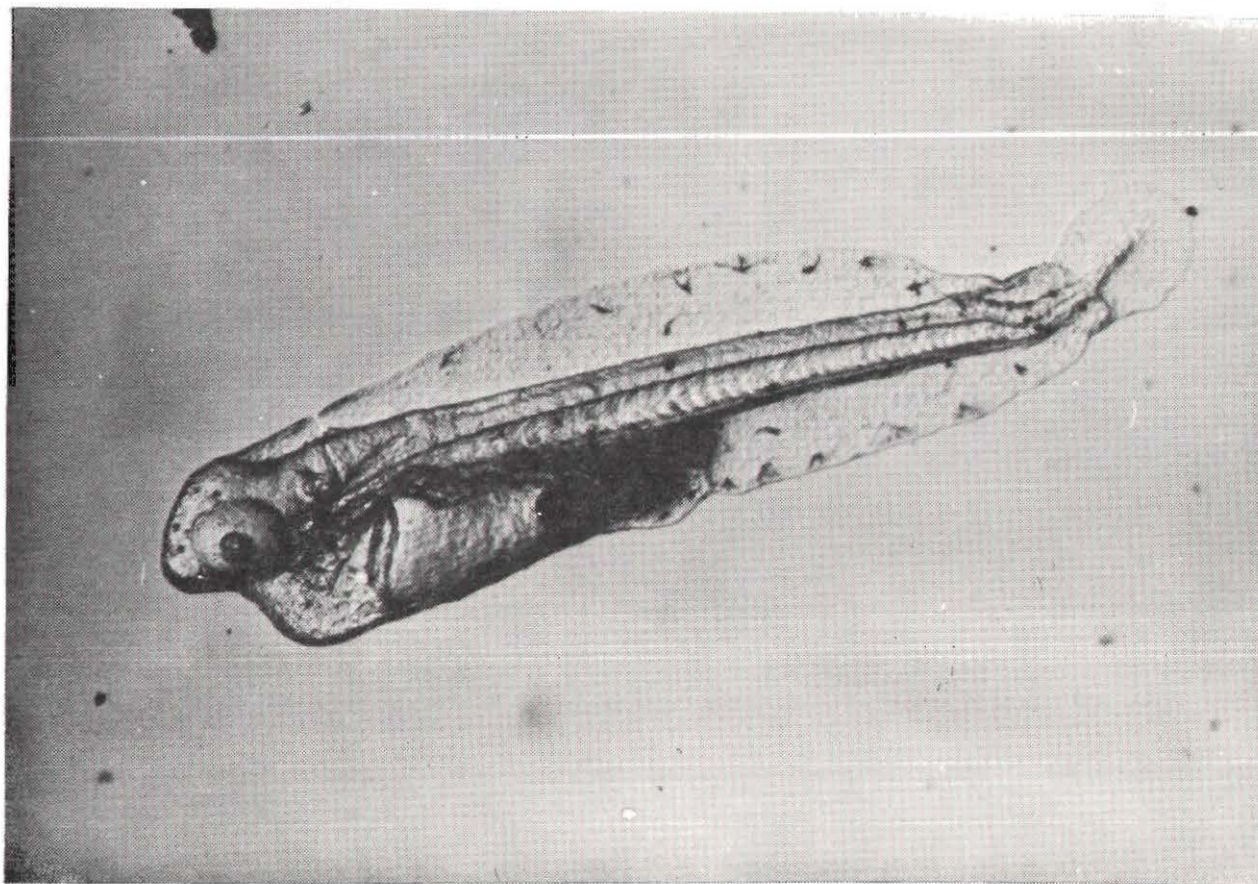
Fig. 10 - Larva exhibiting a tail anomaly
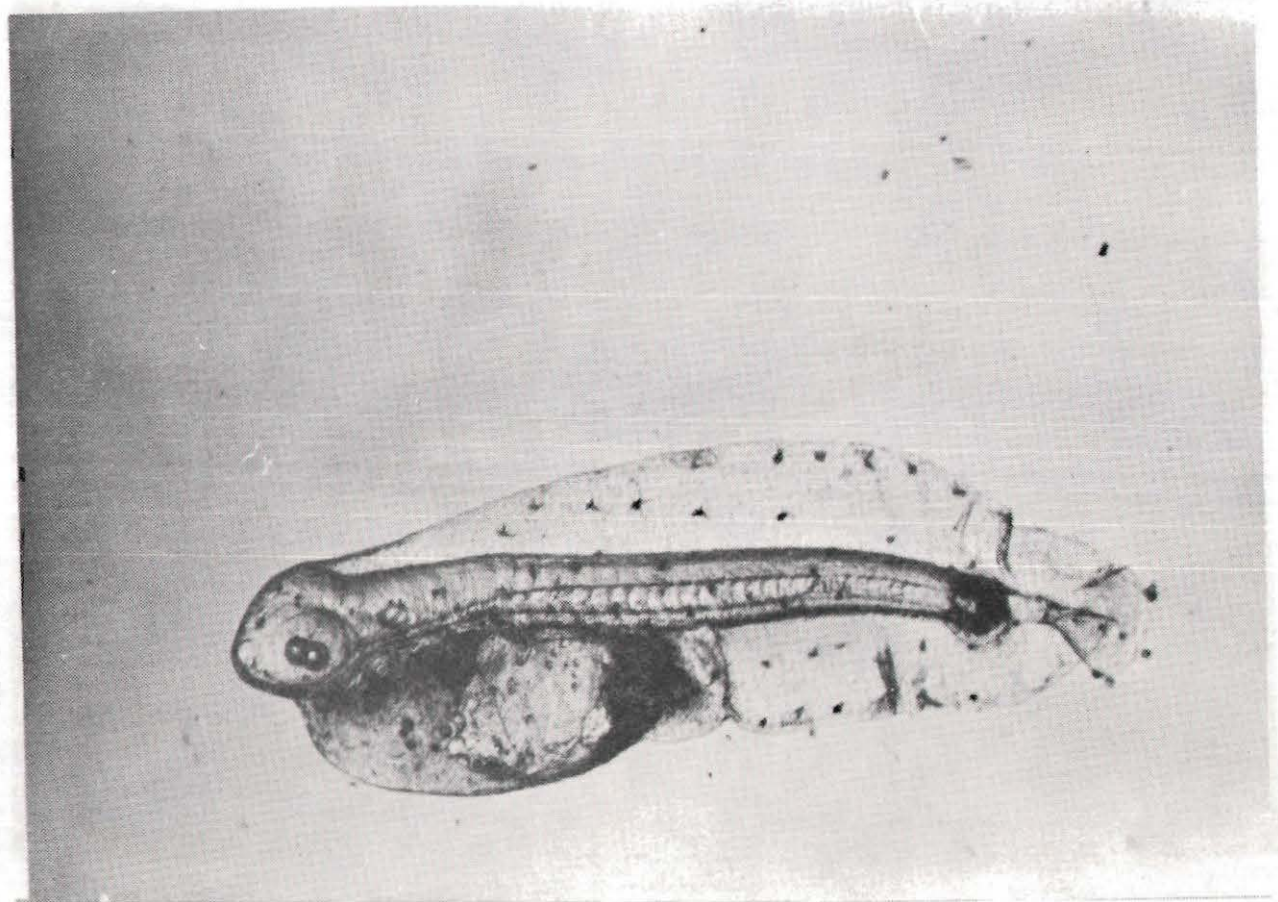
Fig. 11 - Larva exhibiting a tail not fully extended
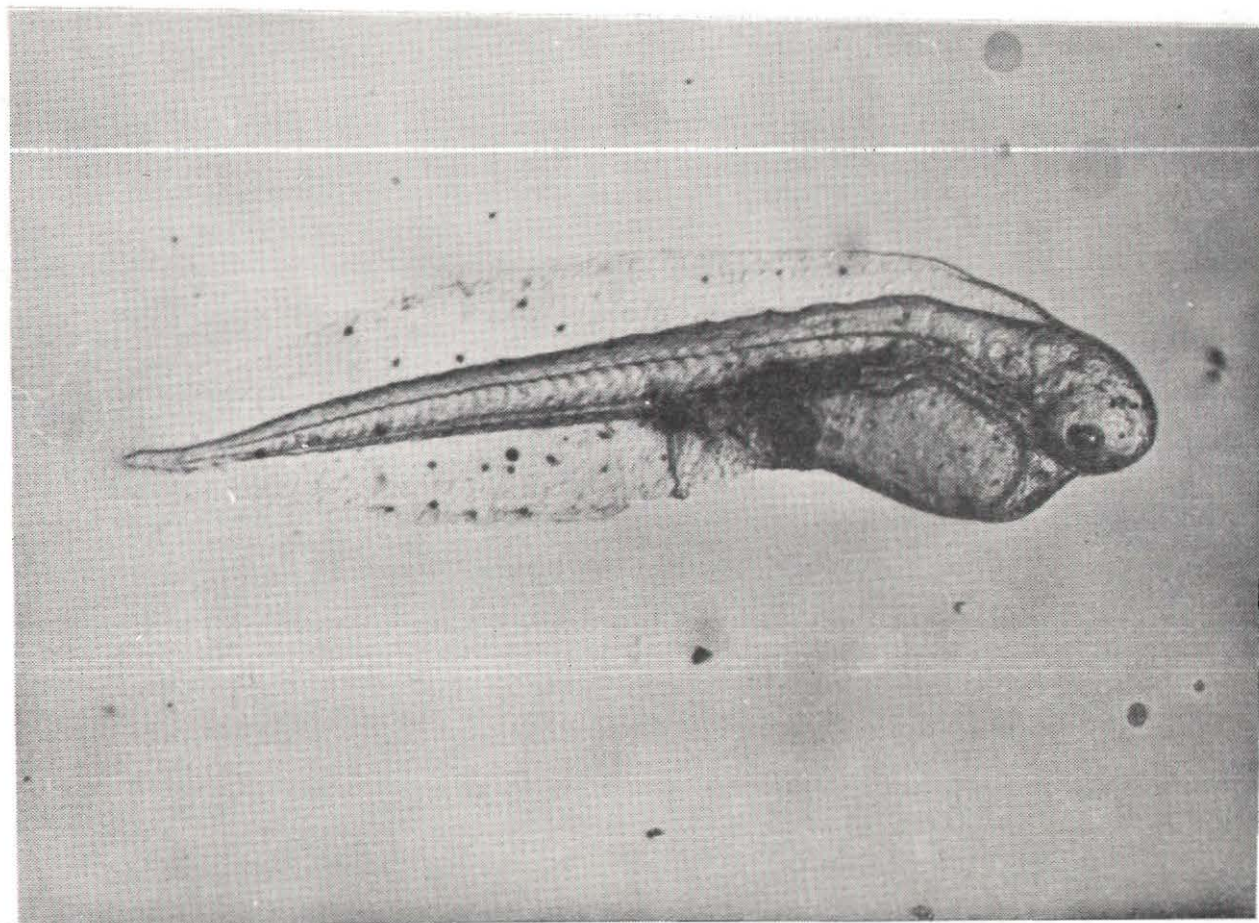
Fig. 12 - Larva exhibiting flexture of the head
region with slight tail anomaly
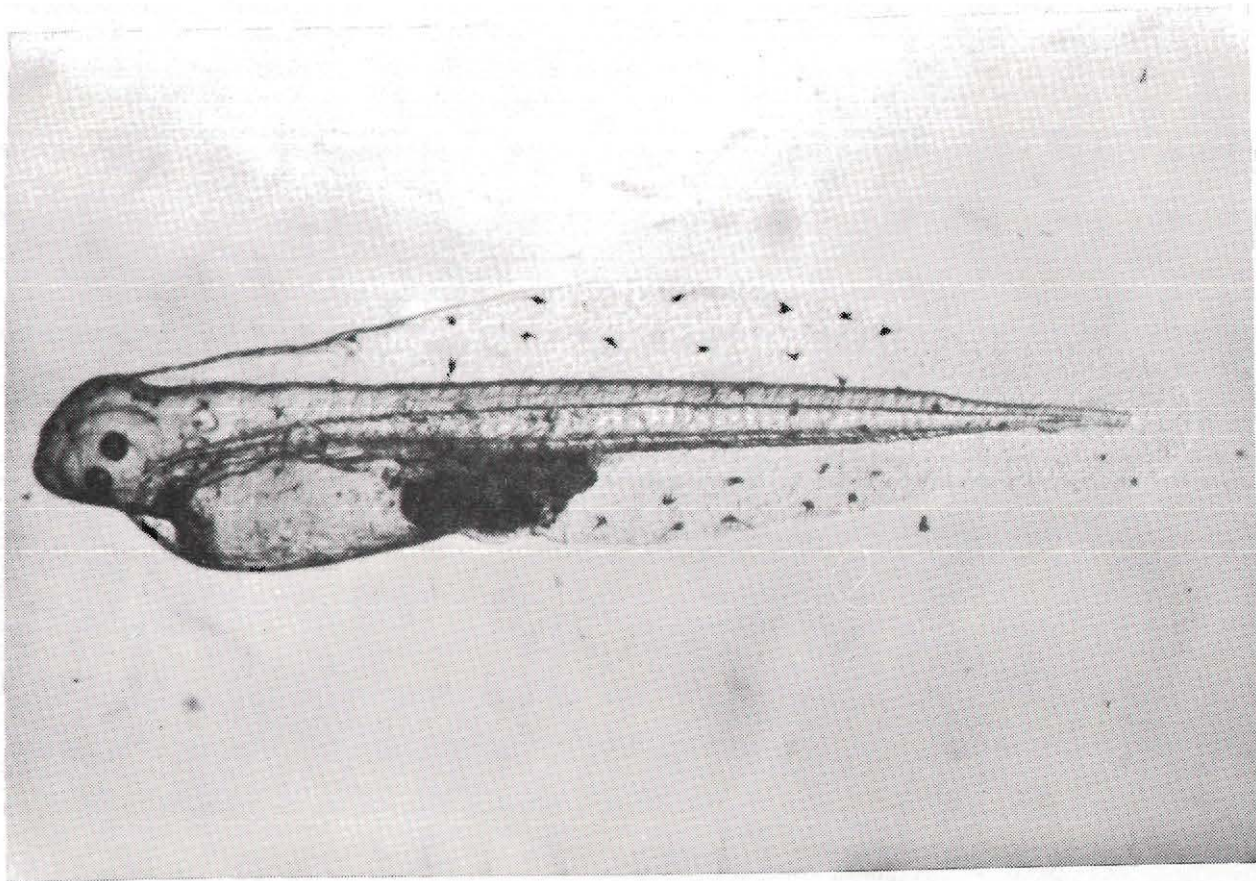
Fig. 13 - Normal larva

Fig. 14 - Non-viable larva

observable vertebral deformities considered were: kyphosis (Fig. 8), lordosis (Fig. 9), tail anomalies (Fig. 10), tail not fully extended (Fig. 11), and flexures of the head region (Fig. 12). Other deformities included abnormal shortness of larvae, yolk-sac disproportional to larval length, and larvae which had just hatched. Larvae having deformities other than those enumerated were considered as a group. Finally, there were the normal larvae which showed no deformities (Fig. 13).

If a larva had more than one of the characteristics enumerated above, each characteristic was counted, the number of larvae exhibiting this trait was listed in parentheses in summary tables; the total number of organisms exhibiting this trait is found to the left of this number; and the difference between the numbers is the number of organisms exhibiting this trait once (Tables 1 and 2). Larvae not having the tail fully extended were also enumerated as having tail anomalies.

Statistical Method

Thermal Mortality

The samples and their controls were segregated, and counted into the following categories: aborted eggs, shocked eggs, and delayed hatch. The larvae resulting from the hatching of the eggs were placed in one of two categories: viable larvae and non-viable larvae. The number of individuals in each category

TABLE 1

CLEAVAGE EXPERIMENT

The ΔT-t Level Counts of Summer Flounder Larvae
Characterized by Abnormality Traits

ΔT-t (°C-Min)	Normal Larvae	Abnormal Larvae	Kyphosis	Lordosis[4]	Tail Anomalies[4]		Head Flexures[4]	
[1]C₁	2	15	3	0	7	(5)	10	
[2]C₂	35	43	3	9	12	(9)	28	
[3]C₁ + C₂	37	58	6	9	19	(14)	38	
8-2	0	17	5	1	9	(9)	11	
8-4	32	56	7	12 (6)	20	(15)	38	
8-8	6	16	2	1 (1)	7	(5)	10	
8-16	7	4	1	2	0	(0)	1	
10-2	2	79	37	1	23	(23)	31	
10-4	14	66	7	10	31	(17)	38	(4)
10-8	27	65	6	7	32	(22)	46	(4)
10-16	10	47	12	0	23	(17)	29	
12-2	13	94	16	1	46	(39)	66	
12-4	12	46	7	4	29	(17)	22	
12-8	2	31	2	4	14	(14)	24	
12-16	0	59	12	3	27	(26)	41	
14-2	11	51	7	2	16	(10)	19	
14-4	3	24	2	5	10	(9)	15	
14-8	3	22	3	4	13	(10)	11	
14-16	0	21	4	4 (2)	12	(12)	13	
16-2	0	40	17	1	17	(17)	22	
16-4	---	---	---	---	---	---	---	
16-8	3	12	3	1	4	(3)	7	
16-16	0	0	0	0	0	(0)	0	
18-2	1	7	1	2	4	(3)	3	
18-4	0	2	1	0	2	(2)	1	
18-8	0	1	0	0	1	(1)	0	
18-16	0	1	0	0	0	(0)	1	
20-2	2	2	0	1	0	(0)	0	
20-4	0	2	0	0	2	(1)	0	
20-8	0	0	0	0	0	(0)	0	
20-16	0	7	4	0	1	(1)	0	

[1]C₁ is the first control, Δ0-0.
[2]C₂ is the second control, Δ0-0.
[3]C₁ + C₂ is the first plus the second control, Δ0-0.
[4]Number of larvae having multiple abnormality traits in parentheses.

TABLE 1 (continued)

Short or Large Yolk-Sac[4]		Have Just Hatched	Tail Not Fully Extended[4]	Others	Total Larvae In Sample
(0)	(0)	0	(0)	None	17
(4)	(0)	0	(1)	None	78
(4)	(0)	0	(1)	None	95
(0)	(0)	0	(0)	None	17
(0)	(0)	0	(0)	None	88
(0)	(1)	0	(0)	None	22
(0)	(0)	0	(0)	None	11
(0)	(0)	7	(0)	None	81
(0)	(0)	0	(0)	1 tail bent > 150°	80
(0)	(0)	0	(0)	None	92
(0)	(0)	0	(0)	None	57
(0)	(0)	4	(0)	3 tail bent > 150°	107
(0)	(0)	0	(0)	None	58
(1)	(0)	0	(0)	1 tail bent > 150°	33
(0)	(0)	1	(0)	None	59
(0)	(0)	0	(0)	16 decomposing; 1 tail bent > 150°	62
(0)	(0)	0	(0)	1 tail bent > 150°	27
(1)	(0)	0	(0)	None	25
(0)	(0)	2	(0)	None	21
(0)	(0)	0	(0)	None	40
---	---	---	---	large number decomposing	---
(0)	(0)	0	(0)	None	15
(0)	(0)	0	(0)	None	0
(0)	(0)	0	(0)	None	8
(0)	(0)	0	(0)	None	2
(0)	(0)	0	(0)	1 V-shape	1
(0)	(0)	0	(0)	None	1
(0)	(0)	1	(0)	None	4
(0)	(0)	0	(0)	None	2
(0)	(0)	0	(0)	None	0
(0)	(0)	0	(0)	None	7

(Tables 3, 4, and 5) was converted to percentages of the total sample (Tables 6, 7, and 8). Table 7 was recomputed to exclude aborted eggs, the naturally occurring mortality, because the eggs had been placed in the hatching boxes soon after fertilization and had not been subjected to a thermal shock for two days (Table 9). Control 1, side B, (C_1B) of the early embryo experiment, has been excluded from consideration in calculating the mean for the controls, because the percentage of non-viable larvae, 17.32% (Table 9), substantially exceeded the percentages of the mean present in the other controls, 4.40 to 4.43% (Table 10). The range of the other control samples vary from 2.82 to 6.82% (Table 9). Two of the subsamples C_2A and $\Delta20$-16 have been re-counted and they are indicated by an asterisk (*) (Tables 4, 9, and 10). Two cases, Case I (C_1A, C_2A, and C_2B) and Case II (C_1A, C_2A*, and C_2B) are now available for analyzing the early embryo data.

Since microscopic examination revealed that the delayed hatch eggs were still viable, the delayed hatch counts were combined with the viable larvae counts to define a measure of "potential" hatching success.

The results were then analyzed using the Chi-square test. The Chi-square test is particularly useful in dealing with enumerative data. It is appropriate for testing whether a set of observed values differs significantly from those which would

TABLE 2

LATE EMBRYO EXPERIMENT

The ΔT-t Level Counts of Summer Flounder Larvae Expressed as
Percentages of Larvae Characterized by Various Abnormality Traits

ΔT-t ($^\circ$C-Min)	Normal Larvae	Abnormal Larvae	Kyphosis	Lordosis	Tail Anomalies[4]		Head Flexures
[1]C_1	440	82	12	1	51	(20)[4]	35
[2]C_2	291	78	4	0	49	(8)	20
[3]$C_1 + C_2$	731	160	16	1	100	(28)	55
8-2	308	43	6	0	20	(5)	19
8-4	---	---	---	---	---	---	---
8-8	289	45	7	2	14	(5)	20
8-16	403	28	0	0	15	(4)	10
10-2	309	49	4	3	28	(4)	18
10-4	215	41	7	2	16	(4)	13
10-8	269	55	0	0	23	(10)	38
10-16	356	118	9	3	26	(9)	88
12-2	300	41	4	1	23	(7)	18
12-4	337	68	10	1	30	(19)	41
12-8	117	42	6	3	21	(9)	18
12-16	86	36	4	0	15	(7)	22
14-2	145	24	3	0	15	(9)	13
14-4	203	62	4	3	17	(13)	47
14-8	81	74	6	1	14	(9)	61
14-16	95	62	4	0	16	(12)	52
16-2	78	33	2	0	9	(7)	29
16-4	141	94	4	1	20	(13)	77
16-8	75	27	0	0	8	(5)	23
16-16	60	106	7	3	38	(21)	76
18-2	11	43	4	3	8	(5)	33
18-4	22	62	9	2	11	(6)	43
18-8	22	82	8	1	17	(16)	72
18-16	37	16	0	0	4	(3)	14
20-2	32	104	4	1	20	(13)	92
20-4	13	39	12	2	11	(9)	22
20-8	3	19	5	0	5	(3)	12
20-16	8	34	3	0	12	(5)	26

[1]C_1 is the first control, Δ0-0.
[2]C_2 is the second control, Δ0-0.
[3]$C_1 + C_2$ is the first plus second control, Δ0-0.
[4]Number of larvae having multiple abnormality traits in parentheses.

TABLE 2 (continued)

Short or Large York-Sac[4]		Have Just Hatched	Tail Not Fully Ex-tended[4]	Others	Total Larvae in Sample
(11)	(1)	2	(9)	None	522
(8)	(1)	1	(7)	2 V-shape	369
(19)	(2)	3	(16)	2 V-shape	891
(5)	(1)	2	(5)	None	351
---	---	---	---	large number decomposing	---
(2)	(0)	2	(2)	None	334
(7)	(5)	1	(2)	1 V-shape	431
(7)	(0)	2	(6)	None	358
(7)	(6)	6	(6)	None	256
(2)	(1)	5	(0)	None	324
(18)	(1)	1	(8)	1 V-shape; 1 tail bent > 150°	474
(5)	(0)	2	(5)	None	341
(9)	(1)	4	(6)	None	405
(7)	(1)	1	(5)	1 tail bent > 150°	159
(1)	(0)	1	(1)	1 tail bent > 150°	122
(7)	(0)	2	(6)	None	169
(9)	(1)	3	(7)	None	265
(5)	(1)	0	(4)	1 tail bent > 150°	155
(4)	(0)	1	(3)	1 decomposing	157
(3)	(0)	0	(3)	None	111
(10)	(0)	0	(9)	1 V-shape; 3 decomposing; 1 tail bent > 150°	235
(3)	(1)	0	(2)	None	102
(5)	(0)	1	(2)	2 V-shape	166
(0)	(0)	0	(0)	None	54
(1)	(0)	3	(0)	None	84
(4)	(0)	0	(4)	1 V-shape	104
(2)	(0)	0	(0)	1 tail bent > 150°	53
(3)	(0)	0	(3)	None	136
(1)	(0)	1	(1)	None	52
(1)	(0)	0	(0)	None	22
(1)	(0)	0	(0)	None	42

Table 3

CLEAVAGE EXPERIMENT

Counts of Summer Flounder Eggs and Larvae for the ΔT-t Levels
Held in Small and Large Tanks (S, L).

ΔT-t (°C-min)	Tank Size	EGGS		LARVAE		Potential Hatch (3) + (4)	Total
		Shocked (2)	Delayed Hatch (3)	Viable (4)	Non-Viable (5)		
[1]C_1	S	17	-	17	-	17	34
[2]C_2	L	195	-	82	43	82	320
8-2	S	51	2	13	8	15	74
8-4	L	169	-	79	26	79	274
8-8	L	96	-	20	21	20	137
8-16	L	33	-	12	15	12	60
10-2	S	174	16	80	11	96	281
10-4	S	115	-	89	4	89	208
10-8	S	78	-	98	2	98	178
10-16	S	225	-	55	3	55	283
12-2	S	127	1	113	6	114	247
12-4	L	79	-	58	2	58	139
12-8	L	171	-	38	2	38	211
12-16	S	79	2	56	5	58	142
14-2	S	45	-	63	2	63	110
14-4	L	17	-	28	1	28	46
14-8	S	24	-	30	1	30	55
14-16	S	121	3	23	1	26	148
16-2	S	161	-	32	15	32	208
16-4	S	232	13	63	6	76	314
16-8	S	65	1	14	2	15	82
16-16	S	29	-	-	-	-	29
18-2	S	125	-	8	33	8	166
18-4	S	209	-	1	8	1	218
18-8	S	58	-	1	1	1	60
18-16	S	184	-	1	1	1	186
20-2	S	126	-	4	1	4	131
20-4	S	306	-	2	-	2	308
20-8	S	75	-	-	2	-	77
20-16	S	307	-	7	-	7	314

[1]C_1 is the first control, Δ0-0.

[2]C_2 is the second control, Δ0-0.

Table 4

EARLY EMBRYO EXPERIMENT

Counts of Summer Flounder Eggs and Larvae with Replication (A,B) for the ΔT-t levels.

ΔT-t ($^\circ$C-min)	EGGS Aborted (1)	Shocked (2)	Delayed Hatch (3)	LARVAE Viable (4)	Non-Viable (5)	Potential Hatch (3) + (4)	Total
$^1C_1$A	333	85	54	1445	46	1499	1963
$^1C_1$B	162	48	9	363	88	372	670
$^2C_2$A	150	25	16	850	49	866	1090
$^2C_2$A*	140	27	16	788	45	804	1016
$^2C_2$B	139	23	4	711	54	715	931
8-2A	3	1	-	31	290	31	325
8-2B	25	5	-	7	130	7	167
8-4A	141	3	-	8	145	8	297
8-4B	77	3	-	105	483	105	591
16-8A	80	4	-	10	525	10	619
16-8B	14	3	-	2	311	2	330
18-2A	205	12	-	35	406	35	658
18-2B	143	2	-	141	504	141	790
18-4A	95	3	-	73	511	73	682
18-4B	210	10	-	83	436	83	739
18-8A	77	10	-	336	188	336	611
18-8B	101	23	-	296	285	296	705
18-16A	223	17	2	998	29	1000	1269
18-16B	34	1	-	581	3	581	619
20-2A	40	9	-	324	54	324	427
20-2B	36	6	-	428	40	428	510
20-4A	32	1	-	372	-	372	405
20-4B	24	5	1	405	6	406	441
20-8A	133	21	3	418	5	421	580
20-8B	98	9	4	865	1	869	977
20-16A	197	24	56	559	2	615	838
20-16A*	188	57	43	571	2	614	861
20-16B	297	36	43	683	3	726	1062

*Sample was recounted

C_1 is the first control, Δ0-0.

C_2 is the second control, Δ0-0.

Table 5

LATE EMBRYO EXPERIMENT

Counts of Summer Flounder Eggs and Larvae for the ΔT-t levels.

ΔT-t (°C-min)	EGGS			LARVAE		Potential Hatch (3) + (4)	Total
	Aborted (1)	Shocked (2)	Delayed Hatch (3)	Viable (4)	Non-Viable (5)		
[1]C[1]	3	1	1	527	8	528	540
[2]C[2]	–	1	1	375	4	376	381
8-2	–	1	1	361	1	362	364
8-4	–	–	–	270	4	270	274
8-8	–	–	–	336	3	336	339
8-16	–	–	2	431	1	433	434
10-2	–	–	–	368	3	368	371
10-4	–	–	–	257	3	257	260
10-8	–	–	–	314	1	314	315
10-16	–	–	3	453	1	456	457
12-2	–	–	1	361	2	362	364
12-4	2	–	–	456	2	456	460
12-8	–	–	–	162	5	162	167
12-16	–	1	–	124	4	124	129
14-2	–	–	2	172	–	174	174
14-4	–	–	3	273	2	276	278
14-8	–	–	2	157	6	159	165
14-16	–	1	–	169	1	169	171
16-2	–	1	2	106	49	108	158
16-4	–	–	1	230	5	231	236
16-8	–	–	1	122	10	123	133
16-16	–	–	1	173	1	174	175
18-2	–	5	1	64	–	65	70
18-4	–	10	1	86	1	87	98
18-8	1	4	–	111	6	111	122
18-16	–	18	–	53	1	53	72
20-2	2	5	–	142	13	142	162
20-4	–	24	1	64	5	65	94
20-8	–	44	–	22	–	22	66
20-16	1	34	2	51	2	53	90

[1]C[1] is the first control, Δ0-0.

[2]C[2] is the second control, Δ0-0.

Table 6

CLEAVAGE EXPERIMENT

Counts of Summer Flounder Eggs and Larvae Expressed as Percentages of
the Total Count for the ΔT-t Levels Held in Small and Large Tanks (S, L).

| ΔT-t (°C min) | Tank Size | EGGS | | LARVAE | | Potential Hatch (3) + (4) |
		Shocked (2)	Delayed Hatch (3)	Viable (4)	Non-Viable (5)	
[1]C_1	S	50.00	-	50.00	-	50.00
[2]C_2	L	60.94	-	25.63	13.44	25.63
8-2	S	68.92	2.70	17.57	10.81	20.27
8-4	L	61.68	-	28.83	9.49	28.83
8-8	L	70.07	-	14.60	15.34	14.60
8-16	L	55.00	-	20.00	25.00	20.00
10-2	S	61.92	5.69	28.47	3.91	34.16
10-4	S	55.29	-	42.79	1.92	42.79
10-8	S	43.82	-	55.06	1.12	55.06
10-16	S	79.51	-	19.44	1.06	19.44
12-2	S	51.42	0.41	45.75	2.43	46.15
12-4	L	56.84	-	41.73	1.44	41.73
12-8	L	81.04	-	18.01	0.95	18.01
12-16	S	55.63	1.41	39.44	3.52	40.85
14-2	S	40.91	-	57.27	1.82	57.27
14-4	L	36.96	-	60.87	2.17	60.87
14-8	S	43.64	-	54.55	1.82	54.55
14-16	S	81.76	2.03	15.54	0.68	17.57
16-2	S	77.40	-	15.39	7.21	15.39
16-4	S	73.89	4.14	20.06	1.91	24.20
16-8	S	79.27	1.22	17.07	2.44	18.29
16-16	S	100.00	-	-	-	-
18-2	S	75.30	-	4.82	19.88	4.82
18-4	S	95.87	-	0.46	3.67	0.46
18-8	S	96.67	-	1.67	1.67	1.67
18-16	S	98.93	-	0.54	0.54	0.54
20-2	S	96.18	-	3.05	0.76	3.05
20-4	S	99.35	-	0.65	-	0.65
20-8	S	97.40	-	-	2.60	-
20-16	S	97.77	-	2.23	-	2.23

[1]C_1 is the first control, Δ0-0.

[2]C_2 is the second control, Δ0-0.

Table 7

EARLY EMBRYO EXPERIMENT

Counts of Summer Flounder Eggs and Larvae Expressed as Percentages
of the Total Count for the ΔT-t Levels.

ΔT-t (°C min)	EGGS			LARVAE		Potential Hatch (3) + (4)
	Aborted (1)	Shocked (2)	Delayed Hatch (3)	Viable (4)	Non-Viable (5)	
C_1A	16.94	4.33	2.75	73.61	2.34	76.36
C_1B	24.18	7.16	1.34	54.18	13.13	55.52
C_2A	13.76	2.29	1.47	77.98	4.50	79.45
C_2A*	13.78	2.66	1.58	77.56	4.43	79.13
C_2B	14.93	2.47	0.43	76.37	5.80	76.80
8-2A	0.92	0.31	-	9.54	77.84	9.54
8-2B	14.97	2.99	-	4.19	77.84	4.19
8-4A	47.47	1.01	-	2.69	48.82	2.69
8-4B	13.03	0.51	-	17.77	81.73	17.77
16-8A	12.92	0.65	-	1.62	84.81	1.62
16-8B	4.24	0.91	-	0.61	94.24	0.61
18-2A	31.15	1.82	-	5.32	61.70	5.32
18-2B	18.10	0.25	-	17.85	63.80	17.85
18-4A	13.93	0.44	-	10.70	74.93	10.70
18-4B	28.42	1.35	-	11.23	59.00	11.23
18-8A	12.60	1.64	-	54.99	30.77	54.99
18-8B	14.33	3.26	-	41.99	40.43	41.99
18-16A	17.57	1.34	0.16	78.65	2.29	78.80
18-16B	5.49	0.16	-	93.86	0.49	93.86
20-2A	9.37	2.11	-	75.88	12.65	75.88
20-2B	7.06	1.18	-	83.92	7.84	83.92
20-4A	7.90	0.25	-	91.85	-	91.85
20-4B	5.44	1.13	0.23	91.84	1.36	92.06
20-8A	22.93	3.62	0.52	72.07	0.86	72.59
20-8B	10.03	0.92	0.41	88.54	0.10	88.95
20-16A	23.51	2.86	6.68	66.71	0.24	73.39
20-16A*	21.84	6.62	4.99	66.32	0.23	71.31
20-16B	27.97	3.39	4.05	64.31	0.28	68.36

*Sample was recounted.

C_1 is the first control, $\Delta 0$-0.

C_2 is the second control, $\Delta 0$-0.

Table 8

LATE EMBRYO EXPERIMENT

Counts of Summer Flounder Eggs and Larvae Expressed as Percentages
of the Total Count for the ΔT-t Levels.

ΔT-t (°C min)	EGGS			LARVAE		Potential Hatch (3) + (4)
	Aborted (1)	Shocked (2)	Delayed Hatch (3)	Viable (4)	Non-Viable (5)	
[1]C$_1$	0.56	0.19	0.19	97.59	1.48	97.78
[2]C$_2$	-	0.26	0.26	98.43	1.05	98.69
8-2	-	0.27	0.27	99.18	0.27	99.45
8-4	-	-	-	98.54	1.46	98.54
8-8	-	-	-	99.12	0.89	99.12
8-16	-	-	0.43	99.31	0.23	99.77
10-2	-	-	-	99.19	0.81	99.19
10-4	-	-	-	98.85	1.15	98.85
10-8	-	-	-	99.68	0.32	99.68
10-16	-	-	0.66	99.13	0.22	99.78
12-2	-	-	0.28	99.18	0.55	99.45
12-4	0.44	-	-	99.13	0.44	99.13
12-8	-	-	-	97.00	2.99	97.00
12-16	-	0.78	-	96.12	3.10	96.12
14-2	-	-	1.15	98.85	-	100.00
14-4	-	-	1.08	98.20	0.72	99.28
14-8	-	-	1.21	95.15	3.64	96.36
14-16	-	0.59	-	98.83	0.59	98.83
16-2	-	0.63	1.27	67.09	31.01	68.35
16-4	-	-	0.42	97.46	2.12	97.88
16-8	-	-	0.75	91.73	7.52	92.48
16-16	-	-	0.57	98.86	0.57	99.43
18-2	-	7.14	1.43	91.43	-	92.86
18-4	-	10.20	1.02	87.76	1.02	88.78
18-8	0.82	3.28	-	90.98	4.92	90.98
18-16	-	25.00	-	73.61	1.39	73.61
20-2	1.24	3.09	-	87.65	8.03	87.65
20-4	-	25.53	1.06	68.08	5.32	69.15
20-8	-	66.67	-	33.33	-	33.33
20-16	1.11	37.78	2.22	56.67	2.22	58.89

[1]C$_1$ is the first control, Δ0-0.

[2]C$_2$ is the second control, Δ0-0.

Table 9

EARLY EMBRYO EXPERIMENT

Counts of Summer Flounder Eggs and Larvae Expressed as Percentages
of the Total Count for T-t Levels from Δ18-16 to Δ20-16.

| | EGGS | | LARVAE | | |
	Shocked (2)	Delayed Hatch (3)	Viable (4)	Non-Viable (5)	Potential Hatch (3) + (4)
C_1A	5.22	3.31	88.65	2.82	91.96
C_1B	9.45	1.77	71.46	17.32	73.23
C_2A	2.66	1.70	90.43	5.21	92.13
C_2A*	3.08	1.83	89.95	5.14	91.78
C_2B	2.90	0.51	89.77	6.82	90.28
18-16A	1.63	0.19	95.41	2.77	95.60
18-16B	0.17	-	99.32	0.51	99.32
20-2A	2.33	-	83.72	13.95	83.72
20-2B	1.27	-	90.30	8.44	90.30
20-4A	0.27	-	99.73	-	99.73
20-4B	1.20	0.24	97.12	1.44	97.36
20-8A	4.70	0.72	93.46	1.12	94.18
20-8B	1.02	0.46	98.41	0.11	98.86
20-16A	3.74	8.74	87.21	0.31	95.94
20-16A*	8.47	6.39	84.84	0.30	91.23
20-16B	4.71	5.62	89.28	0.39	94.90

* Sample was recounted.

C_1 is the first control, Δ0-0.

C_2 is the second control, Δ0-0.

Table 10

Percentage of the Mean of Replicates of the Cleavage, Early Embryo, and Late Embryo Experiments.

ΔT-t (°C-min)	EGGS		LARVAE		Potential Hatch (3) + (4)	Total Mortality (2) + (5)
	Shocked (2)	Delayed Hatch (3)	Viable (4)	Non-Viable (5)		
Cleavage						
Controls	59.89	0.00	27.97	12.15	27.97	72.03
Early Embryo						
Controls (Case I)	3.96	2.20	89.41	4.43	91.61	8.09
Controls (Case II)	4.09	2.24	89.27	4.40	91.51	8.49
18-16	1.10	0.12	96.81	1.96	96.93	3.07
20-2	1.74	0.00	87.34	10.92	87.34	12.66
20-4	0.76	0.13	98.35	0.76	98.48	1.52
20-8	2.26	0.53	96.76	0.45	97.28	2.72
20-16	4.27	7.04	88.34	0.36	95.38	4.62
20-16*	6.47	5.98	87.20	0.35	93.18	6.82
Late Embryo						
Controls	0.22	0.22	97.94	1.30	98.15	1.52

*Sample was recounted.

occur if some specified hypothesis were true (Johnson, 1949). Our hypothesis is that there is no difference between mortalities in the experimental and control groups.

Sublethal Effects

The raw data (Tables 1 and 2) were converted to percentages of the total sample as well as the means of the controls (Tables 11 and 12).

These forms are equally amenable to the Chi-square test. Again, the hypothesis is that there is no difference between abnormalities found in the experimental samples and those found in the controls.

TABLE 11

CLEAVAGE EXPERIMENT

The ΔT-t Level Counts of Summer Flounder Larvae Expressed as
Percentages of Larvae Characterized by Various Abnormality Traits

ΔT-t (°C-Min)	Normal Larvae	Abnormal Larvae	Kyphosis	Lordosis[4]	Tail Anamalies[4]	Head Flexures[4]
[1]C_1	11.76	88.24	17.64	0.00	14.18 (29.41)	58.82
[2]C_2	44.87	55.12	3.84	11.54	15.39 (11.54)	35.90
[3]C_1 + C_2	38.94	61.05	6.32	9.47	20.00 (14.74)	40.00
8-2	0.00	100.00	29.41	5.88	52.94 (52.94)	64.70
8-4	36.36	63.64	7.96	13.64 (6.82)	22.72 (17.04)	43.18
8-8	27.27	72.72	9.09	4.54 (4.54)	31.82 (22.72)	45.46
8-16	63.64	36.36	9.09	18.18	0.00 (0.00)	9.09
10-2	2.46	97.53	45.68	1.24	28.40 (28.40)	38.27
10-4	17.50	82.50	8.75	12.50	38.75 (21.25)	47.50 (5.00)
10-8	29.34	70.65	6.52	7.61	34.78 (23.91)	50.00 (4.34)
10-16	17.54	82.46	21.05	0.00	40.35 (29.82)	50.88
12-2	12.15	87.85	14.95	0.96	42.99 (36.44)	61.68
12-4	20.69	79.31	12.06	6.90	50.00 (29.31)	37.93
12-8	6.06	93.94	6.06	12.12	42.42 (42.42)	72.73
12-16	0.00	100.00	20.34	5.08	45.76 (44.06)	69.49
14-2	17.74	82.26	11.29	3.22	25.80 (16.12)	30.64
14-4	11.11	88.88	7.40	18.52	37.04 (3.33)	55.56
14-8	12.00	88.00	12.00	16.00	52.00 (40.00)	44.00
14-16	0.00	100.00	19.04	19.04 (9.52)	57.14 (57.14)	61.90
16-2	0.00	100.00	42.50	2.50	42.50 (42.50)	55.00
16-4	---	---	---	---	---	---
16-8	20.00	80.00	20.00	6.66	53.33 (46.66)	46.66
16-16	0.00	0.00	0.00	0.00	0.00 (0.00)	0.00
18-2	12.50	87.50	12.50	25.00	50.00 (37.50)	37.50
18-4	0.00	100.00	50.00	0.00	50.00 (50.00)	50.00
18-8	0.00	100.00	0.00	0.00	100.00 (100.00)	0.00
18-16	0.00	100.00	0.00	0.00	0.00 (0.00)	100.00
20-2	50.00	50.00	0.00	25.00	0.00 (0.00)	0.00
20-4	0.00	100.00	0.00	0.00	100.00 (50.00)	50.00
20-8	0.00	0.00	0.00	0.00	0.00 (0.00)	0.00
20-16	0.00	100.00	57.14	0.00	14.28 (14.28)	42.86

[1]C_1 is the first control, Δ0-0.

[2]C_2 is the second control, Δ0-0.

[3]C_1 + C_2 is the means of the controls, Δ0-0.

[4]Percentage of larvae having multiple abnormality traits in parentheses.

TABLE 11 (continued)

Short or Large Yolk-Sac[4]		Have Just Hatched	Tail Not Fully Extended[4]	Others
(0.00)	(0.00)	0.00	(0.00)	None
(5.12)	(0.00)	0.00	(1.28)	None
(4.21)	(0.00)	0.00	(1.05)	None
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(4.54)	0.00	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	8.64	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	1.25 tail bent > 150°
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	3.74	(0.00)	2.80 tail bent > 150°
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	1.70	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	25.80 decomposing; 1.61 tail bent > 150°
(0.00)	(0.00)	0.00	(0.00)	3.70 tail bent > 150°
(4.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	9.52	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	None
---	---	---	---	large number decomposing
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	100% mortality thermal shock
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	100% V-shape
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	25.00	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	None
(0.00)	(0.00)	0.00	(0.00)	100% mortality thermal shock
(0.00)	(0.00)	0.00	(0.00)	None

TABLE 12

LATE EMBRYO EXPERIMENT

The ΔT-t Level Counts of Summer Flounder Larvae Expressed as
Percentages of Larvae Characterized by Various Abnormality Traits

ΔT-t (°C-Min)	Normal Larvae	Abnormal Larvae	Kyphosis	Lordosis	Tail Anamalies[4]		Head Flexures
[1]C_1	84.29	15.71	2.30	0.19	9.77	(3.83)	6.70
[2]C_2	78.86	21.14	1.08	0.00	13.28	(2.16)	5.42
[3]$C_1 + C_2$	82.04	17.96	1.80	0.11	11.22	(3.14)	6.17
8-2	87.75	12.25	1.70	0.00	5.70	(1.42)	5.41
8-4	---	---	---	---	---	---	---
8-8	86.53	13.47	2.10	0.60	4.19	(1.50)	5.99
8-16	93.50	6.50	0.00	0.00	3.48	(0.93)	2.32
10-2	86.31	13.68	1.12	0.84	7.82	(1.12)	5.02
10-4	83.98	16.02	2.73	0.78	6.25	(1.56)	5.08
10-8	83.02	16.98	0.00	0.00	7.10	(3.08)	11.72
10-16	75.10	24.90	1.90	0.63	5.48	(1.90)	18.56
12-2	87.98	12.02	1.17	0.29	6.74	(2.05)	5.28
12-4	83.21	16.79	2.47	0.24	7.40	(4.69)	10.12
12-8	73.58	26.42	3.77	1.88	13.20	(5.66)	11.32
12-16	70.49	29.50	3.28	0.00	12.30	(5.73)	18.03
14-2	85.80	14.20	1.78	0.00	8.88	(5.32)	7.69
14-4	76.60	23.40	1.51	1.13	6.42	(4.90)	17.74
14-8	52.26	47.74	3.87	0.64	9.03	(5.80)	39.36
14-16	60.51	39.49	2.54	0.00	10.19	(7.64)	33.12
16-2	70.27	29.73	1.80	0.00	8.10	(6.30)	26.12
16-4	60.00	40.00	1.70	0.42	8.51	(5.53)	32.76
16-8	73.52	26.47	0.00	0.00	7.84	(4.90)	22.55
16-16	36.14	63.86	4.22	1.80	22.89	(12.65)	45.73
18-2	20.37	79.63	7.40	5.56	14.82	(9.26)	61.11
18-4	26.19	73.81	10.71	2.83	13.10	(7.14)	51.19
18-8	21.15	78.84	7.69	0.96	16.34	(15.38)	69.23
18-16	69.81	30.18	0.00	0.00	7.54	(5.66)	26.42
20-2	23.52	76.47	2.94	0.74	14.70	(9.56)	67.64
20-4	25.00	75.00	23.08	3.84	21.15	(17.30)	42.30
20-8	13.63	86.36	22.72	0.00	22.72	(13.63)	54.54
20-16	19.04	80.95	7.14	0.00	28.57	(11.90)	61.90

[1]C_1 is the first control, Δ0-0.
[2]C_2 is the second control, Δ0-0.
[3]$C_1 + C_2$ is the means of the controls, Δ0-0.
[4]Percentage of larvae having multiple abnormality traits in parentheses.

TABLE 12 (continued)

Short or Large Yolk-Sac[4]		Have Just Hatched	Tail Not Fully Extended[4]	Others
(2.11)	(0.19)	0.38	(1.72)	None
(2.16)	(0.27)	0.27	(1.90)	0.52 V-shape
(2.13)	(0.22)	0.34	(1.80)	0.22 V-shape
(1.42)	(0.28)	0.57	(1.42)	None
---	---	---	---	large number decomposing
(0.60	(0.00)	0.60	(0.60)	None
(1.62)	(1.16)	0.23	(0.46)	0.23 V-shape
(1.96)	(0.00)	0.56	(1.68)	None
(2.73)	(2.34)	2.34	(2.34)	None
(0.62)	(0.30)	1.54	(0.00)	None
(3.80	(0.21)	0.21	(1.68)	0.21 V-shape; 0.21 tail bent > 150°
(1.46)	(0.00)	0.58	(1.46)	None
(2.22)	(0.24)	0.98	(1.48)	None
(4.40)	(0.62)	0.62	(3.14)	0.62 tail bent > 150°
(0.82)	(0.00)	0.82	(0.82)	0.82 tail bent > 150°
(4.14)	(0.00)	1.18	(3.55)	None
(3.40)	(0.38)	1.13	(2.64)	None
(3.22)	(0.64)	0.00	(2.58)	0.64 tail bent > 150°
(2.54)	(0.00)	0.63	(1.91)	0.63 decomposing
(2.70)	(0.00)	0.00	(2.70)	None
(4.26)	(0.00)	0.00	(3.83)	0.42 V-shape; {1.28 decomposing; 0.42 tail bent > 150°
(2.94)	(0.98)	0.00	(1.96)	None
(3.01)	(0.00)	0.60	(1.20)	1.20 V-shape
(0.00)	(0.00)	0.00	(0.00)	None
(1.19)	(0.00)	3.57	(0.00)	None
(3.84)	(0.00)	0.00	(3.84)	0.96 V-shape
(3.77)	(0.00)	0.00	(0.00)	1.88 tail bent > 150°
(2.20)	(0.00)	0.00	(2.20)	None
(1.92)	(0.00)	1.92	(1.92)	None
(4.54)	(0.00)	0.00	(0.00)	None
(2.38)	(0.00)	0.00	(0.00)	None

RESULTS

Thermal Mortality

The percentages of successful hatch, actual or "potential", were each compared with the mortalities, in several categories, using the Chi-square test. The purpose of these comparisons was to see whether statistical evidence of a thermal shock effect existed and to determine at what excess temperature and exposure time it began.

Table 13

Comparisons used in Evaluating Different Mortalities
with Actual and Potential Hatching Success.

Comparison	Actual Hatching Success	Mortality
I A	Viable larvae	Shocked eggs
II A	Viable larvae	Non-viable larvae
III A	Viable larvae	Total mortality (shocked eggs plus non-viable larvae)

Comparison	Potential Hatching Success	Mortality
I P	Viable larvae plus delayed hatch	Shocked eggs
II P	Viable larvae plus delayed hatch	Non-viable larvae
III P	Viable larvae plus delayed hatch	Total mortality (shocked eggs plus non-viable larvae

Three embryological stages were investigated: cleavage (Fig. 15 a), which occurs immediately after fertilization; early embryo (Fig. 15 b), which occurs about two days later; and late embryo (Fig.15c), which occurs three days after fertilization.

a.) CLEAVAGE
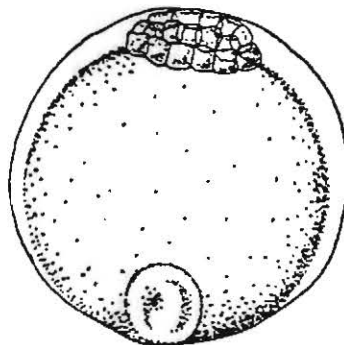
b.) EARLY EMBRYO
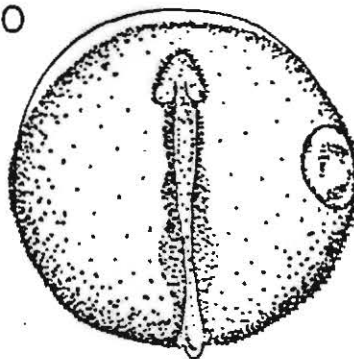
c.) LATE EMBRYO
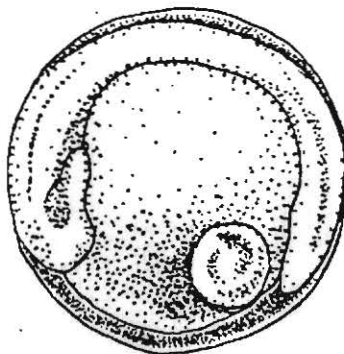
Fig. 15 - Three embryological stages tested

Cleavage Stage Experiment

The acclimation temperature was 16.0°C.

Comparison I A. Viable larvae versus shocked eggs.

Significant differences at the 5% level begin to appear at an excess temperature of 8°C after an exposure period of 8 min. The literature for experiments conducted with eggs in the cleavage stage of development (Frank, 1974; Schubel, 1973; Lauer et al., 1974) suggests that no effect on egg mortality, dependent upon species and acclimation temperature, should be experienced for ΔT's of 8°C and 10°C for exposure times of up to 120 min. The tolerance of the later embryological stages exposed to the varying temperature-time combinations indicates that summer flounder eggs have a greater resistance to thermal shock than species previously reported (see Discussion). The scatter present in the data (Table 14) which is manifested in anomalously high P values--values greater than 3.841 (5% level, d.f. = 1)--probably results from the high percentage of eggs that showed abnormal cleavage patterns.

It is not generally considered an acceptable practice to change the significance level selected *a priori*. However, some of the scatter is eliminated when the significance level is lowered to 0.1%. Therefore this was done for comparisons I A through III P. Significant differences at the 0.1% level suggest onset of thermal

Table 14

CLEAVAGE EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae Compared with
Counts of Shocked Eggs.

ΔT (°C)	Time (min)			
	2	4	8	16
8	2.998	0.000	4.939	0.520
10	0.002	2.734	10.716	3.651
12	4.476	2.188	4.678	1.827
14	13.113	17.127	10.571	6.463
16	5.753	2.567	4.957	37.404
18	17.782	34.615	31.487	35.404
20	27.870	35.220	36.521	30.382

P values > 10.827, significant

P values < 10.827, not significant

0.1% level, d.f. = 1

shock in the region between an excess temperature of 16°C and an exposure period of 8 min and an excess temperature of 16°C and an exposure period of 16 min (Table 14). Hereafter, the temperature-time relationships will be denoted by a ΔT-t abbreviation, which in this instance would read as the region between Δ16-8 and Δ16-16.

Comparison II A. Viable larvae versus non-viable larvae.

Significant differences at the 0.1% level are present at Δ10-8, Δ12-4, Δ14-2, Δ14-4, and Δ14-8. If the effects of thermal mortality are real, one would expect that after some critical excess temperature and exposure time was exceeded all results would be uniformly significant except for the effect of natural variability. The pattern of significant values in Table 15 is so nonuniform that it is questionable whether an effect has been demonstrated.

Comparison III A. Viable larvae versus total mortality (shocked eggs plus non-viable larvae).

Significant differences at the 0.1% level are present for Δ10-8, Δ14-2, Δ14-4, and Δ14-8, but it is not until the onset of the thermal shock effect in the region between Δ16-8 and Δ16-16 that the P values become uniformly significant (Table 16).

Table 15

CLEAVAGE EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Value for Counts of Viable Larvae
Compared with Counts of Non-viable Larvae.

	Time (min)			
ΔT (°C)	2	4	8	16
8	0.455	0.299	3.157	5.507
10	3.445	10.324	15.767	5.021
12	10.117	11.060	4.766	6.615
14	14.617	14.776	13.856	4.471
16	0.018	3.778	2.245	*
18	15.414	5.594	0.468	0.162
20	0.226	0.280	5.250	0.947

*Mathematically non-computable

P values > 10.827, significant

P values < 10.827, not significant

0.1% level, d.f. = 1

Table 16

CLEAVAGE EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae
Compared with Counts of Total Mortality (Shocked Eggs
Plus Non-viable Larvae).

	Time (min)			
ΔT (°C)	2	4	8	16
8	2.729	0.018	5.335	1.742
10	0.116	4.803	15.113	2.012
12	6.916	4.170	2.683	3.205
14	17.558	21.921	14.570	4.231
16	4.662	1.312	3.239	32.518
18	19.549	31.031	27.397	30.778
20	23.692	30.434	32.518	25.841

P values > 10.827, significant

P values < 10.827, not significant

0.1% level, d.f. = 1

Comparison I P.   Viable larvae plus delayed hatch versus shocked eggs.

Significant differences at the 0.1% level suggest that the onset of the termal shock effect is in the region between $\Delta 16$-8 and $\Delta 16$-16 (Table 17).

Comparison II P.   Viable larvae plus delayed hatch versus non-viable larvae.

Significant differences at the 0.1% level are present for $\Delta 10$-8, $\Delta 12$-4, $\Delta 14$-2, $\Delta 14$-4, and $\Delta 14$-8. If the effects of thermal mortality are real, one would expect that after some critical excess temperature and exposure time was exceeded all results would be uniformly significant except for the effects of natural variability. The pattern of significant values in Table 18 is so non-uniform that it is questionable whether an effect has been demonstrated.

Comparison III P.   Viable larvae plus delayed hatch versus total mortality (shocked eggs plus non-viable larvae).

Significant differences at the 0.1% level are present for $\Delta 10$-8, $\Delta 14$-2, $\Delta 14$-4, and $\Delta 14$-8, and it is not until the onset of the thermal shock effect in the region between $\Delta 16$-8 and $\Delta 16$-16 that the P values become uniformly significant (Table 19).

Table 17

CLEAVAGE EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae Plus Delayed
Hatch Eggs Compared with Counts of Shocked Eggs.

	Time (min)			
ΔT (°C)	2	4	8	16
8	1.852	0.000	4.939	0.520
10	0.284	2.734	10.716	3.651
12	4.608	2.188	4.678	2.169
14	13.113	17.129	10.571	5.068
16	5.753	1.178	4.229	37.404
18	17.782	34.615	31.487	35.404
20	27.870	35.220	36.521	30.382

P values > 10.827, significant

P values < 10.827, not significant

0.1% level, d.f. = 1

Table 18

CLEAVAGE EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae Plus Delayed
Hatch Eggs Compared with Counts of Non-Viable Larvae.

ΔT (°C)	Time (min)			
	2	4	8	16
8	0.162	0.299	3.157	5.507
10	4.794	10.324	15.767	5.021
12	10.225	11.060	4.766	6.967
14	14.617	14.776	13.856	5.159
16	0.018	4.990	2.570	*
18	15.414	5.594	0.553	0.192
20	0.179	0.280	5.250	0.947

*Mathematically non-computable

P values > 10.827, significant

P values < 10.827, not significant

0.1% level, d.f. = 1

Table 19

CLEAVAGE EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae Plus Delayed
Hatch Eggs Compared with Counts of Total Mortality (Shocked Eggs Plus
Non-viable Larvae).

	Time (min)			
ΔT (°C)	2	4	8	16
8	1.620	0.018	5.335	1.742
10	0.895	4.803	15.113	2.012
12	7.088	4.170	2.802	3.673
14	17.558	21.921	14.525	3.075
16	4.662	0.368	2.634	32.518
18	19.549	31.031	27.397	30.778
20	23.692	30.434	32.518	25.841

P values > 10.827, significant

P values < 10.827, not significant

0.1%, d.f. = 1

This interpretation is corroborated by Table 20, which shows the data normalized to the means of the hatching success of the controls for the cleavage experiment. We see that the hatching success at a ΔT of 8°C varies from 52.21% to 103.09%. According to the literature (Frank, 1974; Schubel, 1973; Lauer et al., 1974), no effect would be expected until exposure to a ΔT of 10°C exceeds 120 min for acclimation temperatures of up to 16.5°C. For this experiment the range of normalized hatching success data at $\Delta 10$ is 69.51% to 196.88%. Also, according to the literature the ranges of hatching success should not differ at a ΔT of 8°C or a ΔT of 10°C from those obtained in the controls. The range encountered at $\Delta 16\text{-}2$ through $\Delta 16\text{-}8$, 61.04% to 71.73%, appears to be acceptable and the region between $\Delta 16\text{-}8$ and $\Delta 16\text{-}16$ must be the region of the onset of egg mortality (Table 20).

The range of the data normalized to the means of the hatching success of the controls at $\Delta 16$ is quite broad, 71.73%, and one might expect a much narrower range. The aberrant total mortality experienced in $\Delta 16\text{-}16$ may be due, in part, to the late spawn which produced eggs of poor quality and were less able to sustain a thermal shock. The onset of thermal mortality may also occur between $\Delta 16\text{-}16$ and $\Delta 18\text{-}2$ as shown by the late embryo experiments, or between $\Delta 18\text{-}2$ and $\Delta 18\text{-}4$ as suggested by the small range, 1.64% to 5.97%. This is similar to the range for $\Delta 20$, 0.0% to 10.91% (Table 20).

Table 20

CLEAVAGE EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Hatching Success Expressed as a Percentage of the Controls.

ΔT-t	Percent
8-2	62.83
8-4	103.09
8-8	52.21
8-16	71.52
10-2	101.80
10-4	153.01
10-8	196.88
10-16	69.51
12-2	163.59
12-4	149.22
12-8	64.40
12-16	141.03
14-2	204.78
14-4	217.66
14-8	195.06
14-16	55.57
16-2	55.03
16-4	71.73
16-8	61.04
16-16	0.00
18-2	17.24
18-4	1.64
18-8	5.97
18-16	1.93
20-2	10.91
20-4	2.32
20-8	0.00
20-16	7.97

Early Embryo Stage

The acclimation temperature used was 14.5°C.

Comparison I A. Viable larvae versus shocked eggs.

No significant differences at the 5% level are present for excess temperatures and exposure periods used. We conclude that no effect has been demonstrated (Table 21).

Comparison II A. Viable larvae versus non-viable larvae.

No significant differences at the 5% level are present for excess temperatures and exposure periods used. We conclude that no effect has been demonstrated (Table 22).

Comparison III A. Viable larvae versus total mortality (shocked eggs plus non-viable larvae).

Significant differences at the 5% level are present at $\Delta20$-4. This P value is statistically significant in both Cases I and II (Table 23) but can not be biologically, because the percentage of shocked eggs and non-viable larvae are lower (1.52%) than that of the controls (8.09% and 8.49%) (Table 10). We conclude that no biological effect has been demonstrated (Table 23).

Table 21

EARLY EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS

Chi-Square Probability Values for Counts of Viable Larvae Compared
with Counts of Shocked Eggs for Controls 1A, 2A, and 2B (Case I) and
for Controls 1A, 2A*, and 2B (Case II).

	Time (min)			
ΔT (°C)	2	4	8	16
		Case I		
18	-	-	-	1.804
20	0.788	2.426	0.589	0.015
				(0.631)*
		Case II		
18	-	-	-	1.921
20	0.868	2.556	0.663	0.006
				(0.560)*

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*Sample was recounted

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

Table 22

EARLY EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS

Chi-Square Probability Values for Counts of Viable Larvae Compared
with Counts of Non-viable Larvae for Controls 1A, 2A, and 2B
(Case I) and for Controls 1A, 2A*, and 2B (Case II).

	Time (min)			
ΔT (°C)	2	4	8	16
		Case I		
18	-	-	-	1.125
20	2.666	2.881	3.478	3.324
				(3.298)*
		Case II		
18	-	-	-	1.105
20	2.688	2.856	3.454	3.301
				(3.274)*

*Sample was recounted

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

Table 23

EARLY EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS

Chi-Square Probability Values for Counts of Viable Larvae Compared
with Counts of Total Mortality (Shocked Eggs Plus Non-viable Larvae)
for Controls 1A, 2A, and 2B (Case I) and for Controls 1A, 2A*, and
2B (Case II).

ΔT (°C)	Time (min)			
	2	4	8	16
		Case I		
18	-	-	-	2.524
20	0.999	4.890	2.938	0.846
				(0.073)*
		Case II		
18	-	-	-	2.824
20	0.818	5.271	3.257	1.027
				(0.134)*

*Sample was recounted

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

Comparison I P. Viable larvae plus delayed hatch versus shocked eggs.

No significant differences at the 5% level are present for excess temperatures and exposure periods used. We conclude that no effect has been demonstrated (Table 24).

Comparison II P. Viable larvae plus delayed hatch versus non-viable larvae.

No significant differences at the 5% level are present for excess temperatures and exposure periods used. We conclude that no effect has been demonstrated (Table 25).

Comparison III P. Viable larvae plus delayed hatch versus total mortality (shocked eggs plus non-viable larvae).

Significant differences at the 5% level are present at Δ20-4. This P value is statistically significant in both Cases I and II (Table 26) but can not be biologically, because the percentage of shocked eggs and non-viable larvae are lower (1.52%) than that of the controls (8.09% and 8.49%) (Table 10). We conclude that no biological effect has been demonstrated (Table 26).

Table 24

EARLY EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS

Chi-Square Probability Values for Counts of Viable Larvae Plus
Delayed Hatch Eggs Compared with Counts of Shocked Eggs for Controls
1A, 2A, and 2B (Case I) and for Controls 1A, 2A*, and 2B (Case II).

	Time (min)			
ΔT (°C)	2	4	8	16
		Case I		
18	-	-	-	1.736
20	0.739	2.345	0.577	0.002
				(0.532)*
		Case II		
18	-	-	-	1.848
20	0.815	2.475	0.624	0.000
				(0.468)*

*Sample was recounted

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

Table 25

EARLY EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS

Chi-Square Probability Values for Counts of Viable Larvae Plus Delayed
Hatch Eggs Compared with Non-viable Larvae for Controls 1A, 2A, and
2B (Case I) and for Controls 1A, 2A*, and 2B (Case II).

	Time (min)			
ΔT (°C)	2	4	8	16
		Case I		
18	-	~	-	1.063
20	2.821	2.792	3.402	3.534
				(3.463)*
		Case II		
18	-	-	-	1.047
20	2.844	2.767	3.377	3.509
				(3.439)*

*Sample was recounted

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

Table 26

EARLY EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS

Chi-Square Probability Values for Counts of Viable Larvae Plus
Delayed Hatch Eggs Compared with Counts of Total Mortality (Shocked
Eggs Plus Non-viable Larvae) for Controls 1A, 2A, and 2B (Case I)
and for Controls 1A, 2A*, and 2B (Case II).

	Time (min)			
ΔT (°C)	2	4	8	16
		Case I		
18	-	-	-	2.408
20	1.108	4.740	2.837	1.023
				(0.121)*
		Case II		
18	-	-	-	2.697
20	0.919	5.109	3.146	1.223
				(0.197)*

*Sample was recounted

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

Late Embryo Stage

The acclimation temperature used was 14.0 °C.

Comparison I A. Viable larvae versus shocked eggs.

Significant differences at the 5% level are present at
Δ18-2. No significant differences appear for lower excess temper-
atures. For excess temperatures of 18°C and 20°C and exposure
periods of 2, 4, 8, and 16 min, 6 of the 8 P values are signifi-
cant. Thus, it is apparent that the onset of the thermal shock
effect lies in the region between Δ16-16 and Δ18-2 (Table 27).

Comparison II A. Viable larvae versus non-viable larvae.

Significant differences at the 5% level are present at
Δ16-2. No significant differences appear for lower excess temper-
atures. For excess temperatures of 16°C, 18°C, and 20°C and for
exposure periods of 2, 4, 8, and 16 min, only 4 of the 12 P values
are significant. Thus, the effect at Δ16 may be questionable and
the existence of an effect does not rest on very convincing
evidence (Table 28).

Comparison III A. Viable larvae versus total mortality
(shocked eggs plus non-viable larvae).

Significant differences at the 5% level are present at
Δ16-2. No significant differences appear for lower excess

Table 27

LATE EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae Compared with
Counts of Shocked Eggs.

	Time (min)			
ΔT (°C)	2	4	8	16
8	0.004	0.221	0.222	0.223
10	0.223	0.222	0.224	0.222
12	0.223	0.222	0.218	0.323
14	0.222	0.220	0.213	0.222
16	0.392	0.219	0.206	0.222
18	6.729	10.116	2.854	27.797
20	2.772	30.157	97.805	48.088

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

Table 28

LATE EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae Compared with
Counts of Non-viable Larvae.

	Time (min)			
ΔT (°C)	2	4	8	16
8	0.683	0.008	0.081	0.757
10	0.119	0.010	0.605	0.774
12	0.311	0.432	0.667	0.753
14	1.303	0.166	1.148	0.271
16	33.081	0.197	4.590	0.289
18	1.206	0.010	2.307	0.083
20	5.362	4.034	0.441	1.027

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

temperatures of 16°C, 18°C, and 20°C and for exposure periods of 2, 4, 8, and 16 min, 10 of the 12 P values are significant. Thus, Δ16 may be a "critical" excess temperature with regard to egg mortality, but since only 2 of the P values at Δ16 are significant Δ18 appears to be more firmly established and suggests that the region of the onset of the thermal shock effect lies between Δ16-16 and Δ18-2 (Table 29).

>*Comparison I P.* Viable larvae plus delayed hatch versus shocked eggs.

Significant differences at the 5% level are present at Δ18-2. No significant differences appear for lower excess temperatures. For excess temperatures of 18°C and 20°C and for exposure periods of 2, 4, 8, and 16 min, 6 of the 8 P values are significant. Thus, it appears, again, that the effects of the thermal shock are in the region between Δ16-16 and Δ18-2 (Table 30).

>*Comparison II P.* Viable larvae plus delayed hatch versus non-viable larvae.

Significant differences at the 5% level are present at Δ16-2. No significant differences appear for lower excess temperatures. For excess temperatures of 16°C, 18°C, and 20°C and for exposure periods of 2, 4, 8, and 16 min, only 4 of the 12 P values are significant. Once again, it appears that the

Table 29

LATE EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae Compared with Counts of Total Mortality (Shocked Eggs Plus Non-viable Larvae).

ΔT (°C)	Time (min)			
	2	4	8	16
8	0.474	0.002	0.170	0.960
10	0.223	0.054	0.796	0.978
12	0.462	0.602	0.482	1.115
14	1.522	0.285	0.909	0.055
16	33.123	0.100	4.185	0.436
18	3.867	7.942	4.847	25.601
20	7.850	31.945	94.035	46.649

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

Table 30

LATE EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae Plus Delayed
Hatch Eggs Compared with Counts of Shocked Eggs.

	Time (min)			
ΔT (°C)	2	4	8	16
8	0.004	0.221	0.222	0.223
10	0.222	0.221	0.223	0.223
12	0.223	0.222	0.217	0.324
14	0.224	0.222	0.216	0.166
16	0.382	0.219	0.207	0.223
18	6.640	10.026	2.861	27.854
20	2.779	29.845	97.963	46.929

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

effect at Δ16 may be questionable and the existence of an effect does not rest on very convincing evidence (Table 31).

> *Comparison III P.* Viable larvae plus delayed hatch versus total mortality (shocked eggs plus non-viable larvae).

Significant differences at the 5% level are present at Δ16-2. No significant differences appear for lower excess temperatures. For excess temperatures of 16°C, 18°C, and 20°C and for exposure periods of 2, 4, 8, and 16 min, 9 out of the 12 P values are significant. Thus, Δ16 may be a "critical" excess temperature, again, with regard to egg mortality, but since only 2 of the 4 P values at Δ16 are significant Δ18 appears to be more firmly established. Also, the region of the onset of the thermal shock effect lies between Δ16-16 and Δ18-4 (Table 32).

Sublethal Effects

Cleavage Experiment

Significant differences at the 5% level are present at Δ8-2 and all other ΔT-t combinations studied in this experiment, except Δ8-4, Δ8-8, and Δ10-8 which do not differ from their controls. The results are therefore inconclusive as to the region, in terms of ΔT-t combinations, where the vertebral anomalies become significant (Table 33).

Table 31

LATE EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae Plus Delayed
Hatch Eggs Compared with Counts of Non-viable Larvae.

	Time (min)			
ΔT (°C)	2	4	8	16
8	0.684	0.009	0.080	0.760
10	0.118	0.010	0.603	0.779
12	0.311	0.430	0.671	0.757
14	1.316	0.171	1.123	0.269
16	32.653	0.195	4.554	0.292
18	1.222	0.011	2.315	0.084
20	5.377	3.962	0.440	0.955

P values   3.841, significant

P values   3.841, not signigicant

5% level, d.f. = 1

Table 32

LATE EMBRYO EXPERIMENT WITH SUMMER FLOUNDER EGGS.

Chi-Square Probability Values for Counts of Viable Larvae Plus Delayed
Hatch Eggs Compared with Counts of Total Mortality (Shocked Eggs Plus
Non-viable Larvae).

	Time (min)			
ΔT (°C)	2	4	8	16
8	0.474	0.001	0.169	0.964
10	0.221	0.053	0.794	0.984
12	0.463	0.600	0.485	1.121
14	1.537	0.292	0.887	0.001
16	32.692	0.099	4.150	0.440
18	3.793	7.855	4.861	25.657
20	7.871	31.602	94.192	45.475

P values > 3.841, significant

P values < 3.841, not significant

5% level, d.f. = 1

Table 33

CLEAVAGE EXPERIMENT.

Chi-Square Probability Values for Counts for Normal Larvae Compared with Counts of Abnormal Larvae.

ΔT (°C)	Time (min)			
	2	4	8	16
8	48.370	0.143	3.078	12.200
10	40.546	11.356	2.052	11.309
12	18.879	7.977	31.013	48.370
14	11.075	20.649	19.128	48.370
16	48.370	*	8.638	†
18	18.307	48.370	48.370	48.370
20	2.472	48.370	†	48.370

*Sample not evaluated

†100% thermal mortality

P values > 3.841 significant

P values < 3.841 not significant

5% level, d.f. = 1

Late Embryo Experiment

Significant statistical differences at the 5% level are present at Δ8-16, but this P value is not significant biologically. No other significant differences are present until the onset of the sublethal effect in the region between Δ14-4 and Δ14-8. Another region observed is between Δ16-2 and Δ16-4 (Table 34).

Significant differences at the 5% level are also present between normal larvae and larvae whose tail did not fully extend at Δ18-8. No other significant differences were observed for other temperature-time combinations used (Table 35).

Table 34

LATE EMBRYO EXPERIMENT

Chi-Square Probability Values for Counts of Normal Larvae Compared with Counts of Abnormal Larvae.

	Time (min)			
ΔT (°C)	2	4	8	16
8	1.271	*	0.760	6.117
10	0.687	0.133	0.112	1.430
12	1.384	0.048	2.073	3.681
14	0.523	0.902	20.102	11.320
16	3.814	11.801	2.097	43.567
18	76.108	62.810	74.219	4.091
20	68.696	65.395	93.746	79.381

*Sample not evaluated

P values > 3.841 significant

P values < 3.841 not significant

5% level, d.f. = 1

Table 35

LATE EMBRYO EXPERIMENT

Chi-Square Probability Values for Counts of Normal Larvae Compared with Those Larvae Whose Tail Did Not Fully Extend.

ΔT (°C)	Time (min)			
	2	4	8	16
8	0.073	*	0.656	0.970
10	0.012	0.057	1.802	0.000
12	0.062	0.035	0.509	0.231
14	0.482	0.216	0.709	0.470
16	0.338	1.467	0.035	0.122
18	0.445	0.572	6.846	1.517
20	2.297	1.517	0.298	0.416

*Sample not evaluated.

P values > 3.841 significant

P values < 3.841 not significant

5% level, d.f. = 1

DISCUSSION

Thermal Mortality

Laboratory studies on the effects of short-term thermal
shock on fish eggs, have previously been investigated by Schubel
(1973, 1974), Schubel and Auld (1972a, 1972b, 1973, 1974),
Schubel et al. (1976), Frank (1974), Lauer et al. (1974), and
Hopkins and Dean (1975). These investigators subjected fish eggs
to time-temperature histories that may be experienced by organisms
entrained by power plants with once-through cooling. Their
studies generally indicate little or no mortality occurring to
the eggs of *Alosa pseudoharengus* (alewife)(Schubel and Auld,
1972a; Schubel, 1973, 1974), *A. aestivalis* (blueback herring)
(Schubel and Auld, 1973, 1974; Schubel, 1973, 1974; Schubel et al.,
1976), *A. sapidissima* (American shad)(Schubel and Auld, 1972b;
Schubel et al., 1976), *Morone americana* (white perch) (Schubel,
1973, 1974), *M. saxatilis* (striped bass) (Schubel, 1973, 1974;
Schubel and Auld, 1974; Schubel et al., 1976), and *Cyprinus carpo*
(carp) (Frank, 1974) when they were subjected to ΔT's of up to 10°C,
for 10 to 180 min and superimposed on average spawning temperatures.

As the ΔT and exposure time are increased, the probability
of mortality and abnormalities occurring in the development of
fertilized ova are increased. When the ΔT was 12.5°C, Frank (1974)
observed almost complete mortality of carp eggs, base temperature

25°C, for an exposure period of 10 min, in the cleavage and blastual stages of development (0-6 hr post-fertilization) and abnormalities (type not stated) of 13.2% in 1 hr post-fertilized eggs. Although Schubel et al., (1976) did not observe any abnormal morphological development at a ΔT of 15°C, they did observe a marked reduction in the hatching success of blueback herring eggs (base temperature 17.9 - 21.1°C) and American shad eggs (base temperature 20.5°C). Striped bass eggs (base temperature 16.6 - 19.6°C) exposed to a similar ΔT were more resistant to acute thermal shock than the other two species and their hatching success was unaffected. Frank (1974) observed complete mortality of eggs exposed to a ΔT of 15°C in the cleavage and blastula stages, and a relatively high thermal sensitivity (mortality) in the developmental stages associated with blastopore closure and initiation of organogenesis. The late embryo stage of the carp appears more resistant to thermal shock than the other stages, but at Δ15 Frank (1974) observed a sharp rise in the percentage of abnormalities present. At ΔT's of 17.5°C (Frank, 1974) and 20°C (Frank, 1974; Schubel et al., 1976), the eggs of carp and striped bass failed to develop.

Lauer et al. (1974), assessing the effects of a change in the operational mode of Consolidated Edison's Indian Point facility, subjected striped bass eggs in various stages of development, to a ΔT of 8.4°C, from base temperature 19.4°C, and exposure periods of up to 120 min. They concluded that the maximum temperature

(base temperature plus ∆T) exceeded the thermal limits of the most
sensitive egg stages (cleavage and blastula stages, and the stages
preceeding blastopore closure) by approximately 2.8°C for an
exposure period of 30 min and by approximately 1.7°C for an
exposure period of 15 min, but the later developmental stages were
capable of surviving the thermal shock. The most sensitive stages
may be able to tolerate exposure periods of only about 10 min.

Hopkins and Dean (1975) investigated the effects of a
thermal shock of 20°C, base temperature 20°C, for an exposure
period of 5 min, on various developmental stages of the killifish
Fundulus heteroclitus. Their results indicated that the cleavage
stage and the stages before blastopore closure are highly sensi-
tive ones for *Fundulus* eggs. They observed in some eggs, exposed
prior to blastopore closure, that the yolk material had expanded
and protruded through the opening in the blastopore. The symmetry
of the embryonic shield was disturbed within 30 min, becoming a
mass of amorphous tissue.

Smith et al. (1979) constructed a thermal resistance curve
for summer flounder eggs. Their study, based on the time-tempera-
ture data of this study, indicated that the late embryo stages of
development of summer flounder eggs may have slightly greater
resistance to thermal shock than we found. Eggs in the
late embryo stage of development, base temperature 16°C, were ex-
posed for periods of up to 180 min. Their own data showed that an

exposure period of between 15-45 min appears to cause significant differences in mortality when a ΔT of 19°C is superimposed on the base temperature. Natural variations between different spawnings or the effect of parental temperature experience (the parents were subjected to a higher acclimation temperature causing the eggs to have a greater thermal tolerance) may be factors causing differences between studies.

Cleavage Experiment

Much of the variability in the hatching success observed in this experiment can be explained by the low percentage of eggs fertilized, 20-25% compared to 90-95% for the other experiments. Besides the low percentage of fertilization, abnormal cleavage patterns were observed in eggs before they were subjected to thermal shock and may be one of the factors associated with the high percentage of eggs in which development was arrested. The cleavage and blastula stages of development and the stages associated with blastopore closure are highly sensitive to stresses; whether they be metals, organic compounds (pesticides, polychlorinated biphenyls, etc.), or temperature.

The scatter present in the data at the 5% level (P = 3.841) were reduced considerably when comparisons were made using the 0.1% level of significance (P = 10.821). Before the onset of the thermal shock effect, anomalously high P values are present at temperature-time exposures of $\Delta 14$-2 and $\Delta 14$-4, when the Chi-square

test is used to compare viable larvae with shocked eggs. The anomalously high P values can be explained by the greater percentage of viable larvae present in these temperature-time exposures, 57.27 and 60.87% (Table 6) compared with 27.97% (Table 10) found in the controls.

When viable larvae are compared with non-viable larvae, using the Chi-square test, anomalously high P values are present at temperature time exposures of Δ10-8, Δ12-4, Δ14-2, Δ14-4, and Δ14-8. These high P values can be explained by the high percentage of viable larvae, 41.73 to 60.87% and the low percentages of non-viable larvae, 1.12 to 2.17% (Table 6), compared with 27.97% viable larvae and 12.15% non-viable larvae present in the controls (Table 10).

When comparisons are made between viable larvae with total mortality (shocked eggs plus non-viable larvae), using the Chi-square test, anomalously high P values are present at temperature-time exposures of Δ10-8, Δ14-2, Δ14-4, and Δ14-8. The explanation for these high P values has been given above, where comparisons were made between viable larvae and shocked eggs and between viable larvae and non-viable larvae.

When the delayed hatch is compared with the viable larvae and compared with shocked eggs, non-viable larvae, and total mortality (shocked eggs plus non-viable larvae), the same temperature-time exposures are significant and the explanations given

above hold for these combinations.

Early Embryo Experiment

Although most of this experiment was lost through improper handling techniques the samples that were saved suggest that the early embryo stage of development is highly resistant to thermal shock.

The only anomalous P values, at the 5% level, are present in both case I and II, when comparisons are made using the Chi-square test between viable larvae (plus delayed hatch) with total mortality (shocked eggs plus non-viable larvae). This anomalously high P value at Δ20-4 can be explained by the low percentage of eggs affected by the thermal shock, 1.52% (Table 10), compared with a control percentage of 8.09 or 8.49% (Table 10) depending upon which case (I or II) is used in the comparison.

Late Embryo Experiment

For this experiment it appears that this embryological stage is less resistant to a thermal shock than the early embryo, described previously.

Anomalous P values, at the 5% level, occur before the onset of the thermal shock at temperature-time exposures of Δ16-2 and Δ16-8, when viable larvae (plus delayed hatch) are compared with non-viable larvae and total mortality (shocked eggs and non-viable larvae). At Δ16-2 and Δ16-8, the percentages of non-viable

larvae are 31.01 and 7.52% respectively (Table 6) compared to a
control percentage of 1.30% (Table 10). When the total mortality
is compared the percentages are 31.64 and 7.52% (Table 8)
compared to a control percentage of 1.52% (Table 10).

Sublethal Effects

Sublethal effects have been defined by Rosenthal and
Alderdice (1976) "as those responses to environmental change--that
may be induced in one stage of development but expressed in a
later stage of organization or development in terms of reduced
survival potential." The manifestations of sublethal effects
resulting from tissue injury during the course of embryological
development include: deformities of the vertebral column; fin
defects, which result from stress during incubation; yolk-sac
deformities, which include incomplete yolk circulation and patches
of necrotic tissue; various forms of eye deformities, involving
a reduction in the size of one or both eyes and disorganization
of retinal tissue; otic capsule defects, ranging from missing
otoliths to absence of otic capsules, causing larvae to be unable
to maintain equilibrium; and jaw anomalies, from the absence of
the lower jaw to its deformed or delayed formation causing the
newly hatched larvae to be unable to capture organisms necessary
for its survival and growth (Rosenthal and Alderdice, 1976).

The effect of vertebral deformaties on larvae which have

been hatched from thermally stressed eggs with which this study is concerned, depends upon the severity of the deformity. Vertebral deformaties can range from slight flexures, to spiralling, and partial dedifferentiation of the vertebral column. The resulting effect on the larva is a reduction and disorientation of activity, and swimming ability, which can decrease its ability to capture prey, may increase the probability of it being preyed upon by other organisms thus, reducing its potential of survival in the environment (Rosenthal and Alderdice, 1976; Koo and Johnston, 1978).

The only study, where larval deformities resulting from exposures of fish eggs to a thermal shock were analyzed by Koo and Johnston (1978). Koo and Johnston (1978) subjected striped bass eggs and blueback herring eggs to a thermal shock for exposure periods of up to 180 min, and contend that hatching success is not a good indicator in assessing thermal effects since deformities also reduce chances of larval survival. Striped bass eggs (base temperature 17°C and 18°C) subjected to a ΔT of 5°C and exposure periods of 15, 60, 120, and 180 min had, upon hatching, deformities of 0%, 0%, 0%, and 12%, respectively. There were no deformities in the controls. Raising the ΔT to 10°C and using exposure periods of 5, 15, 30, 60, 120, and 180 min, Koo and Johnston (1978) observed deformities of 0%, 6%, 0-17%, 24%, 26%, and 0-60%, while controls had deformities of 0-10%. At a ΔT of 15°C striped bass eggs at hatching had deformities of 7-24%, 8%,

13-20%, 95%, 100%, and 100%, while the controls had deformities ranging between 0-10%, for exposure periods of 5, 15, 30, 60, 120, and 180 min, respectively.

Blueback herring eggs (base temperature 19°C), subjected to a ΔT of 10°C, had deformities upon hatching of 0-20%, 0-25%, and 0-25%, while their controls varied between 0-5%, for exposure periods of 5, 60, and 180 min, respectively. When the ΔT was raised by 5°C to 15°C, the percentage of abnormalities increased to 0-25%, 0-70%, and 100% for similar exposure periods of 5, 60, and 180 min, with control variation of 0-15%. Koo and Johnston (1978) conclude that the percentage and severity of the abnormalities appears to be dependent upon the resistance of the eggs to the thermal dose.

The abnormalities observed in their samples included: shortened bodies, especially the portion caudal to the anus; enlarged fins; uneven resorption of the yolk; occasional splitting off of minute oil droplets (this condition might result from the artificial fertilization of the eggs); and curving and twisting of the spine (Koo and Johnston, 1978).

The swimming behavior of the larvae observed by Koo and Johnston (1978) varied according to the severity of the abnormality. Normal larvae were able to swim almost continuously, while larvae with severe deformaties lost their swimming ability and were limited to sporadic simple jerks, darts, or kicks. Larvae with lesser deformities, those with slightly bent bodies, were only

capable of swimming in a circular path, or making short spurts
with long resting periods.

Cleavage Experiment

Although the cleavage stage of development is highly
susceptible to a thermal shock, the abnormalities encountered in
the lower ΔT's (ΔT's of 8 and 10°C) when a thermal shock is applied
to average spawning temperatures suggests that the vertebral
abnormalities were caused by other factors acting synergistically
with the thermal shock. It is recommended that the cleavage stage
of development be retested.

Late Embryo Experiment

The only anomalous P values, at the 5% level, occurring
before the onset of significant vertebral abnormalities occurred
at $\Delta 8\text{-}16$. The occurrence of this high P value can be explained
by the high percentage of larvae considered normal, 93.50%,
compared with control values of 82.04% (Table 12).

Comparing normal larvae with those larvae in which the
tail did not fully extend, the anomalous P value was past the
region of the onset of the thermal shock (Tables 27 and 32).
It is thought, that if this particular sample had been larger, no
significant differences would have been detected since the number
of larvae that displayed this condition is relatively small
(Table 2).

CONCLUSIONS AND RECOMMENDATIONS

The various embryological stages of summer flounder (cleavage, early embryo, and late embryo) are resistant to thermal shock. The thermal shock effect was observed in the region between $\Delta 16\text{-}8$ ($^\circ$C-min) and $\Delta 16\text{-}16$ for the cleavage experiment and between $\Delta 16\text{-}16$ and $\Delta 18\text{-}2$ for the late embryo experiment. The thermal shock region for the early embryo experiment was found to exceed the highest temperature-time combination used in this study, $\Delta 20\text{-}16$.

Koo and Johnston (1978) proposed that hatching success should not be used as the only criterion for assessing thermal shock effects on fish eggs. Examination of the viable larvae that hatched from eggs subjected to thermal shock for vertebral and other deformities, supports their suggestion. In this study, two of the three stages were examined for sublethal effects. The experimental samples differed significantly from their controls in the region between $\Delta 14\text{-}4$ and $\Delta 14\text{-}8$, and between $\Delta 16\text{-}2$ and $\Delta 16\text{-}4$ for the late embryo stage of development. The results for the larvae hatched from eggs used in the cleavage experiment were inconclusive.

Because of limited data produced by this study, it is recommended that additional experiments be conducted with the various embryological stages and that determinations be made of hatching success and of sublethal effects. Additionally, the larval stages should also be evaluated for both thermal mortality

and sublethal effects. Summer flounder larvae have been found in local estuarine waters of New York (Poole, 1961) and New Jersey (Murawski, 1964, 1965, 1966) and the species is important commercially and recreationally. Larvae hatched from eggs exposed to thermal stress, and larvae exposed to thermal stress, should be cultured through metamorphosis, which occurs at a length of 12 or 13 mm (Smith and Fahay, 1970), since at metamorphosis a number of other abnormalities may become detectable: incomplete eye migration, hooked dorsal fins, presence of left pectoral fin, ambicoloration, partial or complete albinism, and reversals.

REFERENCES

Beck, A.D. and N.F. Lackie 1974. Effects of passage of marine
    animals through power plant cooling water systems.
    Presented at: American Fisheries Society 1974 Annual
    Meeting, Hawaii.

Beck, A.D. and D.C. Miller. 1974. Analysis of inner plant
    passage of estuarine biota. Pages 122-226 *in* Proc. ASCE
    Power Div. Spec. Conf., Boulder, Colorado, August 12-14.

Beck, A.D. and Committee on Entrainment. 1978. Cumulative effects
    a field assessment. Pages 189-210 *in* J.R. Schubel and
    B.C. Marcy, Jr., eds. Power Plant Entrainment: A
    Biological Assessment. Academic Press, Inc., New York.

Bigelow, H.B. and W.C. Schroeder. 1953. Fishes of the Gulf of
    Maine. U.S. Fish Wild. Ser. Fish. Bull. 53(74).

Bumpus, D.F. and L.M. Lauzier. 1965. Surface circulation on the
    continental shelf off eastern North America between
    Newfoundland and Florida. Ser. Atlas Mar. Envir. Amer.
    Geog. Soc. Folio 7, 8 plates.

Clark, J. and W. Brownell. 1973. Electric power plants in the
    coastal zone: Environmental issues. American Littoral
    Society, Spec. Publ. No. 7.

Committee on Entrainment. 1978. Introduction. Pages 1-18 *in*
    J.R. Schubel and B.C. Marcy, Jr., eds. Power Plant
    Entrainment: A Biological Assessment. Academic Press,
    Inc., New York.

Committee on Water Quality Criteria. 1973. Water Quality Criteria
    1972. U.S. Environmental Protection Agency. Ecological
    Research Series. EPA R3-73-033.

Coutant, C.C. 1970. Ecological aspects of thermal pollution.
    I. Entrainment and discharge canal effects. CRC Critical
    Reviews in Environmental Control 1(3):341-381.

Coutant, C.C. 1973. Effects of thermal shock on vulnerability
    of juvenile salmonids to predation. J. Fish. Res. Bd.
    Can. 30(7):965-973.

Frank, M.L. 1974. Relative sensitivity of different embryonic stages of carp to thermal shock. Pages 171-176 *in* J.W. Gibbons and R.R. Sharitz, eds. Thermal Ecology. AEC Symp. Series, Conf. 730505.

Ginsburg, I. 1952. Flounders of the genus *Paralichthys* and related genera in American waters. U.S. Fish Wild. Ser. Fish. Bull. 52(71):267-351.

Gutherz, E.J. 1967. Field guide to the flatfishes of the family *Bothidae* in the western North Atlantic. U.S. Fish Wild. Ser., Circ. 263.

Hopkins, S.R. and J.M. Dean. 1975. The response of developmental stages of *Fundulus* to acute thermal shock. Pages 301-318 *in* F.J. Vernberg, ed. Physiological Ecology of Estuarine Organisms. Belle Baruch Library in Marine Science No. 3.

Johnson, P.O. 1949. Statistical Methods in Research. Prentice-Hall, INc., New York.

Koo, T.S.Y. and M.L. Johnston. 1978. Larvae deformity in striped bass, *Morone saxatilis* (Walbaum), and blueback herring, *Alosa aestivalis* (Mitchill), due to heat shock treatment of developing eggs. Environ. Poll. 16(1):137-149.

Lauer, J., W.T. Waller, D.W. Bath, W. Meeks, R. Heffner, T. Ginn, L. Zubarik, P. Bibko, and P.C. Storm. 1974. Entrainment studies on Hudson River organisms. Pages 37-82 *in* L.D. Jensen, ed. Proc. Sec. Entrain. Intake Screening Workshop, Feb. 5-9, 1973, The John Hopkins University, Baltimore, Md.

Lux, F.E., P.E. Hamer, and J.C. Poole. 1966. Summer flounder... the middle Atlantic flatfish. Marine Resources Atlantic Coast. Atlantic States Marine Fisheries Commission. Leaflet 6.

Marcy, Jr., B.C. 1973. Vulnerability and survival of young Connecticut River fish entrained at a nuclear power plant. J. Fish. Res. Bd. Can. 30(8):1195-1203.

McHugh, J.L. 1966. Management of estuarine fisheries. Pages 133-154 *in* A symposium on estuarine fisheries. American Fisheries Society, Spec. Publ. No. 6.

McHugh, J.L. 1977. Fisheries and fishery resources of New York Bight. U.S. Dept. Comm., NOAA Tech. Rep. NMFS Circ. 401.

McHugh, J.L. and J.J.C. Ginter. 1978. Fisheries. MESA New York Bight Atlas Monograph 16. New York Sea Grant Institute, Albany.

McHugh, J.L. and A.D. Williams. 1976. Historical statistics of fisheries in the New York Bight area. New York Sea Grant Institute, Albany.

Murawski, W.S. 1964. Fluke investigations. New Jersey Federal Aid Project F-15-R-5 (Completion Report Job 3). N.J. Dept. Cons. Econ. Devel.

Murawski, W.S. 1965. Fluke investigations. New Jersey Federal Aid Project F-15-R-6 (Completion Report Job 3). N.J. Dept. Cons. Econ. Devel.

Murawski, W.S. 1966. Fluke investigations. New Jersey Federal Aid Project F-15-R-7 (Completion Report Job 3). N.J. Dept. Cons. Econ. Devel.

Murawski, W.S. 1970. Results of tagging experiments of summer flounder, *Paralichthys dentatus*, conducted in New Jersey waters from 1960 to 1967. Miscel. Rep. 5M., N.J. Dept. Environ. Prot.

Poole, J.C. 1961. Age and growth of the fluke in Great South Bay and their significance to the sport fishery. New York Fish Game J. 8(1):1-18.

Poole, J.C. 1962. The fluke population of Great South Bay in relation to the sport fishery. New York Fish Game J. 9(2):93-117.

Poole, J.C. 1964. Feeding habits of the summer flounder in Great South Bay. New York Fish Game J. 11(1):28-34.

Poole, J.C. 1966. A review of research concerning summer flounder and needs for further research. New York Fish Game J. 13(2):226-231.

Powell, A.B. 1974. Biology of the summer flounder, *Paralichthys dentatus*, in Pamilico Sound and adjacent waters, with comments on *P. lethostigma* and *P. albigutta*. M.S. Thesis. University of North Carolina. Chapel Hill.

Powell, A.B. and F.J. Schwartz. 1977. Distribution of paralichthid flounders (Bothidae: *Paralichthys*) in North Carolina estuaries. Ches. Sci. 18(4):334-339.

Rosenthal, H. and D.F. Alderice. 1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. J. Fish. Res. Bd. Can. 33(9):2047-2065.

Schubel. J.R. 1973. Effects of exposure to time excess temperature histories typically experienced at power plants on the hatching success of fish eggs. Spec. Rep. 32. Chesapeake Bay Institute, The John Hopkins University, Baltimore, Md.

Schubel, J. 1974. Effects of exposure to time-excess temperature histories typically experienced at power plants on the hatching success of fish eggs. Estuarine and Coastal Mar. Sci. 2(1):105-116.

Schubel, J.R. and A.H. Auld. 1972a. Thermal effects of a model power plant on the hatching success of alewife (*Alosa pseudoharengus*) eggs. Spec. Rep. 28. Chesapeake Bay Institute, The John Hopkins University, Baltimore, Md.

Schubel, J.R. and A.H. Auld. 1972b. Thermal effects of a model power plant on the hatching success of American shad, *Alosa sapidissima*, eggs. S.E. Assoc. Game Fish Comm., Proc. 26:644-648.

Schubel, J.R. and A.H. Auld. 1973. Effects of exposure to time-excess temperature histories typically experienced at power plants on the hatching success of blueback herring (*Alosa aestivalis*) eggs. Spec. Rep. 31. Chesapeake Bay Institute, The John Hopkins University, Baltimore, Md.

Schubel, J.R. and A.H. Auld. 1974. Hatching success of blueback herring and striped bass eggs with various time vs. temperature histories. Pages 164-170 *in* J.W. Gibbons and R.R. Sharitz, eds. Thermal Ecology. AEC Symp. Series, Conf. 730505.

Schubel, J.R., T.S.Y. Koo, and C.F. Smith. 1976. Thermal effects power plant entrainment on survival of fish eggs and larvae: A laboratory assessment. Spec. Rep. 52. Chesapeake Bay Institute, The John Hopkins University, Baltimore, Md.

Smigielski, A.S. 1975. Hormone-induced spawning of the summer flounder and rearing of the larvae in the laboratory. Prog. Fish-Cult. 37(1):3-8.

Smith, C.F., J.R. Schubel, M.P. Greges, N. Itzkowitz, S.J. Di Piero J. Longo, and M.A. Morgan. 1979. Thermal resistance of early life history stages of finfish from Long Island waters. Spec. Rep. 26 of the Marine Sciences Research Center, State University of New York at Stony Brook.

Smith, R.W. and F.C. Daiber. 1977. Biology of the summer flounder, *Paralchthys dentatus*, in Delaware Bay. Fish. Bull. 75(4):823-830.

Smith, W.G. 1973. The distribution of summer flounder, *Paralichthys dentatus*, eggs and larvae on the continental shelf between Cape Cod and Cape Lookout, 1965-66. Fish. Bull. 71(2): 527-548.

Smith, W.G. and M.P. Fahay. 1970. Description of eggs and larvae of the summer flounder, *Paralichthys dentatus*. U.S. Fish Wild. Serv. Res. Rep. 75.

Smith, W.G., J.D. Sibunka, and A Wells. 1975. Seasonal distribution of larval flatfishes (Pleuronectiformes) on the continental shelf between Cape Cod, Massachusetts, and Cape Lookout, North Carolina, 1965-66. Dept. of Comm. NOAA Tech. Rep. NMFS SSRF-691.

Woodhead, A.D. 1975. Endocrine physiology of fish migration. Pages 287-382 *in* H. Barnes, ed. Ocean. Mar. Bio. Ann. Rev. 13. George Allen and Unwin Ltd., London.

Woodhead, A.D. and P.M.J. Woodhead. 1965. Seasonal changes in the physiology of the Barents Sea cod, *Gadus morhua*L., in relation to its environment. I. Endocrine changes particularly affecting migration and maturation. Int. Comm. Northwest Atl. Fish. Spec. Publ. No. 6, pp.691-715.

Yocom, T.G. and T.A. Edsall. 1974. Effects of acclimation temperature and heat shock on vulnerability of fry of lake whitefish (*Coregonus clupeaformis*) to predation. J. Fish. Res. Bd. Can. 31(9):1503-1506.