EFFECTS OF TEMPERATURE AND SALINITY ON THE SURVIVAL OF WINTER FLOUNDER EMBRYOS

CAROLYN A. ROGERS[1]

ABSTRACT

A series of experiments was performed to determine the optimum temperature and salinity for incubating winter flounder, *Pseudopleuronectes americanus*, embryos. Eggs in lots of 50 were subjected to a 0.5 to 45‰ salinity range and a 3° to 14°C temperature range in a total of 67 salinity-temperature combinations. Highest proportion of viable hatches occurred at 3°C over a salinity range of 15 to 35‰. At temperatures above 3°C, the optimal range was 15 to 25‰. Viable hatch decreased with increasing temperature.

The winter flounder, *Pseudopleuronectes americanus* (Walbaum), an important species in local New England commercial and sport fishing industries, occurs from Chesapeake Bay to the northern shore of the Gulf of St. Lawrence (Bigelow and Schroeder 1953). The adults disperse into cooler offshore waters as temperatures rise, but move back into embayments and estuaries in the fall. Spawning occurs in shoal waters of these areas from February to mid-May with the maximum in Rhode Island waters occurring in March (Perlmutter 1947; Bigelow and Schroeder 1953; Pearcy 1962). Winter flounder spawn demersal eggs, which range from 0.74 to 0.85 mm in diameter when fertilized. Hatching occurs in 15 to 18 days at 3° to 4°C, the temperature normally encountered in the natural environment (Bigelow and Schroeder 1953).

This paper reports the optimum temperature and salinity ranges for the development and survival of winter flounder embryos and larvae and discusses the relationship between the two factors as it affects embryo development. An earlier study (Scott 1929) indicated some of the effects of temperature and salinity as separate factors on the hatching of winter flounder eggs but presented no data on possible interaction of the two. Forrester and Alderdice (1966) and Alderdice and Forrester (1968, 1971a, b) working on the effects of temperature and salinity on the embryonic development of the English sole, *Parophrys vetulus*; petrale sole, *Eopsetta jordani*; and Pacific cod, *Gadus macrocephalus*, respectively, indicated a relationship between the two factors, which influenced early development, hatching time, and viable hatch.

METHODS AND MATERIALS

Ripening adult winter flounder were captured by trawl on 29 October 1970 at a depth of 23 to 30 m in Block Island Sound. Surface waters were 15°C, and a bottom temperature of 12°C was estimated for that area (Colton and Stoddard 1973). The live fish were transported to the laboratory where they were held in running water aquaria until they were ripe in early February when ambient water temperature was 3°C. The fish were fed clam worms, earthworms, and cut up clam during the holding period. Eggs were stripped into polyethylene dishpans, fertilized, and coated with diatomaceous earth to prevent clumping, according to the technique of Smigielski and Arnold (1972). Fertilized eggs were transferred to incubation baskets and held at 3°C in running seawater (32‰ salinity) for 24 h when normal development could be distinguished. Day 1 embryos were in the early blastoderm stage when the experiments were started. Three separate experiments were run at salinities ranging from 0.5 to 45‰ and at temperatures of 3° to 14°C. Each experiment was run in duplicate.

To avoid bias, all salinities were prepared by adding Instant Ocean[2] salts to normal seawater (32‰) to bring the salinity up to 50‰. Experimental salinities were then made by diluting the stock salinity with distilled water. Each salinity

[1]Northeast Fisheries Center Narragansett Laboratory, National Marine Fisheries Service, NOAA, Narragansett, RI 02882.

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

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was checked with a refractometer to within ±0.15‰ of the test salinity. The test salinities were cooled to the ambient seawater temperature (3°C) at which the eggs were incubated for the first 24 h.

Eggs in lots of 50 were counted into 100-ml polyethylene beakers filled with the test salinities. The beakers were covered with fitted 50-mm plastic disposable culture dish bottoms to eliminate evaporation and placed in thermostatically controlled water baths at the experimental temperatures. Dead eggs or larvae were removed daily and examined for stage of development.

Daily observations were made on the development of embryos. The time of hatching and the duration of the hatching interval were noted so that mean hatching time (time from fertilization to 50% hatch) could be calculated. Abnormal larvae (those with curvature of the spine, abnormal yolk sacs, or enlarged fin folds) were noted and counted as nonviable since their chance of continued survival was considered to be small. Prematurely hatched or aborted larvae were also considered nonviable in calculations. Such larvae were easily recognized since they were short, thickened, often curled, and in no way resembled a normal healthy larva.

Each experiment was terminated when all eggs had either hatched or died, and when the larvae could be judged normal or abnormal. From this information, total percentage hatch (percentages of eggs producing live larvae) as well as percentage viable hatch (percentage producing viable or normal larvae) was calculated. Salinities were checked at the end of each experiment.

The experiment was set up as a factorial design. However, replications at different factor combinations were unequal and there were missing data at 3°C due to equipment malfunction. In view of this, a mean value of the replicates was computed for each factor combination and values for the missing data at 3°C were predicted from the hyperbolic equation describing the actual data at 3°C. The resultant design was a 2 factor, 6 × 12 (6 levels of temperature and 12 levels of salinity) factorial design with no replicates. Duncan's multiple range test (Steel and Torrie 1960) was used to compare the mean survivals for each temperature and salinity condition.

RESULTS

The results of these experiments indicate that winter flounder embryos are euryhaline, with best survival occurring between 10 and 30‰ but with some survival from 5 to 40‰. Hatching occurred at all temperatures tested, but the lower temperatures produced the highest survival. Incubation time and hatching interval were decreased by increased temperatures and higher salinities. Abnormal development occurred particularly at extremes of salinity but was also influenced by temperature.

Effects of Salinity and Temperature on Viable Hatch

Results of the temperature-salinity experiments (Table 1) indicated an optimal salinity range between 15 and 25‰ for temperatures above 3°C and between 15 and 35‰ for 3°C (Figure 1, Table 2). Viable hatch was highest at 3°C and lowest at 14°C with similar survival rates at 5, 7, and 12°C for all salinities. Percentage survival at 10°C follows a similar curve at salinities of 25‰ and above, but was between 15 and 30% lower than that of other temperatures at 20‰ and below. At 3°C, high survival (>78%) occurred from 15 to 35‰, but survival decreased sharply at all other temperatures for salinities above 25‰.

TABLE 1.—Number of winter flounder eggs at each of 67 temperature-salinity combinations. Number of replicates shown in parentheses.

Salinity (‰)	Temperature (°C) 3	5	7	10	12	14
0.5	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
5.0	100 (2)	300 (6)	400 (8)	300 (6)	300 (6)	300 (6)
7.5		100 (2)	100 (2)	100 (2)	50 (1)	100 (2)
10.0	100 (2)	300 (6)	400 (8)	300 (6)	300 (6)	300 (6)
15.0		300 (6)	400 (8)	200 (4)	300 (6)	300 (6)
20.0	100 (2)	300 (6)	400 (8)	300 (6)	300 (6)	300 (6)
25.0		200 (4)	300 (6)	300 (6)	300 (6)	200 (4)
30.0	100 (2)	300 (6)	400 (8)	300 (6)	300 (6)	300 (6)
35.0	100 (2)	300 (6)	400 (8)	300 (6)	300 (6)	300 (6)
37.5		100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
40.0		200 (4)	300 (6)	200 (4)	200 (4)	200 (4)
45.0	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)

Influence of Temperature and Salinity on Total and Viable Hatch

The influence of temperature and salinity is shown in the percentages of mean total hatch and mean viable hatch (Table 3). There is a sharp decrease in mean total hatch and mean viable hatch at temperatures over 3°C, while these means approximate a normal distribution at the salinities tested. The mean percentage of abnormal larvae calculated from total and viable hatch data shows

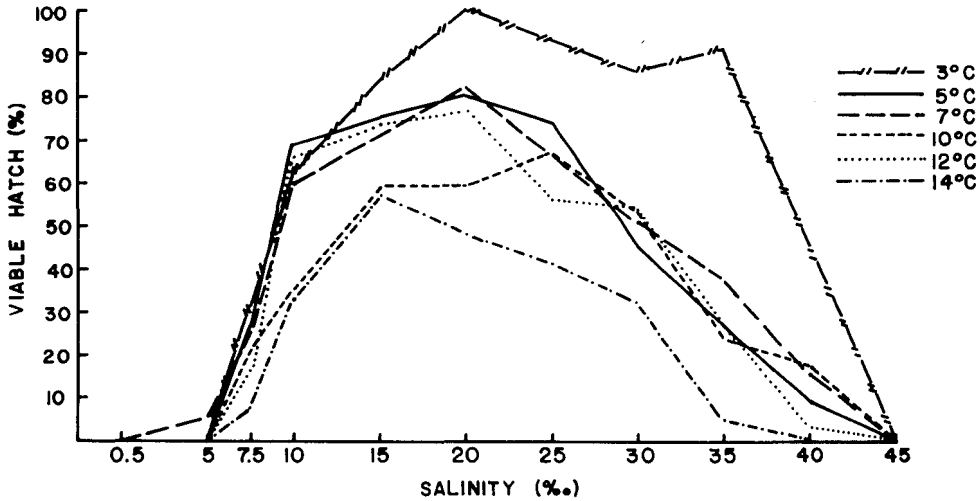

FIGURE 1.—The effects of temperature and salinity on the percent viable hatch of winter flounder embryos.

TABLE 2.—Mean percent total and viable ( ) hatch at the various temperature-salinity combinations.

Salinity (‰)	Temperature (°C) 3	5	7	10	12	14
0.5	0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
5.0	26 (0)	6.3 (0)	14.5 (6.5)	23.0 (0)	0.0 (0)	0.0 (0)
7.5	—	58.0 (26.0)	48.0 (26.0)	49.0 (21.0)	46.0 (17.0)	23.0 (7.0)
10.0	88 (61)	79.7 (65.7)	71.5 (57.8)	59.0 (32.0)	82.7 (65.3)	55.3 (32.7)
15.0	92 (84)	79.3 (75.7)	76.5 (71.0)	71.0 (57.0)	77.0 (69.0)	69.3 (57.3)
20.0	100 (99)	82.3 (79.3)	83.8 (82.0)	70.3 (61.0)	78.3 (68.0)	61.3 (48.7)
25.0	—	75.5 (74.0)	69.3 (66.7)	74.0 (66.5)	62.0 (56.5)	57.5 (42.0)
30.0	74 (64)	54.7 (45.7)	59.8 (51.3)	50.0 (43.7)	63.0 (54.7)	48.7 (32.3)
35.0	84 (67)	31.3 (27.3)	42.8 (37.5)	31.0 (24.0)	47.0 (34.5)	21.0 (7.7)
37.5	—	40.0 (37.0)	86.0 (78.0)	57.0 (52.0)	38.0 (16.0)	5.0 (0)
40.0	—	19.0 (9.5)	63.0 (15.7)	34.0 (17.0)	26.0 (3.5)	0.0 (0)
45.0	0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)

TABLE 3.—Means and ranges for percent total and viable hatches and mean abnormal hatches for each salinity at all temperatures, and each temperature at all salinities.

Item	Mean % total hatch (Range)	Mean % viable hatch (Range)	Mean abnormal hatch[1] (%)
0.5‰	No hatch	No hatch	
5.0‰	12.8(2.3-26.0)	1.6(0-6.5)	11.2
7.5‰	44.8(23.0-58.0)	19.4(7.0-26.0)	25.4
10.0‰	72.7(55.3-88.0)	52.4(32.0-65.7)	20.3
15.0‰	77.5(69.3-92.0)	72.3(57.0-84.0)	5.2
20.0‰	79.3(61.3-100)	73.0(48.7-99.0)	6.3
25.0‰	67.7(57.5-74.0)	61.1(42.0-74.0)	6.6
30.0‰	58.4(48.7-74.0)	48.6(32.3-64.0)	9.8
35.0‰	42.9(21.0-84.0)	33.0(7.7-67.0)	9.9
37.5‰	45.2(5.0-86.0)	36.6(0-78.0)	8.6
40.0‰	15.1(9.5-21.0)	11.4(3.5-17.0)	3.7
45.0‰	No hatch	No hatch	
3°C	77.3(26.0-100)	62.5(0-99.0)	14.8
5°C	51.7(6.3-82.3)	44.0(9.5-79.3)	7.7
7°C	57.3(14.5-86.0)	49.3(6.5-82.0)	8.0
10°C	48.1(2.3-74.0)	37.4(0-66.5)	10.7
12°C	56.3(13.0-82.7)	42.7(3.5-69.0)	13.6
14°C	42.6(5.0-69.3)	28.5(0-57.3)	14.1

[1]Mean abnormal hatch = mean percent total hatches − mean percent viable hatches.

no trend with temperature, but a high percentage of abnormal larvae for salinities of 10‰ and below. Lowest percentages for abnormal larvae were for salinities between 15 and 35‰. The low percentage for 40.0‰ reflects low hatching rates and mortality during embryonic stages and does not reflect values which can be compared with salinities of 37.5‰ and below.

Analysis of variance performed on the survival data indicate that salinity and temperature are both significant factors (Table 4). Because of missing data (Table 1), it was not possible to test for interaction between the two factors; however, by examining the data, especially as it is expressed in Figure 1, it is reasonable to conclude that an interaction does occur. The multiple comparison of means indicates significant differences between hatch means at various temperatures and

TABLE 4.—Analysis of variance for the effects of temperature and salinity on the survival and hatching of winter flounder embryos.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	71	69,248.75		
Salinity	11	51,935.36	4,721.39	31.5**
Temperature	5	9,078.78	1,815.76	12.2**
Residual	55	8,234.61	149.72	

**significant at $P = 0.005$.

TABLE 5.—Duncan's multiple comparison of means for temperature-salinity studies of winter flounder embryos. (Means with similar symbols denote similar mean survival percentages.)[1]

Temperature (°C)	Mean survival (%)	Salinities (‰)	Mean survival (%)
3	56.1√	0.5	0.0√
5	36.2*	5.0	1.1√
7	41.1*√	7.5	21.6*
10	31.4*∞	10.0	53.7°
12	32.4*∞	15.0	69.9†
14	18.9∞	20.0	74.3†
		25.0	67.4†
		30.0	52.6°
		35.0	35.6∞
		37.5	40.3∞
		40.0	15.3*
		45.0	0.0√

[1]$P = 0.05$.

salinities and allows a grouping of each in order of its significance (Table 5). The grouping of the hatch means for variations in both temperature and salinity coincides closely with viable hatch curves illustrated in Figure 1.

Incubation Time and Duration of Hatching Interval

The time to 50% hatch and the total range of hatching time for each temperature and salinity combination are recorded in Table 6. Figure 2 illustrates the time to 50% hatch and the mean incubation time for each temperature and salinity respectively. The mean hatching interval

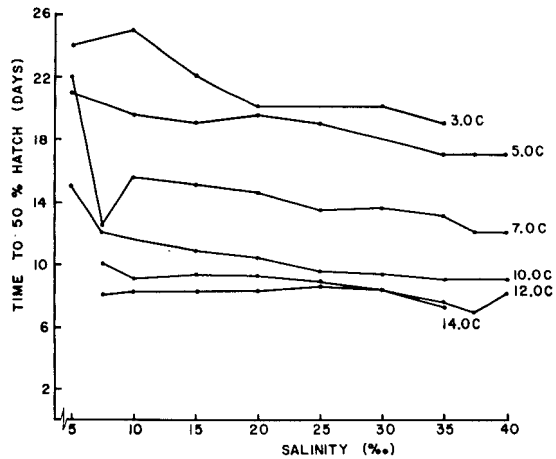

FIGURE 2.—The effects of salinity on the time to 50% hatch of winter flounder embryos.

ranges from 25 days at 3°C (10‰) to 7 days at 12° and 14°C (37.5 and 35‰ respectively). Individual eggs hatched in as few as 5 days in most salinities at 12° and 14°C, but took as long as 31 days at 3°C (10‰). An inverse relationship for temperature with respect to the duration of hatching time is evident.

There is also a trend toward the same inverse relationship with respect to salinity as can be seen in Figure 2 where the time to mean 50% hatch at all temperatures decreased slightly with increasing salinities. This phenomenon of greater hatching time at low salinities was noted in Pacific cod eggs by Forrester and Alderdice (1966). When salinity means versus incubation time is considered by least squares regression, there is a low correlation coefficient and a regression relationship is not applicable (Figure 3). However, temperature means have a high correlation coefficient and there is a strong regression relationship present.

TABLE 6.—Time in days to 50% hatch. Range of hatching interval in days shown in parentheses. NH denotes no hatch.

Temperature (°C)	Salinity (‰) 5.0	7.5	10.0	15.0	20.0	25.0	30.0	35.0	37.5	40.0
3	24		25 (19-31)	22 (19-27)	20 (19-25)		20 (17-25)	19 (16-25)		
5	21	20 (16-20)	20 (17-29)	19 (17-25)	19 (17-29)	19 (22-24)	18 (13-25)	17 (11-25)	16 (14-16)	16 (14-16)
7	22	13 (10-16)	15 (12-23)	15 (12-23)	15 (12-25)	13 (8-17)	14 (8-21)	13 (11-19)	12 (12-14)	12 (8-14)
10	15	12 (10-14)	12 (7-15)	11 (9-14)	10 (7-16)	9 (7-17)	9 (5-13)	9 (8-10)	9 (7-10)	9 (7-10)
12	NH	10 (7-12)	9 (5-10)	9 (7-12)	9 (7-10)	9 (5-10)	8 (5-10)	8 (5-10)	7 (5-10)	8 (5-10)
14	NH	8 (5-10)	8 (5-10)	8 (6-10)	8 (5-10)	8 (5-10)	8 (5-10)	7 (5-10)	NH	NH

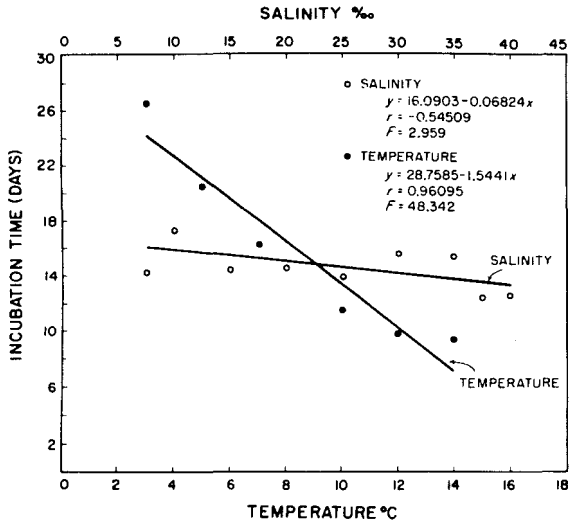

FIGURE 3.—The mean hatching time of winter flounder embryos for each temperature and salinity.

Effects of Temperature and Salinity on Embryonic Development

In each of the three experiments, general observations were made on the eggs, embryos, and larvae (Figure 4). No development occurred in a salinity of 0.5‰; however, the eggs swelled approximately 20% before death occurred. A diameter increase of 8 to 10% was also observed in eggs held at 5‰. Below 10°C, embryos held in 5‰ appeared to develop normally, then died just prior to hatching. At 10°C and above, most of the embryos died during gastrulation. Embryos held in a salinity of 10‰ had the highest mortalities just prior to hatching and at hatching; many larvae were observed dead partly emerged from the chorion. Mortality occurred throughout development at 12° and 14°C.

In salinities between 15 and 30‰, most mortalities occurred just prior to hatching, although

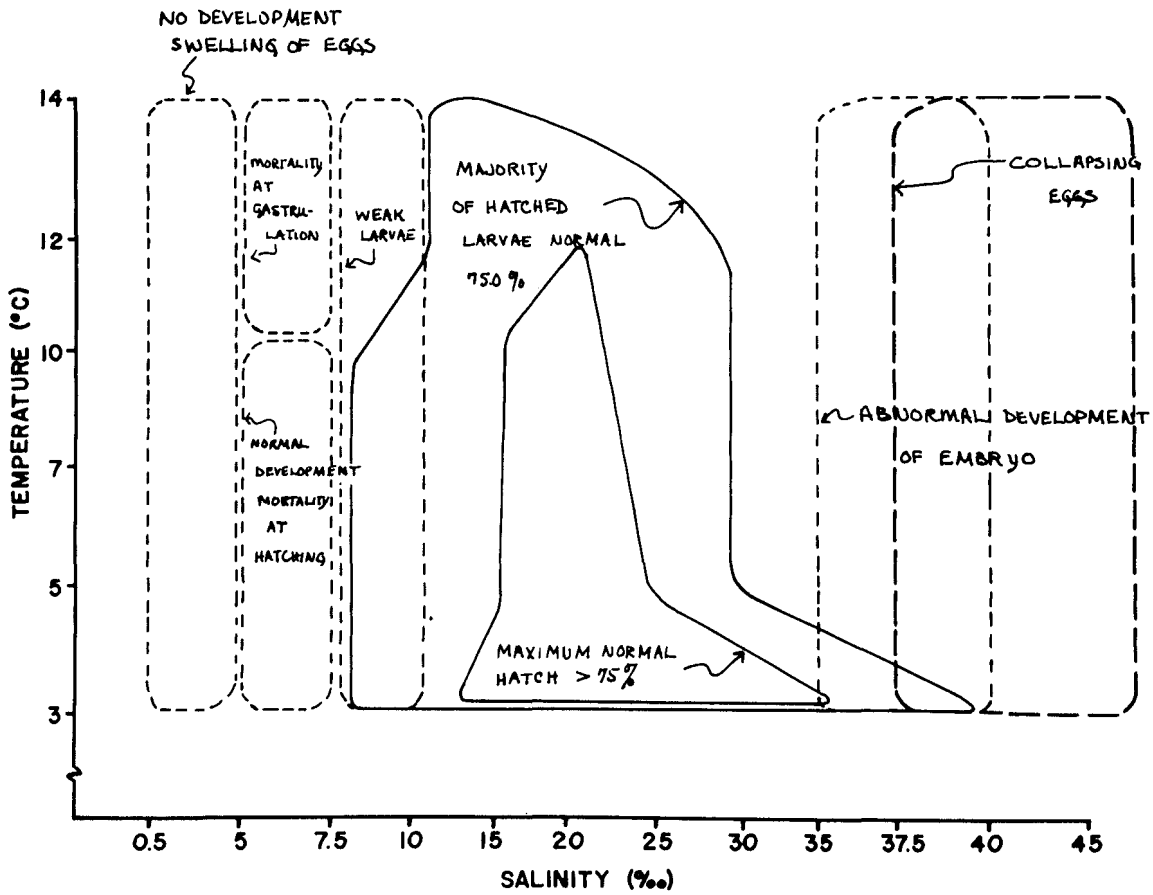

FIGURE 4.—The qualitative effects of temperature and salinity on the development and hatching of winter flounder embryos.

at temperatures of 10°C and above some mortalities usually occurred during gastrulation. At salinities of 35 to 40‰, abnormal development of the embryos was observed. The embryos were shorter and thicker than normal and died just prior to hatching. Collapsing eggs were noted at 37.5‰ and above. Embryos incubated at 40‰ died during gastrulation and throughout development at all temperatures while all embryos held at 45‰ died during gastrulation. At both 40 and 45‰ embryos exhibited shrinkage and often collapsed.

DISCUSSION

The results indicate that although temperature and salinity are both significant, the major effect of increased temperature is to decrease the incubation period, whereas salinity is the factor which has more effect on the successful hatching and survival of winter flounder embryos and larvae (Figure 4, Table 4). It is apparent however, that an interaction between the two does occur since, at the optimum experimental temperature (3°C), the salinity range over which high percentages of viable hatches occurred was extended by 10‰ (Figure 1). At higher than optimal experimental temperatures, the survival curves appear to be dictated primarily by salinity; however, survival occurs over a broad enough range that the embryos and larvae can be described as euryhaline with regard to the natural environment in which they are normally spawned. At all temperatures tested, there was a decrease in incubation time at higher salinities, a phenomenon which was also reported in studies done on *Clupea harengus* (Holliday and Blaxter 1960) and Pacific cod (Forrester and Alderdice 1966). Those authors speculated that the relationships of temperature and salinity with hatching are dependent on conditions that minimize the energy required of the embryos in maintaining osmotic equilibrium with their environment. Salinity also appears to influence the time of embryo mortality. Observations on eggs indicated that mortality usually occurred either at gastrulation, in salinities of 40 and 45‰ at all temperatures, or just prior to hatching in the lower salinities. Battle (1930) noted increased mortality of the four bearded rockling, *Enchelyopus cimbrius*, at hatching in low salinities and she attributed this to poorly developed tail musculature. McMynn and Hoar (1953), working with embryos of the Pacific herring, *Clupea harengus pallasi*, observed that with the closing of the blastopore at the end of grastrulation, embryos had a greater ability to tolerate low salinities. However, many embryos died just prior to hatching or when partly emerged. Holliday (1965, 1969) observed a similar occurrence in cod, *Gadus callarius*, and plaice, *Pleuronectes platessa*. He felt that the low specific gravity of such salinities made it difficult for larvae to free themselves from the chorion so that they died partly emerged. He also maintained that chorions did not rupture as easily at low salinities. This phenomenon is also clearly demonstrated for winter flounder in Table 3. The highest percentages of abnormalities which were aborted or partially hatched occurred at salinities below 15‰.

Results of these laboratory experiments indicate that successful incubation of embryos occurred over a temperature range which exceeded normal spawning season temperatures by as much as 10°C, but coincide quite closely with natural observations for salinity, although there is a shift in survival toward slightly higher salinities than would have been expected. It is possible that the adults, while being held in the laboratory, were conditioned to slightly higher salinities than would have been encountered in a spawning migration into estuaries. This might explain the differences between natural populations and results of laboratory experiments.

Most winter flounder populations move to inshore and estuarine waters to spawn (Perlmutter 1947; Bigelow and Schroeder 1953; Saila 1961), but there are also spawning populations that remain in offshore shoals (Bigelow and Schroeder 1953; Marak et al. 1962). Field observations in two estuaries of Narragansett Bay and in the Bay itself indicate that spawning occurs at salinities ranging from 11 to 32‰. Plankton tows taken in upper Chesapeake Bay produced one egg in 20‰ with maximum numbers of larvae occurring between 6 and 14‰ (Dovel 1971). Salinities in suspected offshore shoal spawning areas range from 32 to 35.5‰ at the bottom (Bumpus 1973), so an overall spawning range from 5 or 6 to 35.5‰ is indicated for natural populations. The normal temperature range for spawning is 0° to 3.3°C with maximum temperatures for any appreciable egg production and spawning being 4.2° to 5.6°C (Bigelow and Schroeder 1953). Since the eggs are demersal and adhesive, they are not subject to transport into areas of unsuitable temperatures; being estuarine, they are subjected instead to

changes in salinity. However, the euryhaline properties of the eggs insure successful incubation and larval development in a constantly varying salinity environment.

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