OBSERVATIONS ON EARLY LIFE STAGES OF ATLANTIC TOMCOD, *MICROGADUS TOMCOD*

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ABSTRACT

In southern New Brunswick, tomcod spawn in streams from late December to mid-January. The benthic eggs hatch and newly hatched larvae drift to sea in mid-March to mid-April at which time ocean temperatures are beginning to increase. Larval migration to sea is probably aided by active swimming of larvae to the surface to fill the swim bladder, which must be filled within 24 hours of hatching. Photopositivity of the larvae may assist in guiding larvae to the surface.

Water content and specific gravity of eggs reared in 0‰ were 2.8 mg and 1.030. Eggs reared at 10-30‰ had about 2.3 mg water per egg. Specific gravity of eggs incubated in 10‰ was constant for 27 days (at 2°-4° C) at 1.038, then decreased to 1.033. This decrease is associated with water uptake of 0.5-0.6 mg per egg and elimination of salt. The specific gravity of eggs incubated in 20‰ declined linearly from 1.044 to 1.037, associated with accumulation of 0.2 mg of water and elimination of a greater salt load. The specific gravity of eggs incubated at 30‰ declined linearly from 1.049 to 1.045, associated with 0.1 mg water uptake and apparently insufficient salt elimination. Water uptake and salt excretion problems are minimized for eggs reared in freshwater, and under the experimental conditions described here. Normal development could not occur in continued exposure to 30‰. In natural spawning areas, the incubation medium is freshwater for most of the total cycle, with seawater invading the area only at extreme high tide. The salinity tolerance of tomcod eggs is compared with that of freshwater and marine fish eggs in general.

Calculation of specific gravity of egg solids may prove a useful indirect way to investigate salt regulation in fish eggs.

The Atlantic tomcod, *Microgadus tomcod* (Walbaum), is an anadromous species of coastal streams from Newfoundland to Virginia. Adults ascend the lower reaches of southern New Brunswick streams in December and January. These spawning migrations form the basis for a recreational ice fishery in some larger rivers. An annual commercial catch of about 200 t is said to be taken from inshore waters of the northwest Atlantic (Scott and Crossman 1973). Local dip net fishermen take numbers of spawners for both human and animal consumption.

Details of the life history of the early stages (e.g., time of hatching, time of descent into saltwater) have been little studied. Leim (1924) observed that eggs would hatch in freshwater or saline water, but larvae would survive only in saline water. Booth (1967) found sperm motility to be maximal in low salinities, and that salinities of 0-15‰ permitted the highest percentages of eggs to develop to the blastula stage. Howe (1971) described the food habits and growth rates of young tomcod in the Weweantic River estuary, Mass.

The early stages of tomcod development have not been studied extensively; therefore, field studies were performed to obtain information on spawning habitat, rates of egg development, and timing of larval descent to saltwater. Tomcod eggs are deposited in areas subject to variable salinities, so the embryonic development and water balance of tomcod eggs reared in several salinities were also investigated to see how the responses of this species compare with those of freshwater and marine species.

METHODS

Field Studies

The mouth and estuary of Frost Fish Creek (frost fish is a local name for tomcod) were chosen as a study area because the stream hosts a large and regular spawning migration of tomcod which is undisturbed except for some local dip net fishing. It is a small stream (2-4 m wide) forming a common estuary with the Digdeguash River in

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southwestern New Brunswick, with a midsummer discharge ca. 80 l/s and a drainage area of 570 ha (Symons and Martin 1978; Symons and Harding[3]). The drainage basin is typical spruce-fir boreal forest with no human habitation. Portions of it farther upstream have been recently logged.

Tomcod spawn in a 10 to 15 m stretch at the head of tide in Frost Fish Creek (Figure 1). This area is freshwater for most of the tidal cycle, but has a variable bottom salinity (depending upon the height of the particular tide) during high tide. Extreme neap tides do not invade the spawning area. The stream substrate in the spawning area varies from ledge to boulders and cobbles. Most of the eggs settle in substrate interstices.

[3]Symons, P. E. K., and G. D. Harding. 1974. Biomass changes of stream fishes after forest spraying with the insecticide fenitrothion. Fish. Res. Board Can. Tech. Rep. 432, 47 p. Fisheries and Environmental Sciences, Fisheries and Oceans Canada, Biological Station, St. Andrews, NB E0G 2X0.

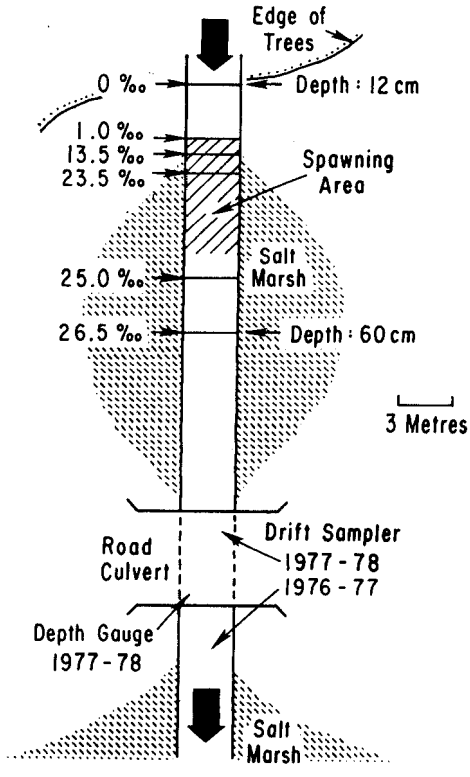

FIGURE 1.—Diagram of tomcod spawning area in Frost Fish Creek in the Digdeguash River estuary, New Brunswick. Depths and salinities are for a "typical" high-tide situation. Salinities were measured at the stream bottom. Hatched area indicates spawning area.

Drift samples were installed downstream of the area of egg deposition (Figure 1) near cessation of spawning (26 Dec. to 2 Jan.) to sample egg and larval drift. The samplers consisted of a galvanized-metal funnel, the narrow opening (5 × 20 cm) facing upstream, with a cloth bag attached to the downstream end (10 × 20 cm). The eggs and larvae accumulated in a 250 ml plastic beaker, with a screened, 2.5 cm diameter hole in one side, clamped to the bag. The sampler was threaded onto an iron rod driven into the stream bottom. A meter stick was installed to measure stream water levels, and stream salinities were measured with a salinity meter. Stream temperatures and drift samples were taken twice weekly at low tide, with the numbers of eggs collected averaged on a per-day basis. Eggs and larvae sampled were preserved in 10% Formalin[4] or 70% ethanol, those preserved in Formalin being cleared later (Galat 1972) to determine degree of development.

One sample of eggs was taken from the area of egg deposition with a Surber sampler in January 1977 to see if development of drifting eggs was the same as those that were not.

Egg Collection

Adults (1 female:2 males) anaesthetized in MS-222 were stripped of eggs and milt in the field. Immediately, the eggs were fertilized by the "dry" method and were washed with stream water 30 s after mixing (temperature at fertilization near 0° C). This water was fresh and was taken from a part of the stream where tomcod were spawning at the time (although spawning may continue into high tide conditions when the water would be of variable salinity). After 1 min the water was changed, and the bottle of eggs was packed in ice and transported to the laboratory. The eggs were transferred to the various incubation salinities 30 min after fertilization. The eggs are weakly adhesive initially, but this adhesiveness disappears if the eggs are separated.

Laboratory Studies

Eggs were incubated in columns of PVC pipe and fittings holding 190 ml of water (Figure 2). Screened floors and lids retained eggs and larvae. Water flowed through the columns at 100 ml/min

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

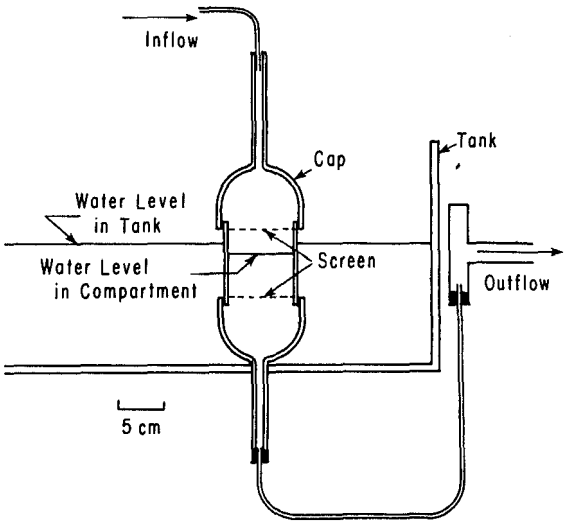

FIGURE 2.—Diagram of incubation chambers used to rear tomcod eggs.

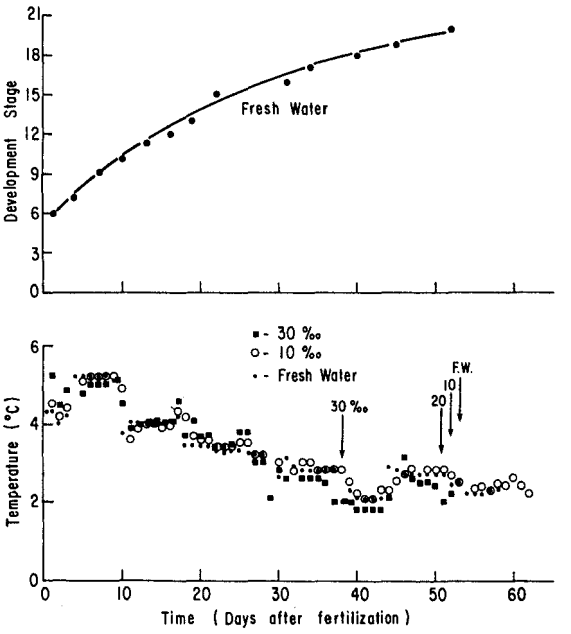

FIGURE 3.—Developmental stages and incubation temperatures for laboratory experiments on tomcod development. Upper panel: Appearance of developmental stages of tomcod eggs incubated in freshwater. Lower panel: Incubation temperatures for tomcod eggs at various incubation salinities. Arrows indicate median hatching dates at each salinity.

(range, 91-111). The columns were immersed in a freshwater bath cooled (2°-4° C) by recirculation of water to refrigerated header tanks. Water flowing to the columns passed through titanium coils in the bath. Water temperature decreased from 4.5° C at the beginning of January to 2.0° C in mid-February (40-d postfertilization), then increased to 2.5° C by the end of February (Figure 3). Eggs were incubated in salinities of 0 (2 columns), 10.1±0.3 (1 column), 20.2±0.6 (1 column), and 30‰ (2 columns). About 250-300 eggs were incubated in each column. Temperature and salinities, by conversion of specific gravity of water with Knudsen's (1962) hydrographical tables, were measured daily.

Columns were checked for egg and larval mortalities every 2-3 d. Every third day, three eggs were removed from each salinity and preserved in 10% Formalin for subsequent study of degree of development. About 100 newly hatched larvae were measured (±0.1 mm) from each salinity and the percentage of deformed larvae noted.

Water content of 10 eggs (combined) from each salinity was measured every fifth day by measuring loss of weight after drying for 16 h at 40° C under vacuum. Specific gravities (sp. gr.) of eggs were measured by glycerol flotation at 10° C as described by Peterson and Metcalfe (1977). Specific gravity of egg solids was calculated from total sp. gr. and water content where solid sp. gr. = egg dry wt/(egg vol. − vol. H_2O), egg vol. = wet wt/sp. gr., and vol. H_2O = water content/sp. gr. of H_2O. Egg diameters were measured microscopically to the nearest 10 μm. Buoyancies of newly hatched larvae, due to air content of the swim bladder, were measured by a Cartesian diver technique (Saunders 1965).

Photic responses of larvae were observed by placing groups of five larvae in a Petri dish, half of which was painted black, on a finger bowl full of ice. Uniform overhead illumination was used.

Statistical Procedures

Differences in water content and dry weights among eggs incubated in the various salinities were tested by one way ANOVA with individual differences detected by means of Duncan's Multiple Range Test. Changes in water content and dry weights during larval development were analyzed by linear regression methods.

RESULTS AND DISCUSSION

Developmental Stages

To assess development of eggs under natural conditions, a series of embryological stages was constructed (Table 1; Figure 3, lower) based on systematically sampled, laboratory-reared eggs. We attempted to make them consistent with those published previously for other species (e.g., Bonnet 1939 for Atlantic cod), although comparisons were difficult in more advanced embryos. For example, Atlantic cod lack a well-differentiated lower jaw at hatching, but it is well developed in tomcod. The stages are also referred to comparable figures in Hardy (1978:278-289) where possible (Table 1). Sampling eggs more frequently would have been useful in some instances; e.g., many anatomical features appeared between days 10 and 13, and are grouped into stage 11. The earliest stages were missed by taking the first sample at 24 h. Stages 3-6 were observed from field samples.

TABLE 1.—Summary of development stages and day of first appearance of anatomical features for tomcod eggs incubated at different salinities. For temperature regime see Figure 3. The stages are for eggs developing in freshwater. Stages 3-5 and 9 were observed in field-collected material only.

Stage	Description	Day of first appearance at: 0‰	10‰	20‰	30‰	Corresponding stage designation by Hardy (1978)
1	Prior to first cleavage	<1	<1	<1	<1	—
2	2 cells	<1	<1	<1	<1	168B
3	4 cells	<1	<1	<1	<1	—
4	8 cells	<1	<1	<1	<1	168C
5	16 cells	<1	<1	<1	<1	—
6	Large celled morula	<1	<1	<1	<1	168E
7	Small celled morula	4	4	4	4	168H
8	Embryonic axis	7	7	7	7	168J
9	Küpfer's vesicle and first somite	—	—	—	—	168E
10	Notochord	10	10	10	10	—
	Optic vesicle	10	10	10	10	169G
11	Eye lens	13	13	13	16	169K
	Ear placode	13	13	13	16	—
	Pericardium	13	13	13	19	—
	Brain lobes differentiating	13	13	13	22	—
	Fin fold	13	13	13	16	—
12	Pectoral fin buds, axial pigmentation	16	16	16	28	170E
13	Eye faintly pigmented	19	19	19	19	170G
14	Gill slit	22	22	22	22	—
	Swim bladder	22	22	25	28	—
15	Nasal placodes	25	25	31	none	—
16	Beginning of pronounced snout	31	31	31	none	171A
17	Lower jaw, mouth not opened	34	34	34	34	171B
18	Mouth can be or is open	37	37	37	—	171C
19	Pigment on lower jaw	45	46	—	—	172A
20	Hatching	—	—	—	—	172A

Irregularities in development were seen in the later stages of development at 30‰. The snout failed to develop normally, and the development of pectoral fin buds and brain lobes was delayed.

Field Observations

Largest numbers of tomcod eggs were sampled by the drift samplers (Figures 4, 5) in the 15- to 20-d period after spawning. The numbers correlated fairly well with stream water level for 1977-78 when water levels were measured (Figure 5). Largest numbers of drifting eggs may also be related to spawning activity rather than stream water levels per se. Typical numbers of eggs col-

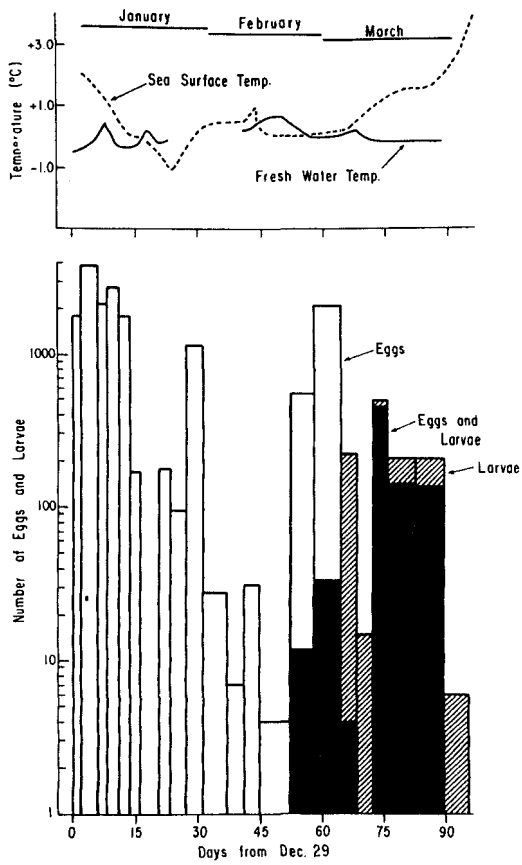

FIGURE 4.—Movements of tomcod eggs and larvae out of Frost Fish Creek. Upper: Environmental conditions and numbers of sampled tomcod eggs and larvae are shown for the 3 mo of egg and larval stream residence in 1976-77. Freshwater temperatures (solid line) and sea surface temperatures (dashed line) for January-April 1976-77. Lower: Histogram of numbers of tomcod eggs and larvae caught in drift samplers. Open bars, eggs; solid bars, eggs and larvae; hatched bars, larvae.

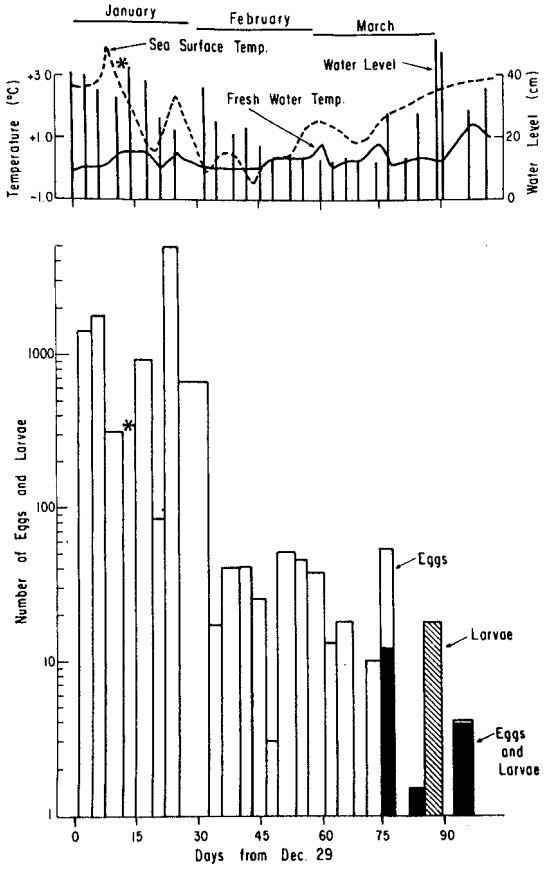

FIGURE 5.—Similar to Figure 4 but for 1977-78. Upper panel includes water levels (vertical bars). Asterisk indicates occurrence of a winter freshet, at which time drift samplers were washed out.

lected ranged from a few hundred to a few thousand per 24 h during these first 15-20 d. In mid-February, stream flow rates decreased as the precipitation accumulated in snow and ice. Typical numbers of eggs sampled/24 h during this period were 10-100. Hatching occurred in March and April (Figures 4, 5). Larvae began to be captured somewhat earlier in 1976-77 and were taken in greater numbers than in 1977-78. This latter phenomenon is thought to be because the sampler was totally submerged in 1977-78, whereas part of it was emergent in 1976-77. Larvae probably emigrate into saltwater near the surface immediately after hatching, as will be discussed in a later section and may have passed over the submerged sampler in 1977-78. Some of the earliest larvae may have hatched in the samplers as a result of warming on the return to the laboratory. These larvae appeared normal and viable. The hatching period in nature corresponded to rising stream water levels in late March in 1977-78. Sea surface temperatures had also risen to 3.0°-4.0° C during fry emigration. Catches of larvae terminated in early to mid-April of both years.

The earliest stages of development obtained in the drift samplers were stage 3 and 4 eggs (Figure 6), owing to lag from spawning to sampling. By the third week of January the embryonic axis was discernible in most eggs sampled. By mid-February, eyes and body axis had become pigmented, nasal placodes and fin fold were appearing, and the tail had curled past the posterior

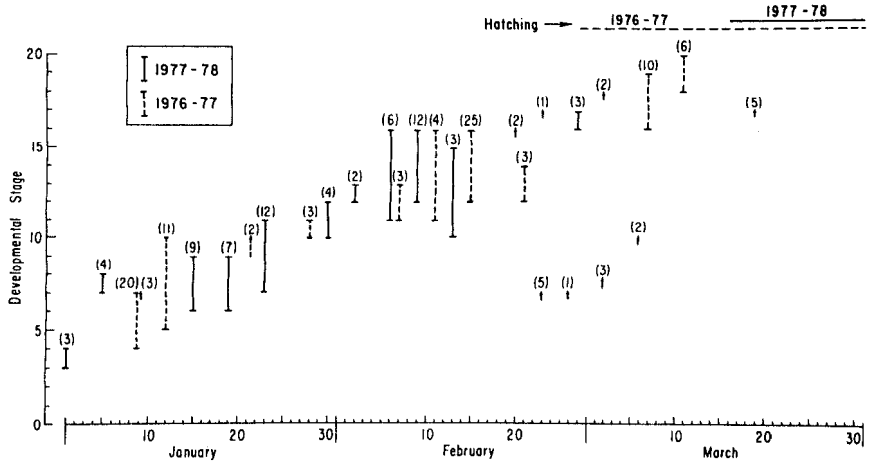

FIGURE 6.—Stages of embryonic development for eggs sampled from Frost Fish Creek with drift samples in 1976-77 and 1977-78. Vertical bars indicate ranges of development observed. Numbers of eggs inspected are in parentheses. Small arrows indicate samples where all eggs were at the same stage.

margin of the eye. Hatching began in late February to mid-March. A series of relatively early stage eggs was also obtained in late February to mid-March 1978 (Figure 6), indicating a possible second spawning of tomcod in late January to early February.

Laboratory Observations

Survival to Hatch and Length at Hatching

Tomcod egg survival to hatching was highest in freshwater (Table 2). Fifty-eight percent of the freshwater eggs hatched, compared with 50, 37, and 13% at 10, 20, and 30‰ salinities, respectively. About half of the mortality at 0 and 10‰ occurred at about day 30 (stage 15). Above 10‰ high mortality also occurred at earlier stages of development.

TABLE 2.—Percentage survival to hatching, total larval length at hatching, and median time to hatch for tomcod eggs incubated at four salinities. Standard deviations are given for larval lengths.

Item	0‰	10‰	20‰	30‰
Percentage egg survival to stage 19	70	73	48	21
Percentage hatched	58	50	37	13
Mean length at hatching (mm)	7.56±0.69	7.25±0.31	6.31±0.44	—
Number of larvae measured	165	104	85	
Time to median hatch (d)	54	51	51	38

Larvae hatched in freshwater were significantly longer than those from higher salinities (7.54 mm for freshwater vs. 7.25 and 6.31 mm at 10 and 20‰, respectively). Larvae at 30‰ had severe spinal curvature and could not be measured accurately. Hatching was earlier at the higher salinities.

The developmental success of tomcod eggs varied with salinity, so various parameters associated with water balance were measured on eggs reared at 0, 10, 20, and 30‰. These parameters are all interdependent so that changes in one may result in concomitant changes in others.

Specific Gravity

The sp. gr. of freshwater (FW) eggs was constant throughout development (Figure 7) at 1.030, implying that weight and volume were not changing or that they were changing in such a way that the sp. gr. was constant. In contrast, eggs incubated at 20 and 30‰ decreased in sp. gr. throughout development. Specific gravity at 10‰ was constant for the first 25 d of incubation, then decreased linearly. The sp. gr. of water at 10, 20, and 30‰ (10° C) are 1.009, 1.017, and 1.024, so that eggs were denser than the incubation medium at all salinities and by approximately the same amount. For example, the sp. gr. of FW eggs is 1.030 compared with 1.000 for freshwater, a difference of 0.03 sp. gr. units. The sp. gr. of 20‰ eggs (extrapolated to 0 time at 20‰ from Figure 7) is 1.045 compared with 1.017 for the incubation medium, again a difference of 0.03 sp. gr. units. Changes in sp. gr. may be associated with changes in water content, loss of solids through metabolism, change of salt content of eggs, or a combination of these factors.

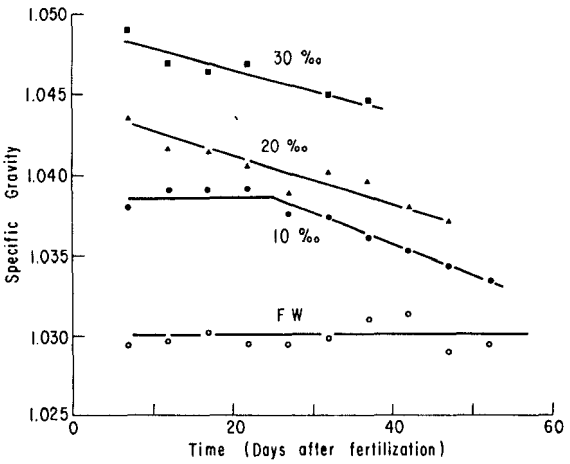

FIGURE 7.—Specific gravity of tomcod eggs incubated at various times from fertilization at various incubation salinities. Each point is based on the mean of measurements made on 10 eggs. Lines fitted by eye. FW = freshwater.

Water Content

Mean water content of FW eggs was 2.83 mg/egg (Table 3) with no trends throughout development. The water content and percentage water content of FW eggs for the first 27 d of development were significantly higher than the values obtained at the other incubation salinities ($P<0.05$, ANOVA, Duncan's Multiple Range Test). The percentage water content increased from 86.4 to 89‰ over the final 25 d of egg development, attributable to decreases in egg dry weight (Tables 4, 5).

There were no significant differences among the percentages of water content of eggs reared at the three higher incubation salinities for the first 27 d

TABLE 3.—Water content (milligrams per egg) and percentage water (parentheses) in tomcod eggs at various incubation salinities and days from fertilization. Each value represents 10 eggs. Sampling periods were fewer at 20 and 30‰ due to earlier hatch.

Days of incubation	0‰	10‰	20‰	30‰
9	2.86(86.7)	2.19(82.3)	2.50(84.7)	2.35(81.9)
12	2.87(87.2)	2.14(84.3)	2.48(83.5)	2.32(82.9)
17	2.72(86.6)	2.36(81.9)	2.47(83.4)	2.54(83.6)
22	2.94(85.5)	2.22(81.5)	2.39(83.9)	2.29(79.8)
27	2.78(86.1)	2.34(83.9)	2.30(82.7)	2.40(82.2)
27-d mean	2.83(86.4)	2.25(82.8)	2.43(83.6)	2.38(82.0)
32	2.78(86.8)	2.40(84.5)	2.41(84.0)	2.42(83.0)
37	2.87(87.5)	2.44(85.3)	2.44(84.5)	2.55(84.7)
42	2.86(89.4)	2.66(86.0)	2.60(86.1)	—
47	2.88(87.8)	2.58(85.4)	2.65(85.8)	—
52	2.76(89.0)	2.91(88.2)	—	—
Newly hatched larvae	1.41(85.5)	—	—	—

The water content of newly hatched FW larvae was 1.41 mg (85.5%), so about half of the water in the FW egg is associated with perivitelline fluid and zona radiata.

Dry Weight

No measurable change in egg dry weight occurred over the first 27 d of incubation (Table 4). A one way ANOVA indicated significant differences among the mean dry weights for the first 27 d of incubation ($F = 3.9$, $P<0.05$). The mean dry weight of 30‰ was significantly greater than those of FW and 20‰ eggs (Duncan's Multiple Range Test).

TABLE 4.—Dry weights (mg) for various incubation salinities and times from fertilization (d). Each value is averaged from 10 pooled eggs. Values to the left of the bracket for the first 27 d of development are means for that period.

Days of incubation	Incubation salinity (‰)			
	0	10	20	30
9	$\pm^{0.45}_{0.03}$ { 0.44	$\pm^{0.47}_{0.05}$ { 0.47	$\pm^{0.47}_{0.02}$ { 0.45	$\pm^{0.52}_{0.04}$ { 0.52
12	0.42	0.40	0.49	0.48
17	0.42	0.52	0.49	0.50
22	0.50	0.50	0.46	0.58
27	0.45	0.45	0.48	0.52
32	0.42	0.44	0.46	0.51
37	0.41	0.42	0.46	0.46
42	0.34	0.40	0.42	—
47	0.36	0.44	0.44	—
52	0.34	0.39	—	—
Newly hatched larvae	0.25	—	—	—

TABLE 5.—Statistical parameters for regressions of percentage water content vs. time (d) and dry wt vs. time (data given in Tables 3, 4). Times are for days 27-52, inclusive. b = slope of regression equation, r = correlation coefficient, df = degrees of freedom.

Regression	Parameter	Incubation salinity (‰)		
		0	10	20
% H_2O vs. time	b(%/d)	0.10	0.17	0.13
	r	0.80	0.91	0.93
	df	4	4	3
	t	2.72	4.53	4.47
	P	~0.05	<0.025	<0.05
Dry wt vs. time	b(mg/d)	−0.0046	−0.0018	−0.0024
	r	0.96	0.70	0.83
	df	4	4	3
	t	3.55	3.92	2.60
	P	<0.025	<0.025	<0.1

of incubation. The water content of 10‰ eggs increased over the last 25 d of incubation at 0.023 mg/egg per d ($P<0.05$, Tables 3, 5), until the water content at hatching approached that of FW eggs. The 20‰ eggs took up water after 27 d incubation at 0.018 mg/egg per d ($P<0.01$), but the water content of these eggs was still lower than for eggs incubated in FW and 10‰. The 30‰ eggs may have taken up slight amounts of water, but the data are insufficient to be tested statistically.

Dry weight decreased significantly over the last 25 d (Tables 4, 5). This decrease was greatest in freshwater, about 0.1 mg/egg compared with 0.07 and 0.04 at 10 and 20‰, respectively. Although sample size is inadequate for statistical analysis, it would appear that the 30‰ eggs lost about 0.05 mg/egg. Yolk content of newly hatched larvae was not measured; however, larvae hatched from higher incubation salinities appear to have more yolk (Figure 8), an observation supported by the fact that hatching is earlier at higher salinities. The lesser amount of yolk of FW eggs is in agreement with the greater loss of solids by these eggs.

About 25% of the dry weight of FW eggs is lost at hatching, and is thus contributed by the chorion and the perivitelline fluid.

Egg Diameter

The diameters of 10 eggs from each incubation salinity were measured at the time intervals indicated in Table 6. There was no indication of any change in egg diameter with length of incubation (Table 6), so that the water uptake at the three

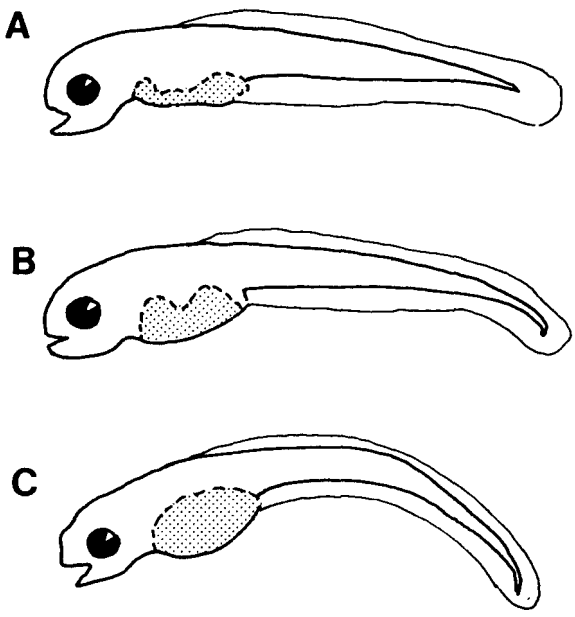

FIGURE 8.—Newly hatched tomcod larvae from various incubation salinities. Note the larger amounts of yolk remaining, the higher the salinity of incubation. A. Freshwater; B. 10‰; C. 20‰. Yolks have shrunk due to (Formalin) fixation effects. Magnifications 13×.

TABLE 6.—Egg diameters (millimeters) for various incubation salinities and incubation times. Each value is the mean of 10 measurements. v = calculated egg volume (microliters). Standard deviations given in parentheses.

Days of incubation	0‰	10‰	20‰	30‰
4	1.85(0.06)	1.79(0.08)	1.77(0.05)	1.81(0.09)
12	1.86(0.04)	1.76(0.07)	1.75(0.06)	1.76(0.04)
17	1.87(0.05)	1.75(0.05)	1.79(0.05)	1.80(0.04)
22	1.89(0.04)	1.77(0.05)	1.76(0.05)	1.78(0.05)
27	1.84(0.07)	1.75(0.05)	1.76(0.05)	1.76(0.04)
32	1.89(0.02)	1.74(0.05)	1.78(0.06)	1.78(0.05)
37	1.90(0.05)	1.77(0.05)	1.78(0.05)	1.78(0.06)
42	1.93(0.05)	1.82(0.04)	1.81(0.05)	—
47	1.84(0.04)	1.81(0.04)	1.76(0.06)	—
52	1.86(0.07)	1.79(0.05)	—	—
$\bar{x}$	1.87	1.78	1.77	1.78
v	3.47	2.86	2.84	2.83

higher salinities toward the end of the incubation period did not lead to measurable swelling, although slight swelling within experimental error probably occurred. The mean diameters of FW, 10, 20, and 30‰ eggs were 1.87, 1.78, 1.77, and 1.78 mm, respectively. The standard deviations for lots of 10 eggs varied from 0.02 to 0.09 mm, and were 0.04-0.05 in most cases. The greater diameter of the FW eggs is no doubt related to the higher water content of these eggs.

Specific Gravity of Egg Solids

The sp. gr. of FW egg solids (lipids included) was constant at 1.27 for the first 27 d of incubation (Figure 9), then rose linearly to about 1.36 just before hatching. This increase in sp. gr. of egg solids may be due to increase in compact tissue, such as cartilage. It could also reflect a rapid increase in embryonic tissue and a corresponding decrease in yolk solids. The sp. gr. of solids of Atlantic salmon alevins also increases during development (Peterson and Metcalfe 1977). This was shown to be due to increase in embryonic mass, the solids of which had a higher sp. gr. than did yolk solids.

The sp. gr. of 10‰ egg solids was identical to that of FW eggs throughout. Apparently, insufficient salt penetrated these eggs to change the solids' sp. gr. measurably. This apparent lack of difference between FW and 10‰ eggs should be accepted with some caution, since an error of 0.01 mg/egg in estimating dry weight (averaged over the first five measurements) could result in a shift in solids' sp. gr. by 0.2 units. Since the dry weight of 10‰ eggs was some 0.02 mg greater than that of FW eggs (Table 4), some salt may well have entered the 10‰ eggs.

The sp. gr.'s of egg solids for 20 and 30‰ eggs during early development were much higher than for the two lower salinities (Figure 9), and they

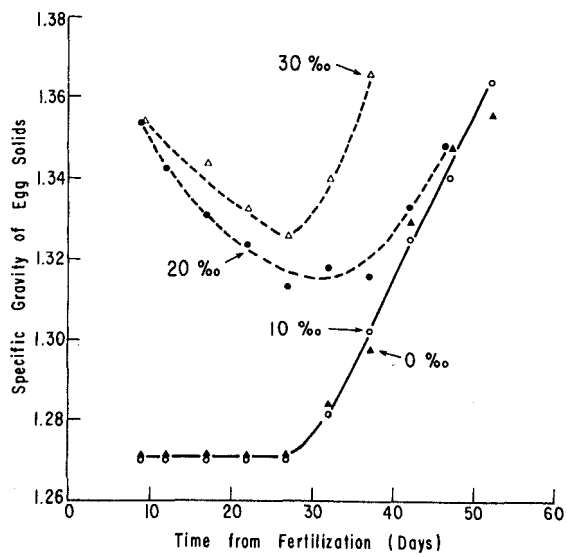

FIGURE 9.—The specific gravity of egg solids (as calculated from water content and egg specific gravity) at various times from fertilization for various incubation salinities. Lines fitted by eye.

decrease with time until a minimum is attained at 27 d postfertilization. The sp. gr. then rose again, as for eggs reared at 0 and 10‰. The decrease in egg solids' sp. gr. from fertilization to day 27 at 20 and 30‰ may be due to more efficient salt elimination as the embryo grows. After 27 d the decrease due to salt elimination is more than balanced by increases due to factors postulated above for FW eggs. Apparently, eggs reared at 20‰ were successful in eliminating salt, because the sp. gr. of their egg solids tends to converge with that for eggs reared at lower salinities. At 30‰, however, the embryos appeared to be unable to eliminate excess salt successfully because the solids' sp. gr. never approached those for eggs reared at lower incubation salinities. The 30‰ eggs hatch earlier than those incubated at lower salinities, perhaps in response to high salt concentrations. As mentioned previously, they were abnormally developed.

The higher sp. gr. of egg solids at 30‰ would require that 15% of the solids be excess salt (using the formula $1.27a + 1.8(1-a) = 1.35$; where a = proportion of egg solids that is not salt; 1.8 = sp. gr. of salt, 1.27 = sp. gr. of FW egg solids, 1.35 = sp. gr. of 30‰ egg solids). This amounts to about 0.08 mg. This is reasonably close to the increase in dry weight of 30‰ eggs over FW eggs (0.07 mg). The decrease in sp. gr. of 30‰ egg solids to its minimum of 1.33 would require the loss of 0.02 mg of salt.

Newly hatched larvae in freshwater that did not have access to the water surface had an sp. gr. of 1.032, which is nearly identical to that of eggs incubated in freshwater. If larvae were permitted access to air at the water surface, the sp. gr. declined within 7 h to 1.01, coincident with ingestion of air into the swim bladder. Newly hatched larvae were observed swallowing air at the surface. The filling of the swim bladder was investigated further with a Cartesian diver technique. Larvae that had access to air floated at a flotation pressure (Saunders 1965) of 154 mm Hg (130-170, $n = 6$) (0.8 atm). Those that had been denied access to air for 24 h failed to float at 675 mm Hg (645-685, $n = 15$), corresponding to 0.1 atm (the greatest vacuum attainable with the apparatus). No air was observed in the swim bladder of these larvae. These larvae were then allowed access to the surface overnight. When tested subsequently, none floated at 675 mm Hg, nor was air observed in the swim bladder. These latter larvae, unlike larvae with air in the swim bladder (that spend most of their time near the surface), stayed on the bottom of the container.

Newly hatched larvae were photopositive as tested in a half-blackened Petri dish. In two trials, 78% (39/50) and 64% (16/25) larvae were observed in the lighted (unpainted) half of the Petri dish. When the dish was kept in darkness, 46% (23/50) and 36% (9/25) larvae were observed in the unpainted half. Larvae were commonly observed to aggregate near the lighted sides of rearing containers.

GENERAL DISCUSSION

The changes that occurred in eggs reared in various salinities will first be summarized:

Eggs reared in freshwater consisted of 2.8 mg water, sufficient for the embryo's needs, being constant throughout development. The egg sp. gr. was also constant despite decrease in solid materials (ca. 0.1 mg)—the egg diameter should therefore decrease slightly (about a 1.7% decrease is required), although this was not observed, as it is within experimental error.

Eggs reared in 10‰ salinity have about 2.2 mg water for the first month of development, but take up an additional 0.5-0.6 mg in the later stages of development, due to the greater water requirements of embryonic tissue. Some of this uptake may also be associated with formation of fluid filled body cavities (Zotin 1965). This water uptake was associated with a decreased egg sp. gr. The 10‰ eggs may have a slight salt load which is probably eliminated in the later developmental stages. The egg dry weight declined by only 0.07 mg, and newly hatched 10‰ larvae may have larger yolks than do those in 0‰ (Figure 9).

Eggs reared in 20‰ salinity had a water content equal to or greater than that of 10‰ eggs in the early stages of development, but had to tolerate a higher salt load (ca. 0.04 mg/egg) as a result. Egg sp. gr. declined throughout development due to salt elimination as the embryo developed and to accumulation of about 0.2 mg water during the later developmental stages. Advanced embryos eliminated much of the initial salt load as the egg solid sp. gr. of late stage eggs is nearly identical to that of eggs reared at lower salinities. The concept of salt elimination seems reasonable, but is subject to some uncertainty in these experiments because the solids associated with the chorion and perivitelline fluid are included in the estimates of

solids sp. gr. These compartments of the egg obviously would have no capacity for elimination of salts. Egg dry weight declined by only 0.04 mg at hatching as newly hatched 20‰ larvae appear to have even more yolk than 10‰ larvae (Figure 8). The 20‰ curve in Figure 9 suggests that salt elimination began very early and increased as the embryo grew. It is probable that at least the early salt elimination had a cellular rather than organ basis.

Eggs reared in 30‰ salinity also had a water content similar to those reared at 10 and 20‰, but the salt load was high. Water accumulation during the later developmental stages was low (ca. 0.1 mg). The sp. gr. of egg solids goes down over the first 27 d of development, indicating some elimination of salt. The pattern during the later stages of development is strikingly different from that at 20‰ in that the solids' sp. gr. again rose to about 1.37 at 37 d of incubation at which point the larva hatched. Problems with salt balance and osmoregulation may have led to the deformities and early hatching. The dry weight of 30‰ eggs decreased only slightly during development.

It has been shown, for the eggs and larvae of some marine organisms, that the salinity in which fertilization and the earliest stage of development occur may influence development and growth of subsequent stages in the life history (Kinne 1962). It is therefore possible that eggs fertilized in water of higher salinity might have responded differently to the various experimental salinities. Booth (1967) obtained data suggesting that fertilization could occur in salinities as high as 15‰. It is notable, however, that the eggs of *Cyprinodon macularius* in Kinne's experiments were allowed to develop 3-6 h in the spawning salinity, and at a higher temperature (27° C) than was the case for the tomcod experiments. It is probable that the eggs of *C. macularius* had developed further before experimentation.

The tomcod's early life history seems adapted to the hydrodynamics of streams in which it spawns. Spawning migrations occur in late December to early January while water levels are still high from the fall freshets. The eggs develop throughout midwinter when water levels are low, thus minimizing loss of eggs from the stream, then hatch when water levels are rising coincident with the early snow melt. The higher water levels during hatching would ensure rapid flushing of larvae into the estuary. Newly hatched larvae probably rise to the stream surface soon after hatching and ingest air into the swim bladder, with possibly the positive response to light facilitating surfacing. This behavior of newly hatched larvae would also ensure rapid flushing into the estuary.

The continuous drift of eggs out of the stream is somewhat puzzling. Most eggs taken in the drift samples were alive and apparently developing normally. These perhaps are eggs which had been deposited where they were likely to be taken up into suspension. In support of this suggestion, egg drift was positively correlated with stream level. Whether these eggs continue to develop would depend in part upon the salinity conditions where they finally settle and the ambient salinity during earlier development. Laboratory results indicate that less than full salinities are required for normal development from fertilization, but the effects of variable salinities on tomcod egg development were not investigated.

The tomcod egg resembled those of freshwater species (rather than marine species) in regard to salt tolerance, assuming that the responses reported here are typical. Eggs of brook trout exhibit increased mortality above 6‰ salinity with total mortality at 12‰ (Sutterlin et al.[5]). Species such as *Abramis* will hatch in salinities up to 20‰, although 2.5-5‰ is optimal (Holliday 1969). With the tomcod, between 20 and 30‰ salinity appears to be the upper limit for production of normal larvae. By way of contrast, eggs of several marine species have been hatched in salinities up to 60‰ (cod, herring, plaice), although optima are usually in the 25-30‰ range (Holliday and Blaxter 1960; Holliday 1965).

Eggs of marine species tend to swell at low salinities (usually <15‰); above this salinity egg diameter is constant (Holliday 1965; Solemdal 1967). Tomcod eggs require salinities of <10‰ for noticeable swelling to occur.

Several parameters measured (water content, dry weight, solids' sp. gr., and egg sp. gr. for 10‰ incubation) begin to change dramatically at about 27 d of incubation. In relation to embryonic development it seems probable the embryonic mass is beginning to increase dramatically at this point, resulting in the noted physiological changes. Perhaps these changes are linked to the high mortality occurring at this stage of development.

[5]Sutterlin, A. M., P. Harmon, and H. Barchard. 1976. The culture of brook trout in salt water. Fish. Mar. Serv. Res. Dev. Tech. Rep. 636, 21 p. Fisheries and Environmental Sciences, Fisheries and Oceans Canada, Biological Station, St. Andrews, NB E0G 2X0.

Zotin (1965) reported that eggs of freshwater teleosts (e.g., loach, zander (*Lucioperca*)) took up no water after water hardening until the chorion began to stretch due to weakening by the hatching enzyme. The mullet egg took up water during the second half of development during which time the perivitelline space first appeared. With the tomcod, water uptake occurred in the latter stages of development in the three higher salinities. It is not known where this water was distributed within the egg, but it was probably incorporated into embryonic tissue.

It is inferred, from calculated specific gravity of egg solids, that tomcod embryos osmoregulated to some degree, becoming more proficient as development proceeded. This may be simply a function of embryonic size, resulting in more osmoregulating tissue. It has been suggested by Holliday (1965) that plaice embryos can regulate osmotic concentration after gastrulation, which occurs in 9 d or less in these tomcod eggs. Holliday (1969) also showed that flounder eggs could regulate yolk sodium from fertilization. Unfortunately, we did not make measurements here before 9 d of incubation.

Holliday and Blaxter (1960) and Forrester and Alderdice (1966) observed development to proceed faster at higher salinities for herring and Pacific cod, respectively. While tomcod hatched earlier at higher salinities, there is little suggestion that development occurred more rapidly. Rather, it appeared that the freshwater larvae grew larger prior to hatching. Some structures were delayed, or never appeared in 30‰ embryos, but this is due to abnormal development at this salinity. Abnormal development has frequently been recorded at abnormally high salinities. Usually the deformities are skeletal as are observed for tomcod, or involved body cavity deformities (Holliday 1965; Alderdice and Forrester 1971).

Although the tomcod is a physoclist species, the pneumatic duct is apparently functional in the newly hatched larva. In <24 h the duct is closed, and the larva can no longer fill the swim bladder by air ingestion. Larval loss of the pneumatic duct has been implied for physoclists generally (Harden Jones 1957). Whether or not the duct is utilized in initial filling of the bladder is apparently quite variable (Johnston 1953; Schwarz 1971).

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