TEMPERATURE EFFECTS ON GROWTH AND YOLK UTILIZATION IN YELLOWTAIL FLOUNDER, *LIMANDA FERRUGINEA*, YOLK-SAC LARVAE

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ABSTRACT

Embryos and yolk-sac larvae of yellowtail flounder, *Limanda ferruginea*, were incubated at 4°, 8°, 10°, and 12° C. Embryos incubated at 8° and 10° C produced significantly larger yolk-sac larvae at hatching than those incubated at 4° and 12° C. Yolk utilization rate was positively correlated with temperature. Growth in length was fastest at 12° C. At yolk-sac absorption there was no significant difference in size among larvae incubated at 8°, 10°, or 12° C. Efficiency of yolk utilization prior to hatching was 86.2, 76.8, 73.5, and 45.9% for 12°, 10°, 8°, and 4° C. Overall yolk utilization efficiency from fertilization to yolk-sac absorption was highest at 12° C (47.1%), intermediate at 8° and 10° C (43.8 and 42.2%), and lowest at 4° C (29.8%). Efficiency decreased during the course of development at all four temperatures.

Based on the experimental results, it appears that sea temperatures between 8° and 12° C would have little, if any, differential effect on larval size at yolk-sac absorption and therefore ability to survive. It also appears that 4° C may be at or near the lower thermal limit for successful reproduction of southern New England yellowtail flounder.

The yellowtail flounder, *Limanda ferruginea*, is an important commercial species in both the New England and Canadian otter trawl fisheries. Yellowtail flounder range from the Gulf of St. Lawrence south to lower Chesapeake Bay (Bigelow and Schroeder 1953). Royce et al. (1959) and Lux (1963) found that there are five relatively distinct stocks within this range with little migration occurring between them: Georges Bank, Cape Cod, Nova Scotian, Newfoundland, and southern New England.

Over the past 35 yr, landings from the southern New England ground have fluctuated widely (Royce et al. 1959; Lux 1964, 1969; Sissenwine 1974), with a sharp decline observed in the late 1940's not accompanied by the usual symptoms of overfishing, i.e., a decline in average size, increased percentage of young fish, or increased growth rate. Royce et al., (1959) suggested the decline was caused by a warming trend inducing a temporary northeastward shift of the population center away from the southern New England grounds. Sissenwine (1974) demonstrated a significant inverse relationship between water temperature and equilibrium catch and recruitment.

The correlation between temperature and yellowtail flounder abundance is an indication that temperature may be causing fluctuations in the fishery. This research was designed to investigate the effect of temperature on growth rate, size at hatching and yolk-sac absorption, and yolk utilization rate and efficiency. Most fisheries biologists agree that early life history stages of fishes are the most vulnerable due to their small size, poor swimming ability, and susceptibility to rapid environmental changes (May 1974a). Because of this, the total set of environmental parameters in which these young fishes develop will largely determine their collective success or failure, and consequently their year-class strength. During larval development, the time when the larva changes from its endogenous source of food (yolk) to exogenous feeding is a "critical period" in the organism's life history (Hjort 1926; Marr 1956; Toetz 1966; May 1974a). Particularly important to successful initiation of exogenous feeding is the size and condition of the larva (Blaxter and Hempel 1963; Braum 1967). To a large extent size and condition will depend on the efficiency with which the organism is able to convert its yolk to larval tissue. Any environmental variable that affects conversion efficiency could affect larval size, and consequently larval ability to begin feeding. Taken over the entire population of larvae, year-class strength could be significantly affected by such environmental influences.

One such variable affecting yolk utilization efficiency is temperature (e.g., May 1974b). Because temperature has been suggested as the dominant environmental variable affecting yellowtail flounder abundance and because other in-

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vestigators have found that temperature can affect yolk utilization efficiency and thereby subsequent size and feeding ability, the current study examines the hypothesis that temperature affects yolk utilization efficiency and size of yellowtail flounder larvae.

METHODS

Adult yellowtail flounder were collected southeast of Block Island, R.I., on 28 March 1979 aboard a commercial fishing vessel. They were placed in a 680 l tank periodically supplied with running seawater and transported to the laboratory where they were held, four to a tank, in 286 l aquaria supplied with a continuous flow of filtered seawater.

To induce ripening, both sexes were anesthetized with tricaine methanesulfonate (MS-222[2]) at a concentration of 1:20,000 g and injected intramuscularly with 2.0 mg of carp pituitary dissolved in marine fish Ringer's solution per kilogram of fish wet weight following Smigielski (1979). Daily injections continued until spawning occurred. Two females of 34.4 and 42.0 cm TL (total length) were anesthetized and their eggs manually stripped into a glass bowl containing 0.45 μm filtered, ultraviolet-treated seawater (34.0‰ salinity, 10.5° C). The eggs were fertilized with milt stripped from two anesthetized males (34.5 and 33.0 cm TL). The fertilized eggs were divided volumetrically among four 6 l black plastic pans containing seawater identical to that in which fertilization had occurred. Twenty-five IU/ml penicillin and 0.02 mg/ml streptomycin were added to each pan as an antibiotic. These pans were placed in temperature-regulating circulation baths, gently aerated, and allowed to equilibrate slowly to the test temperatures. The four test temperatures chosen (4°, 8°, 10°, and 12° C) were maintained at 4.5±0.6°, 8.7±0.6°, 10.3±0.5°, and 12.2±0.6° C (mean ± 1 SD). The temperatures chosen encompass the range over which most eggs and larvae of yellowtail flounder have been collected in nature (Royce et al. 1959; Colton 1972; Smith et al. 1975). Dissolved oxygen and salinity ranged from 7.6 to 8.1 mg/l and 33.0 to 34.0‰. Photoperiod was 12D:12L throughout the experiment.

Measurements of egg and yolk diameters of a random sample of unfertilized eggs (n = 100) were made by ocular micrometer, and egg and yolk volumes calculated.

Prior to weighing, fresh unfertilized eggs were rinsed in three changes of isotonic 0.9% (weight/volume) ammonium formate to remove residual saltwater. Mean dry weight and ash-free dry weight of 390 eggs were determined to the nearest 1.0 μg, using a Perkin-Elmer electrobalance following the method of Laurence (1973). To determine the mean dry weight and ash-free dry weight of yolk per egg it was necessary to subtract mean dry and ash-free dry weight of the egg capsule (zona radiata) from the two values. Twenty-six capsules were removed from embryos just prior to hatching and dry weights and ash-free dry weights were determined by the method previously cited. Mean capsule weights were subtracted from the mean values of dry weight and ash-free dry weight of unfertilized eggs. The difference was taken as the mean dry and ash-free dry weight of yolk per egg. As both mean yolk weight and mean yolk volume were known, it was possible to calculate the dry weight and ash-free dry weight of yolk for any given volume.

Random samples of 25 yolk-sac larvae were removed from each temperature treatment beginning 2 h after hatching, and continuing at 24-h intervals until the experiments were terminated. Yolk-sac measurements were made with an ocular micrometer. The volume of the elliptical yolk sac (V_{ys} in cubic millimeters) was calculated from the formula for a spheroid:

$$V_{ys} = (\pi/6)LH^2 \quad (1)$$

where L is the length (millimeters) and H the height (millimeters) of the yolk sac (Blaxter and Hempel 1963). At each sampling period the length from tip of snout to tip of notochord was measured to the nearest 0.01 mm for each of the larvae sampled using an ocular micrometer. The fish then were rinsed in ammonium formate, and mean dry weights and ash-free dry weights determined as previously described.

Because of inherent variability in micromeasurements, data were smoothed using linear regression to relate ash-free dry weights of yolk-sac larvae and yolk-sac volumes to numbers of hours posthatch at all four temperatures. Ash-free dry weights of yolk-sac larvae and yolk-sac volumes were predicted using regression equations for each 24-h time interval and temperature. Predicted

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

yolk-sac volume (cubic millimeters) then was multiplied by the calculated ash-free dry weight of a cubic millimeter of yolk to yield the ash-free dry weight of the yolk within the yolk sac. This value was subtracted from the predicted ash-free dry weight of the yolk-sac larvae to give the ash-free dry weight of the larval tissue alone. The validity of using the same ash-free dry weight (and caloric value) of yolk throughout the yolk-sac stage has been demonstrated by Lasker (1962).

Temperature effects on yolk utilization rate were determined using analysis of covariance to compare the slopes of the regression lines. The relationship between larval notochord length and hours posthatch was nonlinear. Growth curves, linearized by logarithmic (natural) transformation of the time axis (hours posthatch), were compared by analysis of covariance. Notochord lengths, ash-free dry weights of yolk-sac larvae, and yolk-sac volumes at time of hatching and yolk-sac absorption for the four different temperatures were compared using analysis of variance. Where significant differences were found, the Student-Newman-Keuls (SNK) test was used to locate individual treatment differences.

Caloric values of yolk and larval tissue were determined by wet oxidation following Maciolek (1962). Ten samples of unfertilized ova were used to determine the caloric content of yolk. Caloric content of larval tissue was determined from finely ground samples of larvae after total yolk absorption. Three samples each were done from larvae reared at 8° and 12° C. No calorimetry was attempted at 4° and 10° C since insufficient numbers of larvae were available at yolk-sac absorption.

Yolk utilization efficiency, expressed as a percentage, is defined as the ash-free dry weight of the larva minus its yolk (or its caloric equivalent) at time t, divided by the ash-free dry weight of yolk (or its caloric equivalent) that had been used from fertilization to time t. Details of this method are described elsewhere (Toetz 1966; Laurence 1969). Because variability was high in ash-free dry weight measurements, it was not possible to calculate meaningful daily efficiencies. Thus efficiencies were calculated at ony two points in time—at hatching and at yolk-sac absorption. Efficiencies also were calculated using ash-free dry weights of larvae minus their yolk sacs and ash-free dry weights of yolk utilized, as predicted by linear regressions. These values were used to examine trends in efficiency over time.

TABLE 1.—Summary of size, ash-free dry weight (AFDW), and caloric value of unfertilized yellowtail flounder eggs.

	n	Mean	SD
Egg diameter, mm	100	0.7566	0.0079
Yolk diameter, mm	100	.7324	.0126
Egg volume, mm³	100	.2268	.0071
Yolk volume, mm³	100	.2059	.0105
Total AFDW, mg	390	.0127	.0009
Egg capsule AFDW, mg	26	.0006	.0001
[1]Yolk AFDW, mg	—	.0121	—
Cal/g AFDW of yolk	3	4,268.3	155.48
[1]AFDW, mg, per mm³ yolk	—	.059	—

[1]Since the mean was derived by subtraction, no sample size or standard deviation are given.

RESULTS

Data on size, ash-free dry weight, and caloric value of unfertilized eggs from the females used in this study are given in Table 1. The mean egg diameter (0.75 mm) is slightly smaller than the mean diameters of 0.9 and 0.88 mm reported by Bigelow and Schroeder (1953) and Colton and Marak.[3]

Incubation temperature affected both the length and weight of yolk-sac larvae at time of hatching, but not at yolk-sac absorption (Table 2). At hatching, notochord length was significantly longer in larvae incubated at 8° and 10° C than in those incubated at 12° and 4° C (ANOVA, SNK, $P<0.05$). Among the four temperatures, no significant differences (ANOVA, $P>0.05$) were found between ash-free dry weights of entire yolk-sac

[3]Colton, J. B. Jr., and R. R. Marak. 1969. Guide for identifying the common planktonic fish eggs and larvae of Continental Shelf waters, Cape Sable to Block Island. U.S. Bur. Commer. Fish., Biol. Lab., Woods Hole, Mass., Lab. Ref. 69-9, 43 p.

TABLE 2.—Mean ± standard deviation of lengths, ash-free dry weights, and yolk-sac volume of yellowtail flounder reared at four temperatures. Values connected by vertical lines are not significantly different (ANOVA, SNK, $P>0.05$).

		At hatching			At yolk-sac absorption	
Temperature (° C)	Sample size	Notochord length (mm) at hatching	Yolk-sac volume (mm³)	Ash-free dry weight of yolk-sac larvae (mg)	Ash-free dry weight of yolk-sac larvae (mg)	Notochord length (mm)
4	25	2.117±0.213	0.1155±0.016	0.0086±0.001	—	—
12	50	2.096±0.126	.1213±0.020	.0116±0.002	0.0040±0.001	3.458±0.218
8	25	2.494±0.138	.0958±0.012	.0100±0.002	.0043±0.001	3.542±0.189
10	35	2.419±0.215	.0978±0.014	.0119±0.002	.0042±0.001	3.406±0.190

larvae compared at hatching. Yolk-sac volumes, however, differed significantly. Larvae reared at 12° and 4° C had significantly larger yolk volumes than those reared at 8° and 10° C (ANOVA, SNK, $P<0.05$). Larval tissue weight is taken as the difference between ash-free dry weight of the entire yolk-sac larva and ash-free dry weight of yolk. Since total ash-free dry weight was not significantly different between the four temperatures, it follows that the ash-free dry weight of the larval tissue alone must be significantly greater in those larvae reared at 8° and 10° C. This coincides with the difference seen in length. At yolk-sac absorption there were no significant differences either in length or ash-free dry weight of yolk-sac larvae (ANOVA, $P>0.05$) for the three temperatures where these variables were measured.

Notochord length increased with time after hatching (Table 3). Analysis of covariance revealed that larvae incubated at 12° C grew significantly faster than those at the other temperatures ($P<0.05$). Larvae at 4° C grew at an intermediate rate that was significantly different ($P<0.05$) from fish in other treatments. No significant difference ($P>0.05$) in growth rate was evident between 8° and 10° C larvae. Fish in both of these treatments exhibited the slowest growth rates ($P<0.05$). Regression coefficients of ash-free dry-weight of yolk-sac larvae and yolk-sac volumes vs. hours posthatch were significantly different ($P<0.001$) from zero (Table 3).

Ash-free dry weight of the larva minus its yolk sac at a particular temperature and time was calculated as the difference between the predicted total ash-free dry weight and the predicted ash-free dry weight of the yolk (Tables 4-7). Predicted values indicate that embryo weight increases linearly with time at all temperatures except 8° C (Table 5) where it remains constant.

Temperature effects on yolk utilization rate were examined by comparing the regression coefficients of the four equations for decrease in yolk-

TABLE 3.—Predictive linear equations ($Y = a + bX$) derived from least squares linear fits of the yellowtail flounder data. All equations are based on measurements taken every 24 h between hatching and yolk-sac absorption. AFDW = ash-free dry weight, and ln = natural logarithm.

Variables Y vs. X and temperature (° C)	Sample size	a	95% confidence limit for a	b	95% confidence limit for b	r
Notochord length (mm) vs. ln hours posthatch:						
4	308	1.6446	0.1192	0.27799	0.02564	0.81
8	228	2.2737	.0919	.21710	.02147	.85
10	195	2.2464	.0997	.20723	.02563	.83
12	225	1.8852	.0660	.30749	.01681	.95
AFDW yolk-sac larvae (mg) vs. hours posthatch:						
4	58	.0092	.0008	-.000021	.000005	.79
8	39	.0103	.0009	-.000035	.000008	.86
10	28	.0106	.0016	-.000046	.000016	.81
12	36	.0114	.0011	-.000047	.000010	.88
Yolk-sac volume (mm^3) vs. hours posthatch:						
4	308	.1139	.0028	-.000432	.000017	.95
8	203	.0840	.0036	-.000584	.000036	.94
10	170	.0931	.0034	-.000822	.000050	.95
12	200	.1068	.0054	-.000899	.000067	.92

TABLE 4.—Predicted values of yolk-sac larval weight, yolk-sac volume and weight, larval tissue weight, and calculated efficiencies at 4° C. AFDW = ash-free dry weight.

Hours posthatch	Yolk-sac larvae (mg AFDW)	Yolk-sac volume (mm^3)	Yolk[1] (mg AFDW)	Larval tissue[2] (mg AFDW)	Yolk utilized[3] (mg AFDW)	Efficiency[4]
2	0.0092	0.1131	0.0067	0.0025	0.0054	46.3
24	.0087	.1035	.0061	.0026	.0060	43.3
48	.0082	.0932	.0055	.0027	.0066	40.9
72	.0077	.0828	.0049	.0028	.0072	38.9
96	.0072	.0724	.0043	.0029	.0078	37.2
120	.0067	.0620	.0037	.0030	.0084	35.7
144	.0061	.0516	.0030	.0031	.0090	34.4
168	.0056	.0413	.0024	.0032	.0097	33.0
192	.0051	.0309	.0018	.0033	.0103	32.0
216	.0046	.0205	.0012	.0034	.0109	31.2
240	.0041	.0101	.0006	.0035	.0115	30.4
264	.0036	0	0	.0036	.0121	29.8
288	.0031	0	0	.0031	.0121	25.6

[1]Yolk-sac volume times 0.059.
[2]Yolk-sac larvae minus yolk.
[3]0.0121 minus yolk.
[4]Larval tissue divided by yolk utilized times 100.

TABLE 5.—Predicted values of yolk-sac larval weight, yolk-sac volume, weight, and caloric value, larval tissue weight and caloric value, and calculated efficiencies at 8° C. AFDW = ash-free dry weight.

Hours posthatch	Yolk-sac larvae (mg AFDW)	Yolk-sac volume (mm^3)	Yolk[1] (mg AFDW)	Larval tissue[2] (mg AFDW)	Yolk utilized[3] (mg AFDW)	Efficiency[4]	Caloric value of yolk utilized[5]	Caloric value of larval tissue[6]	Caloric efficiency[7]
2	0.0102	0.0828	0.0049	0.0053	0.0072	73.6	0.0307	0.0167	54.4
24	.0094	.0670	.0040	.0054	.0081	66.7	.0346	.0170	49.1
48	.0086	.0559	.0033	.0053	.0088	60.2	.0376	.0167	44.4
72	.0078	.0419	.0025	.0053	.0096	55.2	.0410	.0167	40.7
96	.0069	.0279	.0016	.0053	.0105	50.5	.0448	.0167	37.3
120	.0061	.0138	.0008	.0053	.0113	46.9	.0482	.0167	34.6
144	.0053	0	0	.0053	.0121	43.8	.0516	.0167	32.4

[1]Yolk-sac volume times 0.059.
[2]Yolk-sac larvae minus yolk.
[3]0.0121 minus yolk.
[4]Larval tissue divided by yolk utilized times 100.
[5]Yolk utilized times 4.2683.
[6]Larval tissue times 3.6959.
[7]Caloric value of larval tissue divided by caloric value of yolk utilized times 100.

TABLE 6.—Predicted values of yolk-sac larval weight, yolk-sac volume and weight, larval tissue weight, and calculated efficiencies at 10° C. AFDW = ash-free dry weight.

Hours posthatch	Yolk-sac larvae (mg AFDW)	Yolk-sac volume (mm^3)	Yolk[1] (mg AFDW)	Larval tissue[2] (mg AFDW)	Yolk utilized[3] (mg AFDW)	Efficiency[4]
2	0.0105	0.0915	0.0054	0.0051	0.0067	76.1
24	.0095	.0734	.0043	.0052	.0078	67.0
48	.0084	.0537	.0032	.0052	.0089	58.4
72	.0073	.0339	.0020	.0053	.0101	52.5
96	.0062	.0142	.0008	.0054	.0113	47.8
120	.0051	0	0	.0051	.0121	42.2

[1]Yolk-sac volume times 0.059.
[2]Yolk-sac larvae minus yolk.
[3]0.0121 minus yolk.
[4]Larval tissue divided by yolk utilized times 100.

TABLE 7.—Predicted values of yolk-sac larval weight, yolk-sac volume, weight, and caloric value, larval tissue weight and caloric value, and calculated efficiencies at 12° C. AFDW = ash-free dry weight.

Hours posthatch	Yolk-sac larvae (mg AFDW)	Yolk-sac volume (mm^3)	Yolk[1] (mg AFDW)	Larval tissue[2] (mg AFDW)	Yolk utilized[3] (mg AFDW)	Efficiency[4]	Caloric value of yolk utilized[5]	Caloric value of larval tissue[6]	Caloric efficiency[7]
2	0.0113	0.1050	0.0062	0.0051	0.0059	86.4	0.0252	0.0188	74.6
24	.0102	.0852	.0050	.0052	.0071	73.2	.0303	.0192	63.4
48	.0091	.0636	.0038	.0053	.0083	63.9	.0354	.0196	55.4
72	.0080	.0420	.0025	.0055	.0096	57.3	.0410	.0203	49.5
96	.0069	.0204	.0012	.0057	.0109	52.3	.0465	.0211	45.4
120	.0057	0	0	.0057	.0121	47.1	.0516	.0211	40.9

[1]Yolk-sac volume times 0.059.
[2]Yolk-sac larvae minus yolk.
[3]0.0121 minus yolk.
[4]Larval tissue divided by yolk utilized 100 times.
[5]Yolk utilized times 4.2683.
[6]Larval tissue times 3.6959.
[7]Caloric value of larval tissue divided by caloric value of yolk utilized times 100.

sac volume (Table 3). Analysis of covariance showed that the rate of decrease was related directly to temperature. All coefficients were significantly different ($P<0.05$) with yolk utilization being fastest at 12° C, followed by 10°, 8°, and finally 4° C.

Efficiency was estimated as ash-free dry weight of yolk converted into ash-free dry weight of larval tissue at all four temperatures. Overall efficiency was considered as the calculated efficiency at time of yolk-sac absorption. Values were lowest at 4° C (29.8%) intermediate at 8° and 10° C (43.8 and 42.2%), and highest at 12° C (47.1%) (Tables 4-7).

Efficiency in terms of calories of yolk converted into calories of larval tissue was calculatecd at 8° and 12° C (Tables 5 and 7). Estimates were obtained by dividing the caloric value of larval tissue at time t by the estimated caloric value of yolk utilized to time t. Calories per milligram ash-free dry weight of larval tissue at yolk-sac absorption were 3.152 ± 0.37 at 8° C and 3.696 ± 0.23 at 12° C. Analysis of variance indicated the two values were not different ($P>0.10$). Efficiencies based on caloric conversions were lower than those based on ash-free dry weight. Larvae reared at 12° C still ranked highest in overall efficiency (40.9%) with

larvae at 8° C showing a somewhat lower overall efficiency (32.4%).

Efficiencies calculated at hatching reflect the efficiency with which yolk was converted to larval tissue during embryological development. These values were consistently higher than overall efficiencies (Tables 4-7) and were similar in ranking. Based upon ash-free dry weight conversions, larvae at 12° C were most efficient (86.2%), followed by those at 10° C (76.8%), at 8° C (73.5%), and, last, at 4° C (45.9%). As for overall efficiencies, caloric efficiencies at hatching were lower than those calculated on an ash-free dry weight basis: 74.7% at 12° C and 54.3% at 8° C.

A decrease in efficiency with continuing development was noted at all four temperatures (Tables 4-7).

DISCUSSION

Mean diameter of eggs used in this study were slightly smaller than mean sizes reported by other investigators (Bigelow and Schroeder 1953; Colton and Marak footnote 3). Many variables can effect egg diameter. Laurence (1969) and Alderdice and Forrester (1974) have demonstrated a relationship between egg diameter and parental size. Egg diameter has also been related to incubation temperature and salinity, as well as time from fertilization (Alderdice and Forrester 1974; Alderdice et al. 1979). In addition, Blaxter and Hempel (1963) found differences in egg diameter between stocks of herring. If any of these variables affect egg size in yellowtail flounder, it appears likely that the results found here may not be comparable with reported values.

Larvae incubated at the intermediate temperatures (8° and 10° C) were significantly larger at hatching than those incubated at the extremes (4° and 12° C). This conflicts with data of Smigielski[4] who found that mean length at hatching was independent of temperature over a 6°-14° C range. One reason for the different findings may be the time at which measurements were taken. Since hatching occurs over a period of about 12-36 h, depending on the temperature, and since growth is rapid, the mean size estimated will depend on the time measurements were taken. Furthermore, Alderdice and Forrester (1974) and Alderdice and Velsen (1971) have shown that larval size at hatching can be different among fish in the same treatment depending on hatching time.

The implications of larval size at hatching may not be great relative to size at yolk-sac absorption. Upon hatching there is no need for larvae to feed actively due to their endogenous yolk supply. Because larger size confers an advantage in swimming ability, which in turn affects feeding ability (Hunter 1972), it follows that the size attained at yolk-sac absorption, when the larvae change to exogenous feeding, is more critical than the size at hatching. Analysis of growth rates from hatching to yolk-sac absorption indicate that larvae at 12° C grew significantly faster than those at other temperatures. Because of this, 12° C larvae attained a size equal to that of 8° and 10° C larvae by yolk-sac absorption. Because of the similarity in size of larvae reared at these three temperatures, it is presumed that they would be equally successful in capturing prey. Although no data were available for 4° C larvae at yolk-sac absorption, their smaller size at hatching, combined with their low conversion efficiency, should result in their being significantly smaller at yolk-sac absorption. The added fact that all larvae in the 4° C treatment died shortly before yolk-sac absorption makes it probable that 4° C is at, or near, the lower temperature limit for successful reproduction in the southern New England yellowtail flounder stock.

Yolk utilization rate, measured by decrease in yolk-sac volume over time, also was affected significantly by temperature; the higher the temperature, the more rapidly yolk was used. This is to be expected since rate of yolk (food) consumption, is one measure of the rate of physiological functions (metabolism and growth) which are temperature-dependent in most ectotherms (Brett 1970). A number of previous studies have shown similar results (e.g., Blaxter 1956; Ryland and Nichols 1967; Fluchter and Pandian 1968).

Calculated efficiencies indicate the number of calories incorporated, or the amount of yolk converted into larval tissue in a particular time interval. The remaining calories, or weight, are lost through the metabolic processes of yolk transformation, maintenance, activity, and excretion. Incubation temperatures in this study were observed to affect both rate of growth and rate of yolk utilization. Since the calculated efficiency will depend on the relationship between these two rates, a change in either rate, relative to the other, will be reflected in a change in efficiency. Because temperature affects both these rates, it is not surpris-

[4]Alphonse Smigielski, Fisheries Biologist, National Marine Fisheries Service, NOAA, Narragansett, RI 02882, pers. commun. November 1979.

ing that several investigators have found a relationship between incubation temperature and yolk utilization efficiency. Laurence (1973) found that overall efficiencies for tautog, *Tautoga onitis*, were 36.3, 25.5, and 25.8% for 16°, 19°, and 22° C. Ryland and Nichols (1967) found that for plaice, *Pleuronectes platessa*, efficiencies were roughly 35-40% at lower temperatures (2.5°-5.0° C) and 43-57% at higher temperatures (6.5°-8.5° C). Working with the Atlantic salmon, *Salmo salar*, Hayes and Pelluet (1945) found that efficiency was low (42%) at temperatures of 0°-5° C, and increased linearly with increasing temperature to 60% at 16° C.

The overall efficiencies in this study, based upon ash-free dry weights, were 43.8, 42.2, and 47.1% for 8°, 10°, and 12° C. The similarity of these values is an indication that within this temperature range, mechanisms are available whereby the increased metabolic demands of the larval tissue, caused by the higher temperatures, are balanced by an increased transfer of energy from the yolk for the building of tissues. The fact that increasing growth rate with temperature is accompanied by an increased rate of yolk utilization lends support to this hypothesis. Blaxter and Hempel (1966) also point out that overall efficiencies can be similar at different temperatures if the interrelationship between rate of rise in metabolic requirements and reduction in development time are balanced over a temperature range. Wood (1932) reported that yolk utilization efficiency in trout was independent of temperature between 7° and 12° C. Marr (1966), however, after recalculating the data, concluded that efficiency was actually higher at 10° C. Johns and Howell (1980) found that efficiencies were similar in summer flounder, *Paralichthys dentatus*, larvae at 16° and 21° C. They noted that the ratio of yolk needed for metabolism to yolk converted to tissue remained constant at the two temperatures, causing efficiencies to be similar. Although none of the investigations on yolk utilization efficiency demonstrates temperature independence, several of the studies show, over a particular section of the temperature range tested, that efficiencies are quite similar. These include work on *S. salar* (Hayes and Pelluet 1945), *Clupea harengus* (Blaxter and Hempel 1966), and *T. onitis* (Laurence 1973).

Larvae incubated at 4° C did not survive to yolk-sac absorption; however, 288 h after hatching, when approximately 2% of the yolk remained, the calculated efficiency was 25.6%. This low value indicates that the energy within the yolk was being largely used for metabolic demands other than growth of larval tissue. The relatively low efficiency of yolk conversion at 4° C adds further support to the conclusion that 4° C is a suboptimal temperature for this stock of yellowtail flounder.

A reduction in efficiency as development proceeded was noted at all four temperatures. Blaxter and Hempel (1966) noted such a decrease in herring larvae and concluded that the reduction was due to the relatively higher metabolic demands of heavier larvae. Although no metabolic measurements were made in this study, it is suspected that the explanation offered by Blaxter and Hempel (1966) applies to these results.

The hypothesis that a deficit in food energy can be caused by yolk exhaustion prior to initiation of exogenous feeding has received considerable attention. Such a deficit has been demonstrated by Lasker (1962) for the Pacific sardine, *Sardinops caerulea*. Laurence (1969, 1973) working with largemouth bass, *Micropterus salmoides*, and tautog found that no such deficit occurred in either species. Yellowtail flounder larvae reared at 8°, 10°, and 12° C in this study, as well as those reared by Smigielski (1979), all possessed darkly pigmented eyes, a functional mouth and jaw apparatus, and a completely formed gut at yolk-sac absorption. These morphological traits strongly indicate that larvae were able to begin feeding at this time. Smigielski (1979) further noted that yellowtail flounder larvae were capable of surviving for several days without food after the yolk reserves were depleted. The larva's apparent capacity to feed at yolk-sac absorption, and its ability to survive temporarily without exogenous food make it unlikely that an energy deficit significantly effects survival. This observation, combined with the fact that larvae reared at 8°, 10°, and 12° C were equal in size at yolk-sac absorption, thus conferring equal feeding and predator avoidance abilities, is an indication that larvae growing at these temperatures would have equal survival potential.

Results of this study indicate that yellowtail flounder eggs and yolk-sac larvae are eurythermal. Smith et al. (1978), studying diel vertical migrations of yellowtail flounder larvae, found that those less than about 4 mm long migrated only short distances, and thus experienced little temperature change. Larger larvae, however, were subjected to as much as a 10° C change (from 5° to

15° C) during the course of their migration, and Smith et al. concluded that yellowtail flounder larvae were "physiologically adapted to wide ranges in temperature." Even though larger yellowtail flounder larvae are apparently rather eurythermal, the fact that their zone of tolerance can be exceeded in nature is demonstrated by the observation of Colton (1959). Colton found many dead yellowtail flounder larvae across a 16 km (10-mi) transect in which the temperature rose from 8° to 20° C in <24 h. This observation does not, however, refute eurythermality in this species since the temperature changes were so extensive and abrupt.

These experimental results indicate that temperatures between 8° and 12° C have little direct effect on survival of yellowtail flounder larvae. This, combined with the observations of Smith et al. (1978) indicate that early stages of yellowtail flounder are eurythermal. Because of this, it seems doubtful if temperature causes the observed fluctuations through direct physiological means. Obviously abrupt thermal changes such as those observed by Colton (1959) could cause mass mortality and therefore poor recruitment of a year class. Perhaps it is through such a mechanism that temperature affects abundance. It is also possible that temperatures tested in this study were not high enough to demonstrate a clear relationship between a high temperature and some physiological response that would affect the larva's ability to survive.

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