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Marta S. Garreton for the degree of Master in Science in Oceanography presented on August 24, 1983.

TITLE: BIOENERGETICS OF YOLK UTILIZATION IN EMBRYOS AND YOLK-SAC LARVAE OF THE SURF-SMELT *HYPOMESUS PRETIOSUS PRETIOSUS* (GIRARD, 1855) UNDER DIFFERENT INCUBATION TEMPERATURES

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Abstract approved: George W. Boehlert

Embryonic dry weight, yolk volume and oxygen consumption of the surf-smelt (*Hypomesus pretiosus pretiosus*) were studied from fertilization to yolk sac absorption. Experiments were conducted under five incubation temperatures encompassing those encountered in beach gravel where eggs are naturally spawned during spring tides of summer months. Yolk utilization rates were independent of the incubation temperature over the range 12° - 22° C. At 6° C the yolk depletion rate was approximately three times lower than the rates calculated for high temperatures. Magnitude of acceleration in converting yolk to body tissue, as reflected by Q_{10}'s, was greatest between 6° and 12° C. Catabolic expenditures of energy, as compared to energy available in the egg, indicated that an energetic deficit did not occur during larval development. Yolk utilization

efficiencies were within the range of 40-70%, similar to those reported for other species of fishes. Similarities in yolk utilization over a wide range of temperatures suggest that physiological mechanisms to compensate for temperature changes are highly developed in surf-smelt. Facultative diapause characterized by retardation of the hatching time was found in laboratory-reared surf-smelt eggs. The factor causing this phenomenon was most likely the lack of water agitation normally present in beach sediments.

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INTRODUCTION

Early stages in the life history of oviparous fishes, from spawning to first exogenous feeding, have a finite supply of energy. The fate of this energy is influenced by physical conditions in the surrounding environment. Temperature has been found to play an important role in the utilization of yolk reserves of developing embryos. Optimum temperatures for the development and growth of the embryo theoretically result in larvae that have optimal physical condition and nutritional status to search for and capture food after yolk sac absorption. Lasker (1962) observed an energy deficit, defined as a complete utilization of yolk reserves prior to the time when larvae are capable of feeding, in embryos of the Pacific sardine (*Sardinops caerulea*). He also noted catabolism of body tissues before larvae were morphologically or behaviorally capable of capturing planktonic prey. Laurence (1973) suggested that such energy deficits resulted in high mortality rates or "critical

periods" in larval life. In species with demersal, intertidal eggs, the developing embryos are exposed to changing temperature regimes. Here, the definition of optimum temperatures and interrelationships with yolk reserves may differ from species exposed to constant temperatures.

The surf-smelt Hypomesus pretiosus pretiosus is biologically interesting due to its peculiar habits of spawning. This fish takes advantage of the breaking surf of the outer sea-coast zone of the Pacific Northwest, where shifting sand and gravel serve as spawing substrate. Spawning activity occurs high on the beach and corresponds with tidal cycles as they affect the beach. (Thompson et al., 1936). The deposition of adhesive eggs occurs in very shallow waters, and the eggs adhere individually to grains of gravel. They are covered by water once or twice daily for a short time; the drained gravel which retains moisture in its interstitial spaces can provide a cooling effect (Misitano, 1977). The location of the spawning on the beach may play an important role due to temperature differences between upper and lower levels, which undoubtedly influences the length of the incubation period and the metabolism of the embryo. Schaefer (1936) working with surf-smelt found that ". . .the incubation period of the eggs may last from 9 to 11 days in the summer months but is much longer at low temperatures."

The geographical distribution of this nearshore species is confined to the Pacific coast of North America. It is common along the

coast of British Columbia (Clemens and Wilby, 1961) and occurs as far south as Monterey Bay, California. It has not been recorded farther west than Sitkalidak Strait in Alaska (MacAllister, 1963).

The main objective of this research is to analyze the influence of temperature upon yolk utilization by surf-smelt embryos and to consider mechanisms of adaptation to variable temperature regimes. The original yolk supply is utilized in two ways; part is transformed into the tissues of the larval body and the remainder is used as a source of energy for total metabolism. The metabolism has three components: first, growth metabolism is the energy expended in the chemical transformation of yolk to body tissue; the second component is maintenance metabolism, the energy used in resting respiration; the third component is active metabolism, providing energy for movement. These components will vary in relation to one another as development proceeds (Blaxter and Hempel, 1966).

Preliminary observations on smelt eggs, during the summer of 1981, suggested that temperature is an important factor in the bioenergetic balance during embryogenesis. Larvae incubated at high temperature were shorter than larvae incubated at low temperature. The different lengths may be caused by differential energy utilization during development. Similar results have been found for other species (Gray, 1928; Laurence, 1969, 1973; Howell, 1980).

Since temperatures of <u>in situ</u> incubation may depend upon sea temperature, solar insolation and tidal height, larvae resulting from

eggs spawned in the upper part of the beach may utilize yolk less efficiently than those incubated at lower levels, where temperatures may be cooler. This study examines the effects of temperature in relation to the ecology of the surf-smelt spawning environment.

MATERIALS AND METHODS

Eggs used in this investigation were collected from intertidal beach gravel in the vicinity of Yachats, Oregon (Lat. 44° 19' N, Long. 124° 06' W) where gravid surf-smelt are found during spawning. Throughout the spawning season of 1981, pebbles with attached, newly spawned eggs were removed from the beach and transported to the O.S.U. Marine Science Center for experimentation.

The spawning area surveyed is a beach of approximately 250 m in length. In the high intertidal, the sediments were composed of particles ranging from 2 to 10 mm in size; most abundant were dark pebbles (1-4 mm) of basaltic origin. In the intertidal (0.0 cm to 169 cm) sand grains predominated. A river mouth is present approximately 1500 meters south of the spawning area.

Surveys were conducted every month during the period of spawning. Data collected included egg densities in beach gravel and measurements of the physical conditions (dissolved oxygen, salinity, and temperature of sea water, and *in situ* temperatures of the substrate at different tidal levels).

Samples of gravel and eggs were taken using a standard sampler of 0.25 m diameter x 0.05 m deep for estimating egg density and cylinders of 54 and 33 cc capacity (cores) to estimate the vertical distribution of eggs in the upper 15 cm of the sediment. Upper, middle and lower zone, defined according to tidal range were sampled

in a transect fashion. Samples, sediment and eggs, from the standard sampler were preserved with 5% formalin and sea water. Bengal Rose, a specific stain for organic matter, was added to facilitate the identification of the eggs among the pebbles. The number of eggs in 100 cc subsamples was counted macroscopically. The material collected in the cores was frozen until analysis was conducted. Immediately after thawing, each "core" was carefully sectioned in two-centimeter portions and stained with Bengal Rose (200 mg/l) dissolved in 5% formalin.

Eggs collected directly from the gravel which were usually viable and resistant to handling and stress from transportation, were used for this study. Eggs from the natural environment appear turgid and transluced to the naked eye. The eggs of surf-smelt have multiple oil droplets and are about one millimeter in diameter. The yolk is heterogeneous and elliptical. Stages of development were based on the nomenclature established by Fischer (1958). Acclimation to the different experimentation temperatures lasted at least 24 hours. The eggs were incubated at 6°, 12°, 15°, 18° and 22° C in ten liter black tanks with aeration. All incubations were carried out in darkness.

During acclimation, the eggs were kept in the same water in which they had been transported. Once acclimation periods were completed, the sea water was partially renewed for the first time.

The sea water for the experiments was obtained from the Yachats area, sterilized in an autoclave and renewed every other day.

Mechanical action caused by the water movement is apparently required for normal embryos to emerge at uniform hatching times within a given incubation temperature. Eggs kept for a long period of time in motionless water, were able to remain in diapause, or delayed hatching, for as much as seven times the normal hatching date (104 days at 6° C). Under experimental conditions lack of agitation by waves and surf causes the larva to emerge at various times. Thus, for this species, the time of hatching was defined as the midpoint between first and last hatching times for each temperature.

The energy budget of an organism with an endogenous source of energy was defined by Johns and Howell (1980) as follows:

$$C = P + R + U$$

where C is the yolk energy consumed, P the portion of energy used for growth, R the portion used for metabolism and U the portion lost through excretion. In this research, the yolk energy consumed (C) and converted to tissue growth (P) was also determined from calculations of the daily changes in their respective weights and were then multiplied by their respective caloric equivalents to yield daily values of energy used or converted to tissue.

Ova, chorions and larvae that had already exhausted their yolk and oil droplet were dried to constant weight in order to determine percentage of carbon, nitrogen and hydrogen using a Perkin Elmer Model 240 B elemental analyzer. The percentage of carbon was used to calculate the caloric contents of the ova, chorions and larvae when yolk is completely utilized. By applying the following equation given by Salonen et al. (1976), the percentage of carbon was converted to calories per gram ash free dry weight of embryo:

$$Cal.g^{-1} \text{ AFDW embryo} = [(0.651 \times \% C) - 12.237] / 4.186 \times 10^{-3}$$

Since unfertilized eggs (ova) are almost entirely yolk, the caloric value of these ova minus caloric value of chorion and adhesive disc was utilized in the energy budget. The chorion and adhesive disc are not a source of energy for the developing embryo. The caloric equivalents estimated correspond to 5546 calories per gram of ash free dry weight for ova and to 4739.7 calories per gram of ash free dry weight for larval tissue with yolk entirely consumed. Energy changes during embryogenesis were measured by direct and indirect calorimetry.

Direct calorimetry (dry weight method) was conducted by calculating energy contents of yolk and embryonic tissue, then measuring yolk volume at intervals through development. In order to determine changes in dry weight at intervals during embryogenesis,

average dry weight and volume measurements on unfertilized eggs were used as a basis for comparison.

The weight of the egg membrane or chorion and that of the adhesive disc was partitioned from the initial dry weight of the ova. The initial mean dry weight of the ovum averaged 134.3 µg. The average dry weight of developing eggs or larvae at any stage was determined by dividing their total weight by the number of eggs or larvae in the sample (usually ten) and subtracting the mean weight of the chorion and adhesive disc.

Embryonic dry weight was measured to the nearest 0.1 µg after drying the eggs at 58° C to constant weight. Prior to weighing, the material was brought to room temperature in a silica gel desiccator. The above method was used for: (1) eggs before fertilization; (2) chorions and adhesive discs; (3) samples of eggs and chorions that were ashed in electric furnace at 500° C for four hours to determine ash content; and (4) eggs after fertilization at intervals during embryogenesis. At each temperature samples of 10 eggs were rinsed in an isomotic solution of 0.9% ammonium formate and blotted dry to absorb excess water.

A conversion factor was determined by dividing the average values of weight by volume of ova. This factor (467 $\mu g/mm^3$) was then applied to the volumetric estimates made upon yolk sac to get yolk weight. The yolk volume was estimated from ocular micrometric measurements of the yolk diameter. In the late embryonic stages, the

yolk length (L) and its height (H) was measured. The formula for volume, $V = (\pi/6)\ L \times H^2$ was applied for the calculations.

Dry weight values of the developing embryo were obtained by subtracting the weight of the yolk from the total embryo plus yolk weight. Calculations on energy budgets were based on the procedures of Toetz (1966) and Laurence (1973).

Indirect calorimetry was conducted by measuring oxygen consumption during embryonic development using standard manometric techniques (Umbreit et al. 1964) with a Gilson differential respirometer. Daily measurements of oxygen consumption started 24 hours after fertilization and lasted until starvation of the hatched larva. These measurements included two replicates of at least 40 eggs for each temperature of incubation. This number was increased when working with earlier stages of development or low temperatures. For all experiments the temperature in the respirometer was the same as incubation temperature. The ova utilized for oxygen consumption measurements were taken from the spawnings from late August, September and October. Measurements were estimated as microliters of oxygen per embryo per hour. The amount of oxygen required throughout the entire period from fertilization to the death of the yolk sac larvae was calculated for each temperature. The resulting values were converted to calories using an oxycalorific equivalent of 0.005 calories per microliter of oxygen (Swift and French, 1954). The oxygen consumption method compares the available

yolk energy at any given time with the sum of the energies of growth increment and metabolism. The indirect energy approach makes feasible the comparisons of the oxygen consumption method with energetic values of yolk obtained from dry weight method calculations.

Yolk utilization efficiencies at intervals during development were calculated by dividing the energy value of growth at a given time interval (t_2) by the energy value of yolk used in that interval.

The efficiency with which yolk is transformed to body tissue was calculated by using the "plastic efficiency coefficient" or P.E.C. (Gray, 1928) which is the ratio of dry weight of the developed embryo to that of the fertilized egg. The same ratio using caloric values rather than dry weight was also applied. This latter was named the "apparent energetic efficienty" by Needham (1931).

Efficiencies were measured from fertilization to maximum weight, to midpoint hatching and to the time point when 75 percent of the yolk had already been exhausted; the latter was called T_{25} because at that point only 25 percent of yolk is left and was the more reliable endpoint for comparisons among samples.

Temperature effects on yolk utilization rate were determined by regression. For each temperature, the variation between rate and time were studied following the general linear approach given by Neter and Wasserman (1974) which includes fitting unrestricted and restricted models and F statistic calculations controlling alpha at

the level 0.5 (F 95%). Models for the five temperatures containing indicator variables and interaction terms were required for both oxygen consumption and yolk utilization rates. The chi square calculations to test for interdependence of density of eggs and height of the beach were based on the procedures stated by Snedecor and Cochran (1980).

RESULTS

Spawning Season. The spawning activity of surf-smelt occured during spring tides and extended from late May to October 1981 with a peak of spawning during August and September. Surf-smelt appeared on the beach in pulses for as long as four hours starting towards the end of the high tide; in most cases the females led the run.

Throughout the spawning season a decrease in mean total length of the spawning population was observed from onset to end of the spawning season. Mean total length values for females were 19 cm, 18.7 and 16.2 cm for onset, middle and end of the spawning season. Slightly lower mean values of 17.7 cm, 17.8 cm and 16 cm were found for males during the same period. The range in total length varied from 13.8 to 21 cm for females and from 13 to 19.5 cm for males. Wide variability in length of mature fish was found at the end of the spawning season, especially among females. A similar pattern was also observed in the size and dry weight of the ova. The difference between minimum and maximum diameters of ova was only 3% of the maximum size at the onset of the spawning season but 12% in October.

Spawning area. A subdivision of the beach was made in the place where most of the material was collected. Three main zones were defined and characterized according to the tidal range. The tidal range was determined by means of graduated poles. The readings obtained for the limits of the three zones are the following: (1)

Lower Zone, comprises from the mean low low tide (MLLW 0.0 cm) to 169 cm above the MLLW; (2) Intertidal Zone, from 169 cm to 279 cm above MLLW; (3) Upper Zone, from 279 cm to 437 cm above MLLW respectively.

In situ readings of temperature were taken sporadically throughout the spawning season. Values for sea water ranged from 10° to 16° C. The maximum was found during two consecutive days in June. The lowest value (10° C) was observed early in a morning during July. In the months of September and October, temperatures were 13° and 13.5°, respectively.

From onset to the end of the spawning season, a decreasing trend in the temperatures of the gravel was noted for the three zones of study in the beach. Maximum and minimum values measured for Upper, Intertidal and Lower zones were: 24° and 15° C, 17° and 11° C and 15° and 10.8° C respectively.

The salinity fluctuated betwen 30 ppm (13°C) and 33 ppm. Oxygen content of the sea water was at saturation levels between 7.75 and 8.4 mg/liter.

Counts of eggs from the standard sampler used to collect pebbles along the three zones and those obtained from the cores placed vertically in the substrate (from surface to approximately 15 cm deep) were made for the four months in study. The average number of eggs ranged from 0.51 to 1.42 egg/cc. Vertical distribution of eggs gave maximal percentages of deposition between 6 and 8 cm (24%) and 8 and 10 cm (21%). Frank and Leggett (1981b) found that the range of egg

densities was 90 to 140 egg/cc in capelin beaches. They reported a maximum depth of egg deposition equal to 10-12 cm. The relationships between density of eggs and height of the beach are shown in Tables 1 and 2. Two hypotheses were tested: (1) the height of the beach is independent of the density of the eggs found in each sampled month and (2) the height on the beach is independent of the density of eggs found in each vertical layer (Tables 1 and 2). Since the calculations of chi square for both null hypotheses of independence gave probabilities greater than 99.9%, the null hypotheses were not rejected.

Unfertilized eggs. The total dry weight including chorion and adhesive disc of all ova measured ranged from 139 µg to 172 µg, with coefficient of variation ($s/\bar{x}$) = 0.66. This research considered only the large ova with consequently the highest dry weights. The caloric content estimated for these ova corresponded to 0.6982 calories per egg or 5546 calories per gram ash free dry weight of yolk. The calculated correction values of chorion plus adhesive disc represents a 25.18% of the total dry weight of the ova (Table 3).

Yolk utilization. The effect of the incubation temperatures upon the rate of decrease in yolk dry weight from fertilization to the death of the yolk-sac larvae was studied by analyzing the regression lines of the yolk ash free dry weight (log transformed values) as a function of time (Figure 1). Similarities in the rate of decrease were established when both terms of the regression lines,

Table 1. A Row x Column table for number of eggs on the vertical distribution in Yachats Beach, classified according to tidal heights on the beach.

Vertical Distribution(cm)		Height on the Beach: Lower	Inter Tidal	Upper	Total[1] #/cc
0-2	f	0.9843	0.8867	0.8587	2.72
	F	1.1597	0.9275	0.6425	
	f-F	-0.1754	-0.0408	0.2162	
2-4	f	1.1047	1.2210	0.5582	2.88
	F	1.2252	0.9799	0.6788	
	f-F	-0.1205	0.2411	-0.1206	
4-6	f	2.1040	1.384	1.1494	4.63
	F	1.9702	1.576	1.0915	
	f-F	0.1338	-0.192	0.0579	
6-8	f	2.6380	2.4670	1.2710	6.37
	F	2.7088	2.1664	1.5007	
	f-F	-0.0708	0.3006	-0.2297	
8-10	f	2.6110	1.065	1.9040	5.58
	F	2.3706	1.896	1.3134	
	f-F	0.2404	-0.831	0.5906	
10-12	f	1.6657	1.800	0.4126	3.93
	F	1.6732	1.338	0.9270	
	f-F	-0.0075	0.522	-0.5144	
Total[2]		11.1077	8.8837	6.1539	26.1453

$$\chi^2 = \Sigma\frac{(f-F)^2}{F} = 1.451 \qquad \chi^2 = 1.451 \text{ df. } 10 \; P > 0.005$$

[1]Total: Sum of the frequencies (f as # egg per cc) of each row (R).
[2]Total: Sum of the frequencies (f as # egg per cc) of each column (C).

$$F = \frac{(\text{row total}) \cdot (\text{column total})}{n(= 26.1453)} \qquad d_f = (R-1)\,(C-1)$$

Table 2. A Row x Column table for density of eggs on the four months sampled in Yachats Beach, classified according to tidal heights on the beach. (Density = number of eggs/cm^3)

Horizontal Distribution (sub levels)		Height on the Beach: Lower	Inter Tidal	Upper	Total[1]
July	f	1.3439	0.8191	0.5152	2.67
	F	1.0201	0.9968	0.6613	
	f-F	0.3238	-0.1777	-0.1461	
August	f	0.6915	1.2020	0.8360	2.72
	F	1.0400	1.0159	0.6740	
	f-F	-0.3485	0.1861	0.1620	
September	f	0.9050	0.9855	0.7131	2.60
	F	0.9917	0.9690	0.6429	
	f-F.	-0.0867	0.0165	0.0702	
October	f	1.4240	1.2580	0.7651	3.44
	F	1.3130	1.2829	0.8512	
	f-F	0.1109	-0.0249	-0.0861	
Total[2]		4.3644	4.2646	2.8294	11.45

$\chi^2 = \Sigma \frac{(f-F)^2}{F} = 0.3906$ $\chi^2 = 0.06$ df = 6 P > 99.5%

[1]Total: Sum of all the frequencies (f as # egg/cc) of each row (R).
[2]Total: Sum of all the frequencies (f as # egg/cc) of each column (C).

$$F = \frac{\text{(row total)} \cdot \text{(Column total)}}{n(=11.4584)} \quad d_f = (R-1)\,(C-1)$$

Figure 1. Yolk-sac and oil droplet ash-free dry weight between fertilization and death of the surf-smelt H. pretiosus for the five incubation temperatures. Arrows indicate the day in which only 25% of yolk is left.

6°C = log Y = 2.052 - 0.021 X r = -0.93
12°C = log Y = 2.192 - 0.082 X r = -0.98
15°C = log Y = 2.298 - 0.084 X r = -0.93
18°C = log Y = 2.035 - 0.068 X r = -0.99
22°C = log Y = 2.284 - 0.088 X r = -0.96

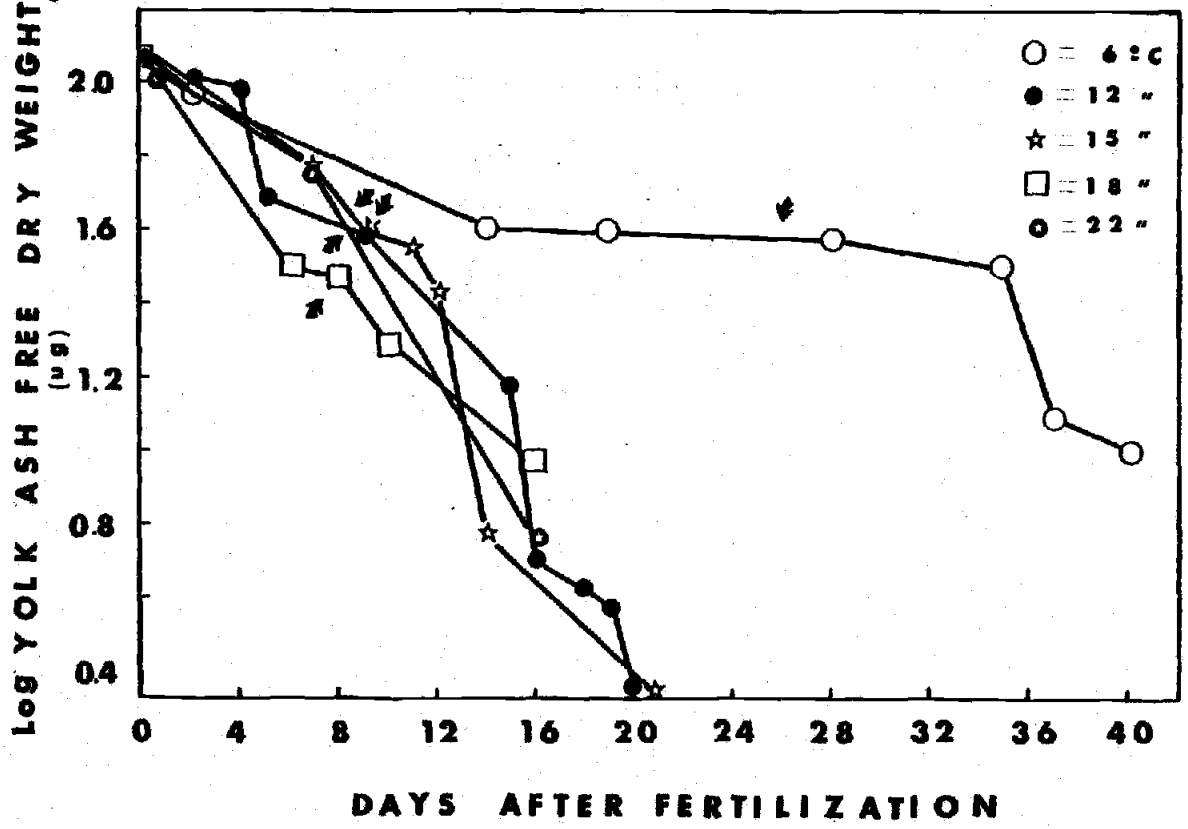


Table 3. Summary of size, ash-free dry weight (A.F.D.W.) and caloric value of the large surf-smelt ova utilized in calorimetry.

	Units	n	Range	Mean	S.D.	C.V.
Egg diameter						
Height	mm	432	0.962-1.09	1.06	0.007	0.001
Width	mm		0.865-0.99	0.934	0.008	0.002
Yolk diameter	mm.	432	0.836-1.010	0.934		
Egg volume	mm^3	29	0.3973-0.5902	0.45		
Yolk volume	mm^3	31		0.29	0.025	0.003
Total dry weight	µg	*432		172.30	0.36	0.001
Total A.F.D.W.	µg	*432		161.50		
Egg membrane	µg	*183		18.10		
Adhesive disc	µg	*194		19.80		
Yolk dry weight	µg	*432		134.30		
Yolk A.F.D.W.	µg	*432		125.90		
$Cal \cdot g^{-1} \cdot AFDW^{-1}$ of yolk	cal.			5546.00		
D.weight conversion factor	$\mu g/mm^3$ of yolk			467.00		
Adhesive disc	%			13.17		
Chorion	%			12.01		
Coefficient of A.F.D.W.	%	432		93.73		
Carbon content	% AFDW			54.45		
Nitrogen content	% AFDW			9.03		
C/N ratio	% AFDW			6.03		

* n =Is the total number analyzed and it is comprised by several samples of 10 or more ova weighted at a common time.

slope and intercept, were not significantly different ($P > 0.05$). The slopes of the regression lines for 12°, 15°, 18° and 22° C were similar indicating similar variation in rates of yolk consumption with age, whereas the slope for 6° C was lower indicating a lower rate of yolk decrement of the yolk ash free dry weight with age.

The rate values were found by dividing the amount of yolk utilized from fertilization to time T_{25}, when 75% of yolk has been depleted, by the number of hours elapsed at each temperature. T_{25} occurred in days 26 at 6°C, 8.5 at 12°C, 9.5 at 15° C, 7.8 at 18° C and 8.9 at 22° C. At 6° C, the depletion rate was approximately 3 times lower than the rates calculated among the high temperatures. The calculated values were 0.15 µg/hr at 6°C, 0.46 µg/hr at 12°C, 0.41 µg/hr at 15°C, 0.50 µg/hr at 18°C and 0.44 µg/hr at 22°C. These values were used to calculate the linear regression for temperature-specific utilization rates (Figure 2). The results indicated that the highest rate of yolk utilization was at an incubation temperature of 18° C.

The van't Hoff coefficient (Q_{10}), calculated from Winberg's (1971) equation ranged from 0.7 to 6.6, similar to the values observed in other biological systems (Rao and Bullock, 1954). The numerical values computed for surf-smelt reveal that the magnitude of the acceleration in converting yolk into tissue is greatest between 6° and 12° C and minimal between 12° and 15° C. The average Q_{10} between 12° and 22° C was 0.95 indicating relative temperature

Figure 2. Temperature-specific yolk-sac absorption rates from fertilization to time point T_{25}.

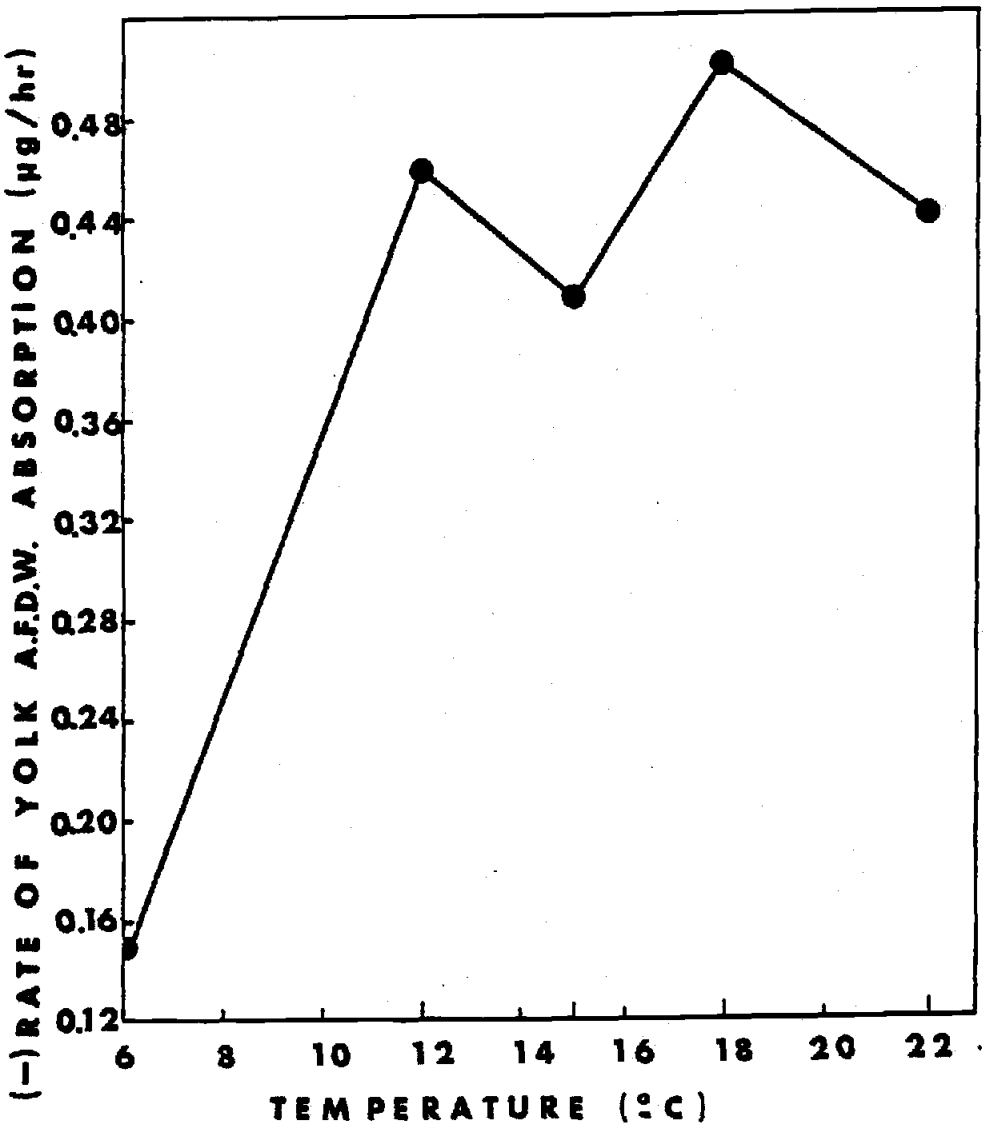
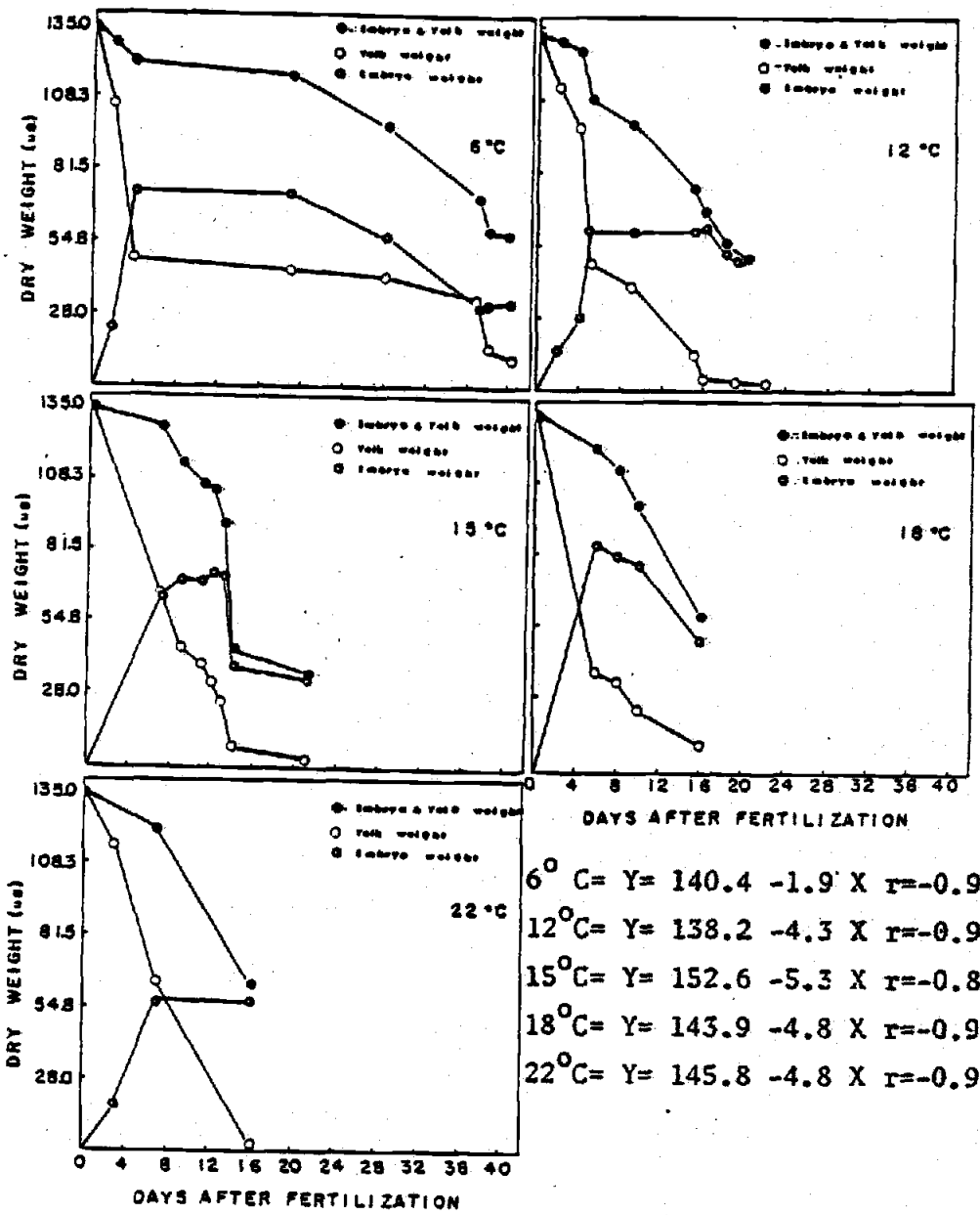


Figure 3. Average tissue dry weight gain and yolk dry weight loss of surf-smelt embryo and yolk-sac larvae during yolk absorption.



6^{o} C= Y= 140.4 -1.9 X r=-0.96

12^{o}C= Y= 138.2 -4.3 X r=-0.99

15^{o}C= Y= 152.6 -5.3 X r=-0.87

18^{o}C= Y= 143.9 -4.8 X r=-0.95

22^{o}C= Y= 145.8 -4.8 X r=-0.95

independence whereas the rate of yolk absorption below these temperatures is very temperature dependent (Table 4).

In order to obtain the corresponding larval tissue value for each temperature, the estimated T_{25} time points were interpolated in the linear regressions of larval tissue (ash-free dry weight or calories) versus days after fertilization. The T_{25} can also be graphically interpolated under the curves of dry weight versus time. (Table 5 Col A) and (Figure 3, a-3).

Direct calorimetry. Throughout embryogenesis, the slowest decrease in dry weight occurred at 6° C. Among the other temperatures, the decrease was similar except at 15° C in which case a higher rate of depletion was observed. These results were based upon slope values obtained fitting the regression lines to the dry weight versus time after fertilization observations (Table 5 Col A) and (Figure 3, a-e). By subtraction of the estimated values of yolk weight (Table 5 Col C) from the embryo plus yolk value, it was possible to obtain the daily gain in embryo weight (Table 5 Col D). This gain in embryo weight is contrasted with the estimates of yolk weight losses at intervals during development (Figure 3, a-e). Contrasting the lowest studied temperature, 6° C, with all other temperatures, it was found that total yolk weight losses differed by a factor of approximately four from fertilization to death of the surf-smelt. Similar comparisons for embryo weight gains gave a factor ranging for 9 to 18 (at 12° and 22° C) during the same time

Table 4. Q_{10} relationships for yolk ash-free dry weight absorption.

Measurement	Temperature Intervals (°C)				
	6	12	15	18	22
Rate of yolk absorption (Yolk ash-free dry wt.) [$\mu g.hr^{-1}$]	3.6	11.11	9.92	12.02	10.52
Q_{10}	6.59	0.686	1.895	0.7168	

Table 5. Dry weight determination of embryos and yolk-sac larvae between fertilization and death for 134.3 µg dry weight of unfertilized ova. (AFDW = Ash free dry weight). *: mean day of hatching.

	DRY WEIGHT (µg)							ENERGY IN CALORIES		
	A	B	C	D	E	F	G	H	I	J
DAYS AFTER FERTILIZATION	EMBRYO PLUS YOLK REMAINING	YOLK REMAINING Initial vol 0.2876 mm³	YOLK COLUMN B times 467 µg/mm³	WEIGHT OF EMBRYOS REMAINING (Col A - Col C)	YOLK AFDW Col C times 93.73%	Larval AFDW Col D times 47.45%	YOLK Col E times 5546 cal/AFDW·g	EMBRYO Col F times 4739.7 cal/AFDW·g	TOTAL Cal (G + H)	LOSS SUCCESSIVE SUBTRACTION Col I
TEMPERATURE 6°C										
0	134.315	0.2876	134.315	0	125.89	0.	0.6982	0.	0.6982	0.00
2	128.055	0.2265	105.77	22.285	99.14	19.48	0.5498	0.0923	0.6421	0.0561
14	121.17	0.1028	48.01	73.16	45.00	63.98	0.2496	0.3032	0.5527	0.08933
19 *	116.33	0.0946	44.18	72.15	41.41	63.09	0.2297	0.2990	0.5286	0.02411
28	98.04	0.0894	41.75	56.29	39.13	49.22	0.2170	0.2333	0.4503	0.07836
37	70.475	0.0758	35.40	35.07	33.17	30.67	0.1823	0.1454	0.3277	0.1226
38	58.155	0.0314	14.67	43.485	13.75	38.03	0.0767	0.1802	0.0256	0.07124
40	57.185	0.0263	12.28	44.905	11.51	39.27	0.0638	0.1861	0.2499	0.00696
104	50.77	0.0000	0.0	50.77	0.0	44.40	0.0	0.2104	0.2104	0.0395
TEMPERATURE 12° C										
0	134.315	0.2876	134.315	0.0	125.89	0.0	0.6982	0.0	0.6982	0.0
2	131.38	0.2446	114.23	17.15	107.07	14.998	0.5938	0.0711	0.6649	0.0333
4	128.24	0.2135	99.708	28.532	93.456	24.95	0.5183	0.1183	0.6366	0.0283
6	110.04	0.1042	48.66	61.38	45.609	53.677	0.2529	0.2544	0.5073	0.1293
9	101.7	0.0874	40.818	60.88	38.259	53.239	0.2122	0.2523	0.4645	0.0428
15	77.56	0.0345	16.104	61.446	15.09	53.730	0.0837	0.2547	0.3384	0.0841
16 *	69.195	0.0138	6.4340	62.761	6.030	54.880	0.0334	0.2601	0.2935	0.0449
18	58.34	0.0118	5.5070	53.080	5.160	46.418	0.0286	0.2200	0.2486	0.0449
19	55.785	0.0107	4.9960	50.790	4.680	44.410	0.0259	0.2106	0.2364	0.0122
20	52.675	0.0069	2.8000	49.870	2.620	43.610	0.0145	0.2067	0.2212	0.0152
TEMPERATURE 15° C										
0	134.315	0.2876	134.315	0.0	125.89	0.0	0.6982	0.0	0.6982	0.0
7	120.575	0.1139	54.914	65.661	60.044	35.671	0.3174	0.2839	0.6013	0.0969
9	114.315	0.0939	43.877	70.438	41.126	61.598	0.2281	0.2919	0.5201	0.08124
11	106.400	0.0811	37.875	68.525	35.500	59.925	0.1969	0.2840	0.4809	0.03916
12	103.720	0.0668	31.2000	72.520	29.240	63.420	0.1622	0.3095	0.4717	0.00919
13	95.420	0.0513	23.9500	71.470	22.450	62.500	0.1245	0.2962	0.4207	0.04210
14 *	43.710	0.0142	6.646	37.060	6.229	32.410	0.0345	0.1536	0.1881	0.23256
21	35.428	0.0059	2.758	32.670	2.580	28.577	0.0143	0.1354	0.1497	0.03844
TEMPERATURE 18° C										
0	134.315	0.2876	134.315	0.0	125.89	0.0	0.6982	0.0	0.6982	0.00
6	121.600	0.0774	36.1520	85.448	33.885	74.724	0.1879	0.3542	0.5421	0.1561
8 *	114.130	0.0717	33.4850	80.645	31.390	70.524	0.1740	0.3343	0.5083	0.0338
10	100.600	0.0484	22.6050	77.995	21.188	68.207	0.1175	0.3233	0.4408	0.0675
16	58.575	0.0222	10.368	48.207	9.718	42.157	0.0539	0.1998	0.2537	0.1871
TEMPERATURE 22° C										
0	134.315	0.2876	134.315	0.0	125.89	0.0	0.6982	0.0	0.6982	0.0
5	131.380	0.2446	114.235	17.145	107.07	14.993	0.5938	0.0711	0.6649	0.0333
7 *	121.345	0.1359	63.4600	57.885	59.480	50.620	0.3298	0.2399	0.5698	0.09512
16	62.735	0.0138	6.43	56.305	6.027	49.239	0.0334	0.2334	0.2668	0.3029

interval. The embryo weight gain showed a negative trend at 15° C and 18° C, with exactly the same rate values (Figure 3 a-e). Embryo weight gain rate values are far lower than the rates of yolk weight losses at similar incubation temperatures. Factors approximately 3 times lower were found at 15°, 18° and 22° C; whereas at 6° C and 12° C, the embryo weight gain rates were approximately 12 and 5 times lower than the yolk weight loss rates respectively. The day on which maximum embryo weight was reached corresponded to days 14, 16, 12, 6 and 7 at 6°, 12°, 15°, 18° and 22° C respectively (Table 5 Col D). The percentage of yolk consumed ranged from 53% (at 22° C) to 95% (at 12° C) at the day of maximum weight attained. At 15°, 18° and 6° C this percentage was 77%, 73% and 64% respectively.

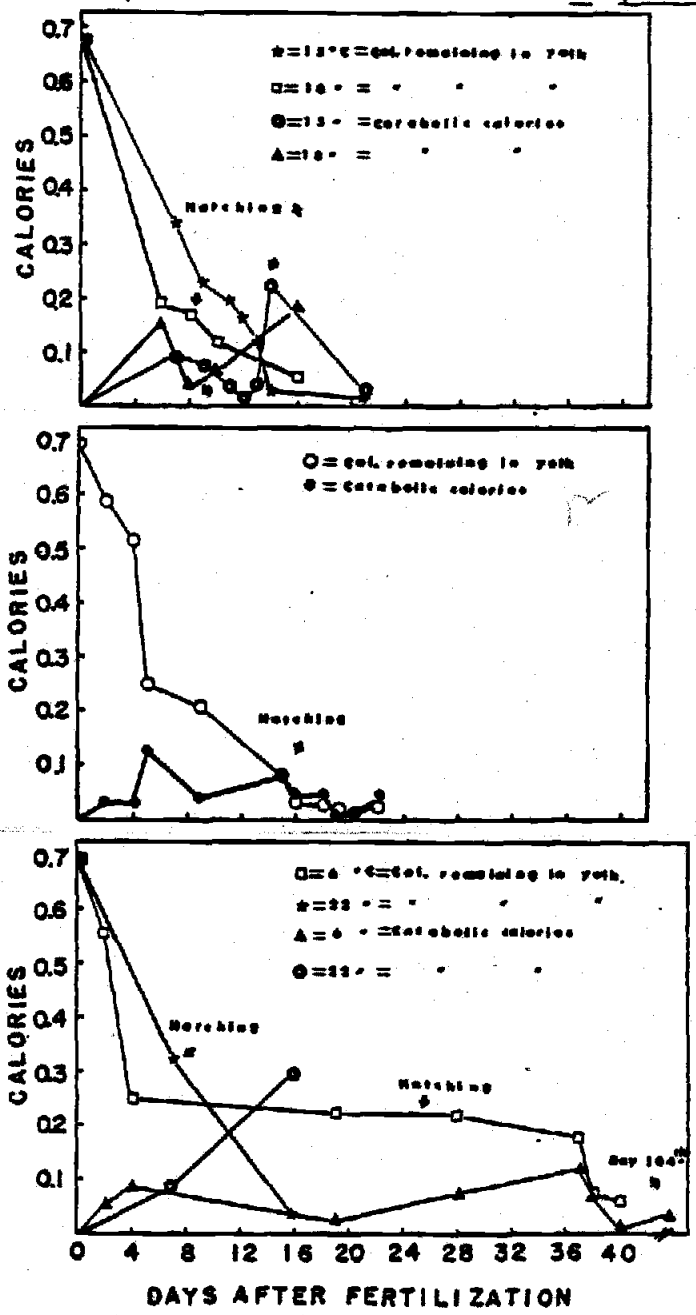
Caloric values of yolk remaining plus embryo combined were also calculated (Table 5 Col I). Average caloric values for ova and larvae when yolk is exhausted were 5546 and 4739.7 calories per gram ash free dry weight respectively. The correction factors defined for ash free dry weight of the ova and the larval tissue were 93.73% and 87.45% respectively. The validity of using the same caloric value for ova before and after fertilization was demonstrated by Lasker (1962). In this research, the same criterion was applied; the starting point for the embryonic yolk once the ash free converting factor and the caloric value have been applied correspond to 0.6982 calories per egg.

The caloric content calculated for the surf-smelt egg, 5546 calories per gram ash free dry weight, is intermediate to values reported in the literature, which range from 4268 cal per gram ash-free dry weight for Limanda ferruginea (Howell, 1980) to 6580 cal per gram ash-free dry weight for Clupea harengus (Pappenhofer and Rosenthal, 1968; cited in Robertson (1974)).

The daily loss of embryo plus yolk dry weight was followed and contrasted with the reduction in yolk weight and the embryo weight gain (Figure 3). Multiplying 5546 calories by the yolk ash free dry weight, the caloric value of yolk was calculated at intervals after fertilization (Table 5 Col G). Similarly, the caloric value of the larval tissue was found by applying the caloric value of the larva (4739.7 cal/g) to the larval ash free dry weight (Table 5 Col H). The caloric value of the embryonic tissue was then added to that of the remaining yolk (Table 5 Col I). Successive subtractions at intervals after fertilization of one's interval sum from the preceding one led to estimates of catabolism (Table 5 Col. J).

Comparisons at intervals after fertilization were made upon catabolic calories and calories remaining in the yolk (Table 5 Col J and G) (Figure 4). This analysis led me to conclude that the average embryo has enough energy to cope with catabolic and developmental requirements until at least one week after midpoint hatching at 18° and 22° C respectively. At 12° and 15° C, the catabolic requirements

Figure 4. Comparison of calories remaining in the yolk with those lost at intervals during development for five incubation temperatures of the surf-smelt H. pretiosus.



determined by direct calorimetry tended to exceed the number of calories remaining in the yolk at around the hatching day.

Indirect Calorimetry. Oxygen consumption was measured at least five hours at each incubation temperature at intervals during embryogenesis (Table 6 Col A). The interval value of oxygen consumption (Table 6 Col B) computed by averaging the hourly mean oxygen consumption between intervals and then multiplying by the number of hours elapsed between those intervals, showed erratic increase as the embryo develops. The effect of age on oxygen uptake was unclear except at, and just after, hatching where there tended to be a peak at each temperature (Figure 5) At 12° and 15° C the larvae seemed to swim more actively, therefore the increased O_2 uptake at hatching may be reflecting an increased activity instead (Figure 6). Lasker and Theilacker (1962) demonstrated differences in O_2 consumption between non-active and active eggs and larvae of the Sardinops caerulea).

By interpolating the assigned time of T_{25} in the hourly mean oxygen consumption (Table 6 Col A) and then adding the resulting value in Col B (Table 6), the total amount of oxygen consumed was found for each temperature from fertilization to T_{25}. This average total volume was then converted to calories and expressed as percentage of the initial number of calories contained in the ovum. The values were 5.5% at 6° and 12° C, 18% at 15°C, 24% at 18° and 23% at 22°C. Similar estimates for the day on which the maximum weight

Table 6. Cumulative oxygen consumption by egg and yolk-sac larvae from fertilization to death for the surf-smelt H. pretiosus. [30]

Days After Fertilization	A Hourly mean Oxygen Consumption(µl)	B Interval Oxygen Consumption(µl)	C Cumulative Oxygen Consumption(µl)	D Percent spent on Respiration**	E Cumulative % spent on Respiration
		TEMPERATURE 6° C			
2	0.0268	0.6432	0.64	0.46	0.46
14	0.0221	7.042	7.68	5.04	5.5
16	0.0777	2.3952	10.08	1.71	7.21
19	0.3855	4.185	14.26	2.99	10.20
21	0.0506	2.1396	16.40	1.53	11.73
28 •	0.0456	8.0808	24.48	5.79	17.52
37	0.1419	20.250	44.73	14.50	32.02
38	0.1587	3.6072	48.34	2.58	34.60
40	0.1366	7.0800	55.42	5.09	39.67
		TEMPERATURE 12° C			
2	0.1107	2.6568	2.66	1.90	1.90
4	0.1384	5.9784	8.64	4.28	6.18
5	0.1228	3.1344	11.76	2.24	8.42
9	0.1263	11.957	23.73	8.56	16.99
10	0.1282	3.0540	26.78	2.18	19.18
12	0.1900	7.6368	34.42	5.46	24.65
15 •	0.0680	9.2880	43.71	6.65	31.30
18	0.2045	9.8100	53.52	7.02	38.32
19	0.3703	6.8976	60.41	4.93	43.26
22	0.3858	27.2196	87.63	19.4	62.75
		TEMPERATURE 15° C			
3	0.0564	1.3536	1.35	0.97	0.97
4	0.0573	1.3644	2.71	0.98	1.90
7	0.1657	8.0280	10.75	5.75	7.70
9	0.3241	11.755	22.50	8.42	16.10
10	0.0749	4.7880	27.29	3.43	19.50
11	0.2650	4.0788	31.37	2.93	22.50
14 •	0.5816	30.478	61.85	22.0	44.00
21	0.7258	109.82	171.6	78.1	122.1
		TEMPERATURE 18° C			
2	0.0517	1.2408	1.24	0.88	0.88
3	0.0754	1.5252	2.77	1.09	1.98
4	0.1318	2.4864	5.25	1.78	3.76
5	0.6040	8.8296	14.08	6.32	10.08
6	0.1530	9.0840	23.17	6.50	16.59
7	0.2379	4.6908	27.86	3.35	19.95
8 •	0.2580	5.9520	33.81	4.26	24.21
16	0.4050	63.6480	97.46	45.58	69.79
		TEMPERATURE 22° C			
5	0.2127	12.7620	12.76	9.14	9.14
7 •	0.0880	7.2168	19.98	5.17	14.31
8	0.2113	3.5916	23.57	2.57	16.88
9	0.4784	8.2764	31.85	5.93	22.81
20	0.3224	105.7056	137.55	75.70	98.50

•: Mean day of hatching.
The percentage of catabolism accounted for by respiration from fertilization to hatching at each temperature were: 21.6% at 6°C; 21% at 12°; 24% at 15°C; 31.5% at 18°C; and 30% at 22°C.
**: The initial number of calories in the ova, 0.6982; represents the hundred percent. To assign percentages, the microliters of oxygen were first expressed in calories multiplying by 0.005.

Figure 5. Mean rate of oxygen consumption at intervals from fertilization to death of H. pretiosus

6°C = Y = 0.0036 X - 0.0082 r = 0.86 12°C = Y = 0.0117 X + 0.048 r = 0.73

15°C = Y = 0.0399 X - 0.1129 r = 0.91 18°C = Y = 0.0208 X + 0.107 r = 0.49

22°C = Y = 0.0091 X + 0.1740 r = 0.36

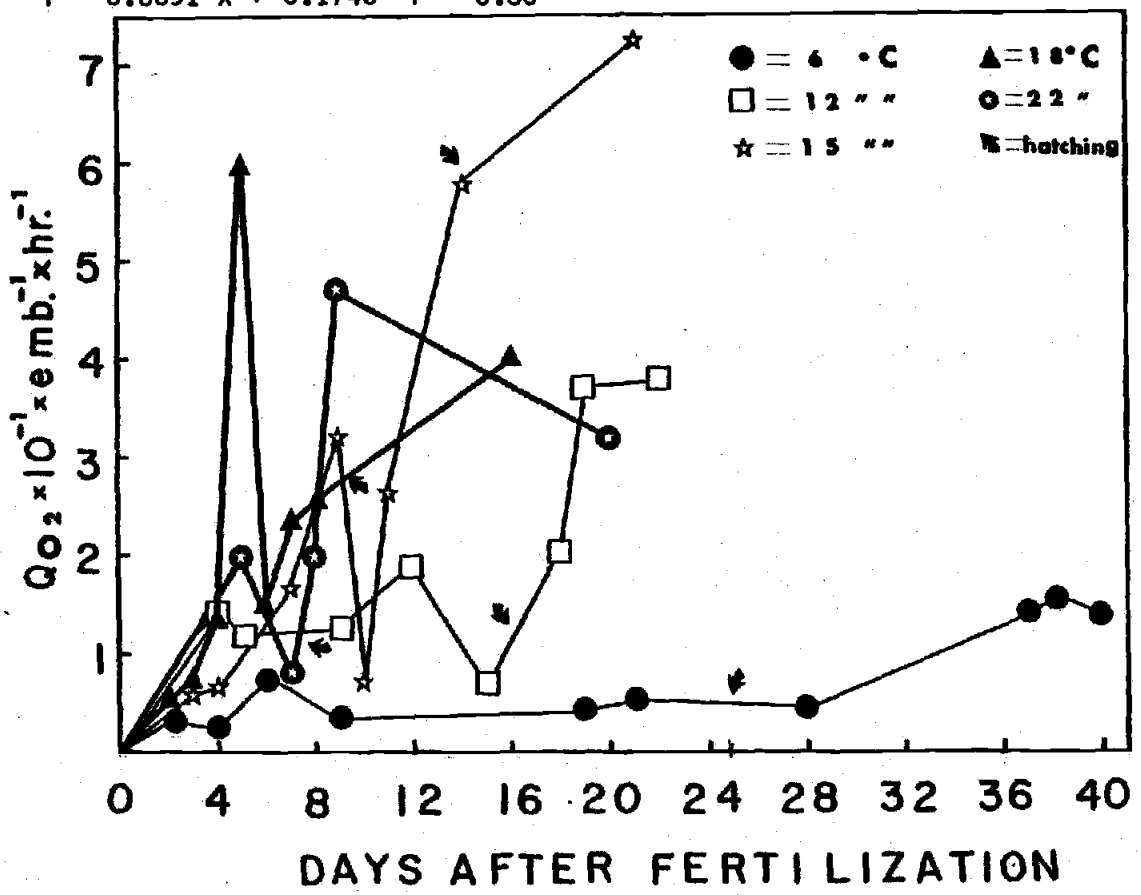
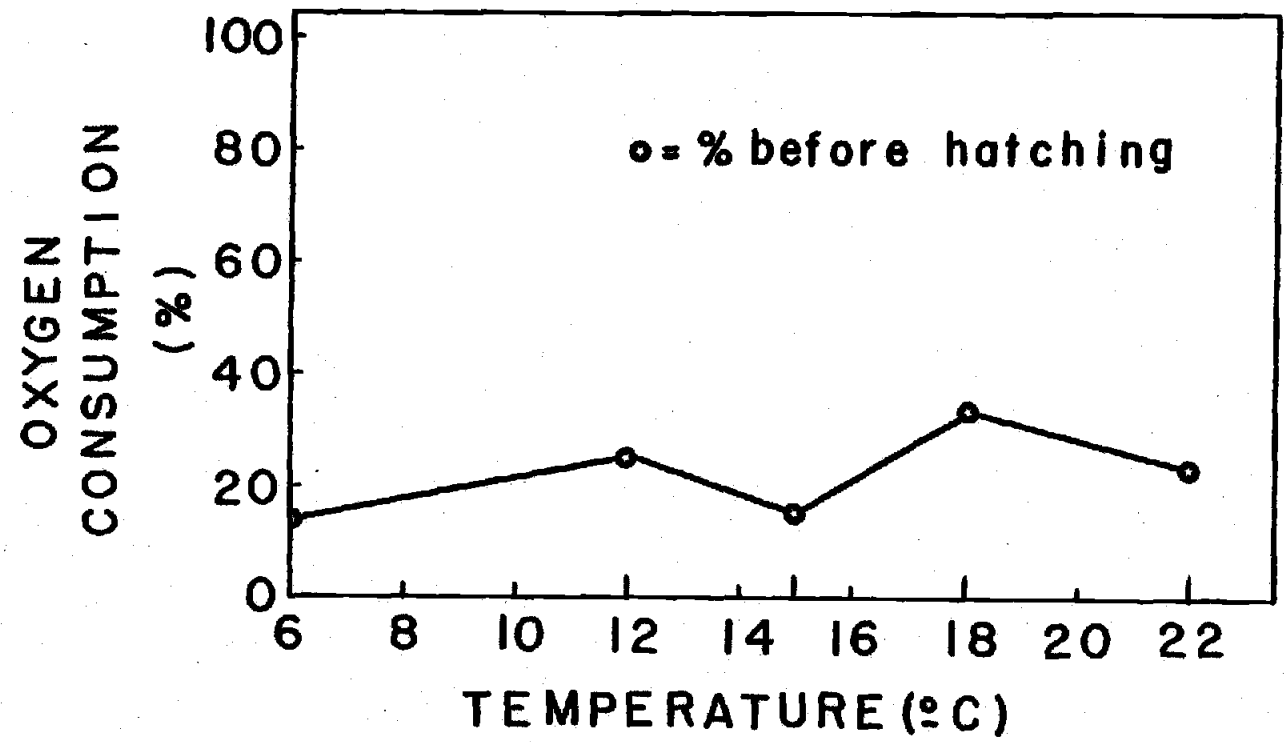


Figure 6. Percentage volume oxygen consumed by embryo and yolk sac larvae from fertilization to midpoint hatching for the surf-smelt H. pretiosus. (100 % = initial number of calories in the ovum).



was achieved gave the following values: 6% at 6° C, 34% at 12° C 30% at 15° C 17% at 18°C and 14% at 22°C.

The catabolic calories (Table 7 Col B) were added to the caloric equivalent of larval tissue to yield the larval energy expenditure (Table 7 Col E and G). The balance of larval expenditure versus yolk availability throughout the observation period from fertilization to death of the yolk-sac larvae remained nearly constant (Table 7 Cols F,G,H). Exceptions were found on days 16 at 18° C and day 5 at 22° C. High oxygen demands at 18° C and high oxygen demands plus a coincident increment in larval growth early in the development, day 5, at 22° C probably accounted for these exceptions. Embryonic growth in terms of ash free dry weight increments and caloric estimates was aberrant and in some case negative, indicating an actual loss. This trend was shown for the five experimental temperatures (Table 7 Col C,D,E). Maximum increment in larval growth was detected earlier in the development at higher incubation temperatures (15°, 18° and 22° C) than at the lower ones, 6° and 12° C (Table 7 Col E).

Comparisons of energy expenditure using dry weight (Table 5 Col G and J) and oxygen consumption methods (Table 7 Col F and G) indicated a departure of values from the available caloric content in the yolk. In cases where an apparent discrepancy was observed, this tended to occur towards the midpoint hatching time. This discrepancy probably does not occur under natural hatching conditions.

Table 7. Energy of embryos and yolk-sac larvae using O_2 consumption method between fertilization and death for 134.3 µg dry weight of unfertilized ova. (The calorific value calculated for yolk is equal to 5546 cal ash-free dry weight^{-1} · g^{-1})

	Metabolism (cal/µl O_2		Ash-Free Dry Weight (µg)		Energy (cal)			
	A	B	C	D	E	F	G	H
Days After Fertilization	Oxygen Consumption [µl embryo^{-1}day^{-1}]	Oxygen Consumption Col A Times 0.005* [cal/day]	Larval Ash-Free Dry Weight [µg]	Larval Growth Increment difference successive Values Col.C	Larval Growth Increment Col D times 4739 cal AFDW^{-1}·g^{-1}	Yolk cal/afdwg	Sum Growth Plus Metabolism Col. 8+E	Cumulative G + F
Temperature 6° C								
0	0.0	0.0	0.0	0.0	0.0	0.69820	0.0	0.6982
2	0.6432	$3.216E^{-3}$	19.48	19.48	0.09233	0.54980	$9.55E^{-2}$	0.6453
14	7.042	$3.52E^{-2}$	63.98	44.5	0.21092	0.24957	$2.46E^{-1}$	0.5911
19 •	4.185	$2.09E^{-2}$	63.09	0.0	0.0	0.22966	$2.09E^{-2}$	0.5921
28	8.0808	$4.040E^{-2}$	49.22	0.0	0.0	0.21700	$4.040E^{-2}$	0.6198
37	20.2500	$1.013E^{-1}$	30.67	0.0	0.0	0.18230	$1.013E^{-1}$	0.6868
38	3.6072	$1.8036E^{-2}$	38.03	7.36	0.03488	0.07626	$5.2916E^{-2}$	0.6330
40	7.0800	$3.54E^{-2}$	29.27	0.0	0.0	0.06380	$3.54E^{-2}$	0.6560
Temperature 12° C								
0	0.0	0.0	0.0	0.0	0.0	0.6982	0.0	0.6982
2	2.6568	$1.328E^{-2}$	14.998	14.998	0.071086	0.5938	$8.437E^{-2}$	0.6782
4	5.9784	$2.98E^{-2}$	24.985	9.952	0.047169	0.5183	$7.7059E^{-2}$	0.6797
5	3.1344	$1.5672E^{-2}$	53.677	28.73	0.13617	0.2529	$1.5184E^{-1}$	0.5662
9	11.457	$5.978E^{-2}$	53.239	0.0	0.0	0.2122	$5.978E^{-2}$	0.5852
15	9.2280	$4.644E^{-2}$	53.73	0.0	0.0	0.0837	$4.644E^{-2}$	0.5027
16 •	12.3840	$6.192E^{-2}$	54.88	1.1	0.00545	0.0334	$6.737E^{-2}$	0.5204
18	9.8100	$4.905E^{-2}$	46.418	0.0	0.0	0.0286	$4.905E^{-2}$	0.5646
19	6.8976	$3.449E^{-2}$	44.41	0.0	0.0	0.0259	$3.449E^{-2}$	0.5963
20	13.7952	$6.898E^{-2}$	43.61	0.0	0.0	0.0145	$6.898E^{-2}$	0.6539
Temperature 15° C								
0	0.0	0.0	0.0	0.0	0.0	0.6982	0.0	0.6982
7	8.0280	$4.014E^{-2}$	55.671	55.671	0.26386	0.3374	$3.04E^{-2}$	0.6414
9	11.755	$5.877E^{-2}$	61.598	5.927	0.02809	0.2281	$8.686E^{-2}$	0.608
11	4.0788	$2.039E^{-2}$	59.925	0.0	0.0	0.1969	$2.039E^{-2}$	0.590
12	- -	- -	63.420	3.495	0.01656	0.1622	$1.656E^{-2}$	0.562
13	- -	- -	62.500	0.0	0.0	0.1245	0.0	- -
14 •	30.478	$1.524E^{-1}$	32.410	0.0	0.0	0.03455	$1.524E^{-1}$	0.615
Temperature 18° C								
0	0.0	0.0	0.0	0.0	0.0	0.6982	0.0	0.6982
6	9.0840	$4.542E^{-2}$	74.724	74.724	0.3542	0.1879	$3.996E^{-1}$	0.5875
8 •	5.9520	$2.976E^{-2}$	70.524	0.0	0.0	0.1740	$2.976E^{-2}$	0.6034
10	-	-	68.207	0.0	0.0	0.1175	-	-
16	63.648	$3.182E^{-1}$	42.157	0.0	0.0	0.0539	$3.182E^{-1}$	0.8015
Temperature 22° C								
0	0.0	0.0	0.0	0.0	0.0	0.69820	0.0	0.6982
5	11.293	$5.646E^{-2}$	14.993	14.993	0.071060	0.59380	$1.275E^{-1}$	0.7213
7 •	7.2168	$3.608E^{-2}$	50.620	35.630	0.168875	0.32988	$2.049E^{-1}$	0.6630

* 0.005: oxycaloric equivalent; see text.

•: average day of hatching

Yolk utilization efficiencies. Throughout embryogenesis the efficiencies of yolk utilization tended to decrease with time (Table 8). This trend was observed in all the experimental temperatures. Optima for efficiency with temperature were variable; at the most reliable endpoint of comparison, T_{25}, the highest efficiencies were calculated at 18° and 15° C. The lowest efficiencies were found at the two extremes experimental temperatures (6° and 22° C). These values were similar (47% at 6° and 45% at 22° C) although rates of yolk absorption differed by a factor of three (Table 9 and Table 4).

Apparent energetic efficiencies of Needham (1931) were calculated by subtracting the percentage of oxygen accounted for by respiration from one hundred. The cumulative volume of oxygen from fertilization to T_{25} was converted to calories by multiplying by 0.005. The resulting value was then divided by the amount of yolk utilized (0.524 calories) until T_{25} (Table 6 Col B,E). This yielded the catabolism accounted for by respiration, the values of which were: 21.6% at 6° C, 21% at 12° C, 24% at 15° C, 31.5% at 18° C and 30% at 22° C. The efficiencies were found by subtracting these values from 100 and corresponded to: 78% at 6° C, 79% at 12°, 76% at 15°C, 69% at 18° C and 70% at 22° C.

Table 8. Efficiencies of yolk utilization by embryo and yolk-sac larvae of surf-smelt Hypomesus pretiosus.

Days After Fertilization	Yolk Energy (cal.)	Larval Growth Increm.	Percent Efficiency
	Temperature 6° C		
0	0.6982	0.0	0.0
2	0.5498	0.0923	62.2
14	0.2495	0.2109	70.2
19	0.2296	0.0	0.0
28	0.2170	0.0	0.0
37	0.1823	0.0	0.0
38	0.0762	0.0348	32.8
40	0.0638	0.0	0.0
	Temperature 12° C		
0	0.6982	0.0	0.0
2	0.5938	0.0710	68.0
4	0.5183	0.0471	62.3
5	0.2529	0.1361	51.3
9	0.2122	0.0	0.0
15	0.0837	0.0	0.0
16	0.0334	0.0054	10.7
18	0.0286	0.0	0.0
19	0.0259	0.0	0.0
20	0.0145	0.0	0.0
	Temperature 15° C		
0	0.6982	0.0	0.0
7	0.3374	0.2638	73.1
9	0.2281	0.0280	25.6
11	0.1969	0.0	0.0
12	0.1622	0.0165	47.5
14	0.1245	0.0	0.0
21	0.0143	0.0	0.0
	Temperature 22° C		
0	0.6982	0.0	0.0
5	0.5938	0.0710	68.0
7	0.03298	0.1688	63.9
16	0.0334	0.0	0.0

Table 9. Efficiencies of yolk utilization based on direct calorimetric estimates from fertilization to hatching and fertilization to starvation for the surf-smelt H. pretiosus for five experimental temperatures. (AFDW: ash free dry weight).

TEMPERATURE (° C)	NOTOCHORD LENGTH (mm)	YOLK-SAC VOLUME (mm^3)	YOLK µg AFDW[1]	LARVAL TISSUE[2] µg AFDW	YOLK UTILIZED[3] µg AFDW	EFFICIENCY[4] (%)	CALORIC VALUE LARVAL TISSUE[5]	CALORIC VALUE[6] YOLK UTILIZED	EFFICIENCY[7] (%)
				AT HATCHING					
6	6.2±0.6	0.092	40.27	56.16	85.62	65.6	0.266	0.475	56
12	6.6±0.4	0.014	6.04	54.88	119.85	45.8	0.260	0.665	39
15	6.3±0.7	0.014	6.22	32.41	119.67	27.1	0.154	0.664	23
18	6.1±0.5	0.060	26.28	69.37	99.61	69.6	0.329	0.552	59
22	5.2±0.3	0.136	59.48	50.62	66.41	76.0	0.240	0.368	65
				AT MAXIMUM WEIGHT					
6	- - -	0.103	44.99	63.98	80.89	79.0	0.303	0.449	68
12	- - -	0.014	6.04	54.88	119.85	45.8	0.260	0.665	39
15	- - -	0.067	29.24	63.42	96.65	65.6	0.301	0.536	56
18	- - -	0.077	33.88	74.72	92.01	81.2	0.354	0.510	69
22	- - -	0.136	59.48	50.62	66.41	76.2	0.239	0.368	65
				AT TIME POINT T_{25}					
6	- - -	0.719	31.47	51.75	94.42	54.81	0.245	0.524	47
12	- - -	0.719	31.47	53.29	94.42	56.44	0.252	0.524	48
15	- - -	0.719	31.47	61.20	94.42	64.82	0.290	0.524	52
18	- - -	0.719	31.47	71.70	94.42	75.94	0.340	0.524	65
22	- - -	0.719	31.47	50.31	94.42	53.29	0.238	0.524	45

[1]Yolk AFDW = volume times 437.7)g AFDW/mm^3. [2]Larval tissue = Yolk-sac larvae AFDW minus Yolk AFDW. [3]Yolk utilized = 125.9 minus yolk AFDW. [4]Efficiency = 100x Larval tissue AFDW/Yolk utilized AFDW. [5]Caloric larval tissue = larval tissue times 4739.7 calories per AFDW · g. [6]Caloric value of yolk = yolk AFDW times 5546 cal/AFDW · g. [7]Efficiency = 100 x calories of larval tissue/calories of yolk utilized.

DISCUSSION

Spawning in fishes is known to be controlled by both endogenous factors (mainly hormones) and exogenous factors. Observations of the spawning event of the surf-smelt clearly suggest moon cycles as another decisive exogenous factor controlling spawning. Spawning in the California grunion, Leuresthes tenuis, which also comes ashore to spawn, is controlled by lunar cycles (Walker, 1952). Although the spawning activity took place from May through October, the surf-smelt may be regarded as summer spawner due to the fact that the major peak in abundance of spawners was observed during August and September. At this time greater scatter in size of the spawners and dry weight content of the ova was also observed. It is interesting to note that surf-smelt fishes lay eggs of different size which may be adaptable to the variety of environmental conditions prevailing at hatching time. Larger eggs may signify a later point of no-return during the search for food of the just hatched yolk-sac larvae or, as suggested by Blaxter and Hempel (1966) and Ware (1975), may relate to conditions of available food and predators.

While the phenomenon of diapause has been described mostly in developing embryos of insects, diapause has also been found in the developmental patterns of annual fishes (Wourms, 1972). The delayed hatching observed in laboratory-reared embryos of the surf-smelt was considered as a facultative diapause III following the terminology

given by Wourms (1972) for Cyprinodont fishes. Factors causing diapause are diverse but mostly constituted by abiotic parameters such as oxygen tension or temperature. In insects, diapause seems more likely to occur under low temperature conditions than at higher ones. For surf-smelt embryos the temperature apparently does not play a decisive role in this respect. Surf-smelt diapause has been attributed to the lack of the external stimuli that result in water agitation in nature. This may indirectly reflect the influence of environment upon larval surf-smelt during their residence in beach gravel. Hatching in the field of a related species (capelin Mallotus villosus) was found to be regulated by a complex of meteorological, hydrographic and biological conditions (Frank and Leggett, 1981b). The pattern of larval emergence of capelin, whose spawning behavior is similar to that of surf-smelt, has also been externally induced. In this case, wind induced wave action disturbs the beach gravel. Surf-smelt eggs that experienced diapause for as long as 104 days at 6° C were able to maximize growth efficiency. This, plus the capability to delay hatching should give surf-smelt larvae some survival advantages. Yolk utilization rate, which is a function of growth efficiency may also account for survival. In this respect, surf-smelt with similar and relatively high growth efficiencies may be in optimal conditions to deal with problems inherent to release to the pelagic environment. Frank and Leggett (1981a) found that onshore winds facilitated the emergence of capelin larvae and that a

low growth efficiency together with absence of onshore winds caused larvae to complete their yolk-absorption prior to release, resulting in death in gravel or the release of severely weakened larvae.

The current idea that larval fishes are able to change their behavior depending on environmental conditions has been demonstrated in several experimental studies. Examples include the capability to cue the patterns of vertical migrations according to intensities of light (Blaxter (1973), and recognition of gradients of temperature, light, turbulence and oxygen (Reynolds and Thompson, 1974). Engraulis mordax larvae were found to actively search for food patches (Hunter and Thomas 1974; Lasker 1975). Facultative diapause regarded as scope for survival might place surf-smelt eggs as another example of this idea. Nevertheless, this capability of the surf-smelt to delay larval emergence highly confounds the result of this investigation, at least in the traditional context that considers hatching time as a reliable endpoint for energetic comparison. Consequently, T_{25} was used as an endpoint in the present work. Independently of experimental temperature, T_{25} was found to closely coincide with the first observed hatching day. Presumably, the observed first day of hatching represents the actual hatching event at sea when gravel is submerged and agitated, and represents the point when embryos are developmentally competent to hatch.

It is worthy to note that the rate of oil droplet decrement is slower than the yolk depletion for the surf-smelt. A similar

phenomenon has been described for Bairdiella icistia (May, 1974) and Leuresthes tenuis (Ehrlich and Muszynski, 1982). Whether this represents an advantage for the larvae seems questionable. Ehrlich and Muszynski (1982) attributed to the oil droplet mainly a buoyancy function with the oil droplet keeping the larva from continuously handing head down. Contrary to this, Rogers and Westin (1981) showed faster digestion of the oil droplet among feeding striped bass larva in comparison to those larvae starved or kept on reduced rations.

Mechanisms to compensate for changes in temperature are highly developed in surf-smelt. The evidence suggests that the rate of yolk consumption showed temperature independence over the range 12°-22° C and the yolk utilization efficiencies appeared relatively similar. Similarities in yolk utilization efficiency over certain temperature range have previously been reported for Salmo salar (Hayes and Pelluet, 1945), Clupea harengus (Blaxter and Hempel, 1966), Tautoga onitis (Laurence, 1973), Limanda ferruginea (Howell, 1980) and Paralichthys dentatus (Johns and Howell, 1980). Temperature ranges utilized to define these similarities in yolk utilization efficiences were narrow. In most of the reported species only four or five degrees were detected between the lowest and highest temperatures of a given range. Exceptions to the above were noted for the Atlantic salmon Salmo salar, where the difference between the lowest and highest segment of the temperature range corresponded to sixteen degrees Celsius. This same difference in temperature range was

utilized for surf-smelt studies (6°-22° C). Coincidently, the efficiency values for surf-smelt are quite close to those found for S. salar at almost equivalent temperatures (47% at 6° C, 65% at 18° C for surf-smelt and 42% at 5° C and 60% at 16° C for S salar).

During growth and morphogenesis while yolk is converted into body tissue there is a loss of dry weight. Based on this fact Gray (1928) defined the "plastic efficiency coefficient" as the ratio of dry weight of body formed versus the original yolk dry weight at fertilization. This ratio has been used as a measure of the efficiency of development at any particular stage and its initial value of 0.63 defined by Gray (1928) has served as a basis for comparisons for numerous species of fishes. The plastic efficiency coefficients for surf-smelt (0.40-0.70) are within the range reported for other species such as that of Sardinops caerulea (Lasker, 1962) which P.E.C. is 0.48 at 14° C and that of Paralichthys dentatus (Johns and Howell, 1980) which P.E.C. is 0.61 at 16° C. Measurements of efficiency by using "apparent energetic efficiencies" (A.E.E.) gave values relatively high for surf-smelt as compared with most of the efficiencies (A.E.E.) reported for a number of previous studies. These, range from 0.26 (at 19° and 22° C) for eggs of Tautoga onitis (Laurence, 1973) to 0.79 (at 14° C) for eggs of the Pacific sardine Sardinops caerulea (Lasker, 1962). The apparent energetic efficiencies calculated for surf-smelt eggs are then closer to those reported by Lasker (1962) for the planktonic eggs of

Sardinops caerulea. Yet both plastic efficient coefficient and apparent energetic efficiency represent reliable estimates of efficiency, a comparison between them will not be valid since the plastic efficiency coefficient has been calculated from a more inclusive direct calorimetric method as noted by Laurence (1969).

The rate of rise in metabolic requirements and the reduction in development time were different over the range 15°-18° C. Winberg (1956) suggested that the apparent constancy of metabolic rate of temperatures close to the preferred one occurs because temperatures below the preferred one stimulates the movement of the fish and a reduction in metabolism is compensated by an increase in motor activity with the net result that over a certain range, metabolism is apparently independent of temperature. It seems that studies on acclimation to different temperatures, as the present one, can clarify how the changes in the metabolic level are affected throughout incubation periods.

Ehrlich and Muszynsky (1982) summarized the findings of several authors concerning decrease in rate of yolk utilization near the upper thermal limits for embryonic development. The surf-smelt is not an exception to this, since among the high temperatures a decrease in the yolk depletion rate was observed for 15° and 22° C.

The expected reduction in efficiency of yolk absorption after hatching will probably be very abrupt due to increased respiration and swimming. Fry et al., (1947) showed that active metabolism

varied 2.5 times the standard one. Lasker and Theilacker (1962) found values of active metabolism three times greater than those for resting metabolism. For the surf-smelt this variation ranged from one (at 6° C) to three times (at 15° C).

Larvae incubated at 22° C were 23% shorter than larvae at any other temperature at both hatching and yolk absorption times. The incubation temperature should bear some relation to larval size at yolk absorption; it is not clear why this decreased size was observed only on larvae reared at 22° C. In this respect, Heming (1982) concluded for chinook salmon that high temperatures reduced the length of the yolk reabsorption period as well as the total quantity of available energy for tissue growth during that period, resulting in shorter larvae. In the plaice Pleuronectes platessa, high temperatures reduced the growth in length of yolk sac larvae and lower temperatures were found to be more efficient in terms of the relationship between growth in length and yolk utilization (Ryland et al. 1975). Smigielski (1979) concluded that the size of yellowtail flounder, Limanda ferruginea at hatching was independent of the incubation temperature. A similar trend was observed for the spanish sardine Sardinops sagax, where length for the range 8.8 to 22° C did not show significant differences at hatching (Garreton and Balbontin, 1982). In most experimental temperatures, except 22° C, the data for surf-smelt seems to agree with the statement that size at hatching is independent of the incubation temperature. In any case, more

critical than the size at hatching is the one at yolk absorption (Howell, 1980). The apparent capability of the surf-smelt to maximize growth during periods of delayed hatching will confer surf-smelt larvae advantage in swimming ability which in turn according to Hunter's (1972) findings will affect the feeding ability at release time.

The results of this research suggest an even distribution of the demersally spawned surf-smelt eggs from lower to upper levels on the beach. Frank and Leggett (1981b) defined a similar pattern in the horizontal distribution of capelin (Mallotus villosus) eggs. Nevertheless, they noticed a tendency of the eggs to accumulate in high tide zones at the start of the spawning activity. Daily brief inundation of the beach where capelin spawns may account for this difference. The maximal occurrence of surf-smelt eggs between 6 and 8 cm in a vertical sense, may prevent eggs from exposure to direct sunlight and desiccation. There is a marked temperature gradient in the substrate where the eggs are naturally reared. A combination of several variables such as solar insolation, sea temperature and tidal height serve to define this in situ temperature. Upper zone with longer periods of exposure and consequently higher temperatures than intermediate and low tide zones will obviously represent the most hostile environment for developing eggs trapped on that location. Twenty-four degrees Celsius represents the maximum lethal temperature for the surf-smelt eggs as also detected by this experimental

research. Similarities in low efficiency values at 6° and 22° C plus the fact that the rate of yolk absorption is temperature independent over the range 12°-22°C, may signify greater tolerance of the intertidal reared eggs to temperature fluctuations over the ones reared at either upper or lowest low tide zones. Although temperature may not greatly influence the rate of yolk absorption in surf-smelt, it plays a decisive role in the duration of the incubation period as have also influenced the incubation period in other species (for e.g. Ahlstrom, 1943; Lasker, 1964; Garreton and Balbontin, 1982). Based on the experimental data of this research, surf-smelt eggs incubated in exposed places will only need approximately one week to hatch, whereas those from low tide zones will require over two weeks. Because of a longer period of incubation, the eggs reared in the low levels of the beach may experience a longer period of exposure to predation than those incubated in the upper zone. On the other hand, the permanent submersion that ensure the immediate incorporation into the pelagic environment at hatching of eggs from low tide levels will probably compensate for eventual mortality due to predation. Timing of hatching of eggs from upper levels may be critical and will probaby have to coincide with periods of inundation and tidal flushing if the egg was to succeed ~~on~~ reaching the marine environment. Delayed hatching, diapause, as experienced by laboratory reared surf-smelt eggs may represent the cue on exposed places lacking tidal flushing

for prolonged periods. (Laboratory-reared eggs were able to delay hatching for as much as two weeks at 22° C). Assuming that the diapause phenomenon occurs in nature, the duration should be very restricted in the upper zones. Experimental conditions demonstrated that under diapause an egg continues its development and it is able to completely exhaust its yolk-sac reserves. Therefore, a prolonged diapause before the incorporation of the larva into the pelagic environment may prevent the larva from finding the first exogenous food due to lack of the necessary energy reserves to cope with metabolic demands, mainly those derived from the onset of the swimming activity. Bearing in mind the causing agent of the experimental diapause, eggs reared in low submerged zones with constant agitation will by no means be in need of experiencing diapause, neither will those inhabiting the intertidal zones. Diapause might occur in some coastal pools though, where constant tidal cycles are more sporadic. It is then a matter of conjecture whether diapause has evolved on an ecological time scale or represents vestiges of an old character. It is worthy to note that considering the fresh-water origin of the fishes, demersal eggs in marine fish are phylogenetically regarded as an old character and that Osmerids are also an ancient group.

Although the significance of the demersal spawning has been tacitly emphasized with all the preceding considerations, the most relevant conclusion of this study has been in showing that

experimentally determined similarities in yolk utilization over a wide range of temperatures can suggest highly developed physiological mechanisms to compensate for temperature changes in surf-smelt. This research has also indicated that energy deficit prior to onset of exogenous feeding capability did not occur in the developing surf-smelt.

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