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Embryo Ecology of the Pacific Surf Smelt, *Hypomesus pretiosus* (Pisces: Osmeridae)[1, 2, 3]

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ABSTRACT: A study of the ecology of developing embryos of the Pacific surf smelt, *Hypomesus pretiosus*, was conducted. Embryos were maintained in the laboratory at 7.6, 12.1, and 17.6°C and the time to specific embryonic stages determined. Embryos held at 7.6°C developed to stage 24, 18 days after collection; those held at 12.1°C hatched after 13 days; at 17.6°C hatching occurred 8.5 days after collection. Embryos maintained at 15°C and salinities of 20, 25, and 30‰ averaged 84% survival. There was no significant difference in survival between the groups (ANOVA, $P = 0.53$).

Field observations indicated that embryos are spawned in patches in the upper intertidal zone near the time of high tide. They are attached to gravel substrates by the zona radiata membrane which ruptures and quickly turns inside out at the time embryos are fertilized. After several days of development, stage 18 to 22 embryos detach from the original spawning substrates and are washed seaward and down into the gravel substrate in the intertidal zone. However, there was no significant difference (ANOVA, $P \geq 0.09$) in the number of eggs found at each of 4 depth strata in the upper, middle, and lower intertidal zones.

THE PACIFIC SURF SMELT, *Hypomesus pretiosus*, is a small fish of the family Osmeridae that occurs from northern California to southern Alaska and utilizes upper intertidal gravel substrates for spawning (Hart 1973). Studies of spawning locations and behavior suggest that a number of physical and geological variables may limit the sites available for reproduction of this species. Within Puget Sound, the known spawning grounds of this species are quite disjunct, with long distances of outwardly suitable-appearing beaches which have apparently never supported surf smelt spawning activity (Penttila 1978).

The inside waters of Washington State are inhabited by ten or more spawning stocks of surf smelt presumed to be more-or-less genetically isolated. The relative youth of the Puget Sound Basin since the last Quaternary glacial retreat has resulted in, as yet, only very small meristic differences between Washington's various disjunct surf smelt populations (Schaefer 1936; Kalambi 1965). Depending on the stocks being considered, surf smelt spawning in Washington State occurs virtually year-round, except for the month of April, with generally a 4 to 5 month spawning season for given stocks.

On the outer coast of the Olympic Peninsula, Washington, *H. pretiosus* spawns on cove beaches that consist of fine gravel and coarse sand (Thompson et al. 1936). Gravel beaches in Puget Sound are also utilized for spawning.

At Utsalady Bay, Washington, summer-spawning *H. pretiosus* always spawned in intertidal areas protected from direct sunlight by the shadows of large trees growing along

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the beach. Spawning runs occurred during daytime and females deposited eggs at an intertidal elevation of approximately 2.5 to 3.5 m above mean low water (MLW). Eggs were buried in the gravel to a depth of 1.0 to 3.0 cm by wave action (Loosanoff 1937).

Our study of winter-spawning *H. pretiosus*, conducted during January 1985, was designed to determine rates of development and salinity tolerance of embryos in laboratory tests. We also studied a spawning beach at Fidalgo Bay, Washington, and determined the distribution of embryos within gravel substrates at locations in the upper, middle, and lower intertidal zones.

MATERIALS AND METHODS

Study Sites

Field observations were made in January 1985 along the eastern shoreline of Fidalgo Bay, Washington, 48°29′ N latitude, 122°35′ W longitude (Figure 1). This area is characterized by pocket beaches with gravel substrates, and a tidal range of 2.8 to 3.0 meters. The beaches where *H. pretiosus* spawned usually had large boulders, diameter 1–2 m, in the extreme upper intertidal zone.* Some areas were shaded by willow trees, *Salix* sp., or big leaf maples, *Acer macrophyllum*. Observations of embryo developmental rates and time to detachment from spawning substrates were made at the University of Washington, Friday Harbor Laboratories (FHL), using naturally spawned embryos collected from Fidalgo Bay. Salinity tolerance tests were conducted at the U.S. Environmental Protection Agency, Environmental Research Laboratory (ERL) in Gulf Breeze, Florida.

Embryo Development

Gravel substrates with attached *H. pretiosus* embryos were collected from the upper intertidal zone at Fidalgo Bay on 11 January 1985, placed in vacuum bottles to prevent abrupt temperature fluctuations and transported to FHL. The substrate/embryo temperature was 9.3°C. Replicate groups of 20 embryos were gently teased from the spawning substrate and examined under a dissecting microscope to confirm stage synchrony. All embryos were in the early high blastula stage. This developmental stage corresponds to stage 8 of Lagler et al. (1977). A synopsis of developmental stages observed in this study and corresponding numerical stages were summarized by Lagler et al. (1977), and hereafter, are identified by the numerical values defined in Table 1.

Gravel substrate with attached stage 8 embryos were distributed among six replicate 250 ml glass containers. Fifty ml of filtered (1.0 μm) seawater, 28–30‰ and 10°C, was added to each pair of containers, which were then covered with plastic closures. This procedure allowed embryos to develop in a moist atmosphere without being inundated. One pair of replicate containers was maintained at 17.6°C in a water bath, after a 6-hour adjustment period from 10°C. A second pair was held at a room temperature of approximately 12.1°C and the third pair was maintained in ambient temperature seawater at approximately 7.6°C, after 6 hours acclimation from 10°C. Samples were taken daily and the developmental stage and viability determined for 15 to 30 embryos from each replicate. These embryos were then discarded. Filtered (1.0 μm) and temperature-adjusted seawater was used to triple rinse developing embryos in respective replicate containers at 2 to 3 day intervals. The photoperiod was 12L:12D; light intensity was 175 lux provided by fluorescent lamps.

Stage 8 embryos for salinity tolerance tests were collected on 10 January 1985. Embryos, attached to gravel spawning substrates were placed in 500 ml vacuum bottles. Approximately 50 ml of seawater, 9.3°C, 30‰, was then added to each bottle to ensure that embryos remained moist during shipment to ERL/Gulf Breeze. Upon arrival at the laboratory on 14 January, stage 15 embryos were gently teased from gravel substrates and randomly distrib-

* The boulders were derived almost totally from highway riprap placement. The March Pt. peninsula's natural geology is eroding Quaternary glacial hills, which feed sand/gravel to the beach system.

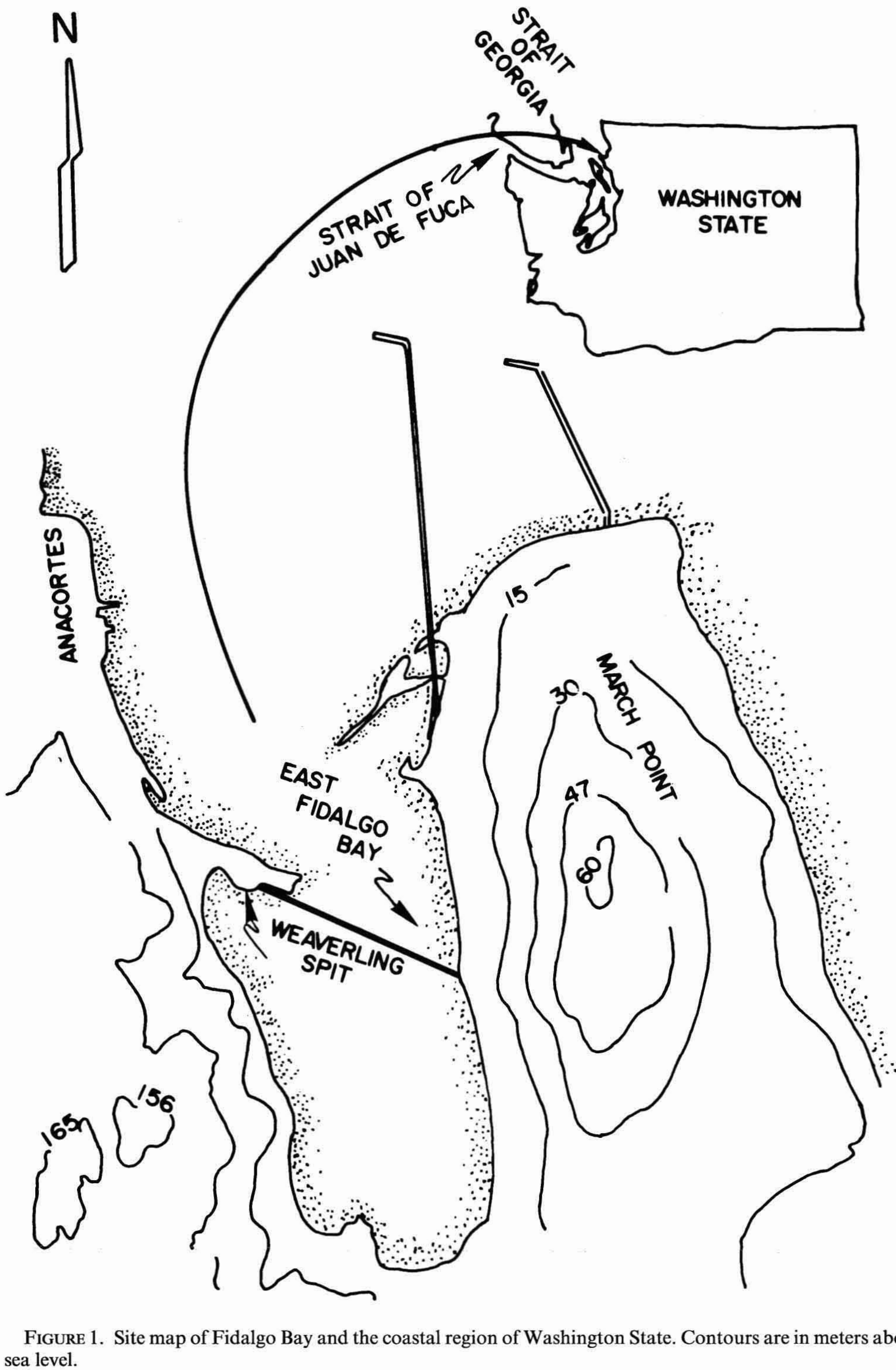

FIGURE 1. Site map of Fidalgo Bay and the coastal region of Washington State. Contours are in meters above mean sea level.

TABLE 1

EMBRYONIC STAGES OBSERVED IN *Hypomesus pretiosus* AND CORRESPONDING NUMERICAL STAGES DESCRIBED BY LAGLER ET AL. (1977)

EMBRYONIC STAGES OBSERVED IN *H. pretiosus*	CORRESPONDING NUMERICAL STAGES DESCRIBED BY LAGLER ET AL. (1977)
Early high blastula	Stage 8
Late high blastula	Stage 9
Early gastrula	Stage 12
Middle gastrula	Stage 13
Late gastrula	Stage 14
Initial formation of rudimentary optic vesicles and first somites	Stage 15
Expansion of forebrain to form optic vesicles	Stage 16
Appearance of 4 to 14 pairs of somites	Stage 18
Pericardium established, nonchambered tube-like heart is visible and pulsing	Stage 20
Motility of developing embryo	Stage 21
Onset of circulation	Stage 22
Melanophores appear in pericardium limited circulation in yolk vessels	Stage 23
Pectoral fin buds apparent	Stage 24
Formation of liver	Stage 25
Prehatch embryo	Stage 31
Newly hatched larva	Stage 32

uted among treatments. Each treatment consisted of triplicate samples of 20 eggs incubated in individual 20 cm diameter glass culture dishes containing 1 l of sterilized seawater. Salinities of 20, 25, and 30‰ were used at temperatures of 15 and 20 $\pm$ 1°C. Embryos were observed at 48-hour intervals until hatching occurred. During each observation, dead embryos (opaque-white) were removed from respective treatments and enumerated. The photoperiod was 12L:12D; light intensity was 175 lux provided by fluorescent lamps. A temperature-compensated refractometer was used to measure salinity. An immersion thermometer with 0.1°C graduations was used to measure water temperature.

Field Observations

Replicate core samples, 5 cm diameter $\times$ 4 cm deep, were taken from the upper intertidal (2.8–3.0 m above MLW), the middle intertidal (1.3–1.6 m above MLW), and the lower intertidal zones (0.2–0.6 m above MLW) on 25 January 1985. Cores were split at 1.0 cm depth intervals to yield strata from 0–1, 1–2, 2–3, and 3–4 cm deep. Respective strata were placed in individual glass containers and the embryos subsequently enumerated and stages identified with a dissecting microscope.

At the time of collection of embryos, concurrent measurements of relative humidity and air temperature were taken with an Atkins Model 90023–30 Digital Psychrometer. Substrate temperatures corresponding to strata depths in core samples were measured with a Bailey Instruments BAT–4 Laboratory Thermometer with a MT 29/1 microprobe.

Statistical Analysis

Statistical analyses were conducted when appropriate (SAS Institute Inc. 1982). Salinity effects on embryo survival were analyzed by one-way analysis of variance (ANOVA) on percentage survival data, using a modification of the arcsin transformation to account for unequal cell sizes (Chanter 1975). A two-way ANOVA, with $\log_{10}$ transformation of the field core data, was used to evaluate the main effect of intertidal zone, depth of strata, and possible interaction on egg numbers. All post-hoc analyses were conducted by the Bonferroni multiple comparison method at $\alpha = 0.05$.

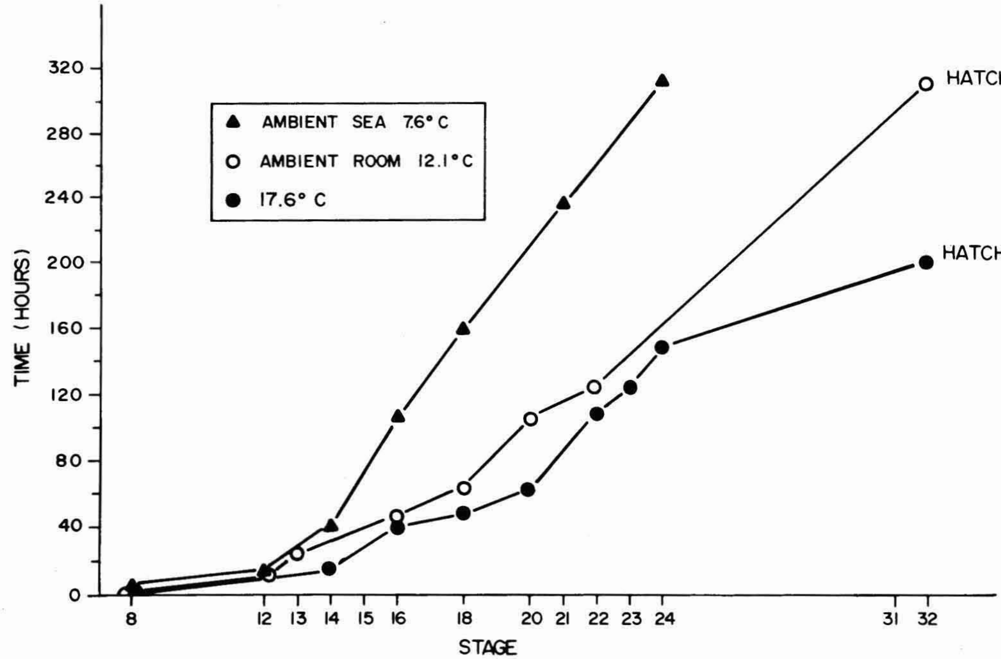

FIGURE 2. Developmental times for *H. pretiosus* embryos held at 7.6, 12.1, or 17.6°C. Stages observed correspond to those outlined in Table 1.

RESULTS

Laboratory Observations

Developmental rates of *H. pretiosus* embryos maintained at ambient seawater temperature ($\bar{x} = 7.6°C$, SD = 0.47, N = 24), ambient room temperature ($\bar{x} = 12.1°C$, SD = 2.6°C, N = 24) and in a heated water bath ($\bar{x} = 17.6°C$, SD = 0.84, N = 24) are shown in Figure 2. Development of embryos proceeded very slowly in ambient temperature seawater and no eggs hatched during 18 days of observation. In contrast, those maintained at ambient room temperature hatched after 13 days. At 17.6°C hatching occurred 8.5 days after collection. The respective percentage survival until stage 32 at 17.6°C and 12.1°C was 77 and 88%. In ambient seawater 7.6°C, survival was 78% through stage 24. Because observations ended on day 18, we were unable to determine the time to hatch of embryos maintained at 7.6°C. No significant differences in survival were found among embryos developed to stage 32 (just after hatching) at 17.6 and 12.1°C, or stage 24 at 7.6°C (ANOVA, P = 0.67).

During intermittent changes of seawater in incubation cups, we noted that *H. pretiosus* embryos detached from the gravel substrates. This detachment occurred after approximately 148 hours at 17.6°C (stages 21 to 23), 200 hours at 12.1°C (stages 18–20), and 310 hours at 7.6°C (stages 22 to 24).

No significant difference (ANOVA, P = 0.53) was found in embryo survival through hatching at 15°C for individuals incubated at salinities of 20, 25, and 30‰ (respective survival, 82, 83, and 87%). Results were generally similar at 20°C for individuals incubated at 20 and 25‰ (respective survival 87 and 78%), but not at 30‰ salinity (survival 47%). However, no significant difference (ANOVA, P = 0.11) was found in survival of embryos hatching at the three test salinities.

Field Observations

Relative humidity and temperature data collected at Fidalgo Bay are summarized in

TABLE 2

ENVIRONMENTAL VARIABLE MEASURED ALONG SHORELINE OF EASTERN FIDALGO BAY ON 25 JANUARY 1985

ELEVATIONS IN CENTIMETERS (cm) ABOVE (+), BELOW (−), OR AT THE SUBSTRATE SURFACE (0)		INTERTIDAL ELEVATION ABOVE MLW: UPPER +2.5–3.0 m SHADED	UPPER +2.5–3.0 m SUNLIGHT	MIDDLE +1.3–1.5 m SUNLIGHT	LOWER +0.2–0.6 m SUNLIGHT
	(Elevation)				
+1 cm	rel. humidity %	98	93	99	96
+1 cm	air temp °C	9.6	9.4	9.3	9.7
0 cm	substrate temp °C	9.2	10.1	9.3	9.5
−1 cm	substrate temp °C	5.0	9.1	9.0	7.5
−2 cm	substrate temp °C	4.9	8.5	8.1	6.0
−3 cm	substrate temp °C	4.9	8.3	7.9	6.0
−4 cm	substrate temp °C	4.8	7.8	7.9	6.0

Table 2. Measurements were taken at two locations in the upper intertidal zone, one shaded and the other in full sunlight. Relative humidity measured 1 cm above the substrate surface in the shaded area was substantially higher than measured at a similar intertidal elevation in full sunlight. While air temperatures were similar at 1 cm, substrate temperatures on and below the surface were higher in areas of the intertidal zone where full sunlight was present. Middle and lower intertidal locations, in full sunlight, had high relative humidities. The middle and lower intertidal substrate temperatures trended downward, both at the surface and below, when compared with the upper intertidal location that was also in full sunlight.

Grain size distributions for surf smelt spawning substrates from March Point, the eastern shoreline of Fidalgo Bay, and Weaverling Spit are shown in Figure 3. These data were collected during the 1981–1983 spawning seasons from areas where observations were made in the current study (Penttila, unpub. data). Samples were taken in the middle to upper intertidal zone (1.3 to 2.5 m above MLW) from the surface to approximately 2.0 cm deep, from representative smelt spawn-bearing beach material.

The relative frequencies of occurrence of several embryonic stages at locations in the intertidal zone in this study are summarized in Figure 4. The frequency distributions were based on the number of embryos in each developmental stage at each intertidal elevation and substrate depth divided by the total number of embryos collected at all elevations and depths. Thus, the relative frequencies of occurrence for respective embryos stages and locations are directly comparable (Simpson et al. 1960).

Most of the embryos collected in the upper intertidal zone at 0–1 cm deep were in stage 9 (late high blastula). This group comprised more than one-fifth of the total number of embryos found. The 1 to 2, 2 to 3, and 3 to 4 cm deep strata in the upper intertial zone yielded a high proportion of embryos with chambered hearts (stage 22) and a few in stages 21 and 23. The stage 22 group was evident in all four strata from the middle intertidal zone, although at a lower relative frequency. Some individuals in stages 16 through 32 were also present in these samples.

While stage 22 embryos were found in all strata from the lower intertidal zone, stage 18 embryos were dominant. A few stage 23 individuals were also present.

Statistical analyses (ANOVA) for the number of eggs at each intertidal location and substrate depth revealed no significant interaction ($P = 0.86$) between locations and depths. Moreover, no significant differences existed between locations in the number of eggs found at each of four depths ($P \geq 0.09$). However, there was a significant difference

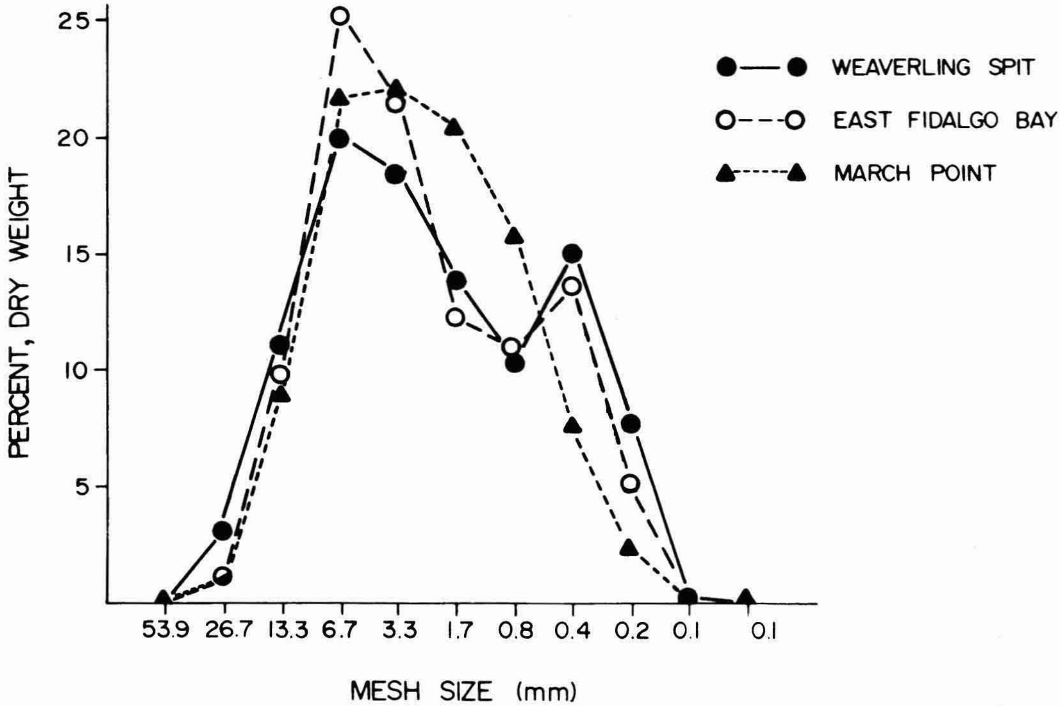

FIGURE 3. Grain size analyses for spawning substrates taken from the upper intertidal zone at March Point, Weaverling Spit, and the eastern shore of Fidalgo Bay.

($P = 0.003$) in the total number of eggs found at each intertidal location. Post-hoc analyses (Bonferroni) indicated a significant difference ($\alpha = 0.05$) between the number of eggs collected from the upper and lower intertidal zone, but the middle intertidal was not different from either the upper or lower intertidal zones.

DISCUSSION

Spawning by surf smelt takes place near the time of high tide. At Utsalady Beach, Washington, Loosanoff (1937) observed reproduction on flood tides that reached 2.5 to 3.5 m above MLW. Daily activity intensified on an increasing high tide series and peaked on late afternoon flood tides, especially when the time of high water coincided with sunset. Penttila (1978) collected ripe surf smelt at Penn Cove, Whidbey Island, and Utsalady Beach, Camano Island, Washington, at biweekly intervals during high tides, shortly before sunset.

We noted spawning patches in the upper intertidal zone at Fidalgo Bay on January 11 and 25. Embryos on both occasions were in stage 8 (early high blastula). It is possible that these patches resulted from spawning during high tides on January 9 and 23. On both dates, high tides of 2.2 to 2.4 m above MLW occurred approximately 30 minutes before sunset.

During the several month-long spawning season of a surf smelt stock, spawning occurs at irregular intervals on any given stretch of beach. Super-imposition of new spawn patches upon older spawn already incubating embryos within the substrate is quite common. In fact, field-sampling of smelt-spawn-bearing beach material on winter spawning grounds in Puget Sound shows most substrates to be incubating from two to four distinct spawning events, with as many as seven to eight different ages noted occasionally (Penttila 1978).

Hypomesus pretiosus eggs are adhesive and

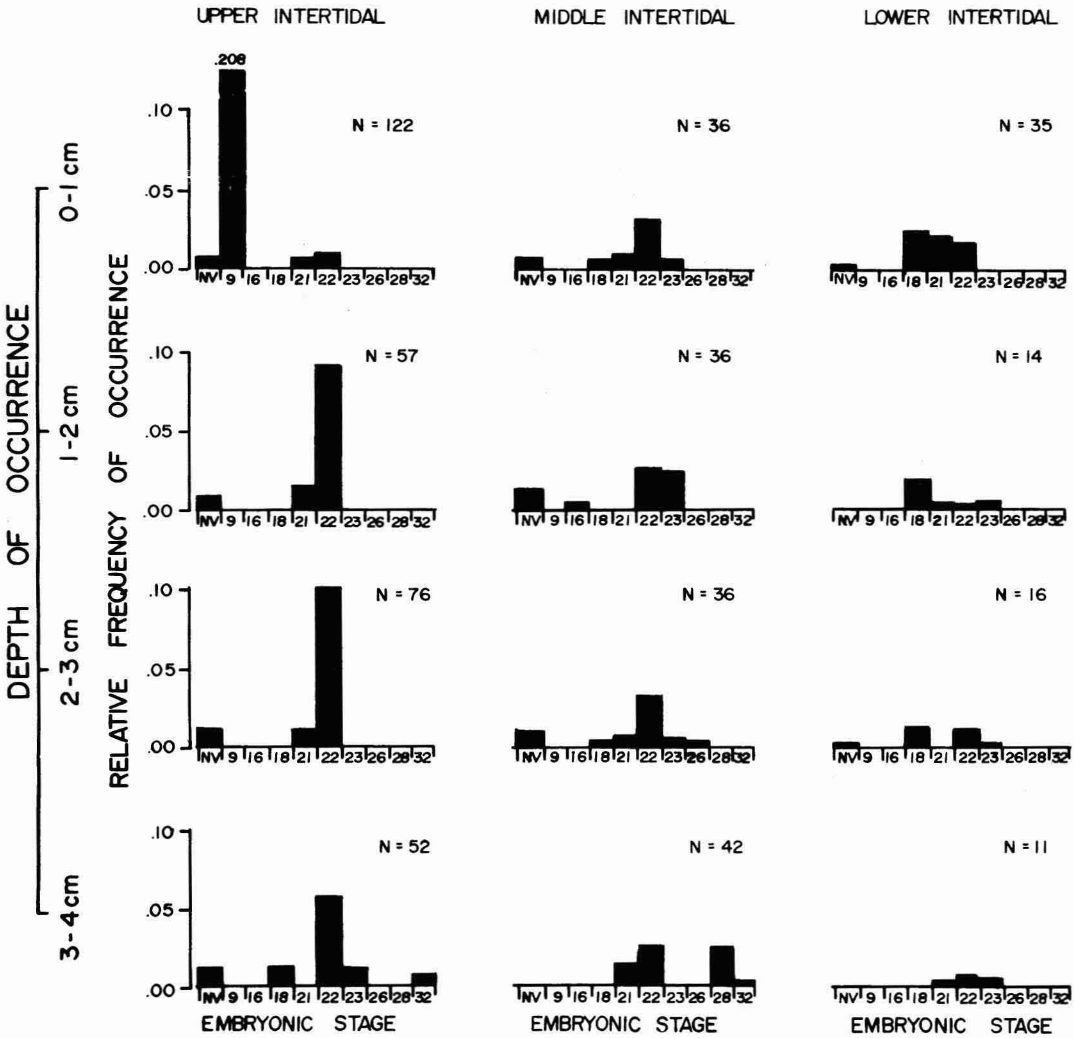

FIGURE 4. Relative frequency of embryonic stages of *H. pretiosus* collected from a pocket beach on the eastern shore of Fidalgo Bay.

readily attach to gravel substrates. Attachment occurs immediately upon fertilization as the zona radiata membrane is ruptured and quickly turns inside out. The inverted zona radiata adheres to any surface that it contacts (Loosanoff 1937). Upon attachment, the zona radiata forms a small pedestal-like structure with the fertilized egg resting at the top.

During laboratory incubation of *H. pretiosus* at 17.6°C, we noted embryo detachment from the gravel substrate at stages 21 to 23. The pedestal (zona radiata) remained attached to the developing embryo for 12 to 24 hours, then disappeared. A similar phenomenon was noted in embryos incubated at a mean room temperature of 12.1°C. However, these embryos were in stages 18 to 20 when detachment occurred. Only a few stage 22 to 24 embryos incubated at an ambient seawater temperature of 7.6°C detached from substrates during this study.

Loosanoff (1937) found that summer-spawned *H. pretiosus* embryos at Utsalady Beach, Washington, deposited on gravel at an elevation of approximately 2.5 to 3.5 m above MLW detached from spawning substrates ten days after fertilization. From his description, it is apparent that these embryos were in an advanced stage of development. Hatching of embryos from Utsalady Beach occurred one day after detachment from gravel substrates. Repeated tests revealed that embryos always hatched on the eleventh day after fertilization. It was also learned that embryos had to be inundated and agitated to stimulate hatching (Loosanoff 1937). A similar need for submergence and agitation to stimulate hatching in the California grunion, *Leuresthes tenuis*, was noted for embryos that had been incubated in sand (Walker 1952). In contrast, David (1939) and Goodman et al. (1985) maintained *L. tenuis* embryos in quiet water for 12 to 15 days, then subjected them to agitation, whereupon hatching occurred.

In this study, *H. pretiosus* embryos were incubated in glass containers that were partially filled with seawater. Detachment of embryos from the gravel substrate resulted in incubation under water during the advanced stages of embryogenesis at 17.6°C and at ambient room temperature of 12.1°C. No hatching was noted among these embryos prior to daily agitation by inversion of the incubation cups. Hatching was noted 10 to 30 minutes after agitation.

The detachment phenomenon and movement of embryos both in a seaward direction and to a lower stratum in intertidal substrates at Fidalgo Bay, Washington, appears to reduce the likelihood of thermal stress and desiccation. The lowest relative humidity and highest substrate temperature were recorded in the upper intertidal zone in sunlight. Relative humidity and temperature were not as extreme at the same elevation in a location shaded by over-hanging willow trees (Table 2).

We noticed that newly spawned eggs occurred most often in patches (1 to 2 m diameter) in the upper intertidal zone where trees or large boulders provided shade. *H. pretiosus* at Utsalady Beach during the summer always deposited eggs in locations shaded from direct sunlight. Loosanoff (1937) noted that eggs unburied and uncovered by water for up to 36 hours were dry and shrunken. However, when placed in seawater these eggs acquired normal shape rapidly, developed successfully, and produced normal larvae.

A study by Penttila (1978) revealed that a preponderance of substrates where spawning occurred at Utsalady Beach ranged between 1 and 7 mm in diameter. Similar substrates were present along the eastern shore of Fidalgo Bay where *Hypomesus* spawned during this study (Figure 3). Sorting and resorting of this material by wave action generally resulted in larger material at the surface. Developing embryos sifted to lower strata where capillary moisture prevented desiccation while allowing adequate aeration. Embryos that were attached to very large gravel or to sand, thus forming light-weight particles with large surface areas, were not sorted to deeper strata and were likely to be exposed to desiccation and thermal stress (Penttila 1978).

Survival ranged from 78 to 87% at five of the six temperature and salinity combinations we tested. However, a decrease in hatchability to 47% was noted for embryos maintained at 20°C and 30‰ salinity. While not statistically different from other salinities tested at 20°C, this combination obviously was stressful to incubating embryos. High and low temperature stress, desiccation, and exposure to rapidly fluctuating salinity during rainfall events and daily tidal inundations are variables that continually challenge *H. pretiosus* embryos. Indeed, embryos from winter (September–March) spawn samples in Southern Hood Canal, Washington, have been observed frosted with ice crystals. Although embryos may be subjected occasionally to weeks of freezing weather during a particularly harsh Puget Sound winter, the net result seems to be a great lengthening of the incubation period (to 90 days or more), but not necessarily higher mortalities (Penttila 1985, pers. comm.). In contrast, summer spawners (June–September) deposit embryos in the upper intertidal zone at Utsalady Beach where they are exposed to daily temperatures ranging from 15.5 to almost 26°C (Loosanoff 1937). Incubating California grunion, *L. tenuis*, embryos were subjected to similar temperatures, 16 to 27°C, at a depth of 7 to 15 cm below the surface; concurrent surface temperatures ranged from 15 to 36°C. Similarly, Middaugh et al. (1983) measured substrate temperatures of 19 to 32°C at a depth of 4 to 8 cm where *L. tenuis* embryos were developing.

The eastern shoreline of Fidalgo Bay is characterized by a relatively short fetch to the west. This limited fetch reduces the height of waves breaking in areas where *H. pretiosus* eggs are deposited. However, some sorting and re-sorting of substrates were observed. It seems likely that initial placement of eggs in shaded microhabitats at the substrate surface, followed by detachment and redistribution to different intertidal locations and substrate depths, plays a major role in reducing the impact of environmental variables on developing embryos along this spawning beach.

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