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# Mass Abundances of Abnormal Pacific Herring Larvae at a Spawning Ground in British Columbia

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# Mass Abundances of Abnormal Pacific Herring Larvae at a Spawning Ground in British Columbia

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*Abstract.*—Abnormalities including contortion of the body and reduction of the jaws and pectoral fins occurred among 2–25% of the yolk-sac Pacific herring larvae *Clupea harengus pallasi* collected in 1985 from Kulleet Bay, a spawning area on Vancouver Island, British Columbia. An additional 4–68% of the post-yolk-sac larvae had underdeveloped lower jaws. Abnormal larvae were distinguished from healthy larvae through discriminant analysis of morphometric characters. The high frequency of larval abnormalities in 1985 may have been related to unseasonably sunny, warm weather during the 14-d incubation of the eggs, which resulted in stress to eggs exposed to sun and warm air during especially low midday tides. Underdeveloped jaws affected feeding by post-yolk-sac larvae, as evidenced by lack of food in the guts and by shallow body depths characteristic of starvation.

Pacific herring *Clupea harengus pallasi* on Canada's British Columbia coast deposit eggs on vegetation in the intertidal zone and below; 80% of the spawn attaches at depths less than 1.5 m below the water level datum (Hourston and Haegele 1980). Intertidally deposited eggs are exposed to drying, direct sunlight, and extremes of air temperature during part of the tidal cycle. These factors may stress eggs and affect their survival or development. Steinfeld (1972) noted higher mortalities of Pacific herring eggs in intertidal zones of Yaquina Bay, Oregon, during periods of warm, dry weather than during cool, damp conditions. Jones (1972) showed that increased intertidal exposure increased egg mortality, reduced the incubation time, and affected hatching size. He proposed that hypoxia, desiccation, and air–water temperature differentials are the primary factors affecting Pacific herring spawn exposed during the tidal cycle, although he did not measure those factors.

During sampling in 1985, we found concentrations of moribund Pacific herring larvae at the tide line in Kulleet Bay, Vancouver Island, British Columbia. Many abnormal larvae occurred in the plankton samples taken at 1–5-m depths during the period larvae were hatching. Herein, we describe these abnormalities and quantify the occurrence of abnormal larvae in Kulleet Bay during April 14–21, 1985. We used multivariate morphometric analysis, which has become a widely used method in larval research (e.g., McGurk 1985a, 1985b), to quantitatively assess phenetic differences among abnormal and healthy larvae. We examine environmental stresses that may have caused the abnormalities, and the effects of the deformities on larval feeding.

## Methods

Pacific herring larvae were sampled during peak hatching at three stations in Kulleet Bay, southeastern Vancouver Island. A 333-$\mu$m-mesh, 0.75-m-diameter plankton net with a flowmeter was towed horizontally at 1 and 5 m depths for 0.5–3.0 min at 0.5–0.8 m/s. In the morning of each day, 10 samples were collected, as detailed in Purcell and Grover (1990). The resulting samples were immediately preserved with formaldehyde buffered with sodium borate (final concentration, 5% formalin). Larvae were counted from subsamples made with a Folsom plankton splitter, and examined for gut contents and gross morphological abnormalities with a dissecting microscope.

Larvae were divided visually into five groups for morphometric analysis: newly hatched, contorted yolk-sac, healthy yolk-sac, abnormal post-

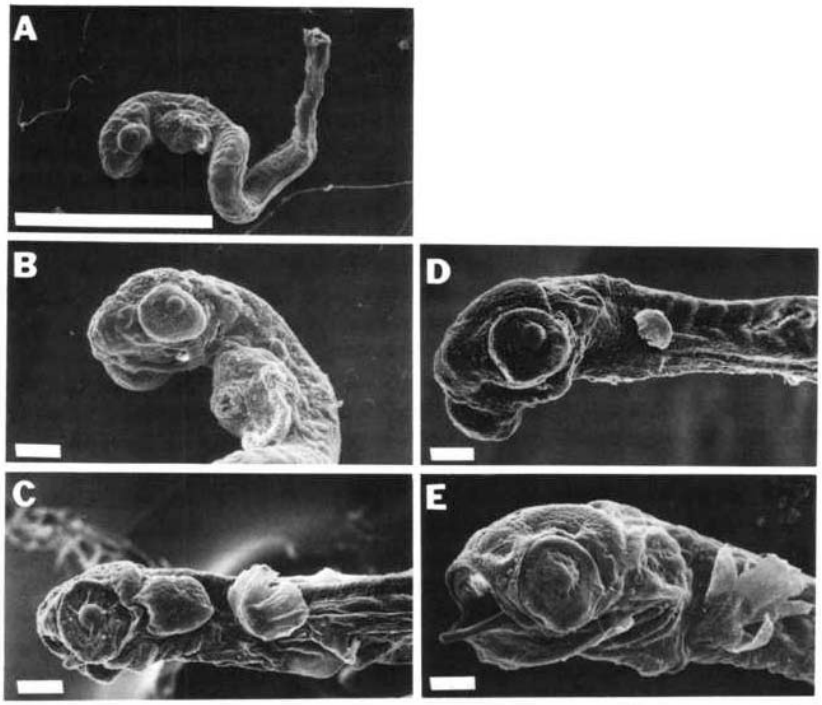


FIGURE 1.—Scanning electron micrographs (SEM) of Pacific herring larvae. (A) Contorted yolk-sac larva with reduced mandible, missing pectoral fins, and shrivelled yolk sac. (B) Same larva, anterior end. (C) Healthy yolk-sac larva, anterior. Yolk was removed before preservation for SEM. (D) Emaciated post-yolk-sac larva with reduced mandible and pectoral fins, anterior. (E) Healthy post-yolk-sac larva with well-formed mandible and pectoral fins, anterior. Scale bars: A, 1,000 μm; B–E, 100 μm.

yolk-sac, and healthy post-yolk-sac. Newly hatched larvae had more lightly pigmented eyes than older yolk-sac larvae. Contorted yolk-sac larvae had poorly formed lower jaws, missing or reduced pectoral fins, shrivelled yolk sacs, and highly contorted bodies (Figure 1A, B). By contrast, healthy yolk-sac larvae had well-formed lower jaws and full yolk sacs (Figure 1C). Abnormal post-yolk-sac larvae had missing or poorly formed lower jaws and thin bodies (Figure 1D). Healthy post-yolk-sac larvae had well-developed jaws and robust appearances (Figure 1E).

In order to evaluate the morphometric distinctness of these groups of larvae, six measures were made with a dissecting microscope and ocular micrometer: (1) standard length, snout to end of notochord; (2) body depth at the anus, exclusive of the gut; (3) body depth at the pectoral fins, exclusive of the gut; (4) head height, measured vertically through the center of the eye; (5) head width, measured across the dorsal surface of the head including the eyeballs; and (6) length and depth of yolk sac, if present. The degree of separation between the larval groups was calculated with BMDP: 7M (Biomedical Programs) stepwise discriminant analysis of the first five morphometric characters. The analysis calculates vectors (canonical variates) that represent underlying dimensions of variation that best separate two or more groups. The first component represents size. Then the program chooses new variates—shape components—that are linear combinations of the original variables (Reyment et al. 1984). The results were plotted on a rectangular coordinate system. Logarithmic transformation of our data, commonly done for multivariate normality, produced plots similar to those from untransformed data, so the latter was used for analysis. The probabilities of correct classification (the percentages of larvae placed visually in what turned out to be the correct mathematical grouping) were calculated directly from individual squared Mahalanobis distances ($D^2$), obtained by multiplying the vector of coefficients that constitute the linear discriminant function by the vector of differences between two group means.

Larvae used for scanning electron microscopy were transferred from 5% formaldehyde solution to an ethanol dehydration series. Then specimens

were dried in a Bomar SPC 900 critical-point drying chamber, mounted on stubs and coated with a 60:40 gold:palladium alloy in a Varian-VE10 vacuum evaporator, and examined in an AMRay 1000A scanning electron microscope.

In the absence of data on environmental conditions in Kulleet Bay during the 14-d incubation of Pacific herring eggs, we gathered information from sources at nearby locations. Air temperature and cloud cover were obtained from hourly meteorological observations made at the Nanaimo Airport, Vancouver Island. Tidal heights were taken from tables of the Canadian Hydrographic Services for Port Atkinson, British Columbia. Water temperature and salinity at 1 m depth were taken daily at Chrome Island, Vancouver Island; those data were provided by the Institute of Ocean Sciences, Sidney, British Columbia.

Microzooplankton was sampled with a 0.5-m-diameter, 63-$\mu$m-mesh plankton net that was dropped mouth-first to the 5-m depth and retrieved, filtering 2 $m^3$ of water. In the laboratory, these formalin-preserved samples were diluted with sea water to a 1-L volume, and prey of Pacific herring larvae (copepod nauplii, tintinnids, radiolarians, and bivalve veligers) were counted in three to five subsamples taken with 5- or 10-mL Hensen stempel pipettes.

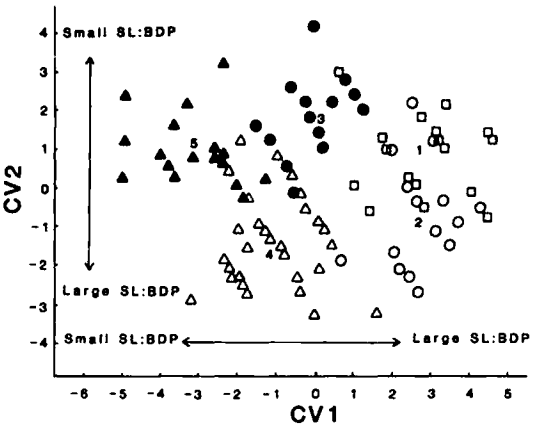


FIGURE 2.—Scores for the first two canonical variates (CV) for five groups of larval Pacific herring. **Open squares**, newly hatched yolk-sac larvae; **open circles**, contorted yolk-sac larvae; **closed circles**, healthy yolk-sac larvae; **open triangles**, abnormal post-yolk-sac larvae; **closed triangles**, healthy post-yolk-sac larvae. The first standardized canonical vector for standard length (SL), body depth at anus (BDA), and body depth at pectorals (BDP) is (−0.59, 0.02, −0.62). The second standardized canonical vector is (0.91, 0.01, −0.88). Numbers denote group means.

## Results

Malformed larvae had smaller body dimensions than apparently well-formed larvae (Table 1). The discriminant analysis showed clear mathematical separation between groups (Figure 2). The first standardized canonical vector (size) had a large negative component for standard length (SL) and body depth at the pectoral fins (BDP). Separation of the groups by age was effected by those two variables. Older larvae had smaller ratios of SL to BDP (they were fatter) than younger larvae. On the second canonical vector (shape), contrast of the same variables separated the abnormal groups, which were relatively long and thin, from the healthy groups. The probabilities of correct visual classification of the larvae ranged from 60 to 90% (Table 1).

TABLE 1.—Mean morphometric measurements (SD in parentheses) of Pacific herring larvae collected in Kulleet Bay in April 1985. Percentages of correct classification represent larvae visually placed in the correct mathematical grouping determined by multivariate discriminant analysis. $N$ = numbers of larvae examined.

| Larval group ($N$) | Standard length, mm | Body depth at | | Head | | Yolk-sac | | Percent correct classification |
|---|---|---|---|---|---|---|---|---|
| | | Pectorals, mm | Anus, mm | Height, mm | Width, mm | Depth, mm | Width, mm | |
| Newly hatched (20) | 6.02 (0.55) | 0.53 (0.09) | 0.41 (0.06) | 1.11 (0.07) | 1.58 (0.09) | 1.15 (0.19) | 1.83 (0.26) | 60.0 |
| Healthy yolk-sac (36) | 7.79 (0.53) | 0.69 (0.08) | 0.45 (0.04) | 1.17 (0.11) | 1.58 (0.08) | 0.97 (0.22) | 2.13 (0.24) | 86.7 |
| Contorted yolk-sac (20) | 5.38 (0.47) | 0.61 (0.09) | 0.41 (0.03) | 1.05 (0.10) | 1.52 (0.13) | 0.49 (0.18) | 0.89 (0.27) | 78.6 |
| Healthy post-yolk-sac (36) | 8.53 (1.00) | 0.94 (0.07) | 0.48 (0.05) | 1.18 (0.13) | 1.62 (0.11) | | | 90.0 |
| Abnormal post-yolk-sac (43) | 7.02 (0.57) | 0.89 (0.09) | 0.42 (0.04) | 1.11 (0.12) | 1.57 (0.09) | | | 84.4 |

TABLE 2.—Occurrences of contorted Pacific herring yolk-sac larvae and post-yolk-sac larvae with underdeveloped jaws in Kulleet Bay. Numbers of larvae examined (*N*) directly reflect the proportions of yolk-sac and post-yolk-sac larvae in the samples.

| Date (1985) | Abnormal yolk-sac larvae: % (*N*) | Abnormal post-yolk-sac larvae: % (*N*) | Abnormal larvae/m$^3$: mean (SD) |
|---|---|---|---|
| Apr 14 | 6.6 (561) | 39.3 (112) | 7.3 (10.2) |
| Apr 15 | 25.4 (681) | 67.6 (151) | 44.6 (46.9) |
| Apr 16 | 18.9 (455) | 26.0 (173) | 35.0 (34.4) |
| Apr 17 | 1.8 (545) | 22.1 (122) | 9.9 (13.8) |
| Apr 18 | 2.5 (643) | 18.6 (188) | 19.8 (24.8) |
| Apr 19 | 1.9 (206) | 8.7 (277) | 0.7 (0.9) |
| Apr 20 | 4.1 (172) | 6.0 (215) | 0.9 (0.9) |
| Apr 21 | 7.9 (165) | 4.5 (201) | 0 |

TABLE 3.—Heights of midday low tide,[a] daytime high air temperatures, and cloud covers[b] during the 14-d incubation of Pacific herring eggs in Kulleet Bay, 1985.

| Days after spawning | Tide[a] (m) | Temperature (°C) | Cloud cover[b] |
|---|---|---|---|
| 1 | | 16.1 | 2 |
| 2 | | 10.9 | 2 |
| 3 | 2.3 | 6.4 | 2 |
| 4 | 1.9 | 9.8 | 2 |
| 5 | 1.6 | 12.2 | 2 |
| 6 | 1.2 | 16.6 | 1 |
| 7 | 0.9 | 17.5 | 2 |
| 8 | 0.8 | 16.9 | 0 |
| 9 | 0.8 | 18.2 | 0 |
| 10 | 0.9 | 15.8 | 2 |
| 11 | 1.0 | 15.1 | 2 |
| 12 | 1.2 | 14.5 | 2 |
| 13 | 1.4 | 11.4 | 2 |
| 14 | | 14.6 | 2 |

[a] Tidal height above the reference datum at low water, between the hours of 1000 and 1830.

[b] Cloud cover: 0 = no cloud cover; 1 = light cover; 2 = complete cover.

The abnormal larvae formed large percentages of the Pacific herring larvae in Kulleet Bay in 1985 (Table 2). Densities of these larvae were high, up to 45/m$^3$, which equalled 33% of the larvae captured. Yolk-sac larvae with apparently underdeveloped lower jaws but without contorted bodies were collected; however, they were not quantified because of uncertainty in determining "normal" jaw size of very young larvae. Post-yolk-sac larvae with underdeveloped jaws occurred in average densities of 24/m$^3$. Contorted post-yolk-sac larvae were never seen, probably because of high early mortality. Densities of contorted larvae did not differ between the 1-m and 5-m samples, indicating that these larvae were not dead and trapped at the water surface (analysis of variance, $P = 0.52$). Similarly, densities of all deformed larvae did not differ between 1 and 5 m ($P = 0.41$).

We examined environmental data from 1983–1987 to determine whether unusual conditions existed in 1985 that could have stressed the developing eggs and caused the high proportions of abnormal larvae that year. The lowest midday low tides (< 1 m above the local reference datum), the highest air temperatures, and the clearest skies recorded in those years during the period of intertidal egg incubation coincided during a few days in 1985 (Table 3). During those days, the daytime low tides occurred between 1300 and 1500 hours; the two high tides occurred between 0530 and 0630 and between 2200 and 2330 hours. Therefore, many eggs would have been exposed to sun and high air temperatures during the entire day. During incubation in other years, midday low tides generally were more than 1 m above datum, daytime high air temperatures were several degrees cooler than in 1985, and skies were usually overcast (Table 4). Water temperatures and salinities were similar in all years, and were within normal ranges for the area (Table 4; L. F. Giovando, personal communication). We conclude that the combination of low midday tides and sunny, warm weather during incubation may have produced conditions that stressed the developing eggs in 1985.

We examined gut contents of post-yolk-sac larvae to determine if feeding was impaired by underdevelopment of the lower jaws. Up to 48% of the healthy larvae contained prey (Table 5), averaging 1.4–3.5 prey/larva each day. By contrast, none of the larvae with underdeveloped jaws contained prey (Table 5). We conclude that healthy post-yolk-sac larvae fed well, and that larvae with deformed jaws were unable to feed.

## Discussion

High densities of abnormal Pacific herring larvae occurred at 1–5-m depths in Kulleet Bay. To our knowledge, mass abundances of abnormal wild larvae have not been reported previously. Bibliographies by Dawson (1964, 1971) listed only a few studies dealing with larval fishes, but more than 1,200 papers on morphological abnormalities in adult fishes. Nankee (1981) reported physical anomalies in 0.6% of 12,000 larvae in 13 fish species collected over 3 years. Abnormalities of the vertebral column and jaws accounted for 11.8% and 10.5%, respectively, of the anomalies he observed.

One of us (J.E.P.) collected samples near the

TABLE 4.—Heights of midday low tide,[a] daytime high air temperatures, water temperatures and salinities at the 1-m depth, and cloud cover during 14-d incubations of Pacific herring eggs in Kulleet Bay, 1983–1987.

| Date | Tide[a] (m): mean (SD) | Air temperature (°C): mean (SD) | Days ≥ 14°C | Cloudless days | Water temperature (°C): mean (SD) | Salinity (‰): mean (SD) |
|---|---|---|---|---|---|---|
| Mar 8–22, 1983 | 2.1 (0.7) | 12.2 (2.4) | 3 | 1 | 9.3 (0.4) | 27.2 (0.6) |
| Mar 22–Apr 5, 1984 | 1.8 (0.6) | 11.2 (2.0) | 0 | 0 | 9.4 (0.5) | 27.8 (0.3) |
| Apr 1–14, 1985 | 1.3 (0.5) | 14.0 (3.4) | 9 | 2 | 9.2 (0.2) | 29.1 (1.1) |
| Mar 16–30, 1986 | 1.6 (0.8) | 11.3 (2.6) | 1 | 0 | 8.2 (0.2) | 29.0 (0.5) |
| Mar 17–23, 1987 | 1.4 (0.5) | 11.2 (1.4) | 0 | 0 | 8.4 (0.4) | 28.8 (0.4) |

[a] Tidal height above the reference datum at low water, between the hours of 1000 and 1830.

time Pacific herring larvae hatched at the same stations in Kulleet Bay in 1983 through 1987. Contorted yolk-sac larvae occurred only in 1985, and post-yolk-sac larvae with missing or greatly reduced jaws also occurred only in 1985. The same sampling equipment and methods were used in all years, so the larval abnormalities seen in 1985 cannot be attributed to sampling artifact. McGurk (1985a) found that the effects of towing on the morphometry of Pacific herring larvae were independent of the starved or fed condition of the larvae; therefore, we assume that sampling affected all larvae in our study similarly.

The proportions of abnormal larvae reported here from samples in the upper 5 m of the water column may not represent proportions in the whole population for at least two reasons: the abundance of the abnormal larvae below 5 m is not known, and tow speeds of 0.5–0.8 m/s could have permitted healthy post-yolk-sac larvae to avoid the net, thereby increasing the apparent proportions of unhealthy larvae in the samples. Nevertheless, large numbers of deformed larvae were present in Kulleet Bay in 1985.

Laboratory studies have shown that extremes of temperature, salinity, dissolved oxygen, and ultraviolet radiation reduce the survival of, and induce abnormalities in, fish larvae (Alderdice and Velsen 1971; Hunter et al. 1979; Alderdice and Hourston 1985); additions of heavy metals, petroleum hydrocarbons, pesticides, and other toxic substances also have these effects (Rosenthal and Alderdice 1976; Laale 1981; Blaxter and Hunter 1982). Within certain limits of these factors, survival is high and abnormalities occur infrequently, but as any of these factors approach or reach tolerance limits, both mortality and the number of abnormal larvae increase (Alderdice and Forrester 1968; von Westernhagen 1970; Alderdice and Velsen 1971; reviewed in Blaxter and Hunter 1982; McQuinn et al. 1983; Alderdice and Hourston 1985). The most frequently observed deformities are to the trunk, tail, eye and mouth areas, and circulatory system. Deformities found in nature have been similar to those produced in laboratory experiments (Nankee 1981). Cause and effect relationships are lacking for larvae in situ, although abnormalities in older fish have been linked to pollution (Matsusato 1973; Mehrle et al. 1982).

TABLE 5.—Daytime feeding of post-yolk-sac Pacific herring larvae in Kulleet Bay. The numbers of larvae examined are in parentheses.

| Date (1985) | Healthy larvae with food: % (*N*) | Abnormal larvae with food: % (*N*) |
|---|---|---|
| Apr 14 | 2.9 (68) | 0 (44) |
| Apr 15 | 10.2 (49) | 0 (102) |
| Apr 16 | 12.5 (128) | 0 (45) |
| Apr 17 | 23.2 (95) | 0 (27) |
| Apr 18 | 29.4 (153) | 0 (35) |
| Apr 19 | 46.2 (253) | 0 (24) |
| Apr 20 | 48.0 (202) | 0 (13) |
| Apr 21 | 35.9 (192) | 0 (9) |

Other studies have implicated factors that could affect the hatching success of Pacific herring eggs. Tester (1942) reported high egg mortality (50–100%) for late herring spawns in late March and early April at several locations along the southeast coast of Vancouver Island. He proposed that high pH or low $CO_2$ or high $O_2$ concentrations in the water may have been the cause, but Kelly (1946) showed that Pacific herring eggs tolerate a wide range of pH and $CO_2$ at saturation $O_2$ levels. Multiple layers of spawn may reduce the oxygen supply to the lower layers and increase mortality (Galkina 1971; Taylor 1971; Alderdice and Hourston 1985). Galkina (1971) pictured abnormal larvae from multilayered spawns that resembled the contorted larvae from Kulleet Bay. However, intertidal spawns were only one or two layers thick in Kulleet Bay in 1983–1987 (Purcell and Grosse, personal observations), and Hourston et al. (1984) found that viability of newly hatched larvae was

not related to spawn thicknesses found naturally in British Columbia.

Alderdice and Velsen (1971) found reduced viability of the hatch from continuously submerged Pacific herring eggs incubated at combinations of temperature and salinity above and below the normal ranges. In substantial numbers of newly hatched larvae, "the lower jaw was entirely absent at hatching or rudimentary in form" at combinations of low salinity (7.8‰) and low temperature (4.0°C), and at moderate salinity (25‰) at both low (4.0°C) and high (14.0°C) temperatures. The abnormalities observed in our study were similar to those described by Alderdice and Velsen.

We propose that the abnormalities in Pacific herring larvae seen in 1985 were induced by stresses on eggs resulting from unusually warm, sunny weather and especially low midday tides. Anthropogenic causes of the larval deformities are unlikely because there are only a few houses along the shores of Kulleet Bay.

The abnormal post-yolk-sac Pacific herring larvae from Kulleet Bay had severely underdeveloped lower jaws, and they contained no prey. These larvae had shallow body depths at the pectoral fins, characteristic of starving larvae (McGurk 1985b). McGurk (1985b) found that head width was most indicative of starving hatchery-reared larvae, but this was not a distinguishing character of deformed wild larvae in our discriminant analysis. This difference may be due to the difficulties of comparing wild and reared larvae (McGurk 1985a). The natural microzooplankton prey of the larvae were dense enough in 1985, averaging 40.8/L (SD, 21.5: Purcell and Grover 1990) to promote good feeding by the larvae (e.g., Kiørboe et al. 1985). We believe that underdevelopment of the lower jaw impaired feeding, and caused many Pacific herring to starve to death in Kulleet Bay that year.



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