

Abnormalities in Larvae from the Once-Largest Pacific Herring Population in Washington State Result Primarily from Factors Independent of Spawning Location

P. K. HERSHBERGER* AND N. E. ELDER

Marrowstone Marine Station, U.S. Geological Survey, Biological Resources Discipline,
616 Marrowstone Point Road, Nordland, Washington 98358, USA

J. WITTOUCK

School of Aquatic and Fishery Sciences, University of Washington,
Box 355100, Seattle, Washington 98195, USA

K. STICK

Washington Department of Fish and Wildlife, Region 4, La Conner District Office,
Post Office Box 1100, La Conner, Washington 98257, USA

R. M. KOCAN

School of Aquatic and Fishery Sciences, University of Washington,
Box 355100, Seattle, Washington 98195, USA

Abstract.—Among larvae from populations of Pacific herring *Clupea pallasii* in Washington State, those from Cherry Point have consistently demonstrated abnormalities indicative of distress, including low weights and lengths at hatch, increased prevalences of skeletal abnormalities, and shorter survival times in food deprivation studies. The biomass of adult, prespawm Pacific herring at Cherry Point declined from 13,606 metric tons in 1973 to a record low 733 metric tons in 2000. However, correlation of larval abnormalities with adult recruitment was weak, indicating that the larval abnormalities did not directly cause the decline. Larval abnormalities originated primarily from factors independent of conditions at the spawning location because they were not reproduced by incubation of foreign zygotes along the Cherry Point shoreline but were reproduced after the development of indigenous zygotes in controlled laboratory conditions. Although the precise cause of the abnormalities was not determined, recent zoographic trends in elevated natural mortality among adult Pacific herring and resulting reduced age structures may be involved.

In Washington State, 18 sympatric populations of Pacific herring *Clupea pallasii* maintain temporal and geographical separation in their spawning patterns (Bargmann 1998). Spawning for all but one of these populations occurs from January through the beginning of April, with the peak occurring in February and March. Spawn timing at Cherry Point, a stretch of shoreline in northwest Washington that supports the once-largest population of Pacific herring in the state, occurs from mid-May through the beginning of June, markedly later than the other sympatric populations (Lemberg et al. 1997). Pacific herring spawning events are initiated by males, often releasing enough milk to result in water discoloration, or “white water.” Females spawn very quickly afterwards, deposit-

ing eggs that adhere to subtidal vegetation (Hay 1985). After fertilization, eggs incubate 10–21 d prior to hatch (Hourston and Haegele 1980). Newly hatched larvae subsist on endogenous yolk for 5–6 d until they switch to exogenous feeding; metamorphosis from larvae to juveniles occurs after 2–3 months (Hay 1985). Juveniles continue feeding, increasing body mass and condition until they recruit as iteroparous adults after 2–3 years.

For management purposes, each spawning population in Washington State is classified as a distinct “stock”; hereafter, each stock in Washington will be referred to as a “spawning population” due to the current lack of published genetic differentiation studies. State fishery managers currently classify most Pacific herring spawning populations as “healthy” or “moderately healthy,” based primarily on recent abundance estimates compared with long-term mean abundance data. However, at Cherry Point, the biomass of spawn-

* Corresponding author: phershberger@usgs.gov

Received December 23, 2003; accepted September 9, 2004
Published online March 29, 2005

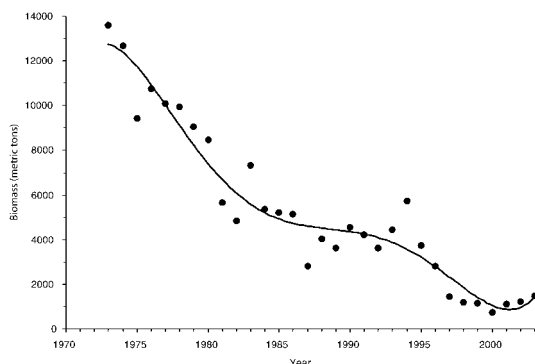


FIGURE 1.—Biomass of prespawning Pacific herring at Cherry Point from 1973 to present (data from Washington Department of Fish and Wildlife). Biomass estimates were determined from a combination of hydroacoustic and egg deposition surveys.

ing Pacific herring declined from 13,606 metric tons in 1973 to a record low 733 metric tons in 2000 (Figure 1). Consequently, the status of this spawning population is currently classified as “critical” by state forage fish managers (Lemberg et al. 1997).

The cause(s) of the decline in the Pacific herring spawning population at Cherry Point remain undetermined, but overfishing was not likely a primary factor. From 1981 (when spawning biomass was estimated at 5,642 metric tons) through 1996 (when spawning biomass was estimated at 2,800 metric tons), annual fishery landings at Cherry Point were negligible, averaging 159 metric tons. Unlike with the overexploitation of New Zealand snapper *Pagrus auratus*, which resulted in census populations below the effective population size (Hauser et al. 2002), it is unlikely that Pacific herring biomass at Cherry Point was below the effective population size (Ferguson and Danzmann 1998) when commercial harvests ceased in 1996 because the biomass of Pacific herring in many other Washington spawning populations has remained relatively stable at levels below 2,800 metric tons (Washington Department of Fish and Wildlife, unpublished data).

Industrial activities along the Cherry Point shoreline, including petroleum offloading and processing and aluminum smelting, represent possible sources of environmental contaminants. Early life history stages of Pacific herring are extremely sensitive to polynuclear aromatic hydrocarbons, with exposure of eggs to aqueous concentrations as low as 0.7 $\mu\text{g/L}$ resulting in larval malformations, genetic damage, mortality, decreased size, and in-

hibited swimming performance (Carls et al. 1999). Timing of contaminant exposure relative to developmental stage of embryos is critical because the first few cleavage stages of a fertilized egg are generally the most likely to be killed by toxic substances, and the predifferentiated developmental stages are most sensitive to teratogenesis (McKim 1985). Further, exposure of crude oil-challenged Pacific herring larvae to brief periods of sunlight results in significantly elevated toxicity compared with those exposed to control lighting (Barron et al. 2003).

These studies were conducted to determine the survival potential of larval Pacific herring from Cherry Point compared with that of other spawning populations and whether the observed larval abnormalities are caused by embryonic exposure to adverse environmental conditions at Cherry Point.

Methods

Collections of wild spawn.—To compare the survival potential of larval Pacific herring from spawning populations in Washington State, metrics of larval health (including hatch weights, skeletal abnormalities, and yolk deficiencies) were quantified. Naturally spawned Pacific herring eggs adhering to submerged vegetation were raked from subtidal sites at known spawning locations during 1999–2002 (Table 1; Figure 2); each collection consisted of roughly thousands to tens of thousands of fertilized eggs. Eggs and associated vegetation were transported to the Marrowstone Marine Station and incubated in flow-through, 265-L tanks supplied with sand-filtered, ultraviolet (UV)-treated ambient seawater. Egg incubation temperatures ranged from 7.9°C to 9.5°C (non-Cherry Point eggs) and 8.8–12.0°C (Cherry Point eggs) in 2000, and from 7.8°C to 9.3°C (non-Cherry Point eggs) and 9.5–10.6°C (Cherry Point eggs) in 2001; ambient water temperatures were not recorded during 1999 or 2002, but water temperatures were likely warmer for Cherry Point eggs due to the seasonally delayed spawn timing (Table 1). On the day of larval hatch and 5–7 d posthatch, subsamples of larvae from each collection were euthanatized in tricaine methanesulfonate (MS-222) and preserved in 5% formalin.

For analytical purposes, multiple collections of eggs from the same spawning population were combined, and metrics of larval health were compared between spawning populations. Replicate pools of approximately 20 newly hatched larvae from each collection location were dried at 70°C

TABLE 1.—Dates and locations where naturally spawned Pacific herring eggs were collected from 1999 to 2002. For analytical purposes, multiple collections of eggs from the same spawning population were combined. Temperatures were not recorded from egg collections marked “ND.”

Year	Spawning population	Egg collection date	Number of sites sampled	Ambient incubation temperature (°C)	
1999	Holmes Harbor	Apr 21	2	ND	
	Cherry Point	May 25	1	ND	
2000	Fidalgo Bay	Feb 15	1	ND	
		Mar 1	1	7.9–8.5	
	Portage Bay	Mar 3	1	7.9–8.5	
	Semiahmoo Bay	Mar 6	1	8.3–8.5	
	Port Gamble Bay	Mar 9	1	8.3–8.5	
	Samish Bay	Mar 10	1	8.5–8.6	
	Discovery Bay	Mar 15	1	8.6–8.8	
	Qulicene Bay	Mar 16	1	8.6–8.8	
	Holmes Harbor	Apr 4	2	8.6–9.2	
	Cherry Point	May 4	1	9.8–11.7	
		May 15	2	ND	
	2001	Wollochet Bay	Feb 2	1	7.7–7.8
			Feb 9	1	7.9–8.1
		Semiahmoo Bay	Feb 20	1	7.8–8.4
Feb 14			1	7.8–8.4	
Portage Bay		Feb 21	1	7.9–8.4	
Qulicene Bay		Mar 1	1	7.8–8.6	
Port Gamble Bay		Mar 1	1	7.8–8.6	
		Mar 15	1	8.1–8.4	
Cherry Point		May 3	1	9.5–10.5	
		May 5	1	9.5–10.5	
		May 11	1	9.5–10.5	
2002	Similk Bay	Feb 11	2	ND	
		Feb 13	1	ND	
	Port Gamble Bay	Feb 27	1	ND	
		Mar 28	1	ND	
		Feb 15	1	ND	
	Portage Bay	Feb 15	1	ND	
	Kilisu Harbor	Feb 27	1	ND	
	Semiahmoo Bay	Mar 18	1	ND	
	Qulicene Bay	Mar 27	1	ND	
	Discovery Bay	Apr 3	1	ND	
	Holmes Harbor	Apr 3	1	ND	
	Cherry Point	May 7	1	ND	
		May 21	1	ND	
		Jun 2	1	ND	

for 17 h and weighed on an analytical balance. Total length, to the nearest 0.01 mm, was recorded with an electronic digital caliper for 30 to 90 newly hatched larvae from each spawning population. Prevalence of skeletal abnormalities from each spawning population was determined by examining larvae (5–7 d posthatch) for spinal curvatures (10× magnification); larvae were recorded as abnormal when spinal curvature exceeded 90° from either the dorsal–ventral or lateral axis. The prevalence of larvae with no yolk was determined by examining newly hatched larvae from each egg collection site.

Prevalences of skeletal abnormalities and yolk deficiencies were compared by a chi-square (χ^2) test followed by Tukey’s test for multiple comparisons. Unless otherwise noted, larval lengths

and dry weights were statistically compared using analysis of variance (ANOVA), followed by Tukey’s test when applicable. Statistical significance was assigned to comparisons with $P \leq 0.05$.

Survival potentials of larval Pacific herring from Washington spawning populations were compared by determining the mean day to death among food-deprived larvae during 2001 and 2002. From each egg collection location, replicate 38-L aquaria were each loaded with approximately 100 newly hatched larvae and supplied with flow-through seawater (double sand filtered, particle filtered to 5 μm , and UV treated). Food was withheld from larvae in the aquaria; larval mortalities in each replicate aquarium were counted and removed daily.

In situ egg exposures.—To determine whether



FIGURE 2.—Map of Puget Sound and the Strait of Georgia, depicting locations where naturally spawned Pacific herring eggs were collected from 1999 to 2002.

larval abnormalities were caused by embryonic exposure to adverse environmental conditions at Cherry Point, two in situ Pacific herring egg exposures were conducted along the spawning shoreline (1999 and 2002). As a source of Pacific herring gametes for the respective exposures, sexually mature Pacific herring were captured by gill net at Cherry Point (May 14, 1999) and Port Gamble (March 13, 2002). Mature gametes from eight females and five males (1999) or 20 females and 12 males (2002) were pooled in separate containers. For both exposures, several hundred pooled eggs were evenly distributed over 10-cm² nylon screens (swatches) and fertilized with pooled milt for 1 h. To deter predators, fertilized egg swatches were placed inside individual cassettes constructed of 15 cm lengths of 10.2-cm-diameter ABS plastic pipe capped with 0.32-cm nylon mesh. During 1999, five replicate egg cassettes were attached to anchor–float lines at each of six stations (CP 1–6) along the Cherry Point shoreline. During 2002, triplicate cassettes were attached to anchor–float lines at each of three positions along the Cherry Point (CP 1–3) and Kilisut Harbor (KH 1–3) shorelines. Egg incubation locations in Kilisut Harbor served as field reference sites for the 2002 exposure. During both exposure periods, cassettes were positioned approximately 9 ft from the bottom in 18 ft of water (mean low water). Incubation of the Pacific herring eggs at the in situ stations proceeded for 5 d (1999) or 12 d (2002), after which egg cassettes were retrieved from the field and transferred to the Marrowstone Marine Station. Each replicate swatch of eggs was then removed from the respective cassette, gently rinsed with clean seawater, and transferred into individual, flow-through, 38-L aquaria supplied with filtered, UV-sterilized seawater for hatching. During both exposures, laboratory controls consisting of nine replicate egg swatches and associated cassettes were returned to the Marrowstone Marine Station immediately after fertilization and incubated in flow through aquaria supplied with filtered, UV-treated seawater throughout their entire embryonic development. To confirm that spawning methods and egg exposure protocols did not compromise the health of larvae used in the in situ exposure studies, control larvae from the in situ studies were compared with naturally spawned larvae from the respective egg donor spawning populations (Cherry Point in 1999 and Port Gamble Bay in 2002).

Within 24 h of peak hatch, subsamples of larvae from each replicate were euthanatized with an overdose of MS-222 and preserved in 5% formalin

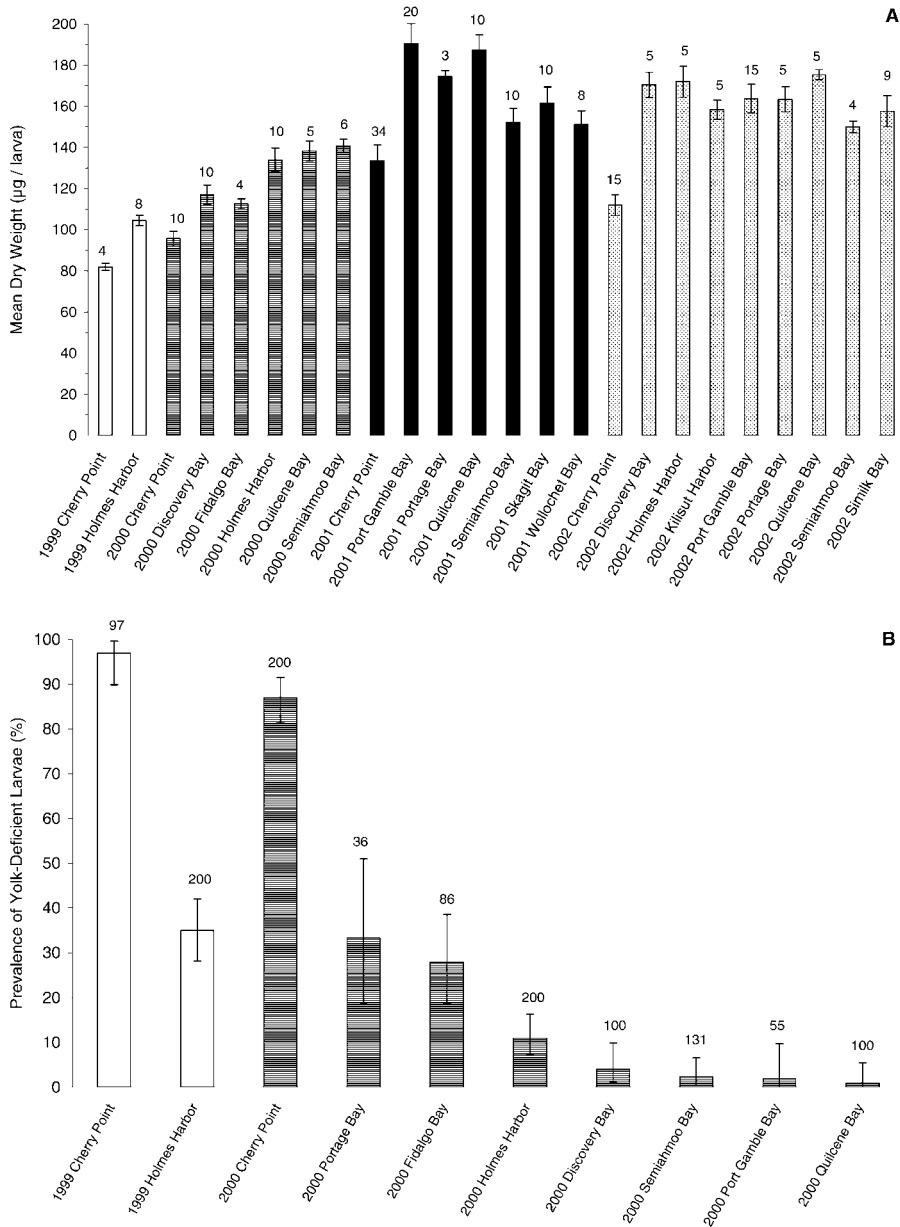


FIGURE 3.—Panel (A) shows the mean dry weight of newly hatched Pacific herring larvae from collections of naturally deposited eggs from 1999 to 2002. Error bars indicate SDs from the mean; the numerals above the bars indicate the numbers of 20-larvae pools used to determine the means. The mean weight of larvae from Port Gamble Bay (128.5 g) in 2000 is not displayed because only two replicates were weighed. Larvae from Cherry Point weighed significantly less than those from all other Washington stocks examined each year. Panel (B) shows the prevalence of yolk-deficient, newly hatched Pacific herring larvae from collections of naturally deposited eggs in 1999 and 2000. Error bars indicate 95% confidence intervals; the numerals above the bars indicate the numbers of larvae examined. Larval results from Samish Bay in 2000 (20%) are not displayed because of the small sample size ($n = 10$). The prevalences at Cherry Point were significantly greater than those at all other spawning locations examined each year.

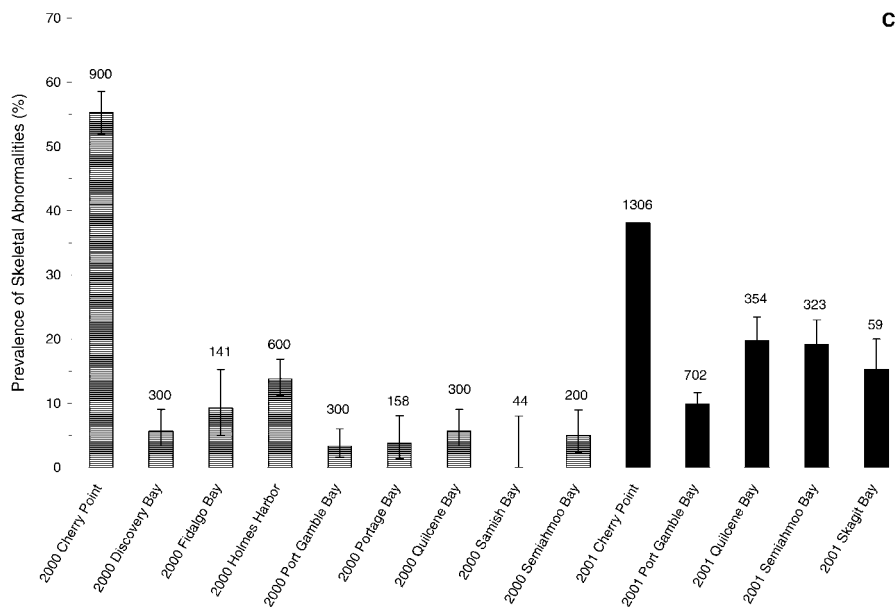


FIGURE 3 Continued.—Panel (C) shows the prevalence of gross skeletal abnormalities among larval Pacific herring from collections of naturally deposited eggs in 2000 and 2001. Error bars indicate 95% confidence intervals; the numerals above the bars indicate the numbers of larvae examined. The prevalences at Cherry Point were significantly greater than those at all other spawning locations examined each year.

for later evaluation. The remaining hatched larvae were similarly sampled after 5–7 d. Preserved larvae were analyzed for mean dry weight, mean total length (2002 only), and percent of larvae demonstrating gross skeletal abnormalities as described previously.

Prevalences of larval skeletal abnormalities from replicate aquaria were arcsine transformed and statistically compared using ANOVA, followed by Dunnett's test for multiple comparisons, when applicable. Larval dry weights (1999 and 2002) and lengths (2002) were statistically compared using ANOVA. Statistical significance was assigned to comparisons with $P \leq 0.05$.

Results

Collections of Wild Spawn

The mean dry weight of newly hatched larvae from Cherry Point in 1999 ($82.0 \mu\text{g}$) was significantly less (t -test: $P < 0.001$) and the prevalence demonstrating yolk deficiencies (97%) was significantly greater ($P < 0.01$) than those of larvae from Holmes Harbor ($104.5 \mu\text{m}$ and 35%; Figure 3A, B). Larval lengths and prevalences of gross skeletal abnormalities were not recorded, and food deprivation studies were not conducted in 1999.

Larvae from Cherry Point in 2000 demonstrated elevated prevalences of abnormalities compared

with those from all other sampled spawning populations. The mean dry weight of larvae from Cherry Point ($95.6 \mu\text{g}$) was significantly less ($P < 0.05$) than that of larvae from all other spawning locations, which ranged from $112.5 \mu\text{g}$ in Fidalgo Bay to $140.8 \mu\text{g}$ in Semiahmoo Bay (Figure 3A). Additionally, prevalences of yolk deficiencies (87%) and skeletal abnormalities (55%) among larvae from Cherry Point were significantly greater ($P < 0.001$) than those of larvae from all other spawning locations examined (1–33%, and 0–14%, respectively; Figure 3B, C). The mean total length of newly hatched larvae from Cherry Point (7.66 mm) was within the range of that from other Washington spawning populations (6.96–8.89 mm). Food deprivation studies were not conducted in 2000.

Larvae from Cherry Point demonstrated elevated prevalences of abnormalities again in 2001. The mean dry weight of larvae from Cherry Point ($133 \mu\text{g}$) was significantly less ($P < 0.001$) than that of larvae from all other spawning locations, which ranged from $151 \mu\text{g}$ in Wollochet Bay to $191 \mu\text{g}$ in Port Gamble Bay (Figure 3A). Prevalence of larvae demonstrating skeletal abnormalities was greatest among those from Cherry Point (38.1%); significantly fewer abnormalities ($P < 0.05$) occurred among larvae from Port Gamble Bay, Quilcene Bay,

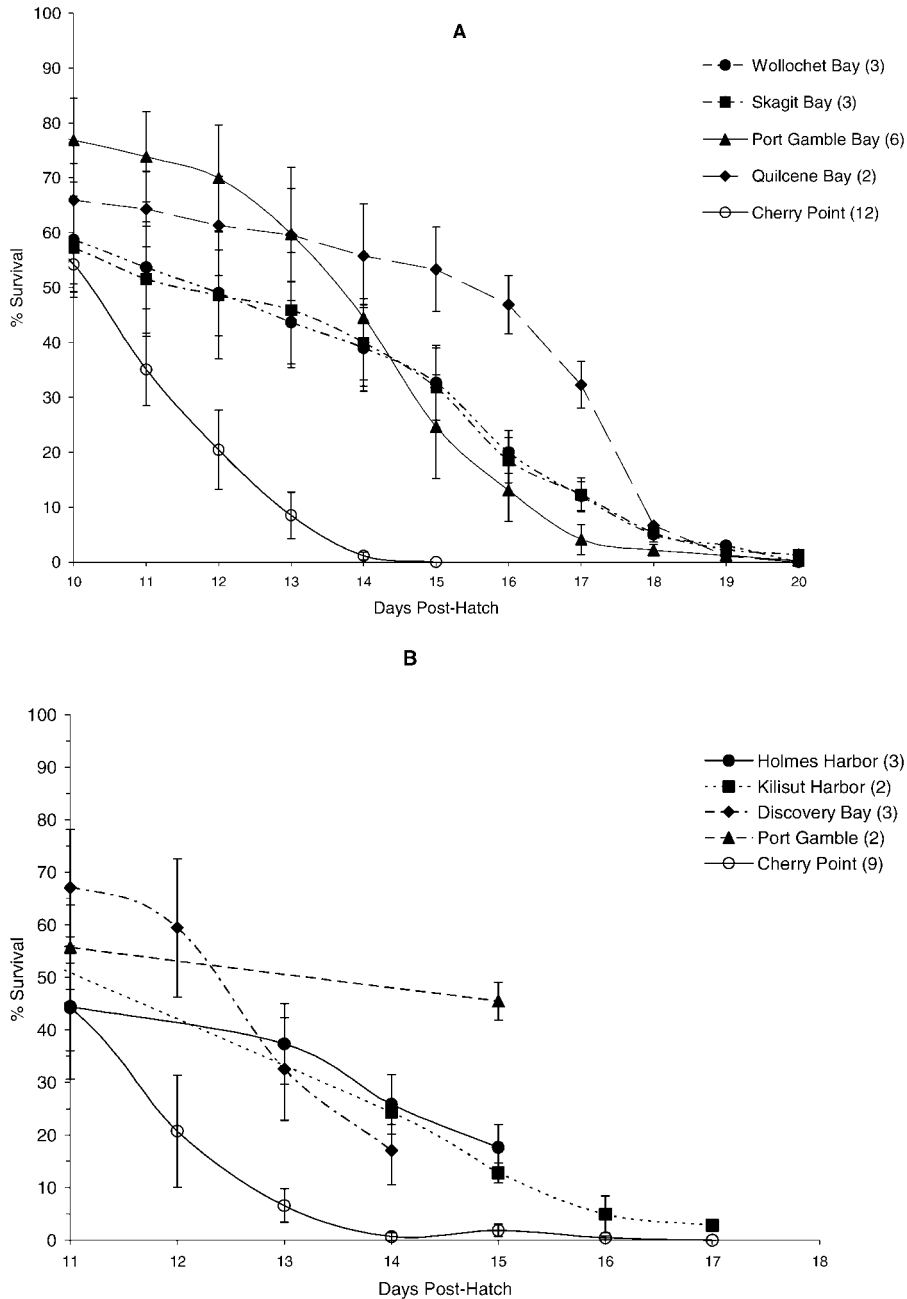


FIGURE 4.—Survival of food-deprived larvae from (A) 2001 and (B) 2002 Pacific herring spawning populations. Error bars indicate SDs from the mean. The numerals in the legend indicate the numbers of replicate aquaria, each initially containing approximately 100 larvae.

and Semiahmoo Bay (9.8–19.8%; Figure 3C). Although prevalence of skeletal abnormalities among larvae from Skagit Bay (15.3%) was within the range of larvae from other non-Cherry Point spawning populations (Figure 3C), statistical difference

from Cherry Point did not occur ($P < 0.10$), probably because of the small sample size ($n = 59$). In the absence of exogenous food, larvae from Cherry Point died sooner than did those from all other spawning locations in Washington (Figure 4A).

Among newly hatched larvae from Cherry Point, prevalence of yolk deficiencies (2%) and mean total length (7.29 mm) were within the ranges of larvae from other Washington spawning populations (0–19% and 6.89–7.86 mm).

Cherry Point larvae again demonstrated elevated prevalences of abnormalities in 2002. The mean dry weight of larvae from Cherry Point (112 μg) was significantly less ($P < 0.001$) than that of larvae from all other spawning locations, which ranged from 150 μg in Semiahmoo Bay to 175 μg in Quilcene Bay (Figure 3A). The mean total length of newly hatched larvae from Cherry Point (5.96 mm) was significantly less ($P < 0.01$) than that of larvae from all other spawning locations, which ranged from 6.39 mm in Quilcene Bay to 8.76 mm in Kilisut Harbor. In the absence of exogenous food, larvae from Cherry Point died sooner than did larvae from all other sampled locations (Figure 4B). However, prevalences of skeletal abnormalities (7%) and yolk deficiencies (3%) were within ranges of larvae from other sample locations (1–35% and 0–17%, respectively).

In Situ Egg Exposures

In situ egg exposures conducted during 1999 indicated that abnormal larvae at Cherry Point resulted primarily from factors independent of conditions at the spawning location. The mean dry weight at hatch among Cherry Point larvae that underwent early embryonic development in situ (73–80 $\mu\text{g}/\text{larva}$) was similar to that of laboratory control cohorts that underwent complete embryonic development in the laboratory (76 $\mu\text{g}/\text{larva}$; Figure 5A). Additionally, the mean dry weight of in situ larvae was similar to that of larvae from naturally spawned eggs collected from Cherry Point in 1999 (82 $\mu\text{g}/\text{larva}$) but significantly less ($P < 0.05$) than that of larvae from naturally spawned eggs at Holmes Harbor (104.5 $\mu\text{g}/\text{larva}$), the only non-Cherry Point spawning population of Pacific herring examined during 1999 (Figure 3A). However, elevated prevalences of skeletal abnormalities occurred among larvae that underwent early embryonic development in situ (24.4–46.6%) compared with laboratory control cohorts (23%; Figure 5B). Significant differences from controls ($P < 0.01$) occurred at the southern extreme of the study range (stations CP-1, CP-2, and CP-3).

In situ egg exposures conducted during 2002 supported the 1999 studies, indicating that smaller larvae from Cherry Point resulted from factors independent of conditions at the spawning location. The mean dry weight of larvae hatching from ar-

tificially fertilized Port Gamble Bay Pacific herring eggs that underwent early embryonic development at Cherry Point (173 μg) was similar to that of control cohorts which underwent complete embryonic development in the laboratory (169 $\mu\text{g}/\text{larva}$) and similar to that of cohorts that underwent early embryonic development in Kilisut Harbor (176 μg), the field reference site (Figure 6). Additionally, the mean dry weight of larvae from the in situ studies was similar to that of larvae from naturally deposited eggs at their natal spawning location (164 μg in Port Gamble Bay) but significantly greater ($P < 0.001$) than that of larvae from naturally deposited eggs at Cherry Point (112 μg , Figure 3A). The mean total length of larvae that underwent early embryonic development at Cherry Point (8.13–8.42 mm) was similar to that of control cohorts that underwent complete embryonic development in the laboratory (8.36 mm) and similar to that of cohorts that underwent early embryonic development in Kilisut Harbor (7.97–8.26 mm), the field reference site. Additionally, the mean total length of larvae from the in situ studies was similar to that of larvae from naturally deposited eggs at their natal spawning location (7.48 mm in Port Gamble Bay) but significantly greater ($P < 0.001$) than that of larvae from naturally deposited eggs at Cherry Point (5.96 mm). Further, the prevalence of skeletal abnormalities among larvae from laboratory control replicates (6.1%) was similar to that of cohorts that underwent early embryonic development at Cherry Point (8.7%) and Kilisut Harbor (6.4%).

Discussion

On an annual basis, larvae from Cherry Point consistently demonstrated abnormalities indicative of a distressed population, including low weights at hatch (1999–2002; Figure 3A), shorter lengths (2002), increased prevalences of skeletal abnormalities (2000–2001; Figure 3C), and shorter survival times in food deprivation studies (2001–2002; Figure 4). In situ egg exposures indicated that these abnormalities were not related to conditions at the spawning location (Figures 5, 6).

Although the specific cause of the larval abnormalities was not determined, no evidence exists that they resulted from vertical transfer of environmental contaminants from the parents. Among adult, prespawn Pacific herring at Cherry Point, levels of PCBs, which can cause lower length and weight of progeny (Black et al. 1988), and polycyclic aromatic hydrocarbon metabolites in bile were no higher than those detected in Pacific her-

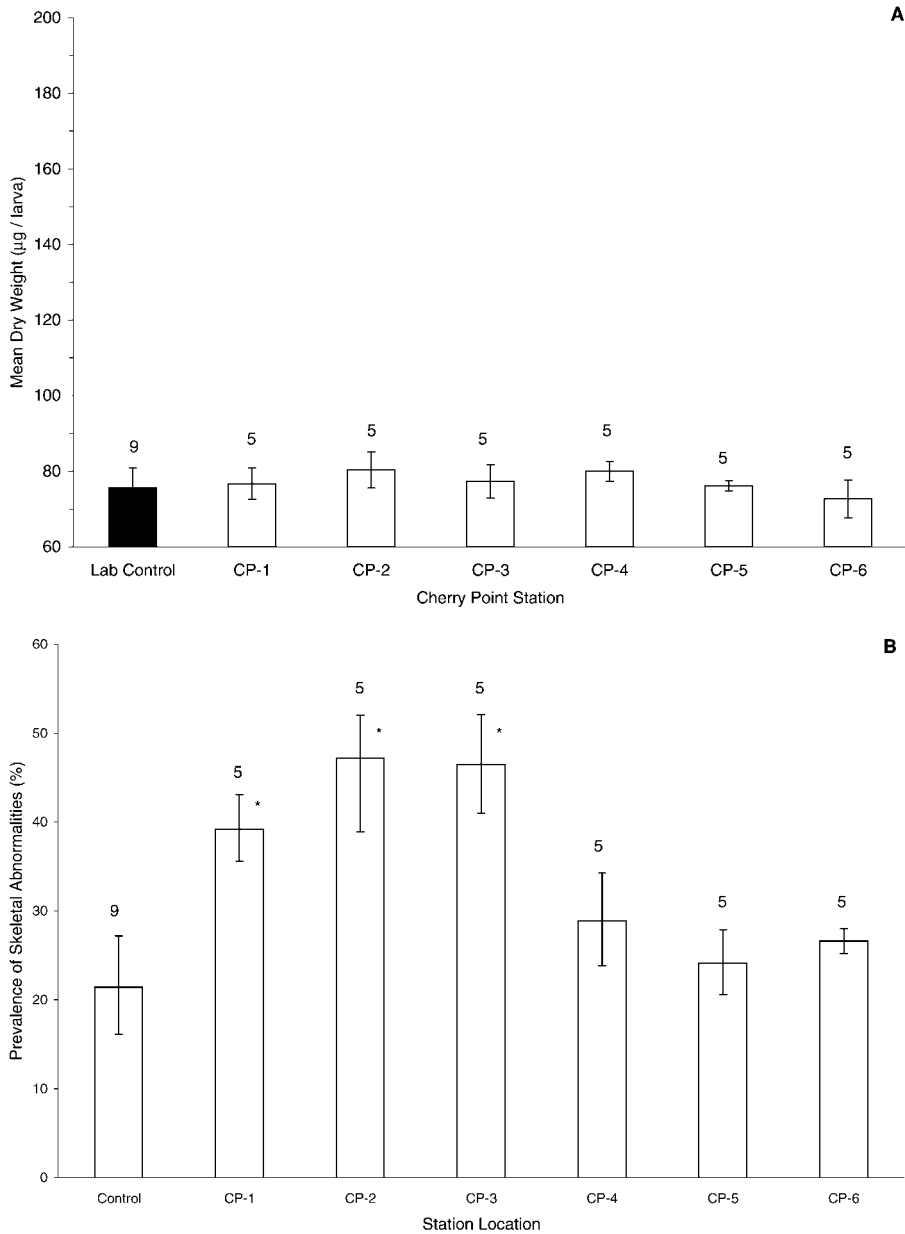


FIGURE 5.—Panel (A) shows the mean dry weight of newly hatched Pacific herring larvae that underwent early embryonic development at Cherry Point in 1999. Error bars indicate one SD from the mean; numerals above the bars indicate the number of 20-larvae pools used to determine the mean. Significant difference from laboratory controls did not occur at any stations. Panel (B) shows the prevalences of skeletal abnormalities among larvae from 1999 in situ egg exposure. Error bars indicate one SD from the mean; numerals indicate the prevalence of skeletal abnormalities detected among the 100 larvae hatching from each egg swatch. Significant differences from laboratory controls occurred at stations marked with an asterisk.

ring from other spawning populations in Washington (S. O’Neil, Washington Department of Fish and Wildlife, personal communication). Additionally, Pacific herring from Cherry Point are believed

to spend the majority of their adult lives foraging in open-water areas, removed from anthropogenic influences typically associated with contaminant sources, and adults only venture near the indus-

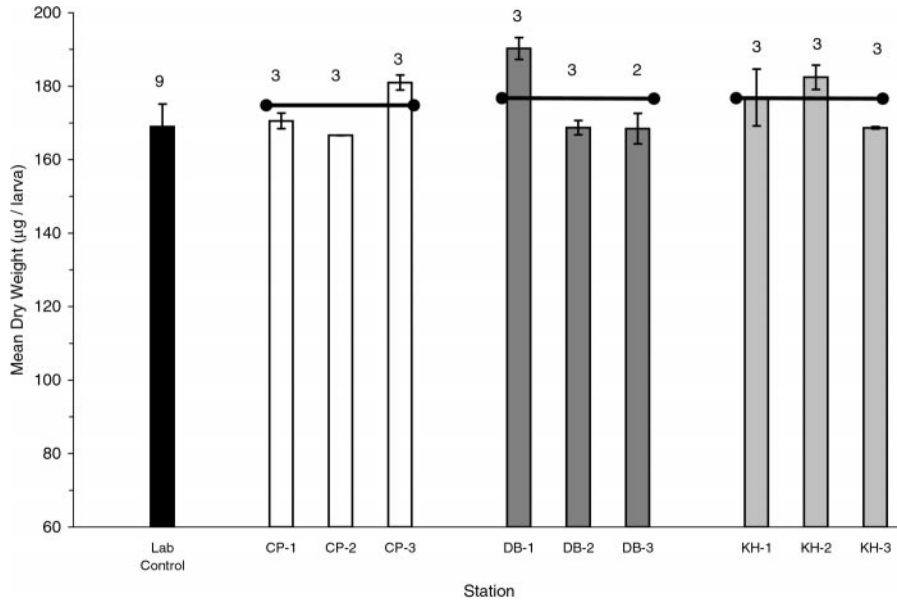


FIGURE 6.—Mean dry weight of newly hatched Pacific herring larvae from 2002 in situ egg exposures. Error bars indicate SDs from the mean; the numerals above the bars indicate the numbers of 20-larvae pools used to determine the means. The barbells represent the mean dry weight of larvae from all of the samples in the respective localities. Significant differences from laboratory controls did not occur at any station.

trialized shoreline for a few hours during the active spawning events.

Elevated prevalences of larval abnormalities at Cherry Point may be the result of recent trends in age structures of adult Pacific herring. The median age of Pacific herring populations in Washington decreased from age 5+ cohorts in the 1970s to age 2–3 (newly recruited) cohorts in recent years (unpublished scale annuli data from Washington Department of Fish and Wildlife). At Cherry Point in 2002, 49% of the spawning biomass consisted of age-2 cohorts. Among Pacific herring older spawning females tend to produce larger eggs and subsequently larger larvae than do younger, smaller adults (Hay 1985). Similarly, in rockfishes older females produce larvae that withstand starvation longer, grow faster, and are more likely to survive than larvae from younger parents (Berkeley et al. 2004a, 2004b; Palumbi 2004). Additionally, microsatellite DNA investigations indicate that Pacific herring from Cherry Point are genetically distinct from other spawning populations in Puget Sound and the Straight of Georgia (Small et al. 2004). Thus, it is possible that genetic separation at Cherry Point results in incomplete sexual maturation of age-2 “adults,” unlike age-2 cohorts from other Pacific herring spawning populations in Washington that likely produce healthy eggs.

This hypothesis is supported by temporally delayed spawn timing at Cherry Point (May), which is later than that of all other Pacific herring spawning populations in Washington (predominantly January–March) and similar to the spawn timing of Pacific herring populations in Alaska, which generally mature at age 3–5 (Hay 1985). Therefore, eggs from age-2 females at Cherry Point may not be completely mature, and their fertilization may result in the development of larvae with gross signs of developmental abnormalities that were reported in these studies. Furthermore, incomplete maturation of age-2 cohorts at Cherry Point can also account for the interannual variability in larval abnormalities (Figure 3), with variability likely resulting from the relative proportion of age-2 females contributing to the spawning population each year.

In addition to primary contributing factors originating independently of the spawning location, secondary site-related factors were responsible for some abnormalities. Elevated prevalences of skeletal abnormalities at the southern extreme of the study range during the 1999 in situ egg exposures were likely caused by suboptimal environmental conditions along the spawning shoreline. It is possible that these conditions originated from industrial activities along the Cherry Point shoreline

because the three southernmost stations were located on the south side of a petroleum off-loading pier and either side of a similar pier used for an aluminum smelting operation. Exposure of fertilized Pacific herring eggs to levels of petroleum hydrocarbons as low as 0.01 mg/L can cause low weight at hatch and DNA damage to developing Pacific herring embryos, while higher levels can result in physical defects and premature hatch to larvae (Kocan et al. 1996). Additionally, it is possible that elevated ambient water temperatures during the 1999 study were responsible for the skeletal abnormalities. The 1999 egg incubations were conducted during the natural spawning period at Cherry Point (May 14–19) when in situ temperatures ranged from 8.9°C to 11.0°C; the 2002 exposures were conducted 2 months earlier (March 13–25), when temperatures at Cherry Point ranged from 7.3°C to 7.5°C. Finally, it is possible that any local factors responsible for the occurrence of skeletal abnormalities during the 1999 exposure subsided in 2002. This hypothesis is supported by prevalences of skeletal abnormalities among larvae from naturally deposited Pacific herring eggs at Cherry Point that decreased steadily from 55.3% in 2000 to 7% in 2002. However, since the decline of the Pacific herring biomass at Cherry Point, the geographical range of egg deposition along the shoreline has retracted to a relatively small area located well north of this portion of the study range, and ecological significance of the experimentally elevated larval abnormalities are likely minimal.

Regardless of the cause(s) of the larval abnormalities, the resulting decreased survival potential did not likely cause the biomass decline in adult Pacific herring at Cherry Point; rather, we contend that they are an indication of a population in distress. All indications from spawning year 2000, including record low biomass of adult Pacific herring (733 metric tons) and elevated levels of larval abnormalities (significantly lower hatch weights, 87% demonstrating yolk deficiencies, and 55% demonstrating skeletal abnormalities), were that the larvae from 2000 would not recruit into the spawning population in 2002. However, the biomass of the spawning population at Cherry Point increased 65%, to 1,330 metric tons in 2002, with 49% of the spawning population consisting of age-2 cohorts from the unhealthy 2000 larval class.

Reasons for the decline in the spawning population at Cherry Point may be a reflection of increased natural mortalities among adult Pacific herring in Washington. The mean estimated annual

mortality (exclusive of commercial fishing) among adult Pacific herring in Washington increased from 20% in the late 1970s and early 1980s to 64–87% during 1996–1999 (Bargmann 1998). At Cherry Point, natural mortality has been constant at around 70% over the past several years. The elevated natural mortality among adult cohorts resulted in decreased median ages of adult Pacific herring, and currently most spawning populations in Washington are sustained on newly recruited, age-2 cohorts that die prior to spawning recurrently. Causes of the elevated adult mortality and resulting decreased median age of Pacific herring in Washington remain undetermined, but future studies are planned to determine whether gametes produced by the age-2 cohorts at Cherry Point are completely viable, or whether they develop into larvae with developmental abnormalities.

Acknowledgments

Pacific herring egg collection and transport were provided by Mark O'Toole, Pat McAllister, Darcy Wildermuth, Angela Harris, and Paul Clarke (Washington Department of Fish and Wildlife). Laboratory investigations were conducted at the Marrowstone Marine Station through the support of Jim Winton, Lyman Thorsteinson and Frank Shipley at the Western Fisheries Research Center (U.S. Geological Survey, Biological Resources Division). Access to remote field locations was facilitated by stakeholders at the Sandy Point Marina and Port Gamble-S'Klallam tribe. We thank Kerry Naish (University of Washington, School of Aquatic and Fishery Sciences) for enlightening discussions regarding possible genetic effects to depleted, wild populations. Funding was provided by the Washington State Department of Natural Resources (FY00-183), a Washington State Sea Grant (R/F-141), and the Marine Ecosystem Health Program (University of California-Davis).

References

- Bargmann, G. 1998. Forage fish management plan: a plan for managing the forage fish resources and fisheries of Washington. Washington Department of fish and Wildlife, Olympia.
- Barron, M. G., M. G. Carls, J. W. Short, and S. D. Rice. 2003. Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska north slope crude oil to Pacific herring eggs and larvae. *Environmental Toxicology and Chemistry* 22:650–660.
- Berkeley, S., C. Chapman, and S. Sogard. 2004a. Maternal age as a determinant of larval growth and survival in marine fish *Sebastes melanops*. *Ecology* 85:1258–1264.

- Berkeley, S. S., M. A. Hixon, R. J. Larson, and M. S. Love. 2004b. Fisheries sustainability via protection of age structure and spatial distribution of fish populations. *Fisheries* 29(8):23–32.
- Black, D. E., D. K. Phelps, and R. L. Lapan. 1988. The effect of inherited contamination on egg and larval winter flounder, *Pseudopleuronectes americanus*. *Marine Environmental Research* 25:45–62.
- Carls, M. G., S. D. Rice, and J. E. Hose. 1999. Sensitivity of fish embryos to weathered crude oil, part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasii*). *Environmental Toxicology and Chemistry* 18:481–493.
- Ferguson, M. M., and R. G. Danzmann. 1998. Genetic disorders. Pages 19–36 in J. F. Leatherland and P. T. K. Woo, editors. *Fish diseases and disorders*, volume 2. Noninfectious disorders. CABI Publishing, New York.
- Hauser, L., G. J. Adcock, P. J. Smith, J. H. Bernal Ramirez, and G. R. Carvalho. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences of the USA* 99:11742–11747.
- Hay, D. E. 1985. Reproductive biology of Pacific herring (*Clupea harengus pallasii*). *Canadian Journal of Fisheries and Aquatic Sciences* 42(Supplement 1): 111–126.
- Hourston, A. S., and C. W. Haegele. 1980. Herring on Canada's Pacific coast. *Canadian Special Publication Fisheries and Aquatic Sciences* 48.
- Kocan, R. M., J. E. Hose, E. D. Brown, and T. T. Baker. 1996. Pacific herring embryo sensitivity to Prudhoe Bay petroleum hydrocarbons: laboratory evaluation and in situ exposure at oiled and unoled sites in Prince William Sound. *Canadian Journal of Fisheries and Aquatic Sciences* 53:2366–2375.
- Lemberg, N. A., M. F. O'Toole, D. E. Pentilla, and K. C. Stick. 1997. Washington Department of Fish and Wildlife 1996 forage fish stock status report. Washington Department of Fish and Wildlife, Fisheries Management Division, Olympia.
- McKim, J. M. 1985. Early life stage toxicity tests. Pages 58–95 in G. M. Rand and S. R. Petrocelli, editors. *Fundamentals of aquatic toxicology*. Hemisphere, New York.
- Palumbi, S. R. 2004. Why mothers matter. *Nature* 430: 621–622.
- Small, M. P., J. Loxterman, and S. Young. 2004. A microsatellite DNA investigation of Pacific herring (*Clupea pallasii*) population structure in Puget Sound, Washington. Washington Department of Fish and Wildlife, Olympia.