Reproductive success of Pacific herring, *Clupea pallasi*, in Prince William Sound, Alaska, six years after the *Exxon Valdez* oil spill

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Abstract.—The *Exxon Valdez* oil spill occurred just prior to the spring migration of Pacific herring, *Clupea pallasi*, from offshore feeding grounds to nearshore spawning areas in Prince William Sound (PWS), Alaska. Most or all of the life stages of herring in PWS may have been exposed to oil after the March 1989 spill. Delayed impacts from the spill were suspected as one possible cause in the unprecedented crash of the adult herring population in 1993 and stimulated studies to assess reproductive success. In spring 1995, mature herring were collected from four sites in PWS and from three uncontaminated sites in southeast Alaska (SE) to determine if reproductive impairment was evident in PWS herring six years after the spill. Herring were artificially spawned and their eggs were reared in a laboratory until hatching. Observed response parameters included fertilization success, hatching times, hatching success, as well as larval viability, swimming ability, and spinal abnormalities. Responses of all year classes combined or those restricted to the same year class did not differ significantly between regions ($P>0.50$); the best and worst responses generally occurred in the SE. Within each site, response of the 1989 year class (most likely impacted by the oil spill in PWS) generally did not differ significantly from any other year class. To verify macroscopic observations, a subset of larvae from the 1989 year class was also inspected microscopically for yolk and pericardial abnormalities, and yolk volume was measured—but no significant regional differences were observed for any of these morphological categories. Based on the parameters examined in this study, evidence of reproductive impairment of Pacific herring in PWS by the spill was not detected in 1995, and the chances of detecting any oil-related effects against natural background variation appeared to be negligible.

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The *Exxon Valdez* oil spill (EVOS) in Prince William Sound (PWS), Alaska, occurred just a few weeks prior to the Pacific herring, *Clupea pallasi*, spawning season. Most or all of the life stages of herring in PWS may have been exposed to oil after the March 1989 spill. Biologically available hydrocarbons were present in the upper water column of PWS for several weeks following the spill (Short and Harris, 1996a), and residual oil may have persisted in some areas into 1990 (Short and Harris, 1996b). An estimated 40–50% of the egg biomass in PWS was deposited within the oil trajectory (Brown et al., 1996a). The failure of the 1989 year class to recruit to the fishery and the subsequent crash of the 1993 population (Meyers et al., 1994) suggested that the early life stages of herring were impacted either from exposure of prespawning adults or from direct exposure of eggs and larvae. Thus, as fish exposed to oil were recruiting into the fishery (20% by age 3, 80% by age 4, 100% by age 5; Funk[1]), the herring population crashed, and recovery was minimal through the 1996 season (Wilcock[2]). Genetic damage, physical deformities, and small size were reported for newly hatched larvae following the spill (Brown et al., 1996a; Hose et al., 1996; Norcross et al., 1996; Marty et al., in press), but long-term effects remain unknown. In a preliminary study in 1992, Kocan et al. (1996b) observed decreased reproductive success in herring from an oil-contaminated area in PWS compared with an uncontaminated area; results were inconclusive, however, because only two sites were compared. Delayed effects from the spill were suspected as one possible cause of the population decline and stimulated the

[1] Funk, F. In prep. Age-structured assessment of Pacific herring in Prince William Sound, Alaska and forecast of abundance for 1994. Regional Information Report, Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division, PO Box 25526, Juneau, AK 99802-5526.

[2] Wilcock, J. 1996. Alaska Department of Fish and Game. Commercial Fisheries Management and Development Division, PO Box 669, Cordova, AK 99574. Personal commun.

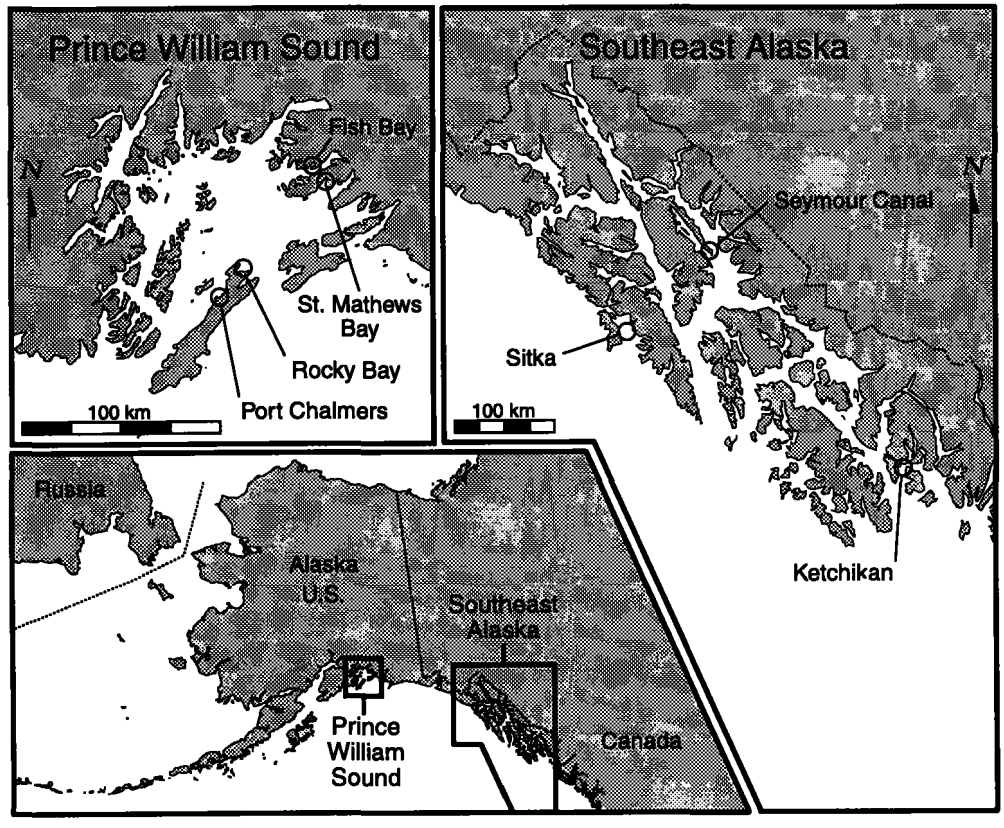

Figure 1

Collection sites of mature Pacific herring in Prince William Sound and southeast Alaska in spring 1995.

need for more definitive studies to assess the reproductive success of herring.

The purpose of this study was to determine if reproductive impairment, a possible result of the spill, was evident in PWS herring six years after the spill. There were two major focuses in the study: 1) a comparison of reproductive success between regions (PWS and southeast Alaska [SE]) and 2) a comparison of reproductive success between year classes within sites, particularly the 1989 year class (most likely impacted by the oil spill) with other year classes in PWS.

Sites sampled within PWS included all areas where spawning occurred in 1995; spawning was absent in areas that were heavily contaminated with oil in 1989. For example, Naked Island, which was in the middle of the spill trajectory, had 22 km of spawned eggs in 1989 (Brown et al., 1996a) but none in 1995. Although some have speculated that herring home to the same general spawning area each year (Zijlstra, 1963; Hourston, 1982), site fidelity is poorly understood. Thus, the herring we sampled in PWS in 1995 may or may not have been exposed to oil at some earlier time in their life history (as adults, eggs, or larvae).

Methods

Herring were collected at four sites in PWS and at three sites in SE (Fig. 1); all sites had been used for spawning in previous years. Two of the sites in PWS (St. Mathews Bay and Fish Bay) were not directly contaminated by the oil spill, whereas the other two sites (Port Chalmers and Rocky Bay) were at least lightly contaminated. Shortly after the spill, elevated hydrocarbon levels were detected in mussels at Rocky Bay (Brown et al., 1996b) and in seawater at Port Chalmers (Carls[3]). Additionally at Port Chalmers, concentrations of oil metabolites in bile of adult herring sampled in spring 1990 were similar to metabolite concentrations observed in 1989. This finding suggested continued contamination (Brown et al., 1996b). Herring were collected in St. Mathews Bay on 7 April, in Fish Bay on 14 April, at Port Chalmers on 30 April, and in Rocky Bay on 1 May 1995. In SE, herring were collected in waters off Sitka on 29–30 March, in waters near Ketchikan on 11 April, and in Seymour Canal on 13 May 1995.

[3] Carls, M. G. 1996. Prince William Sound oil database. Auke Bay Laboratory, National Marine Fisheries Service, NOAA, 11305 Glacier Hwy., Juneau, AK 99801.

Mature herring were captured during or just prior to spawning at all sites, sorted by size, and artificially spawned. Capture gear included gill net, cast net, and purse seine. Fish were chilled immediately after capture and transported within two hours to a field laboratory, except Seymour Canal fish, which were transported directly to Auke Bay Laboratory (ABL). To approximate the different age classes present, fish were sorted by sex and size (usually in 10-mm increments; e.g. 220–230 mm fork length). Six or more size classes were usually identified at each site. From each size class, 25 females were artificially spawned with males of the same size; generally 3 males contributed sperm for all 25 crosses. Size classes of fish were processed at random. Each fish was assigned an identification number, measured to the nearest mm (fork length), and weighed to the nearest 0.1 g (wet weight). To determine age, three scales were removed from the left side of each spawned fish near the posterior margin of the dorsal fin, placed on a glass slide, and covered with a second slide.

For spawning, testes were removed, sealed in a plastic bag and maintained in chilled seawater until use; ovarian membranes were cut longitudinally, and eggs were removed with a hydrocarbon-free stainless steel spatula similar to that used by Brown.[4] From each female, approximately 150 eggs were deposited with a gentle swirling motion onto a 25 × 75 mm glass slide placed on the bottom of a shallow plastic dish filled with seawater. Each slide was then placed in a staining rack and suspended in its own 1-L beaker of seawater. Milt was prepared from collected testes by cutting sections from each into small segments; segments plus a small amount of seawater were mixed with a spatula. A few milliliters of the milt mixture were added to each beaker containing eggs. Eggs and milt remained in contact for 5 min; the milt was then poured off, and the eggs were gently rinsed in seawater. Slides were kept in staining racks and maintained in ambient seawater with constant aeration until they were transported to ABL by air. To transport the eggs, staining racks were placed in plastic seawater-filled containers, which were then placed in coolers with blue ice.

Slides with eggs from each site were randomly distributed among twelve 600-L tanks with flow-through seawater. Slides were suspended from monofilament line attached to a pivoting overhead framework designed to cause slow egg movement (1 rpm) through the water. During the first 16–18 days of incubation, all slides were maintained in the seawater bath. A few days before hatching, each slide was isolated in a 1-L glass jar that contained seawater and that was surrounded by flowing seawater. Lighting was natural, supplemented by overhead fluorescent light during daylight hours. Seawater flow was approximately 1 L/min at 3.9°C, warming to 7.1°C with normal seasonal change. Salinity was 32 ±1 ppt.

Reproductive success of female herring was defined as the production of physically and functionally normal larvae. Key reproductive parameters included hatching success and larval viability, swimming ability, and spinal abnormalities. These four parameters were sensitive to oil in laboratory studies (Carls et al.[5]). Other parameters examined included fertility and hatching times. Fertility was not considered a key parameter because it may have been negatively influenced by unavoidable handling conditions at the different sites and by variable periods in the storing of gametes prior to spawning. Hatching times were not considered a key parameter because they were strongly influenced by seasonal increases in water temperature.

Fertilization success and stage of development were determined 1 to 10 days after spawning. Excess eggs were removed from all slides by scraping—i.e. those along slide margins susceptible to mechanical damage and clumps of eggs not directly exposed to water. This process was accomplished in water with minimal exposure to air.

Isolated eggs were inspected every two days to determine onset of hatching. Once hatching began, larvae were counted and assessed daily for swimming ability and gross physical deformities. Without exposing eggs to air, we changed the seawater in each jar every two days prior to hatching and daily after hatching began. All hatched larvae were collected, anesthetized with tricaine methanesulfonate, and preserved in 10% phosphate buffered formalin. Approximately the first and last 10% of larvae hatched from each female were preserved in separate bottles. Live larvae were preserved separately from dead larvae. After hatching was completed, remaining eggs were inspected; infertile eggs and dead embryos were counted.

A subset of preserved larvae was scored for yolk-sac edema, pericardial edema, and yolk volume. Ten females from the 1989 year class were randomly selected from each site, and 10 larvae per female were randomly selected from the central portion of hatched eggs for analysis. At Fish Bay, only five females from

[4] Brown, E. D. 1995. Alaska Department of Fish and Game. Commercial Fisheries Management and Development Division, PO Box 669, Cordova, AK 99574. Personal commun.

[5] Carls, M. G., D. M. Fremgen, J. E. Hose, S. W. Johnson, and S. D. Rice. In prep. Effects of incubating herring (*Clupea pallasi*) eggs in water contaminated with weathered crude oil. Auke Bay Laboratory, National Marine Fisheries Service, NOAA, 11305 Glacier Hwy., Juneau, AK 99801.

the 1989 year class were present; therefore the number of larvae analyzed per female was doubled. Sitka and St. Mathews Bay were excluded because of an insufficient number of females from the 1989 year class. Lateral views of larvae were displayed digitally, and specimens were rotated to align eyes in order to minimize variance. Yolk shapes were generally elliptical; major and minor axes were measured perpendicular to the body axis. Yolk volume was estimated from these linear measures according to the method of Hourston et al. (1984). Yolk-sac edema was indicated if the anterior margin of the yolk membrane was bounded by an area of clear fluid. Pericardial edema was scored if the pericardium was unusually large or convex ventrally.

Data processing and statistics

To assess the general health of parent fish, condition factor (K) was calculated for each female according to the method of Bagenal and Tesch (1978):

$$K = \frac{100(W)}{FL^b},$$

where W = somatic wet weight in g;
FL = fork length in cm; and
b = the value determined by site from length-weight regressions.

Gonad weight was substracted from body weight to avoid variation in spawning condition.

Times of hatching among sites, which were temperature dependent, were compared by using peak hatching times as the estimator. Peak hatching day was defined as the day the most larvae hatched from eggs of a given female; if two hatch peaks of equal magnitude occurred, the first peak was reported. Mean incubation temperature for eggs from each female was calculated by weighting mean water-bath temperatures by the number of eggs hatched daily. This method avoided possible under or over estimates of mean incubation temperature caused by early or late hatching of eggs as seasonal temperature increased.

Most observations were expressed as percentages. The denominator used to calculate percentages varied by response parameter (Table 1). Percentages of eggs fertile and initially dead were based on the total number of eggs counted near the beginning of the experiment. Percentages of eggs that hatched were based on the total number of hatched larvae plus the number of dead eggs determined at the endpoint. The number of hatched larvae was subdivided into number live, moribund, and dead. Hearts of moribund larvae were beating, but these larvae were incapable of movement. Accordingly, percent live was the number of living larvae (excluding moribund larvae) divided by the total number hatched. Swimming of live larvae was categorized as effective, ineffective, or incapable. Effective swimmers were active, frequented the water column, and avoided capture. Ineffective swimmers were generally more lethargic than effective swimmers and were more likely to be found on jar bottoms. Incapable swimmers were unable to swim in a straight line and were often capable only of spasmodic twitching. Swimming of moribund and dead larvae was, by definition, nonexistent; thus the number of live larvae was used as the denominator for swimming categories. Because larvae quickly became distorted after death, spinal aberrations were assessed only in live and moribund larvae. Percentage of spinal abnormalities, therefore, was the number of larvae with spinal aberrations divided by the sum of live and moribund larvae.

Table 1

Description of key response parameters used to evaluate reproductive impairment in Pacific herring collected from Prince William Sound and southeast Alaska. Herring were collected in 1995, artificially spawned, and reared in a laboratory until hatching. Moribund larvae were alive (heart beating) but incapable of swimming.

Parameter (%)	Description
Hatch	100 · (total number of eggs that hatched) /(total number of eggs that hatched + total number of dead eggs)
Live (viable)	100 · (total number of live larvae excluding moribund larvae) / (total number of eggs that hatched)
Effective swimmers	100 · (total number of effective swimmers) / (total number of live larvae excluding moribund larvae)
Spinal abnormalities	100 · (number of live + moribund larvae with spinal defects) / (total number of live + moribund larvae)

One-way analysis of variance (ANOVA) was used to examine differences among sites, among age classes, and between regions. Each reproductive parameter was tested separately by individual age class and for all age classes combined; percentage data were arc-sine transformed and corrected for small n as necessary (Snedecor and Cochran, 1980). To account for variance among sites, the F-test comparison between regions was:

$$F = \frac{MS_{between\ regions}}{MS_{among\ sites}}$$

where MS = mean square.

Somatic weight, FL, and K were analyzed similarly. Age-class responses within site were compared because in PWS different age classes were potentially exposed to varying levels of oil (Table 2). When the overall ANOVA was significant ($P \leq 0.05$), a priori multiple comparisons were used to identify which ages differed:

$$F = \frac{MS_{between\ age\ classes}}{MS_{error}}.$$

Maternal age was used as the standard in all age comparisons because ages frequently differed in the male and female crosses. Age-3 and age-4 herring were not exposed to oil at any life stage in PWS; therefore they were combined as site-specific controls. Few older age fish were captured; thus, ages ≥age 9 were combined and reported as 9+.

Because cold storage of adult fish (mean time of fish capture to mean spawning time) varied among sites (0.7–12.9 h), we also examined the possible effect of storage time on all reproductive parameters with storage time as a covariate in the ANOVA. Storage times up to 7 h did not significantly affect any of the key reproductive parameters ($P \geq 0.376$, except $P=0.084$ for % live larvae). We repeated the ANOVA for regional differences with storage times in the model as a covariate and restricted the analysis to include only those fish sampled within the same time period (≤7 h).

Scored yolk-sac edema was analyzed with the Kruskal-Wallis nonparametric test (SAS Institute Inc., 1989). Yolk-sac edema was also re-expressed as a percentage by female, arcsin-transformed, and analyzed by ANOVA. Yolk volume was analyzed by ANOVA.

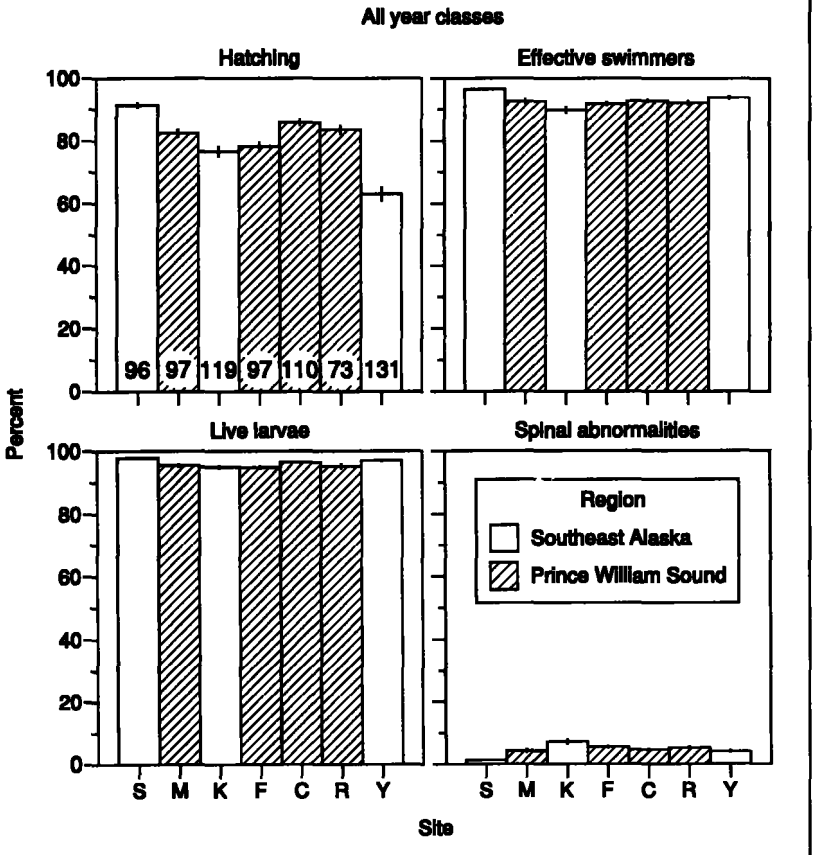

Figure 2

Mean (±SE) percent hatching, live, effective swimmers, and spinal abnormalities of larval Pacific herring by site and region in Alaska, 1995. S = Sitka, M = St. Mathews Bay, K = Ketchikan, F = Fish Bay, C = Port Chalmers, R = Rocky Bay, Y = Seymour Canal; sites in chronological order of spawning date. Sample size is shown in hatching graph. No significant differences existed between regions for any reproductive parameter ($P>0.50$).

Results

Regional comparison

Herring sampled from all sites appeared healthy and showed no obvious external signs of disease. For fish of the same age, there were no significant differences in FL ($P \geq 0.09$), weight ($P \geq 0.09$), or condition factor ($P \geq 0.41$) between regions. For herring in PWS, mean FL ranged from 196 to 260 mm and mean weight from 60.7 to 151.7 g, whereas in SE, mean FL ranged from 198 to 253 mm and mean weight from 65.1 to 140.4 g (Table 3).

For all age classes combined, reproductive success of herring did not differ significantly between regions ($P>0.50$); the best and worst responses generally occurred in SE, whereas PWS sites were intermediate (Fig. 2). Statistical power of these tests was high (≥0.99) and remained high for most analyses. Restricting the analysis to fish stored for ≤7 h did not alter the overall results; no significant ($P>0.39$) regional differences existed for any reproductive parameter. In SE, mean responses ranged from 63 to 91% for hatching success, 95 to 98% for live larvae, 90 to 96% for effective swimmers, and 1 to 7% for

Table 2

Age, year class, and possible oil exposure for Pacific herring collected in Prince William Sound, Alaska, in 1995. The *Exxon Valdez* oil spill occurred in March 1989.

Age	Year class	Possible oil exposure
3	1992	no direct oil exposure of any life stage
4	1991	no direct oil exposure of any life stage
5	1990	all life stages possibly exposed to residual oil
6	1989	all life stages likely exposed to oil
7	1988	juveniles at time of spill
8	1987	juveniles or immature at time of spill
9+	1986	mature—reproductive at time of spill

Table 3

Fork length (mm) and somatic weight (g) of mature female Pacific herring captured in southeast (SE) and Prince William Sound (PWS), Alaska, in spring 1995. Values are mean ($\bar{x}$) and ± standard error; sample size = n.

		Age (yr)								
		3	4	5	6	7	8	9	10	11
Fork length										
SE	$\bar{x}$	198	211	217	221	236	234	241	236	253
	±	1.9	2.9	2.0	1.2	1.3	3.5	8.4	3.5	7.7
	n	86	21	49	94	95	15	3	2	3
PWS	$\bar{x}$	196	219	225	236	242	260	259	259	260
	±	1.1	2.3	1.1	1.7	1.0	3.2	1.3	2.2	2.9
	n	81	18	65	25	149	10	16	7	13
Weight										
SE	$\bar{x}$	65.1	79.1	87.2	91.7	112.9	112.7	117.5	108.3	140.4
	±	1.8	3.2	2.1	1.7	1.9	5.1	4.6	6.4	14.7
	n	81	21	49	93	94	15	3	2	3
PWS	$\bar{x}$	60.7	90.3	95.0	107.4	121.0	149.0	147.8	148.9	151.7
	±	1.2	2.2	1.3	2.7	1.5	8.3	3.3	6.8	5.1
	n	80	18	65	25	149	10	16	7	13

spinal abnormalities. In PWS, mean responses ranged from 78 to 86% for hatching success, 95 to 96% for live larvae, 92 to 93% for effective swimmers, and 4 to 6% for spinal abnormalities. Among all sites, reproductive success was consistently best at Sitka (e.g. highest hatching success=91% and fewest spinal abnormalities=1%) and worst at Seymour Canal or Ketchikan (e.g. lowest hatching success=63% and most spinal abnormalities=7%) (Fig. 2). Of the sites in PWS, reproductive success was usually best at St. Mathews Bay or Port Chalmers (e.g. highest hatching success=86% and fewest spinal abnormalities=4%) and worst at Fish Bay (e.g. lowest hatching success=78% and most spinal abnormalities=6%) (Fig. 2). Similarly, when reproductive success was estimated for each age class individually, regional differences were not significant ($P>0.50$). This finding was true for all age comparisons—ages 3 to 9+. For example, age-6 (1989 year class) herring in PWS did not differ significantly from those in SE (Fig. 3). Among all sites where more than four age-6 fish were collected (excluding St. Mathews Bay and Sitka), hatching success ranged from 66% (Seymour Canal) to 91% (Port Chalmers), live larvae from 95% (Fish Bay) to 98% (Port Chalmers), effective swimmers from 93% (Rocky Bay) to 96% (Port Chalmers), and spinal abnormalities from 2% (Port Chalmers) to 6% (Fish Bay).

No significant regional differences were observed in progeny of the 1989 year class scored for physical condition. Only one larva of 500 had pericardial edema. Analyzed with the Kruskal-Wallis test, the site with the most yolk-sac edema (Port Chalmers) was significantly different from that with the least (Ketchikan), but there was no regional trend. Percentages of larvae with yolk-sac edema were low

(≤16%), and differences among sites and between regions were not significant ($P \geq 0.348$) (Fig. 4).

Yolk volume in larvae from the 1989 year class did not differ significantly ($P=0.486$) between regions but may have been related to incubation temperature. The largest and smallest mean yolk volumes were observed in PWS but closely overlapped those in SE (Fig. 4). Although scatter was high ($r^2=0.13$), yolk volumes declined significantly ($P<0.001$) as temperature increased. It is possible, however, that site differences and incubation temperature were confounding factors.

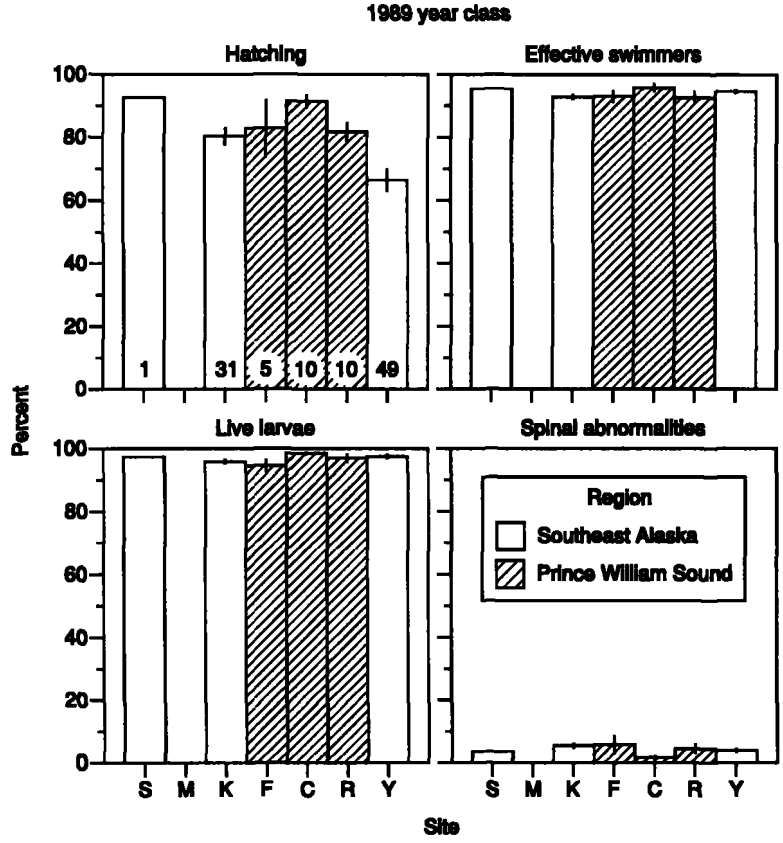

Figure 3

For the 1989 year class, mean (±SE) percent hatching, live, effective swimmers, and spinal abnormalities of larval Pacific herring by site and region in Alaska, 1995. The 1989 year class, sampled in Prince William Sound in 1995, was more likely exposed to oil as eggs or larvae than were other year classes. S = Sitka, M = St. Mathews Bay, K = Ketchikan, F = Fish Bay, C = Port Chalmers, R = Rocky Bay, Y = Seymour Canal. Sample size is shown in hatching graph. Progeny of the 1989 year class did not differ significantly between regions for any reproductive parameter ($P>0.50$).

Comparison among age classes within sites

Reproductive success differed significantly among some age classes at Sitka, Ketchikan, Port Chalmers, and Rocky Bay but not among age classes at St. Mathews Bay, Fish Bay, and Seymour Canal (Figs. 5–8). The few significant differences we observed were highly variable, inconsistent among sites, and no pattern existed for the 1989 year class. For example, at Rocky Bay, age-3 and age-4 fish had a significantly lower percentage of live larvae than age-5, age-6, and age-7 fish (Fig. 6), whereas at Sitka, age-3 and age-4 fish had a significantly higher percentage of effective swimmers and a significantly lower percentage of spinal abnormalities than age-7 fish (Figs. 7 and 8).

Other parameters

Hatching times decreased steadily with increasing incubation temperature (Fig. 9). For Sitka, the first site sampled, peak hatching occurred about 33 d after start of incubation at a mean temperature of about 4.5°C, whereas at Seymour Canal, the last site sampled, peak hatching occurred about 26 d after start of incubation at a mean temperature of about 6.0°C.

Fertility did not differ significantly ($P>0.50$) between regions for all ages combined or when the comparison was restricted to fish of the same age. For all ages combined, fertility in SE ranged from 80% at Seymour Canal to 96% at Sitka; in PWS, fertility ranged from 88% at Fish Bay to 94% at St. Mathews Bay.

Discussion

Six years after the spill, reproductive impairment was not detected in PWS herring. This conclusion was reached by comparing reproductive success of fish collected in PWS and SE and among age classes within specific sites. Specifically, hatching success, larval viability, and fertility did not differ significantly between PWS and SE, including response of the 1989 year class. In fact, discrimination of responses between regions was not possible because the best and worst responses were usually found in

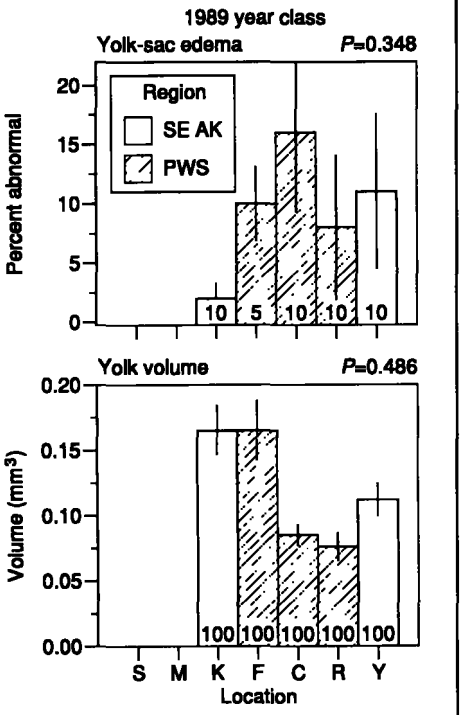

Figure 4

For the 1989 year class, mean (±SE) percent yolk-sac abnormalities (edema) and yolk volume for larval Pacific herring by site and region in Alaska, 1995. Sample size is shown in each bar. S = Sitka, M = St. Mathews Bay, K= Ketchikan, F = Fish Bay, C = Port Chalmers, R = Rocky Bay, Y = Seymour Canal. No significant differences existed between regions for either parameter ($P \geq 0.348$).

one region (SE). Therefore, the chances of detecting any oil-related effects against the natural background variation were negligible when herring were compared between regions. Although responses among some age classes within Port Chalmers and Rocky Bay were occasionally significant, these differences were highly variable, did not indicate reproductive impairment of the 1989 year class, and were inconsistent between sites.

Of the four key reproductive parameters we examined, spinal defects were particularly important because exposure of herring eggs to oil frequently causes spinal defects (Linden, 1978; Kocan et al., 1987; Rice et al., 1987; Pearson et al.[6]), that could result in reduced swimming ability and long-term survival. Spinal defects, however, can also occur naturally as a result of other environmental factors. In our study, herring from an uncontaminated site, Ketchikan, had the highest percentage of spinal defects (7%). Ketchikan samples were collected at least 40 km from any urban area, and it is unlikely that these fish were exposed to industrial or other urban pollutants. Whether the incidence of spinal defects at Ketchikan was just random noise or a response to some underlying environmental factor is impossible to determine, but it is evidence that similar results could occur in PWS without implicating oil as a cause. In fact, a 10% incidence of gross abnormalities was observed in PWS herring 23 years prior to the spill (Smith and Cameron, 1979).

Reproductive success of herring in PWS was consistently better in 1995 than that reported in earlier studies. For example, we observed a mean hatching success of 78–86% compared with 53% in 1976 (Smith and Cameron, 1979), 62%[7] in 1989 (McGurk et al.[8]), 85% in 1990 (McGurk et al.[9]), 59–79% in 1991 (Kocan et al., 1996a), and 19–56% in 1992 (Kocan et al., 1996b). The viable hatching[10] that we observed in PWS (79%) also exceeded previously reported percentages; 53%[11] in 1989 (McGurk et al.[8]), 57% in 1990 (McGurk et al.[9]), 35–37%[12] in 1991 (Kocan et al., 1996a), and 13–33%[12] in 1992 (Kocan et al., 1996b). Incidence of spinal abnormalities in PWS was about 5% in our study compared with 7% in 1989 (McGurk

[6] Pearson, W. H., D. L. Woodruff, S. L. Kiesser, G. W. Fellingham, and R. A. Elston. 1985. Oil effects on spawning behavior and reproduction in Pacific herring (*Clupea harengus pallasi*). Final Report OF-1742 to American Petroleum Inst., Battelle Marine Res. Lab., Sequim, WA, 108 p. [API publication 4412.]

[7] To avoid desiccation effects, and because egg survival was significantly less in the +1.5-m collections in the McGurk et al.[8] data set, these data were not included in this comparison. Estimated egg survival was 59% when the +1.5-m data were included.

[8] McGurk, M., D. Warburton, T. Parker, and M. Litke. 1990. Early life history of Pacific herring: 1989 Prince William Sound herring egg incubation experiment. Final report, contract number 50ABNC-7-00141, Triton Environmental Consultants LTD., No. 120-13511 Commerce Parkway, Richmond, British Columbia, Canada V6V 2L1.

[9] McGurk, M., T. Watson, D. Tesch, B. Mattock, and S. Northrup. 1991. Viable hatch of Pacific herring eggs from Prince William Sound and Sitka Sound, Alaska, in 1990. Report number 2060/WP 4269, Triton Environmental Consultants LTD., No. 120-13511 Commerce Parkway, Richmond, British Columbia, Canada V6V 2L1.

[10] To conform with McGurk et al.[8,9], % viable hatch was defined as 100 [(no. live larvae – no. abnormal larvae)/(no. hatched eggs)] × (no. eggs hatched/no. eggs total). The value defined by Kocan et al. (1996, a and b) as % viable larvae is nearly synonymous with % viable hatch. Our % live larvae (Table 1) included abnormal larvae, but McGurk et al.[8,9] excluded abnormal larvae in their definition of % viable larvae (% viable = 100 (no. live larvae – no. abnormal larvae)/no. hatched).

[11] As previously, +1.5-m data were not included; estimated % viable hatch was 50% when these data were included.

[12] Percent viable larvae values reported by Kocan et al. (1996, a and b) should be increased by 2% to approximate percent viable hatch.

et al.[8]). Although procedural differences between earlier studies and ours may partially account for differences in assessment of reproductive success, the responses we observed in 1995 were consistently the best.

To interpret the effects of the spill on herring in PWS, it is necessary to understand the life stage exposed and the magnitude and duration of exposure. Which life stages were impacted, and to what extent, however, is largely a matter of conjecture. Adult fish may have encountered oil before, during, or after spawning, but determining what percentage of the population was significantly impacted is impossible. Metabolites of aromatic hydrocarbons were detected in adult herring (Haynes et al.[13]), but sample sizes were very low. Nematode prevalence in adult body cavities differed significantly between contaminated and uncontaminated areas (Moles et al., 1993), also indicating adult exposure. The duration and magnitude of oil exposure of herring eggs and larvae is also unknown. After hatching, herring larvae from both contaminated and uncontaminated sites may have been exposed to oil as they passively traversed the spill trajectory. For example, some of the largest concentrations of larvae in June were found in the southwest portion of PWS, well within the oil trajectory (Norcross et al., 1996). By inference, juvenile herring occupying the same nearshore habitat used by juvenile salmonids may have also been exposed to oil: such exposure was documented in juvenile pink and chum salmon (Carls et al., 1996).

Response of wild herring to an oil spill can be partially inferred from laboratory studies. For example, exposure of mature herring to hydrocarbons in the laboratory did not cause discernible damage in progeny, including fertility, viability, and larval swimming, physical, and genetic abnormalities (Rice et al., 1987; Carls et al.[14]). In contrast, the early life

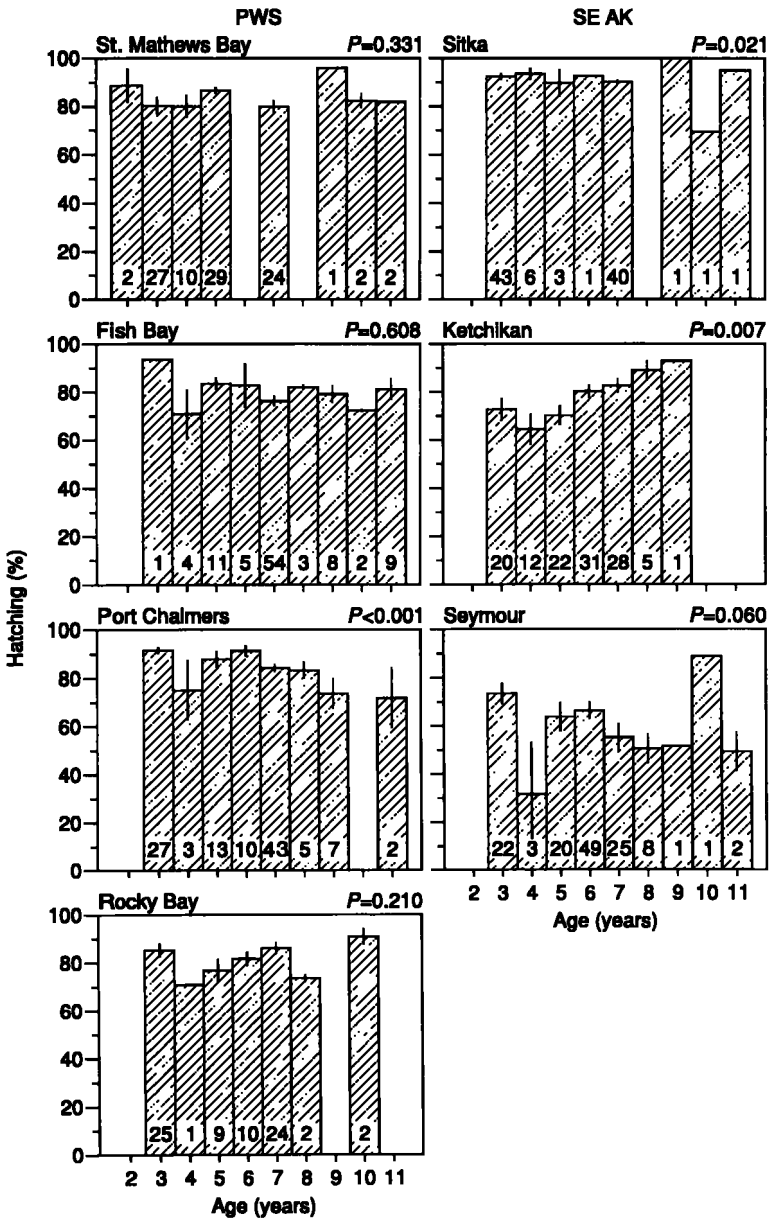

Figure 5

Mean (±SE) percent hatching of larval Pacific herring by female parent age, site, and region in Alaska, 1995. Sample size is shown in each bar. Overall *P*-value from ANOVA is listed above each graph. Significant differences were Ketchikan, ages 3 and 4 < age 6, 7, and 8 ($P \leq 0.015$); Port Chalmers, ages 3 and 4 > age 9+ ($P=0.050$).

[13] Haynes, E., T. Rutecki, M. Murphy, and D. Urban. 1995. Impacts of the *Exxon Valdez* oil spill on bottomfish and shellfish in Prince William Sound, *Exxon Valdez* oil spill state/federal natural resource damage assessment final report (fin/shellfish study no. 18). Auke Bay Laboratory, National Marine Fisheries Service, 11305 Glacier Hwy., Juneau, AK 99801.

[14] Carls, M. G., D. M. Fremgen, J. E. Hose, D. Love, and R. E. Thomas. 1995. The impact of exposure of adult pre-spawn herring (*Clupea harengus pallasi*) on subsequent progeny. Chapter 2 *in* Carls et al., *Exxon Valdez* oil spill report, restoration project 94166, annual report; the impact of exposure of adult pre-spawn herring (*Clupea harengus pallasi*) on subsequent progeny, p. 29–49. Auke Bay Laboratory, NMFS, NOAA, 11305 Glacier Hwy., Juneau, AK 99801.

stages of herring are more susceptible to the effects of oil according to laboratory (Linden, 1978; Pearson et al., 1985; Carls, 1987; Kocan et al., 1987; Rice et al., 1987) and field studies (Brown et al., 1996a; Norcross et al., 1996). Abnormal larvae have poor survival potential (Kocan et al., 1996a), and thus the exposure of eggs and larvae to oil in PWS may have resulted in increased mortality. Furthermore, the same oil concentrations that caused significant genetic damage also caused significant physical damage in developing embryos (Carls et al.[5]); thus early death would likely preclude recruitment of genetically damaged individuals to spawning populations.

Although genetic damage was detected in larvae collected in the oil-contaminated areas of PWS in 1989 (Hose et al., 1996; Brown et al., 1996a), we did not inspect larvae for genetic damage. Concomitant laboratory measurements of larvae that had been artificially contaminated indicated that genetic response was not a more sensitive measure of oil exposure than the parameters we examined (Carls et al.[5]). In addition, artificial exposure of prespawning adults to relatively high oil concentrations (58 ppb, initial PAH) did not cause genetic defects in artificially spawned progeny (Carls et al.[14]). Other defects observed in larvae from PWS in 1989 included physical damage, assessed by scored indices (Hose et al., 1996). Carls et al.[5] observed that two of these indices, pericardial edema and finfold condition, were more sensitive to oil damage than was the genetic response. Because we did not detect significant pericardial abnormalities in larvae from PWS six years after the spill, it is likely that the genetic condition of these larvae has not been adversely affected.

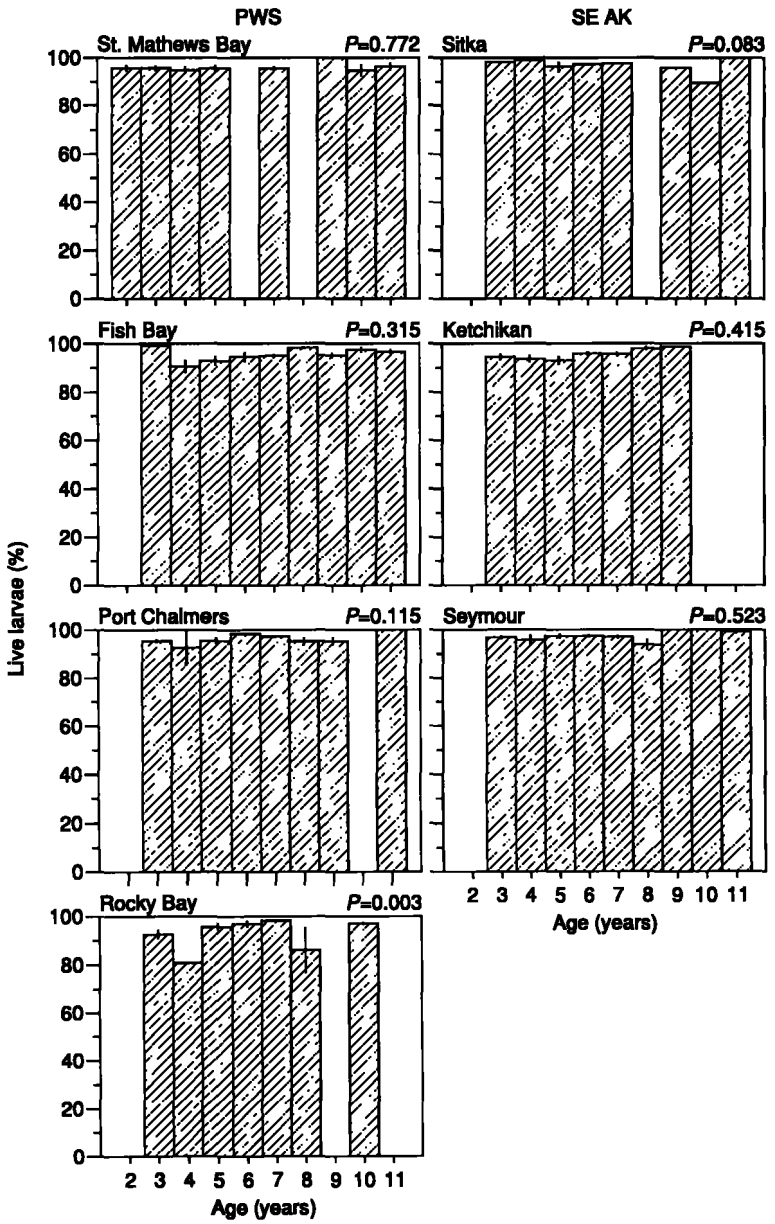

Figure 6

Mean (±SE) percent live larvae of Pacific herring by female parent age, site, and region in Alaska, 1995. Sample sizes are indicated in Figure 5. Overall *P*-value from ANOVA is listed above each graph. Significant differences were Rocky Bay, ages 3 and 4 < age 5, 6, and 7 ($P \leq 0.047$).

The failure of the 1989 year class of herring in PWS to recruit to the spawning population may have been partly attributable to the spill, but it is impossible to separate oil effects from other natural factors. At the sites we sampled in PWS, the 1989 year class usually represented <4.0% of the spawning population (ADF&G[15]). Larval survival in PWS was reduced an estimated 52% in 1989 as a result of the spill (Brown et al., 1996a); such loss supports inferences of poor survival that are based on laboratory obser-

[15] ADF&G (Alaska Department of Fish and Game). 1995. Herring test fishery data. Commercial Fisheries Management and Development Division, PO Box 669, Cordova, AK 99574.

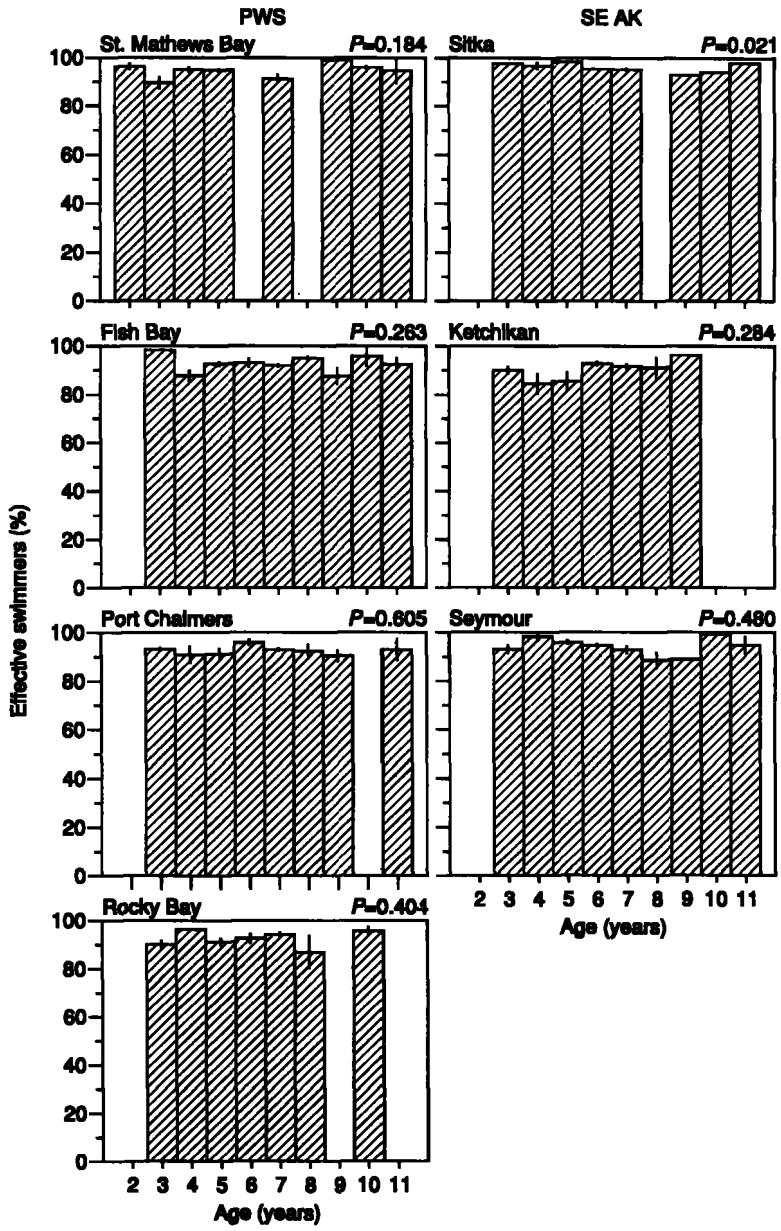

Figure 7

Mean (±SE) percent effective swimmers of larval Pacific herring by female parent age, site, and region in Alaska, 1995. Sample sizes are indicated in Figure 5. Overall *P*-value from ANOVA is listed above each graph. Significant differences were Sitka, ages 3 and 4 > age 7 (P=0.011).

vation. Natural environmental conditions, however, can also cause a high degree of variability in herring recruitment (Stevenson, 1962; Anthony and Fogarty, 1985). For example, the 1989 year class at Sitka also represented a small proportion of the spawning population in 1995 (<2%; ADF&G[16]); therefore factors other than oil are important determinants of cohort size.

Whether or not herring in PWS were ever reproductively impaired by the EVOS is unknown, but the time lapse between the spill and our study probably precluded any detection of reproductive impairment.

[16] ADF&G (Alaska Department of Fish and Game). 1995. Herring test fishery data. Commercial Fisheries Management and Development Division, 304 Lake St., Room 103, Sitka, AK 99835.

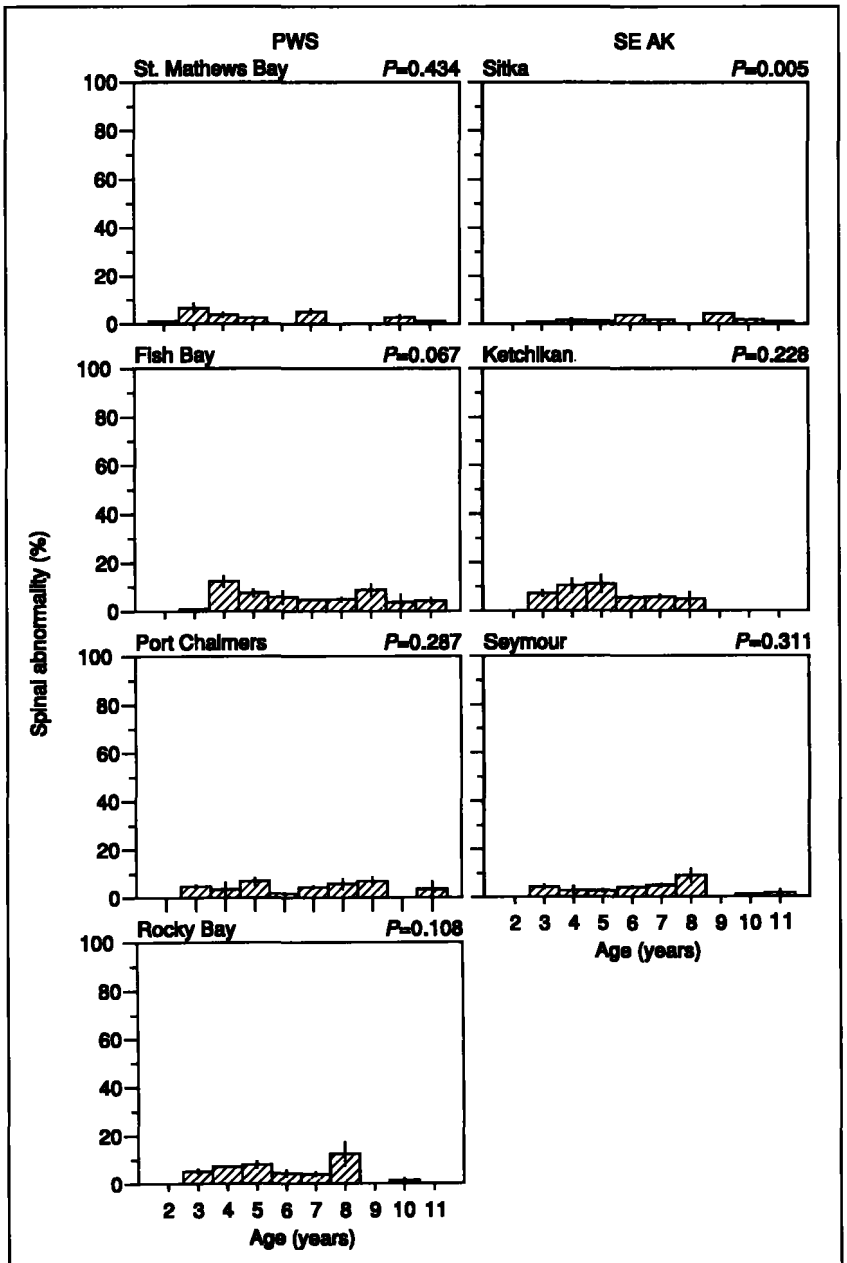

Figure 8

Mean (±SE) percent spinal abnormalities of larval Pacific herring by female parent age, site, and region in Alaska, 1995. Sample sizes are indicated in Figure 5. Overall *P*-value from ANOVA is listed above each graph. Significant differences were Sitka, ages 3 and 4 < age 7 (P=0.024).

Measurable effects likely declined most rapidly during the first year as the most adversely affected individuals died. Although oil-related abnormalities were observed in larvae immediately following the spill, both developmental and genetic damage progressively decreased with time (Brown et al., 1996a) and were undetectable in 1990 and 1991 (Hose et al., 1996). The extent of spawning-site fidelity in herring is poorly understood, but unaffected individuals from other geographic areas have probably joined remaining, less affected spawners, diluting possible residual effects. The disease epidemic observed in PWS in 1993 (Meyers et al., 1994) may have removed additional marginal spill survivors. Thus, it is not particularly surprising that reproductive impairment was not detected in 1995.

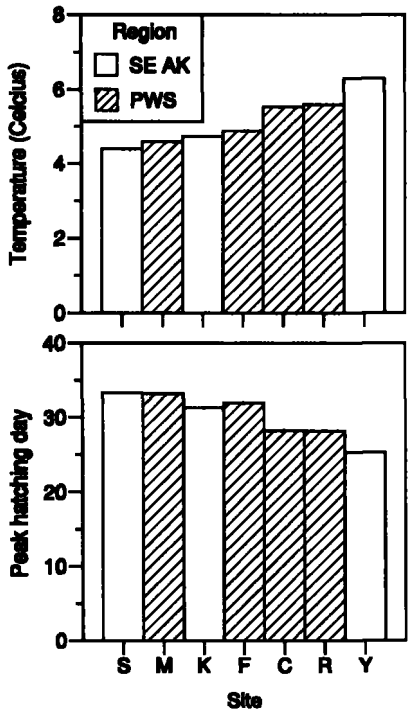

Figure 9

Peak hatching day and mean (±SE) incubation temperature (°C) for eggs spawned from mature Pacific herring by site and region in Alaska, 1995. S = Sitka, M = St. Mathews Bay, K = Ketchikan, F = Fish Bay, C = Port Chalmers, R = Rocky Bay, Y = Seymour Canal; sites are in chronological order of spawning date.

Understanding the long-term implications of exposure of Pacific herring to oil in PWS was the principal objective of this research. Regardless of the life stage of herring and the likelihood of possible oil exposure, herring we sampled in PWS in 1995 appeared to be reproductively fit and similar to herring in SE. Although herring stocks are still depressed in PWS, factors other than reproductive impairment are probably limiting recovery.

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