*Exxon Valdez* Oil Spill State/Federal Natural Resource Damage Assessment
Final Report

# Injury to Prince William Sound Herring Following the *Exxon Valdez* Oil Spill

Fish/Shellfish Study Number 11
Final Report

Evelyn D. Brown
Timothy T. Baker

Alaska Department of Fish and Game
Division of Commercial Fisheries Management and Development
P.O. Box 669
Cordova, Alaska 99574

December 1998

*Exxon Valdez* Oil Spill State/Federal Natural Resource Damage Assessment
Final Report

# Injury to Prince William Sound Herring Following the *Exxon Valdez* Oil Spill

Fish/Shellfish Study Number 11
Final Report

Evelyn D. Brown
Timothy T. Baker

Alaska Department of Fish and Game
Division of Commercial Fisheries Management and Development
P.O. Box 669
Cordova, Alaska 99574

December 1998

Injury to Prince William Sound Herring Following the *Exxon Valdez* Oil Spill

Fish/Shellfish Study Number 11
Final Report

**Study History:** Fish/Shellfish Study Number 11 was initiated as a State/Federal Natural Resource Damage Assessment project as part of the 1989 *Exxon Valdez* oil spill study plan and continued through the 1992 work plan. Most components of this report were published in October 1996 in the *Canadian Journal of Fisheries and Aquatic Sciences,* vol. 53(10), followed by one article published in August 1997 in the *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 54(8). Permission to reproduce these articles was granted by the publisher, National Research Council of Canada, Research Press.

**Abstract:** Studies were conducted in 1989 through 1991 to determine injury to herring in Prince William Sound following the *Exxon Valdez* oil spill. About half the herring eggs deposited in 1989 were exposed to *Exxon Valdez* oil. Survival to hatch was not impacted, but there were substantial sublethal effects (acceleration of embryo development, morphological deformities and cytogenetic abnormalities) in newly hatched larvae and reduced survival from hatch to free swimming. These correlated well with oil exposure in adjacent mussels and paralleled experimental laboratory observations. The relationship of oil to histopathological lesions in feeding larvae from oiled areas was supported by laboratory studies. An estimated 40 – 80 % of the 1989 year class was impacted by oil at toxic levels. Egg/larval mortality was twice as great in oiled areas and larval growth half that of unoiled areas. Adult reproductive tissue damage could not be tied to oil. Severe focal hepatocellular necrosis in 20% of adult herring examined from oiled areas (none in unoiled areas) was probably due to viral hemorrhagic septicemia induced by exposure of carrier fish to *Exxon Valdez* oil, and consistent with later laboratory studies. Polynuclear aromatic hydrocarbons, probably *Exxon Valdez* oil in origin, were detected in adult herring tissues.

**Keywords:** *Clupea pallasi*, *Exxon Valdez* oil spill, Pacific herring, Prince William Sound, viral hemorrhagic septicemia.

**Project Data:**

*Description of data* - Diver estimates of numbers of egg per quadrat for egg deposition and egg loss estimates, preserved (formalin) samples of eggs for laboratory evaluation of sublethal damage, frozen samples of whole mussels for hydrocarbon content analysis, samples of whole herring for length, weight, roe maturity and fecundity (egg) cts./female, preserved samples of herring tissue for histopathological analysis, frozen samples of herring tissue for hydrocarbon content, live eggs for shipment to the Vancouver Aquarium for artificial rearing and hatching, collections of live eggs for egg mortality estimates, electronic copies of ocean temperature data, samples of preserved herring larvae for histopathological work, growth estimates, and morphometric work, samples of live larvae for counts (density estimates), samples of herring stomachs for gross stomach content.
*Format* - Lotus spreadsheets (WK1 and WK2 files), R:BASE database files (*.rb! files), WordPerfect files (*.wp! files) and SAS output files (*.sas are basically ASCII text

output). *Custodian* - Stored on the network machine at ADFG, CFMD, Cordova in zipped files. *Availability* - Available in electronic form, all forms (disk, ftp or email).

**Citation:**

Brown, E.D., and T.T. Baker. 1998. Injury to Prince William Sound herring following the *Exxon Valdez* oil spill, *Exxon Valdez* State/Federal Natural Resource Damage Assessment Final Report (Fish/Shellfish Study Number 11), Alaska Department of Fish and Game, Division of Commercial Fisheries Management and Development, Cordova, Alaska.

## Table of Contents

# An introduction to studies on the effects of the *Exxon Valdez* oil spill on early life history stages of Pacific herring, *Clupea pallasi*, in Prince William Sound, Alaska

**Evelyn D. Brown, Brenda L. Norcross, and Jeffrey W. Short**

## Pacific herring and the oil spill in Prince William Sound

In late March and early April large numbers of adult Pacific herring (*Clupea pallasi*) annually migrate to spawn in nearshore areas of Prince William Sound (PWS) (Fig. 1). Spawning commences generally by mid-April and embryos incubate in natal areas for approximately 24 days (E.D. Brown, unpublished data). Hatching begins in May, and transported by local currents, the pelagic larval herring become part of the plankton biomass in PWS. Thus, it is during the spring when adult herring and their offspring are most vulnerable to predation, weather patterns, ocean conditions, and human activities (Blaxter et al. 1982).

On March 24, 1989, the tanker vessel *Exxon Valdez* spilled 42 000 000 L of Prudhoe Bay crude oil after grounding on Bligh Reef in the northeastern part of PWS (Fig. 1). The oil spill followed a trajectory that was predictable on the basis of local currents, winds, and the oceanography of PWS (Royer et al. 1990; Venkatesh 1990; Niebauer et al. 1994). The spill trajectory overlapped the route of adult herring spawning migration and spawning locations, thus creating a significant risk of exposure to adults and eggs. Potential oil contamination effected the closure of the commercial spring herring fisheries in 1989 (Brady et al. 1991).

On the basis of knowledge of oil effects, herring damage assessment studies were initiated. The objective of these studies was to determine the effects of the *Exxon Valdez* oil (EVO) spill on PWS herring by using both long-term (prespill) fisheries base-line data and detailed information collected from oiled and unoiled sites following the spill. The early life stages are the most sensitive to the toxic effects of oil (Rice et al. 1987*a*, 1987*b*) and therefore were emphasized in damage assessment studies. Critical processes such as growth and reproduction were targeted when possible in an attempt to link effects at the organismal level with population effects. However, one of the most challenging tasks for damage assessment was the establishment of the level of exposure at each of the life stages examined. To understand how oil spread through the PWS ecosystem and how herring were potentially exposed, it is important to understand the oceanography of the region.

PWS is a complex fjord-type estuary (Schmidt 1977) located on the northern margin of the Gulf of Alaska at 60°N, 146°W covering about 8800 $m^2$ and having 3200 km of shoreline (Grant and Higgens 1910) (Fig. 1). PWS acts as a small inland sea wide enough to have appreciable horizontal, cyclonic circulation (Niebauer et al. 1994). Large-scale surface currents vary seasonally, driven by regional winds and buoyancy forcing.

The annual cycles of temperature and salinity in PWS waters are determined by freshwater input, by seasonal shifts in large-scale weather patterns, and by interaction with the surface and deep water of the bordering open ocean (Niebauer et al. 1994). Active exchange of water occurs with water from the open Gulf of Alaska via the Alaska Coastal Current (ACC) entering through Hinchinbrook Entrance and transiting the sound counterclockwise before exiting through Montague Strait (Schmidt 1977) (Fig. 1). Following a transition in weather patterns in late April and May, a relaxation of coastal downwelling occurs that reduces or reverses the flow through Hinchinbrook Entrance and allows stratification of surface waters within the sound (Niebauer et al. 1994). Water temperatures increase dramatically by May because of increased solar radiation and decreased mixing. Interannual differences in seasonal cycles are determined by large-scale atmospheric processes affecting the adjacent Gulf of Alaska (Salmon 1992).

The spill occurred under highly unusual conditions that retained the oil within PWS to a far greater degree than would be expected at any other time in recent history. During late March and early April of 1989 the seasonal circulation cycle was at a minimum while the freshwater discharge in 1989, which drives the residual currents, was the lowest in the 59-year discharge history (Royer et al. 1990). Regional wind forcing, intensely strong from 1976 to 1988, relaxed in 1989 (Salmon 1992), resulting in the sound acting more like a lake than in preceding years. The combination of a normal seasonal low of circulation and unusual weather patterns created the unique set of circumstances affecting the retention of oil in PWS.

## Effect of the spill

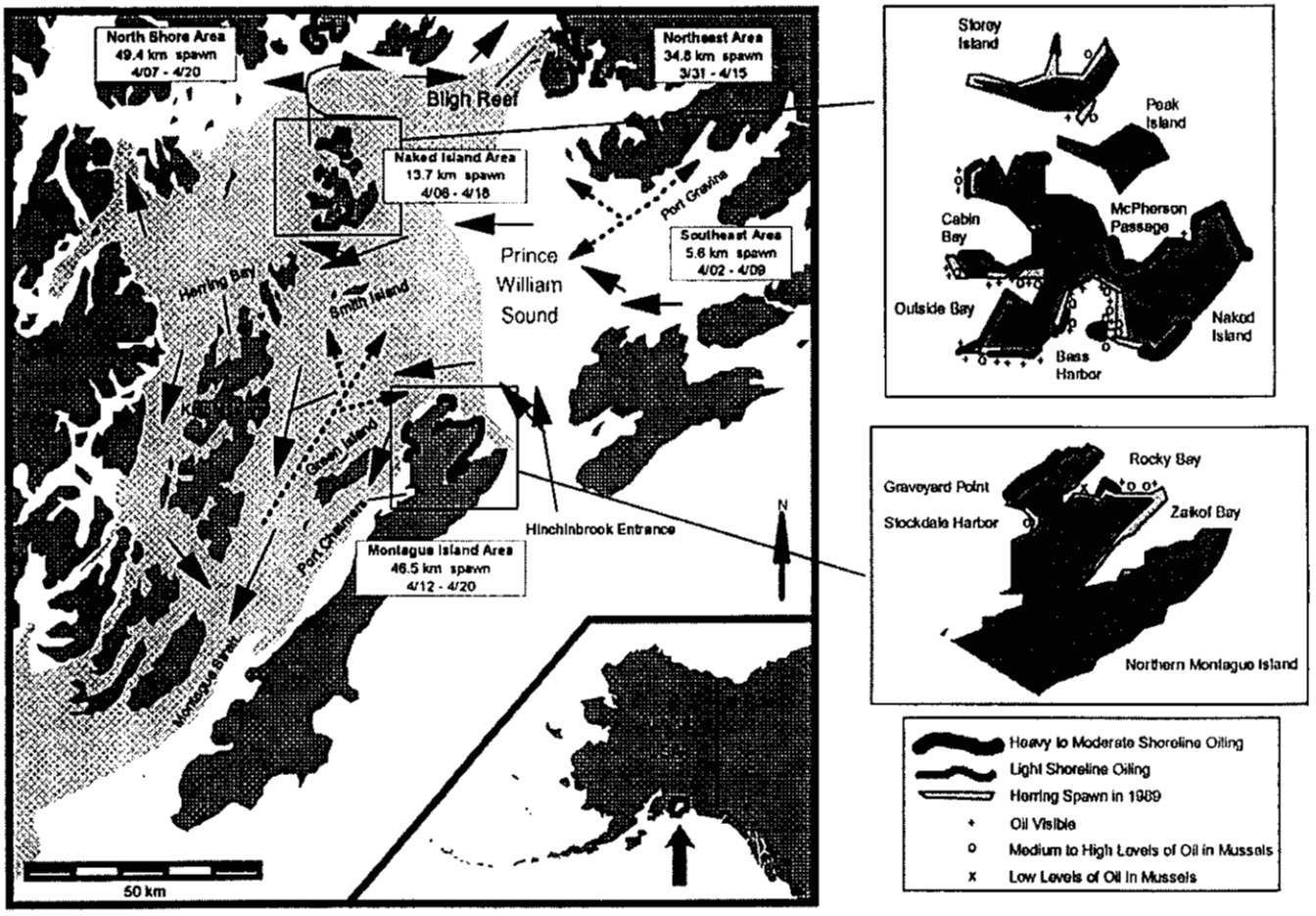
*Exxon Valdez* oil (EVO) followed a trajectory that mirrored the

Received April 1, 1996. Accepted August 13, 1996.
J13384

**E.D. Brown**[1] **and B.L. Norcross.** Institute of Marine Science, School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks, AK 99775–7220, U.S.A.
**J.W. Short.** National Marine Fisheries Service, Alaska Fisheries Science Center, Auke Bay Laboratory, Box 210155, Auke Bay, AK 99821, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: brown@grizzly.pwssc.gen.ak.us

**Fig. 1.** A composite map of the general circulation pattern in Prince William Sound (arrows), oil spill trajectory (lightly shaded area; Galt et al. 1991), direction of annual adult herring spawning migration (broken lines) and herring spawning areas in 1989 (dark shaded areas adjacent to shorelines). Ranges of spawning dates and kilometres of shoreline receiving spawn in 1989 are listed for each of the five areas (southeast, northeast, North Shore, Naked Island, and Montague Island). Enlargements of the two oiled areas, Naked Island and Montague Island, denote beach segments with herring spawn, shoreline oiling from beach surveys (Gundlach et al. 1990), oil in mussel tissue (Short and Harris 1996*a*), and locations of visible oil observed during the herring survey (Brown et al. 1996).




main counterclockwise ocean circulation pattern in PWS (Royer et al. 1990; Venkatesh 1990) (Fig. 1). On March 24, 1989, crude oil leaching from the grounded tanker *Exxon Valdez* began forming a large pool to the south and west of the vessel. Within the 1st week following the spill this pool was tracked by aerial and satellite surveys (Dean et al. 1990; Gundlach et al. 1990), moved to the southwest surrounding the Naked Island group, and began to exit PWS through Montague Strait. A week and a half after the spill, oil was moving steadily out of PWS and had covered much of the area traditionally used by adult herring for spawning (Biggs et al. 1992; Brown et al. 1996) (Fig. 1).

Oil sheens that had broken off from the main body of oil were observed floating eastward across the northern bays of Montague Island opposite from the main pattern of ocean circulation. Although the predominant flow is inward through Hinchinbrook Entrance, down-fjord winds that converge in the central sound can cause flow out through Hinchinbrook Entrance (Royer et al. 1990; Niebauer et al. 1994). Such flow was observed with an acoustic Doppler current profiler (ADCP) in April 1989 (Norcross et al. 1991; Niebauer et al. 1994).

Of the 42 000 000 L spilled, only about 10% was carried out of PWS as floating oil, whereas an estimated 40–45% became beached within PWS (Wolfe et al. 1995). Approximately 634 km of shoreline was lightly to heavily oiled in 1989, and 24 km remained heavily oiled through 1990. An unknown proportion of the oil was dispersed throughout the water column to a depth of at least 25 m and was available to organisms during the summer following the spill in 1989 (Short and Harris 1996*a*).

Petroleum hydrocarbons in nearshore and offshore waters of PWS were highest just after the spill and declined continuously thereafter (Short and Harris 1996*a*, 1996*b*). Dissolved polynuclear aromatic hydrocarbons (PAH) in seawater were found to a depth of 5 m and at concentrations up to 6.24 ± 0.63 $\mu g \cdot L^{-1}$ in an area bounded by northwest Montague Strait, northern Knight Island, and Smith Island for 1–2 weeks after the spill (Short and Harris 1996*b*). While concentrations of particulate EVO hydrocarbons were highest just after the spill, they also remained high in PWS 1–2 months after the spill, especially at sites near heavily oiled beaches (Short and Harris 1996*a*).

**Table 1.** Shoreline segments of spawn, average density of eggs within each segment, total eggs estimated within each segments, determination of oil exposure to segment, and estimated percentages of exposed beach segments with spawn and herring eggs.

| | Area | Location | Station numbers | Spawn (km)[a] | Average density of eggs/m$^2$[a] | Total eggs (billions)[a] | Within trajectory[b] | DEC oiled shoreline[c] | Visual oil observed | Oil in mussel tissue[d] |
|---|---|---|---|---|---|---|---|---|---|---|
| Unoiled | Southeast | Sheep Bay | None | 5.6 | 22 300 | 3 | No | No | No | None taken |
| Unoiled | Northeast | Tatitlek Narrows, Valdez Arm | None | 34.8 | 182 800 | 437 | No | No | No | At reference level |
| Unoiled | North shore | Fairmont Bay and Island | C1–C6 | 49.4 | 354 200 | 1061 | No | No | No | Low in 1989, below reference in 1990 |
| Oiled | Naked Island | | | | | | | | | |
| | | Subareas | O1–O16 | 22.0 | 338 400 | 598 | Yes | Partially | Yes | Low to high |
| | | Bass Harbour | O1–O10 | | | | Yes | Light | Yes | Low to high |
| | | Outside Bay | O11–O12, O14 | | | | Yes | Light | Yes | Moderate to high |
| | | Cabin Bay | O13 | | | | Yes | Light | Yes | High |
| | | Storey Island | O15–O16 | | | | Yes | No | Yes | Moderate |
| Oiled | Montague Island | Subareas | O17–O19 | 46.5 | 209 700 | 1031 | Yes | Partially | Yes | Low to high |
| | | Stockdale Harbor and Graveyard Point | None | 20.8 | 45 200 | 181 | Yes | Light to heavy | Yes | Low to moderate |
| | | Rocky Bay | O17–O19 | 12.0 | 373 700 | 503 | Yes | Very light | Yes | Low to high |
| | | Zaikof Bay | None | 13.8 | 243 900 | 382 | Partially | No | No | None taken |
| PWS totals | | | | 158.3 | 257 900 | 3130 | | | | |
| Percentage of total unoiled | | | | 56.7% | | 48.0% | | | | |
| Percentage of total oiled including Zaikof Bay | | | | 43.3% | | 52.0% | | | | |
| Percentage of total oiled excluding Zaikof Bay | | | | 34.6% | | 41.0% | | | | |

[a]Alaska Department of Fish and Game, Cordova, unpublished data; methodology in Biggs and Funk (1988).
[b]Galt et al. (1991).
[c]Gundlach et al. (1990).
[d]Short and Harris (1996*a*).

Oiled beaches acted as oil reservoirs contributing to subsurface oil concentrations (Short and Harris 1996*a*). Mean concentrations of EVO PAH in mussels deployed in cages in May of 1989 near heavily oiled beaches in Herring Bay (Fig. 1) ranged from 815 to 5740 ng·g wet mass$^{-1}$ (3200 to 45 200 ng·g dry mass$^{-1}$) compared with EVO-PAH concentrations of 61 to 425 ng·g wet mass$^{-1}$ (818 to 3420 ng·g dry mass$^{-1}$) at the southwestern border of PWS where oil exited the sound. Background concentrations of the PAH compounds used to define EVO-PAH ranged up to 18 ng·g wet mass$^{-1}$, median 8 ng·g wet mass$^{-1}$ (162 ng·g dry mass$^{-1}$, median 73), in mussels from Admiralty Island in southeast Alaska before deployment (Short and Harris 1996*a*). Native mussels on the beaches adjacent to oiled sites contained 2–10 times more oil in 1989 than their submerged caged counterparts. By 1990, PAH concentrations had declined considerably from the 1989 levels and were highest (<200 ng·g wet mass$^{-1}$, 1810 ng·g dry mass$^{-1}$) at stations adjacent to heavily oiled beaches.

EVO concentrations in adjacent native bay mussels were used to estimate site-specific exposure in herring eggs (Brown et al. 1996; Hose et al. 1996) because they were a more sensitive measure of oiling and less subjective than visual observations (Short and Harris 1996*a*). Using chemistry results from herring eggs, EVO was detected only at Cabin Bay on Naked Island where whole oil was observed adhering to their surface (Brown et al. 1996). Using results from native bay mussels, EVO was detected on most herring spawning beaches inside the oil trajectory. In 1989, mean (± SE) concentrations of EVO-PAH in mussels within the oil trajectory (753 ± 22 ng·g wet mass$^{-1}$; dry mass figures not available) were significantly higher than at unoiled areas (49 ± 8 ng·g wet mass$^{-1}$) (Brown et al. 1996) (Fig. 1). PAH concentrations in mussels from unoiled sites in the North Shore Area in 1989 (Fig. 1) were consistent with PAH concentrations at other sites not affected by the spill (Short and Babcock 1996). The majority of the PAH detected at unoiled sites in 1989 was naphthalene (Short and Babcock 1996). In 1990, oil concentrations in mussels from control sites were below prespill reference levels (Brown et al. 1996; Short and Harris 1996*a*).

For the purposes of the damage assessment work, we assumed the unoiled sites were not affected. Tarballs and sheens were visually observed at two of the Rocky Bay sites on Montague Island, which both appeared to be outside of the trajectory mapped from aerial surveys; at two of the Bass Harbor sites and on Naked Island located within oil booms; and near the Cabin Bay site on Naked Island (Brown et al. 1996) (Fig. 1 and Table 1). These visual observations often corresponded to the higher levels of oiling according to mussel tissue results. Hydrocarbon levels in mussels sampled at some spawning areas in 1990 were still elevated, but in general were much lower than in 1989.

Since we have no direct measurements of the percentage of

the spawning population that encountered oil, we can only speculate on exposure of prespawning adults to oil. During migration, dissolved, particulate, or oil–water droplets and surface sheens of weathering oil were present. Ingestion of oiled particles was not a likely avenue for oil exposure in the prespawning adults because they generally do not feed during this period (Rice et al. 1987*b*). However, external contact with surface and subsurface suspended oil was likely for at least part of the population.

The only indication of the extent of exposure in prespawning adult herring was from histopathological observations in field-caught herring; these observations were comparable to oil exposure experiments in laboratory studies. Severe lesions, including fin erosion (Moles et al. 1993), were found in 20% of adult herring sampled from oiled areas and 0% from unoiled areas. Adult herring from oiled areas were relatively void of gut parasites compared with herring from unoiled areas (Moles et al. 1993). In the laboratory, prespawning adult herring were exposed to 0.68–1.20 parts per million (ppm) total aromatics for 12 days, after which time lesions, fin erosion, and voiding of parasites similar to those detected in the field-caught herring were observed (Moles et al. 1993).

Herring did not spawn on the most heavily oiled beaches but did spawn on shorelines considered lightly to moderately oiled (Gundlach et al. 1990) (Fig. 1). During peak herring spawning in April 1989, the composite oil trajectory overlapped 34.6–43.3% of the total shoreline used for spawning, with oiled areas having higher egg densities (Brown et al. 1996). The EVO trajectory overlapped 41.0–52.0% of the total egg population of PWS (Fig. 1 and Table 1). Suspended or particulate oil adhering to the surface of herring eggs is the most likely exposure route after a spill (Pearson et al. 1985) such as that from the *Exxon Valdez*. Surface films of oil, which were present during incubation of herring eggs in oiled spawning areas, are known to be 10 to 10 000 times more toxic than subsurface dissolved oil (Cross et al. 1987; Kocan et al. 1987). Also, oil adhering to eggs was directly observed in two independent studies (Pearson et al. 1993; Brown et al. 1996) (Fig. 1). Therefore, measurable oil effects on herring embryos were most likely caused by coating from the surface film of oil during tidal cycles, suspended oil–water droplets, or particulate matter adhering to the egg surface.

The distribution of planktonic herring larvae overlapped the distribution of oil to a greater degree than that of the demersal herring embryos. Peak concentrations of herring larvae were caught in nearshore areas in PWS in May (McGurk and Brown 1996) and offshore areas in June (Norcross and Frandsen 1996; Norcross et al. 1996). By this time, dissolved PAH concentrations in the water column were low; thus, direct contact with suspended oiled particles or ingestion of oiled particles or prey was the most likely source of exposure. Ingestion of oiled prey was considered to be the source of oil exposure in growth-impaired immature pink salmon captured in oiled areas (Sturdevant et al. 1996; Wertheimer and Celewycz 1996).

By 1990 very little oil was found in herring spawning areas. However, oil exposure continued for at least part of the herring population in oil-affected areas. Smith Island had significant amounts of oil as measured in mussel tissue, but this area contained less than 10% of the total eggs deposited. In addition, adult herring caught in the spring of 1990 from Port Chalmers on Montague Island had amounts of oil metabolites in their bile similar to the amounts found in herring sampled in 1989, indicating recent exposure at levels similar to those in 1989 (Manen 1993). This bay is adjacent to areas where, according to historical fisheries (Donaldson et al. 1992) and hydroacoustic surveys (DeCino et al. 1994), herring aggregate during the winter. This bay is also typically used for spawning in the spring (Biggs et al. 1992).

## Studies of oil spill injury to Prince William Sound herring

The overall goal of the herring studies was to describe any injuries to PWS herring, including population effects, caused by the spill. The *Exxon Valdez* oil spill necessitated thorough examination of the effects of oil on Pacific herring in the field and comparison with effects observed in laboratory studies. Although larval studies were conducted, most of the research effort was focused on spawning herring and their progeny because (*i*) spawning herring are sensitive to petroleum compounds and are unable to metabolize them (Rice et al. 1987*a*), (*ii*) oil is lipophilic, concentrating in the lipid-rich ova (Rice et al. 1987*a*, 1987*b*), and (*iii*) oil uptake by adult females can affect their eggs (Struhsaker 1977) and newly hatched larvae.

Spill studies conducted from 1989 through 1992 concentrated on sublethal effects. In addition, during 1993 and 1994, pathology studies were the focus because of a documented outbreak of viral hemorrhagic septicemia in PWS herring (Meyers et al. 1994). Because exposure to oil can cause histopathological damage including mutagenic effects in marine fish and can affect immunocompetency, growth, and reproductive success (Capuzzo 1987), studies are continuing in these areas. We recognized that the most sensitive measure of oil injury in the early life stages would be the mutagenic evaluations (Kocan et al. 1982) and therefore concentrated on genetic damage. The series of five papers that follow examines oil injury to PWS herring. A second set of papers addressing the effects of oil on embryo survival, the histopathology of larval and adult herring following the spill, and the relationship between the spill and the disease-related herring population crash in 1993 is currently being prepared for publication.

## Acknowledgments

National Marine Fisheries Service personnel Stanley Rice, Adam Moles, Mark Carls, Sid Korn, and Chris Broderson provided advice and assistance and guided sample collections for hydrocarbons and damage assessment research. Prince William Sound Science Center oceanographer David Salmon assisted with the oceanographic data interpretation. John Wilcock and Steve Fried of the Alaska Department of Fish and Game provided assistance in the oil spill studies of Pacific herring and critical comments on this paper. This is contribution 1623 of the Institute of Marine Science, University of Alaska, Fairbanks. This project was funded by the *Exxon Valdez* Oil Spill Trustee Council, Nature Resource Damage Assessment Study No. 11, but the views expressed are those of the individual investigators and do not necessarily represent the conclusions of the Council.

## References


Biggs, E.D., Haley, B.E., and Gilman, J.M. 1992. Historic database for Pacific herring of Prince William Sound, 1973–1991. Regional information report No. 2C91-11. Alaska Department of Fish and Game, Anchorage, Alaska.

Blaxter, J.H.S., Russell, F.S., and Yonge, M. 1982. The biology of clupeoid fishes. Adv. Mar. Biol. **20**.

Brady, J.A., Morstad, S.M., Simpson, E., and Biggs, E.D. 1991. Prince William Sound management area 1989 annual finfish management report. Regional information report No. 2C90-07. Alaska Department of Fish and Game, Division of Commercial Fisheries, Cordova, Alaska.

Brown, E.D., Baker, T.T, Hose, J.E., Kocan, R.M., Marty, G.D., McGurk, M.D., Norcross, B.L., and Short, J.W. 1996. Injury to the early life history stages of Pacific herring in Prince William Sound after the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. No. 18. pp. 448–462.

Capuzzo, J.M. 1987. Biological effects of petroleum hydrocarbons: assessments from experimental results. *In* Long-term environmental effects of offshore oil and gas development. *Edited by* D.F. Boesch and N.N. Rabalais. Elsevier Applied Science Publishers, London. pp. 343–410.

Cross, J.N., Hardy, J.T., Hose, J.E., Hershelman, G.P., Antrium, L.D., Gossett, R.W., and Crecelius, E.A. 1987. Contaminant concentrations and toxicity of sea-surface microlayer near Los Angeles, California. Mar. Environ. Res. **23**: 307–323.

Dean, K.G., Stringer, W.J., Groves, J.E., Anlnas, K., and Royer, T.C. 1990. The Exxon-Valdez oil spill: satellite analyses. *In* Oil Spills Management and Legislative Implications: Proceedings of the Conference, May 15–18, 1990, Newport, Rhode Island, U.S.A. *Edited by* M.L. Spaulding and M. Reed. American Society of Civil Engineers, New York. pp. 492–502.

DeCino, R., Wilcock, J.W., Patrick, V., Thorne, R., and Thomas, G. 1994. Acoustic estimate of herring biomass in the Green Island area of Prince William Sound, Alaska. Final report to Cordova District Fishermen United, Box 939, Cordova, Alaska.

Donaldson, W., Morstad, S., Simpson, E., and Biggs, E. 1992. Prince William Sound management area 1991 annual finfish management report. Regional information report No. 2A92-09. Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage, Alaska.

Galt, J.A., Lehr, W.J., and Payton, D.L. 1991. Fate and transport of the *Exxon Valdez* oil spill. Environ. Sci. Technol. **25**: 202–209.

Grant, U.S., and Higgens, D.F. 1910. Reconnaissance of the geology and mineral resources of Prince William Sound, Alaska. U.S. Geol. Surv. Bull. No. 443.

Gundlach, E.R., Bauer, J., Bayliss, R., Provant, S., and Kendziorek, M. 1990. Response to the Exxon Valdez oil spill by the Alaska Department of Environmental Conservation. *In* Oil Spills Management and Legislative Implications: Proceedings of the Conference, May 15–18, 1990, Newport, Rhode Island, U.S.A. *Edited by* M.L. Spaulding and M. Reed. American Society of Civil Engineers, New York. pp. 471–478.

Hose, J.E., McGurk, M.D., Marty, G.D., Hinton, D.E., Brown, E.D., and Baker, T.T. 1996. Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphological, cytogenetic, and histopathological assessments, 1989–1991. Can. J. Fish. Aquat. Sci. **53**: 2355–2365.

Kocan, R.M., Landholt, M.L., and Sabo, K.M. 1982. Anaphase aberrations: a measure of genotoxicity in mutagen-treated fish cells. Environ. Mutagen. **4**: 181–189.

Kocan, R.M., Westernhagen, H.V., Landolt, M.L., and Furstenberg, G. 1987. Toxicity of sea-surface microlayer: effects of hexane extract on Baltic herring (*Clupea harengus*) and Atlantic cod (*Gadus morhua*) embroys. Mar. Environ. Res. **23**: 291–305.

Manen, C.A. 1993. Hydrocarbon analytical support services and analysis of distribution and weathering of spilled oil. Natural Resource Damage Assessment Technical Services study 1, final status report. National Oceanic and Atmospheric Administration, Washington, D.C.

McGurk, M.D., and Brown, E.D. 1996. Egg–larval mortality of Pacific herring in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. **53**: 2343–2354.

Meyers, T.R., Short, S., Lipson, K., Batts, W.N., Winton, J.R., Wilcock, J., and Brown, E. 1994. Association of viral hemorrhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA. Dis. Aquat. Org. **19**: 27–37.

Moles, A., Rice, S.D., and Okihiro, M.S. 1993. Herring parasite and tissue alterations following the *Exxon Valdez* oil spill. *In* Proceedings of the 1993 International Oil Spill Conference, May 3–5, 1993, Tampa, Fla. U.S. Coast Guard, American Petroleum Institute, and Environmental Protection Agency, U.S.A. pp. 325–328.

Niebauer, H.J., Royer, T.C., and Weingartner, T.J. 1994. Circulation of Prince William Sound, Alaska. J. Geophys. Res. **99**: 14, 113–114, 126.

Norcross, B.L., and Frandsen, M. 1996. Distribution and abundance of larval fishes in Prince William Sound, Alaska during 1989 after the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. No. 18. pp. 463–486.

Norcross, B.L., Holladay, B., Frandsen, M., and Vallarino, M. 1991. Injury to larval fish in Prince William Sound. Final report to Alaska Department of Fish and Game, OSIAR division. Institute of Marine Science, University of Alaska, Fairbanks, Alaska.

Norcross, B.L., Hose, J.E., Frandsen, M., and Brown, E.D. 1996. Distribution, abundance, morphological condition, and cytogenetic abnormalities of larval herring in Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. **53**: 2376–2387.

Pearson, W.H., Woodruff, D.L., Kiesser, S.L., Fellingham, G.W., and Elston, R.A. 1985. Oil effects on spawning behavior and reproduction in Pacific herring (*Clupea harengus pallasi*). API publication No. 4412. American Petroleum Institute, Washington, D.C.

Pearson, W.H., Moksness, E., and Skalski, J.R. 1995. A field and laboratory assessment of oil spill effects on survival and reproduction of Pacific herring following the *Exxon Valdez* oil spill. *In* *Exxon Valdez* oil spill: fate and effects in Alaska waters. *Edited by* P.G. Wells, J.N. Butler, and J.S. Hughes. American Society for Testing and Materials, Philadelphia, ASTM STP 1219. pp. 626–661.

Rice, S.D., Babcock, M.M., Brodersen, C.C., Gharrett, J.A., and Korn, S. 1987*a*. Uptake and depuration of aromatic hydrocarbons by reproductively ripe Pacific herring and the subsequent effect of residues on egg hatching and survival. *In* Pollution physiology of estuarine organisms. *Edited by* W.B. Vernberg, A. Calabrese, F.P. Thurberg, and F.J. Vernburg. University of South Carolina Press, Columbia, S.C. pp. 139–154.

Rice, S.D., Babcock, M.M., Brodersen, C.C., Carls, M., Gharrett, J.A., Korn, S., Moles, A., and Short, J.W. 1987*b*. Lethal and sublethal effects of the water soluble fraction of Cook Inlet crude oil on Pacific herring (*Clupea harengus pallasi*) reproduction. NOAA Tech. Memo. No. NMFS F/NWC-111. National Oceanic and Atmospheric Administration, Auke Bay, Alaska.

Royer, T.C., Vermersch, J.A., Weingartner, T.J., Niebauer, H.J., and Muench, R.D. 1990. Ocean circulation influencing the *Exxon Valdez* oil spill. Oceanography, **3**: 3–10.

Salmon, D.K. 1992. On interannual variability and climate change in the North Pacific. Ph.D. thesis, University of Alaska, Fairbanks, Alaska.

Schmidt, G.M. 1977. The exchange of water between Prince William Sound and the Gulf of Alaska. M.Sc. thesis, University of Alaska, Fairbanks, Alaska.

Short, J.W., and Babcock, M.M. 1996. Prespill and postspill concentrations of hydrocarbons in mussels and sediments in Prince William Sound. Am. Fish. Soc. Symp. No. 18. pp. 149–166.

Short, J.W., and Harris, P.M. 1996*a*. Petroleum hydrocarbons in caged mussels deployed in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. No. 18. pp. 29–39.

Short, J.F., and Harris, P.M. 1996*b*. Chemical sampling and analysis of petroleum hydrocarbons in the near-surface seawater of Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. No. 18. pp. 17–28.

Struhsaker, J.W. 1977. Effects of benzene (a toxic component of petroleum) on spawning Pacific herring, *Clupea harengus pallasi*. Fish. Bull. **75**: 43–49.

Sturdevant, M.V., Wertheimer, A.C., and Lum, J.L. 1996. Diet of juvenile pink and chum salmon in oiled and non-oiled nearshore habitats in Prince William Sound, 1989 and 1990. Am. Fish. Soc. Symp. No. 18. pp. 578–592.

Venkatesh, S. 1990. Model simulations of the drift and spread of the *Exxon Valdez* oil spill. Atmos.-Ocean, **28**: 90–105.

Wertheimer, A.C., and Celewycz, A.G. 1996. Abundance and growth of juvenile pink salmon in oiled and non-oiled locations of western Prince William Sound after the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. No. 18. pp. 518–532.

Wolfe, D.A., Krahn, M.M., Casillas, E., Sol, S., Thompson, T.A., Lunz, J., and Scott, K.J. 1995. Toxicity of intertidal and subtidal sediments contaminated by the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. No. 18. pp. 121–139.

# Egg–larval mortality of Pacific herring in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill

**Michael D. McGurk and Evelyn D. Brown**

**Abstract:** We tested the hypothesis that instantaneous daily rates of egg–larval mortality of Pacific herring, *Clupea pallasi*, were greater at two oil-exposed sites than at two nonexposed sites. Egg–larval mortality is defined as the ratio of larval density at hatch to mean egg density divided by the number of elapsed days between the dates of the two estimates. There were significant differences between sites in egg density, small differences between sites in mean larval length, and no oil-related differences between sites in the rates of larval growth and loss. However, mean egg–larval mortality in the two oiled areas was twice as great as in the two nonoiled areas, and larval growth rates were about half those measured in populations from other areas of the north Pacific Ocean. These findings support the hypothesis of oil injury to herring embryos and larvae.

**Résumé** : Nous avons vérifié l'hypothèse selon laquelle, chez le hareng du Pacifique (*Clupea pallasi*), les taux quotidiens instantanés de mortalité des larves par rapport à la densité des masses d'oeufs sont supérieurs dans deux stations polluées par le pétrole à ce qu'ils sont dans deux stations non polluées. La mortalité des larves par rapport à la densité des masses d'oeufs est le rapport entre la densité des larves à l'éclosion et la densité moyenne des oeufs, divisé par le nombre de jours qui se sont écoulés entre les deux estimations. Il existait des différences significatives entre les stations quant à la densité des masses d'oeufs et de petites différences quant à la longueur moyenne des larves, et il n'existait aucune différence attribuable au pétrole quant aux taux de perte et de croissance des larves. Toutefois, dans les deux secteurs pollués, la mortalité moyenne des larves par rapport à la densité des masses d'oeufs était le double de ce qu'elle était aux deux autres stations, et le taux de croissance des larves était environ la moitié de ceux mesurés dans d'autres secteurs du Pacifique nord. Ces résultats confirment l'hypothèse selon laquelle le pétrole a des effets nocifs sur les embryons et les larves de hareng.
[Traduit par la Rédaction]

## Introduction

On March 24, 1989, the oil tanker *Exxon Valdez* struck Bligh Reef and spilled 42 000 000 L of Prudhoe Bay crude oil into Prince William Sound, Alaska. It was the largest spill to date in U.S. coastal waters, and it coincided with spawning of the local stock of Pacific herring, *Clupea pallasi*. In reaction to the spill, state and federal agencies mounted what may be the most comprehensive environmental impact assessment ever conducted on a marine oil spill. This article describes one part of this assessment: the effect of the oil spill on herring egg–larval mortality. This aspect of herring population dynamics was studied because hatching is a critical period in the life history of fishes and one that is affected by exposure of embryos to pollutants (von Westernhagen 1988). Also, egg–larval mortality is less confounded by problems of dispersal of fish between oiled and nonoiled areas of the sound than other population parameters because herring eggs are fixed to the substrate.

Received October 23, 1992. Accepted March 11, 1993.
J11670

**M.D. McGurk.** Triton Environmental Consultants Ltd., 120 – 13 511 Commerce Parkway, Richmond, BC V6V 2L1, Canada.
**E.D. Brown.**[1] Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division, P.O. Box 669, Cordova, AK 99574, U.S.A.

[1] Author to whom all correspondence should be addressed at the Institute of Marine Science, School of Fisheries and Ocean Sciences, University of Alaska–Fairbanks, Fairbanks, AK 99775-7220, U.S.A. e-mail: MMcGurk@Triton-env.com

We first briefly describe the timing and trajectory of the oil slick and the timing and distribution of herring spawn in the sound. Then, we compare egg–larval mortality between oiled and control areas.

### Trajectory of the oil slick

During the first 60 h after grounding of the *Exxon Valdez* the weather was clear and calm, and the spilled oil was confined to a 13 km long slick in the center of the sound southwest of Bligh Reef (Fig. 1). Shortly thereafter, a violent storm moved the slick rapidly in a southwesterly direction. By the end of the 4th day after the spill, March 27, the slick was 60 km long and had begun passing through the Naked Island archipelago and down the west and east shores of Knight Island. At this time, and over subsequent days, oil washed ashore and fouled beaches and intertidal habitat. By the end of the 7th day, March 30, the oil slick began to exit the sound through Montague Strait but not before some oil had spread eastwards around the northern tip of Montague Island. The slick never approached the eastern or northern shores of the sound. This trajectory confirmed the counterclockwise pattern of surface water circulation in the sound (Royer et al. 1990; Norcross et al. 1996).

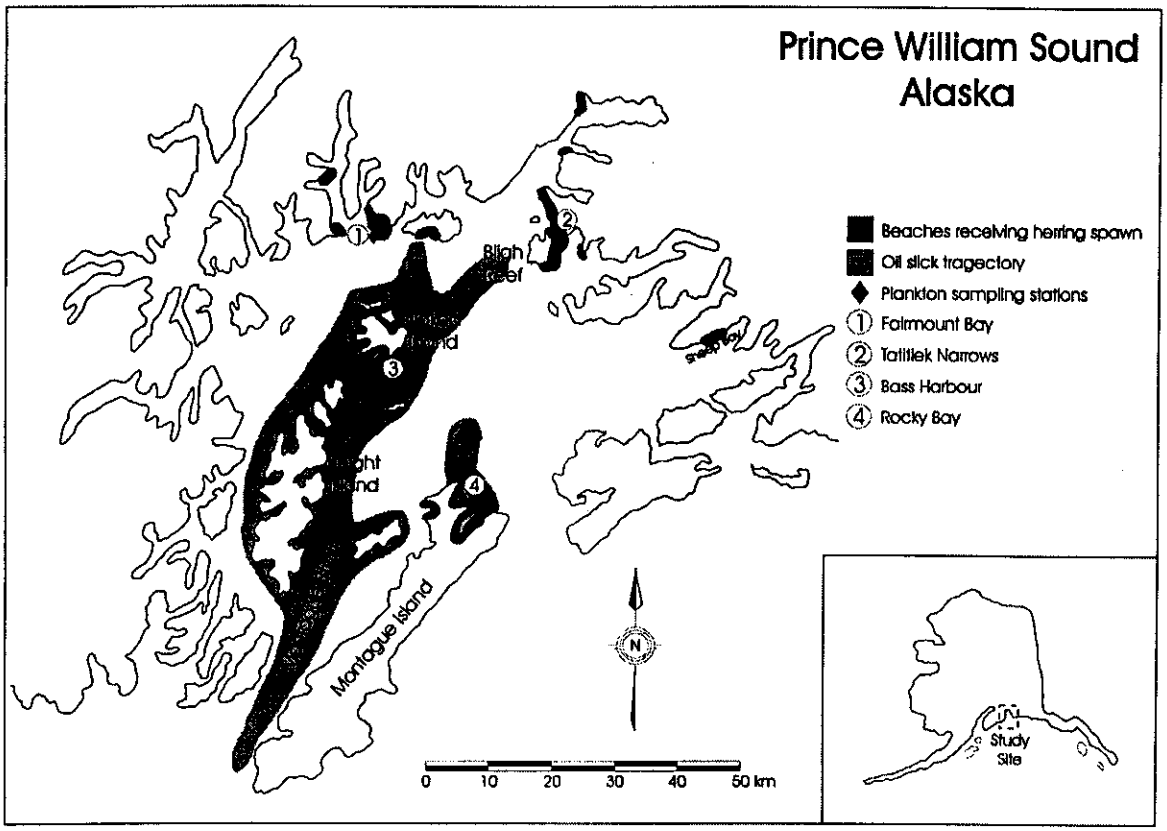
### Timing and distribution of herring spawning

Aerial surveyors first observed herring massing near their spawning grounds between March 29 and April 1 (Alaska Department of Fish and Game 1991). Spawners were concentrated in four major areas: the northeast area centered on Tatitlek Narrows, the north area centered on Fairmount Bay, the Naked Island archipelago including Bass Harbor, and the



Can. J. Fish. Aquat. Sci. 53: 2343–2354 (1996).

**Fig. 1.** Map of Prince William Sound, Alaska, showing the southwesterly trajectory of the *Exxon Valdez* oil slick, the major herring spawning areas, and the plankton stations.




northern end of Montague Island including Rocky Bay (Fig. 1). One minor concentration was observed in Sheep Bay. Spawning began on March 31 and ended on April 20, depositing 158 linear km of spawn on vegetation in the lower intertidal and upper subtidal zones. This was equivalent to 52 000 metric tons (t) of spawners, on the basis of scuba surveys of egg density. Eggs incubated for about 20 days at an average surface water temperature of 7°C (Alderdice and Velsen 1971), before hatching in late April and early May. Therefore, herring spawned in the sound in 1989 while concentrations of dissolved hydrocarbons in the central and southwestern sound were at their highest levels (Neff 1991; Short and Harris 1996). Incubating embryos and free-swimming larvae in the southwestern part of the sound were exposed to hydrocarbon concentrations significantly above background levels.

## Methods

### Study design

The primary objective of this study was to estimate and compare egg–larval mortality between oiled and nonoiled areas of the sound. Secondary objectives flowed from the requirements of estimating egg–larval mortality and involved analyses of ancillary variables, such as depth profiles of temperature and salinity, width of egg beds and the depth distribution of eggs, mean length of larvae, rate of growth of larval length, and rate of reduction in larval density at plankton stations.

To measure egg–larval mortality required estimating egg density, larval density at hatch, and the duration of the egg–larval period. Egg density was measured by scuba surveys. Larval density at date was measured by towed plankton nets. Larval density at hatch was estimated by regressing larval density on larval age and extrapolating the regression back to an age of 0 days. Dates at capture of larvae were converted to ages of larvae with the use of a mean hatch date, which was estimated in two ways: (*i*) as the date at which a regression of larval length on date predicted mean length at hatch, and (*ii*) as the date predicted from the fraction of larvae in a sample that carried a yolk sac. The duration of the egg–larval period was the period between the date of the scuba survey of egg density and the mean hatch date. Therefore, estimation of egg–larval mortality also required estimation of larval growth rates and loss rates, which meant that they were available to be tested for an oil effect. We also took the opportunity to test larval length and larval density at date for an oil effect. Finally, temperature and salinity profiles of the study areas were measured to test for differences between areas not related to oil that may affect interpretation of egg–larval mortalities.

The northeast and north areas were chosen as nonoiled control areas and the Naked Island archipelago and the northern tip of Montague Island were chosen as oiled treatment areas (Fig. 1). This simple design of one treatment and one control, each with two replicates, allowed the use of analysis of variance (ANOVA) techniques when testing for an oil effect.

Simultaneous with our study, Norcross et al. (1996) measured the distribution, abundance, size, and morphological and cytological condition of herring larvae at 20 plankton stations in offshore areas of the sound.

### Herring egg density

As part of its program of estimating annual herring spawning biomass in Prince William Sound, the Alaska Department of Fish and Game conducted scuba surveys of herring egg density in 1989. In each of four main spawning areas, biologists laid between 20 and 51 transects

perpendicular to the shoreline. At each transect, a scuba diver swam down a lead line from the upper end to the lower end of the band of littoral vegetation. Pacific herring eggs are almost always laid on vegetation (Haegele et al. 1981). Every 5 m, the diver visually estimated the number of eggs within a 0.1-$m^2$ quadrat. At one place on each transect, the diver removed and preserved all of the eggs and vegetation in a quadrat. In the laboratory, eggs were removed from vegetation and counted, and counts were used to calibrate the visual egg counts for each diver.

The width of the band of spawn on each transect was the distance between the highest intertidal and lowest subtidal (or furthest offshore) quadrat where eggs occurred. Spawn width was ln-transformed because it was highly skewed owing to the presence of a few transects with very wide bands of spawn.

Mean egg density of each spawning area was calculated from mean egg densities of those quadrats that could have possibly contributed to the population of herring larvae that was sampled at a nearby plankton station. The choice of quadrats was guided by topography; in areas of open water, such as in Tatitlek Narrows or off Fairmount Island, all transects were included. In embayments, such as at Bass Harbor and Rocky Bay, only transects within the embayment were included. This rationale was somewhat arbitrary, but there was no other procedure that guaranteed a more accurate and precise estimate of the average egg density of an area. Egg density was ln-transformed to normalize its distribution; therefore, only non-zero egg densities were used to calculate mean ln(egg density) for each area.

**Plankton samples**

Plankton stations were located <1 km from herring egg survey transects. A site off the western shore of Fairmount Island (60°52.30′N, 147°28.80′W) in the north area was chosen because it was within 1 km of the spawning beaches of Fairmount Bay, but deep enough (>30 m) to safely perform oblique plankton nets tows (Fig. 1). Similar reasons guided the choice of a second control site in the northeast area about 0.5 km off Black Point in the middle of Tatitlek Narrows (60°54.40′N, 146°45.99′W), and oiled sites inside Bass Harbor on Naked Island (60°37.40′N, 147°24.50′W) and inside Rocky Bay on Montague Island (60°21.25′N, 147°04.00′W).

We conducted seven 2-day cruises of the sound each week from May 1 to June 22, 1989. Herring larvae were not expected to enter the water column in maximum numbers until early May, on the basis of the dates of spawning and the relationship between temperature and the duration of herring egg incubation (Alderdice and Velsen 1971). Sampling stopped in late June when we could no longer catch sufficient numbers of herring larvae because most had grown to a size that allowed them to avoid plankton nets.

The same sampling protocol was followed at each of the plankton stations. Temperature and salinity were measured at 2-m intervals from the surface to a depth of 30 m with a conductivity–temperature meter. Herring larvae were captured with a bongo net that had a mouth diameter of 60 cm, a length of 3 m, and a mesh width of 0.333 or 0.505 mm. The net was towed at a speed of about 1 m/s in a double oblique pattern to 30 m, a maximum depth chosen because Pacific herring larvae are almost always found within the upper 30 m of the water column (Stevenson 1962). The 0.333-mm mesh was used for the first two cruises, when populations of herring larvae contained many newly hatched larvae, because herring larvae <8 mm long are extruded through 0.505-mm mesh (Colton et al. 1980). The 0.505-mm mesh was used for the remaining five cruises. The volume of water filtered by all bongo nets was measured with a flowmeter placed in one of the two mouths. Two replicate tows were made at each station on each cruise. The catch of the first tow was preserved in 5% seawater formalin, and the catch of the second tow was drained and preserved in 37% isopropyl alcohol in fresh water. Formalin-preserved larvae were used in analyses of larval size and density, and alcohol-preserved larvae were used for otolith analyses (not reported here) and for analysis of density.

All fish larvae were picked from the plankton samples except for several samples that contained so many larvae (> 10 000) that they had to be split before sorting. All herring larvae were identified and counted. One hundred herring larvae were randomly chosen from each formalin-preserved sample; the presence or absence of a yolk sac was recorded, and standard length was measured to the nearest 0.1 mm. Density of herring larvae was calculated as the number per square metre of surface area. Only non-zero densities were analyzed because zero counts did not represent true zero densities, but rather the fact that the density of the larvae was lower than the lower detection limit of the bongo nets. Larval density was not corrected for underestimation because of net avoidance (McGurk 1992).

**Analyses of variance**

ANOVA was the primary method of testing for differences between oiled and nonoiled areas of the sound. To test the significance of variation in water temperature or salinity between areas, cruises, and depths we used a three-factor ANOVA:

$$(1)\quad Y_{ijkl} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + \varepsilon_{ijkl},$$

where $Y_{ijkl}$ is the $l$th observation of temperature or salinity ($n$ = 4 stations × 7 cruises × 16 depths − 6 outliers = 442), $\mu$ is the grand mean, $A_i$ is the fixed effect of the $i$th area, $B_j$ is the fixed effect of the $j$th cruise, $C_k$ is the fixed effect of the $k$th depth (observations divided into three arbitrary depth classes: 0–9, 10–19, and 20–30 m), bracketed terms are interactions, and $\varepsilon_{ijkl}$ is the error. This ANOVA and all statistical analyses of this study were done with SPSS software (SPSS Inc. 1988). All terms in ANOVA with probabilities <0.05 were assumed to be significant, and those <0.001 highly significant.

To test the significance of oil to larval length, we used a mixed-model three-factor ANOVA:

$$(2)\quad L_{ijkl} = \mu + A_i + B_j + C_k + (AC)_{ik} + \varepsilon_{ijkl},$$

where $L_{ijkl}$ is the $l$th larval length (mm) ($n$ = 1370), $A_i$ is the fixed effect of the $i$th oil treatment, $B_j$ is the random effect of the $j$th station, and $C_k$ is the fixed effect of the $k$th cruise. There was no interaction between station and treatment or between station and cruise because there can be no interactions between fixed and random variables.

Larval length was also examined with a repeated-measures ANOVA:

$$(3)\quad L_{ij} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ij},$$

where $L_{ij}$ is the mean larval length (mm) ($n$ = 4 stations × 5 cruises with non-zero catches = 20) measured on the $j$th cruise at the $i$th oil treatment, $A_i$ is the fixed effect of the $i$th oil treatment, and $B_j$ is the fixed effect of the $j$th cruise. The effect of station within treatment could not be tested because there was only one mean length measured at each station on each cruise. For our purposes, the critical term in eqs. 2 and 3 was the interaction of oil and cruise.

To test the effect of oil on density of herring larvae, we used both types of ANOVA. In eq. 2, $\ln(N)_{ijkl}$ was substituted for $L_{ijkl}$, where $N$ is the $l$th density (number per square metre). In eq. 3, $\ln(N)_{ij}$ was substituted for $L_{ij}$, where $N$ is the $j$th geometric mean density of herring larvae measured at a station during a cruise. Density was ln-transformed to normalize its distribution.

**Dates at hatch**

Two methods were used to estimate mean dates of hatch. The first method was to determine the date at which the mean length of herring larvae at a station was 8.8 mm, the length at which 100% of all herring larvae were yolked. This critical length was estimated from a regression of mean length on the fraction of yolked larvae in a sample, using only samples with at least one yolked larva (Fig. 4). The date at which mean larval length was 8.8 mm was estimated by regressing larval length, $L$ (mm), on day of year (DOY) as

$$(4)\quad L = 8.8 + G(\mathrm{DOY} - \mathrm{DOY}_0),$$

**Table 1.** Mean day of year of hatch of the major cohort of herring larvae at four plankton stations (day 121 = May 1).

| Aging method | Bass Harbor | Fairmount Bay | Rocky Bay | Tatitlek Narrows |
|---|---|---|---|---|
| Length at date[a] | 127 | 121 | 130 | 124 |
| Fraction yolked[b] | 130 | 125 | 131 | 124 |
| Mean | 128 | 123 | 130 | 124 |

[a] $DOY_0$ from Fig. 5.

[b] Age (days) = $40.9T^{-0.84}(1-f)$, where $T$ is the average temperature (°C) of the upper 30 m measured on the same day as the sample was collected, and $f$ is the fraction of yolk sac larvae in the sample. Data from Alderdice and Velsen (1971).

where $G$ is the rate of growth (millimetres per day) and $DOY_0$ is the DOY at hatch. Equation 4 was fit separately to lengths at each site with nonlinear regression.

The second method of aging was based on the fraction of yolk sac larvae in a sample, which is an index of age because the number of days from hatch to exhaustion of yolk decreases with temperature (Alderdice and Velsen 1971; McGurk et al. 1993) (Table 1).

### Loss of herring larvae

Total loss of larvae, including dispersal and mortality, was estimated as the slope of the regression of $\ln(N)$ on age:

$$(5) \quad \ln(N) = \ln(N_0) - Zt,$$

where $N_0$ is the density (number per square metre) at hatch ($t = 0$ days) and $Z$ is the coefficient of total larval loss (days$^{-1}$). Equation 5 was fit separately to larval densities of each site with linear regression.

### Egg–larval survival and mortality

Egg–larval survival was the ratio of the density of newly hatched herring larvae to the density of herring eggs:

$$(6) \quad \ln(s) = \ln(N_0) - \ln(N_e),$$

where $s$ is the egg–larval survival, $N_0$ is the density (number per square metre) of newly hatched herring larvae estimated from eq. 5, and $N_e$ is the mean density (number per square metre) of herring eggs estimated from scuba surveys. All statistical analyses of survival were conducted on ln-transformed ratios because $N_0$ and $N_e$ were log-normally distributed. Following standard practice (Neter and Wasserman 1974), the variance (var) of $\ln(s)$ was calculated as

$$(7) \quad \mathrm{var}(\ln(s)) = \mathrm{var}(\ln(N_0)) + \mathrm{var}(\ln(N_e)) - 2\mathrm{cov}(\ln(N_0), \ln(N_e)).$$

Covariance (cov) was assumed to be zero over the range of $\ln(N_0)$ and $\ln(N_e)$ encountered in this study, which is a conservative assumption because it means that the variance of $\ln(s)$ was always overestimated. The amount of overestimation was relatively small; this was verified by calculating the between-sample covariance, or

$$(8) \quad var_{\ln(N_0),\ln(N_e)} = \sum^{i=n} \ln(N_0)_i \ln(N_e)_i - n \ln(N_0) \ln(N_e),$$

where $\ln(N_0)$ and $\ln(N_e)$ are means and $n$ is the number of areas. This variance was only 2–12% of the variance of $\ln(s)$ for each area.

The unbiased sample size of $\ln(s)$ was the sum of the number of quadrats used to calculate mean $\ln(N_e)$ and the number of larval densities used to estimate $\ln(N_0)$ minus two. The period between a scuba survey of egg density and mean hatch date varied between areas, so it was necessary to standardize $\ln(s)$ as an egg–larval mortality rate, $Z_{el}$ (days$^{-1}$):

$$(9) \quad Z_{el} = (1/t)\ln(s),$$

where $t$ is the number of days between the mid-date of the scuba survey and the date of hatch. Following standard practice for the variance of a ratio (Neter and Wasserman 1974), the variance of $Z_{el}$ was

$$(10) \quad \mathrm{var}(Z_{el}) = (\ln(s)/t)^2(\mathrm{var}(\ln(s))/\ln(s)^2 + \mathrm{var}(t)/t^2 + 2\mathrm{cov}(\ln(s), t)/\ln(s)t).$$

$\mathrm{Cov}(\ln(s), t)$ was assumed to be zero because egg–larval survival and timing of the scuba surveys were independent of each other. Variance of $t$ was

$$(11) \quad \mathrm{var}(t) = \mathrm{var}(D_h - D_s) = \mathrm{var}(D_h) + \mathrm{var}(D_s) - 2\mathrm{cov}(D_h, D_s),$$

where $D_s$ is the mid-date of the scuba survey of egg density and $D_h$ is the date at hatch. Covariance of dates was assumed to be zero because the dates were independent of each other. Variance of $D_s$ was zero because $D_s$ was known exactly, and variance of hatch date was approximated by the sum of squared differences between the date at hatch and the range of all possible dates. Therefore, eq. 11 reduced to

$$(12) \quad \mathrm{var}(t) = (1/n_h) \sum^{j=n_h} (D_{h,j} - D_h)^2,$$

where $n_h$ is the number of dates within the range of hatching dates and $D_{h,j}$ is the $j$th date within the range of hatching dates. This was an underestimate of variance because it assumed that all dates were equally possible. The sample size of $Z_{el}$ was the same as that for $\ln(s)$.

## Results

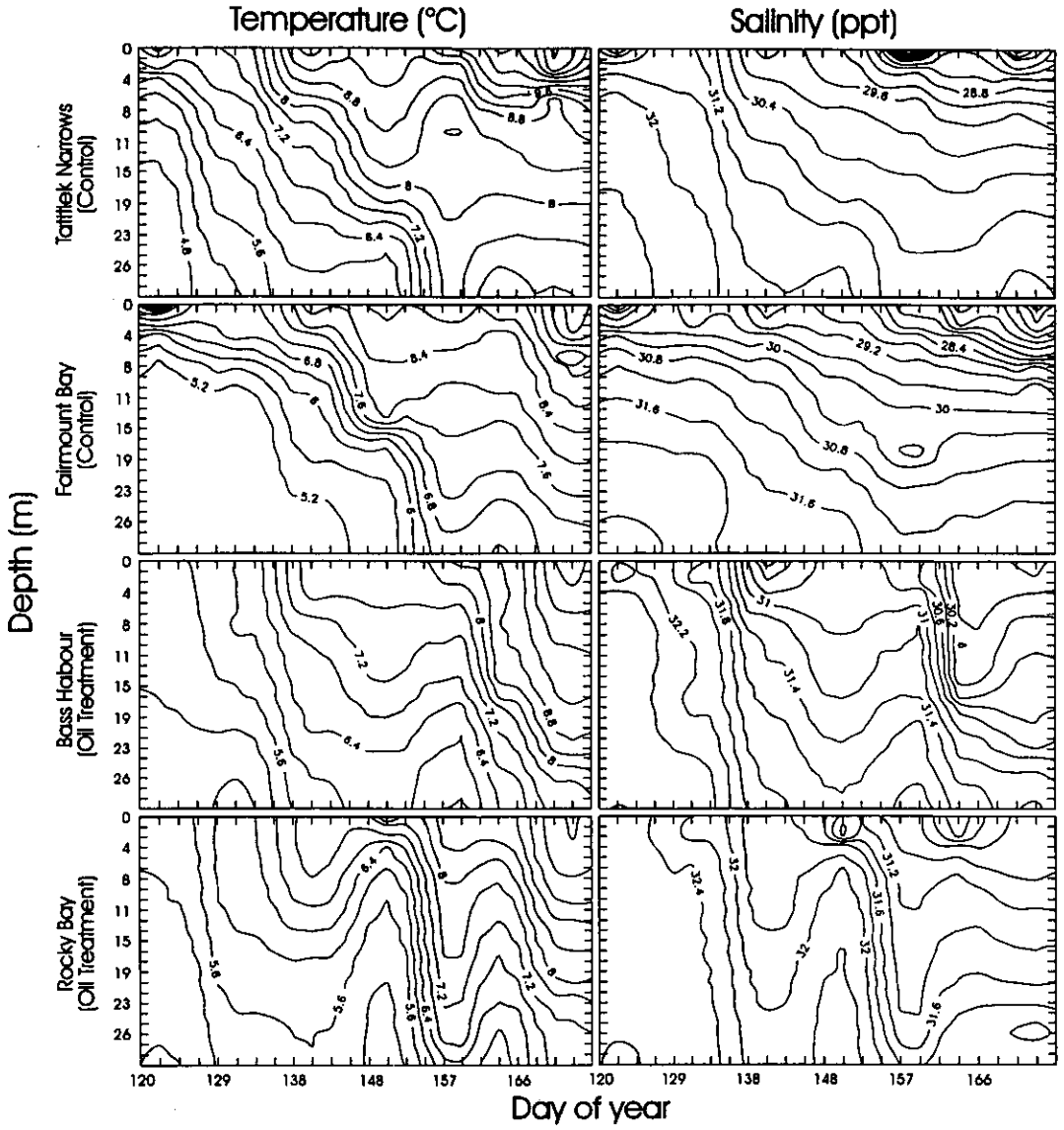
### Temperature and salinity

All terms in the three-factor ANOVA of temperature were highly significant. Tatitlek Narrows had a higher mean temperature (7.4°C) than Rocky Bay (6.6°C), but there were no differences in mean temperature between Rocky Bay, Bass Harbor (6.9°C), and Fairmount Bay (6.9°C) (Fig. 2). Mean temperature increased from 5.1°C on May 2–3 to 8.7°C on June 20–22, Bass Harbor and Tatitlek Narrows having faster warming rates than Fairmount Bay and Rocky Bay. Mean temperature decreased from 8.8°C at the surface to 5.8°C at 30 m, Fairmount Island and Tatitlek Narrows having steeper temperature gradients than Bass Harbor or Rocky Bay.

For salinity, all terms in eq. 1 were highly significant except for area–cruise and area–cruise–depth interactions. Bass Harbor and Rocky Bay had higher mean salinity (31.58 parts per thousand (ppt) and 31.69 ppt, respectively) than Fairmount Bay (30.43 ppt) and Tatitlek Narrows (30.75 ppt). Mean salinity decreased from 31.65 ppt on May 2–3 to 30.35 ppt on June 20–22, and increased from 28.77 ppt at the surface to 31.91 ppt at 30 m, the two control stations having steeper salinity depth gradients than the two oil treatment stations.

These results demonstrate the strong influences of water depth and of the distance between stations and the entrance to the Gulf of Alaska on the physical environment of herring eggs and larvae. Both control stations were shallower than the oiled stations, so they tended to warm up faster. Control stations were also closer to the glaciers on the northern shore of the sound and farther from Hinchinbrook Entrance than oiled stations. The glaciers pump fresh water into the nearshore zone whereas Hinchinbrook Entrance is the major entrance route into the sound for cold, saline water from the Gulf of Alaska. At least one period of mixing, perhaps driven by storms, occurred in June at each of the oiled sites, but none occurred at the control sites. Thus, oiled stations were always colder, more saline, and more mixed than control stations.

**Fig. 2.** Depth–time contour plots of temperature and salinity at four plankton stations in Prince William Sound, 1989.




### Timing of herring spawning and hatching

Aerial surveys for the presence of milt patches near spawning beaches indicated that most spawning occurred between April 8 and 15, the midpoints of the spawning period falling between April 11 and 13 (Table 2). Scuba surveys of egg density were conducted 10–16 days later. Hatch dates of larvae occurred 9–12 days after the mid-dates of the scuba surveys (Tables 1 and 2).

### Egg density

The egg density of 900 quadrats from 87 transects ranged from 100 to 4 600 152 eggs/m$^2$. The geometric mean egg density for Tatitlek Narrows (75 508 eggs/m$^2$) was significantly lower than the geometric mean egg density for the three other areas (Fairmount Bay, 191 894 eggs/m$^2$; Bass Harbor, 243 970 eggs/m$^2$; Rocky Bay, 208 147 eggs/m$^2$) (ANOVA: $P < 0.001$) (Table 3).

### Spatial distribution of eggs

The spatial distribution of eggs differed between the four areas. This was important for evaluating between-area differences in egg–larval mortality because it confounded natural and anthropogenic effects. Mean ln(width (m)) of herring spawn was different among areas (ANOVA: $0.001 < P < 0.01$). Tukey's range test showed that this difference was due to greater width at Rocky Bay (geometric mean width 83 m) than at Tatitlek Narrows (geometric mean width 36 m) or at Fairmount Bay (geometric mean width 38 m) (Table 4). The geometric mean spawn width of 42 m at Bass Harbor was not significantly different from the spawn width at Rocky Bay, which implied that Rocky Bay had an average beach gradient lower than that of Fairmount Bay and Tatitlek Narrows because spawn width is inversely related to beach gradient. Because egg loss owing to scouring by wave action decreases with increasing depth, low-gradient beaches will have greater rates of scouring than high-gradient beaches, all other factors being equal. On this basis, the two control areas would be expected to have lower egg–larval mortality than Rocky Bay, which is the same result expected from a direct relationship between egg–larval mortality and exposure to the *Exxon Valdez* oil spill.

**Table 2.** Dates of spawning, scuba survey of egg density, and hatch for four herring spawning areas of Prince William Sound in 1989.

| Area | Herring spawning | | Egg survey | | Date of hatch | |
|---|---|---|---|---|---|---|
| | Range | Midpoint | Range | Midpoint | Range | Mean |
| Control | | | | | | |
| Tatitlek Narrows | April 8–15 | April 11 | April 21–22 | April 22 | May 4 | May 4 |
| Fairmount Bay | April 11–15 | April 13 | April 23–25 | April 24 | May 1–5 | May 3 |
| Oiled | | | | | | |
| Bass Harbor | April 8–15 | April 11 | April 26 | April 26 | May 7–9 | May 8 |
| Rocky Bay | April 12–15 | April 13 | April 29 | April 29 | May 10–11 | May 10 |

**Table 3.** Herring egg–larval mortality at four areas in Prince William Sound.

| | $\ln(N_e)$ | $\ln(N_0)$ | $\ln(s)$ | $s$ | $t$ (days) | $Z_{el}$ (day$^{-1}$) |
|---|---|---|---|---|---|---|
| **Control: Tatitlek Narrows** | | | | | | |
| Mean | 11.2320 | 8.9861 | −2.2459 | 0.1058 | 12 | 0.1872 |
| SD | 2.0020 | 2.4618 | 3.1731 | | 1 | 0.2770 |
| $n$ | 168 | 10 | 176 | | | 176 |
| **Control: Fairmount Bay** | | | | | | |
| Mean | 12.1647 | 10.4929 | −1.6718 | 0.1879 | 9 | 0.1858 |
| SD | 1.7185 | 2.4093 | 2.9594 | | 2 | 0.3314 |
| $n$ | 413 | 10 | 421 | | | 421 |
| **Oiled: Bass Harbor** | | | | | | |
| Mean | 12.4048 | 7.4385 | −4.9663 | 0.0070 | 12 | 0.4139 |
| SD | 1.9606 | 3.1579 | 3.7170 | | 1 | 0.3117 |
| $n$ | 124 | 10 | 132 | | | 132 |
| **Oiled: Rocky Bay** | | | | | | |
| Mean | 12.2460 | 6.9941 | −5.2519 | 0.0052 | 11 | 0.4774 |
| SD | 1.7438 | 4.3176 | 4.6564 | | 3 | 1.6261 |
| $n$ | 195 | 8 | 201 | | | 201 |

Distribution of herring spawn between intertidal and subtidal (≤mean lower low water) habitats also varied between areas, ranging from 48.8% subtidal at Tatitlek Narrows to 75.4% subtidal at Bass Harbor (Table 4). There is little doubt that mortality of herring eggs is greater in the intertidal zone than in the subtidal zone owing to greater probability of desiccation and bird predation (Cleaver and Frannett 1946; Outram 1958; Steinfeld 1971; Taylor 1971; Palsson 1984; Johannessen 1986; Haegele and Schweigert 1989, 1991; Haegele 1993*a*, 1993*b*). Therefore, egg–larval mortality may decrease directly with increasing percent subtidal eggs, which implies that Tatitlek Narrows should have had the highest egg–larval mortality followed in descending order by Rocky Bay, Fairmount Bay, and Bass Harbor.

**Table 4.** Width of herring spawn and percentage of eggs in subtidal habitat at four herring spawning areas of Prince William Sound in 1989.

| Area | ln(spawn width (m)) | | | Percent subtidal[a] |
|---|---|---|---|---|
| | Mean | SD | $n$ | |
| Control | | | | |
| Tatitlek Narrows | 3.5747 | 0.7773 | 22 | 48.8 |
| Fairmount Bay | 3.6510 | 0.8096 | 45 | 72.0 |
| Oiled | | | | |
| Bass Harbor | 3.7297 | 1.1596 | 9 | 75.4 |
| Rocky Bay | 4.4172 | 0.7142 | 11 | 60.6 |

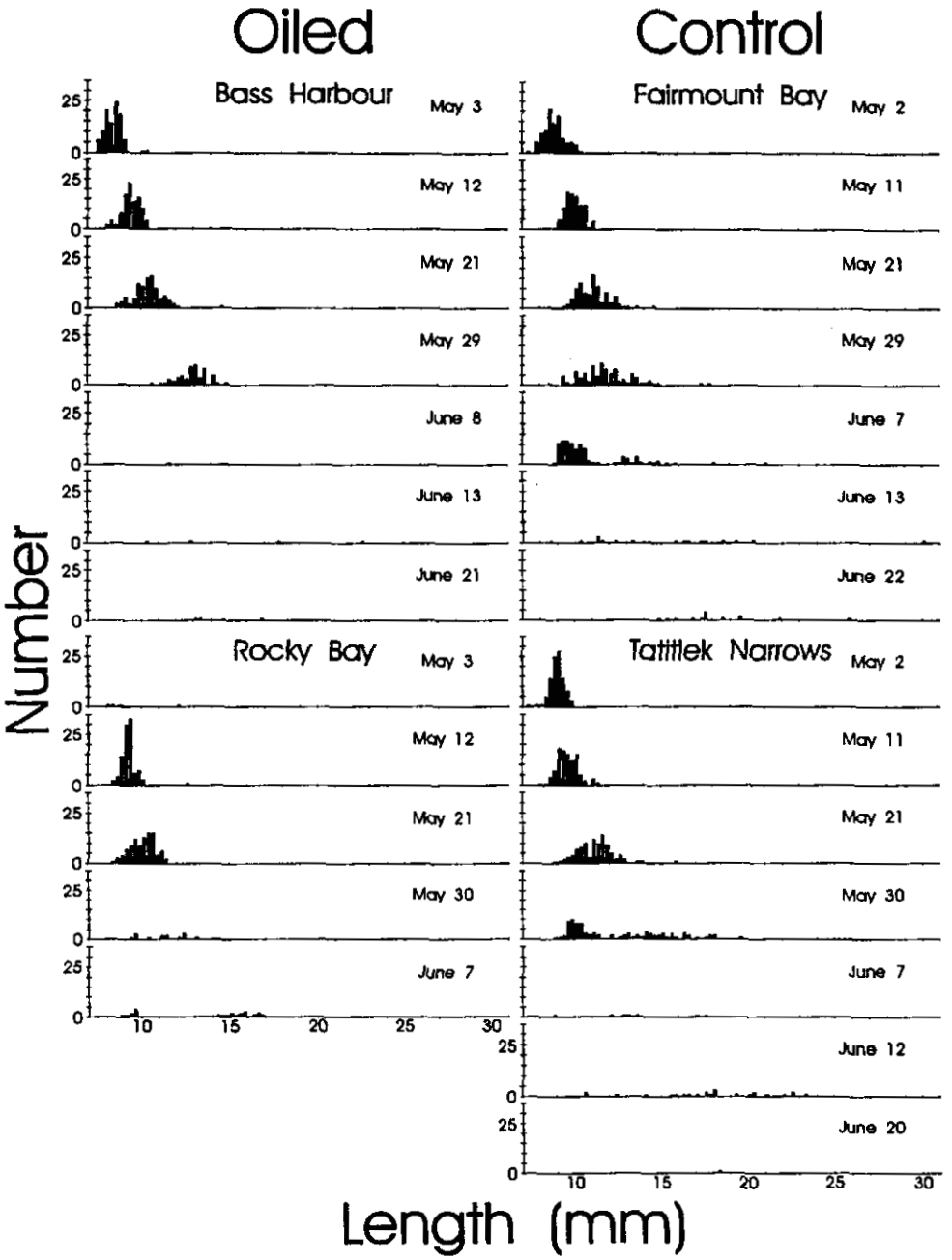
[a]Measured from all transects, not only those used for calculation of egg–larval mortality.

### Size and age of herring larvae

Herring larvae were captured on all seven sampling dates at three of the sites; larvae were not captured at Rocky Bay after June 7 (Fig. 3). One mode in the length frequencies was apparent at each site throughout most of May, but two modes appeared at Tatitlek Narrows on May 30 and at Fairmount Bay and Rocky Bay on June 7. Only one mode was observed at Bass Harbor throughout the study. None of the fish in the smaller of the two modes carried a yolk sac, suggesting that multiple modes may not have been due to hatching of a second cohort but to splitting of larval populations into slow- and fast-growing subgroups.

An ANOVA of larval length (eq. 2) showed that oil treatment was significant ($P = 0.003$) because the control larvae were longer (10.9 ± 2.6 mm (mean ± SD), $n = 955$) than the oiled larvae (10.1 ± 1.8 mm, $n = 607$). Cruise number and the interaction of cruise number and oil treatment were highly significant. These results were not changed if the analysis was restricted to the first five cruises in which larvae were captured at all stations. Repeated-measures ANOVA of the mean length of the first five cruises (eq. 3) showed that cruise number was the only highly significant factor. However, the nonsignificance of the oil treatment must be interpreted with caution because it had only two degrees of freedom. These results may be interpreted as evidence for an impact of oil on larval growth, or they may reflect differences in temperature-dependent growth between stations.

**Fig. 3.** Length–frequency distributions of herring larvae captured at four plankton stations in Prince William Sound, 1989.

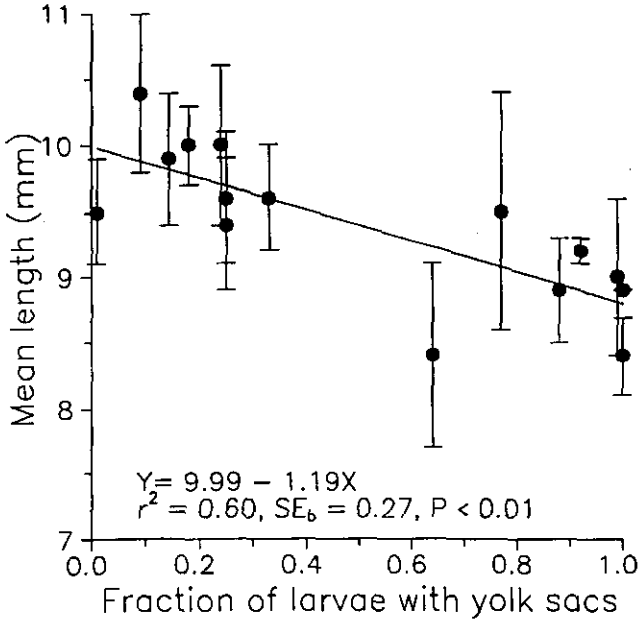



To estimate growth, mean length at hatch of herring larvae was first estimated to be 8.8 mm from a regression of mean length on the fraction of yolked fish (Fig. 4). Growth was well-described by a linear regression of length on day of year (Fig. 5). Growth ranged from 0.10 to 0.17 mm/day, and dates at hatch ranged from May 1 (DOY = 121) to May 10 (DOY = 130). There was no apparent effect of oil on growth rates; although the highest growth rate was measured at a control site, Tatitlek Narrows, the two oiled stations had the second and third highest growth rates. Nor was there an apparent effect of temperature. Although the greatest growth rate was measured at the warmest site, Tatitlek Narrows, the lowest growth rate was measured at an intermediate temperature.

**Larval density and loss**

Larval density varied (ANOVA: $P < 0.001$) with cruise number, but not with oil treatment or the interaction of oil treatment and cruise number. Similar results were obtained by repeated-measures ANOVA of mean larval density; cruise number was highly significant and oil treatment was not significant. Cruise number was significant because larval density followed a dome-shaped relationship with age at all stations (Fig. 6). The ascending limb of the catch curve represented recruitment of newly hatched larvae to the plankton station owing to a combination of hatching over several days and dispersal of larvae from the egg beds towards the plankton station. Ages of maximum larval density were close to zero at

**Fig. 4.** Regression of mean length (±1 SD) of samples of herring larvae from Prince William Sound, 1989, on the fraction of each sample that carried a yolk sac. Mean length at 100% yolked, 8.8 mm, is mean length at hatch.




all stations, indicating that larvae were fully recruited to the plankton stations within several days of hatching. The descending limb of the catch curve represents loss of larvae from the plankton station owing to dispersal and mortality.

To estimate average rate of loss of larvae from each station, larval densities were regressed on age for all ages greater than zero. There were no significant differences in density of newly hatched larvae or in total loss rate between sites. Estimated density of newly hatched larvae ranged from 1090 larvae/m$^2$ at Rocky Bay to 36 059 larvae/m$^2$ at Fairmount Bay (Table 3). Loss rates of larvae ranged from 0.21 to 0.25 day$^{-1}$, more than twice as great as the average mortality rate of 0.08 day$^{-1}$ reported for other populations of Pacific herring larvae (McGurk 1993).

### Egg–larval mortality

Subtracting ln($N_0$) from ln($N_e$) gave egg–larval survival rates that were higher in the two control areas than in the two oiled areas ($t$ test: $P < 0.001$). Dividing ln($s$) by the duration of the egg–larval period gave egg–larval mortalities that were lower in the two control areas than in the two oiled areas ($t$ test: $P < 0.001$). $Z_{el}$ was not significantly different between the two control areas, and it was not significantly different between the two oiled areas. Mean egg–larval mortality at oiled areas, 0.446 day$^{-1}$, was more than twice as great as mean egg–larval mortality at control areas, 0.186 day$^{-1}$.

## Discussion

This study found that herring egg–larval mortality was greater in oiled areas of Prince William Sound than in nonoiled areas, supporting the hypothesis that exposure of herring eggs to the *Exxon Valdez* oil led to a decrease in the survival and hatching success of late-stage embryos. This is not conclusive evidence of an oil effect on herring because an unknown portion of the differences in egg–larval mortality between oiled and control sites was caused by differences in the magnitude of egg desiccation, predation, and wave scouring. We cannot eliminate these factors because we have no independent information on the natural loss rates of herring eggs in 1989.

### Larval density at hatch

Larval density at hatch was the limiting factor in comparing egg–larval mortality; because of the three variables involved (egg density, larval density, and duration of the egg–larval stage), it was estimated with the least precision. The coefficients of variation (CV = 100 × SD/mean) of ln(larval density) ranged from 23 to 62%, several times greater than the CVs of the egg–larval period (range 8–27%) and the CVs of mean ln(egg density) (range 14–18%). This was partly due to fewer observations of larval density (range 8–10/site) than egg density (range 124–413/site) and partly due to several unavoidable sources of error inherent in prediction of larval density at hatch.

Error was introduced because larval density varied with time because of dispersal of larvae as well as mortality. We have no quantitative information on dispersal of herring larvae in Prince William Sound in 1989 that could be used to remove this effect. Norcross et al. (1996) reported that distribution of herring larvae in the sound in 1989 was in agreement with the general counterclockwise pattern of surface circulation in the sound. However, correction for dispersal of larvae would require numerical estimates of the rates of advection and diffusion of larvae at each site.

Error was also introduced because of variation in the proportions of the larval populations that were vulnerable to capture in plankton nets. Avoidance of plankton nets increases with larval length and decreases with towing speed and mesh size (McGurk 1992). Therefore, changes in towing speed and the distribution of larval lengths near a station, the change in mesh size from 0.333 to 0.505 mm after the first two cruises, and even changes in turbidity of the water may have affected catchability of larvae and estimated larval density. We have no satisfactory means of correcting for these changes in net avoidance, except to note that they would be minimal in newly hatched herring larvae.

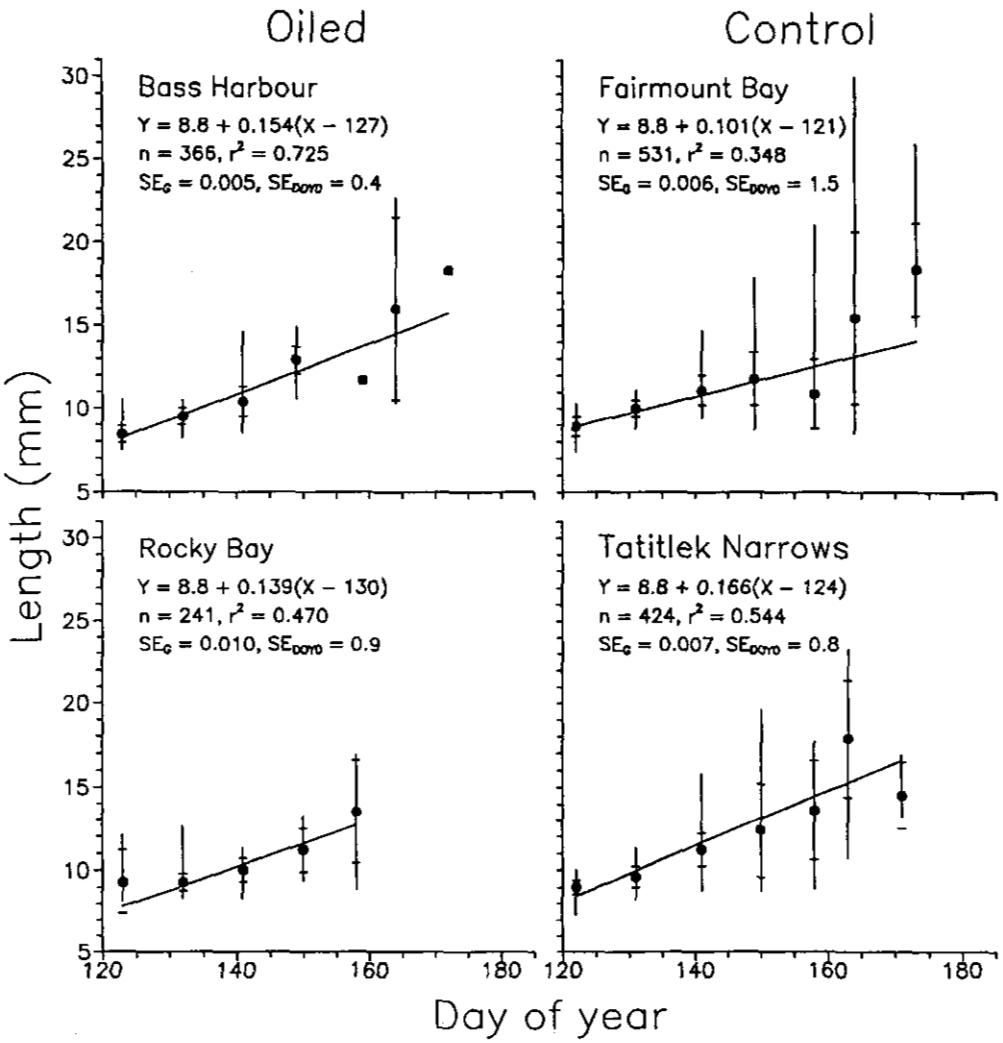
Error would also have been introduced into estimates of density at hatch if the populations of larvae consisted of more than one cohort. However, the evidence for more than one cohort at each site is ambiguous.

Finally, error was introduced to estimates of density at hatch by using a constant loss rate when actual loss rates changed with time. The right-hand limbs of all four catch curves show a slight curvilinearity that suggests loss rates decreased with age. Mortality of herring decreases with body weight to the power −0.40 (McGurk 1993), and dispersal of larvae would also decrease with age as larvae began to exhibit schooling behavior (McGurk 1987*b*). However, the curvilinearity of the catch curves is not strong enough to justify assuming anything other than a constant loss rate.

### Egg–larval mortality

Herring egg–larval mortality in Prince William Sound can be compared with expected rates of egg mortality in pristine environments. McGurk (1993) regressed published estimates of

**Fig. 5.** Growth of herring larvae at four stations in Prince William Sound, 1989. Solid circles are mean lengths at date, horizontal bars are ±1 SD of the mean, and vertical bars are ranges. Solid lines are nonlinear regressions of mean length on day of year (DOY) minus DOY of hatch, $DOY_0$, a free parameter in regression equation 4.



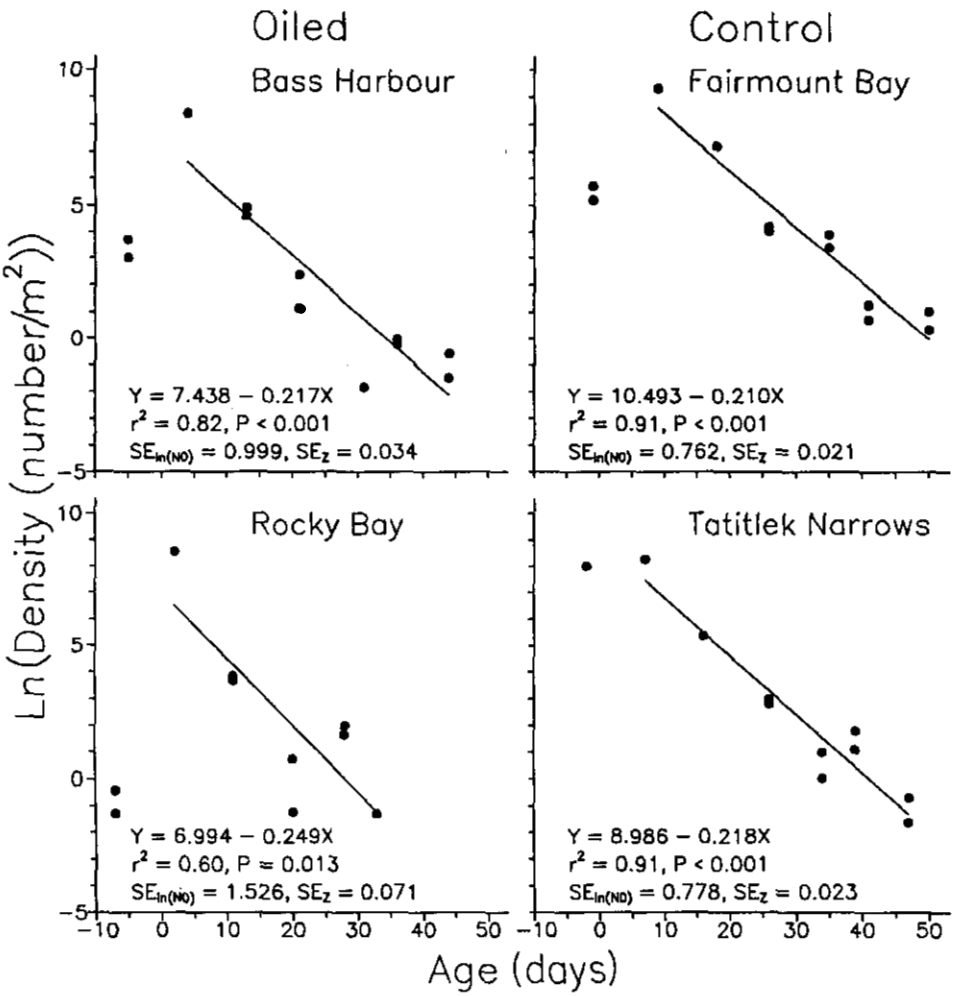

natural mortality of Pacific herring larvae and adults on dry body weight. By extrapolating this regression to a range of dry egg weights of 211–290 μg, he estimated that the average natural mortality rate of Pacific herring eggs ranges from 0.12 to 0.14 $day^{-1}$. Therefore, egg–larval mortality in Prince William Sound was 50% greater than expected at the two control sites and 215–270% greater than expected at the two oiled sites. However, egg–larval mortality may have been overestimated owing to underestimation of larval density caused by net avoidance at both control and oiled sites. According to McGurk's (1992) regression model of night–day catch ratios of herring larvae, a plankton net with a mesh width of 0.333 mm towed at 1 m/s catches only 48% of the 8.8 mm long larvae in its path. Therefore, if density of newly hatched herring larvae is doubled, then avoidance-corrected egg–larval mortality rates were 0.126 $day^{-1}$ for Tatitlek Narrows and 0.104 $day^{-1}$ for Fairmount Bay control sites, rates similar to expected egg mortality rates for the species. At the oiled sites, however, rates of 0.353 $day^{-1}$ for Bass Harbor and 0.411 $day^{-1}$ for Rocky Bay were 170–216% greater than the expected rate of natural mortality, a result that supports the oil-injury hypothesis.

### Growth of herring larvae

Comparisons of larval growth between sites within Prince William Sound and between the sound and other locations in the Pacific Ocean provide less certainty about an oil injury. On one hand, the difference in mean length of larvae between oiled and control sites was only 0.8 mm, too small to be considered strong evidence for an oil effect. There were also no differences in growth rate between sites that were clearly due to oil exposure, and the prey field for herring larvae was similar between oiled and nonoiled areas of the sound.

On the other hand, growth rates of 0.10–0.17 mm/day are about half the growth rates of wild populations of Pacific herring larvae (0.21–0.41 mm/day: Stevenson 1962; Iizuka 1966; Jones 1978; McGurk 1987*a*; McGurk et al. 1993). Norcross et al. (1996) reported a similarly low rate of growth, 0.15 mm/day, for herring larvae captured in offshore stations of Prince William Sound in May and June of 1989 and argued

**Fig. 6.** Regressions of density of herring larvae against age for four stations in Prince William Sound, 1989.




that it was evidence for a direct toxicological link between oil exposure and potential for herring larvae growth. This interpretation was supported by the Norcross et al. (1996) finding of deformed herring larvae. However, this raises the question of how growth rates of larvae from nonoiled areas could be as low as those from oiled areas; either there was immediate and rapid mixing of larvae from all areas or exposure to oil was far more widespread than was revealed by the trajectory of the oil slick. Either scenario implies that separation of oiled versus nonoiled sites may have been less definite than expected.

### Loss of herring larvae

Rates of loss of herring larvae were not significantly different between stations. However, comparison of loss rates is a weak test of an oil effect because loss rates included unknown rates of dispersal as well as mortality. Dispersal may have been greater at oiled stations than at control stations because of greater mixing at oiled stations. For the same reason, loss rates cannot be compared with natural mortality rates of Pacific herring larvae reported by other authors.

### Cohort structure or slow- and fast-growing larvae?

Comparison of rates of growth and loss of herring larvae may have been confounded by the assumption of only one cohort of larvae at each of the four spawning areas. Multiple spawnings separated by 2–4 weeks are a basic feature of the reproductive ecology of both Pacific and Atlantic herring (*Clupea harengus*) (Lambert 1984, 1987; Lambert and Ware 1984; Haegele and Schweigert 1985; Hay 1985). The oldest and largest fish tend to spawn first and the youngest and smallest fish spawn in subsequent waves. Ware and Tanasichuk (1989) showed that multiple waves of spawning of Pacific herring occur because gonad growth depends on the weight of the fish, so heavier, older fish mature earlier than smaller, younger fish. Multiple cohorts of Pacific herring larvae have been reported from British Columbia (Stevenson 1962; Robinson and Ware 1988; McGurk 1989), southeast Alaska (McGurk et al. 1993), and Hokkaido Island, Japan (Iizuka 1966).

The assumption of only one cohort was reasonable in the absence of observation of a second wave of spawners by aerial surveyors or scuba divers. Also, no yolked larvae were captured after mid-May. However, the length–frequency distributions clearly exhibit two modes at Tatitlek Narrows on May 30 and at Fairmount Bay and Rocky Bay on June 7.

One reasonable explanation for these observations is that only one cohort hatched at each site, but that it split into two groups within 1 month of hatch: a group of slow growers and a group of fast growers. Slow growers would presumably be

the deformed larvae reported by Norcross et al. (1996) and fast growers would be fish with normal growth potential. This scenario is speculative, but the production of two modal groups of larvae from a single spawning event is not unknown. Wespestad and Moksness (1990) reported such a phenomenon in Pacific herring reared from the egg to larval metamorphosis in large enclosures.

Regardless of whether low growth rates are interpreted as an effect of exposure of embryos or larvae to oil or as the result of mixing of multiple cohorts, it would have little or no effect on the dates of hatch estimated from the growth curves because the predicted lengths at date were indistinguishable from the mean lengths of the first samples taken in May, samples that were indisputably composed of one cohort.

**Summary**

We found no significant differences between oiled and nonoiled sites in the rates of growth or loss of herring larvae. Interpretation of these results as evidence for an oil effect on herring larvae is confounded by different temperature and salinity regimes at each station, by possible differences in rates of dispersal of larvae among stations, and by some uncertainty about the identification of stations as oiled or nonoiled. However, we found significantly higher egg–larval mortality rates at oiled sites compared with control sites. This finding is less confounded by problems of identifying oiled and nonoiled areas than for the other parameters investigated because eggs are fixed to the substrate and newly hatched larvae are not old enough to disperse great distances. Also, we found low growth rates of larvae compared with populations of Pacific herring larvae from pristine areas. Evidence provided by Norcross et al. (1996) of morphological and cytological deformations in herring larvae captured in Prince William Sound in 1989 combined with our findings indicate that the *Exxon Valdez* oil spill may have had a significant negative impact on the proportion of herring eggs that survived hatching.

## Acknowledgments

This study was funded by the Alaska Outer Continental Shelf Region of the Minerals Management Service, U.S. Department of the Interior, Anchorage, Alaska, under contract number 14-35-0001-30562, through an interagency agreement with the National Ocean Service, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and by the Alaska Department of Fish and Game under contract Nos. IHP-90-055 and IHP-93-011. We gratefully acknowledge the assistance of L. Jarvela, R. Meyer, P. Harmon, V. Komori, D. Warburton, D. Tesch, and M. Galbraith. Earlier drafts of this paper were read by T. Baker, D. Cushing, D. Hay, M. Paine, and several anonymous reviewers. The conclusions of this article are those of the authors and may not reflect the opinions of other individuals or organizations involved in assessment of the impact of the *Exxon Valdez* oil spill.

## References

Alaska Department of Fish and Game. 1991. Prince William Sound area finfish management report, 1989. Regional information report No. 2C90-07. Alaska Department of Fish and Game, Cordova, Alaska.

Alderdice, D.H., and Velsen, F.P.J. 1971. Some effects of salinity and temperature on early development of Pacific herring (*Clupea pallasi*). J. Fish. Res. Board Can. **28**: 1545–1562.

Cleaver, F.C., and Frannett, D.M. 1946. The predation by sea birds upon the eggs of the Pacific herring (*Clupea pallasi*) at Holmes Harbor during 1945. Biological report No. 46B. Washington (State) Department of Fisheries, Olympia, Wash.

Colton, J.B., Jr., Green, J.R., Byron, R.R., and Frisella, J.L. 1980. Bongo net retention rates affected by towing speed and mesh size. Can. J. Fish. Aquat. Sci. **37**: 606–623.

Haegele, C.W. 1993*a*. Seabird predation of Pacific herring, *Clupea pallasi*, spawn in British Columbia. Can. Field-Nat. **107**: 73–82.

Haegele, C.W. 1993*b*. Epibenthic invertebrate predation of Pacific herring, *Clupea pallasi*, spawn in British Columbia. Can. Field-Nat. **107**: 83–91.

Haegele, C.W., and Schweigert, J.F. 1985. Distribution and characteristics of herring spawning grounds and description of spawning behaviour. Can. J. Fish. Aquat. Sci. **42**(Suppl. 1): 39–55.

Haegele, C.W., and Schweigert, J.F. 1989. Egg loss from Pacific herring spawns in Barkley Sound in 1988. Can. Manuscr. Rep. Fish. Aquat. Sci. No. 2037.

Haegele, C.W., and Schweigert, J.F. 1991. Egg loss in herring spawns in Georgia Strait, British Columbia. *In* Proceedings of the International Herring Symposium, Anchorage, Alaska, October 23–25, 1990. Alaska Sea Grant report No. 91-01. Alaska Sea Grant, Fairbanks, Alaska. pp. 309–322.

Haegele, C.W., Humphreys, R.D., and Hourston, A.S. 1981. Distribution of eggs by depth and vegetation type in Pacific herring (*Clupea harengus pallasi*) spawnings in southern British Columbia. Can. J. Fish. Aquat. Sci. **38**: 381–386.

Hay, D.E. 1985. Reproductive biology of Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. **42**(Suppl. 1): 111–126.

Iizuka, A. 1966. Studies on the early life history of herring (*Clupea pallasi* C. et V.) in Akkeshi Bay and Lake Akkeshi, Hokkaido. Bull. Hokkaido Natl. Fish. Res. Inst. **31**: 18–63.

Johannessen, A. 1986. Recruitment studies of herring (*Clupea harengus* L.) in Lindaspollene, western Norway. Fiskeridir. Skr. Ser. Havunders. **18**: 139–240.

Jones, J.D. 1978. Growth of Pacific herring in Auke Bay, Alaska, in 1975 and 1976. M.Sc. thesis, University of Alaska Southeast, Juneau, Alaska.

Lambert, T.C. 1984. Larval cohort succession in herring (*Clupea harengus*) and capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. **41**: 1552–1564.

Lambert, T.C. 1987. Duration and intensity of spawning in herring (*Clupea harengus*) as related to the age structure of the mature population. Mar. Ecol. Prog. Ser. **39**: 209–220.

Lambert, T.C., and Ware, D.M. 1984. Reproductive strategies of demersal and pelagic spawning fish. Can. J. Fish. Aquat. Sci. **41**: 1565–1569.

McGurk, M.D. 1987*a*. Age and growth of Pacific herring larvae based on length-frequency analysis and otolith ring number. Environ. Biol. Fishes, **20**: 33–47.

McGurk, M.D. 1987*b*. Spatial patchiness of Pacific herring larvae. Environ. Biol. Fishes, **20**: 81–89.

McGurk, M.D. 1989. Advection, diffusion and mortality of Pacific herring larvae *Clupea harengus pallasi* in Bamfield Inlet, British Columbia. Mar. Ecol. Prog. Ser. **51**: 1–18.

McGurk, M.D. 1992. Avoidance of towed nets by herring larvae: a model of night–day catch ratios based on larval length, net speed and mesh width. J. Plankton Res. **14**: 173–182.

McGurk, M.D. 1993. Allometry of herring mortality. Trans. Am. Fish. Soc. **122**: 1035–1042.

McGurk, M.D., Paul, A.J., Coyle, K.O., Ziemann, D.A., and Haldorson, L.J. 1993. Relationships between prey concentration and growth, condition and mortality of herring larvae in an Alaskan subarctic embayment. Can. J. Fish. Aquat. Sci. **50**: 163–180.

Neff, J.M. 1991. Water quality in Prince William Sound and the Gulf of Alaska. Arthur D. Little, Inc., Cambridge, Mass.

Neter, J., and Wasserman, W. 1974. Applied linear statistical models. Richard D. Irwin, Inc., Homewood, Ill.

Norcross, B.L., Hose, J., Frandsen, M., and Brown, E.D. 1996. Distribution, abundance, morphological condition, and abnormalities of larval herring in Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. **53**: 2376–2387.

Outram, D.M. 1958. The magnitude of herring spawn losses due to bird predation on the west coast of Vancouver Island. Fish. Res. Board Can. Prog. Rep. Pac. Coast Stn. No. 111. pp. 9–13.

Palsson, W.A. 1984. Egg mortality upon natural and artificial substrata within Washington State spawning grounds of Pacific herring (*Clupea harengus pallasi*). M.Sc. thesis, University of Washington, Seattle, Wash.

Robinson, S.M.C., and Ware, D.M. 1988. Ontogenetic development of growth rates in larval Pacific herring, *Clupea harengus pallasi*, measured with RNA–DNA ratios in the Strait of Georgia, British Columbia. Can. J. Fish. Aquat. Sci. **45**: 1422–1429.

Royer, T.C., Vermersch, J.A., Weingartner, T.J., Neibauer, H.J., and Muench, R.D. 1990. Ocean circulation influencing the *Exxon Valdez* oil spill. Oceanography (Washington, D.C.), **3**: 3–10.

Short, J.W., and Harris, P.M. 1996. Chemical sampling and analysis of petroleum hydrocarbons in near-surface seawater of Prince William Sound after the *Exxon Valdez* oil spill. *In* Proceedings of the *Exxon Valdez* oil spill Symposium. *Edited by* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright. Am. Fish. Soc. Symp. 18. pp. 17–28.

SPSS Inc. 1988. SPSS/PC+ V2.0 base manual and advanced statistics. SPSS Inc., Chicago, Ill.

Steinfeld, J.D. 1971. Distribution of Pacific herring spawn in Yaquina Bay, Oregon, and observations on mortality through hatching. M.Sc. thesis, Oregon State University, Corvallis, Oreg.

Stevenson, J.C. 1962. Distribution and survival of herring larvae (*Clupea harengus pallasi* Valciennes) in British Columbia waters. J. Fish. Res. Board Can. **19**: 735–810.

Taylor, F.H.C. 1971. Variation in hatching success in Pacific herring (*Clupea pallasi*) eggs with water depth, temperature, salinity and egg mass thickness. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, **160**: 34–41.

von Westernhagen, H. 1988. Sublethal effects of pollutants on fish eggs and larvae. *In* Fish physiology. Vol. XI. Part A. *Edited by* W.S. Hoar and D.J. Randall. Academic Press, New York. pp. 253–407.

Ware, D.M., and Tanasichuk, R.W. 1989. Biological basis of maturation and spawning waves in Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. **46**: 1776–1784.

Wespestad, V.G., and Moksness, E. 1990. Observations on growth and survival during the early life history of Pacific herring *Clupea pallasi* from Bristol Bay, Alaska, in a marine ecosystem. Fish. Bull. **88**: 191–200.

# Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphological, cytogenetic, and histopathological assessments, 1989–1991

**Jo Ellen Hose, Michael D. McGurk, Gary D. Marty, David E. Hinton, Evelyn D. Brown, and Timothy T. Baker**

**Abstract**: Following the *Exxon Valdez* oil spill in Prince William Sound, Alaska, in March 1989, Pacific herring (*Clupea pallasi*) larvae were evaluated for sublethal damage. From 1989 to 1991, egg masses were collected from oiled and unoiled beaches and incubated to hatch. Newly hatched herring larvae were assessed for morphological (skeletal, craniofacial, and finfold) deformities, cytogenetic abnormalities (anaphase–telophase aberrations), and histopathological lesions. In 1989, herring larvae from both oiled areas (Rocky Bay on Montague Island and Naked Island) had significantly more morphological deformities and cytogenetic abnormalities than did larvae from the unoiled location (Fairmont Bay). The extent of morphological and cytogenetic damage was correlated with oil exposure in adjacent native bay mussels. Larvae had no oil-related histopathological lesions. In 1990 and 1991, oil-related developmental and genetic effects were undetectable.

**Résumé** : Nous avons évalué les dégâts sublétaux subis par des larves de hareng du Pacifique (*Clupea pallasi*) dans les eaux du golfe du Prince-William (Alaska), à la suite du déversement de pétrole, en mars 1989, causé par le naufrage de l'*Exxon Valdez*. De 1989 à 1991, nous avons prélevé des masses d'oeufs à des plages polluées et non polluées, et nous avons assuré leur incubation jusqu'à l'éclosion. Nous avons examiné les larves nouvellement écloses pour voir si elles étaient atteintes de malformations morphologiques (du squelette, de la région crânofaciale et du repli cutané des nageoires), d'anomalies cytogénétiques (aberrations associées aux stades de l'anaphase et de la télophase) et de lésions histopathologiques. En 1989, les larves de hareng provenant des deux secteurs contaminés (la baie Rocky sur l'île Montague et l'île Naked) présentaient significativement plus de malformations morphologiques et d'anomalies cytogénétiques que les larves provenant du secteur non contaminé (la baie Fairmont). L'importance des dégâts morphologiques et cytogénétiques a été mise en corrélation avec l'exposition au pétrole chez des moules indigènes de la baie. Les larves ne manifestaient pas de lésions histopathologiques pouvant être associées au pétrole. En 1990 et en 1991, nous n'avons pas pu détecter d'effets génétiques et d'anomalies du développement attribuables au pétrole.
[Traduit par la Rédaction]

## Introduction

Pacific herring (*Clupea pallasi*) are highly sensitive to petroleum (Rice et al. 1979). Adult herring had the lowest 96-h $LC_{50}$ to crude oil (1 part per million (ppm) total aromatics) of 39 Alaskan marine species tested. The 12-day $LC_{50}$ value for herring eggs was 1.5 ppm total aromatics (Rice et al. 1987), although embryonic toxicity to oil is frequently manifested as malformations observable after hatching (Linden 1975, 1978; Pearson et al. 1985; Carls and Rice 1989; Kocan et al. 1996). Total hydrocarbon concentrations from 4 to 761 parts per billion (ppb) resulted in increased frequencies of abnormal herring larvae following exposure of eggs to undispersed oil. Oil may induce other sublethal effects in fish, including histopathological changes (Cameron and Smith 1980; Solangi and Overstreet 1982) and genetic damage (mutagenicity) in embryos (Longwell 1977). Because certain aromatic constituents of crude oil may be genotoxic (Dipple 1982), mutagenicity assessments in target organisms are important, although infrequently performed in field situations (U.S. National Research Council 1985).

The first evaluation of mutagenicity in the marine environment found cytogenetic damage coupled with high mortality in a limited sample of fish eggs collected near the *Argo Merchant* oil spill (Longwell 1977). Ambient oil concentrations were not measured. Examination of dividing chromosomes revealed higher incidences of abnormalities during the telophase portion of mitosis (chromosome bridges and chromosomes

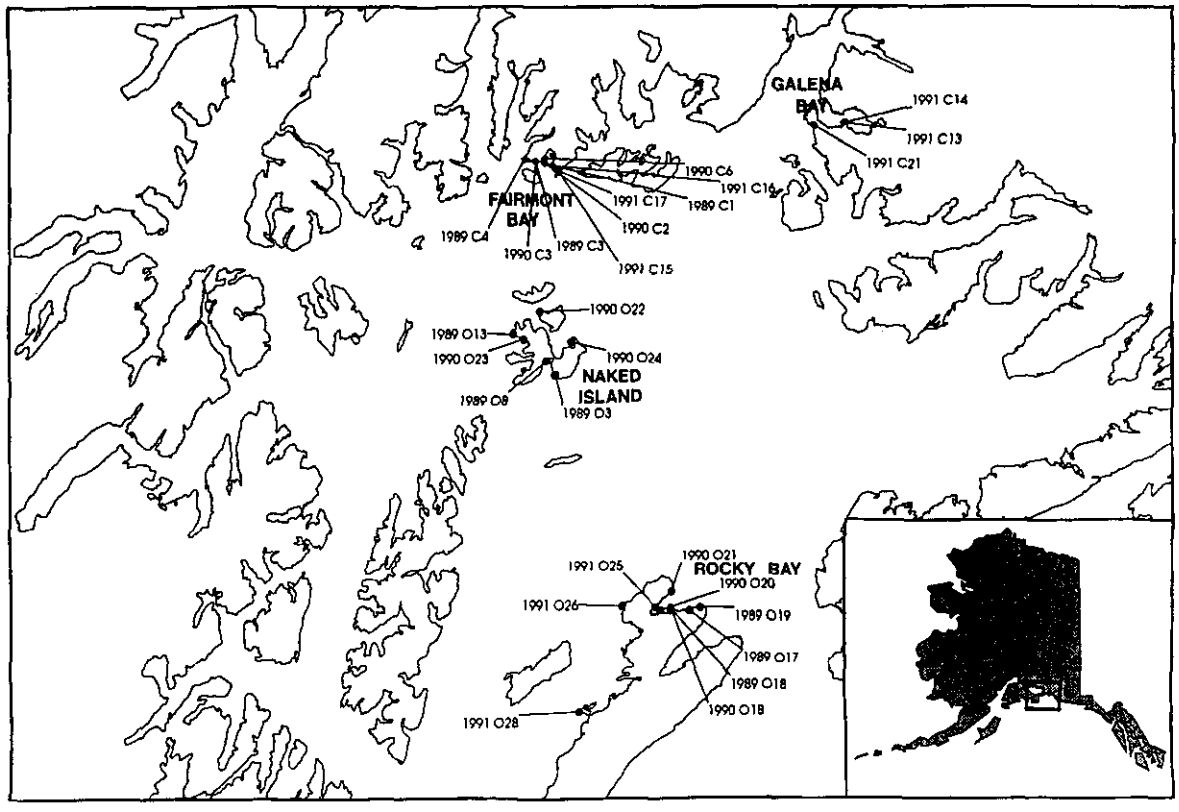
Received November 8, 1993. Accepted March 11, 1996.
J12152

**J.E. Hose.**[1] Department of Biology, Occidental College, 1600 Campus Road, Los Angeles, CA 90041, U.S.A.
**M.D. McGurk.** Triton Environmental Consultants, Ltd., Richmond, BC V6V 2L1, Canada.
**G.D. Marty and D.E. Hinton.** Department of Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616, U.S.A.
**E.D. Brown.** Institute of Marine Science, School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks, AK 99775–7220, U.S.A.
**T.T. Baker.** State of Alaska Fish and Game, Anchorage, AK 99518, U.S.A.

[1] Author to whom all correspondence should be sent at the following address: 2590 Softchess Place, Arroyo Grande, CA 93420, U.S.A.

**Fig. 1.** Sampling sites for egg incubation studies in Prince William Sound, Alaska, U.S.A., 1989–1991. Insert shows location of Prince William Sound within Alaska. Sites beginning with C are unoiled (control) stations; those with O are oiled stations.




failing to orient on the spindle) and lower mitotic activity in oiled embryos. Recently, a strong correlation was observed between the frequency of chlorophyll-deficit mutations in Puerto Rican mangrove trees and the concentration of polycyclic aromatic hydrocarbons (PAH) (Klekowski et al. 1994). Mutation frequencies were tripled in areas with >40 ppm PAH in the underlying oiled sediments. Further evidence for a relationship between genotoxicity and pollution has been obtained from investigations of embryonic Atlantic mackerel (Longwell and Hughes 1980), sea urchins (Long et al. 1990), and bivalve molluscs (Stiles et al.1991) and adult fish (Hughes and Hebert 1991). Fish embryos and larvae are particularly amenable for studying genotoxicity because dividing cells are abundant and because effects can be related at the organismal (external morphology), tissue–cell (histopathology), and chromosomal (cytogenetic) levels (Longwell and Hughes 1980).

In March 1989, a few weeks before the Pacific herring spawning season, oil spilled from the tanker *Exxon Valdez* drifted onto beaches in Prince William Sound (PWS), Alaska. Although at least some of these adults were exposed to *Exxon Valdez* oil (EVO) in certain staging areas, population abundance and egg biomass were not affected through 1992 (Brown et al. 1996). Herring eggs, however, may be vulnerable to oil exposure because they are adherent, demersal, and typically spawned onto sea grass or kelp in the intertidal and subtidal zones (depths of 0–20 m) (Smith and Cameron 1979). In 1989, approximately half of the egg biomass was deposited within the oil trajectory, and an estimated 40–50% sustained exposure to EVO (Brown et al. 1996). The objective of this study was to determine if potential embryonic exposure to EVO resulted in sublethal injury to herring larvae. Eggs were collected from oiled and unoiled PWS beaches and hatched in the laboratory. Larvae were evaluated for morphological defects and cytogenetic damage (1989–1991) and for histopathological changes (1989, 1990).

## Materials and methods

### Sites

In 1989, variable numbers of sites were sampled at three locations: 5 sites at Fairmont Bay (unoiled, C1–C5), 3 at Rocky Bay on Montague Island (oiled, O17–O19), and 12 on Naked Island (oiled, O1–O4, O8, O10–O16) (Fig. 1). Procedures for designating areas as unoiled or oiled in 1989 have been described previously (Brown et al. 1996). The 1989 designations were maintained in subsequent years to allow comparisons between previously unoiled and oiled locations. At each site, three replicate samples of eggs were removed from each of three depths. For morphological–cytogenetic analysis, sample sites and corresponding codes were Fairmont Bay (C1, C3, C4), Naked Island (O3, O8, O13), and Rocky Bay (O17–O19). The depths sampled were –1.5, 0, and 1.5 m mean low tide at all sites except for C3, O8, and O13, where –4.6, –1.5, and 0 m were used.

In 1990, four areas were sampled: Fairmont Bay (C2, C3, C6), Naked Island (O22–O24), Rocky Bay (O18, O20, O21) (Fig. 1), and Sitka Sound, an unoiled area in southeast Alaska (C18–C20). Sampling sites in 1991 were Fairmont Bay (C15–C17) and Rocky Bay (O25, O26, O28). Because herring did not spawn at Naked Island in 1991, a second unoiled site, Galena Bay (C13, C14, C21), was evaluated instead. In 1990 and 1991, three samples of eggs were collected from each of three depths (–4.6, –1.5, and 0 m).

**Sample collection**

Egg masses gathered by divers 9–16 days after spawning were packaged and flown to the laboratory (Triton Environmental Consultants, Richmond, British Columbia, Canada). Each sample was coded with a random number, placed inside a cone of Vexar mesh in a 1-L plastic jar containing seawater (14 parts per thousand (ppt) salinity), aerated, and maintained at 9°C until hatching commenced, usually 1 week later. Once a day, the water was poured into a glass bowl and newly hatched larvae were counted, a process that took about 10 min. Larvae were then preserved in 3.3% formalin in seawater. The remaining egg mass was then returned to fresh seawater and the process repeated daily until all eggs had either hatched or died. Inadvertent excessively vigorous aeration in 1989 resulted in death and postmortem decay (autolysis) of many newly hatched larvae before fixation. In subsequent years, aeration rates were reduced and rearing conditions optimized.

**Larvae for analysis**

Collection depths were analyzed as shallow, middle, and deep. For morphological and cytogenetic analysis of 1989 larvae, individuals were pooled from the three replicates to obtain a target sample size of 30–50 larvae per site–depth permutation (>100 per site, >300 per location). Although many of the 1989 larvae were autolyzed, only those that appeared well fixed were selected for analysis. Selection criteria were an unbroken yolk sac and an absence of bacterial colonies on pectoral fins. In 1990 and 1991, 10 larvae were randomly chosen from each of the replicates from every site–depth permutation (270 per location). Sitka Sound and Galena Bay larvae were analyzed only for morphological deformities.

For histopathology in 1989, larvae from 20 sites were examined for lesions. Regardless of gross appearance, five larvae were randomly chosen for most site–depth permutations (about 15 larvae per site, 282 larvae total). In addition, 26 larvae were selected from other samples because they had external deformities when examined under a dissecting microscope. In 1990, five randomly chosen larvae from one replicate per depth were examined for histopathological lesions (10 or 15 larvae for each of 12 sites, 183 larvae total). An additional six larvae from other replicates were examined because they had gross abnormalities.

**Morphological deformities**

Larvae were examined at 40× magnification for external abnormalities. Three malformation categories were scored: skeletal, craniofacial, and finfold. On the basis of Middaugh et al. (1988), deformities were assigned a graduated severity index (GSI) score as follows: 0, normal; 1, slight defect of size or structure; 2, moderate defect or multiple slight defects; or 3, severe defect or multiple moderate defects. Skeletal malformations consisted of notochord bends and stunting. Craniofacial defects included jaw abnormalities (size reduction, abnormal structure or absence), ocular malformations (reduced eye diameter (microphthalmia), pigment irregularities, etc., but not ocular protrusions), and otic deformities (size reduction or absence). Because histopathological examination showed that in individuals with ocular protrusions, microphthalmia resulted from retinal herniation during postmortem decay, only individuals without visible protrusions (herniation) were scored as microphthalmic. Finfold defects were a reduction in thickness or absence of the fin. Total GSI scores were the sum of the three individual malformation scores.

Statistical analyses for the GSI data followed methods of Middaugh et al. (1988) consisting of Kruskal–Wallis analysis of variance combining ranked data for all three locations, followed by pairwise testing against the pooled Fairmont Bay values. All locations had significant depth effects; however, depth-related trends were dissimilar among locations. Therefore, data were also analyzed separately by depth. The Fairmont Bay data were compared with data from each of the six oiled sites.

**Cytogenetic evaluation**

Larvae were randomized, and the methods of Longwell and Hughes (1980) were followed except that fins were evaluated instead of embryos or yolk sac tissues. The pectoral fin was chosen because it has been previously shown to be damaged by oil exposure (Linden 1975; Carls and Rice 1989), it remains throughout the life of the fish (unlike the yolk sac), and it contains a germinal layer between muscle cells of the fin base and the elongating ray structure. One pectoral fin from each larva was placed on a microscope slide, and a few drops of acetic orcein stain (19 parts saturated orcein in 45% acetic acid plus 1 part propionic acid) were applied for 10 min. Fins (from two individuals per slide) were covered with a 20 × 50 mm glass cover slip, gently flattened, and sealed.

Genetic toxicity can be manifested as a decreased ability of cells to successfully divide; therefore, toxic effects could include a depressed mitotic rate and (or) increased mitotic aberrations (Longwell and Hughes 1980). Both end points were evaluated using larval fins. The germinal layer provided adequate numbers of dividing cells and anaphase–elophase mitotic configurations. In addition, the elongating rays contained dividing cells.

Fins were coded and examined at 1000× using blind review. All mitotic configurations in the fin were counted, yielding an estimate of the mitotic rate (MIT). The mitotic aberration rate was calculated by examining all anaphase–telophase mitotic figures (AT) for chromosomal or chromatid breaks and for spindle defects. The criteria of Kocan et al. (1982) were used to categorize aberrations into translocation bridges, attached or acentric fragments, stray and lagging chromosomes, or multipolar spindles. Numbers of normal and aberrant AT were recorded from the entire fin. The percentage of anaphase aberrations (%AAT) was the total number of aberrant AT divided by the total number of AT (usually >100) in that sample. Numbers of micronucleated cells (interphase cells with evidence of prior chromosome breakage or spindle defects, Schmid (1976)) were noted. Cytogenetically abnormal fins were defined as having fewer than eight mitotic figures, >20% AAT per individual, or at least two micronucleated cells. The threshold of eight mitoses was derived from examination of fins with normal and deformed appearances; most normal fins contained more than eight mitoses. The 20% anaphase aberration rate threshold was chosen by rounding Kocan et al.'s (1982) control base line up to the nearest 10%. Percentages of cytogenetically abnormal larvae (%GA) were calculated for each sample and location. Differences between depths and locations were assessed using analysis of variance tests with Student–Newman–Keuls multiple range tests for MIT and $G$ statistics (group and pairwise, the latter using adjusted $\alpha$ values) for %AAT and %GA.

**Histopathology**

Larvae were preserved within 24 h after hatching and later processed for histopathological examination using the methods of Marty et al. (1990). Individuals were oriented as flat as possible in dorsal or ventral recumbency, and one larva was embedded per block, identified by an assigned random number. Each larva was sectioned at 5 μm; at approximately 80- μm intervals through each block, groups of three or four serial sections were saved and stained with hematoxylin and eosin, yielding 12–30 sections per larva. Sections were mounted and read in numerical order using blind examination, and most major organs were found in at least one of the sections. Selected larvae with gross lesions (26 in 1989, 6 in 1990) were embedded in glycol methacrylate, and sections 4 μm thick were saved at 25-μm intervals, yielding 10–15 sections per block.

Each larval section was examined for extent of autolysis, tissue artifacts, and lesions in major organs: gonad, retina, brain, heart, branchial arch, skin, skeletal muscle, kidney, gastrointestinal tract, yolk, liver, and spleen. Lesions were scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe) in relation to similar lesions. Each larva received a score for hepatic glycogen, lens vacuolation, and myofiber degeneration or necrosis; other lesions when present were recorded. For

**Table 1.** Significant correlations between sublethal end points and 1989 mussel tissue hydrocarbon concentrations.

| End point | EVO-PAH | ARO | NAPH | FLUOR | PHENA | DIBENZ | CHRYS | PHYTANE | BAP |
|---|---|---|---|---|---|---|---|---|---|
| Morphologic | | | | | | | | | |
| Skeletal GSI | ** | ** | ** | * | ** | ** | ** | ** | ** |
| Craniofacial GSI | | | | | | | | | * |
| Jaw reduced | | | | | | | | | |
| Jaw abnormal | | | | | | | | | |
| Jaw absent | | | | | | | | | |
| Jaw abnormal + absent | | | | | | | | | |
| Microphthalmia | * | * | | | | | | | |
| Otics absent | * | * | * | * | * | * | * | * | |
| Otics reduced | | | | | * | * | | | |
| Otics absent + reduced | * | * | * | * | * | * | * | * | |
| Finfold GSI | | | * | | * | * | * | | * |
| Total GSI | * | * | * | | * | * | * | | ** |
| Cytogenetic | | | | | | | | | |
| No. of mitoses per fin | | | | | | | | | |
| % anaphase aberrations | ** | ** | * | ** | ** | ** | ** | * | * |
| % cytogenetically abnormal | ** | ** | * | * | ** | ** | ** | * | * |

Note: EVO-PAH, *Exxon Valdez* oil polynuclear aromatic hydrocarbons; ARO, aromatic hydrocarbons; NAPH, naphthalenes; FLUOR, fluorenes; PHENA, phenanthrenes; DIBENZ, dibenzothiophenes; CHRYS, chrysenes; BAP, benzo[*a*]pyrene; *, $0.01 < p < 0.05$; **, $p < 0.01$).

statistical analysis, scores for oil levels, autolysis, artifact, and lens vacuolation were compared using Spearman correlation coefficients, $\alpha = 0.05$.

### *Exxon Valdez* oil analyses

Correlation analysis was performed between all morphological and cytogenetic responses shown in Table 1 and 1989 site-specific measurements of polynuclear aromatic hydrocarbons characteristic of *Exxon Valdez* oil (EVO-PAH) (Brown et al. 1996). Herring eggs were collected and analyzed for EVO-PAH measurements, but the amount of tissue was insufficient to provide adequate detection of EVO (J. Short, National Marine Fisheries Service, Juneau, AK, personal communication). Therefore, hydrocarbon concentrations in native bay mussels (*Mytilus trossulus*) were used as a biological integrator of oil exposure (Short and Rounds 1996). This approach, although allowing correlations to be made with a larger chemistry data base, does have several limitations. Mussel EVO concentrations should be considered only a rough estimate of EVO exposure in herring eggs because (*i*) the relationship between EVO uptake in mussels and herring eggs has not been established, (*ii*) mussels were collected at only one depth (usually near 0 m) whereas herring eggs were collected at three depths throughout their tidal range, (*iii*) it is generally accepted that mussels cannot metabolize petroleum hydrocarbons whereas fish embryos can metabolize them at low rates (Goksøyr et al. 1991), and (*iv*) the analyte patterns in mussels and herring eggs may differ because of variable modes of exposure and uptake. Accumulation of hydrocarbons by demersal fish eggs follows octanol–water partition coefficients and standard kinetics (Marty et al. 1990). Maternal contributions to herring egg hydrocarbon concentrations in 1989 are not known but expected to be small.

Resident mussels were collected adjacent to each site at the time of egg collection in 1989 (–0.7 m except at –0.3 m for sites O8, O18, and O19) and 1990 (–0.5 m except 0 m for site O20). The summed PAH concentrations (expressed as nanograms EVO-PAH/gram wet tissue weight) represent a mean of three (1989) or three to seven (1990) measurements per site. Correlation coefficients (Pearson *r* values) were calculated between each morphological or cytogenetic end point and $\log_{10}$ transformations of (*i*) EVO-PAH concentrations and (*ii*) mean concentrations of several classes of aromatic hydrocarbons. The threshold for statistical significance was $\alpha = 0.05$.

## Results

### Morphology

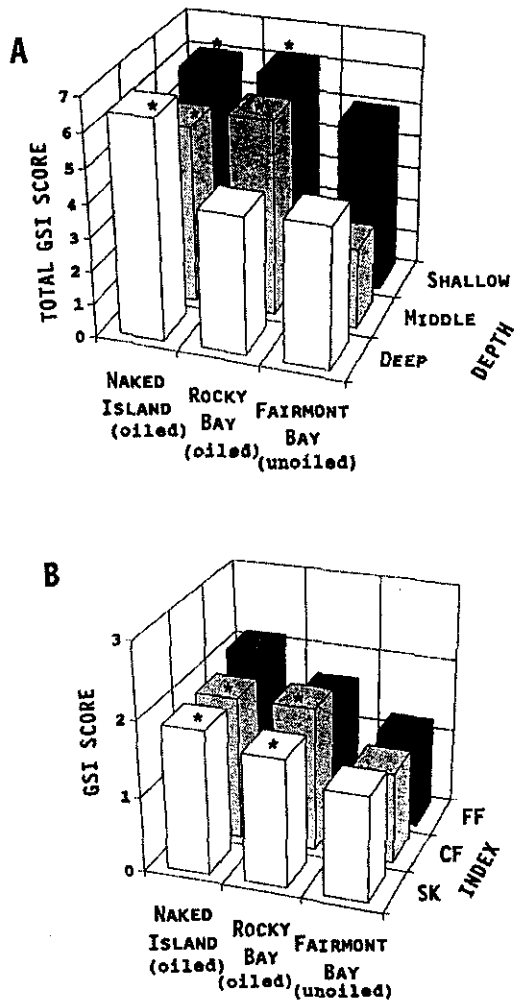
Total GSI scores from Naked Island were significantly greater than those from Fairmont Bay at every depth tested ($p < 0.01$, Fig. 2A). Most total GSI scores at the three Naked Island sites were also higher than the Fairmont Bay scores ($p < 0.01$). At Rocky Bay, pooled scores and individual site scores were higher for the shallow and middle depths but not for the deep stratum. Deformities of Naked Island larvae were more severe than those from Rocky Bay only at the deep stratum.

Results using the individual GSI scores paralleled those from the total GSI scores (Fig. 2B). At all depths, the severity of skeletal defects in Naked Island and Rocky Bay larvae was statistically greater than in Fairmont Bay fish. For most site–depth permutations, skeletal GSI scores were higher at each of the oiled sites. Compared with Fairmont Bay, finfold malformations were more severe at all Naked Island depth strata and for only the middle depth at Rocky Bay. Individual oiled sites displayed an identical trend for finfold GSI scores.

Craniofacial GSI scores from Naked Island and Rocky Bay larvae were higher than those of Fairmont Bay fish (Fig. 2B). By location, differences were highly significant ($p < 0.01$) at all depths except for the deep stratum at Rocky Bay. Larvae at all Naked Island sites had elevated craniofacial scores as did those at the shallow and middle depths at Rocky Bay sites. Certain types of craniofacial defects had higher incidences at the oiled locations. Incidences of abnormal or absent lower jaws were significantly greater at one or both oiled locations, but the proportion of larvae with reduced jaw size was not statistically significant (Fig. 3A). Microphthalmia (not associated with retinal herniation) was most common in Naked Island larvae (6.3 ± 4.9% (mean ± SE) versus 0.2 ± 0.2% at Fairmont Bay and 2.3 ± 1.2% at Rocky Bay). Percentages of reduced and absent otic capsules were higher in larvae at both oiled locations (Fig. 3B).

In 1990 and 1991, virtually no morphological differences

**Fig. 2.** (A) Total graduated severity index (GSI) scores by depth (deep, middle, and shallow) in 1989. Scores significantly different ($p < 0.05$) from Fairmont Bay values are marked with an asterisk. (B) Graduated severity index (GSI) scores for skeletal (SK), craniofacial (CF), and finfold (FF) defects in 1989. Scores significantly different from Fairmont Bay values are marked with an asterisk.



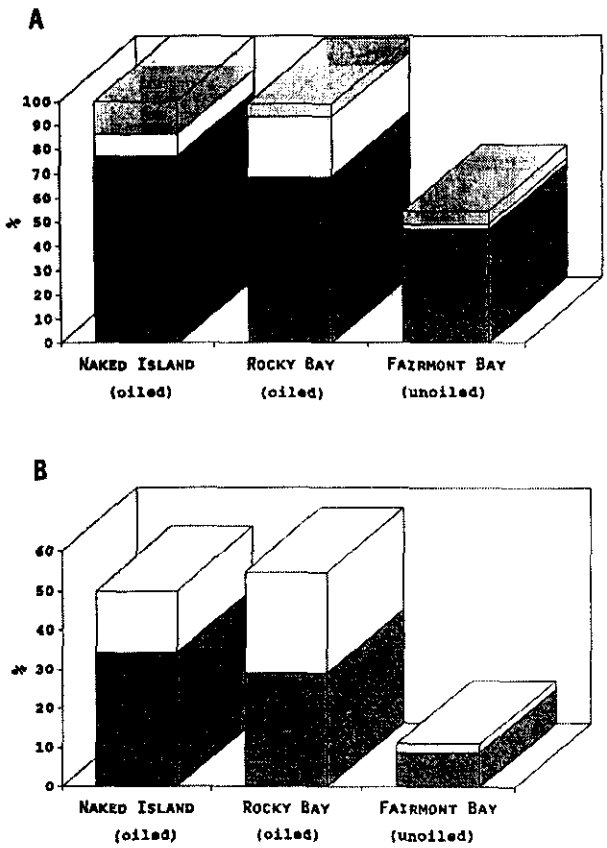

were observed in larvae from unoiled and previously oiled areas (Fig. 4). In 1990, larval GSI scores were similar at both unoiled areas, Fairmont Bay and Sitka Sound in southeast Alaska. The only significant difference at previously oiled areas relative to Fairmont Bay was a slight increase in larval deformities (craniofacial and total GSI scores) at Naked Island.

In 1991, GSI measurements were remarkably similar between Fairmont Island and Rocky Bay (Fig. 4) except for a greater finfold GSI score at Rocky Bay. Larvae from Galena Bay (an unoiled area) had significantly higher skeletal, craniofacial, and total scores than Fairmont Bay or Rocky Bay fish. However, the observed 31% increase in the total GSI score was lower than the percent difference between Fairmont Bay and oiled areas in 1989.

### Cytogenetic evaluation

For every depth, the number of mitoses per fin from the pooled

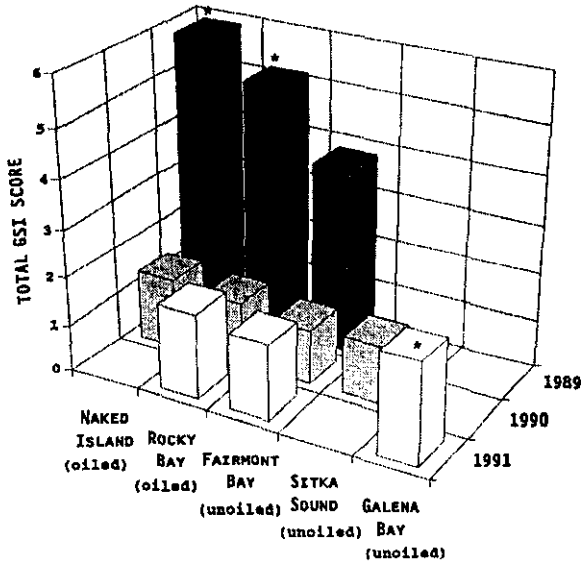
**Fig. 3.** (A) Incidence of reduced jaw size (dark grey), abnormal jaw (light grey), and absent lower jaws (white) in 1989. (B) Incidence of reduced otic capsule size (dark grey) and absent otic capsules (white) in 1989.




Fairmont Bay data averaged above eight, the lower threshold for normality. Anaphase aberration rates averaged from 8 to 13% for all depths and were thus within the expected range of 0–20% (Fig. 5A). These two end points were combined to designate each larva as cytogenetically normal or abnormal; an abnormal larva had either a low mitotic rate (MIT < 8) or evidence of genetic damage (%AAT > 20%). The percentage of cytogenetically abnormal Fairmont Bay larvae averaged 48%, ranging from 31% (middle depth) to 61% (deep) (Fig. 5B).

The cytogenetic data paralleled morphological results in that (*i*) larvae from both oiled areas were more adversely affected than Fairmont Bay larvae and (*ii*) Naked Island larvae had more damage than those from Rocky Bay. The anaphase aberration rates at Naked Island were 2–6 times above their respective Fairmont Bay values (Fig. 5A). Significantly higher percentages of cytogenetically abnormal larvae were present at all depths from Naked Island (90% versus Fairmont Bay's 48%) (Fig. 5B). All three sites exhibited significantly elevated genotoxicity. At Rocky Bay, the cytogenetic data were more variable. Only the deep and middle depths had significantly higher percentages of anaphase aberrations and cytogenetically abnormal larvae than did Fairmont Bay. Larvae from two sites on the outer edge of Rocky Bay (O17 and O19) had significant genetic damage. Cytogenetic end points from the sheltered site (O18) were similar to the pooled Fairmont Bay values. The mean anaphase aberration rate at site O18 was

**Fig. 4.** Total graduated severity index (GSI) scores in 1989–1991. Scores significantly different ($p < 0.05$) from the corresponding Fairmont Bay values are marked with an asterisk.




24%, and the percentage of cytogenetically abnormal larvae was 78%.

In 1990, cytogenetic end points were similar at Fairmont Bay and Naked Island, with anaphase aberration rates of 18–19% (Fig. 6A), and 42–43% cytogenetically abnormal larvae (Fig. 6B). Rocky Bay had a significantly elevated anaphase aberration rate (33%) and a higher percentage of cytogenetically abnormal larvae (67%).

In 1991, cytogenetic end points were almost identical between Fairmont Bay and Rocky Bay. Anaphase aberration rates were 22%, significantly higher than the 1989 rate of 10% (Fig. 6A). Similarly, percentages of cytogenetically abnormal larvae (57–58%) were significantly higher than in 1989 (Fig. 6B).

## Histopathology

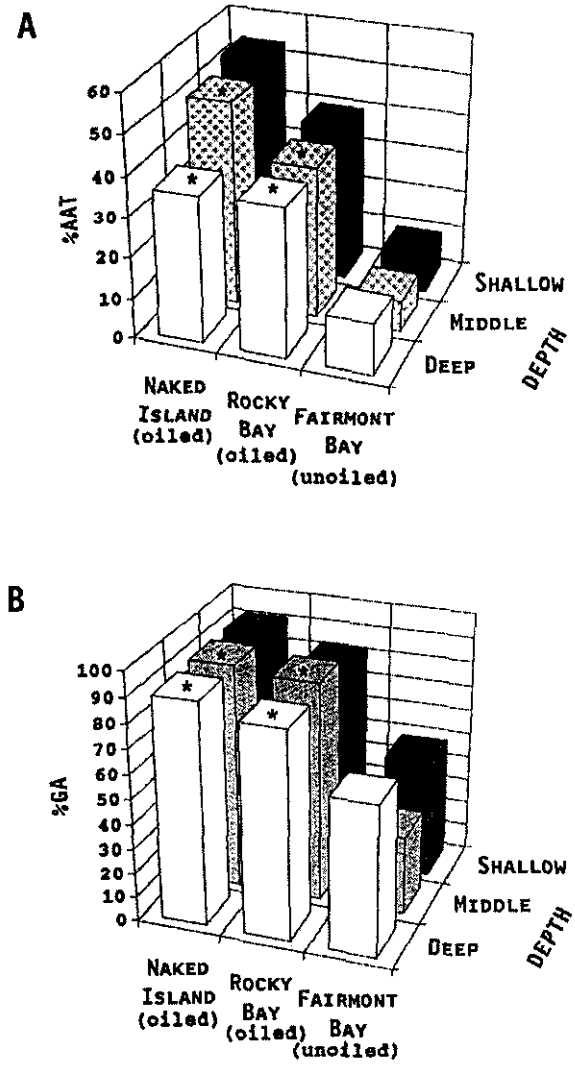
No oil-related histopathological lesions were observed in larvae sampled in 1989 or 1990. In 1989, autolysis was moderate or severe in 58 of 282 (21%) of the larvae examined.

Lesions identified grossly as protrusions or swellings in the eye contained lens vacuolation. Whereas the normal lens is composed of concentric layers of acellular protein surrounded by a lens capsule (Fig. 7A), the vacuolated lens was comprised of layers of protein separated by clear fluid (Fig. 7B). Statistically, lens vacuolation was not correlated with EVO exposure, but it was highly correlated with both autolysis and section artifact ($p < 0.001$, Spearman correlation coefficient).

Two larvae with grossly identified eye masses and microphthalmia had retinal herniation and vacuolated lenses. The normal intact retina was composed of uniform layers of retinal cells surrounded by a prominent, smooth, continuous, pigmented epithelium (Figs. 7A, 7B). By comparison, posthemiation retinal layers were irregular and the pigmented epithelium was rough and discontinuous (Fig. 7C).

In 1990, only 2 of 183 (1.1%) had moderate to severe autolysis. None of the larvae had lens vacuolation. Scores for autolysis or artifact were not correlated with relative hydrocarbon levels. Wild-caught larvae were not examined in 1991.

**Fig. 5.** (A) Percentage of anaphase aberrations (%AAT) by depth (deep, middle, and shallow) in 1989. Scores significantly different ($p < 0.05$) from Fairmont Bay values are marked with an asterisk. (B) Percentage of cytogenetically abnormal larvae (%GA) by depth (deep, middle, and shallow) at Naked Island, Rocky Bay, and Fairmont Bay in 1989. Fairmont Bay was unoiled; Naked Island and Rocky Bay were oiled. Scores significantly different from Fairmont Bay values are marked with a star.




## *Exxon Valdez* oil measurements

Mean EVO-PAH concentrations in resident mussels at the unoiled sites (87 ± 28 ppb) were less than at the six oiled sites (1176 ± 410 ppb) (Fig. 8). Values for the oiled sites were highly variable, from within the range of the unoiled sites (112 ppb at Rocky Bay site O18) to a high of 2501 ppb at Naked Island site O3. Means for Naked Island were slightly higher than for Rocky Bay, although the distributions overlapped (1275 ± 660 versus 1077 ± 630 ppb). The relative PAH concentrations at Naked Island sites O3, O8, and O13 and Rocky Bay sites O17 and O19 were consistent with corresponding relative PAH concentrations in EVO. PAH detected at Fairmont

Bay sites C3 and C4 as well as Rocky Bay site O18 did not match the EVO fingerprint and consisted primarily of naphthalene, possibly from diesel fuel.

Site-specific hydrocarbon concentrations were correlated with overall means of the sublethal end points (Table 1). There was a significant direct relationship between EVO-PAH concentrations and the anaphase aberration rate ($r^2 = 0.88$, $p < 0.01$). Using Mantel's test (Douglas and Endler 1982), the association between these two variables was also highly significant ($p < 0.001$). In addition, EVO-PAH concentrations were correlated with skeletal GSI scores, percent cytogenetically abnormal larvae ($p < 0.01$), and the total GSI score ($0.01 < p < 0.05$). Each chemical class (naphthalenes to chrysenes, phytane) was correlated with three or four end points. The chemical variables were all highly intercorrelated ($p < 0.01$).

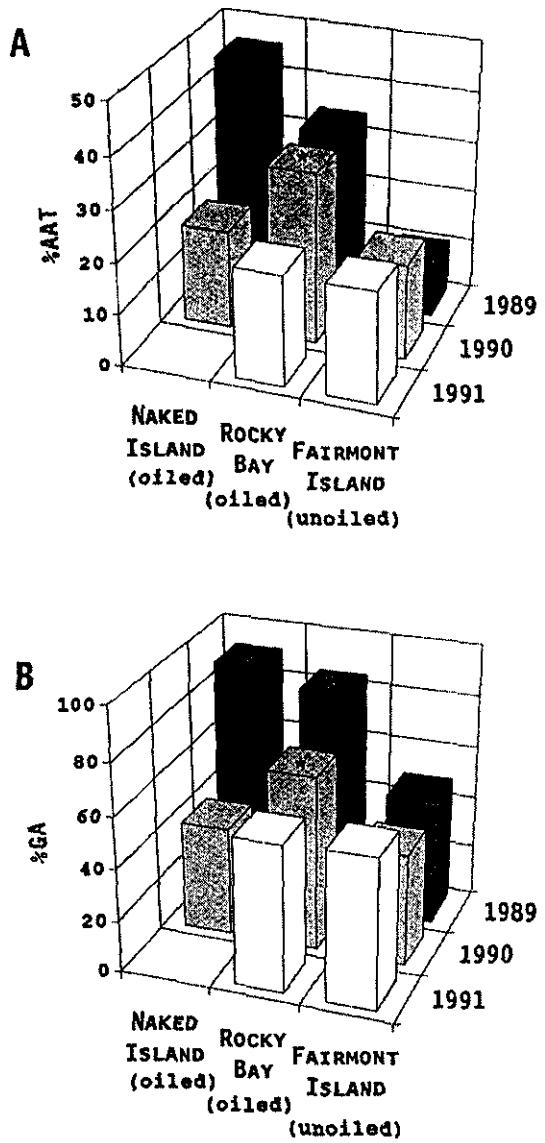
Hydrocarbons were measured in 1990 at all of the Fairmont Bay and Naked Island egg incubation sites and at a Rocky Bay site close to the 1989 sampling area (O2) (Fig. 8). The low EVO-PAH concentrations at Fairmont Bay demonstrate that oil had not dispersed into this area in 1990. Compared with measurements in 1989, all previously oiled sites showed large reductions in hydrocarbon concentrations. By 1990, EVO-PAH concentrations at all sites declined below the level of detection (<60 ppb). Therefore, the biological effects observed in 1990 (a slight increase in larval deformities at Naked Island and elevated genotoxicity at Rocky Bay) could not be attributed to residual EVO.

## Discussion

In 1989, herring eggs from beaches contaminated with EVO produced larvae with morphological and genetic damage similar to that documented in parallel laboratory exposures of herring to Prudhoe Bay crude oil (Kocan et al. 1996) and previously for herring and other fishes (Pearson et al. 1985; Rice et al. 1987; Carls and Rice 1989). The severity of skeletal bends, craniofacial malformations, and finfold reductions were elevated at oiled areas in 1989; higher incidences of abnormal or missing jaws and malformed fins were also observed. These defects are general responses to stress and are typical of oil-contaminated herring larvae (Linden 1975; Lönning 1977; Linden 1978; Smith and Cameron 1979; von Westernhagen 1988). They may also be induced by extreme physicochemical conditions (e.g., temperature, sunlight, and (or) salinity) (Purcell et al. 1990), but the climatic conditions were not excessive at any of the 1989 PWS sites where herring had severe larval deformities. Other craniofacial defects (otic and ocular defects as well as the absence or reduction of larval finfolds) were also more frequent at oiled PWS locations but were not reported in naturally stressed larvae (Purcell et al. 1990). In contrast, another 1989–1990 study of PWS herring found no correlation between EVO exposure and a number of biological response variables, including incidences of larval malformations (Pearson et al. 1995). However, site-specific malformation rates were not presented; therefore, that data set cannot be compared with our measurements.

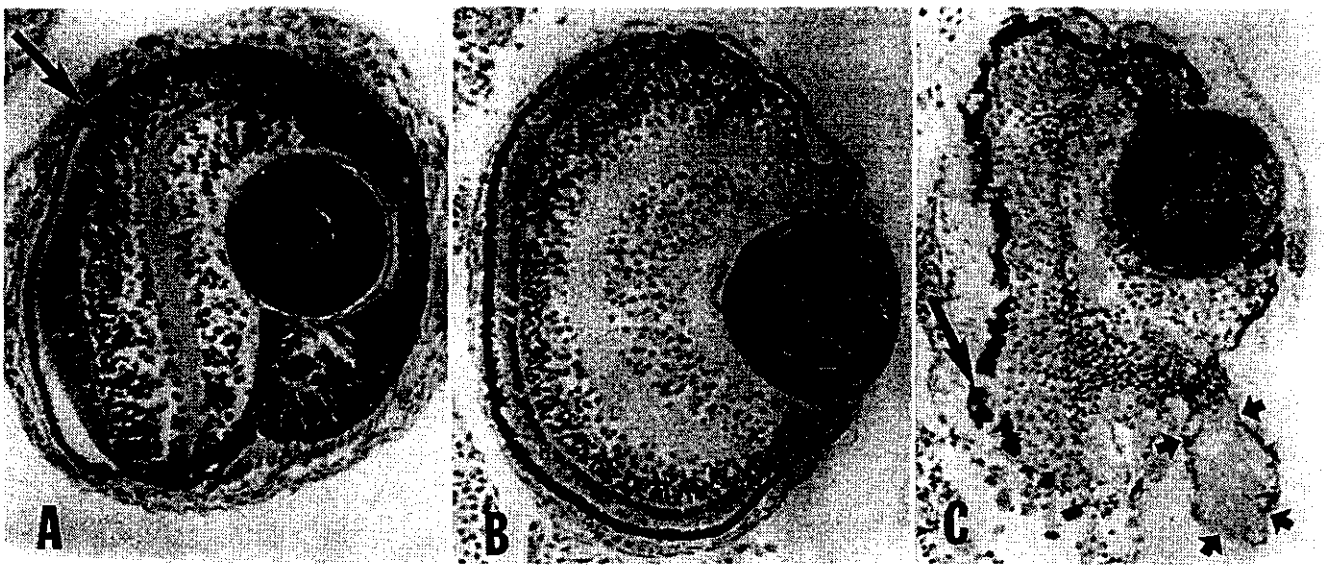
Two observations support the association of the observed defects with EVO exposure: (*i*) malformation incidences were directly correlated with 1989 EVO-PAH concentrations in adjacent mussels, a rough estimate of EVO exposure in herring eggs (Table 1); and (*ii*) identical defects were induced in larvae

**Fig. 6.** Percentage of anaphase aberrations (%AAT) in 1989–1991. Scores significantly different ($p < 0.05$) from Fairmont Bay values are marked with an asterisk. (B) Percentage of cytogenetically abnormal larvae (%GA) in 1989–1991. Scores significantly different from Fairmont Bay values are marked with an asterisk.




exposed to Prudhoe Bay crude oil – water dispersion in a dose-dependent fashion (Kocan et al. 1996). Skeletal and total GSI scores were significantly related to EVO concentrations, and the correlation with finfold GSI scores was of borderline significance ($p = 0.06$). Many of these defects were also related to various aromatic components of EVO. Skeletal malformations were highly correlated with every aromatic constitutent examined; finfold defects were correlated with several. Craniofacial malformations were marginally correlated only with the log benzo[*a*]pyrene concentration ($p = 0.04$). However, two types of craniofacial defects (otic and microphthalmia) were correlated with EVO-PAH or individual aromatic constituents. Although the incidence of abnormal lower jaws was clearly elevated at both oiled locations, the proportion of larvae with reduced jaw size was not statistically significant. The

**Fig. 7.** Comparison of normal and abnormal eyes. Plane of section varies for each larva. Glycol methacrylate embedment, hematoxylin and eosin stain, magnification 250×. (A) Normal: pigmented retinal epithelium (long arrow) has a regular contour, and the lens is composed of concentric layers of acellular protein. (B) Vacuolated lens: lens protein is expanded by translucent fluid, a postmortem change. (C) Microphthalmia and vacuolated lens: pigmented retinal epithelium (long arrow) has an irregular contour because the retina has partly herniated through the globe (short arrows), a postmortem change.




high incidence of jaw reduction could be related to natural stresses early in incubation (Purcell et al. 1990) and may have obscured potential differences caused by EVO exposure. Excessively vigorous laboratory aeration in 1989 might also have induced hatching before the jaw was fully developed, but high incidences of reduced or absent jaws were also observed in free-swimming larvae from both inside and outside of areas considered to be oiled (Norcross et al. 1996). The extent of parental exposure to petroleum hydrocarbons during migration through the oil trajectory to spawning sites has not been resolved, but it would be expected that exposure would have to be very high to produce malformations in the offspring (Rice et al. 1987). Thus, although the association between EVO concentrations at spawning beaches and jaw reduction is not statistically significant, correlations with the other observed morphological defects remain strong.

The relationship between EVO in resident mussels and actual EVO concentrations in herring eggs has not been established. EVO-PAH levels in 1989 mussels (ranging from 237 to 2502 ppb) are difficult to relate to published studies, many of which use water-soluble fractions. However, these EVO concentrations generally appear to be lower than those reported to cause malformations or death in fish larvae (Lönning 1977; Linden 1978; Stene and Lönning 1984; Rice et al. 1987; Middaugh et al. 1988; Carls and Rice 1989) with two exceptions. Histopathological damage was present in fish embryos exposed to 54 ppb crude oil (Hawkes and Stehr 1982), and Pearson et al. (1985) found increased incidences of abnormalities when herring eggs were exposed to 4 ppb undispersed crude oil. Many of the morphological indices in our study were highly correlated with high molecular weight aromatics and dibenzothiophenes, compounds that are present in parent crude oil but rarely tested in aquatic toxicology. These relationships are consistent with the suggestion by Pearson et al. (1985) that the amount of oil adhering to herring eggs may be the major determinant of the frequency of abnormal larvae and the observation that particulate oil was bioavailable to the subsurface marine fauna the summer following the spill (Short and Rounds 1996). Indeed, the microscopic oil particles adhering to fish eggs near spills (Longwell 1977) may reduce metabolism, leading to the nonspecific morphological abnormalities reported here. Adherence of EVO to herring eggs was not quantified.

Although histopathological examination did not reveal significant oil-specific tissue or cellular alterations, histopathology was needed to characterize certain gross findings. For example, one ocular abnormality observed under a dissecting microscope (ocular protrusions with microphthalmia) proved histologically to result from lens vacuolation and herniation of the retina through a ruptured globe. Lens vacuolation was highly correlated with autolysis but not with EVO levels. The vacuolated lenses in both these cases of microphthalmia provided further evidence for the role of autolysis in forming ocular masses through retinal herniation. In another study, Lönning (1977) reported microphthalmia in marine fish larvae exposed to crude oil; however, histopathological work was not done, and the nature of that microphthalmia was undefined. Because of the confusion regarding the pathogenesis of ocular malformations, it is suggested that all future oil toxicity studies with herring larvae include histopathology to confirm putative effects observed grossly.

Genetic damage, as measured by the anaphase aberration rate and the percentage of cytogenetically abnormal larvae, proved to be a very useful indicator of oil exposure in 1989. Both end points were significantly related to every chemical variable, including the EVO-PAH concentration (Table 1). The lowest correlation for the anaphase aberration rate was with total naphthalenes, which are not genotoxic (Klopman et al. 1985). The correlation coefficient increased with the number of aromatic rings (naphthalene with two rings was

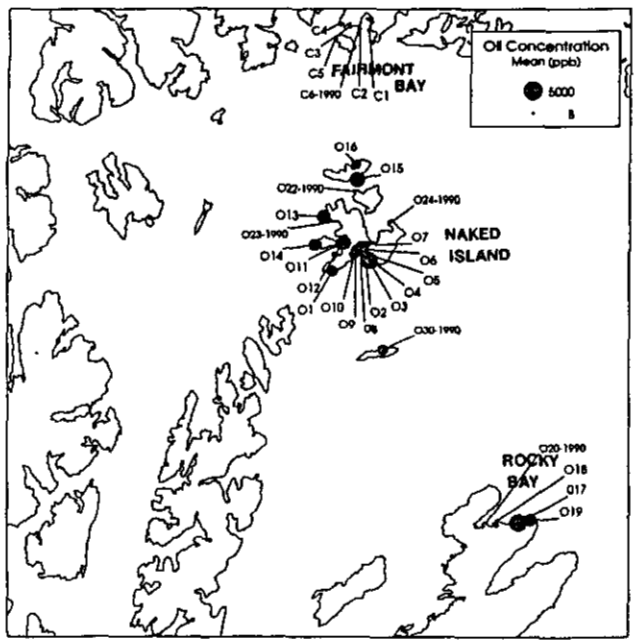
lowest, phenanthrene with three rings was intermediate, chrysene with four rings was highest). Black et al. (1983) also found that the toxicity of aromatic compounds to fish embryos increased with the number of aromatic rings. The association of anaphase aberrations with PAH concentrations reflects the genotoxic (mutagenic) activity of some constituents. Unsubstituted naphthalene, anthracene, and phenanthracene are not genotoxic but certain methylated derivatives of the three-ring PAH have slightly mutagenic activities (Dipple 1982; Flesher and Myers 1991) and would be expected to covary with the measured analytes. Unsubstituted fluorenes and dibenzothiophenes are also not genotoxic but the mutagenic potential is unknown for their methylated derivatives, which were present in much greater concentrations than their unsubstituted parents. Mussel concentrations of measured analytes with known genotoxic action were very low (<2 ppb benzo[*a*]pyrene), but water column benzo[*a*]pyrene concentrations of ≥0.5 ppb have been shown to induce anaphase aberrations in marine embryos (Hose et al. 1983). Concentrations of genotoxic components in herring eggs probably were also in the low ppb range, but assimilation into larval body tissues during yolk sac absorption could produce transiently higher PAH levels. The period surrounding yolk sac absorption has been shown to be toxicologically stressful (Guiney et al. 1980; von Westernhagen 1988), and the highest anaphase aberration rates were observed around the time of yolk sac absorption in herring trawled from Naked Island and Rocky Bay (Brown et al. 1996).

Although the correlation between EVO concentrations and genetic damage appears to be strong, other confounding factors could conceivably contribute to the observed pattern. For instance, in situ egg–larval mortality was higher at Rocky Bay and Bass Harbor on Naked Island, but these areas also had different beach characteristics, which implied greater mortality than at Fairmont Bay and the northeast spawning areas (Galena Bay) (McGurk and Brown 1996). Elevated rates of genetic damage might result from differential morbidity–mortality as well as from oil exposure. Temperature and salinity can also influence cytogenetic measurements (Longwell and Hughes 1980), although their relationships have not been clearly defined. These factors are not expected to produce the wide variability observed in 1989 between oiled and unoiled locations because all eggs were incubated in identical conditions prior to hatching.

All sublethal measurements showed improvement by 1990; this reduction in toxicity is consistent with the substantial decrease in EVO concentrations at the sites we examined. The minor morphological lesions in Naked Island larvae and elevated anaphase aberration rates at Rocky Bay could not be attributed to residual EVO. Historically, Rocky Bay has been contaminated by low levels of hydrocarbons from ship traffic (Karinen et al. 1993), but EVO-PAH concentrations in 1989 were two or three orders of magnitude above the historical base line for aromatic hydrocarbons. Other possible contaminants such as fuel oil and metals were not analyzed in this study.

By 1991, all indices had returned to probable base-line levels (Figs. 4, 6). This statement must be qualified because the lack of spawning at Naked Island in 1991 precluded 3rd-year comparisons. However, data from Naked Island in 1990 were similar to those obtained at Fairmont Bay in 1991. Another study (Kocan et al. 1996) attempted to resolve this problem by deploying laboratory-spawned eggs at previously oiled Naked Island sites. They concluded, in contrast to the results presented here, that certain sublethal effects (such as growth reductions) were found at previously oiled sites 2 years after the spill. Because of site to site variability inherent in evaluating field-collected embryos, the egg deployment technique may be preferable for future oil spill studies.

**Fig. 8.** Prince William Sound collection sites for herring eggs and mussels and relative petroleum hydrocarbon levels (parts per billion of mean *Exxon Valdez* oil polynuclear aromatic hydrocarbons), 1989–1990. Measurements from 1989 are marked with a site number only, those from 1990 are marked with a site number followed by –1990.




Although the data from Fairmont Bay and Rocky Bay were strikingly similar in 1991, both the Fairmont Bay morphological and cytogenetic end points were elevated over those of the previous 2 years and even over the 1990 Naked Island values. It seems likely that low temperatures and numerous storm events in 1991 affected herring development; egg mortality was higher than in either of the 2 previous years (E.D. Brown, unpublished data). Environmental variables probably alter the base line of sublethal indicators; therefore, multiple-year assessments are needed. Because some juvenile and adult herring from oiled areas had been exposed to oil (Brown et al. 1996), an alternative but untested hypothesis is that cumulative genetic damage from parental oil exposure resulted in embryonic genotoxicity in subsequent spawns.

The data presented here provide evidence that adverse sublethal effects in herring larvae are produced by exposure to levels of petroleum hydrocarbons present immediately following an oil spill. Although considered to be sublethal defects, certain morphological defects can potentially be lethal during the larval stage, depending on severity. An organism's ability to capture food can be impaired by severe skeletal bends, stunting, and nonfunctional jaws (Purcell et al. 1990). Because larvae respire through the finfold, a reduction in its width would decrease respiratory potential (von Westernhagen 1988). These conditions would be expected to limit larval growth.

Because of the greater severity of these defects at oiled PWS areas, a concomitant decrease in survival could be predicted. Although a threshold for lethal genetic damage has not been determined for larval fish, the high rate of genetic damage at heavily oiled stations combined with severe morphological malformations would mitigate against survival through the stressful larval period. The observation that fish in oiled areas had reduced survival rates during the transition from egg to larva is therefore consistent with these predictions, although results could not solely be attributed to oil exposure (McGurk and Brown 1996). Severely malformed larvae would not have hatched or would have died soon after hatching; incompetent larvae would have been unable to swim up into the plankton and begin feeding. Growth rates of pelagic larvae were reduced at oiled sites, which also had lower temperatures than did control sites (Brown et al. 1996). Increased genetic damage was present at some sites within the oil trajectory into June (Norcross et al. 1996). Using site-specific estimates of the 1989 PWS instantaneous mortality rates, 58% fewer larvae were produced than expected (Brown et al. 1996). However, a difference of this magnitude in a weak year-class such as that of 1989 cannot be detected at the population level. Therefore, measurements of egg to larval survival and sublethal effects in larvae may be better assessments of toxicity than are traditional estimates of the fishery population such as egg production and subsequent recruitment.

## Acknowledgements

We thank Pamela Morris and Neil Willits for guidance in statistical analyses, Nancy McKrell for assistance with microscopic analyses, Wendy Widmann and Kellie Henninger for preparation of histological sections, and Jeffrey Short for the chemical analyses.

## References

Black, J.A., Birge, W.J., Westerman, A.G., and Francis, P.C. 1983. Comparative toxicology of aromatic hydrocarbons. Fundam. Appl. Toxicol. **3**: 353–358.

Brown, E.D., Baker, T.T., Hose, J.E., Kocan, R.M., Marty, G.D., McGurk, M.D., Norcross, B.L., and Short, J.F. 1996. Injury to the early life history stages of Pacific herring in Prince William Sound after the *Exxon Valdez* oil spill. *In* Exxon Valdez Oil Spill Symposium Proceedings. *Edited by* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright. Am. Fish. Soc. Symp. **18**: 448–462

Cameron, J.A., and Smith, R.L. 1980. Ultrastructural effects of crude oil on early life stages of Pacific herring. Trans. Am. Fish. Soc. **109**: 224–228.

Carls, M.G., and Rice, S.D. 1989. Abnormal development and growth reductions of pollack *Theragra chalcogramma* embryos exposed to water-soluble fractions of oil. Fish. Bull. **88**: 29–37.

Dipple, A. 1982. Polynuclear aromatic carcinogens. *In* Polynuclear aromatic hydrocarbons: physical and biological chemistry. *Edited by* M. Cooke, A.J. Dennis, and G.L. Fisher. Springer Verlag, New York. pp. 246–265.

Douglas, M.E., and Endler, J.A. 1982. Quantitative matrix comparisons in ecological and evolutionary investigations. J. Theor. Biol. **99**: 777–795.

Flesher, J.W., and Myers, S.R. 1991. Rules of molecular geometry for predicting carcinogenic activity of unsubstituted polynuclear aromatic hydrocarbons. Teratog. Carcinog. Mutagen. **11**: 41–54.

Goksøyr, A., Solberg, T.S., and Serigstad, B. 1991. Immunochemical detection of cytochrome P450IA1 induction in cod larvae and juveniles exposed to a water soluble fraction of North Sea crude oil. Mar. Pollut. Bull. **22**: 122–127.

Guiney, P.D., Lech, J.J., and Peterson, R.E. 1980. Distribution of a polychlorinated biphenyl during early life stages of rainbow trout (*Salmo gairdneri*). Toxicol. Appl. Pharmacol. **53**: 521–529.

Hawkes, J.W., and Stehr, C.M. 1982. Cytopathology of the brain and retina of embryonic surf smelt (*Hypomesus pretiosus*) exposed to crude oil. Environ. Res. **27**: 164–178.

Hose, J.E., Puffer, H.W., Oshida, P.S., and Bay, S.M. 1983. Developmental and cytogenetic abnormalities induced in the purple sea urchin by environmental levels of benzo(*a*)pyrene. Arch. Environ. Contam. Toxicol. **12**: 319–325.

Hughes, J.B., and Hebert, A.T. 1991. Erythrocyte micronuclei in winter flounder (*Pseudopleuronectes americanus*): results of field surveys during 1980–1988 from Virginia to Nova Scotia and in Long Island Sound. Arch. Environ. Contam. Toxicol. **20**: 474–479.

Karinen, J.F., Babcock, M.M., Brown, D.W., MacLeod, W.D., Jr., Ramos, L.S., and Short, J.W. 1993. Hydrocarbons in intertidal sediments and mussels from Prince William Sound, Alaska, 1977–1980: characterization and probable sources. NOAA Tech. Memo. No. NMFS-AFSC9. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Washington, D.C.

Klekowski, E.J., Jr., Corredor, J.E., Morell, J.M., and Del Castillo, C.A. 1994. Petroleum pollution and mutation in mangroves. Mar. Pollut. Bull. **28**: 167–176.

Klopman, G., Namboodiri, K., and Kalos, A.N. 1985. Computer automated evaluation and prediction of the IBALL index of carcinogenicity of polycyclic aromatic hydrocarbons. *In* Molecular basis of cancer. *Edited by* R. Rein. Alan R. Liss, New York. pp. 287–298.

Kocan, R.M., Landolt, M.L., and Sabo, K.M. 1982. Anaphase aberrations: a measure of genotoxicity in mutagen-treated fish cells. Environ. Mutagen. **4**: 181–189.

Kocan, R.M., Hose, J.E., Brown, E.D., and Baker, T.T. 1996. Pacific herring (*Clupea pallasi*) embryo sensitivity to Prudhoe Bay petroleum hydrocarbons: laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound. Can. J. Fish. Aquat. Sci. **53**: 2366–2375.

Lindén, O. 1975. Acute effects of oil and oil/dispersant mixture on larvae of Baltic herring. Ambio, **4**: 130–133.

Lindén, O. 1978. Biological effects of oil on early development of Baltic herring *Clupea harengus membras*. Mar. Biol. (Berlin), **45**: 273–283.

Long, E.R., Buchman, M.F., Bay, S.M., Breteler, R.J., Carr, R.S., Chapman, P.M., Hose, J.E., Lissner, A.L., Scott, J., and Wolfe, D.A. 1990. Comparative evaluation of five toxicity tests with sediments from San Francisco Bay and Tomales Bay, California. Environ. Toxicol. Chem. **9**: 1193–1214.

Longwell, A.C. 1977. A genetic look at fish eggs and oil. Oceanus, **20**: 46–58.

Longwell, A.C., and Hughes, J.B. 1980. Cytologic, cytogenetic and developmental state of Atlantic mackerel eggs from sea surface waters of the New York Bight, and prospects for biological monitoring with ichthyoplankton. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, **179**: 275–291.

Lönning, S. 1977. The effects of Ekofisk oil and oil products on marine fish larvae. Astarte, **10**: 37–48.

Marty, G.D., Nuñez, J.M., Lauren, D.J., and Hinton, D.E. 1990. Age-dependent changes in toxicity of *N*-nitroso compounds to Japanese medaka (*Oryzias latipes*) embryos. Aquat. Toxicol. **17**: 45–62.

McGurk, M.D., and Brown, E.D. 1996. Egg–larval mortality of Pacific herring in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. **53**: 2343–2354.

Middaugh, D.P., Hemmer, M.J., and Lores, E.M. 1988. Teratological

effects of 2,4-dinitrophenol, "produced water" and naphthalene on embryos of the inland silverside *Menidia beryllina*. Dis. Aquat. Org. **4**: 53–65.

Norcross, B.L., Hose, J.E., Frandsen, M., and Brown, E.D. 1996. Distribution, abundance, morphological condition, and cytogenetic abnormalities of larval herring in Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. **53**: 2376–2387.

Pearson, W.H., Woodruff, D.L., Kiesser, S.L., Fellingham, G.W., and Elston, R.A. 1985. Oil effects on spawning behavior and reproduction in Pacific herring (*Clupea harengus pallasi*). API publication No. 4412. American Petroleum Institute, Washington, D.C.

Pearson, W.H., Moksness, E., and Skalski, J.R. 1995. A field and laboratory assessment of oil spill effects on survival and reproduction of Pacific herring following the *Exxon Valdez* spill. *In Exxon Valdez* oil spill: fate and effects in Alaska waters. *Edited by* P.G. Wells, J.N. Butler, and J.S. Hughes. American Society for Testing and Materials, Philadelphia, Pa. ASTM, STP 1219. pp. 626–661.

Purcell, J.E., Grosse, D., and Glover, J.J. 1990. Mass abundances of abnormal Pacific herring larvae at a spawning ground in British Columbia. Trans. Am. Fish. Soc. **119**: 463–469.

Rice, S.D., Moles, A., and Karinen, J.F. 1979. Sensitivity of 19 Alaska marine species to Cook Inlet crude oil and No. 2 fuel oil. *In* Proceedings of the 1979 Oil Spill Conference (Prevention, Behavior, Control, Cleanup), March 19–21, 1979, Los Angeles, CA. American Petroleum Institute, Washington, D.C. pp. 549–554.

Rice, S.D., Babcock, M.M., Broderson, C.C., Carls, M.G., Gharrett, J.A., Korn, S., Moles, A., and Short, J.W. 1987. Lethal and sublethal effects of the water-soluble fraction of Cook Inlet crude oil on Pacific herring (*Clupea harengus pallasi*) reproduction. NOAA Tech. Memo. No. NMFS F/NWC-111. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Washington, D.C.

Schmid, W. 1976. The micronucleus test for cytogenetic analysis. Chem. Mutagen, **4**: 31–53.

Short, J.W., and Rounds, P. 1996. Petroleum hydrocarbons in caged mussels deployed in Prince William Sound, after the *Exxon Valdez* oil spill. *In Exxon Valdez* oil spill symposium proceedings. *Edited by* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright. Am. Fish. Soc. Symp. **18**: 29–39.

Smith, R.L., and Cameron, J.A. 1979. Effect of water soluble fraction of Prudhoe Bay crude oil on embryonic development of Pacific herring. Trans. Am. Fish. Soc. **108**: 70–75.

Solangi, M.A., and Overstreet, R.M. 1982. Histopathological changes in two estuarine fishes, *Menidia beryllina* (Cope) and *Trinectes maculatus* (Bloch and Schneider), exposed to crude oil and its water-soluble fractions. J. Fish Dis. **5**: 13–35.

Stene, A., and Lönning, S. 1984. Effects of 2-methylnaphthalene on eggs and larvae of six marine species. Sarsia, **69**: 199–203.

Stiles, S., Choromanski, J., Nelson, D., Miller, J., Greig, R., and Sennefelder, G. 1991. Early reproductive success of the hard clam (*Mercenaria mercenaria*) from five sites in Long Island Sound. Estuaries, **14**: 332–342.

U.S. National Research Council (Ocean Studies Board). 1985. Oil in the sea: inputs, fates and effects. National Academy Press, Washington, D.C.

von Westernhagen, H. 1988. Sublethal effects of pollutants on fish eggs and larvae. *In* Fish physiology, the physiology of developing fish. Vol. 11A. Eggs and larvae. *Edited by* W.S. Hoar and D.J. Randall. Academic Press, New York. pp. 253–346.

# Pacific herring (*Clupea pallasi*) embryo sensitivity to Prudhoe Bay petroleum hydrocarbons: laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound

**Richard M. Kocan, Jo Ellen Hose, Evelyn D. Brown, and Timothy T. Baker**

**Abstract**: Pacific herring (*Clupea pallasi*) embryos were exposed to oil–water dispersions of Prudhoe Bay crude oil in artificial seawater. The original extract contained 9.67 mg/L high molecular weight and 64 mg/L low molecular weight hydrocarbons. From this stock, concentrations of oil–water dispersions were made ranging from 9.67 to 0.01 mg/L. Pilot studies demonstrated that over 85% of the low molecular weight hydrocarbons evaporated during the first 24 h of each exposure. Seawater with and without oil–water dispersions showed constant values of 10.2–10.4 mg $O_2$/L, a pH of 8.4, and salinity of 29.7–30.3‰. Genetic damage was the most sensitive biomarker for oil exposure, followed by physical deformities, reduced mitotic activity, lower hatch weight, and premature hatching. Oil had its greatest effect on the blastodisc and gastrula stages. Embryos deployed in Prince William Sound 3 years after the *Exxon Valdez* oil spill yielded a greater proportion of abnormal ($p < 0.002$) and lower weight ($p < 0.01$) larvae at previously oiled sites than at unoiled sites. Although there is no unequivocal evidence that oiled sites pose a long-term hazard to herring embryo or larval survival, a more comprehensive in situ study is warranted in light of the data obtained during this study.

**Résumé** : Des embryons de hareng du Pacifique (*Clupea pallasi*) ont été exposés à des dispersions de pétrole de la baie Prudhoe dans une eau de mer synthétique (DPE). L'extrait original contenait 9,67 mg/L d'hydrocarbures de masse moléculaire élevée et 64 mg/L d'hydrocarbures de faible masse moléculaire. Cet extrait a servi à la préparation de DPE dont la concentration en pétrole variait entre 9,67 et 0,01 mg/L. Des études pilotes ont montré que plus de 85% de la fraction légère des hydrocarbures s'évapore dans les 24 premières h de chaque exposition. Dans l'eau de mer, avec ou sans pétrole dispersé, les paramètres suivants gardaient une valeur constante : oxygène dissous entre 10,2 et 10,4 mg $O_2$/L, pH à 8,4 et salinité entre 29,7 et 30,3‰. Les dégâts génétiques constituent le biomarqueur le plus sensible de l'exposition au pétrole; viennent ensuite les malformations physiques, l'activité mitotique réduite, un poids inférieur à la moyenne au moment de l'éclosion et l'éclosion prématurée des oeufs. Le pétrole exerce le plus ses effets aux stades du blastodisque et de la gastrulation. Les embryons installés dans les eaux du golfe du Prince-William 3 ans après le déversement de pétrole qui s'est produit lors de l'échouage de l'*Exxon Valdez* ont donné une proportion supérieure de larves anormales ($p < 0,002$) et de poids inférieur ($p < 0,01$) dans les sites qui avaient été contaminés, par comparaison à des sites intacts. Il n'existe pas de preuve catégorique que les sites pollués par le pétrole exposent les embryons de hareng à des dangers à long terme ou menacent la survie des larves, mais les résultats obtenus dans le cadre de ces travaux justifient la tenue d'une étude in situ de portée plus étendue. [Traduit par la Rédaction]

## Introduction

The oil spill that resulted from the grounding of the tanker *Exxon Valdez* on March 24, 1989, coincided with the return of adult Pacific herring (*Clupea pallasi*) to Prince William Sound (PWS) spawning areas. These fish were at risk of exposure to petroleum hydrocarbons, as were their eggs and larvae for the next several months, and possibly for several years. Histopathological examination of adult herring collected shortly after the spill demonstrated lesions consistent with oil exposure (Marty et al. 1993). Likewise, naturally spawned herring eggs collected from oiled sites in 1989 produced significantly more physically deformed and genetically damaged larvae than did eggs collected from unoiled sites (Hose et al. 1996). Such sublethal effects from environmental pollution are believed to lead to reduced survival potential at some later

Received August 11, 1993. Accepted March 11, 1996.
J12048

**R.M. Kocan.**[1] University of Washington, School of Fisheries, Box 355100, Seattle, WA 98195, U.S.A.
**J.E. Hose.**[2] Occidental College, Biology Department, Los Angeles, CA 90041, U.S.A.
**E.D. Brown.** Alaska Department of Fish and Game, Box 669, Cordova, AK 99574–0669, U.S.A.
**T.T. Baker.** Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99518–1599, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: kocan@fish.washington.edu
[2] *Present address:* 2590 Softchess Place, Arroyo Grande, CA 93420, U.S.A.

period in the organism's life cycle (Rosenthal and Alderdice 1976).

Embryos and larvae present at the time of the spill were exposed to petroleum hydrocarbons (Brown et al. 1996) and would be entering the breeding population in 1993 for the first time as 4 year olds. Laboratory studies were used to simulate oil exposure of these embryos and larvae and thereby determine if there was any significant damage that could affect their survival and ultimate recruitment into the breeding population. To do this, herring were manually spawned onto an artificial substrate, then exposed to a range of Prudhoe Bay crude oil concentrations. Embryos were then evaluated for survival, hatching, normal larval development, biomass, and sublethal genetic damage. The objective of the laboratory component of this study was to evaluate the toxicity of Prudhoe Bay crude oil in seawater for developing herring embryos and larvae under controlled laboratory conditions.

A field component of the study was conducted using herring embryos derived from the same source as the laboratory component. These embryos were exposed, in situ, at previously oiled and unoiled sites and then hatched in the laboratory where they were evaluated for survival and development. The objective of the field component was to determine if there were residual toxic effects at previously contaminated sites in PWS.

## Materials and methods

### Preparation of embryos for the field and laboratory components

There were three major components: (*i*) an in situ or field exposure component in 1991, (*ii*) a continuous exposure laboratory component in 1991, and (*iii*) an intermittent (pulse) exposure laboratory component in 1992. All three components used artificially spawned and fertilized Pacific herring embryos as subjects. For the field component conducted in 1991, embryos were fertilized and placed in cassettes deployed at selected sites for a period of in situ incubation. For the two laboratory components conducted in 1991 and 1992, embryos were fertilized and prepared for immediate shipment to Seattle.

Ripe spawning herring from PWS were used as a source of eggs and sperm. The 1991 spawners were primarily 6- and 7-year-old fish taken from commercial fishery impoundments near Tatitlek Narrows, an unoiled area in the northwest corner of PWS. The 1992 spawners were taken with gill nets during spawning events in Boulder Bay, an unoiled site adjacent to Tatitlek Narrows; these fish were predominantly age 4. Eggs were artificially spawned onto glass slides in PWS seawater (29‰). Acid-cleaned glass microscope slides were placed on the bottom of a 4-L Tupperware container and covered with 6 in. (15.2 cm) of 6°C seawater, and eggs were broadcasted over the slides by hand. To minimize interfemale variability, eggs from six to eight females were randomly distributed to all slides at the desired egg density per slide. Sperm from three to four males was then pooled and used to fertilize the eggs. After 1 h, several slides were examined microscopically to verify that a desired fertilization rate of at least 90% was attained.

### Field component

Sites used in 1989 for damage assessment studies and new sites in the northeast area of PWS were selected for the in situ field component to test for potential site differences resulting from previous oil contamination. Unoiled sites C3, C4, and C6 in Fairmont Bay (1989 study sites; Brown et al. 1996) and C7 and C8 in Tatitlek Narrows (new sites) were not in the trajectory of the oil spill. Oiled sites O1–O2 in Rocky Bay on northern Montague Island and O3–O5 on Naked Island (1989 study sites) were directly hit by the oil plume 2 years earlier. Although in 1991 spawn was not present in Fairmont Bay and at the previously oiled sites selected for this experiment, all selected sites had contained natural herring spawn at the time of the spill in 1989.

After preparation of embryos in 1991 six slides were loaded into cassettes, placed in PVC pipe containers (Fig. 1), and transported to the deployment sites. The cassettes were held on shipboard in seawater that was exchanged every hour until deployment. Overnight, cassettes were hung overboard in mesh bags to ensure adequate oxygenation and prevent fluctuations in incubation temperature. Embryos were deployed at unoiled sites (C3, C4, C6, C7, C8) 8–24 h after fertilization and at oiled sites (O1–O5) 72 h after fertilization. At each site except C4, two cassettes were deployed at each of two depths (–1.5 and –4.5 m relative to mean low water or 0 m). Embryos remained in place for 8–12 days, then were retrieved, placed in clean filtered seawater, and transported to the University of Washington where they were evaluated by (*i*) site (oiled vs. unoiled) and (*ii*) depth (–1.5 and –4.5 m) for physical deformities, hatching success, embryo mortality, and weight.

### Embryo transport to the laboratory

In both 1991 and 1992, slides containing fertilized eggs from PWS were placed into an inert plexiglass carrier to prevent slide to slide contact during transit, submerged in seawater in a Tupperware container, aerated with $O_2$, and placed in a cooler containing wet ice for transport back to the University of Washington in Seattle. For embryos shipped immediately after fertilization in 1991 and 1992, the total elapsed time from fertilization to arrival at the university was 8 h and the water temperature on arrival was 6.5°C. For embryos allowed to incubate in situ for 8–12 days in the 1991 field component, 8 h elapsed from the time cassettes were removed from the study sites to the time of arrival at the university. Once at the university, the embryos were placed into 29‰ artificial seawater in an environmental chamber, and the water temperature was allowed to rise slowly to 8°C. This temperature was maintained at 8 ± 0.5°C for the remainder of the study.

### Laboratory components

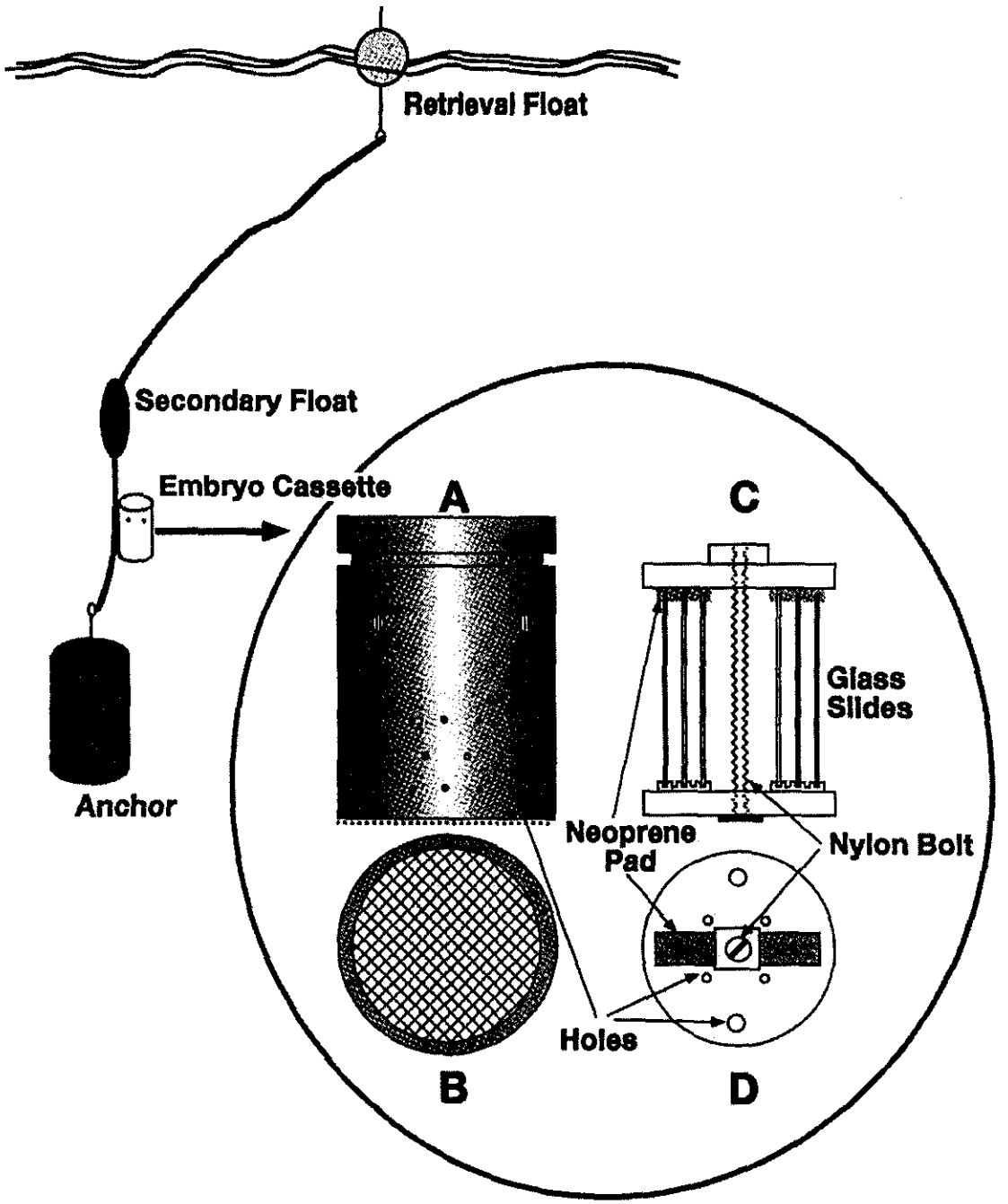
*Transport effect*

To control for possible effects resulting from transport of embryos from PWS to Seattle, ripe Pacific herring from Puget Sound were collected locally by gill net and transported to the University of Washington where they were spawned in the environmental chamber described above. These were then incubated concurrently with and under the same conditions as the PWS embryos. They were also exposed to oil–water dispersions (OWD) and compared with PWS embryos for differences in development or response that might be attributed to transport between Alaska and Washington.

*Preparation of oil and water mixtures*

Prudhoe Bay crude oil taken directly from the *Exxon Valdez* was supplied by the National Marine Fisheries Service, Auke Bay, Alaska. For toxicity tests, an OWD was prepared by shaking for 5 min 40 mL of crude oil with 1 L of 29‰ artificial seawater (Instant Ocean) in a 2-L separatory funnel at 8°C. This mixture was allowed to separate in the funnel for 18 h. The funnel was tapped lightly several times during separation to release oil droplets adhering to the glass. The lower aqueous layer was then drained into a glass-stoppered bottle, designated as 100% OWD, and diluted with control (no oil) seawater to yield a range of dilutions from 100 to 0.1%. A new stock solution and dilutions were prepared every 48 h until the embryos began hatching at about 18 days after fertilization. Microscopic examination (60×) of the incubation water and exposed embryos revealed no visible oil droplets in the OWD preparation.

**Fig. 1.** Apparatus and deployment system used for exposing herring embryos in situ: (A) 7.5 cm PVC cylinder that holds cassette and slides; (B) top and bottom view of PVC cylinder showing netting; (C) cassette with slides (each cassette holds six 2.5 × 7.5 cm glass slides, each of which can hold up to 300 eggs); (D) top and bottom view of cassette. The secondary float was used to keep the apparatus suspended above the bottom during periods of low tides.




*Chemical analysis of oil–water dispersions*

Chemical analyses of OWD were based on the total gas chromatographic peaks observed for low molecular weight (LMW; C6–C12) gasoline-range hydrocarbons, and high molecular weight (HMW; C12–C28) diesel-range hydrocarbons. LMW samples were analyzed by gas chromatography – flame ionization detection (purge & trap technique) with a bromobenzene standard and the HMW samples were analyzed by gas chromatography – flame ionization detection using methylarachidate as a standard. Both procedures are modified Environmental Protection Agency (EPA) method 8015 and were carried out by Analytical Resources Inc. of Seattle, Washington, on samples prepared at the University of Washington. The oil–water mixtures more closely resemble OWD than water-soluble fraction (WSF), although various authors use the term WSF to describe what Anderson et al. (1974) more accurately described as OWD. Analyses were performed on two separate seawater extracts of crude oil, which were split into two equal aliquots. One aliquot was sealed immediately after extraction ($t_0$) in a glass-stoppered, acid-washed flask. The second was allowed to stand uncovered with gentle aeration at 8°C for 48 h ($t_{48}$), simulating the conditions under which the herring embryos were

exposed. The final analyses were then performed on two $t_0$ and two $t_{48}$ samples. These values were used to convert percent OWD to milligrams per litre of water-accommodated petroleum hydrocarbons. Because as much as 90% of LMW hydrocarbons can volatilize within 24 h (Anderson et al. 1974), the more stable HMW values were used to calculate dilutions. Consequently, the values given are the minimum concentrations of HMW hydrocarbons to which embryos were exposed.

*Effect of oil–water dispersion on physical characteristics of seawater*

Using a Corning M90 oxygen–pH meter and Atago S10 refractometer to determine whether OWD had an effect on the $O_2$ concentration, pH, or salinity of the incubation seawater (independent of the presence of herring embryos), two groups of glass vessels containing 300 mL of OWD covering the full range of exposure dilutions were monitored for 48 h, one with aeration (+) and one without (–). This was done to ensure that any observed embryotoxic effects were not the result of changes in physical or chemical characteristics of the seawater created by interaction with OWD.

*Embryo exposure to oil–water dispersion*

For the continuous exposure to OWD, herring embryos 8–10 h after fertilization were placed into 400-mL glass vessels containing 300 mL of OWD ranging from 100 to 0.1%. Each concentration sample consisted of two vessels containing five slides each. Three vessels with five slides each received only artificial seawater (controls). The exposure and control water was changed every 48 h through 18 days after fertilization. Larvae were allowed to hatch into uncontaminated artificial seawater so that only effects of embryonic exposure would be measured. A parallel set of slides containing Puget Sound herring embryos was incubated along with the PWS embryos to evaluate transport effects (see previous section).

For the intermittent (pulse) exposure component conducted in 1992, the sensitivity of different embryonic stages to OWD was evaluated. PWS herring embryos were subjected to a single 36-h pulse exposure of OWD. Beginning 24 h after fertilization (i.e., early blastodisc) and every 24 h thereafter, a different group of embryos was exposed to OWD for 36 h. This resulted in four groups of embryos being exposed to OWD beginning at 24, 48, 72, and 96 h after fertilization. On the basis of previous continuous exposures, we determined that concentrations of OWD above 10% would be required to ensure that an obvious toxic effect would be observed following such a short exposure period. The concentrations selected were 2.4 and 9.67 mg/L, (i.e., 25 and 100% OWD). At the end of each exposure period the embryos were washed and incubated to hatching in flowing natural seawater, which produced a complete water exchange every 6 h.

Both continuous and intermittent exposures were carried out in 300 mL of seawater (29–30‰) at 8°C. Embryos were gently aerated for the entire exposure period with an aquarium air pump that delivered approximately 60 bubbles/min to each exposure vessel through a fine-tipped pipette. The combination of a high-humidity, low-temperature environmental room and a low rate of aeration resulted in minimal evaporative loss over 48 h. Before the embryos hatched, the slides were removed from oil-contaminated seawater and placed into clean seawater. When hatching began, all larvae were collected twice daily, fixed in 5% Formalin, labeled, stored, and later evaluated.

*Embryo–larval evaluation*

Embryos were evaluated for hatching success (i.e., percent live hatch) and larvae were evaluated for genotoxicity, physical deformities, and differences in weight. Percent hatching or hatching success was the total number of larvae hatched live divided by the number of fertile eggs. Percent viable larvae, or the percentage of morphologically and functionally normal larvae, was determined by the following equation:

$$(1)\quad \%\ \text{viable larvae} = \frac{\text{no. of live larvae} - \text{no. of abnormal larvae}}{\text{no. of fertile eggs}} \times 100.$$

*Scoring gross morphological abnormalities*

When eye pigmentation was evident in the embryos (7–8 days after fertilization), two slides from each treatment group were microscopically examined and the number of infertile, dead, and live eggs was recorded. After all of the exposure groups had hatched and the larvae were preserved, the total number of live-hatched larvae was counted. The preserved larvae were then evaluated for gross physical defects. Those having craniofacial, eye, spinal, fin, and pericardial – yolk sac defects were enumerated.

*Scoring genotoxic damage*

The germinal layer between the muscle cells and developing ray structure of the pectoral fin of the newly hatched herring larvae proved to be a good source of mitotic cells (Hose et al. 1996). Twenty-five larvae from each treatment and control group were selected without apparent bias from a pool of 100 larvae preserved in Formalin for cytogenetic analyses. Pectoral fins were dissected from the larvae and placed on clean microscope slides in a drop of 45% acetic acid for post-fixation. Acetic acid was replaced with 19 parts saturated orcein in 45% acetic acid plus 1 part proprionic acid and allowed to stain for 30 min. Stained fins were examined under 1000× magnification for the presence of mitotic cells and abnormal anaphase–telophase mitotic configurations using the criteria described by Kocan et al. (1982).

*Larval weights*

Within 24 h of hatching, larvae were collected from each incubation vessel, fixed in 5% Formalin, blotted dry on filter paper, and dehydrated in a desiccator for 5 days. Larvae were then weighed daily and placed back in the desiccator until the weights stabilized. Twenty normal and 20 abnormal larvae were weighed from each exposure group, and the results were compared with weights obtained from three groups of control larvae. Because many of the larvae were severely contorted and otherwise deformed, it was not possible to obtain lengths that were as reliable as dry weights.

## Results and discussion

### Laboratory components

*Concentration of crude oil in seawater*

Following 18 h separation time, the lower aqueous layer of the oil–water mixture appeared clear and free of visible oil. This was drained from the separatory funnel without disturbing the surface oil layer and used for chemical analysis and exposure dilutions. The mean concentration of LMW (gasoline range, C6–C12) components decreased from 64 parts per million (ppm) at $t_0$ to 9.67 mg/L at $t_{48}$, a loss of 54.3 mg/L (84.9%). The mean concentration of HMW (diesel range, C12–C28) components was 9.6 mg/L at $t_0$ and 10.2 at $t_{48}$, indicating a stable concentration. Because the HMW components were stable over time (9.67 ± 2 mg/L), this value was used to calculate the $EC_{50}$ for the embryo toxicity titrations, recognizing that the volatile LMW components were also present at varying concentrations during each 48-h replenishment period. These values were comparable to those obtained by Anderson et al. (1974) for OWD from two different sources of crude oil.

The marine environment is often characterized by disturbances of the sea surface owing to wind, wave, and current action, which produces both soluble and particulate components

**Table 1.** Dissolved oxygen concentration, pH, and salinity values of seawater containing petroleum hydrocarbons, with (+) and without (–) aeration.

| | $O_2$ concn. (mg/L) | pH | Salinity (‰) |
|---|---|---|---|
| Controls ($N = 6$) | 10.47±0.19 | 8.4±0.13 | 29.7±0.55 |
| OWD (+) ($N = 12$) | 10.39±0.18 | 8.4±0.15 | 29.9±1.03 |
| OWD (–) ($N = 12$) | 10.24±0.18 | 8.3±0.16 | 30.3±0.83 |

Note: Values are given as the mean ± SD, for the mean of all hydrocarbon concentrations for 96 h.

in the mixture. OWD probably more closely resemble the type of oil–water mixture that might occur following an oil spill than does a true water-soluble fraction (U.S. National Research Council 1985), which is an artificial preparation designed more for accurate laboratory replications than to simulate conditions that occur in the marine environment.

*Physical characteristics of seawater and oil–water dispersions*

There was no demonstrable change, with and without aeration, in the $O_2$ concentration, pH, or salinity of OWD seawater, without herring embryos (Table 1). Our previous experience and that reported by other investigators indicate that a high level of dissolved oxygen is critical for successful hatching of herring larvae (Holliday et al. 1964). Because the presence of petroleum hydrocarbons in seawater does not alter oxygen concentration, pH, or salinity, any changes in these are probably due to the biological activity of the embryos rather than the presence of oil.

Several investigators have exposed herring embryos to petroleum hydrocarbons with and without aeration, and in most cases it appeared that oxygen concentration at the time of hatching was extremely critical. Holliday et al. (1964) demonstrated a constant increase in $Qo_2$ for developing embryos and a 100–150% increase in $O_2$ consumption during hatching. McQuinn et al. (1983) demonstrated high field mortality rate of embryos associated with rapid loss of oxygen when photosynthesis ceased at night. Our results (Table 1) demonstrate that the OWDs do not directly affect pH, $O_2$ concentration, or salinity in the absence of living embryos. Consequently, increased activity and respiratory rate of the embryos at the time of hatching could reduce dissolved oxygen levels and account for the elevated mortality observed by some investigators. Ideally, the dissolved oxygen concentration should be optimized to ensure true experimental toxicity when bioassays are being conducted with herring embryos and larvae in static or static-renewal exposure systems. Depletion of oxygen by embryos is probably less critical in a flow-through system.

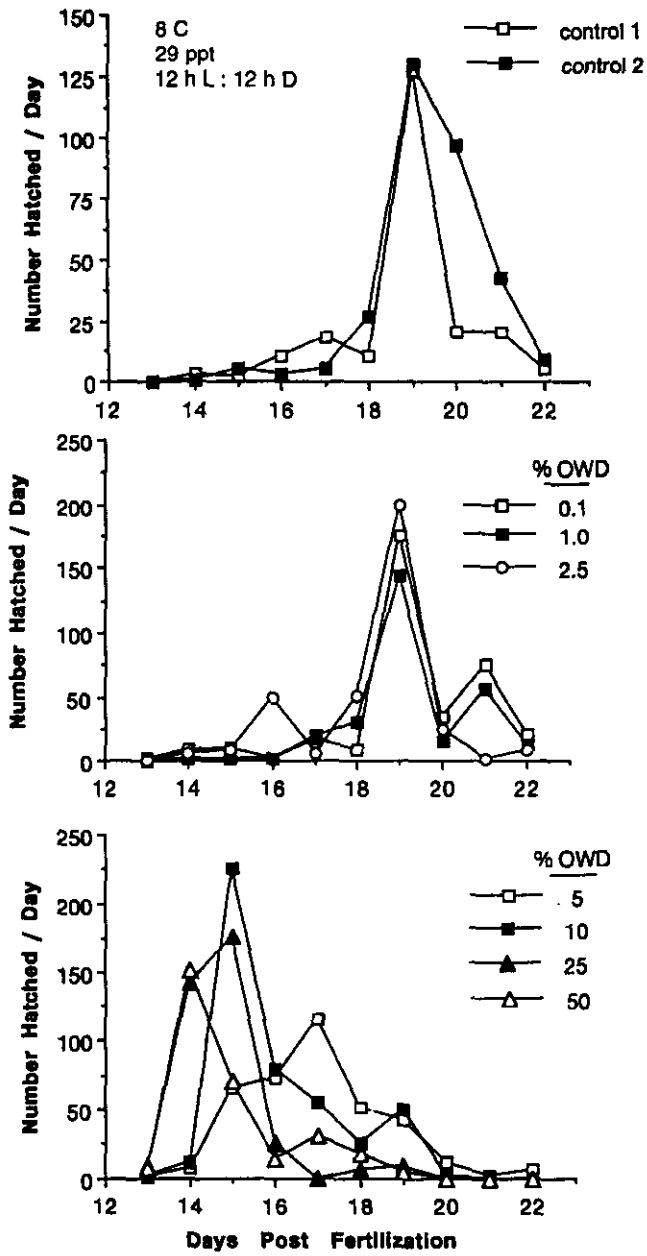
*Transport effects*

No significant differences were observed for percent hatch, percent normal larvae, or larval weight between PWS and Puget Sound herring embryos incubated in parallel under identical conditions ($p > 0.5$; ANOVA). Because no transport effects were observed, only PWS embryos were used for the remainder of the study.

*Continuous embryonic exposure to oil–water dispersions (1991)*

*Hatching dynamics:* Exposure of embryos to >0.24 mg/L

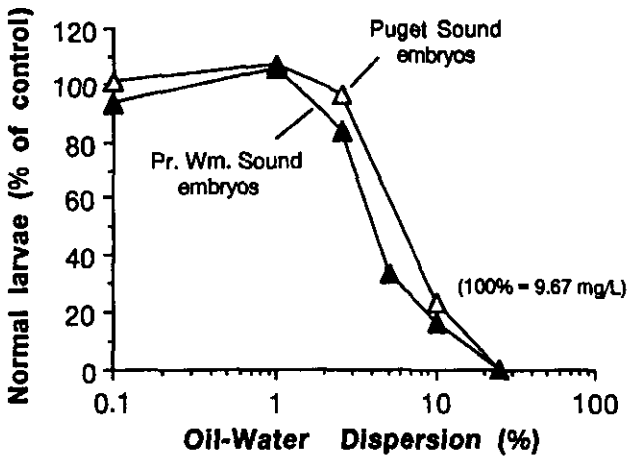
**Fig. 2.** Hatching dynamics of Prince William Sound herring embryos (*Clupea pallasi*) exposed to oil–water dispersions (OWD) of Prudhoe Bay crude oil from fertilization to hatching.




(2.5% OWD) resulted in a 4- to 5-day premature hatch (mean = 15 days) relative to the controls and lower OWD concentrations (mean = 19–20 days). Exposure to 0.48 mg/L (5% OWD) resulted in a peak hatch on day 17, intermediate between the normal and precocious hatch groups (Fig. 2).

Perturbation of mean hatching day has been reported by several authors, but the effect is not consistent from study to study. Kocan et al. (1987) reported precocious hatching in Baltic herring embryos exposed to petroleum hydrocarbons in sea-surface microlayer; other authors reported no difference in hatching time (Smith and Cameron 1979; Pearson et al. 1985), and others reported both precocious and delayed hatching, depending on the source of the crude oil and exposure concentration

**Fig. 3.** Comparative concentration-response curve for physical deformities showing no difference between herring embryos from Prince William Sound, Alaska, and Puget Sound, Washington, following exposure to oil–water dispersions of Prudhoe Bay crude oil ($p > 0.05$; $t$ test).




(Linden 1978). It appears that there are factors that have not yet been identified that can perturb hatching in the presence of toxic substances.

*Physical defects in post-hatch larvae:* There was a significant increase in the proportion of physically deformed larvae between 0.24 and 0.97 mg/L OWD; however, embryo survival and live hatch were not affected (Table 2). Physical deformities included spinal defects (scoliosis or lordosis), optic malformations (protruding or missing lens, cyclopia, deformed eyeball), maxillary and mandibular malformations, and enlargement of the pericardial region. All physical abnormalities were pooled for the purpose of this study (i.e., total gross abnormalities). By combining all types of morphological abnormalities, both fatal and nonfatal abnormalities were distinguished from normal (e.g., viable) larvae. There was no significant difference ($p > 0.5$; ANOVA) in the response to exposure to Prudhoe Bay crude oil between Puget Sound embryos and Prince William Sound embryos (Fig. 3).

Significant post-hatching larval abnormalities were reported by Linden (1978), who saw increases in the number of malformed herring larvae after exposure to light fuel oil (3.1 mg/L), Venezuelan crude oil (5.4 mg/L), and Tuimazan crude oil (2.6 mg/L). Herring embryos exposed to 0.68 ppm WSF for 48 h exhibited a significant increase in gross abnormalities (Smith and Cameron 1979). Pearson et al. (1985) concluded that the concentrations of total saturated hydrocarbons and total hydrocarbons were the best indicators of larval abnormalities, a conclusion consistent with the findings of this study. Conversely, no abnormal larvae were observed by Rice et al. (1987) when they exposed herring larvae to <5 ppm Prudhoe Bay WSF for 12 days.

Other herring embryo studies demonstrated high mortality rates following exposure to crude oil. Smith and Cameron (1979) reported no decrease in hatching success of embryos exposed to 0.68 mg/L WSF for up to 48 h, but 100% mortality occurred when exposures lasted for 6 days. Rice et al. (1987) reported mortality rates of up to 100% in embryos exposed for

**Table 2.** Results of continuous exposure of Prince William Sound herring embryos (*Clupea pallasi*) to varying concentrations of oil–water dispersions (OWD) of Prudhoe Bay crude oil, from fertilization to hatching.

| % OWD | Petroleum hydrocarbon concn. ($mg \cdot L^{-1}$)[a] | Live hatch[b] | % hatch | % normal |
|---|---|---|---|---|
| | | **Controls** | | |
| 0 | 0 | 175 | 61.4 | 59 |
| 0 | 0 | 166 | 75.8 | 51 |
| 0 | 0 | 203 | 83.2 | 54 |
| Mean±SD | 0 | 181±19.3 | 73.5±11.1 | 55±4.0 |
| | | **Exposed** | | |
| 0.1 | 0.01 | 237 | 72.9 | 51 |
| 1.0 | 0.10 | 198 | 79.2 | 58 |
| 2.5 | 0.24 | 180 | 84.9 | 46 |
| 5.0 | 0.48 | 136 | 86.1 | 18 |
| 10 | 0.97 | 200 | 92.2 | 9 |
| 25 | 2.41 | 137 | 74.9 | 0 |
| 50 | 4.83 | 164 | 72.9 | 0 |
| 100 | 9.67 | 157 | 70.4 | 0 |
| Mean±SD | — | 176±34.7 | 79.2±7.8 | — |

**Note:** There were three replicates of untreated controls.

[a]Dilutions (mg/L) calculated from high molecular weight value (100% OWD = 9.67 mg/L).

[b]Number of hatched per treatment.

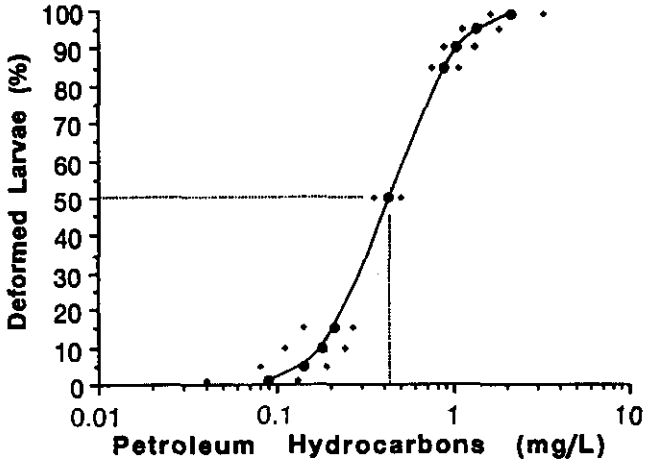
12 days ($LC_{50}$=1.5 mg/L) but no mortality in embryos exposed for 2 days ($LC_{50}$ > 5.3 mg/L). Linden (1978) observed no effect on hatching when embryos were exposed to 1 mg/L or less but over 25% embryo mortality at 10 mg/L for two different crude oils. Pearson et al. (1985) reported similar results, except that their exposures lasted only 24 h.

The data from Table 2 were analyzed using the Environmental Protection Agency's probit analysis program (Environmental Protection Agency 1985) and resulted in an $EC_{50}$ of 0.43 mg/L (4.46%) of the HMW components of OWD. A concentration-response curve with confidence limits was constructed by converting probit units to percent physical deformities (Fig. 4).

*Genotoxicity:* Mitotic activity and anaphase–telophase aberrations were the most sensitive biomarkers indicative of exposure to OWD. The number of mitotic cells per fin declined significantly at and above 0.24 mg/L ($p < 0.05$; one-way ANOVA); anaphase–telophase aberrations doubled at 0.01 mg/L OWD and increased directly with dose for all concentrations above 0.01 mg/L ($p < 0.01$) (Table 3). These observations are particularly significant because damage was evident in post-hatch larvae, while exposure occurred during embryo development.

The long-term consequence of genetic damage to any population is two-fold. First, genetic damage to somatic cells is believed to be the mechanism for neoplasia (cancer) induction and physical defects. Second, genetic damage to germ cells (sperm and ova) can result in mutations capable of being passed on to subsequent generations. The presence of recessive mutations in a population presents an insidious threat that might not manifest itself until several generations later when the cause of the genetic damage is no longer present. Reduced

**Fig. 4.** Concentration-response curve showing $EC_1$ to $EC_{99}$ values and 95% confidence limits for physical deformities in herring larvae exposed to oil–water dispersions from fertilization to hatching (18 days). Values were obtained by converting probit units to percent response.




fertility, embryo death, physical and biochemical defects, and behavioral modification are some of the possible consequences of mutant gene amplification in a population (Brusick 1980).

*Larval weights:* Normal OWD-treated larvae weighed less (75 ± 11 μg) than normal controls (120 ± 25 μg) ($p < 0.05$; ANOVA). Although normal and abnormal larvae both occur in treated and control groups, only the physically normal OWD-exposed larvae experienced significant weight loss (Fig. 5). The weights of the normal untreated control larvae were comparable to the weights reported by Schnack (1981) for normal herring larvae (114 μg). Mean weights of unexposed and oil-exposed abnormal larvae were 69 ± 18 and 89 ± 12 μg, respectively. Other studies on the effects of oil on herring larval size report similar results, although most data are presented as length rather than dry weight. Linden (1978) and Smith and Cameron (1979) reported that embryos exposed to oil produced significantly shorter larvae than did controls, and embryos exposed to the Prudhoe Bay water-soluble fraction also developed into shorter larvae than did controls (Pearson et al. 1985).

Inhibition of mitosis or damage to mitotic cells are possible mechanisms to explain the reduced weight observed in normal OWD-treated larvae. A reduction in mitotic rate as well as cell death would result in fewer cells being produced in a given amount of time, resulting in slower growth and smaller larvae. An equally plausible explanation would be an increase in the energy demand required to metabolize and excrete the petroleum hydrocarbons, thus consuming yolk material that might otherwise be used for embryo growth (Kocan and Landolt 1984; Stahl and Kocan 1986).

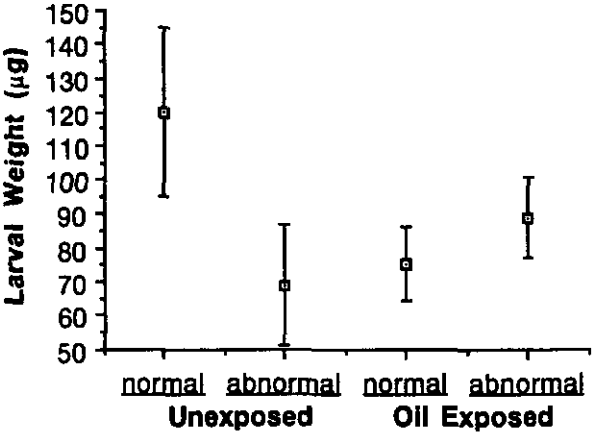
Regardless of the mechanism responsible for the observed weight loss in oil-exposed larvae, it has been demonstrated that individuals with higher hatching weights have an increased survival potential relative to smaller individuals, even if they both appear to be otherwise normal (Crowder et al. 1992).

**Table 3.** Genotoxic damage and mitotic activity in herring larvae (*Clupea pallasi*) continuously exposed as embryos to oil–water dispersions of Prudhoe Bay crude oil.

| Concentration (mg/L) | Mitoses/fin | % anaphase aberrations |
|---|---|---|
| 0.00 | 7.6 | 8.3 |
| 0.01 | 8.1 | 16.1 |
| 0.10 | 7.6 | 18.0[a] |
| 0.24 | 5.5[a] | 25.4[a] |
| 0.48 | 4.4[a] | 24.0[a] |
| 0.97 | 6.8[a] | 26.6[a] |
| 2.42 | 3.9[a] | 29.6[a] |
| 4.84 | 5.2[a] | 39.6[a] |
| 9.67 | 1.1[a] | 57.1[a] |

[a]Significantly different from control ($p < 0.01$).

**Fig. 5.** Mean weight of newly hatched herring larvae exposed continuously to oil–water dispersions as embryos.



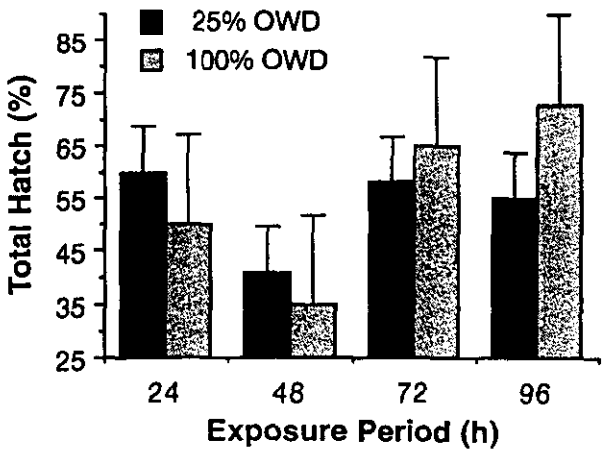

*Intermittent (pulse) exposure (1992)*

Pulse exposure of embryos to OWD for 36 h revealed the blastula and gastrula stages to be most sensitive. The hatching success of unexposed control herring embryos was 63%, whereas OWD-exposed embryos exposed at 24 and 48 h after fertilization (blastula–gastrula) exhibited a distinct dose response with a nearly 40% decrease in live hatch ($p < 0.05$, Fisher's test; Fig. 6). Embryos appeared to be resistant to the lethal effects of petroleum hydrocarbons by 72 h after exposure under laboratory conditions.

Unlike the embryos exposed continuously to OWD in 1991, embryos exposed intermittently to OWD in 1992 showed a significantly increased mortality rate rather than physical defects. One possible explanation is that embryos were derived from different gene pools in 1991 and 1992. The 1991 embryos were from a mixed population of primarily 6- and 7-year-old spawners, whereas the 1992 embryos were produced almost exclusively from 4 year olds. It is not clear at this time whether this difference resulted from a genetic difference in year-classes, previous adult exposure to toxins, or some other unknown variable.

Regardless of the type of response observed, these data demonstrate that the age of the embryo at the time of exposure could influence the toxic response and consequently the degree to which subsequent life stages might be affected, a phenomenon originally predicted by Rosenthal and Alderdice (1976).

**Fig. 6.** Herring embryo developmental stage sensitivity following exposure to oil–water dispersions (OWD) of Prudhoe Bay crude oil. Exposure periods began at 24, 48, 72, and 96 h after fertilization, and lasted for 36 h.




The differences in embryo mortality, hatching success, and physical malformations for laboratory-reared herring reported here and by other investigators probably reflect the different techniques used for incubation, exposure, oil source, and other variables such as parental stock and possibly the presence of competing marine organisms in the exposure water during embryo incubation. Investigations specifically designed to detect differential spawning success between age-classes and subpopulations of spawners could answer these questions as well as explain differences in productivity among year-classes.

The results of this study demonstrate that Pacific herring embryos and larvae are adversely affected by exposure to Prudhoe Bay crude oil and responses range from sublethal genetic damage to physical deformities and death. These experimental data, which relate pathological response to dose and time, can be used by fisheries managers to predict the extent of damage in the field following disasters such as the *Exxon Valdez* oil spill. By comparing experimental laboratory data with data obtained from field samples following such events, it should be possible to predict the proportion of larvae that would be affected, provided field data on oil concentration and exposure time were collected.

## Field component

*Hatching success*

Hatching success was generally lower in embryos incubated in situ (58.8% at unoiled sites; 68.4% at previously oiled sites) than embryos incubated in the laboratory (73.5% for controls; 79.2% for oil treatments). There did not appear to be any depth effect at either the oiled or unoiled sites (Table 4). Differences in hatching success between the field and laboratory may represent differences in procedure or environmental conditions during incubation.

*Abnormal larvae*

A greater proportion of morphologically normal larvae hatched from embryos exposed at unoiled sites (63.3%) than at oiled sites (51.3%) ($p < 0.002$; $t$ test). Although the percentage of normal larvae was significantly lower at previously oiled sites, the rates observed generally fell within the ranges observed for the control or lowest exposure treatments (0.10–1.0 mg/L) of the dose-response experiments performed in the laboratory. Depth had no effect on the production of abnormal larvae from oiled or unoiled sites (Table 4).

*Viable larvae*

Percent viable larvae (eq. 1) did not differ significantly between the oiled and unoiled sites (Table 4). Greater numbers of abnormal larvae at oiled sites were offset by lower hatching success at unoiled sites, making percentages of viable larvae similar. These conflicting data may be due to the numerous variables encountered under field conditions. The differences observed between data collected under controlled laboratory conditions and those obtained from the field emphasize the need to understand how procedural methodology and physical conditions during incubation affect hatching success, larval weights, and production of viable larvae.

*Larval weights*

The mean dry weights of both normal and abnormal larvae from the oiled sites were significantly lower than the weights of larvae from unoiled sites ($p < 0.01$; $t$ test) (Table 5), a finding similar to that observed in the laboratory. If lower hatching weights affect larval survival, then eq. 1 is an overestimate of the percent viable larvae and should be modified to include reduced survival potential for smaller larvae (Crowder et al. 1992).

Because there was no unequivocal evidence for continuing exposure to petroleum at the oiled sites (Brown et al. 1996), differences in larval weights could not be directly linked to previous oiling of the sites. Future studies should examine survival of variable-weight larvae under different physical conditions to better understand how weight at hatch affects viability and whether persistent effects of petroleum remain at oiled sites for extended periods.

## Conclusions

During continuous exposure of herring embryos to OWD (0.01–0.97 mg/L), genetic damage was the most sensitive biomarker for oil exposure, with a statistically significant increase in damage at 0.10 mg/L and a biologically significant increase at 0.01 mg/L. This was followed by (*i*) physical deformities (significant ≥ 0.24 mg/L); (*ii*) reduced mitotic activity (significant ≥ 0.24 mg/L); (*iii*) lower hatch weight (all OWD concentrations); and (*iv*) premature hatch, which was effected at >0.24 mg/L. Percent successful hatch and percent live hatch were not significantly affected at any concentration of OWD.

During intermittent 36-h exposure of herring embryos at different developmental stages, the blastodisc (24 h after fertilization) and gastrula (48 h after fertilization) were the most sensitive to the OWD. Live hatch was reduced by nearly 40% in embryos exposed at these two stages compared with embryos exposed later in development.

Embryos collected from similar sources and placed at previously oiled and unoiled sites 2 years after the spill exhibited variable rates of hatching success and production of viable larvae. Because there was little evidence to confirm continuing exposure at the oiled sites and because the range in the percentage of normal larvae observed generally fell within the

**Table 4.** Percent hatch and percent normal and viable larvae following in situ exposures of herring embryos at previously oiled and unoiled sites in Prince William Sound, 3 years after the *Exxon Valdez* oil spill.

(A) Results by depth and site.

| | Unoiled sites (C) | | | | | Oiled sites (O) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | % hatch | | % normal | | | % hatch | | % normal | |
| Site | −1.5 m | −4.5 m | −1.5 m | −4.5 m | Site | −1.5 m | −4.5 m | −1.5 m | −4.5 m |
| 3 | 64.8 | 71.8 | 66.5 | 78.2 | 1 | 64.4 | ne | 55.2 | ne |
| 4 | 56.4 | ne | 67.1 | ne | 2 | 66.3 | 70.5 | 53.3 | 45.7 |
| 6 | 58.9 | 59.1 | 33.7 | 7.6 | 3 | 68.8 | ne | 29.2 | ne |
| 7 | 61.8 | 54.2 | 50.5 | 70.2 | 4 | 68.5 | 68.7 | 65.3 | 47.9 |
| 8 | 54.9 | 47.8 | 67.9 | 63.1 | 5 | 70.5 | 69.4 | 54.7 | 59.1 |
| Mean±SD | 59.3±4.0 | 58.2±10.1 | 57.1±14.9 | 70.9±6.3 | | 67.7±2.4 | 69.5±0.9 | 51.5±13.4 | 50.9±7.20 |

(B) Summary.

| | Unoiled sites (mean±SD) | Oiled sites (mean±SD) | $p^a$ |
|---|---|---|---|
| % hatch | 58.8±6.9 | 68.4±2.1 | <0.01 |
| % normal | 63.3±12.6 | 51.3±10.1 | <0.002 |
| % viable | 37.2±10.0 | 35.1±10.8 | ns |

**Note:** Percent hatch and percent viable were calculated from total fertile eggs. Percent normal was calculated from total live hatch. ne, no exposure; ns, not significant.

[a]Statistic: *t* test.

**Table 5.** Mean dry weights of morphologically normal and abnormal herring larvae exposed in situ at two depths at unoiled and oiled sites 3 years after the *Exxon Valdez* oil spill in Prince William Sound.

(A) Results by depth and site.

| | Unoiled sites[a] (C) | | | | | Oiled sites[a] (O) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | −1.5 m | | −4.5 m | | | −1.5 m | | −4.5 m | |
| Site | Normal | Abnormal | Normal | Abnormal | Site | Normal | Abnormal | Normal | Abnormal |
| 3 | 52[a] | 71 | 66 | 112 | 1 | 66 | 81 | ne | ne |
| 4 | 83 | 138 | ne | ne | 2 | 63 | 101 | 97 | 56 |
| 6 | 78 | 106 | 104 | 98 | 3 | 93 | 82 | ne | ne |
| 7 | 119 | 143 | 135 | 83 | 4 | 48 | 71 | 51 | 86 |
| 8 | 103 | 96 | 106 | 61 | 5 | 94 | 86 | 89 | 84 |
| Mean±SD | 87±26 | 111±30 | 102±28 | 88±22 | | 72±20 | 84±11 | 79±24 | 75±17 |

(B) Summary.

| | Unoiled sites (mean±SD) | Oiled sites (mean±SD) | $p^a$ |
|---|---|---|---|
| % hatch | 94±25 | 75±19 | 0.01 |
| % abnormal | 101±26 | 81±12 | 0.01 |
| Total | 97±26 | 78±17 | 0.01 |

**Note:** ne, no exposure.

[a]Values are given as micrograms per larva and represent the mean of 20 individuals per site.

[b]Statistic: *t* test.

range observed in control or low oil exposure treatments in the laboratory, the in situ results may reflect natural variability resulting from different physical and chemical conditions encountered during incubation rather than oil exposure. More comprehensive studies designed to determine the extent of variability inherent at different sites, including the effect of temperature, salinity, solar radiation, and nearshore ocean circulation patterns, need to be carried out before the effects of previous oiling can be separated from effects caused by the natural conditions at different sites.

## Recommendations

Our ability to confidently distinguish oil effects from natural biological variation was compromised owing to the lack of background data with which to compare the extensive data collected since the grounding of the *Exxon Valdez* in March 1989. Damage assessments could be estimated more accurately in the future if background data from sensitive marine areas were collected in advance of any catastrophic event. The collection of base-line data should include field studies conducted in situ to better define the causes and extent of natural

variability as well as laboratory studies that define the effects of oil under different conditions on growth and survival of herring embryos and larvae.

## Acknowledgments

This study was funded by Alaska Department of Fish and Game contracts IHP-91-043 and IHP-92-039, and National Institute of Environmental Health Sciences grant ES-04696. The technical assistance of T. Mehl, M. Greene, L. Bolles, J. Wilcock, J. Gilman, J. Allen, and N. McKrell and the cooperation of M. Carles and D.A. Moles are appreciated.

## References

Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E., and Hightower, G.M. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. (Berlin), **27**: 75–88.

Brown, E.D., Norcross, B.L., and Short, J.W. 1996. An introduction to studies on the effects of the *Exxon Valdez* oil spill on early life history stages of Pacific herring, *Clupea pallasi*, in Prince William Sound, Alaska. Can. J. Fish. Aquat. Sci. **53**: 2337–2342.

Brusick, D. 1980. The consequences of genotoxic effects in humans and other animals. *In* Principles of genetic toxicology. *Edited by* D. Brusic. Plenum Press, New York. pp. 45–68.

Crowder, L.B., Rice, J.A., Miller, T.J., and Marschall, E.A. 1992. Empirical and theoretical approaches to size-based interactions and recruitment variability in fishes. *In* Individual-based models and approaches in ecology. *Edited by* D.L. DeAngelis and L.J. Gross. Chapman & Hall, New York. pp. 237–255.

Environmental Protection Agency. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. Toxicity data analysis. Sect. 11. EPA/600/4-85/013. U.S. Environmental Protection Agency, Cincinnati, Ohio. pp. 70–78.

Holliday, F.G.T, Blaxter, J.H.S., and Lasker, R. 1964. Oxygen uptake of developing eggs and larvae of the herring (*Clupea harengus*). J. Mar. Biol. Assoc. U.K. **44**: 711–723.

Hose, J.E., McGurk, M.B., Marty, G.D, Hinton, D.E, Brown, E.D., and Baker, T.T. 1996. Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphological, cytogenetic, and histopathological assessments, 1989–1991. Can. J. Fish. Aquat. Sci. **53**: 2355–2365.

Kocan, R.M., and Landolt, M.L. 1984. Alterations in patterns of excretion and other metabolic functions in developing fish embryos exposed to benzo(*a*)pyrene. Helgol. Meeresunters. **37**: 493–504.

Kocan, R.M., Landolt, M.L., and Sabo, K.M. 1982. Anaphase aberrations: a measure of genotoxicity in mutagen-treated fish cells. Environ. Mutagen. **4**: 181–189.

Kocan, R.M., Westernhagen, H.V., Landolt, M.L., and Furstenberg, G. 1987. Toxicity of sea-surface microlayer: effects of hexane extract on Baltic herring (*Clupea harengus*) and Atlantic cod (*Gadus morhua*) embryos. Mar. Environ. Res. **23**: 291–305.

Linden, O. 1978. Biological effects of oil on early development of the Baltic herring *Clupea harengus membras*. Mar. Biol. (Berlin), **45**: 273–283.

Marty, G.D., Okihiro, M.S., and Hinton, D.E. 1993. Herring histopathology report on the *Exxon Valdez* oil spill. Progress report No. IHP-93-016. Alaska Department of Fish and Game, Anchorage, Alaska.

McQuinn, I.H, Fitzgerald, G.J., and Powles, H. 1983. Environmental effects on embryos and larvae of the Isle Verte stock of Atlantic herring *Clupea harengus pallasi*). Nat. Can. Que. **110**: 343–355.

Pearson, W.H., Woodruff, D.L., Kiesser, S.L., Fellingham, G.W., and Elston, R.A. 1985. Oil effects on spawning behavior and reproduction in Pacific herring (*Clupea harengus pallasi*). API publication No. 4412. American Petroleum Institute, Washington, D.C.

Rice, S.D., Babcock, M.M., Brodersen, C.C., Carls, M.G., Gharrett, J.A., Korn, S., Moles, A., and Short, J.W. 1987. Lethal and sublethal effects of the water-soluble fraction of Cook Inlet crude oil on Pacific herring (*Clupea harengus pallasi*) reproduction. NOAA Tech. Memo. No. NMFS F/NWC-111. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Washington, D.C.

Rosenthal, H., and Alderdice, D.F. 1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. J. Fish. Res. Board Can. **33**: 2047–2065.

Schnack, D. 1981. Studies on the mortality of Pacific herring larvae during their early development, using artificial in situ containments. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, **178**: 135–142.

Smith, R.L., and Cameron, J.A. 1979. Effect of water soluble fraction of Prudhoe Bay crude oil on embryonic development of Pacific herring. Trans. Am. Fish. Soc. **108**: 70–75.

Stahl, R.G., and Kocan, R.M. 1986. Influence of age on patterns of uptake and excretion of polycyclic aromatic hydrocarbons in the rainbow trout embryo. *In* Aquatic toxicology and environmental fate. Vol. 9. ASTM STP 921. *Edited by* T.M Poston and R. Purdy. American Society for Testing and Materials, Philadelphia, Pa. pp. 287–303.

U.S. National Research Council. 1985. Oil in the sea: inputs, fates and effects. Chap. 3. National Academy Press, Washington, D.C.

# Distribution, abundance, morphological condition, and cytogenetic abnormalities of larval herring in Prince William Sound, Alaska, following the *Exxon Valdez* oil spill[1]

**Brenda L. Norcross, Jo Ellen Hose, Michele Frandsen, and Evelyn D. Brown**

**Abstract**: Pacific herring (*Clupea pallasi*) larvae were collected throughout Prince William Sound in May, June, and July following the *Exxon Valdez* oil spill of March 1989. Movement of herring larvae is related to current patterns within the sound, and deformed larvae were found both inside and outside of areas considered to be oiled. Herring may have been exposed to oil as embryos in contaminated spawning areas and as larvae encountering the oil trajectory. Although it was impossible to estimate the effects of exposure to *Exxon Valdez* oil, many larvae exhibited symptoms associated with oil exposure in laboratory experiments and other oil spills. These included morphological malformations, genetic damage, and small size. Growth between May and June 1989 was the lowest ever reported for field-caught larval herring. Jaw malformations and genetic damage were highest in May and were elevated through the western area of Prince William Sound, which overlapped the oil trajectory. In June 1989, jaw development was normal, but genetic damage persisted. In contrast, in May 1995, jaw and cytogenetic development were normal and significantly different from those in larvae in 1989.

**Résumé** : Les auteurs ont prélevé des larves du hareng du Pacifique (*Clupea harengus*) à différents endroits dans le golfe de Prince-William au cours des mois de mai, juin et juillet 1989, à la suite du déversement de pétrole de l'*Exxon Valdez* en mars précédent. Les déplacements de larves de hareng dépendent en partie des courants dans le golfe, et des larves difformes ont été trouvées à l'intérieur comme à l'extérieur des secteurs qu'on jugeait être contaminés. Les harengs ont pu être exposés au pétrole alors qu'à l'état d'embryons, ils se trouvaient dans des aires de ponte contaminées ou qu'à l'état de larves, ils ont été pris dans la trajectoire du pétrole. Même s'il était impossible d'évaluer les effets de l'exposition au pétrole déversé, les auteurs ont constaté que de nombreuses larves présentaient des symptômes associés à l'exposition au pétrole lors d'essais en laboratoire ou observés à l'occasion d'autres déversements. Ce sont notamment des défauts morphologiques, des dommages génétiques et la petite taille des sujets. Entre mai et juin 1989, la croissance mesurée était la plus faible jamais signalée chez des larves de hareng capturées dans leur milieu. C'est en mai qu'il y avait le plus de difformités de la mâchoire et de dommages génétiques; le nombre de sujets atteints de la sorte est resté élevé partout dans la partie ouest du golfe de Prince-William balayée par la nappe de pétrole. En juin 1989, le développement de la mâchoire était redevenu normal, mais les dommages génétiques persistaient. En mai 1995, par contre, le développement cytogénétique et celui de la mâchoire étaient normaux et différaient significativement de ce qu'ils étaient en 1989.
[Traduit par la Rédaction]

## Introduction

Pacific herring (*Clupea pallasi*) spawned in Prince William Sound (PWS), Alaska, from 31 March through 20 April 1989 (Biggs et al. 1993) immediately following the grounding of the tanker *Exxon Valdez* on 24 March, which spilled 42 000 000 L (11 000 000 gallons) of Prudhoe Bay crude oil into PWS. In PWS, Pacific herring are demersal spawners that primarily lay their eggs on kelp in subtidal waters. Pacific herring and Baltic herring (*Clupea harengus membras*) are adversely affected when exposed to oil during spawning, egg, or larval stages (Eldridge et al. 1977, 1978; Lindén 1978; Smith and Cameron 1979; Struhsaker 1977; Urho 1991). The inshore spawning strategy of herring makes them vulnerable to oil pollution (Rice et al. 1987).

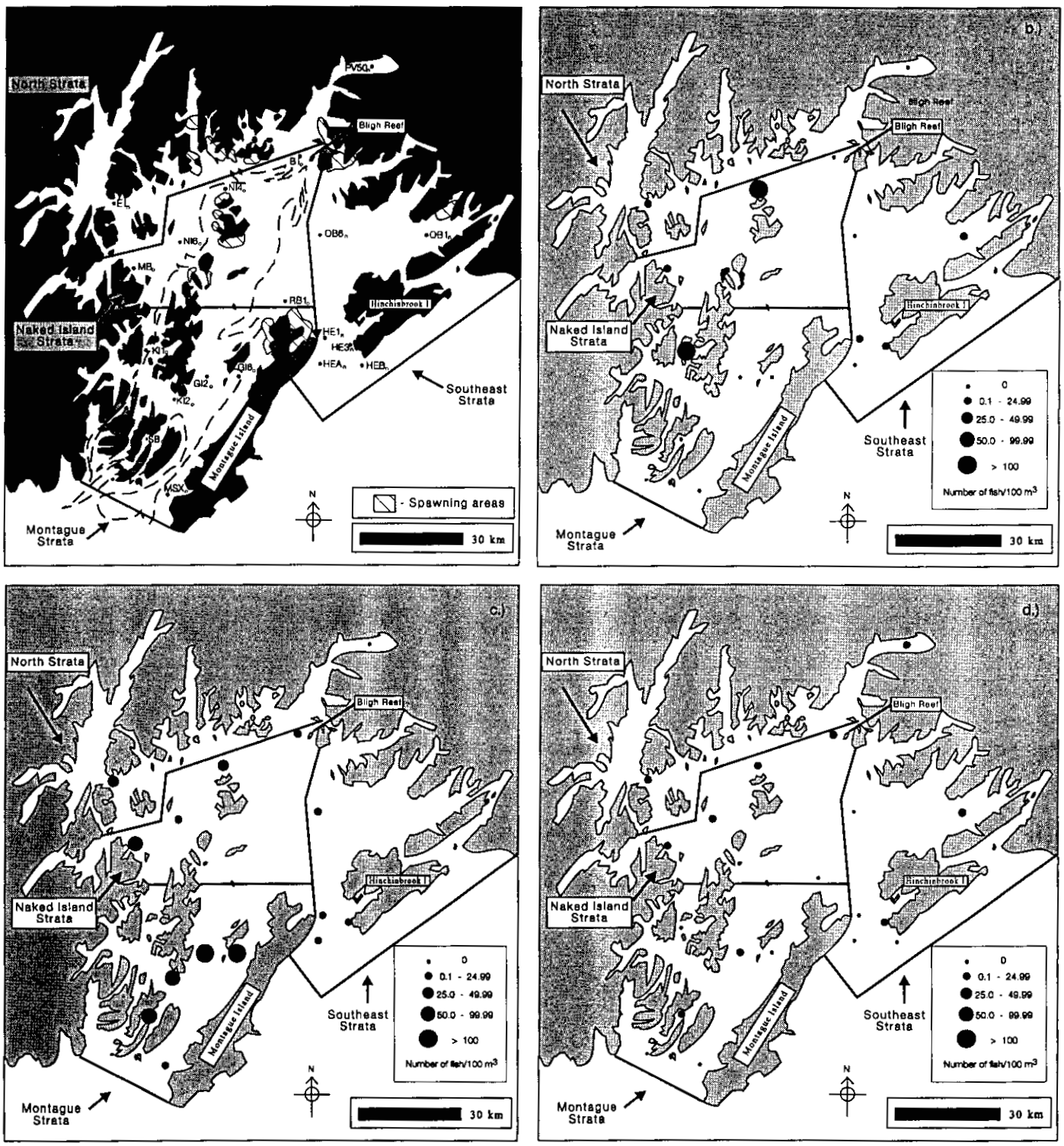
Hydrocarbon exposure of fish eggs can be acutely toxic (Rice et al. 1979; Smith and Cameron, 1979) or can lead to sublethal effects (Kuhnhold 1977; Solbakken et al. 1984; Rice et al. 1987) including increased frequencies of abnormalities (Struhsaker 1977; Smith and Cameron 1979; Pearson et al. 1985). The effects of oil on fish larvae depend on a complex interaction of factors: spatial and temporal distribution of spawning, developmental stage, population dynamics of species, spill location, duration of exposure, hydrodynamics of the

Received October 6, 1993. Accepted September 19, 1996.
J12114

**B.L. Norcross**[2] **and M. Frandsen.** Institute of Marine Science, School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks, AK 99775–1080, U.S.A.
**J. Hose.**[3] Department of Biology, Occidental College, 1600 Campus Road, Los Angeles, CA 90041, U.S.A.
**E.D. Brown.** Division of Commercial Fisheries Management and Development, Alaska Department of Fish and Game, Cordova AK 99574, U.S.A.

[1] Contribution No. 1543 of the Institute of Marine Science, University of Alaska.
[2] Author to whom all correspondence should be addressed. e-mail: norcross@ims.alaska.edu
[3] Present address: 2590 Softchess Place, Arroyo Grande, CA 93420, U.S.A.

**Fig. 1.** (*a*) Prince William Sound stations and strata divided into oiled (Naked Island and Montague Strait) and nonoiled (southeast and north) including herring spawning areas and trajectory of oil spilled by the *Exxon Valdez* on the basis of overflight surveys in 1989; distribution of herring larvae standardized to number of larvae per 100 $m^3$ of water filtered in (*b*) May 1989, (*c*) June 1989, and (*d*) July 1989.




area (Spaulding et al. 1985; Foyn and Serigstad 1987), and environmental factors such as temperature and salinity (Rice et al. 1979).

Oceanographic and meteorologic factors influence the direction and rate of transport of both fish larvae (Norcross and Shaw 1984) and spilled oil (Spaulding et al. 1983). The general circulatory pattern of surface waters within PWS in which water flows in through Hinchinbrook Entrance and out through Montague Strait (Royer et al. 1990) would result in herring larvae within the open waters of the sound encountering the

trajectory of the *Exxon Valdez* oil (Fig. 1). However, the subsurface movement of oil in droplets and suspended oil particles is different and more widespread than the surface oil trajectory (Short and Harris 1996); without more precise knowledge of subsurface oil dispersal in PWS following the spill, oiled and nonoiled larvae cannot be distinguished reliably.

The first objective of this study was to document the distribution, abundance, and size of herring larvae and postlarvae in PWS during the spring and summer of 1989. The second objective was to evaluate the herring larvae collected in 1989 for morphometric and cytogenetic abnormalities. Finally, morphological and cytogenetic abnormalities measured in 1989 larvae were compared with those in larvae collected in 1995, to assess potentially useful distinctions between oiled and nonoiled individuals.

## Methods

### Field collections

In response to the *Exxon Valdez* oil spill, four 1-week cruises were conducted on 5–11 April, 5–11 May, 1–6 June, and 7–14 July 1989. Stations were chosen to represent a cross section of all geographic areas and oceanographic conditions. The stations were post-stratified into four regions for analysis on the basis of the oil spill trajectory, oceanography of the sound, and spawning locations of herring. The strata partitioned PWS geographically into oiled (Naked Island and Montague) and nonoiled (southeast and north) areas (Fig. 1). Station names are designated with a subscript of o in the oiled Naked Island and Montague strata and n in the nonoiled southeast and north strata. The stations that were occupied during the first cruise were also sampled during subsequent cruises (weather permitting) and additional stations were sampled as the season progressed.

In 1989, larvae were collected with a 1-$m^2$ Tucker trawl rigged with two or three 505-μm mesh nets towed at 1 $m \cdot s^{-1}$. A TSK or General Oceanics flow meter was mounted in the mouth of the net to determine the volume of water filtered. Conductivity–temperature–depth (CTD) profiles were taken at all stations in addition to a self-contained CTD (SeaBird Electronics SBE-19) being attached to the net. During the first three cruises, depth intervals of 1–25, 25–50, 50–100, and 0–100 m were sampled obliquely or horizontally. Owing to wire constraints on board the R/V *Alpha Helix*, no deeper samples were obtained. During the July cruise (National Oceanic and Atmospheric Administration ship *John Cobb*), the same depth increments were sampled in the upper 100 m, as well as an increment of 100–400 m. At a given station, each depth increment was sampled only once. After each tow, all nets were immediately rinsed with seawater, and samples were sieved and preserved. Plankton was preserved in alcohol (100% ethanol or isopropyl alcohol) or 10% formalin. The preservatives were replaced after 24, 48, and 72 h.

In 1995, larvae were collected as morphologic and cytogenetic controls, for comparison with 1989 larvae. Vertical hauls were made to 20–50 m, depending on the depth of the site, with a 333-μm mesh zooplankton net. Samples were preserved in 10% formalin buffered with seawater. Samples were collected at four stations: nearshore and offshore locations off both the north and west sides of Montague Island. Collection sites in 1995 differed from those in 1989 because herring have not spawned in the northern part of PWS since 1990 (Donaldson et al. 1993).

### Laboratory analysis

Whole plankton samples were sorted using dissecting microscopes to isolate fish larvae. After sorting, larvae were stored in isopropyl alcohol. Quality control of the sorting technique was monitored by using stratified random sampling to select 10% of the jars sorted by each individual. These jars were resorted to determine sorting error. An error rate of 10% (number remaining/number originally sorted) was considered acceptable. If the error rate for one person was consistently greater than 10%, all of the samples sorted by that person were resorted.

Larval fish were identified to the lowest taxonomic level according to Matarese et al. (1989). Standard length was measured to the nearest 0.1 mm using a Wild MZ3 dissecting microscope and BioQuant image analysis system (Fowler 1988) interfaced to a computerized data base containing the cruise information. Samples were preserved for more than 3 years prior to identification and measurement of larvae; lengths were not corrected for preservative shrinkage, condition factors could not be estimated, and morphological estimates could be made only of craniofacial malformations, not of fin-fold damage (Hose et al. 1996). Fish that could not be identified owing to disintegration (<10%) were discarded. Abundance was standardized as number of herring larvae per 100 $m^3$ of water filtered.

### Morphological examination

Morphological deformities were assessed without knowledge of sampling site, using the graduated severity index (GSI) method described in Hose et al. (1996). Because of possible confusion between effects caused by handling and actual condition, only craniofacial malformations (eye and jaw defects) were evaluated. Each larva was examined using a Nikon SMZ-2B dissecting microscope and the severity of craniofacial deformities was assigned a score: 0, no effect; 1, slight defect in structure or size; 2, moderate defect in structure or size or multiple slight defects; 3, severe defect in structure or size or multiple moderate defects. Thus, a larva with a slightly reduced lower jaw would be assigned a score of 1 whereas an individual with both jaws missing would be given a 3. A larva with a score of 3 was considered to be severely deformed. For stations with more than 25 larvae, a random subsample of 25 larvae was scored. Only stations with ≥10 larvae were evaluated, as a sample size of 10 generally yields adequate statistical power in laboratory studies of cytogenetic defects.

All larvae used in analysis of morphological and cytogenetic abnormalities were collected from plankton tows and thus were independent of those used in evaluation of field-collected eggs (Hose et al. 1996).

### Cytogenetic examination

Pectoral fins were removed and stained with acetic orcein. Dividing cells were examined with no prior knowledge of site location for cytogenetic abnormalities using the techniques of Hose et al. (1996) and Kocan et al. (1982). All mitotic configurations in the fin were counted. The mitotic rate was calculated for each station as the mean number of mitoses per fin. Anaphase–telophase mitotic figures were evaluated for chromosome and chromatid breakage and spindle abnormalities. The anaphase aberration rate was the overall percentage of aberrant configurations at each station. On the basis of several data sets (Hose et al. 1996; Kocan et al. 1996), healthy larvae usually had a mitotic rate of more than eight and approximately 10% anaphase aberrations. Cytogenetically abnormal larvae had fewer than eight mitotic figures, more than 20% anaphase aberrations, or at least two micronucleated cells, a different measurement of chromosome breakage (Schmid 1976; Hose et al. 1996). Station-specific percentages of cytogenetically abnormal larvae were calculated. From previous studies, the percentage of cytogenetically abnormal larvae at nonoiled sites varies between 40 and 60% (Hose et al. 1996; Kocan et al. 1996). Individuals exhibiting autolysis or postmortem decay (Hose et al. 1996) were not evaluated; none of the samples had a significant proportion of autolyzed larvae.

To increase the sample size for statistical purposes, data from stations that had similar cytogenetic values within the same strata were combined for the June cruise ($GI_o = GI2_o$ and $GI6_o$). No larvae were analyzed from the July cruise.

**Table 1.** Depth distribution of herring larvae by month.

| | 0–50 m | | 50–100 m | | *N* | |
|---|---|---|---|---|---|---|
| Month | Larvae (%) | Effort (%) | Larvae (%) | Effort (%) | Larvae | Tows |
| May | 98 | 77 | 2 | 23 | 321 | 13 |
| June | 99 | 83 | 1 | 17 | 1619 | 24 |
| July | 63 | 42* | 2 | 23* | 56 | 44 |

Note: Effort indicates percentage of tows taken at the depth increments indicated. *N* indicates total number of larvae captured and total number of tows taken.<R*Additionally 14% were between 0 and 100 m and 21% were at depths >100 m.

### Statistical analysis

Differences in variables were assessed by $\chi^2$ analysis and analysis of variance (ANOVA) procedures. Values of the morphometric and cytogenetic data were compared using $\chi^2$ analysis to determine differences between GSI levels. The nonparametric Kruskal–Wallis ANOVA was used to compare differences in juvenile fish lengths for 1989 between GSI levels by month of collection. Nonparametric procedures were selected because variances between GSI levels were not equal and some levels had few data points. When an ANOVA comparison was significant at $p < 0.05$, the Mann–Whitney *U* test was applied to determine differences between the individual GSI levels. The number of mitotic divisions and the anaphase aberration rate were compared using mixed-model ANOVA procedures, with GSI as a random factor, as assumptions were not violated by these data. Mitotic division counts were $\ln(x + 1)$ transformed and percent (decimal) anaphase aberration rates were transformed using the arcsine(square root($x$)) transformation. The Tukey multiple comparison test for unequal sample sizes was applied when the *F* statistic was significant at $p < 0.05$. In some cases, an *F* statistic was significant but the Tukey tests showed no difference so a Newman–Keuls test was applied. In 1989, some GSI levels had low sample sizes, particularly GSI class 0, and these classes were not considered in some comparisons between May 1989 and other months or years.

## Results

### Water temperature

In April the open waters of PWS were nearly isothermal at 4°C. By May the upper 10–20 m had warmed to 5–7°C, while the lower layers were 4–5°C. Warming progressed with the layer above the seasonal thermocline reaching 6–10°C in June, but the lower layers remained at 4–5°C. In July, the warm surface layer had deepened to 20–50 m and warmed to 6–15°C. The deeper waters also warmed to 5–6°C. The stations nearest the Gulf of Alaska were cooler and better mixed than those well within the sound.

### Distribution, abundance, and length of larvae

As expected, herring larvae were present at stations in both oiled and nonoiled strata. No herring larvae were captured in the open waters of PWS in April because most hatching had not yet occurred. Herring larvae represented 2% of all fish larvae taken in May (Norcross and Frandsen 1996); they were found at 7 of the 15 stations sampled but were most abundant at 2 stations in the western portion of the sound (Fig. 1*b*). The largest catches were taken at stations within the oil trajectory. One station with high herring abundance was just north ($NI4_o$) of Naked Island (247 larvae/100 m$^3$) and another was west ($KI1_o$) of Knight Island (108 larvae/100 m$^3$). By June, herring larvae represented 42% of the larval fish catch and were distributed throughout the sound. They were collected at 14 of the 15 stations sampled and were most abundant (69–733 larvae/100 m$^3$) in the oiled Montague strata ($GI2_o$, $GI6_o$, $KI2_o$, and $SB_o$) in the waters flowing out of the sound (Fig. 1*c*). In June comparatively few larvae were collected in the nonoiled areas near Esther Island ($EI_n$) and eastern PWS ($OB6_n$, $HE1_n$, $HE3_n$, and $HEA_n$). In July herring represented 3% of the catch. They were evenly distributed around the sound and captured at 11 of the 18 stations sampled (Fig. 1*d*). All stations had a very low abundance of herring (<2.4 larvae/100 m$^3$) in July compared with values for May and June.

Ninety-eight percent of the 1989 herring larvae captured over three cruises were captured in the upper 50 m of the water column, 1% were captured between 50 and 100 m, and 1% were captured between 100 and 400 m (Table 1). Further analyses were conducted on the total number of herring larvae captured at a station without regard to depth of capture.

Growth of herring larvae between the May and June cruises was estimated (Fig. 2). Yolk-sac larvae constituted 32% of the May collection of herring but no yolk-sac larvae were captured in the later cruises. Average modal growth over the 27 days between the mid-dates of the May and June collections was 4.0 mm or 0.15 mm/day. Using mean sizes of 8.2 mm in May

**Fig. 2.** Percentage of herring larvae by 1.0 mm length increments for May, June, and July 1989.

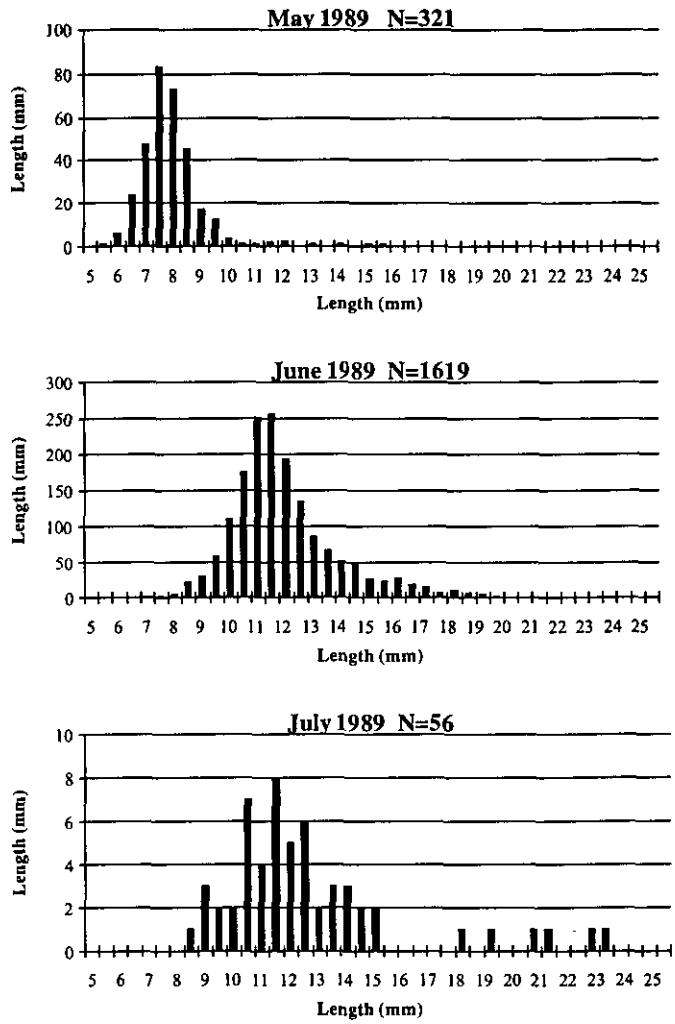


**Table 2.** Graded severity index (GSI) averaged for each month and percentages of each score within each month.

| Cruise | No. of larvae | GSI (mean ± SE) | GSI score (%) 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|---|
| 1989 | | | | | | |
| May | 76 | 2.45±0.09 | 2.6 | 9.2 | 28.9 | 59.2 |
| June | 279 | 0.79±0.05 | 43.7 | 36.2 | 17.6 | 2.5 |
| July | 50 | 1.32±0.14 | 22.0 | 38.0 | 26.0 | 14.0 |
| 1995 | | | | | | |
| May | 99 | 1.80±0.12 | 23.2 | 16.2 | 20.2 | 40.4 |

and 12.2 mm in June yields an identical estimated growth rate of 0.15 mm/day. Modal size did not change from June to July, but mean size increased to 13.0 mm.

### Morphological malformations

While herring larvae were most abundant in the western portion of the sound (Figs. 1*b*–1*d*), GSI values averaged between 1 and 2 throughout PWS. Larvae without craniofacial defects (GSI = 0) comprised 35%, those with slight defects (GSI = 1) 30%, those with moderate defects (GSI = 2) 20%, and those with severe defects (GSI = 3) 15% of the May–June total (Table 2). Virtually all malformations consisted of absent, reduced, or abnormal jaws. Moderately to severely deformed larvae (GSI ≥ 2) were found at six of the seven stations where herring were collected in May (Table 3) and slightly deformed larvae were found at the other station ($OB1_n$). The GSI value for the Orca Bay station ($OB1_n$) in the southeast stratum was lower ($p < 0.01$) than for all stations except the one in the north stratum near Esther Island ($EI_n$). Though 20% of the larvae collected in June were moderately to severely deformed, the average GSI score at any one station did not exceed 1.56 (Tables 2, 3). One station to the west of Naked Island ($NI6_o$) with a low GSI value (0.09) was significantly different from high values at the station near Bligh Island ($BI_o$) in the Naked Island stratum and the station at Evans Island ($SB_o$) in the Montague stratum (Table 3). In July the average GSI score was 3 for the two fish taken at the Hinchinbrook Entrance station ($HE3_n$) in the southeast stratum (Table 3); however, owing to low numbers of herring captured in July, there was no significant difference among stations.

Average GSI scores and the proportion of severely deformed larvae (GSI = 3) were highest in May, lowest in June, and intermediate in July 1989 (Table 2). There was no difference ($\chi^2 = 0.66$, $p = 0.88$) between oiled (Naked Island and Montague strata) and nonoiled (southeast and north strata) areas of the sound nor among the four strata ($\chi^2 = 15.68$, $p = 0.07$) when GSI values were averaged over all three cruises. However, monthly GSI scores were higher for oiled areas than for nonoiled areas: May, $\chi^2 = 31.68$, $p < 0.001$; June, $\chi^2 = 8.84$, $p = 0.03$; and July, $\chi^2 = 36.44$, $p < 0.001$. Average GSI values were lower in May 1995 than in May 1989 ($t = 3.46$, $p < 0.01$) (Table 2). In May 1995 there was a highly significant proportion of low (0 and 1) GSI values ($\chi^2 = 36.29$, $p < 0.005$).

For each cruise the smaller fish consistently had the highest GSI scores (Fig. 3). For fish with no malformations (GSI score = 0), mean length increased over the three cruises. At intermediate values of GSI (1–2), mean length increased between the May and June cruises despite malformations, but appeared to drop from the June to the July cruise. Fish with a GSI score of 2 appeared to increase their mean length more between May and June than did those fish with GSI scores of 0, 1, and 3 (Fig. 3). When length was examined with respect to GSI score within a month, the shorter larvae had higher GSI values. Mean lengths in June steadily decreased from 14.7 mm for a score of 0 to 13.4 mm for a score of 1, to 13.2 mm for a score of 2, to a low of 10.0 mm for a GSI score of 3 (Fig. 3). In May there was no significant difference in length between GSI scores of 0 and 1 nor between scores of 2 and 3, but there were significant differences between all other GSI values (Table 4). In June there were significant differences in lengths between all GSI scores except 1 and 2.

### Cytogenetic aberrations

Cytogenetic differences among depth strata were tested for stations $GI_o$ and $KI2_o$ in June. There was no significant difference between the percentage of cytogenetically abnormal larvae (%GA) at 0–25 m (58.3%) and 25–50 m (30.4%) ($\chi^2 = 2.55$, $n = 35$, $p > 0.05$) nor between aberration rates (%AAT) at the same two depths (9.4 and 10.6%; $\chi^2 = 0.05$, $n = 40$, $p > 0.05$) at $GI_o$. There was also no difference at $KI2_o$ between the percentage of cytogenetically abnormal larvae ($\chi^2 = 0.02$, $n = 25$, $p > 0.05$) or anaphase aberration rates ($\chi^2 = 1.34$, $n = 19$, $p > 0.05$) at 0–25 m (%GA = 58.3%; %AAT = 24.2%) and at 25–50 m (%GA = 56.2%; %AAT = 16.1%). This, combined with the depth distribution of herring larvae, all within the upper 50 m (Table 1), confirmed that depth was not a significant factor. Thus, herring from different depth strata were combined in all analyses.

Herring collected in June from the nonoiled north strata ($EI_n$) had a normal mitotic rate, the lowest anaphase aberration rate, and a relatively low percentage of cytogenetically abnormal larvae (Table 5). Four stations ($KI2_o$, $SB_o$, $MB_o$, and $NI4_o$) had significantly different anaphase aberration rates from that of $EI_n$ (Table 5). Two of those stations ($SB_o$ and $NI4_o$) also had significantly greater percentages of cytogenetically abnormal larvae. At $SB_o$, larvae had a low mitotic rate compounding the low percentage of successful cell divisions. Larvae from $NI4_o$ both in May and June contained micronucleated cells, indicating genetic damage in previous cell divisions.

When compared with pooled values from May 1995 (Table 5), May 1989 larvae had a lower ($t = 5.00$, $p < 0.001$) mitotic rate and a higher ($\chi^2 = 26.01$, $p < 0.005$) anaphase aberration rate, and a higher ($\chi^2 = 36.2$, $p < 0.005$) percentage of them were cytogenetically abnormal. Mitotic rates for May 1995 were similar to those in June 1989; however, the anaphase aberration rates and percentage of cytogenetically abnormal larvae were lower in May 1995 than in June 1989.

Significant differences were found when comparisons were made among fish in which both GSI level and cytogenetic analysis, using the number of mitotic divisions and the anaphase aberration rate, were evaluated via one-way ANOVA (Table 6). In 1989 there was a significant difference among GSI values for the number of mitotic divisions (Table 7). By a Tukey test, the number of mitotic divisions for a GSI value of 0 was found to differ from 1 ($p = 0.029$), from 2 ($p = 0.002$), and from 3 ($p = 0.003$). Likewise, there was a difference in the anaphase aberration rate between GSI values (Table 7). Using a Newman–Keuls test, there was a significant difference in anaphase aberration rate between a GSI value of 3 and 0

**Table 3.** Geographical distribution of herring larvae in Prince William Sound in 1989.

| | May | | | June | | | July | | |
|---|---|---|---|---|---|---|---|---|---|
| | Length (mm) | GSI | *N* | Length (mm) | GSI | *N* | Length (mm) | GSI | *N* |
| North strata | | | | | | | | | |
| $Ei_n$ | 9.24±0.37 | 2.00±0.58 | 4 | 15.10±0.33 | 0.68±0.16 | 25 | 14.40±1.08 | 1.20±0.33 | 10 |
| $PV50_n$ | — | — | — | — | — | — | 12.55±0.58 | 1.86±0.26 | 7 |
| Southeast strata | | | | | | | | | |
| $OB6_n$ | — | — | — | 15.71±2.81 | 1.00±0.00 | 2 | — | — | — |
| $OB1_n$ | 10.52±0.95 | 1.56±0.38 | 9 | — | — | — | 11.43±0.37 | 1.50±0.22 | 18 |
| $HE1_n$ | 6.68±0.02 | 3.00±0.00 | 2 | 17.97±0.92 | 0.00±0.00 | 2 | — | — | — |
| $HE3_n$ | 9.28±1.17 | 2.71±0.29 | 7 | 15.74±0.72 | 0.29±0.18 | 7 | 9.17±1.54 | 3.00±0.00 | 7 |
| $HEA_n$ | — | — | — | *18.96±0.60* | *0.00±0.00* | F4 | — | — | — |
| Naked Island strata | | | | | | | | | |
| $Mb_o$ | 7.85±0.79 | 2.33±0.33 | 3 | 14.83±0.38 | 0.68±0.16 | 28 | 15.14±0.19 | 1.33±0.33 | 3 |
| $NI4_o$ | 7.89±0.12 | 2.68±0.11 | 25 | 15.47±0.36 | 0.82±0.16 | 22 | 14.17±3.68 | 0.00±0.00 | 3 |
| $NI6_o$ | — | — | — | 12.56±0.33 | *0.09±0.09* | 11 | *13.08±0.60* | *0.75±0.25* | 4 |
| $Bi_o$ | — | — | — | 12.79±1.01 | 1.56±0.29 | 9 | 19.21±3.83 | 0.33±0.33 | 3 |
| Montague strata | | | | | | | | | |
| $KI1_o$ | 8.45±0.20 | 2.50±0.11 | 26 | — | — | — | — | — | — |
| $KI2_o$ | — | — | — | 15.26±0.32 | 0.82±0.12 | 44 | — | — | — |
| $GI2_o$ | — | — | — | 11.40±0.23 | 0.89±0.14 | 28 | — | — | — |
| $GI6_o$ | — | — | — | 13.72±0.63 | 1.09±0.20 | 22 | — | — | — |
| $Sb_o$ | — | — | — | 11.85±0.19 | 0.92±0.12 | 50 | — | — | — |
| $MSX_o$ | — | — | — | 13.59±0.36 | 0.64±0.14 | 25 | — | — | — |

Note: Lengths are given as the mean ± standard error (total number of larvae evaluated for GSI). GSI values are also given as the mean ± standard error (total number of larvae evaluated).

**Fig. 3.** Average length (±1 SE) of herring by GSI score and time of collection.

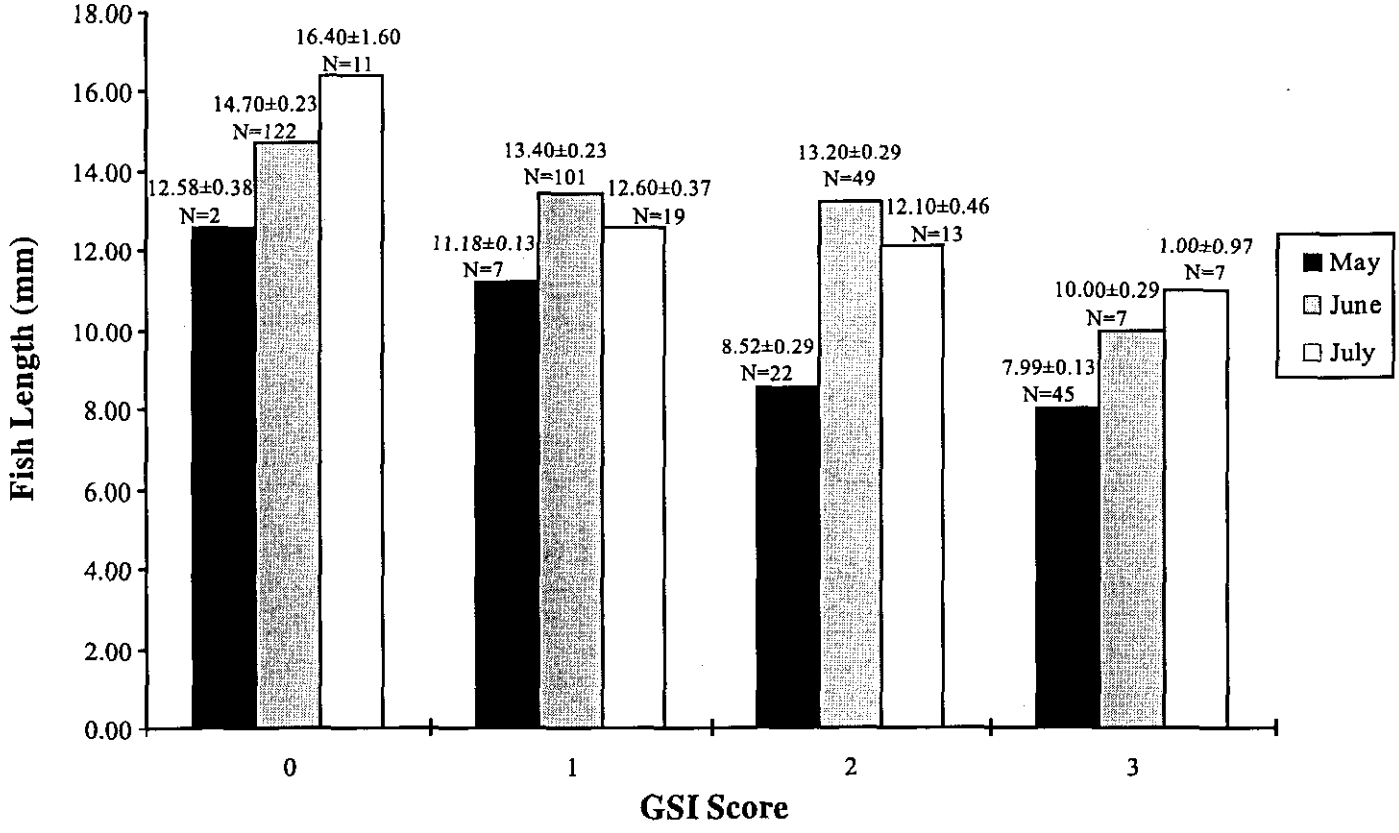


**Table 4.** Results ($p$ values) from Kruskal–Wallis ANOVA comparisons of juvenile herring lengths.

| | May | June | July |
|---|---|---|---|
| ANOVA | <0.001 | <0.001 | 0.105 |
| 0 versus 1 | 0.380 | <0.001 | nt |
| 0 versus 2 | 0.028 | <0.001 | nt |
| 0 versus 3 | 0.017 | <0.001 | nt |
| 1 versus 2 | 0.003 | 0.573 | nt |
| 1 versus 3 | <0.001 | <0.001 | nt |
| 2 versus 3 | 0.109 | <0.001 | nt |

Note: Mann–Whitney $U$ tests were performed between GSI levels when an ANOVA comparison was significant ($p < 0.05$). nt, not tested.

($p = 0.005$), 1 ($p = 0.007$), and 2 ($p = 0.04$). There was no significant difference between cytogenetic measurements for GSI values in 1995.

Comparisons of the same measurements of GSI and cytogenetic analysis using two-way ANOVA procedures between months and year showed significant differences; May and June 1989 differed in the number of mitotic divisions (Table 7), but not in the anaphase aberration rate. There were no differences among GSI values between the two months and the interaction effect was not significant. Similarly there was a significant difference for number of mitotic divisions only between months May 1989 and May 1995, but there were no significant differences for anaphase aberration rates over those periods (Table 7). Comparisons between June 1989 and May 1995 reveal significant differences between GSI values for the number of mitotic divisions and between months for the anaphase aberration rate.

## Discussion

### Distribution

The highest concentrations of herring larvae in 1989 were adjacent to or downstream from oiled spawning sites (Brown et al. 1996*b*) and within the area of the oil spill trajectory (Fig. 1). Herring spawned in the east, north, and central portions of PWS but larvae were primarily found in the southwest portion. This is the first distribution study of herring larvae in the open waters of PWS. The larval distributions found were in agreement with the general oceanographic circulation of PWS (Niebauer et al. 1994; Royer et al. 1990; Venkatesh 1990). Though herring spawned in April (McGurk and Brown 1996), it was not until the beginning of May that herring larvae were found in open waters of PWS in limited concentrations.

As noted for Pacific herring in British Columbia, the initial patchiness of herring caused by concentrations of demersal eggs is rapidly reduced as larvae are released into the water column and experience turbulent diffusion (McGurk 1987). By June, herring larvae were much more abundant, distributed throughout the sound and found in greatest numbers in the waters flowing out of the sound in the Montague Strait region. Larvae from all spawning locations would be expected to pass through this region in a manner analogous to the flow of oil, making it impossible to determine the origin of larval fishes collected in the Montague stratum in 1989. By July most herring larvae had either been transported out of the sound or had grown beyond a size that could be captured by the plankton net. They were no longer concentrated in the Montague Strait region. The few larvae that were found in July were evenly distributed around the sound.

### Growth

One cohort of herring larvae can be clearly followed from May to June (Fig. 2). This cohort came from the major spawning that occurred between 31 March and 20 April, 1989, and hatched between 30 April and 10 May, 1989 (McGurk et al. 1990). Though a second mode appeared in nearshore waters between 29 May and 13 June, 1989 (McGurk et al. 1990), there is no evidence from spawning data (Brown et al. 1996*b*) of a large second cohort and no additional cohorts of herring larvae are apparent in offshore waters. This is supported by yolk-sac larvae appearing in May in our collections but not in June or July. If the larvae collected in June were a different cohort than the larvae collected in May, we would expect distinct peaks in larval lengths to appear in the June sample. If the smaller fish in June represent a second cohort, the entire first cohort is no longer represented in the collections.

Our estimated growth rate of 0.15 mm/day represents one of the lowest values recorded for Pacific herring; however, the estimate of growth rate is based solely on changes in modal and mean size over the 27 days between the May and June cruises and thus may be imprecise. Growth rates for Pacific herring in Alaskan waters have been recorded as 0.31–1.48 mm/day (Wespestad and Moksness 1990), 0.30 mm/day (McGurk et al. 1993), and 0.18–0.29 mm/day (McGurk 1984*a*). Yolk sacs were present in 32% of the May herring, which suggested recent hatching, as did the small size of larvae. The small lengths of herring larvae in June (no yolk-sac larvae found) are more difficult to explain naturally, though that may be due to net avoidance (McGurk 1992) and (or) preservation shrinkage (McGurk 1985). While slow larval growth may have occurred in PWS (there are no previous data for comparison), this seems unlikely as rates this low are rarely reported for Pacific or Atlantic herring. We do know that the mitotic rate in May 1995 was about twice that in May 1989 and similar to that in June 1989. The few herring larvae captured in July were probably those with stunted development that remained too small to avoid net capture, which increases for larvae >10 mm (McGurk 1992).

Exposure of herring eggs to petroleum retards hatching and development (Struhsaker et al. 1974; Lindèn 1978; Pearson et al. 1985; Rice et al. 1987; von Westernhagen 1988) and reduces subsequent growth of herring larvae (Lindén 1978; Smith and Cameron 1979; Rice et al. 1987). Sublethal concentrations of benzene depress embryonic growth (Eldridge et al. 1977; Lindén 1978). Exposure of eggs to Prudhoe Bay crude oil for 12 h or more reduces the size of newly hatched larvae (Smith and Cameron 1979; Rice et al. 1987). Feeding and swimming abilities are inhibited when herring larvae are exposed to oil (Struhsaker et al. 1974; Carls 1987). Larvae may subsequently regain their ability to feed, but length remains less than that observed in unexposed larvae. Larvae hatched in uncontaminated waters may also be adversely affected by encountering an oil spill during larval drift (von Westernhagen 1988). One of the major effects of oil is reduced larval activity, making the larvae more susceptible to predation. Because swimming capacity determines the success of pursuit and capture of prey, feeding is reduced. There is a reduction of larval

**Table 5.** Cytogenetic abnormalities of herring larvae at oiled (o) and nonoiled (n) stations in 1989 and nearshore (ns) and offshore (os) stations in 1995.

| | No. of mitotic figures | | Anaphase aberrations | | | Cytogenetic evaluation | | |
|---|---|---|---|---|---|---|---|---|
| | *N* | Mean ± SD | No. NAT | No. AAT | %AAT | No. N | No. A | %GA |
| | | | | **May 1989** | | | | |
| $NI4_o$ | 21 | 5.3±2.5 | 15 | 8 | 34.8 | 1 | 20 | 95.2 |
| | | | | **June 1989** | | | | |
| $GI_o$ | 35 | 10.7±3.8 | 124 | 14 | 10.1 | 21 | 14 | 40.0 |
| $KI2_o$ | 40 | 14.3±8.8 | 117 | 31 | 20.9* | 17 | 23 | 46.0 |
| $SB_o$ | 27 | 5.3±2.1 | 36 | 12 | 25.0* | 3 | 24 | 88.8* |
| $MB_o$ | 25 | 10.8±5.6 | 63 | 22 | 25.9* | 9 | 16 | 64.0 |
| $NI4_o$ | 22 | 10.6±5.3 | 55 | 16 | 22.5* | 7 | 15 | 68.2* |
| $EI_n$ | 25 | 10.1±6.0 | 85 | 7 | 7.6 | 15 | 10 | 40.0 |
| | | | | **May 1995** | | | | |
| N. $Mont._{ns}$ | 25 | 11.8±4.6 | 58 | 3 | 4.9 | 20 | 5 | 20.0 |
| N. $Mont._{os}$ | 26 | 11.1±4.4 | 23 | 0 | 0.0 | 19 | 7 | 26.9 |
| W. $Mont._{ns}$ | 25 | 11.2±3.6 | 41 | 1 | 2.4 | 20 | 5 | 20.0 |
| W. $Mont._{os}$ | 23 | 9.6±3.1 | 25 | 2 | 7.4 | 15 | 8 | 34.8 |

*Note*: *N*, number larvae examined; No. NAT, number of normal anaphase–telophase mitotic figures; No. AAT, number of abnormal anaphase–telophase mitotic figures; %AAT, anaphase aberration rate; No. N, number of cytogenetically normal individuals; No. A, number of cytogenetically abnormal individuals; %GA, percentage of cytogenetically abnormal individuals; N. Mont. and W. Mont., north and west sides of Montague island.

*Significantly ($p < 0.05$) different from $Ei_n$.

**Table 6.** Means, standard deviations, and *N* (in parentheses) for the number of mitotic divisions (No. MIT) and percent anaphase aberration rate (%AAT) for 1989 and 1995.

| Month | GSI 0 | GSI 1 | GSI 2 | GSI 3 |
|---|---|---|---|---|
| | | **No. MIT** | | |
| May 1989 | 0.0±0.00 (0) | 6.0±0.00 (1) | 7.0±3.16 (4) | 6.8±2.28 (12) |
| June 1989 | 13.4±7.20 (72) | 10.1±5.77 (55) | 9.3±5.91 (30) | 7.0±0.00 (1) |
| May 1995 | 13.1±4.95 (20) | 10.2±3.24 (13) | 9.1±1.89 (13) | 10.7±3.46 (34) |
| | | **%AAT** | | |
| May 1989 | 0.0±0.00 (0) | 0.0±0.00 (0) | 41.7±50.01 (4) | 38.9±42.25 (12) |
| June 1989 | 14.9±17.90 (72) | 16.4±23.00 (55) | 22.9±30.97 (30) | 33.3±0.00 (1) |
| May 1995 | 5.1±12.56 (20) | 0.0±0.00 (13) | 0.0±0.00 (13) | 1.6±6.57 (34) |

growth in oiled waters and the negative effects of the oil are exacerbated by starvation (von Westernhagen 1988).

Although the small sizes of larvae collected are consistent with oil exposure to developing eggs in May and low growth rates are consistent with exposure of larvae to oil, reduced size of herring larvae at hatching and low growth rates also can result from a number of other factors, including decreased time to hatching, increased water temperatures during incubation, and decreased prey density. While water temperatures >13°C (Alderdice and Velsen 1971) can reduce length at hatch of Pacific herring, water temperatures in the upper 20 m near the major herring spawning areas of PWS in 1989 were 5–8°C (Brown et al. 1996*b*) and in open waters of the sound they were 5°–7°C at the beginning of May 1989, well below temperatures that reduce the size of larvae. Growth of Pacific herring is depressed to 0.09–0.10 mm/day (Haegele and Outram 1978) and 0.11–0.23 mm/day (McGurk 1984*b*) under adverse feeding conditions, but there is no evidence of reduced prey density in PWS in 1989 (Willette 1993; McGurk and Brown 1996); thus, reduced food availability does not explain the low growth rate of herring larvae. Decreased time to hatching can produce premature larvae (Hay 1986) and there was a significant decrease in time to hatching observed in eggs collected from oiled versus nonoiled beaches in 1989 (McGurk et al. 1990). Thus, there was no known natural environmental factor that caused reduced larval size or growth in 1989.

### *Morphological malformations*

Although craniofacial and mandibular malformations naturally occur at incidences up to 25% in yolk sac larval herring and 68% in post yolk-sac larvae (Struhsaker et al. 1974; Nanke 1981), exposure to crude oil can increase both the frequency and severity of defects (von Westernhagen 1988; Urho 1991). In May 1989, both the incidence and severity of craniofacial malformations were significantly higher than in May 1995, observations consistent with oil exposure; May 1989 malformations were also more severe than in laboratory-hatched larvae from 1990 or 1991 (Hose et al. 1996). As in newly hatched larvae from laboratory experiments, craniofacial defects in 1989 and 1995 larvae from this study consisted primarily of reduced jaw size with lower incidences of abnormal and missing jaws. In May 1989, GSI scores of the pelagic yolk-sac larvae reported here were higher than those of newly hatched larvae from laboratory incubations (Hose et al. 1996). GSI scores increased during the approximately 1-week period between hatching and collection of pelagic yolk-sac larvae in

**Table 7.** One- and two-way ANOVA comparisons of number of mitotic divisions (No. MIT) and percentage aberration rate (%AAT) between GSI values for May and June 1989 and May 1995.

| | No. MIT | | | %AAT | | |
|---|---|---|---|---|---|---|
| | df | *F* | *p* | df | *F* | *p* |
| **One-way comparisons of GSI levels for each year** | | | | | | |
| 1989 | 3, 193 | 10.002 | <0.001* | 3, 171 | 3.214 | 0.024* |
| Summary | | GSI 0 > 1, 2, 3 | | GSI 3 > 0, 1, 2 | | |
| 1995 | 3, 95 | 2.172 | 0.096 | 3, 76 | 2.063 | 0.112 |
| Summary | | No differences | | | No differences | |
| **Two-way ANOVA comparisons between May and June 1989** | | | | | | |
| Month | 1, 2 | 96.042 | 0.010* | 1,2 | 0.002 | 0.966 |
| GSI | 3, 113 | 0.102 | 0.903 | 3, 97 | 1.205 | 0.304 |
| Interaction | 3, 113 | 0.012 | 0.988 | 3, 97 | 0.560 | 0.573 |
| Summary | | June > May | | | No differences | |
| **Two-way ANOVA comparisons between May 1989 and May 1995** | | | | | | |
| Year–month | 1, 2 | 399.14 | 0.002* | 1, 2 | 6.246 | 0.130 |
| GSI | 3, 94 | 0.203 | 0.817 | 3, 71 | 1.837 | 0.167 |
| *Interaction* | 3, 94 | 0.041 | 0.960 | 3, 71 | 1.589 | 0.211 |
| Summary | | May 1995 > May 1989 | | | No differences | |
| **Two-way ANOVA comparisons between June 1989 and May 1995** | | | | | | |
| Year–month | 1, 3 | 8.889 | 0.059 | 1, 3 | 15.787 | 0.029* |
| GSI | 3, 264 | 4.423 | *0.005** | 3, 230 | *0.536* | *0.659* |
| Interaction | 3, 264 | 0.271 | 0.846 | 3, 230 | 1.059 | 0.367 |
| Summary | | GSI 0 > 1, 2, 3 | | | June 1989 >May 1995 | |

*Significant value.

May 1989 (Brown et al. 1996*a*; Hose et al. 1996). This difference probably reflects the incomplete development of the jaws at hatching; therefore, detection of jaw deformities may be facilitated in older, pelagic larvae with greater jaw development. Although this finding was not statistically significant, newly hatched larvae from oiled sites in 1989 had higher incidences of reduced jaw size; however, incidences of abnormal or absent lower jaws were significantly elevated at oiled sites (Hose et al. 1996). Reductions, absences, and malformations of the jaws are characteristic of oil exposure (Lindén 1978; Smith and Cameron 1979) but also may result from incubation at temperatures above (14°C) or below (4.0°C) the normal range, dessication, salinity, and exposure to ultraviolet light (Purcell et al. 1990). However, most PWS herring eggs were deposited in subtidal areas and water temperatures in PWS in spring 1989 were not sufficiently extreme to cause the jaw malformations seen in the herring larvae in this study.

In oil-exposed larvae, one or both jaws may be abnormal, reduced, or completely absent (von Westernhagen 1988; Hose et al. 1996). The absence of jaw rudiments could only occur as a result of embryonic exposure during the critical period for jaw organogenesis. Similarly, jaw malformations are induced during the embryonic stage, although after hatching, abnormalities may become more pronounced as developing structures elongate or fail to differentiate (Carls and Rice 1988). However, as the lower jaw is normally shorter than the upper jaw at hatching and in succeeding days grows to project forward beyond the upper jaw (Alderdice and Velsen 1971), reduced jaws in larvae could result from either embryonic oil exposure or an overall depression of normal growth if larvae were exposed to toxic levels of oil. Fish larvae and fry are generally more sensitive to petroleum hydrocarbons than are eggs (Korn and Rice 1981; Carls and Rice 1988), and growth can be reduced following ingestion of oil-contaminated prey (Schwartz 1985). A high proportion of 1989 larvae (46%) had severe craniofacial defects such as the absence of one or both jaws. Our group has estimated that between 40 and 50% of the spawning beaches in 1989 sustained some level of oiling (Brown et al. 1996*a*, 1996*b*); however, possible oil exposure during the larval period cannot be estimated by geographic location, duration, or dose.

Malformed jaws consistently were present in smaller herring larvae over all cruises. The small lengths of larvae captured in May are probably due in part to poor growth because of a physical inability to feed. Time from hatching to irreversible starvation is 28–37 days for herring larvae in water at 6–8°C (McGurk 1984*b*), a temperature range experienced in PWS during May 1989. In the month between the May and June cruises these severely affected larvae may have died and been removed from the population. This premise is supported by the lower GSI values in June when only 20% of the larvae were severely deformed. Thus, the overall condition of the remaining larvae improved. Only a few deformed larvae remained to be captured in July because the healthier larvae should have grown sufficiently to evade the net.

### Cytogenetic aberrations

Cytogenetic evaluations of PWS herring larvae support the findings of the morphological analyses. In 1989 when the GSI value was high, the mitotic rate was low, the anaphase aberration rate was high, and the percentage of abnormal larvae was high (Table 6). Virtually all herring captured in May at $NI4_o$ in the Naked Island stratum were cytogenetically abnormal; damage was significantly higher than in larvae from nonoiled sites (Hose et al. 1996; Brown et al. 1996*a*) or in experimentally reared larvae (Kocan et al. 1996). In 1995, a high GSI value was associated with a high mitotic rate and a low anaphase aberration rate, a pattern quite different from that seen in 1989

(Table 6). Thus, though larvae may hatch in an undeveloped stage (Hay 1986), they can have quite normal cytogenetic measurements as in our 1995 larvae. For larvae that experience cytogenetic effects during the embryonic stage, jaws may develop and result in low GSI scores and the number of mitotic divisions may increase; however, the rate of anaphase aberrations remains high as seen in June 1989 larvae.

The extent of genetic abnormality measured here is less than that described in newly hatched larvae (Hose et al. 1996) but strikingly similar to that measured in post-hatch larvae collected near oiled beaches in PWS (Brown et al. 1996*a*). Identical anaphase aberration rates were measured in larvae captured near- and off-shore from Naked Island in May and June. As in the study of nearshore larvae, the degree of genetic abnormalities declined from May to June, although June anaphase aberration rates were still significantly elevated over nonoiled values throughout most of the western sound. This reduction might be due to several factors: death of severely affected larvae, mixture with larvae from nonoiled areas, or a diminution of genetic damage during later larval stages. Our results suggest that severely deformed larvae are moribund and therefore were lost from the population.

Genetic effects (reduced mitotic rate and elevated anaphase aberration rate) were more pronounced in larvae from May 1989 than in those from 1990–1995 (Hose et al. 1996). In both pelagic larvae and laboratory-hatched larvae from oiled areas in May 1989, mitotic rates were approximately half of those found in subsequent years at similar locations. Anaphase aberration rates in May 1989 larvae from oiled areas (34–46%) were also greater than in 1990–1995. Aberration rates declined at previously oiled sites from 19–32% in 1990 laboratory-hatched larvae to 5% in 1995 pelagic larvae. All other studies of unexposed herring have yielded anaphase aberration rates below 10% (Kocan et al. 1996; M. Carls, National Marine Fisheries Service, Juneau, Alaska, personal communication; J.E. Hose, unpublished data).

Many lines of evidence support the contention that exposure to *Exxon Valdez* oil caused larval damage in 1989. The types of damage reported in this study, jaw defects and genetic effects, are identical to those consistently observed following laboratory exposure to oil–water dispersions of crude oil (Lindén 1978; Smith and Cameron 1979; Kocan et al. 1996). Such elevated anaphase aberration rates have not been observed in PWS or Alaskan herring since 1989 (Hose et al. 1996). Both morphological malformations and genetic damage corresponded to quantitative differences in spatial oil exposure and temporally decreased on a monthly scale during spring 1989 and annually from 1989 through 1995 (Brown et al. 1996*a*).

### Herring larvae and circulation of Prince William Sound

The malformation incidences we found in spring 1989 were relatively high, particularly in May (97%) compared with May 1995 measurements (77%) and published reports (Purcell et al. 1990). If shoreline oiling was highly discrete, as suggested by subtidal mussel bioaccumulation data (Brown et al. 1996*b*), it would appear contradictory that deformed larvae were widespread throughout PWS in 1989. Closer examination of the oceanography of the sound (Brown et al. 1996*b*) provides an explanation of this. Strong northerly winds immediately following the spill played a significant role in pushing the oil out of PWS and covering much of the Naked Island and Montague strata with oil (Royer et al. 1990). With reduced wind the oil would affect the western islands and shore (Royer at al. 1990; Venkatesh 1990). These variations in winds may explain how in May and possibly July herring larvae at unoiled Esther Island ($Ei_n$) were transported to that site from oiled areas around Naked Island.

An alternative explanation, that unoiled larvae might have been exposed to oil in the water column or to oil-contaminated prey, may account for the larvae exhibiting less severe craniofacial defects such as jaw reduction. Thus, as movement of herring larvae is related to currents within PWS, it is not surprising that herring hatching from nonoiled areas are found within the oil trajectory and the herring hatching from oiled areas traverse nonoiled waters. This movement confounds the conclusive demonstration of oil damage in post-hatch herring larvae. However, effects observed in these larvae, including reduced growth, malformations, and genetic damage, were identical to effects in younger embryos and larvae collected from oiled beaches (Hose et al. 1996; Brown et al. 1996*a*) and in laboratory exposures to *Exxon Valdez* oil (Kocan et al. 1996). The long-term consequences of the observed effects to the survival of the 1989 year-class cannot be predicted with any certainty, but the magnitude and severity of these effects have prompted further monitoring.

## Acknowledgments

We thank the crews of the R/V *Alpha Helix* and the National Atmospheric and Oceanic Administration ship *John Cobb* as well as the numerous undergraduate students who sorted samples and assisted at sea. Thanks to Tim Baker for statistical assistance and J.M. Castellini, B. Holladay, M. McGurk, A. Moles, R. Wilbur, and two anonymous referees for review of the manuscript, and to A. Blanchard who assisted with statistics. Collections for this study were funded by the State of Alaska Natural Resource Damage Assessment (NRDA) study No. 19 (contract No. EN1103435) and analysis was funded by NRDA study No. 11 (Reciprocal Services Agreement No. 1121167). Additional funding was provided by the University of Alaska Natural Resources Fund and the Institute of Marine Science. This project was funded by the *Exxon Valdez* Oil Spill Trustee Council, but the views expressed are those of the individual investigators and do not necessarily represent the conclusions of the Council. This is contribution No. 1624 of the Institute of Marine Science, University of Alaska, Fairbanks, and contribution No. PP.093 of the Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division, Juneau, Alaska.

## References

Alderdice, D.F., and Velsen, F.P.J. 1971. Some effects of salinity and temperature on early development of Pacific herring (*Clupea pallasi*). J. Fish. Res. Board Can. **28**: 1545–1562.

Biggs, E.D., Haley, B.E., and Gilman, J.M. 1993. Historic database for Pacific herring in Prince William Sound, Alaska, 1973–1991. Regional information report No. 2C91-11. Alaska Department of Fish and Game, Anchorage, Alaska.

Brown, E.D., Baker, T.T., Hose, J.E., Kocan, R.M., Marty, G.D., McGurk, M.D., Norcross, B.L., and Short, J. 1996*a*. The *Exxon Valdez* oil spill and Pacific herring in Prince William Sound: a

summary of injury to the early life history stages. *In Exxon Valdez* Oil Spill Symposium Proceedings. *Edited by* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright. Am. Fish. Soc. Symp. No. 18. pp. 448–462.

Brown, E.D., Norcross, B.L., and Short, J.W. 1996*b*. An introduction to studies on the effects of the *Exxon Valdez* oil spill on early life history stages of Pacific herring, *Clupea pallasi*, in Prince William Sound, Alaska. Can. J. Fish. Aquat. Sci. **53**: 2337–2342.

Carls, M.G. 1987. Effects of dietary and water-borne oil exposure on larval Pacific herring (*Clupea harengus pallasi*). Mar. Environ. Res. **22**: 253–270.

Carls, M.G., and Rice, S.D. 1988. Sensitivity differences between eggs and larvae of walleye pollock (*Theragra chalcogramma*) to hydrocarbons. Mar. Environ. Res. **26**: 285–297.

Carls, M.G., and Rice, S.D. 1988. 1989. Abnormal development and growth reduction of pollock *Theragra chalcogramma* embryos exposed to water-soluble fractions of oil. Fish. Bull. **88**: 29–37.

Donaldson, W., Morstad, S., Simpson, E., Wilcock, J., and Sharr, S. 1993. Prince William Sound management area 1992 annual finfish management report. Regional information report No. 2A93-12. Alaska Department of Fish and Game, Anchorage, Alaska.

Eldridge, M.B., Echeverria, T., and Whipple, J.A. 1977. Energetics of Pacific herring (*Clupea harengus pallasi*) embryos and larvae exposed to low concentrations of benzene, a monoaromatic component of crude oil. Trans. Am. Fish. Soc. **106**: 452–461.

Eldridge, M.B., Echeverria, T., and Korn, S. 1978. Fate of $^{14}$C-benzene in eggs and larvae of Pacific herring (*Clupea harengus pallasi*). J. Fish. Res. Board. Can. **35**: 861–865.

Fowler, D.A. 1988. BQ system IV. R & M Biometrics, Inc., Nashville, TN.

Foyn, L., and Serigstad, B. 1987. Age dependent sensitivity of oil on fish larvae, used in assessment of potential oil pollution damages on fish resources. ICES statutory meeting, Marine Environmental Quality Commission. ICES C.M 1987/E:12. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Haegele, C.W., and Outram, D.N. 1978. The effects of diet and ration on the growth and survival of Pacific herring (*Clupea harengus pallasi*) larvae. Tech. Rep. Fish. Mar. Serv. Can. No. 767.

Hay, D.E. 1986. Effects of delayed spawning on viability of eggs and larvae of Pacific herring. Trans. Am. Fish. Soc. **115**: 155–161.

Hose, J.E., McGurk, M.D., Marty, G.D., Brown, E.D., and Baker, T.T. 1996. Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphological, cytogenetic, and histopathological assessments, 1989–1991. Can. J. Fish. Aquat. Sci. **53**: 2355–2365.

Kocan, R.M., Landholt, M.L., and Sabo, K.M. 1982. Anaphase aberrations: a measure of genotoxicity in mutagen-treated fish cells. Environ. Mutagen. **4**: 181–189.

Kocan, R.M., Hose, J.E., Brown, E.D., and Baker, T.T. 1996. Pacific herring (*Clupea pallasi*) embryo sensitivity to Prudhoe Bay petroleum hydrocarbons: laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound. Can. J. Fish. Aquat. Sci. **53**: 2366–2375.

Korn, S., and Rice, S. 1981. Sensitivity to, and accumulation and depuration of, aromatic petroleum components by early stages of coho salmon (*Onchorhynchus kisutch*). Rapp. P.-V. Reun. Cons. Int. Explor. Mer, **178**: 87–92.

Kuhnhold, W.W. 1977. The effect of mineral oils on the development of eggs and larvae of marine species: a review and comparison of experimental data in regard to possible damage at sea. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, **171**: 175–183.

Lindén, O. 1978. Biological effects of oil on early development of the Baltic herring, *Clupea harengus membras*. J. Mar. Biol. Assoc. U.K. **45**: 273–283.

Matarese, A.C., Kendall, A.W., Jr., Blood, D.M., and Vinter, B.M. 1989. Laboratory guide to early life history stages of northeast Pacific fishes. NOAA Tech. Rep. No. NMFS 80.

McGurk, M.D. 1984*a*. Ring deposition in the otoliths of larval Pacific herring, *Clupea harengus pallasi*. Fish. Bull. **82**: 113–120.

McGurk, M.D. 1984*b*. Effects of delayed feeding and temperature on the age of irreversible starvation and on the rates of growth and mortality of Pacific herring larvae. J. Mar. Biol. **84**: 13–26.

McGurk, M.D. 1985. Effects of net capture on the postpreservation morphometry, dry weight, and condition factor of Pacific herring larvae. Trans. Am. Fish. Soc. **114**: 348–355.

McGurk, M.D. 1987. The spatial patchiness of Pacific herring larvae. Environ. Biol. Fishes, **20**: 81–89.

McGurk, M.D. 1992. Avoidance of towed plankton nets by herring larvae: a model of night–day catch ratios based on larval length, net speed and mesh width. J. Plankton Res. **14**: 173–182.

McGurk, M.D., and Brown, E.D. 1996. Egg–larval mortality of Pacific herring in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. **53**: 2343–2354.

McGurk, M., Warburton, D., Parker, T., and Litke, M. 1990. Early life history of Pacific herring: 1989 Prince William Sound herring egg incubation experiment. Final report. OCS Environ. Assess. Prog. 71. pp. 239–347. U.S. Department of Commerce, NOAA-OMA-OAD, Alaska Office, Anchorage, AK 99513-7543, U.S.A.

McGurk, M.D., Paul, A.J., Coyle, K.O., Ziemann, D.A., and Haldorson, L.J. 1993. Relationships between prey concentration and growth, condition, and mortality of Pacific herring, *Clupea pallasi*, larvae in an Alaskan subarctic embayment. Can. J. Fish. Aquat. Sci. **50**: 163–180.

Nanke, R.L. 1981. Observations on deformed fish larvae in Long Island Sound and Niantic Bay, Connecticut. Rapp. P.V. Reun. Cons. Int. Explor. Mer, **178**: 355–356.

Niebauer, H.J., Royer, T.C., and Weingartner, T.J. 1994. Circulation of Prince William Sound, Alaska. J. Geophys. Res. **99**: 113–114.

Norcross, B.L., and Frandsen, M. 1996. Distribution and abundance of larval fishes in Prince William Sound, Alaska during 1989 after the *Exxon Valdez* oil spill. *In Exxon Valdez* Oil Spill Symposium Proceedings. *Edited by* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright. Am. Fish. Soc. Symp. No. 18. pp. 463–486.

Norcross, B.L., and Shaw, R.F. 1984. Oceanic and estuarine transport of fish eggs and larvae: a review. Trans. Am. Fish. Soc. **113**: 153–165.

Pearson, W.H., Woodruff, D.L., Kiesser, S.L., Fellingham, G.W., and Elston, R.A. 1985. Oil effects on spawning behavior and reproduction in Pacific herring (*Clupea harengus pallasi*). API publication No. 4412. American Petroleum Institute, Washington, D.C.

Purcell, J.E., Grosse, D., and Grover, J.J. 1990. Mass abundances of abnormal Pacific herring larvae at a spawning ground in British Columbia. Trans. Am. Fish. Soc. **119**: 463–469.

Rice, S.D., Moles, A., Taylor, T.L., and Karinen, J.F. 1979. Sensitivity of 39 Alaskan marine species to Cook Inlet crude oil and No. 2 fuel oil. *In* 1979 Oil Spill Conference (Prevention, Behavior, Control, Cleanup) Proceedings. American Petroleum Institute, Washington, D.C. pp. 549–554.

Rice, S.D., Babcock, M.M., Brodersen, C.C., Gharrett, J.A., and Korn, S. 1987. Uptake and depuration of aromatic hydrocarbons by reproductively ripe Pacific herring and the subsequent effect of residues on egg hatching and survival. *In* Pollution physiology of estuarine organisms. *Edited by* W.B. Vernberg, A. Calabrese, F.P. Thurberg, and F.J. Vernberg. University of South Carolina Press, Columbia, S.C. pp. 139–154.

Royer, T.C., Vermersch, J.A., Weingartner, T.J., Niebauer, H.J., and Muench, R.D. 1990. Ocean circulation influencing the *Exxon Valdez* oil spill. Oceanography, **3**(2): 3–10.

Schmid, W. 1976. The micronucleus test for cytogenetic analysis. Chem. Mutagens, **4**: 31–53.

Schwartz, J.P. 1985. Effects of oil-contaminated prey on the feeding and growth rate of pink salmon fry (*Onchorhynchus gorbuscha*). *In* Marine pollution and physiology: recent advances. *Edited by* F.J. Vernberg, F.P. Thurberg, A. Calabrese, and W. Vernberg. University of South Carolina Press, Columbia, S.C. pp. 459–476.

Short, J.W., and Harris, P.M. 1996. Petroleum hydrocarbons in caged mussels deployed in Prince William Sound after the *Exxon Valdez* oil spill. *In Exxon Valdez* oil spill symposium proceedings. *Edited by* S.D. Rice, R.B. Spies, D.A. Wolfe, and B.A. Wright. Am. Fish. Soc. Symp. No. 18. pp. 29–39.

Smith, R.L., and Cameron, J.A. 1979. Effect of water soluble fraction of Prudhoe Bay crude oil on embryonic development of Pacific herring. Trans. Am. Fish. Soc. **108**: 70–75.

Solbakken, J.E., Tilseth, S., and Palmork, K.H. 1984. Uptake and elimination of aromatic hydrocarbons and a chlorinated biphenyl in eggs and larvae of cod, *Gadus morhua*. Mar. Ecol. Prog. Ser. **16**: 297–301.

Spaulding, M.L., Saila, S.B., Lorda, E., Walker, H., Anderson, E., and Swanson, J.C. 1983. Oil-spill fishery impact assessment model: application to selected Georges Bank fish species. Estuarine Coastal Shelf Sci. **16**: 511–541.

Spaulding, M.L., Reed, M., Anderson, E., Isaji, T., Swanson, J.C., Saila, S.B., Lorda, E., and Walker, H. 1985. Oil spill fishery impact assessment model: sensitivity to spill location and timing. Estuarine Coastal Shelf Sci. **20**: 41–53.

Struhsaker, J.W. 1977. Effects of benzene (a toxic component of petroleum) on spawning Pacific herring, *Clupea harengus pallasi*. Fish. Bull. **75**: 43–49.

Struhsaker, J.W., Eldridge, M.B., and Echeverria, T. 1974. Effects of benzene (a water-soluble component of crude oil) on eggs and larvae of Pacific herring and northern anchovy. *In* Pollution and physiology of marine organisms. *Edited by* F.J. Vernberg and W.B. Vernberg. Academic Press, New York. pp. 253–284.

Urho, L. 1991. Impact of an oil spill on herring stock. *In* Proceedings of the International Herring Symposium, Anchorage, Alaska, October 23–25, 1990. Alaska Sea Grant report No. 91-01. Alaska Sea Grant, University of Alaska, Fairbanks, Alaska. pp. 569–582.

Venkatesh, S. 1990. Model simulations of the drift and spread of the *Exxon Valdez* oil spill. Atmos.-Ocean, **28**: 90–105.

von Westernhagen, H. 1988. Sublethal effects of pollutants on fish eggs and larvae. *In* Fish physiology. Vol. XIA. *Edited by* W.S. Hoar and D.J. Randall. Academic Press, New York. pp. 253–346.

Wespestad, V.G., and Moksness, E. 1990. Observations on growth and survival during the early life history of Pacific herring *Clupea pallasi* from Bristol Bay, Alaska, in a marine mesocosm. Fish. Bull. **88**: 191–200.

Willette, M. 1993. Impacts of the *Exxon Valdez* oil spill on the migration, growth, and survival of juvenile pink salmon in Prince William Sound. *In* Abstracts of the *Exxon Valdez* Oil Spill Symposium, Anchorage, Alaska, February 2–5, 1993. General Services Administration, Region Nine Printing Plant, Juneau, Alaska. pp. 112–114.

# Reproductive success and histopathology of individual Prince William Sound Pacific herring 3 years after the *Exxon Valdez* oil spill

**R.M. Kocan, G.D. Marty, M.S. Okihiro, E.D. Brown, and T.T. Baker**

**Abstract**: Adult Pacific herring (*Clupea pallasi*) collected in 1992 from a site previously oiled by the *Exxon Valdez* oil spill exhibited a lower percent hatch and produced fewer morphologically normal larvae than fish from a previously unoiled site. Possible explanations for these reproductive differences include (*i*) exposure to residual oil, (*ii*) homing of previously oil-injured fish, (*iii*) homing of different strains of herring, and (*iv*) physical or chemical characteristics of each exposure site unrelated to oil. Differences in microscopic tissue lesions were also observed and were marginally significant between sites. Granulomatous inflammation occurred only in females from previously oiled sites, and this plus increased splenic congestion were negatively correlated to production of normal larvae. Scores for macrophage aggregates in spleen, liver, and kidney were greater in fish from previously oiled sites, particularly in males, but differences were related to age rather than exposure history. Because most of the lesions related to reproductive success were acute or subacute, differences in tissue damage could not be directly related to previous oil exposure.

**Résumé** : Chez des harengs du Pacifique adultes (*Clupea pallasi*) prélevés en 1992 dans un site qui avait été contaminé par la nappe de pétrole déversée par l'*Exxon Valdez*, le pourcentage d'éclosion des oeufs et la proportion de larves normales sur le plan morphologique étaient inférieurs à ce qu'ils étaient chez des poissons provenant d'un autre site qui n'avait pas été contaminé. Voici des explications possibles : (*i*) exposition à du pétrole résiduaire, (*ii*) retour de poissons qui avaient déjà été contaminés par le pétrole, (*iii*) retour de différentes souches de hareng et (*iv*) caractéristiques chimiques ou physiques propres à chaque site qui sont sans rapport avec le déversement de pétrole. Nous avons également noté des différences, marginalement significatives entre les sites, relativement à des lésions tissulaires microscopiques observées chez les sujets. L'inflammation granulomateuse n'a été observée que chez des femelles capturées dans des sites qui avaient été mazoutés. Ce phénomène, couplé à une congestion accrue de la rate, est en corrélation négative avec la production de larves normales. Les indices des agrégats de macrophages dans la rate, le foie et le rein sont supérieurs chez les poissons provenant des sites qui avaient été mazoutés, particulièrement les mâles, mais les différences observées sont attribuables à l'âge plus qu'à l'exposition au pétrole. Puisque la plupart des lésions ayant un lien avec le succès de la reproduction étaient aiguës ou subaiguës, il était impossible d'attribuer directement à une exposition passée au pétrole les différences observées en termes de lésions tissulaires. [Traduit par la Rédaction]

## Introduction

The *Exxon Valdez* oil spill in March 1989 coincided with one of the largest Pacific herring (*Clupea pallasi*) spawning events ever observed in Prince William Sound. The embryos and larvae produced by that event were potentially exposed to petroleum hydrocarbons derived from Prudhoe Bay crude oil, as were 1-year-old herring that were rearing in the sound at the same time. Because many effects of exposure to toxic substances are sublethal and not immediately observed, we believed that close observation of exposed embryos and larvae, as well as evaluation of the reproductive success of exposed fish, would reveal any long-term toxic effects that might not otherwise have been apparent.

This project was designed to evaluate the reproductive potential of individual female herring from oiled and unoiled sites in Prince William Sound 3 years after the *Exxon Valdez* oil spill. The primary target fish were 4 year olds that were potentially exposed to Prudhoe Bay crude oil as 1 year olds at the time of the *Exxon Valdez* oil spill and were returning as first-time spawners in 1992. Previous studies demonstrated a difference in embryo survival, larval abnormalities, larval weight, and genetic damage between oiled and unoiled sites from 1989 through 1992 (Kocan et al. 1996; McGurk and Brown 1996; Hose et al. 1996). The objective of this study was to determine whether first-time spawners that were collected at previously oiled and unoiled sites exhibited differences in reproductive success but we did not specifically address cause and effect relationships.

To evaluate potential reproductive impairment, females from previously oiled and unoiled sites were collected and their individual reproductive success was compared by individually spawning each fish and maintaining their eggs in separate vessels until they hatched. Adults were also examined

Received March 21, 1995. Accepted March 11, 1996.
J12830

**R.M. Kocan.**[1] School of Fisheries, Box 355100, University of Washington, Seattle, WA 98195, U.S.A.
**G.D. Marty and M.S. Okihiro.** Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, University of California, Davis, CA 95616, U.S.A.
**E.D. Brown.** Alaska Department of Fish and Game, Box 669, Cordova, AK 99574, U.S.A.
**T.T. Baker.** Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99518, U.S.A.

[1] Author to whom all correspondence should be addressed.
e-mail: kocan@fish.washington.edu

for differences between reproductive success and microscopic tissue damage (e.g., histopathology). Several possibilities were considered that might explain any observed effects: (*i*) residual toxicity of oil remaining in previously oiled areas, (*ii*) delayed effects of oil on first-time spawners, (*iii*) strain differences among spawning groups, and (*iv*) environmental differences unrelated to oil.

## Methods

### Collection and spawning

Ripe, actively spawning male and female herring were collected by gill net from one unoiled site in Tatitlek Narrows (Boulder Bay) on 11 April 1992 and two oiled sites, designated Rocky Bay-a and Rocky Bay-b on Montague Island on 21 and 22 April. The fish were packed in a cooler to maintain their body temperature near that of the water and transported to Cordova, Alaska, by air where they were given identification numbers and artificially spawned onto glass slides. Eggs from each of 25 females were distributed into separate containers of seawater, each containing six glass slides. Approximately 300 eggs attached to the surface of the slides and these were fertilized with the pooled sperm of five males from the same capture site. The entire process from field collection of fish to completion of artificial spawning was completed within 6 h. The containers and slides were then placed into a cooler, aerated with $O_2$, and transported by air to the University of Washington's Friday Harbor Laboratories for incubation. When embryos arrived at Friday Harbor they were 24 h old and in the early blastodisc stage of development. Water temperature was maintained at 6°C from the time of collection until their arrival at Friday Harbor, where the temperature was allowed to rise to the local ambient seawater temperature of 9°C over 6 h.

Condition factor ($K$) of adults was calculated from the length ($L$) and weight ($w$) measurements by the following equation (Gunderson and Myers 1989):

$$(1) \quad K = (w/L^3) \times 100$$

### Embryo–larval evaluation

Developing eggs from each female were placed in flowing seawater chambers for incubation and were microscopically examined for fertilization, embryo survival (percent hatch), and normal-live larvae. Fertilization success was determined by examining the eggs for the presence of a perivitelline space. Percent hatch was determined by

$$(2) \quad \%\ \text{total hatch} = \frac{\text{no. of live larvae}}{\text{no. of fertile eggs}} \times 100$$

Normal-live (i.e., viable) larvae was determined by

$$(3) \quad \%\ \text{normal larvae} = \frac{\text{no. of live larvae} - \text{no. of abnormal larvae}}{\text{no. of fertile eggs}} \times 100$$

Physical defects (abnormal larvae) were categorized as (*i*) spinal deformities (scoliosis and lordosis), (*ii*) craniofacial deformities (mandibular and maxillary), (*iii*) optic abnormalities, and (*iv*) pericardial edema. Because none of these defects were considered to be pathognomonic, they were pooled and the data evaluated in relation to female:female variability and oiled versus unoiled sites.

### Histopathology

Following spawning, the fish were killed with an overdose of tricaine methane sulfonate (MS-222, Finquel) and examined for standard length and wet weight. Scales were removed for age determination, and liver, kidney, and spleen were fixed in 10% neutral buffered formalin for histopathology. Fixed tissues were shipped to the University of California at Davis, where random numbers were generated for further processing and blind study. Tissues were processed routinely into paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Slides were read in ascending order on the basis of the assigned random number. Lesions were semiquantitatively ranked using a four-point scale: none (0), mild (1), moderate (2), or severe (3). For each lesion, mean scores (±SE) were calculated for males and females from each site. For quality control, each spleen section was scored for extent of autolysis and sectioning artifact. Scores for sectioning artifact were based on the extent that artifacts made photography or interpretation of lesions difficult.

Lesion scores were first summarized through use of a principal components analysis. For a given set of scores, the first four principal components were used as a summary for most of the variability in the raw data. The principal components were then tested for systematic site and age effects using a multivariate analysis of variance (MANOVA). All of the variables were combined in another MANOVA to determine the overall significance of capture site. For females only, the relation between percent normal progeny and lesion scores was examined using Spearman correlation coefficients. These correlations were calculated either without or with an adjustment for possible site effects (through use of a partial correlation). This allowed us to distinguish between relations that were strictly driven by site differences, and those that also exist on a within-site level. Comparisons were classified as insignificant ($p > 0.05$), significant ($p \leq 0.05$), or highly significant ($p \leq 0.01$).

## Results

### Spawner age

All samples consisted primarily of 4-year-old, first-time spawners, with a few fish from older year-classes. When data from the older fish were removed from results for percent hatch and normal larvae, the overall results remained the same; therefore, the older fish were included in the final analyses to maintain a constant sample size of 25 fish/site. All three populations were similar in age composition (Table 1).

### Fertilization

Fertilization success for the three groups ranged from 94.2 to 96.8% (Table 1). The number of fertile eggs represented the maximum potential number of live embryos that could potentially produce normal larvae.

### Length, weight, and condition

There were no significant weight differences among the three groups of females ($p = 0.13$; ANOVA); however one group from an oiled site (Rocky Bay-b) was longer than both the Boulder Bay group and the Rocky Bay-a group ($p < 0.01$; $t$-test). Boulder Bay spawners had a higher condition factor than either Rocky Bay-a or -b ($p < 0.01$; ANOVA), however, the value for Rocky Bay-a was also higher than that of Rocky Bay-b ($p < 0.01$) (Table 1).

### Hatching success and percentage of morphologically normal larvae

The percent total hatch (eq. 2) from the two oiled sites was less than that from the unoiled site ($p < 0.001$; $t$-test). However, the two Rocky Bay groups were also different from each other ($p < 0.01$) (Table 1). The percentage of normal larvae (eq. 3) produced by females from Boulder Bay, the unoiled site, was higher (31.8%) than that from either oiled site (Rocky Bay-a, 13%; Rocky Bay-b, 25%) ($p < 0.01$; $t$-test).

**Table 1.** Morphometric and reproductive success data for Pacific herring captured from three sites in Prince William Sound, Alaska.

| | Boulder Bay (unoiled) | | Rocky Bay-a (oiled) | | Rocky Bay-b (oiled) | |
|---|---|---|---|---|---|---|
| | Females | Males | Females | Males | Females | Male |
| Standard length (mm) | 198.8±2.0 | 194.4±1.8 | 202.2±2.6 | 200.8±4.8 | 206.4±1.91 | 214.1±3.7 |
| Weight (g) | 100.4±3.3 | 94.4±2.2 | 103.8±4.2 | 102.2±7.8 | 97.9±2.2 | 112.6±5.5 |
| Age (years) | 4.4±0.2 | 4.4±0.3 | 4.8±0.3 | 5.4±0.6 | 4.4±0.2 | 5.8±0.6 |
| Condition factor ($K$) | 1.27±0.09 | 1.28±0.12 | 1.24±0.10 | 1.26±0.12 | 1.11±0.08 | 1.15±0.09 |
| Fertility (%) | 96.8±0.5 | | 96.7±0.5 | | 94.2±0.5 | |
| Hatch (%) | 56.0±2.6 | | 19.0±1.8 | | 38.0±2.0 | |
| Normal larvae (%) | 31.8±2.4 | | 13.1±1.3 | | 25.0±1.4 | |

Note: Values are given as the mean ± SE. Collection dates: Boulder Bay, 11 April 1992; Rocky Bay-a, 21 April 1992; Rocky Bay-b, 22 April 1992.

**Table 2.** Histopathology lesion scores for Pacific herring captured at three sites in Prince William Sound, Alaska.

| | Boulder Bay (unoiled) | | Rocky Bay-a (oiled) | | Rocky Bay-b (oiled) | |
|---|---|---|---|---|---|---|
| | Females | Males | Females | Males | Females | Males |
| Liver lesions | | | | | | |
| Sample size | 25 | 9 | 25 | 10 | 24 | 10 |
| Lesion scores | | | | | | |
| Hepatocellular glycogen depletion | 3.0±0.0 | 3.0±0.0 | 3.0±0.0 | 2.9±0.1 | 3.0±0.0 | 3.0±0.0 |
| Lipidosis | 1.2±0.2 | 1.0±0.2 | 0.6±0.1 | 0.3±0.2 | 1.1±0.2 | 0.6±0.2 |
| Macrophage aggregates | 1.3±0.2 | 1.3±0.2 | 1.5±0.2 | 1.8±0.3 | 1.2±0.1 | 2.0±0.3 |
| Single cell necrosis | 0.8±0.2 | 0.6±0.2 | 0.3±0.1 | 0.2±0.1 | 0.5±0.1 | 0.3±0.2 |
| Focal necrosis | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.2±0.1 |
| Hepatocellular megalocytosis | 0.9±0.2 | 1.0±0.2 | 1.0±0.2 | 1.1±0.3 | 0.8±0.1 | 0.8±0.2 |
| Coccidiosis (*Goussia clupearum*) | 0.7±0.2 | 1.1±0.5 | 0.9±0.2 | 0.7±0.2 | 0.7±0.2 | 0.7±0.3 |
| Kidney lesions | | | | | | |
| Sample size | 24 | 4 | 24 | 9 | 21 | 9 |
| Lesion scores | | | | | | |
| Macrophage aggregates | 1.3±0.2 | 1.1±0.2 | 1.4±0.2 | 1.7±0.3 | 1.2±0.1 | 1.9±0.3 |
| Lymphocytes | 0.0±0.0 | 0.2±0.1 | 0.2±0.1 | 0.3±0.2 | 0.1±0.1 | 0.2±0.1 |
| Thickened glomerular basal lamina | 0.2±0.1 | 0.0±0.0 | 0.0±0.0 | 0.3±0.2 | 0.0±0.0 | 0.1±0.1 |
| Interstitial fibrosis | 0.2±0.1 | 0.2±0.1 | 0.0±0.0 | 0.1±0.1 | 0.2±0.1 | 0.0±0.0 |
| Spleen lesions | | | | | | |
| Sample size | 19 | 8 | 24 | 7 | 25 | 10 |
| Lesion scores | | | | | | |
| Autolysis | 0.1±0.1 | 0.0±0.0 | 0.0±0.0 | 0.1±0.1 | 0.0±0.0 | 0.0±0.0 |
| Artifact | 0.9±0.1 | 1.1±0.1 | 1.1±0.1 | 1.1±0.1 | 1.0±0.0 | 1.1±0.1 |
| Congestion | 0.4±0.2 | 1.3±0.5 | 1.1±0.2 | 1.1±0.5 | 1.1±0.2 | 1.0±0.4 |
| Macrophage aggregates | 1.3±0.2 | 1.3±0.3 | 1.6±0.2 | 1.6±0.4 | 1.4±0.1 | 1.8±0.3 |
| Granulomatous inflammation | 0.0±0.0 | 0.0±0.0 | 0.1±0.1 | 0.1±0.1 | 0.1±0.1 | 0.0±0.0 |
| *Ichthyophonus* | 0.1±0.1 | 0.0±0.0 | 0.2±0.1 | 0.0±0.0 | 0.1±0.1 | 0.1±0.1 |
| Serosal cell thickening | 1.0±0.1 | 0.9±0.3 | 0.8±0.1 | 0.7±0.2 | 0.8±0.1 | 0.7±0.2 |
| Ellipsoid hypertrophy | 0.8±0.1 | 1.0±0.0 | 0.9±0.1 | 1.1±0.1 | 1.1±0.1 | 1.6±0.2 |
| Focal arteriolar intimal hypertrophy | 0.4±0.1 | 0.1±0.1 | 0.3±0.1 | 0.3±0.2 | 0.4±0.1 | 0.3±0.2 |

Note: Lesion scores are given as the mean ± SE. Collection dates: Boulder Bay, 11 April 1992; Rocky Bay-a, 21 April 1992; Rocky Bay-b, 22 April 1992.

## Histopathology

Overall site differences were not significant (MANOVA) for lesions scored in liver, kidney, and spleen (Table 2); however, age differences were highly significant (Table 3). Because of missing values, only 83 of 105 fish were used when lesions from all three organs were analyzed. When scores for splenic lesions were ignored, 96 fish were used in the analysis; then, site differences were significant and age differences remained highly significant (Table 3). For both analyses, overall differences between the unoiled site (Boulder Bay) and the first sample from the oiled site (Rocky Bay-a) were significant, whereas other site comparisons were not.

Age differences were highly significant for the first principal component, in which macrophage aggregates from kidney, liver, and spleen contributed most to variability. Scores for macrophage aggregates were higher in older fish. Age differences were not significant for any other principal component.

Parental females with splenic congestion and granulomatous inflammation produced significantly fewer normal larvae than did fish without these conditions (Spearman's correlation coefficient) (Table 4) (Fig. 1). Among the five female herring with severe splenic congestion, none produced more than 20% normal larvae. Only five female herring, all from Rocky Bay (oiled), had granulomatous inflammation in the spleen; the

**Table 3.** Multiple analysis of variance (MANOVA) to test the influence of site, age, and oil exposure history in Pacific herring adults captured from three sites in Prince William Sound, Alaska.

| Effect or method | *n* | Relation | Significance | Significant principal component (and most important lesions[a]) |
|---|---|---|---|---|
| MANOVA (all variables) | 83 | Site | ns | na |
| | | Unoiled vs. oiled-a | * | 3rd (splenic serosal cell thickening and artifiact) |
| | | Unoiled vs. oiled-b | * | 2n (thickened glomerular basal lamina) splenic congestion) |
| | | Oiled-a vs. oiled-b | ns | None |
| | | Age | ** | 1st (renal, hepatic, and splenic macrophage aggregates) |
| MANOVA (liver and kidney only) | 96 | Site | * | na |
| | | Unoiled vs. oiled-a | * | 3rd (hepatocellular single cell necrosis, renal fibrosis) and 4th (hepatic coccidiosis and hepatocellular lipidosis) |
| | | Unoiled vs. oiled-b | ns | None |
| | | Oiled-a vs. oiled-b | ns | None |
| | | Age | ** | 1st (hepatic and renal macrophage aggregates) |

**Note**: Significance is identified as $p \geq 0.05$ (ns), $p < 0.05$ (*), or $p < 0.01$ (**). Overall site comparisons did not depend on individual principal components (na).

[a]The first principal component contributes most to variability, whereas the second and later components contribute progressively less to variability.

**Table 4.** Results of correlation analysis to test the relation of lesions and percent viability.

| Lesion | *n* | *r* |
|---|---|---|
| **Spearman's correlation coefficient** | | |
| Hepatocellular lipidosis | 74 | 0.23* |
| Hepatocellular single cell necrosis | 74 | 0.23* |
| Splenic artifact | 68 | –0.33** |
| Splenic congestion | 68 | –0.38** |
| *Splenic granulomatous inflammation* | 68 | –0.24* |
| **Partial Spearman's correlation coefficient, with site differences factored out** | | |
| Splenic congestion | 60 | 0.39** |
| Splenic granulomatous inflammation | 60 | –0.25* |

**Note**: Significant *r* values are identified as $p < 0.05$ (*) or $p < 0.01$ (**). Lesions not listed were not significant.

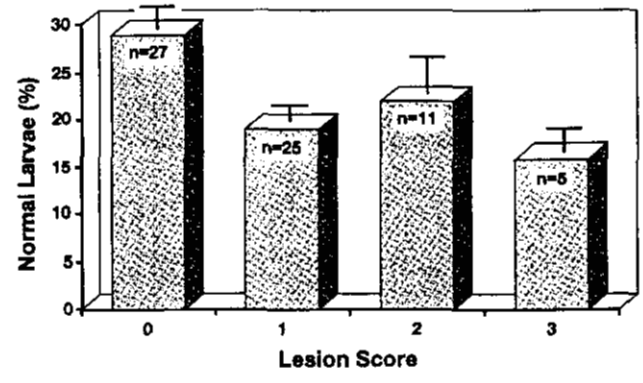
mean percentage of normal larvae among affected fish was 14.2%, and none were greater than 20%. None of the herring with granulomatous inflammation had severe congestion. Scores for hepatocellular single cell necrosis and lipidosis were positively correlated with numbers of normal larvae, and mean scores for these lesions were lowest in fish from Rocky Bay, the oiled site (Tables 2 and 4). However, neither single cell necrosis nor lipidosis was significantly correlated to numbers of normal larvae when Spearman's partial correlations were done, indicating that differences were driven by site effects rather than within-site variability.

Six herring (5.7%) had resting spores of *Ichthyophonus hoferi* in at least one organ. Kidneys from three herring (3.1%) had pansporoblasts of the myxosporean *Ortholinea orientalis* in archinephric ducts. Fish from both oiled and unoiled sites were infected with both organisms.

## Discussion

Spawning herring from previously oiled sites in Rocky Bay produced significantly fewer normal larvae than herring from an unoiled site (Boulder Bay), and lower reproductive potential was significantly correlated with several histopathological lesions. The 25 spawners from Boulder Bay produced a mean

**Fig. 1.** Splenic congestion in female Pacific herring compared with percentage of normal larvae (mean ± SE) among resultant progeny. Congestion was scored as none (0), mild (1), moderate (2), or severe (3).




total hatch of 56%, significantly higher than that of either of the groups from Rocky Bay (19 and 38%). This success rate is similar to that reported for herring by other investigators: 42% by Rosenthal and Hourston (1982), 53% by Smith and Cameron (1979), and 78–85% by Rice et al. (1987). The percentage of normal larvae (33%) produced by fish from Boulder Bay was also significantly higher than for the Rocky Bay sites (13 and 25%). There was no difference in fertility rate among the fish from oiled and unoiled sites.

Histopathology provided minimal evidence that lesions were related to previous oiling; however, several lesions were positively correlated with increased larval abnormalities. Although site differences were significant in some of the analyses, mean scores for lesions that contributed most to variability, e.g., hepatocellular lipidosis and single cell necrosis, were lowest in the group with the fewest normal larvae (Rocky Bay-a). Hepatic lipidosis has been associated with exposure to petroleum hydrocarbons in the laboratory (Eurell and Haensly 1981; Solangi and Overstreet 1982), whereas Haensley et al. (1982) described decreased hepatocellular lipids in fish after the *Amoco Cadiz* oil spill. Part of the difficulty in interpreting hepatic lipidosis is that females in spawning condition

often have small lipid vacuoles that are normal in hepatocytes producing vitellogenin for transfer to oocytes (van Boheman et al. 1981). Hepatocellular single-cell necrosis has not been previously described in relation to hydrocarbon exposure, and the reason for lower mean scores in herring from previously oiled sites is unknown.

Splenic granulomatous inflammation and severe congestion were good biomarkers for predicting abnormality in the larval progeny. Granulomatous inflammation was indicative of subacute to chronic inflammation, whereas congestion was probably an acute cardiovascular alteration; however, causes were not determined. Causes of splenomegaly in mammals include chronic and acute infections (Valli 1985), but causes in fish are poorly understood. Another lesion type, macrophage aggregates, increases with age, starvation, and exposure to toxicants (Blazer et al. 1987; Wolke 1992). Unlike granulomatous inflammation in these fish, macrophage aggregates were chronic lesions that probably took several months to develop and may last for several years. In our study, increased scores for macrophage aggregates in herring from previously oiled sites were related to age but were unrelated to previous oiling history.

Although histopathological damage could not be conclusively correlated to previous oiling, the decreased reproductive success observed in both groups of fish from the oiled site suggests that the oiling history of the site may have contributed to reproductive impairment. A similar pattern of toxicity was reported by Kocan et al. (1996) for herring embryos incubated in situ at these sites in 1991.

There are several possible explanations for results. These include, but are not restricted to the following: (*i*) residual oil at the previously oiled sites, (*ii*) previously injured fish homing to the same sites, (*iii*) different strains of fish homing to the same sites, and (*iv*) physical or chemical features specific to each site.

Owing to the low solubility of petroleum hydrocarbons, residual oil present in Prince William Sound in 1992 would most likely have been associated with sediments rather than the water column, and it could have been made available to the spawning fish through contaminated prey items. It is doubtful, however, that this would have affected the reproductive potential of herring, since it has been experimentally demonstrated that spawn survival is not affected in adult herring recently exposed to oil (Rice et al. 1987).

If first-time spawners had been genetically damaged by oil as 1 year olds in 1989, then subsequently homed to the same sites in 1992, this could explain the differences in reproductive success at the previously oiled sites.

The best available experimental data support the contention that herring home to the same spawning areas from year to year. This opens the possibility that herring damaged during an event such as the *Exxon Valdez* oil spill can continue to display damage at the same sites over a period of years. Hourston (1982) reported a degree of homing of 77–94% for relatively isolated populations of Pacific herring, and 66–96% for 18 artificially established management units in British Columbia. The same type of homing behavior was reported for Atlantic herring (Wheeler and Winters 1984), for which homing rates were reported to be as high as 90%. Homing studies have not been carried out on Prince William Sound herring, but if they have a similar degree of homing, then spawning fish observed in this study may have encountered oil at these same sites in 1989 when they were 1 year old.

Another possibility that would explain the reproductive differences may be strain differences among spawning populations. If Prince William Sound herring home to any extent as suggested above, then the differences observed in reproductive success may reflect reproductive differences in different strains of spawning herring.

The physical and chemical differences among the various sites may also have influenced the reproductive success of each population studied. It seems unlikely, however, that natural environmental conditions would be more detrimental to aquatic organisms than 11 000 000 gallons (41 635 000 L) of crude oil deposited during the peak of their spawning season, or genetic differences among strains of herring.

Studies addressing the issue of natural differences in herring reproductive success have focused on temperature, salinity, and desiccation effects on the developing embryos (Alderdice and Hourston 1985). By comparison, all embryos in this study were incubated under identical laboratory conditions, with variables being derived solely from individual females.

The mechanism(s) underlying the reproductive differences in herring from different sites observed in this study are not known at this time. To determine the precise cause of the observed differences, a study must be designed to address cause and effect relationships for reproductive impairment. However, in lieu of other causes for the observed reproductive impairment, the effect of crude oil from the 1989 *Exxon Valdez* oil spill should not be ruled out as at least being partly responsible for the observed injury to herring reproduction.

The effects of crude oil on fish reproduction have been reported in several studies where exposure of spawning herring to crude oil components decreased hatching success relative to that of unexposed herring (Struhsaker 1977; Whipple et al. 1981). A similar finding for starry flounder (*Platichthys stellatus*) was described by Spies et al. (1985), and laboratory studies have generally supported field observations. English sole (*Parophrys vetulus*) exposed to benzo[*a*]pyrene, a component of crude oil, produced eggs with significantly lower hatching success and a higher rate of larval abnormalities than unexposed cohorts (Hose et al. 1981). However, Rice et al. (1987) found no adverse effects on the hatching success of herring eggs produced by females that had accumulated high levels of aromatic hydrocarbons in their tissues just prior to spawning.

The design of the study reported here does not give a mechanism for the observed reproductive damage in herring. However, the evidence suggests that genetic damage, direct exposure to hydrocarbons, or site-related strain differences should be considered as possible causes of the observed reproductive impairment.

## Acknowledgments

This study was funded by Alaska Department of Fish and Game contracts IHP-91-043, IHP-92-039, and IHP-93-016 and National Institute of Environmental Health Sciences grant ES-04696. Technical assistance was provided by T. Mehl, L. Bolles, J. Wilcock, J. Gilman, J. Allen, and N. Willits.

## References


Alderdice, D.F., and Houston, A.S. 1985. Factors influencing development and survival of Pacific herring (*Clupea harengus pallasi*) eggs and larvae to beginning of exogenous feeding. Can. J. Fish. Aquat. Sci. **42** (Suppl. 1): 56–68.

Blazer, V.S., Wolke, R.E., Brown, J., and Powell, C.A. 1987. Piscine macrophage aggregate parameters as health monitors: effect of age, sex, relative weight, season and site quality in largemouth bass (*Micropterus salmoides*). Aquat. Toxicol. **10**: 199–215.

Eurell, J.A.C., and Haensly, W.E. 1981. The effects of exposure to water soluble fractions of crude oil on selected histochemical parameters of the liver of the Atlantic croaker, *Micropogon undulatus* L. J. Fish Dis. **4**: 187–194.

Gunderson, D.R., and Myers, K.W. 1989. Age and growth. *In* Harvesting life from water. *Edited by* F.G. Johnson and R.R. Stickney. Kendall/Hunt Publishing Co., Dubuque, Iowa. pp. 122–131.

Haensly, W.E., Neff, J.M., Sharp, J.R., Morris, A.C., Bedgood, M.F., and Boem, P.D. 1982. Histopathology of *Pleuronectes platessa* L. from Wrac'h and Aber Benoit, Brittany, France: long-term effects of the *Amaco Cadiz* crude oil spill. J. Fish Dis. **5**: 365–391.

Hose, J.E., Hannah, J.B., Landolt, M.L., Miller, B.S., Felton, S.P., and Iwaoka, W.T. 1981. Uptake of benzo(*a*)pyrene by gonadal tissue of flatfish (F. Pleuronectidae) and its effects on subsequent egg development. J. Toxicol. Environ. Health, **7**: 991–1000.

Hose, J.E., McGurk, M.D., Marty, G.D., Hinton, D.E., Brown, E.D., and Baker, T.T. 1996. Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphological, cytogenetic, and histopathological assessments, 1989–1991. Can. J. Fish. Aquat. Sci. **53**: 2355–2365.

Hourston, A.S. 1982. Homing by Canada's west coast herring to management units and divisions as indicated by tag recoveries. Can. J. Fish. Aquat. Sci. **39**: 1414–1422.

Kocan, R.M., Hose, J.E., Brown, E.D., and Baker, T.T. 1996. Pacific herring (*Clupea pallasi*) embryo sensitivity to Prudhoe Bay petroleum hydrocarbons: laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound. Can. J. Fish. Aquat. Sci. **53**: 2366–2375.

McGurk, M.D., and Brown, E.D. 1996. Egg–larval mortality of Pacific herring in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. **53**: 2343–2354.

Rice, S.D., Babcock, M.M., Brodersen, C.C., Carls, M.G., Gharrett, J.A., Korn, S., Moles, A., and Short, J.W. 1987. Lethal and sublethal effects of the water-soluble fraction of Cook Inlet crude oil on Pacific herring (*Clupea harengus pallasi*) reproduction. NOAA Tech. Memo. No. NMFS F/NWC-111. U.S. Department of Commerce, National Oceanic and Atmospheric Admistration, Washington, D.C.

Rosenthal, H., and Hourston, A.S. 1982. Observations on viability of eggs from small fish of a local herring stock. Canadian. Tech. Rep. Fish. Aquat. Sci. No. 1090.

Smith, R.L., and Cameron, J.A. 1979. Effect of water soluble Prudhoe Bay crude oil on embryonic development of Pacific herring. Trans. Am. Fish. Soc. **108**: 70–75.

Solangi, M.A., and Overstreet, R.M. 1982. Histopathological changes in two estuarine fishes, *Menidia beryllina* (Cope) and *Trinectes maculatus* (Bloch and Schneider), exposed to crude oil and its water soluble fractions. J. Fish Dis. **5**: 13–35.

Spies, R.B., Rice, D.W., Jr., Montagna, P.A., and Ireland, R.R. 1985. Reproductive success, xenobiotic contaminants and hepatic mixed-function oxidase (MFO) activity in *Platichthys stellatus* populations from San Francisco Bay. Mar. Environ. Res. **117**: 121–127.

Struhsaker, J.W. 1977. Effects of benzene (a toxic component of petroleum) on spawning Pacific herring, *Clupea harengus pallasi*. U.S. Natl. Mar. Fish. Serv. Fish. Bull. **75**: 43–49.

Valli, V.E.O. 1985. The hematopoietic system. *In* Pathology of domestic animals. *Edited by* K.V.F. Jubb, P.C. Kennedy, and N. Palmer. Vol. 3. 3rd ed. Academic Press, Orlando, Fla. pp. 84–236.

van Boheman, C.G., Lambert, J.G.D., and Peute, J. 1981. Annual changes in plasma and liver in relation to vitellogenesis in the female rainbow trout, *Salmo gairdneri*. Gen. Comp. Endocrinol. **44**: 94–107.

Wheeler, J.P., and Winters, G.H. 1984. Homing of Atlantic herring (*Clupea harengus harengus*) in Newfoundland waters as indicated by tagging data. Can. J. Fish. Aquat. Sci. **41**: 108–117.

Whipple, J.A., Eldridge, M.B., and Benville, P., Jr. 1981. An ecological perspective of the effects of monocyclic aromatic hydrocarbons on fishes. *In* Biological monitoring of marine pollutants. *Edited by* F.J. Venberg, A. Calabrese, F. Thurberg, and W. Vernberg. Academic Press, New York. pp. 483–551.

Wolke, R.E. 1992. Piscine macrophage aggregates: a review. Annu. Rev. Fish Dis. **2**: 91–108.

# Histopathology and cytogenetic evaluation of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill

**Gary D. Marty, J. E. Hose, Michael D. McGurk, Evelyn D. Brown, and David E. Hinton**

**Abstract**: Following the 1989 *Exxon Valdez* oil spill in Prince William Sound, Alaska, USA, Pacific herring (*Clupea pallasi*) larvae sampled from oiled sites had ascites, pericardial edema, and genotoxic damage. Laboratory study confirmed that these lesions were consistent with oil exposure. Pacific herring larvae were trawled from two oiled and two unoiled sites in Prince William Sound in May 1989. Larvae from oiled sites were shorter, had ingested less food, had slower growth (oiled, 0.07–0.10 mm/day; unoiled, 0.15–0.18 mm/day), and had higher prevalence of cytogenetic damage (oiled, 56–84%; unoiled, 32–40%) and ascites (oiled, 16%; unoiled, 1%) than from unoiled sites. In the laboratory experiment, Pacific herring eggs were exposed to an oil–water dispersion of Prudhoe Bay crude oil (initial concentrations of 0.0, 0.10, 0.24, 0.48, and 2.41 mg/L) and sampled for histopathology <24 h after hatching. Effects were significant at the 0.48 mg/L dose (Dunnett's procedure, $P < 0.05$). Lesions included ascites; hepatocellular vacuolar change; and degeneration or necrosis of skeletal myocytes, retinal cells, and developing brain cells. Lesions in field-sampled larvae were consistent with higher mortality rates documented in larvae from oiled sites.

**Résumé** : Après le déversement pétrolier de l'*Exxon Valdez* qui a eu lieu en 1989 dans le golfe du Prince-William, Alaska, É.-U., des larves de hareng du Pacifique (*Clupea pallasi*), prélevées dans des sites exposés au pétrole présentaient des ascites, de l'oedème péricardique et des lésions génotoxiques. Une étude en laboratoire a confirmé qu'il y avait compatibilité entre ces lésions et l'exposition au pétrole. Des larves de hareng du Pacifique ont été récoltées par chalutage dans deux sites exposés au pétrole et dans deux sites non exposés dans le golf du Prince-William en mai 1989. Les larves provenant des sites exposés étaient plus courtes, avaient ingéré moins d'aliments, avaient une croissance plus lente (site exposé, 0,07–0,10 mm/jour; site non exposé, 0,15–0,18 mm/jour) et présentaient une fréquence plus élevée de lésions cytogénétiques (site exposé, 56–84 %; site non exposé, 32–40 %) et d'ascites (site exposé, 16 %; site non exposé, 1 %) que les larves provenant des sites non exposés au pétrole. Au cours d'une expérience de laboratoire, des oeufs de hareng du Pacifique ont été exposés à une dispersion pétrole–eau de pétrole brut de Prudhoe Bay (concentrations initiales de 0,0, 0,10, 0,24, 0,48 et 2,41 mg/L) et ont été examinés pour déceler des manifestations histopathologiques moins de 24 h après leur éclosion. Les effets étaient statistiquement significatifs à la dose d'exposition de 0,48 mg/L (méthode de Dunnett, $P < 0,05$). Parmi les lésions figuraient des ascites, des modifications vacuolaires hépatocellulaires et une dégénérescence ou une nécrose des myocytes squelettiques, des cellules rétiniennes et des cellules cérébrales en développement. Les lésions chez les larves échantillonnées sur le terrain correspondaient avec les taux de mortalité élevés documentés chez les larves provenant des sites exposés au pétrole.
[Traduit par la Rédaction]

## Introduction

Fish embryos and larvae are the most sensitive life-history stage to toxicant exposure (Weis and Weis 1989). When the *Exxon Valdez* oil spill occurred in Prince William Sound (PWS), Alaska, U.S.A., in late March 1989, Pacific herring (*Clupea pallasi*) were beginning to congregate in shallow bays for their annual mass spawning in April. Previous studies had shown that Pacific herring embryos and larvae were susceptible to low concentrations of petroleum hydrocarbons (Smith and Cameron 1979; Cameron and Smith 1980; Pearson et al. 1985; Carls 1987), but oil-related damage had not been demonstrated

Received May 7, 1996. Accepted February 17, 1997.
J13465

**G.D. Marty**[1] **and D.E. Hinton.** Department of Anatomy, Phisiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, CA, 95616, U.S.A.
**J.E. Hose.** Occidental College, Biology Department, Los Angeles, CA, 90041, U.S.A.
**M.D. McGurk.** Triton Environmental Consultants Ltd., 120–13511 Commerce Parkway, Richmond, BC V6V 2L1, Canada.
**E.D. Brown.** Institute of Marine Science, School of Fisheries and Ocean Science, University of Alaska, Fairbanks, AK 99775-7220, U.S.A.

[1] Author to whom all correspondence should be addressed. e-mail: gdmarty@ucdavis.edu

under field conditions. Because contamination of Pacific herring embryos with *Exxon Valdez* oil seemed likely, several studies were initiated to determine effects of the spill on embryonic and larval development.

In two experiments, Pacific herring eggs were removed from oiled and unoiled sites in PWS and incubated in clean water in the laboratory. In one experiment, newly hatched larvae had oil-related cytogenetic damage and morphologic defects but no histologic lesions (Hose et al. 1996). In the other experiment, newly hatched larvae had no oil-related morphologic lesions, but cytogenetic and histopathologic analysis were not done (Pearson et al. 1995). In a laboratory experiment, Pacific herring eggs were exposed to an oil–water dispersion from fertilization to hatch, and newly hatched larvae were sampled for examination (Kocan et al. 1996). Significant oil-related and dose-dependent changes included premature hatching, low weight, and genotoxicity. These three experiments were limited in their ability to detect oil-induced damage in the 1989 year-class because exposure was terminated before hatch or immediately after hatch. In PWS, however, Pacific herring were potentially exposed to petroleum hydrocarbons throughout embryonic and larval development. Therefore, Pacific herring larvae were trawled from the nearshore environment in oiled and unoiled sites during May and early June of 1989. Estimated larval mortality in oiled areas was two times higher than in unoiled areas (McGurk and Brown 1996), but without more detailed study, increased mortality could not be definitively attributed to oil exposure.

In this paper, trawled larvae from McGurk and Brown (1996) were examined for cytogenetic and histologic lesions, and laboratory-exposed larvae from Kocan et al. (1996) were examined for histologic lesions. Significant histopathologic lesions in fish larvae potentially exposed to an oil spill have not previously been reported. We describe ascites as the most important lesion in larvae from oiled sites in PWS and confirm through laboratory study that this lesion can be caused by exposure to petroleum hydrocarbons. We also link oil exposure to delayed development in field-exposed larvae and to premature hatching of laboratory-exposed fish.

## Methods

### Environmental oil exposure

Pacific herring larvae examined from PWS were a subset of the larvae analyzed for growth and mortality by McGurk and Brown (1996). To summarize, Pacific herring larvae were captured using plankton trawls in two oiled areas and two unoiled areas in PWS. The designation of sites as oiled or unoiled was based on visual evidence of shoreline oil and the presence of polynuclear aromatic hydrocarbons characteristic of *Exxon Valdez* oil in intertidal mussels (Brown et al. 1996). Mean hatch date was estimated by McGurk and Brown (1996) for each site:

(1) Fairmont Bay, unoiled site, sampled on May 12 and 21, 1989; estimated hatch date, May 3;

(2) Tatitlek Narrows unoiled site, sampled on May 11 and 21, 1989; estimated hatch date, May 4;

(3) Bass Harbor oiled site, sampled on May 12 and 21, 1989; estimated hatch date, May 8; and

(4) Rocky Bay oiled site, sampled on May 12 and 21, 1989; estimated hatch date, May 10.

Spawning occurred at all sites between April 8 and 15; oiled sites were about 1°C colder, 1‰ more saline, and the water column was more mixed than control stations (McGurk and Brown 1996).

Larvae were preserved in 5% seawater formalin (1 part 37% formaldehyde diluted into 19 parts undiluted seawater taken from the sea surface at the side of the boat), and 25 larvae from each site and each date (200 total) were randomly selected for gross morphology, cytogenetics, and histopathology. Craniofacial and finfold abnormalities were evaluated using methods described in Hose et al. (1996). Graduated severity index scores assigned to each variable reflected no defect (score = 0), mild defect in structure or size (score = 1), moderate defect in structure or size (score = 2), or severe or multiple defect(s) in structure or size (score = 3). A pectoral fin was removed and evaluated for cytogenetic abnormalities using Hose et al.'s (1996) method of enumerating the numbers of mitotic cells and apoptotic (pyknotic) cells per fin. Prevalences of cytologically abnormal fins (those with abnormal cells) and cytogenetically abnormal fins (high anaphase aberration rate or low mitotic rate) were determined.

McGurk and Brown (1996) estimated growth from body length measurements of Pacific herring larvae captured from at least five cruises at each site from May 3 through June 12, 1989. To evaluate whether larvae from the two cruises per site used in this study had significant differences in growth not detected in the broader study, body length was measured independently of McGurk and Brown (1996) using an Olympus Cue-2 Image Analyzer. Growth at each site was estimated by subtracting the mean length of the first sample from the mean length of the second sample and dividing by the number of days between samples. Methods for histopathology were described by Hose et al. (1996), except that all larvae for histopathology were embedded in glycol methacrylate (GMA) instead of paraffin. Each GMA-embedded larva was serially sectioned at 5 μm, and sections were saved at 20-μm intervals so that between 12 and 30 sections of each larva were examined.

For histopathologic examination, sections of each larva were scanned at 100× magnification for major organs: gonad, retina, brain, heart, branchial arch, skin, skeletal muscle, kidney, gastrointestinal tract, yolk sac, liver, and spleen. Gonads were undifferentiated and gender could not be determined. The extent of liver glycogen was ranked and scored as minimum (no obvious hepatocellular clear, pale areas; score = 1), moderate (extent of hepatocellular clear, pale areas less than nuclear area; score = 2), or abundant (volume of hepatocellular clear, pale areas greater than nuclear area; score = 3). Yolk and the surrounding yolk sac were classified as abundant (sectional area of brightly eosinophilic, homogeneous, proteinaceous yolk was greater than the sectional area of the surrounding yolk sac cells; score = 3), moderate (sectional area of yolk less than yolk sac; score = 2), minimal (only the weakly staining yolk sac remained; score = 1), or absent (no yolk or yolk sac; score = 0).

Lesions were scored as none (0), mild (1), moderate (2), or severe (3). Lesions included (1) myodegeneration and necrosis; (2) hepatic vacuolar change; (3) individual hepatocellular necrosis; (4) lens vacuolation; (5) retinal degeneration or necrosis; (6) brain degeneration or necrosis, (7) proteinaceous ascites; and (8) histiocytic peritonitis. We used "ascites" to describe accumulation of fluid around viscera within the body cavity; the lesion is morphologically the same as described by Spitsbergen et al. (1991) as "subcutaneous edema" (J.M. Spitsbergen, Oregon State University, Corvallis, OR 97331, personal communication). For quality control, each larva was examined for extent of autolysis and tissue artifact and scored using the same semiquantitative scale. The relative amount of food in the gastrointestinal tract was scored as none (0), minimal (1), moderate (2), or abundant (3).

### Laboratory oil exposure

Laboratory-exposed Pacific herring larvae were a subset of the larvae analyzed for hatching, morphologic abnormalities, and genotoxicity by Kocan et al. (1996). To summarize, Pacific herring eggs were continuously exposed to an oil–water dispersion (OWD) of *Exxon Valdez* crude oil. Initial concentrations of high molecular weight (C12–C28) diesel range hydrocarbons were 0, 0.1, 0.24, 0.48, 0.97,

**Table 1.** Multiple analysis of variance (MANOVA) on histopathology and cytogenetic scores in Pacific herring larvae sampled from Prince William Sound, Alaska, after the *Exxon Valdez* oil spill.

| Effect or relation | Significant principal component (most important lesion scores)[a] | Level of significance |
|---|---|---|
| | **Histopathology scores** | |
| Oil | Overall comparison (MANOVA) | *** |
| Oil | First (yolk, gastrointestinal food, tissue artifact, ascites)[a] | *** |
| Oil | Third (hepatocellular degeneration, vacuolar change, and glycogen; gastrointestinal food) | *** |
| Site (oil) | Overall comparison (MANOVA) | *** |
| Site (oil) | Third (hepatocellular degeneration, vacuolar change, and glycogen; gastrointestinal food) | ** |
| Site (oil) | Fourth (histiocytic peritonitis, myodegeneration and necrosis, tissue artifact) | *** |
| | **Cytogenetic scores and length** | |
| Oil | Overall comparison (MANOVA) | *** |
| Oil | First (cytogenetically abnormal, craniofacial malformations, number of mitotic figures per fin, fin fold malformations, body length) | *** |
| Oil | Second (number of apoptotic cells per fin, cytologically abnormal) | * |
| Site (oil) | Overall comparison (MANOVA) | ns |

Note: Analysis tested the influence of exposure status (oil) and the influence of site as a nested factor within exposure status (site (oil)). Because of missing values, 183 of 200 fish were used to analyze histopathology scores, and 158 of 200 fish were used to analyze cytogenetic scores. Significances are as follows: ns, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Overall comparisons (MANOVA) did not depend on individual principal components. Principal components not shown were not significant.

[a]The first principal component contributes most to variability, whereas the second and later principal components contribute progressively less to variability. Within a significant principal component, lesions are listed in descending order of importance.

and 2.41 mg/L (100% OWD = 9.67 mg/L; Kocan et al. 1996). Larvae were sampled immediately after hatch to allow direct comparisons with other postspill field studies in which sampling occurred within 24 h of hatching (Pearson et al. 1995; Hose et al. 1996). Because laboratory techniques for feeding larvae had not been developed at the time of the experiment, larvae were not held beyond 24 h posthatch; therefore, same-age comparisons of laboratory-exposed larvae with field trawls of planktonic larvae were not done. Every 24 h, all larvae in each container (i.e., those that had hatched in the previous 24 h) were anesthetized in tricaine methanesulfonate and transferred to 5% neutral buffered formalin. Later, fixed larvae were checked for abnormalities under a dissecting microscope, and a pectoral fin was removed for cytogenetic analysis (Kocan et al. 1996). Three groups of larvae were analyzed for histopathologic lesions:

(1) random selection, paraffin embedment (50 larvae): 10 control larvae and 10 larvae each from the 0.10, 0.24, 0.48, and 2.41 mg/L OWD exposures;

(2) nonrandom selection, paraffin embedment (22 larvae): five larvae each from the 0.10, 0.24, 0.48, and 2.41 mg/L OWD exposures and two larvae from the 0.97 mg/L OWD exposure; and

(3) nonrandom selection, GMA embedment (16 larvae): seven control larvae and two or three larvae at each of the 0.24, 0.48, 0.97, and 2.41 mg/L OWD exposures.

Nonrandom samples were taken to determine the histologic features of observations made with the dissecting microscope; results from nonrandom samples were not used for statistical analysis. Because fish reared in the highest OWD doses hatched earlier, age at sampling (days postfertilization) decreased with dose: controls (19–20 days old); 0.10 and 0.24 mg/L (19 days old); 0.48 mg/L (16–17 days old); and 2.41 mg/L (14–15 days old).

All 88 larvae were assigned a unique random number for further processing and blind study. For paraffin-embedded larvae, groups of three or four serial sections were saved at approximately 25-μm intervals through each larva. Larvae embedded in GMA were processed the same as larvae sampled from PWS. Nearly all paraffin sections had moderate artifact, but section quality of the GMA-embedded larvae was superior.

### Statistical analysis

Results from the field and laboratory experiments were analyzed separately using similar methods. Lesion scores were first summarized through use of a principal components analysis (Ehrenberg 1975). For a given set of scores, the first four principal components were used as a summary for most of the variability in the raw data. The principal component factor scores were then tested for systematic effects of oil exposure using a multivariate analysis of variance (MANOVA; Johnson and Wichern 1992). After the MANOVA, univariate ANOVA models were run on each of the individual principal components (Winer et al. 1991). In the field study, sample site was treated as a nested factor within oiled status to separate out differences between sites that might be unrelated to oil exposure. To compare histologic changes, Spearman's rank correlation coefficients were calculated on eigenvalues derived from principal components analysis. Cytogenetic results were analyzed separately from histopathology scores. In the laboratory experiment, Dunnett's comparison was used to test each exposure group against the control, and Spearman's correlation coefficients were calculated to compare OWD dose to histologic changes. Comparisons in both experiments were categorized as not significant ($P > 0.05$), significant ($P \leq 0.05$), or highly significant ($P \leq 0.01$). To demonstrate trends in the data, mean scores (±SE) were graphed for all lesions. Because the probability of type-I error increases with multiple analyses, ANOVA was not done on scores for individual lesions.

## Results

### Environmental oil exposure

Pacific herring larvae from oiled sites had cytogenetic and histopathologic lesions that were highly significant when compared with larvae from unoiled sites, and several variables contributed to the differences (Table 1). Site as a nested factor within exposure group was highly significant for histopathology but not cytogenetic scores.

Ascites was the most consistent lesion associated with oil exposure, affecting 16 of 100 larvae from oiled bays but only 1 of 97 larvae from unoiled sites. In fish with ascites, the peritoneal cavity was distended by pale eosinophilic fluid that sometimes also expanded the pericardial space (pericardial edema or hydropericardium; Fig. 1A). By comparison, normal larvae had minimal clear pericardial fluid (Fig. 1B). All fish with pericardial edema also had ascites. Fish with ascites usually had abundant yolk, and yolk scores were correlated with scores for ascites (Spearman's correlation, $r^2 = 0.30$, $P = 0.0001$).

In addition to ascites, larvae from oiled sites had several histologic and cytogenetic changes consistent with delayed

**Fig. 1.** Micrographs of Pacific herring larvae. Fish are oriented with the head to the left; hematoxylin and eosin stain. (A) Parasagittal section of the anterior half of a larva from Bass Harbor (oiled site) with severe ascites (a) around abundant yolk (y), and moderate pericardial edema surrounding the heart (h). Scale bar = 200 μm. (B) Parasagittal section of a normal larva from Bass Harbor (oiled site) in which the heart (h) is surrounded by minimal clear fluid (f). Scale bar = 150 μm. (C) Mild histiocytic peritonitis in a larva from Tatitlek Narrows (unoiled site). Scattered histiocytes (large arrow) partly surround the resorbing yolk sac epithelium (small arrows), which is elongate and contains multiple, large, basophilic, condensed nuclei. Scale bar = 80 μm. (D) Foregut of a larva exposed to oil in the laboratory contains large numbers of bacteria (arrows) surrounded by degenerating cells; note homogeneous proteinaceous ascites ventral to the foregut and surrounding the scale bar. Scale bar = 150 μm. (E) Liver with no hepatocellular cytoplasmic vacuoles. Scale bar = 30 μm. (F) Liver with mild hepatocellular cytoplasmic vacuoles; magnification same as (E).

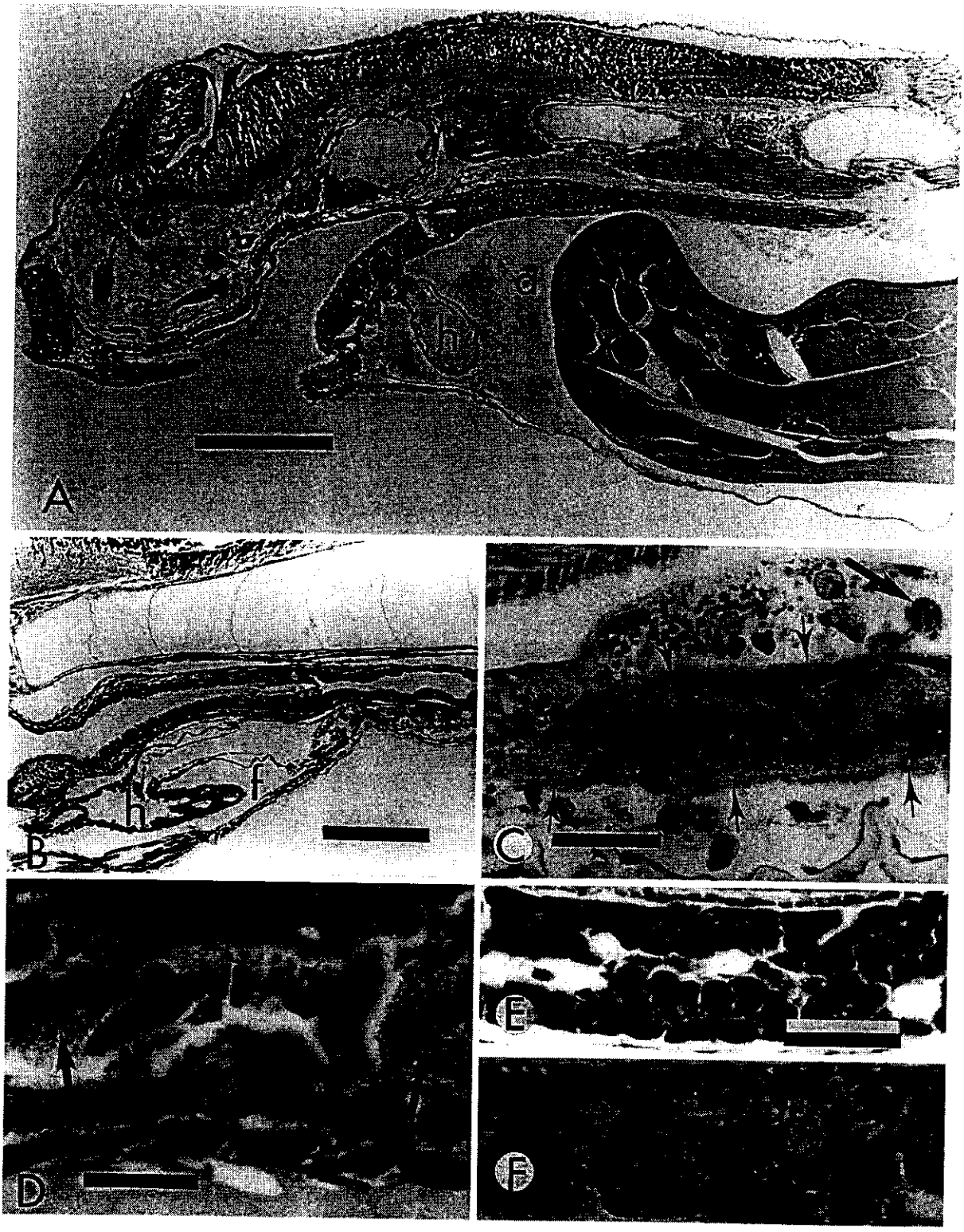
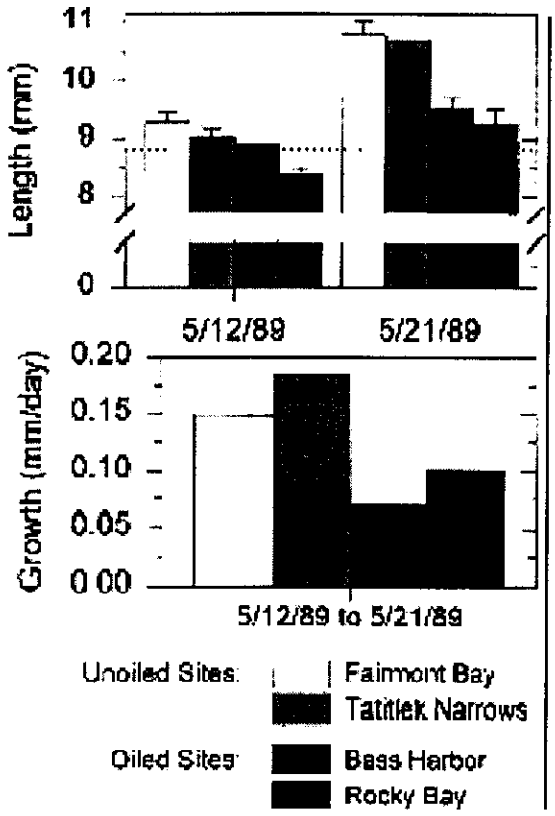


**Fig. 2.** Mean total length and estimated growth of Pacific herring larvae sampled from Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Each sample included 22–25 larvae. The reference line in the top graph is at 8.8 mm, the estimated length at hatch for these larvae (McGurk and Brown 1996); the reference line in bottom graph highlights growth differences between unoiled and oiled sites. Error bars are 1 SE.




development and growth (Figs. 2–4). Fish from oiled sites were shorter and grew slower than fish from unoiled sites (Fig. 2). Sectional area of the yolk sac was used as the best histologic marker of development. As expected, mean yolk scores decreased from the first to the last sample in larvae from all sites. However, yolk scores were consistently greater in fish from oiled sites (Fig. 3). Likewise, the amount of food in the gastrointestinal tract increased during the study, but the increase was less in larvae from oiled sites. Larvae from Rocky Bay never had food in their gastrointestinal tract (Fig. 3). Digestion of food in the gastrointestinal tract usually was minimal, and identifiable food consisted of eggs, copepod larvae, and an unidentified metazoan (probably a decapod) that had abundant striated muscle (Fig. 4). Three of the 61 larvae with moderate to abundant yolk also had food material in their gastrointestinal tract; two were from an oiled site and one was from an unoiled site. As larvae increased in size, hepatocellular glycogen and cytoplasmic vacuolation increased. Hepatocellular glycogen was minimal in all larvae from the first set of samples (Fig. 1E). In the last set of samples, most larvae had minimal glycogen, but four fish from unoiled sites had moderate amounts of glycogen. Cytoplasmic vacuoles in hepatocytes were multiple, fairly well demarcated, and 2–10 μm in diameter. Vacuole morphology was most consistent with lipid (Fig. 1F), but the presence of lipid could not be confirmed because lipids were lost during tissue processing. Although cytoplasmic vacuoles were significantly correlated with increasing length, they were not significantly correlated with increasing scores for gastrointestinal food.

Histiocytic peritonitis was the other development-associated histologic change that was delayed in fish from oiled sites. Histiocytic peritonitis occurred when yolk stores were depleted and the yolk sac epithelium was being resorbed through a process of apoptosis, or programmed cell death (score = 1, Fig. 3). The resorbing yolk sac epithelium was often surrounded by small numbers of foamy macrophages (histiocytes), producing a mild peritonitis (Fig. 1C). Histiocytic peritonitis was absent from larvae with abundant yolk and in larvae that had completed yolk sac absorption.

Myodegeneration and necrosis was primarily a site effect (Table 1). The lesion was more frequent and severe in fish from the last set of samples, and scores were greatest for fish from Tatitlek Narrows (Fig. 3). Degenerating myofibers had clear, irregular, cytoplasmic vacuoles, hypereosinophilic cytoplasm, and pyknotic nuclei (Fig. 5B).

Among morphologic, cytologic, and cytogenetic changes, oil-related differences were most significant for cytogenetic and morphologic changes (Table 1, Fig. 6). Finfold and craniofacial lesions occurred in all samples, but mean scores were consistently greater in fish from oiled sites (Fig. 6). Prevalence of cytologic lesions contributed significantly to oil-related differences (Table 1), but differences in prevalence of cytologic lesions were less consistent than morphologic changes. All samples had some fish with cytogenetic damage, anaphase aberrations were more prevalent in fish from oiled sites, regardless of developmental stage (Fig. 6). Among other subcellular changes, increased numbers of mitotic figures and apoptotic cells in fish from unoiled sites were consistent with increased growth rates in larvae from unoiled sites.

### Laboratory oil exposure

As in field samples, ascites was the most consistent and sensitive oil-related lesion in larvae exposed to oil during development in the laboratory. A high proportion of larvae were affected at initial doses as low as 0.24 mg/L (Fig. 7), although the lowest dose resulting in significant overall effects was 0.48 mg/L. Ascites prevalence was only 10% for the control and 0.1 mg/L treatments, 60% in each of the 0.24 and 0.48 mg/L treatments, and 100% in the 2.41 mg/L treatment. Ascites was more highly correlated with oil dose than any other variable (Table 2).

Several other variables contributed to highly significant oil-related differences, and increases in mean scores for significant lesions were dose dependent (Table 3, Fig. 7). The second variable most highly correlated with oil dose was hepatocellular vacuolar change (Table 2); no control fish had this lesion (Fig. 7). Hepatocytes in affected larvae had irregular, poorly demarcated, cytoplasmic vacuoles, and nuclei often were pyknotic. Vacuole morphology was consistent more with hydropic degeneration (fluid accumulation) than with the morphology of lipid vacuoles in hepatocytes of field-sampled fish. Most laboratory-exposed fish with vacuolar change also had moderate autolysis.

Autolysis was most common in larvae from the two highest doses (Fig. 7), and its correlation with oil dose was highly significant (Table 2). However, autolysis was not among the

**Fig. 3.** Significant histopathology scores in Pacific herring larvae sampled from Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Each sample included 22–25 larvae. Scales vary, but the reference line score is the same in every graph. Error bars are 1 SE.

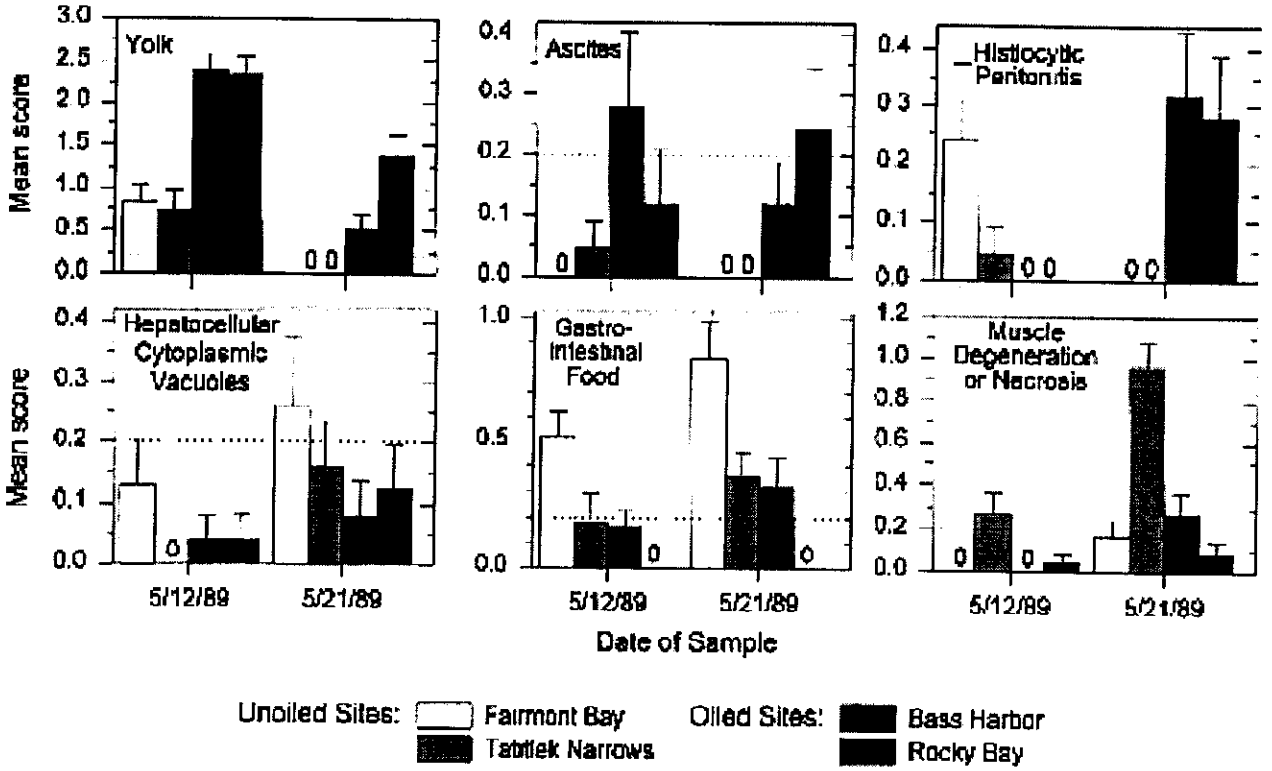



**Fig. 4.** Minimally digested food in the intestinal tract of Pacific herring larvae from Fairmont Bay (unoiled site), hematoxylin and eosin stain. (A) Four organisms (probably larvae of a mollusc). Scale bar = 150 μm. (B) Two eggs. Scale bar = 50 μm. (C) A probable copepod nauplius. Scale bar = 50 μm.

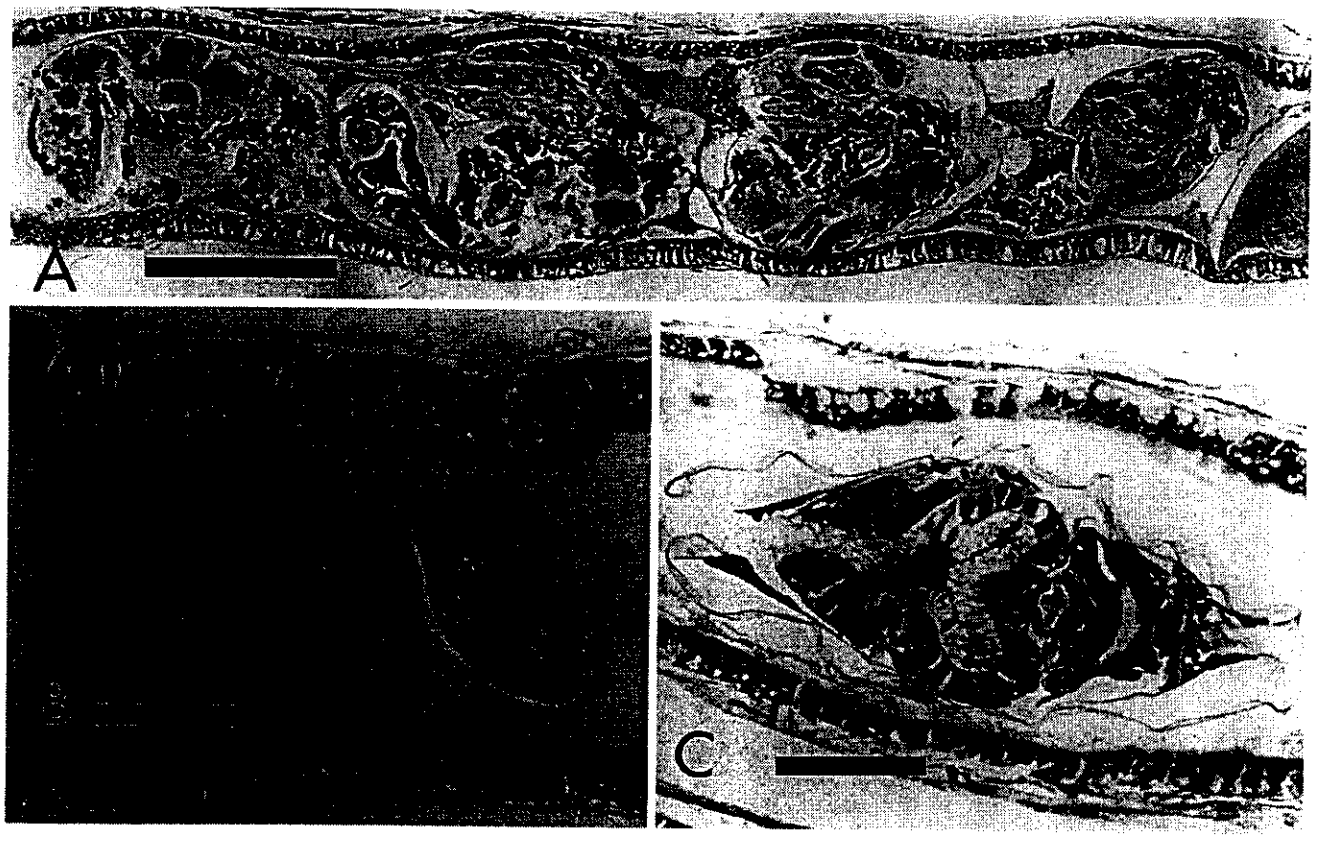


**Fig. 5.** Epaxial muscle of Pacific herring larvae, hematoxylin and eosin stain. Scale bar = 80 μm. (A) Normal skeletal muscle fibers were parallel and not vacuolated. (B) Degenerative and necrotic myofibers (arrows) were irregular, with cytoplasmic vacuoles and pyknotic nuclei (larva was exposed oil in the laboratory).

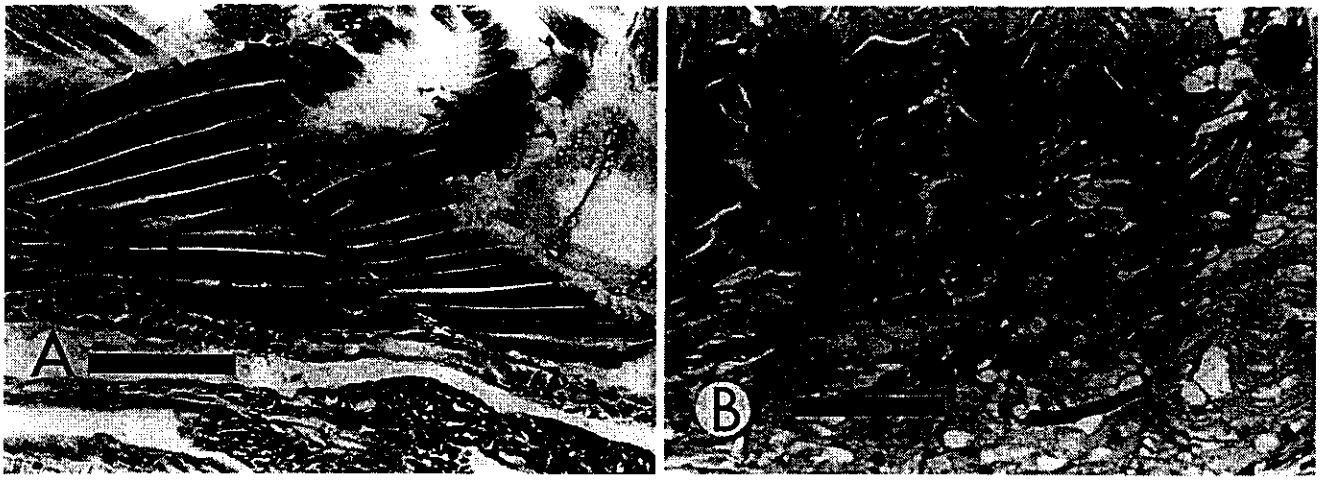
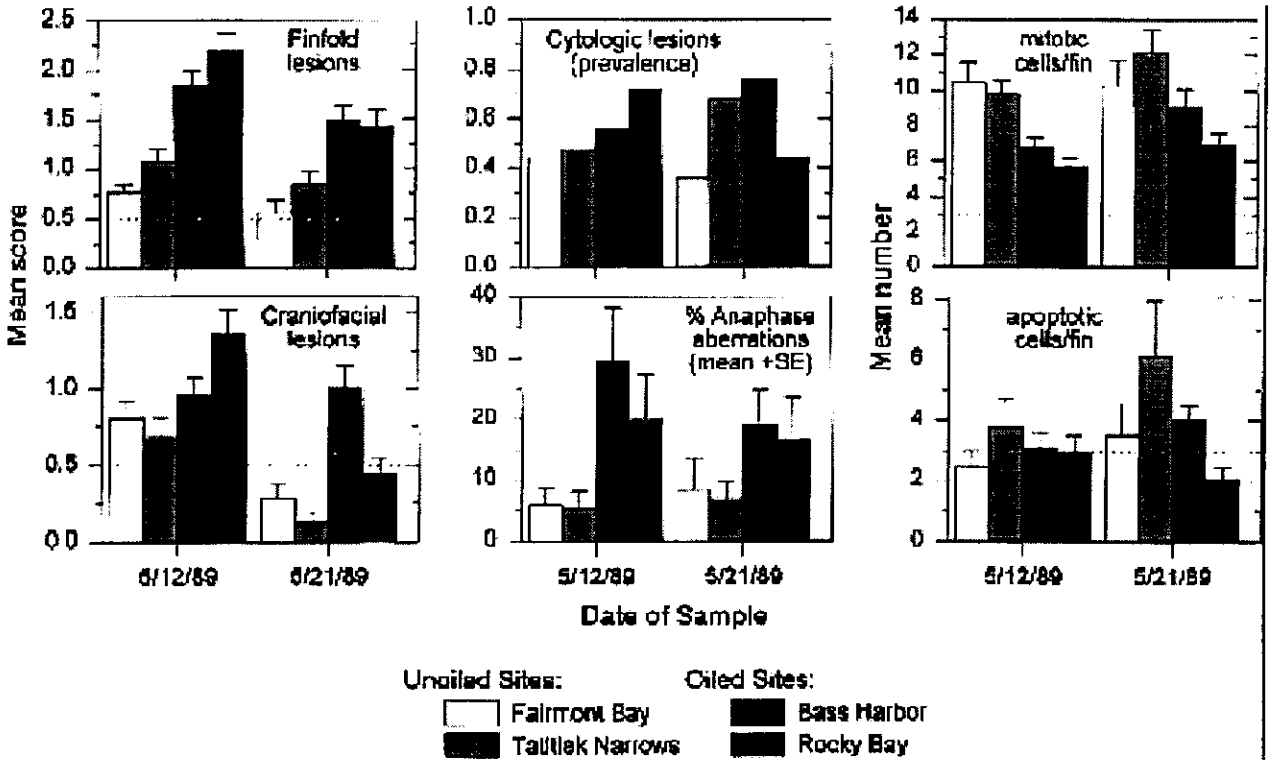



**Fig. 6.** Significant gross, cytologic, and cytogenetic results in Pacific herring larvae sampled from Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Each sample included 22–25 larvae. Scales vary, but the reference line value is the same within each column of graphs. Error bars are 1 SE.




three lesions that contributed most to variability within significant principal components (Table 3). Larvae that were partly autolyzed often had bacterial colonies in the digestive tract, and colonies were occasionally infiltrated by macrophages or surrounded by degenerating cells (Fig. 1D).

Other significant lesions included degeneration and necrosis of skeletal myocytes, retinal cells, and brain cells (Table 3). Myodegeneration and necrosis was similar to that described in field-sampled larvae (Fig. 5). Degeneration and necrosis within the retina and brain were uncommon. In affected foci, individual cells or small clusters of cells had pyknotic nuclei and condensed hypereosinophilic cytoplasm. Lens vacuolation was the same as described by Hose et al. (1996), and all six larvae with lens vacuolation were moderately or severely autolyzed. Also, among the 38 fish selected nonrandomly for examination, 11 had lens vacuolation, and all but one of these was moderately or severely autolyzed.

Oil-related changes associated with delayed development were not as pronounced in these larvae as they were in field-sampled larvae. Instead, lesions in exposed fish were

Fig. 7. Histopathology scores from Pacific herring larvae exposed to an oil–water dispersion during embryonic development. Ten larvae were examined from each dose. The reference line is included to clarify differences in bar length. Error bars are 1 SE.

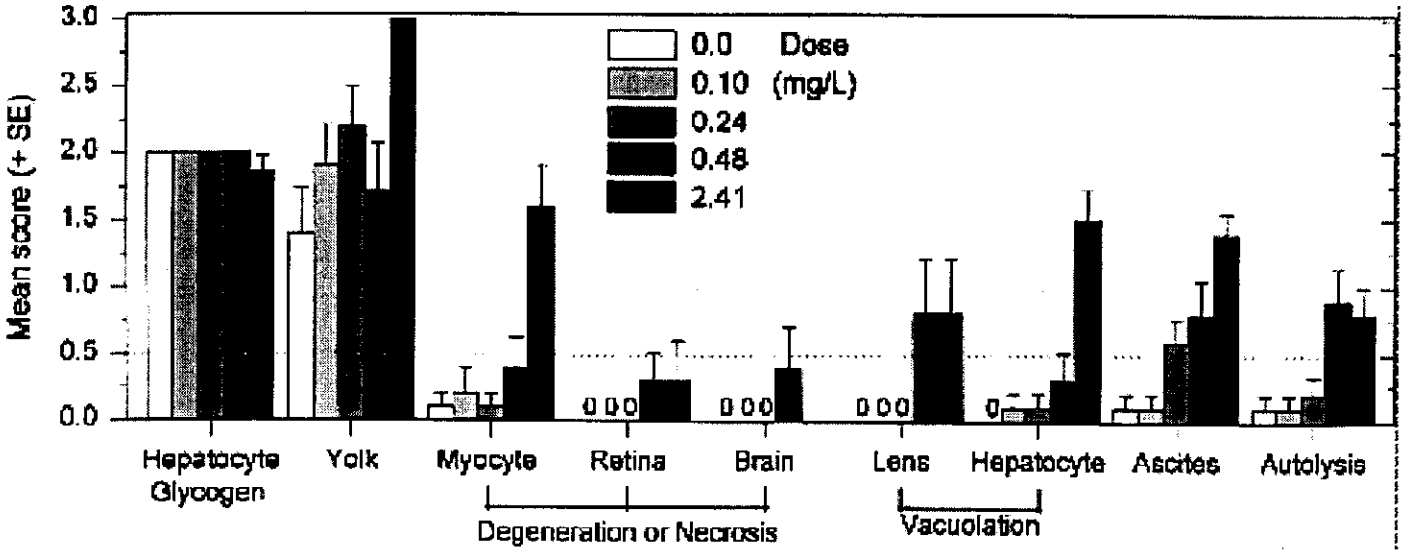



**Table 2.** Correlation between scored variables (lesions) and oil dose in newly hatched Pacific herring larvae exposed to an oil–water dispersion during embryonic development.

| Scored variable (lesion) | $r^2$ | Significance |
|---|---|---|
| Ascites | 0.68[a] | ***[b] |
| Hepatocellular cytoplasmic vacuoles | 0.58 | *** |
| Autolysis | 0.53 | *** |
| Myodegeneration or necrosis | 0.53 | *** |
| Yolk | 0.43 | *** |
| Lens vacuolation | 0.39 | *** |
| Retinal degeneration or necrosis | 0.24 | ns |
| Hepatocellular glycogen | –0.22 | ns |
| Brain degeneration and necrosis | 0.14 | ns |

[a]Spearman correlation coefficients on eigenvalues derived from principal components analysis ($n = 47$–$50$ for each determination).

[b]Significances are as follows: ns, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

**Table 3.** Multiple analysis of variance (MANOVA) on histopathology scores in Pacific herring larvae exposed to an oil–water dispersion of petroleum hydrocarbons during early development in the laboratory.

| Effect or relation | Significant principal component (and most important lesion scores)[a] | Level of significance |
|---|---|---|
| Oil (categorical) | Overall comparison (MANOVA) | *** |
| Oil (categorical) | First (hepatocellular vacuolar change, ascites, and myodegeneration or necrosis)[a] | *** |
| Oil (categorical) | Second (hepatocellular glycogen, retinal degeneration or necrosis, and myodegeneration or necrosis) | * |
| Oil (linear) | Overall comparison (MANOVA) | *** |
| Oil (linear) | First (hepatocellular vacuolar change, ascites, and myodegeneration or necrosis) | *** |
| Oil (linear) | Third (lens vacuolation, brain degeneration, or necrosis) | * |

Note: Analysis tested the influence of oil exposure as a categorical effect or as a linear effect. Because of missing values, 43 of 50 fish were used in the analysis. Significances are as follows: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Overall comparisons (MANOVA) did not depend on individual principal components. Principal components not shown were not significant.

[a]The first principal component contributes most to variability, whereas the second and later principal components contribute progressively less to variability. Within a significant principal component, lesions are listed in descending order of importance.

consistent with premature hatching. For example, yolk reserves tended to increase with dose, and differences were most prominent between the control and high-dose groups (Fig. 7). All exposure groups had moderate amounts of hepatocellular vacuolation attributed to glycogen stores, but one high-dose fish had minimal hepatocellular glycogen (Fig. 7). In general, the distribution of lesions in the 38 nonrandomly selected larvae was the same as in the randomly selected larvae, with dose-dependent increases in ascites, hepatocellular vacuolar change, and all degenerative and necrotic lesions compared in Fig. 7.

## Discussion

In both field and laboratory experiments, ascites was the most significant lesion related to oil exposure. Ascites was clearly related to oil exposure under controlled conditions in the laboratory, but because of variable environmental conditions, the link between ascites and oil exposure in field-sampled larvae was less clear. Were hydrocarbon concentrations in PWS high enough to explain the development of ascites? Several studies have reported toxic effects of the water soluble fraction of crude oil on Pacific herring embryonic development, and levels required to produce toxicity range from 0.1 to 1.0 mg/L (Smith and Cameron 1979; Cameron and Smith 1980; Kocan et al. 1996). In PWS after the oil spill, concentrations of polynuclear aromatic hydrocarbons (PAH) in surface water (depth up to 5 cm) from oiled sites averaged 0.9 μg/L in late April and early May 1989 (Neff and Stubblefield 1995); PAH

concentrations at a depth of 1 m were 0.5 to 1.0 μg/L in mid-April, but at those depths, PAHs were detectable only near heavily oiled beaches by early May (Short and Harris 1996). Neff and Stubblefield (1995) used water samples from PWS in 1989 to perform standard acute and chronic toxicity tests on three species of marine animals. Based on previously published values and lack of toxicity in their samples, they concluded that "it is likely that the safety factor (ratio of effects concentration to environmental concentration) for marine organisms in PWS following the spill was in excess of 100 and possibly as high as 10 000 during April and May 1989" (Neff and Stubblefield 1995).

However, recent laboratory studies provide new evidence that indigenous fish species are sensitive to PAH concentrations of 0.2–5 μg/L when exposure continues throughout embryonic and early larval development. In these studies, ascites was one of the most sensitive markers of PAH exposure. Pink salmon (*Oncorhynchus gorbuscha*) incubated in gravel substrate were exposed from fertilization through emergence to an initial PAH concentration of 31.8 μg/L that decreased to less than 3 μg/L after day 50 of incubation. Ascites developed in 2.6% of exposed fish (114 of 4380), but none of 3722 fish from control incubators developed ascites (Marty et al. 1997). In Pacific herring exposed to PAH from fertilization to hatching, 20% of larvae developed ascites after exposure to 0.3 μg/L PAH, and 0.7 μg/L PAH was sufficient to cause ascites in 50% of exposed fish (M.G. Carls, U.S. National Marine Fisheries Service, Auke Bay, Alaska; personal communication). Therefore, ascites prevalence of 16% in Pacific herring larvae from oiled sites in PWS is consistent with continuous laboratory exposure to less than 0.3 μg/L PAH: concentrations well within documented PAH levels in PWS in April and May 1989 (Neff and Stubblefield 1995; Short and Harris 1996). Increased sensitivity in these recent studies provides strong evidence that toxicity of multiring PAHs is greater than the toxicity of low molecular weight hydrocarbons that predominate in water-soluble fractions or oil–water dispersions.

Within each experiment, affected larvae had several lesions other than ascites. From oiled sites in PWS, several lesions were associated with delayed or prolonged development. By comparison, larvae exposed to oil in the laboratory had several degenerative or necrotic lesions, but developmental differences were less distinct and related to premature hatching. Skeletal myodegeneration or necrosis in laboratory-exposed fish was most common after exposure to the highest oil dose but, in field samples, was most common at a reference site; therefore, myodegeneration in larvae from PWS cannot be exclusively attributed to oil exposure.

Hydrocarbon exposure was the most likely cause of ascites and pericardial edema in our studies. Because these lesions are not specific for hydrocarbon exposure, other causes of ascites must be ruled out. Atlantic herring (*Clupea harengus*) larvae reared in hypotonic (7‰) or hypertonic (35‰) salt water developed pericardial edema and ascites (Dushkina 1973). However, all measured or extrapolated salinities at sample sites were greater than 25‰ (McGurk and Brown 1996, Fig. 2), and the chance that larvae were exposed to salinities <10‰ was minimal. Pericardial edema has been described in many developmental studies with several fish species and different toxicants (Waterman 1940; Weis and Weis 1974; Dial 1978; Gillespie and Baumann 1986; Middaugh et al. 1988; Marty et al. 1990), but ascites is less often described. Ascites was the main lesion associated with blue-sac disease in wild lake trout (*Salvelinus namaycush*) larvae, and ascites also occurred in lake trout larvae exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as fertilized eggs (Symula et al. 1990; Spitsbergen et al. 1991). Despite detailed study of water samples from PWS, no toxicants other than oil-related PAH were identified that could explain the high prevalence of ascites in fish from oiled sites in PWS. In addition to physical and chemical causes of ascites, biological causes must also be considered. Viral hemorrhagic septicemia virus has recently been isolated from adult Pacific herring in several locations in Alaska, British Columbia, and Washington (Meyers et al. 1994; Meyers and Winton 1995), and related viruses cause ascites in young salmonids (Wolf 1988). Viral contribution to the pathogenesis of ascites in larval Pacific herring in 1989 cannot entirely be ruled out, but larvae lacked hemorrhagic lesions that have been associated with viral hemorrhagic septicemia in older fish (Meyers et al. 1994), and a viral cause of ascites seems unlikely.

Ascites and pericardial edema probably also contributed to the pathogenesis of delayed development and degenerative or necrotic lesions. Edema (and ascites) can develop by several mechanisms (Cotran et al. 1989), but the pathogenesis in this study was not determined. Regardless of the mechanism of edema formation, increased fluid pressure around the heart and major vessels probably restricted atrial stroke volume, leading to decreased venous return and subsequently, decreased blood flow to tissues. If blood flow to tissues decreased, then developing organs would have received fewer nutrients, resulting in decreased growth and development.

Decreased growth in larvae from oiled sites is consistent with findings in three other laboratory studies with Pacific herring. First, embryonic exposure to the water-soluble fraction of Prudhoe Bay crude oil for 48 h resulted in pericardial edema, decreased growth, and jaw lesions (Smith and Cameron 1979). Second, larval exposure to the water-soluble fraction of crude oil in food resulted in decreased growth (Carls 1987). In the same study, oil-contaminated prey (6 mg/L) caused larval mortality but did not affect feeding or growth of survivors. Thirdly, embryonic exposure to 45 mg/L benzene (an aromatic component of crude oil) resulted in delayed development, irregular heartbeat, and fin and jaw lesions, and larval exposure to 12 mg/L decreased feeding and growth (Struhsaker et al. 1974). Note that Carls (1987) found that the water-soluble fraction he used contained 95% monoaromatic and only 5% diaromatic hydrocarbons, so direct comparisons of total aromatic hydrocarbons in these laboratory studies with PAHs in the water column of PWS in 1989 are not possible.

Increased yolk volume in larvae from the highest laboratory exposure group was consistent with premature hatch. These larvae hatched up to 3 days earlier than controls but weighed significantly less (Kocan et al. 1996). Weight differences provided evidence that energy was going to oxidative activity rather than growth. Induction of cytochrome P450 enzymes has been hypothesized as a cause of increased metabolism in oil-exposed fish larvae (Moles et al. 1987), but specifics of this hypothesis have not been critically evaluated. Alternatively, cardiovascular dysfunction in the high-dose group might have increased anaerobic metabolism resulting in less efficient use of yolk stores. Oil-associated differences in yolk volume were

more distinct in larvae from the field experiment, but the exact age of these fish was unknown and the possibility that younger fish were sampled from oiled sites cannot be eliminated. Water temperatures were consistently about 1°C lower at oiled sites, but this small difference probably was not enough to explain all differences in developmental rates between oiled and unoiled sites. Interestingly, feeding by fish larvae that still have yolk has been readily demonstrated in laboratory studies, but has been difficult to corroborate in field surveys (Heming and Buddington 1988). In our study, three Pacific herring larvae had gastrointestinal food and moderate to abundant yolk; these findings may have been enhanced by immediate fixation of whole larvae, embedment in GMA, and examination of parasagittal step sections.

In severe cases of pericardial edema and ascites, blood flow is sufficiently restricted that tissues become necrotic, eventually fish die, and tissues autolyze and are colonized by bacteria. In observations of living embryos and larvae (Waterman 1940; G.D. Marty and J.E. Hose, unpublished data), bacterial growth and necrosis can involve a large fraction of the body even when the rudimentary heart continues pulsatile movement; in these cases, blood flow is poor or has ceased. Hence, the exact time of death is often difficult to pinpoint in larval fish. In the laboratory study, colonies of bacteria probably grew after death in some larvae, but inflammatory cells admixed with the bacteria in these larvae were evidence that some bacterial growth occurred before death. Malformed Pacific herring embryos often die during hatching (R.M. Kocan, University of Washington, Seattle, WA 98195, personal communication). Larvae for the laboratory experiment were not closely examined under a microscope before being placed in fixative, and several larvae probably were dead before fixation. Some of these larvae might have been incorrectly been placed into the "% live hatch" category by Kocan et al. (1996); however, because most autolyzed larvae were also malformed, the "% normal" category as reported by Kocan et al. (1996) probably was minimally affected by postmortem changes. Problems differentiating necrosis from autolysis could be eliminated in future studies if all larvae slated for histopathology are examined for an active heart beat before they are preserved.

Lesions in Pacific herring larvae exposed to oil have also been described in lake trout larvae exposed to chlorinated dioxin during embryonic development (Spitsbergen et al. 1991). Lake trout larvae often died near the time of hatch, and survivors developed severe ascites and pericardial edema, resulting in cessation of blood circulation in the yolk sac and body (Spitsbergen et al. 1991). Necrosis of the retina, brain, and spinal cord occurred in morbid embryos and sac fry, and all larvae in the highest dose group had arrested development after hatching. In another study, surf smelt (*Hypomesus pretiosus*) embryos had necrotic neurons in the forebrain and neuronal layer of the retina after exposure to 54 or 113 µg/L water-soluble fraction of Cook Inlet crude oil for 15–21 days (Hawkes and Stehr 1982).

In the laboratory experiment, mean scores for ascites were greater in fish exposed to 0.24 mg/L than in controls, but overall increases in histopathologic lesions were not statistically significant until initial OWD concentrations reached 0.48 mg/L. Therefore, histopathology was less sensitive for detecting oil-related lesions than was cytogenetic analysis (0.10 mg/L) and equally as sensitive as examination for gross physical deformities (Kocan et al. 1996). In the field experiment, histopathologic, cytogenetic, and morphologic examination all yielded significant oil-related differences. Comparison of the two experiments is limited because of differences in age at sampling and probable differences in exposure, but prevalence and severity of lesions in larvae from oiled sites were roughly equivalent to lesions in larvae from the laboratory exposed to 0.24 mg/L. For example, both groups of fish had ascites and pericardial edema, but neither group had degenerative and necrotic lesions in the brain and retina. Eggs that were spawned in oiled sites in PWS in 1989, but later moved to clean water in the laboratory for incubation until hatching, had significant oil-related cytotoxicity but not ascites (Hose et al. 1996). The relatively high prevalence of autolysis in that experiment, and resultant rupture of the yolk sac, may have masked the identification of ascites, which requires an intact body wall. Alternatively, Pearson et al. (1995) did not observe oil-related differences in prevalence of pericardial edema, indicating that longer exposure might have been needed to cause ascites and other histologic lesions. Methods have recently been developed for long-term culture of Pacific herring larvae and juveniles in the laboratory (R.M. Kocan, personal communication), and additional laboratory studies are planned to determine the role of early oil exposure on long-term survival and growth.

In the field experiment, growth was an important component of significant oil-related effects in the subset of larvae examined histologically in this study, but oil-related effects on growth were not significant for estimates from a larger series of trawls in PWS (McGurk and Brown 1996). At least two explanations are possible. First, subsamples of larvae for histopathology were from a much smaller range (May 11–21) than were all larvae sampled by McGurk and Brown (May 3–30), and subtle differences in growth may have been significant only within the shorter range. Second, McGurk and Brown (1996) estimated growth based on the assumption that newly hatched larvae were 8.8 mm long. However, Kocan et al. (1996) found that oil-exposed eggs hatched earlier and had a lower dry weight than control fish; therefore, the assumption may be incorrect that Pacific herring throughout PWS were the same size at hatch. In juvenile pink salmon sampled from PWS after the *Exxon Valdez* oil spill, growth was less in oiled areas than in unoiled areas (Weidmer et al. 1996; Willette 1996), and oil-related decreases in growth were confirmed in the laboratory (Schwartz 1985; Carls et al. 1996). Juvenile Pacific herring were often caught with pink salmon in oiled sites in 1989 (Wertheimer and Celewycz 1996). It is, therefore, reasonable to conclude that estimated growth differences in Pacific herring sampled for histopathology were real. Also, lack of food material in any of 50 larvae examined from Rocky Bay is consistent with decreased growth in fish from this oiled site. In laboratory experiments, starved Atlantic herring larvae ceased to grow (Ehrlich et al. 1976); therefore, growth of Pacific herring from Rocky Bay ( 0.10 mm/day) is evidence that available food had been captured and moved through the digestive system before the larvae were sampled.

If a large proportion of a population is exposed to contaminants during early life stages, impacts on subsequent recruitment may be significant (Sindermann 1993). Estimates on how much of the 1989 Pacific herring year-class in PWS was affected by the oil spill vary from 4% (Pearson et al. 1995) to 50% (Brown et al. 1996). Pacific herring spawning events in

PWS in 1989 were among the largest recorded, but through 1996, recruitment of the 1989 year-class into the fishery was among the lowest of any year-class since reliable estimates of year-class size were made in the early 1970s (J. Wilcock, Alaska Department of Fish and Game, Cordova, personal communication). Although larval lesions and mortality might have contributed to poor recruitment, recruitment of the 1989 year-class was also poor in Sitka Sound (G.D. Marty and Alaska Department of Fish and Game, unpublished data), which was used as a reference site for the PWS Pacific herring population (Pearson et al. 1995; Hose et al. 1996). Therefore, oceanographic variables probably were more significant in limiting recruitment of the 1989 year-class in PWS than was the oil spill.

In summary, Pacific herring larvae sampled from oiled sites after the 1989 *Exxon Valdez* oil spill had significant morphologic, histologic, and cytogenetic lesions, and the relation of these lesions to oil exposure was supported by laboratory study. Furthermore, histopathology and cytogenetic analysis were useful for detecting subtle lesions in wild fish larvae, and use of these techniques is recommended for damage assessment of future toxic spills. McGurk and Brown 1996) estimated that egg–larva mortality at the oiled sites was twice that of the two unoiled areas, and the high prevalence of ascites in larvae from oiled areas is consistent with those estimates.

## Acknowledgments

We thank Neil Willits for statistical analysis and Wendy Widmann for histotechnology. The research described in this paper was supported by the *Exxon Valdez* Oil Spill Trustee Council through contracts with the Alaska Department of Fish and Game. However, the findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the Trustee Council.

## References

Brown, E.D., Baker, T.T., Hose, J.E., Kocan, R.M., Marty, G.D., McGurk, M.D., Norcross, B.L., and Short, J. 1996. Injury to the early life history stages of Pacific herring in Prince William Sound after the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. **18**: 448–462.

Cameron, J.A., and Smith, R.L. 1980. Ultrastructural effects of crude oil on early life stages of Pacific herring. Trans. Am. Fish. Soc. **109**: 224–228.

Carls, M.G. 1987. Effects of dietary and water-borne oil exposure on larval Pacific herring (*Clupea harengus pallasi*). Mar. Environ. Res. **22**: 253–270.

Carls, M.G., Holland, L., Larsen, M., Lum, J.L., Mortensen, D.G., Wang, S.Y., and Wertheimer, A.C. 1996. Growth, feeding, and survival of pink salmon fry exposed to food contaminated with crude oil. Am. Fish. Soc. Symp. **18**: 608–618.

Cotran, R.S., Kumar, V., and Robbins, S.L. 1989. Pathologic basis of disease. 4th ed. W.B. Saunders, Philadelphia, Pa.

Dial, N.A. 1978. Methylmercury: some effects on embryogenesis in the Japanese medaka, *Oryzias latipes*. Teratology, **17**: 83–92.

Dushkina, L.A. 1973. Influence of salinity on eggs, sperm and larvae of low-vertebral herring reproducing in the coastal waters of the Soviet Union. Mar. Biol. **19**: 210–223.

Ehrenberg, A.S.C. 1975. Data reduction: analysing and interpreting statistical data. Wiley, New York.

Ehrlich, K.F., Blaxter, J.H.S., and Pemberton, R. 1976. Morphological and histological changes during the growth and starvation of herring and plaice larvae. Mar. Biol. **35**: 105–118.

Gillespie, R.B., and Baumann, P.C. 1986. Effects of high tissue concentrations of selenium on reproduction by bluegills. Trans. Am. Fish. Soc. **115**: 208–213.

Hawkes, J.W., and Stehr, C.M. 1982. Cytopathology of the brain and retina of embryonic surf smelt (*Hypomesus pretiosus*) exposed to crude oil. Environ. Res. **27**: 164–178.

Heming, T.A., and Buddington, R.K. 1988. Yolk absorption in embryonic and larval fishes. *In* Fish physiology. Vol. 11A. *Edited by* W.S. Hoar and D.J. Randall. Academic Press, San Diego, Calif. pp. 407–446.

Hose, J.E., McGurk, M.D., Marty, G.D., Hinton, D.E., Brown, E.D., and Baker, T.T. 1996. Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphologic, cytogenetic, and histopathological assessments, 1989–1991. Can. J. Fish. Aquat. Sci. **53**: 2355–2365.

Johnson, R.A., and Wichern, D.W. 1992. Applied multivariate statistical analysis. 3rd ed. Prentice Hall, Englewood Cliffs, N.J.

Kocan, R.M., Hose, J.E., Brown, E.D., and Baker, T.T. 1996. Pacific herring (*Clupea pallasi*) embryo sensitivity to Prudhoe Bay petroleum hydrocarbons: laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound. Can. J. Fish. Aquat. Sci. **53**: 2366–2375.

Marty, G.D., Núñez, J.M., Laurén, D.J., and Hinton, D.E. 1990. Age-dependent changes in toxicity of *N*-nitroso compounds to Japanese medaka (*Oryzias latipes*) embryos. Aquat. Toxicol. **17**: 45–62.

Marty, G.D., Short, J.W., Dambach, D.M., Willits, N.H., Heintz, R.A., Rice, S.D., Stegeman, J.J., and Hinton, D.E. 1997. Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome-P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. Can. J. Zool. **75**: 989–1007.

McGurk, M.D., and Brown, E.D. 1996. Egg–larva mortality of Pacific herring in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. **53**: 2343–2354.

Meyers, T.R., and Winton, J.R. 1995. Viral hemorrhagic septicemia virus in North America. Annu. Rev. Fish Dis. **5**: 3–24.

Meyers, T.R., Short, S., Lipson, K., Batts, W.N., Winton, J.R., Wilcock, J., and Brown, E. 1994. Association of viral hemorrhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA. Dis. Aquat. Org. **19**: 27–37.

Middaugh, D.P., Hemmer, M.J., and Lores, E.M. 1988. Teratological effects of 2,4-dinitrophenol, "produced water" and naphthalene on embryos of the inland silverside *Menidia beryllina*. Dis. Aquat. Org. **4**: 53–65.

Moles, A., Babcock, M.M., and Rice, S.D. 1987. Effect of oil exposure on pink salmon, *Oncorhynchus gorbuscha*, alevins in a simulated intertidal environment. Mar. Environ. Res. **21**: 49–58.

Neff, J.M., and Stubblefield, W.A. 1995. Chemical and toxicological evaluation of water quality following the *Exxon Valdez* oil spill. *In* *Exxon Valdez* oil spill: fate and effects in Alaskan waters. *Edited by* P.G. Wells, J.N. Butler, and J.S. Hughes. ASTM STP 1219. American Society for Testing and Materials, Philadelphia, Pa. pp. 141–177.

Pearson, W.H., Woodreff, D.L., Kiesser, S.L., Fellingham, G.W., and Elston, R.A. 1985. Oil effects on spawning behavior and reproduction in Pacific herring (*Clupea harengus pallasi*). API Publ. No. 4412. American Petroleum Institute, Washington, D.C.

Pearson, W.H., Moksness, E., and Skalski, J.R. 1995. A field and laboratory assessment of oil-spill effects on survival and reproduction of Pacific herring following the *Exxon Valdez* oil spill. *In* *Exxon Valdez* oil spill: fate and effects in Alaskan waters. *Edited by* P.G. Wells, J.N. Butler, and J.S. Hughes. ASTM STP 1219. American Society for Testing and Materials, Philadelphia, Pa. pp. 626–661.

Schwartz, J.P. 1985. Effect of oil-contaminated prey on the feeding and growth rate of pink salmon fry (*Oncorhynchus gorbuscha*). *In* Marine pollution and physiology: recent advances. *Edited by* F.J. Vernberg, F.P. Thurberg, A. Calabrese, and W. Vernberg. University of South Carolina Press, Columbia. pp. 459–476.

Short, J.W., and Harris, P.M. 1996. Chemical sampling and analysis of petroleum hydrocarbons in near-surface seawater of Prince William Sound after the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. **18**: 17–28.

Sindermann, C.J. 1993. Interactions of pollutants and disease in marine fish and shellfish. *In* Pathobiology of marine and estuarine organisms. *Edited by* J.A. Couch and J.W. Fournie. CRC Press, Boca Raton, Fla. pp. 451–482.

Smith, R.L., and Cameron, J.A. 1979. Effect of water soluble fraction of Prudhoe Bay crude oil on embryonic development of Pacific herring. Trans. Am. Fish. Soc. **108**: 70–75.

Spitsbergen, J.M., Walker, M.K., Olson, J.R., and Peterson, R.E. 1991. Pathologic alterations in early life stages of lake trout, *Salvelinus namaycush*, exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as fertilized eggs. Aquat. Toxicol. **19**: 41–72.

Struhsaker, J.W., Eldridge, M.B., and Echeverria, T. 1974. Effects of benzene (a water-soluble component of crude oil) on eggs and larvae of Pacific herring and northern anchovy. *In* Pollution and physiology of marine organisms. *Edited by* F.J. Vernberg and W.B. Vernberg. Academic Press, New York. pp. 253–284.

Symula, J., Meade, J., Skea, J.C., Cummings, L., Colquhoun, J.R., Dean, H.J., and Miccoli, J. 1990. Blue-sac disease in Lake Ontario lake trout. J. Great Lakes Res. **16**: 41–52.

Waterman, A.J. 1940. Effects of colchicine on the development of the fish embryo, *Oryzias latipes*. Biol. Bull. (Woods Hole), **78**: 29–34.

Weidmer, M., Fink, M.J., Stegeman, J.J., Smolowitz, R., Marty, G.D., and Hinton, D.E. 1996. Cytochrome P450 induction and histopathology in pre-emergent pink salmon from oiled streams in Prince William Sound, Alaska. Am. Fish. Soc. Symp. **18**: 509–517.

Weis, J.S., and Weis, P. 1989. Effects of environmental pollutants on early fish development. Rev. Aquat. Sci. **1**: 45–73.

Weis, P., and Weis, J.S. 1974. Cardiac malformations and other effects due to insecticides in embryos of the killifish, *Fundulus heteroclitus*. Teratology, **10**: 263–268.

Wertheimer, A.C., and Celewycz, A.G. 1996. Abundance and growth of juvenile pink salmon in oiled and non-oiled locations of western Prince William Sound after the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. **18**: 518–532.

Willette, M. 1996. Impacts of the *Exxon Valdez* oil spill on the migration, growth, and survival of juvenile pink salmon in Prince William Sound. Am. Fish. Soc. Symp. **18**: 533–550.

Winer, B.J., Brown, D.R., and Michels, K.M. 1991. Statistical principles in experimental design. 3rd ed. McGraw-Hill, New York.

Wolf, K. 1988. Infectious hematopoietic necrosis. *In* Fish viruses and fish viral diseases. *Edited by* K. Wolf. Cornell University Press, Ithaca, N.Y. pp. 83–114.

# Histopathology of Adult Pacific Herring in Prince William Sound, Alaska, after the *Exxon Valdez* Oil Spill

by Gary D. Marty[1*], Mark S. Okihiro[1], Evelyn D. Brown[2], David Hanes[3], and David E. Hinton[1]

[1]Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, One Shields Ave., Davis, CA, 95616-8732, U.S.A.

[2]Institute of Marine Science, School of Fisheries and Ocean Science, University of Alaska, Fairbanks, AK 99775-7220, U.S.A.

[3]Dept. of Biology, Sonoma State University, Rohnert Park, CA 94928, U.S.A.

*Author to whom all correspondence should be addressed. e-mail: gdmarty@ucdavis.edu

**Abstract**: Pacific herring (*Clupea pallasi*) sampled from oiled sites in Prince William Sound, Alaska, USA, three weeks after the 1989 *Exxon Valdez* oil spill had multifocal hepatic necrosis and significantly increased tissue concentrations of polynuclear aromatic hydrocarbons (PAH). By comparison, Pacific herring from reference sites in 1989, and from all sites in 1990 and 1991 did not have hepatic necrosis or increased PAH concentrations. Adult Pacific herring were sampled for histopathology of liver, spleen, and kidney from oiled and reference sites in April (1989 and 1991) and October (1990 and 1991). Increased scores for macrophage aggregates contributed to significant differences in 1990, but these differences probably resulted from sampling older fish from the oiled site. Naphthalenes were the predominant PAH in all tissue samples. The development of hepatic necrosis and the predominance of naphthalenes in samples from 1989 is consistent with recent laboratory study in which crude oil exposure resulted in dose-dependent expression of viral hemorrhagic septicemia virus (VHSV). We conclude that Pacific herring were exposed to *Exxon Valdez* oil in 1989, and that development of hepatic necrosis in exposed fish probably was a result of VHSV expression.

## Introduction

The effects of crude oil exposure on organs of adult fish have been documented in several laboratory studies (Hawkes 1977; Payne et al. 1978; Malins 1982; Solangi and Overstreet 1982; Khan 1995), but histopathology of the effects of environmental exposure to oil spills has rarely been documented (Haensly et al. 1982; Khan 1990). When the *Exxon Valdez* oil spill occurred in Prince William Sound, Alaska, U.S.A., in late March 1989, Pacific herring (*Clupea pallasi*) were beginning to congregate in shallow bays for their annual mass spawning in April. Because of the potential for contamination of Pacific herring with *Exxon Valdez* oil, study was initiated to determine effects of the spill. In 1989, we found that fish from oiled sites had hepatic necrosis and evidence of hydrocarbon exposure, but fish from reference sites did not. Study was continued through 1991 to determine the persistence of exposure-related lesions. Some of the histopathology results from Pacific herring sampled in 1989 were reported previously (Moles et al. 1993), and in that report fish from oiled sites had increased hepatic necrosis, more severe hepatic coccidian parasites (*Goussia* [*Eimeria*] *clupearum*), and decreased numbers of larval herring worms (*Anisakis simplex*) in the peritoneal cavity. These changes were attributed to oil exposure, but hydrocarbon analyses from field-sampled fish were not available.

Since Moles et al. (1993) was published, important new information has become available which may explain their results. First, hydrocarbon analysis of Pacific herring tissues is now complete, and results are reported herein. Second, annual study of Pacific herring in Prince William Sound has documented the variability of various microscopic lesions, particularly pigmented macrophage aggregates (Kocan et al. 1996; Elston et al.

1997; Marty et al. 1998). And third, viral hemorrhagic septicemia virus (VHSV) was isolated from Pacific herring in Prince William Sound in 1993 (Meyers et al. 1994). VHSV has subsequently been associated with multifocal hepatic necrosis in three different studies (Kocan et al. 1997; Marty et al. 1998; M.G. Carls, U.S. National Marine Fisheries Service, Auke Bay, Alaska, unpublished data).

Adult Pacific herring were sampled from Prince William Sound after the *Exxon Valdez* oil spill, in 1989, 1990, and 1991. The objective of this report is to describe microscopic lesions in these fish, and to present mechanisms by which these lesions could be related to crude oil exposure, natural variability, and viral hemorrhagic septicemia. Also, the Pacific herring population of Prince William Sound declined over 80% from an estimated $9.8 \times 10^7$ kg in 1992 to $1.5 \times 10^7$ kg in 1994. Because fish sampled in the present study were from year classes that were still alive during this population collapse, these results provide important information on parasite and lesion prevalence before the epizootic. Even though epizootics are relatively common on a global scale (Sindermann 1990; Hyatt et al. 1997), detailed pre-epizootic information has rarely been available.

**Methods**

Adult Pacific herring were captured in oiled and reference areas in Prince William Sound using purse seines (Table 1), except for the October 1991 sample from Knowles head, which was trawled. Dates and locations varied for each year because of fish movements, weather conditions, and limited time for study design in 1989. Age (from scales), body weight, and standard length were determined for all but the 1989 and October 1991 samples. When most samples were collected, VHSV had never been isolated from Pacific

Table 1 near here

herring, and virology was not a standard method used in toxic spill damage assessment; therefore, virus isolation was not attempted on any fish sampled from 1989 through 1991. Fish sampled in 1996 were not a part of this study, but are reported here solely to demonstrate the relation of age to pigmented macrophage aggregates in the trunk kidney.

## Histopathology

Tissues were preserved in Bouin's (1989 samples) or 10% neutral buffered formalin (1990, 1991, and 1996 samples) and shipped to the University of California, Davis. Liver, trunk kidney, and spleen were examined from all samples except in 1989 (when gonads were examined, but spleens were not). Tissues from each fish were assigned a unique random histopathology number for blind study, processed routinely into paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Lesions were scored using a four-point scale as none (0), mild (1), moderate (2), or severe (3). For quality control, autolysis and artifact in each spleen were scored on the same four-point scale. Ranking of lesions was often based on the number of inflammatory cells or organisms (e.g., resting spores of *Ichthyophonus hoferi*) per 100× or 400× field on a binocular light microscope. Degenerative and necrotic lesions (e.g., hepatocellular necrosis) were scored based on the extent of organ involvement (Marty et al. 1998). After all organs were examined and lesions scored, codes were revealed, and results were subjected to statistical analysis.

Lesion scores from each year were analyzed separately using similar methods (Marty et al. 1997). Lesion scores were first summarized using principal components analysis. For a given set of scores, the first four principal components were used as a summary for most of the variability in the raw data. The principal component factor scores

were then tested for systematic effects of oil exposure using multivariate analysis of variance (MANOVA; Johnson and Wichern 1992). After the MANOVA, univariate ANOVA models were run on each of the individual principal components (Winer et al. 1991). For each analysis, gender and age (or length) effects were also determined. Because the probability of Type I error increases with multiple analyses, ANOVA was not done on scores for individual lesions. Differences in sample prevalence of major parasites were compared using a chi-square test with a 2×2 contingency table.

Hydrocarbon analysis

Concentrations of polynuclear aromatic hydrocarbons (PAH) were determined by gas chromatography followed by mass spectrometry for aromatics or flame ionization detection for aliphatics. Analysis was done by the Geochemical and Environmental Research Group at Texas A&M University, College Station, Texas, USA. Experimentally determined method detection limits depended on sample weights, and generally were 1 ppb in tissue. Concentrations below method detection limits were treated as 0. The accuracy of the hydrocarbon analyses was about 15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation was usually less than about 20%, depending on the PAH. Total PAH (TPAH) concentrations were calculated by summing concentrations of individual PAH. Tissue concentrations are reported on a wet-weight basis. Not all tissues were collected the same in 1989 and 1990; in 1989, "viscera" was restricted to liver and intestinal ceca, but in 1990 "viscera" included liver and the entire gastrointestinal tract.

Statistical analysis of hydrocarbon results was limited to tissues common to both

years (ovary and viscera). TPAH concentrations in ovaries and viscera were independently analyzed with two-way ANOVA (year, site, and year × site). For statistical analysis, differences in viscera collections between 1989 and 1990 were ignored. Comparisons of lesion scores and TPAH concentrations were categorized as not significant ($P > 0.05$), significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$).

## Results

### Histopathology

Table 2 near here

In 1989 (Table 2), four Pacific herring from oiled sites (20%) had moderate to severe hepatocellular necrosis, whereas none of the herring from unoiled sites had more than mild necrosis. All four affected fish had single cell necrosis of hepatocytes (or apoptosis) characterized by granular condensed cytoplasm and nuclear pyknosis (Marty et al. 1998). Three of these fish also had moderate or severe, multifocal, coagulative necrosis characterized by rounding up of individual hepatocytes, cytoplasmic hypereosinophilia, and nuclear karyorrhexis and karyolysis (Fig. 1). Necrotic foci were randomly distributed and sometimes coalesced into large areas of necrosis. In some areas, necrotic foci were bordered by moderate to large numbers of macrophages that were often in aggregates. Despite these differences, sample size was small and site differences in 1989 were not significantly different with respect to oiling history of the sample sites (MANOVA). Fish from all sites had several other changes, but none were clearly related to potential oiling of the sample sites: 1) glycogen depletion (all fish); 2) lipidosis; 3) pigmented macrophage aggregates; and 4) karyomegaly, bi- and tri-nucleated hepatocytes (Table 2).

Fig. 1 near here

Oil-related differences in lesion scores were significant in 1990 but not in 1991. None of the Pacific herring sampled in 1990 or 1991 had hepatic necrosis. Instead, the primary tissue change used to separate the sites was pigmented macrophage aggregates (Table 3). In 1990, mean scores for pigmented macrophage aggregates were greater in fish sampled from the previously oiled site. Note, however, that fish from the oiled site were older than fish from the reference site (mean age = 5.6 vs. 2.3 years), and mean scores for pigmented macrophage aggregates within each age were similar to mean scores for same-aged fish sampled in October 1996 (Table 4). Also, age differences were significant for 1990 and 1991 samples (MANOVA), and scores for pigmented macrophage aggregates contributed most to significant differences. Gender differences were never significant.

Table 3 near here

Table 4 near here

Prevalence of major parasites followed two general trends from 1989 to 1991 (Table 5). First, prevalence of *Goussia clupearum* (an hepatic myxosporean) and *Ichthyophonus hoferi* (a disseminated fungus) were stable in 1989 and 1990, but significantly decreased in 1991. And second, prevalence of the renal myxosporeans *Ortholinea orientalis* and *Sphaerospora* sp.? significantly increased from 1990 through 1991. Testis was examined only in 1989: 93% of the males had the testicular coccidian *Eimeria sardinae*.

Table 5 near here

Hydrocarbon analysis

Tissue TPAH concentrations were significantly greater in fish collected from oiled areas in 1989 than in tissues collected from reference areas in 1989 and from all areas in 1990. Naphthalenes represented 95-97% of TPAH above method detection limits in these tissues (Tables 6 and 7). In 1989, PAH concentrations tended to be greater in the viscera than in

Table 6&7 near here

the gonad.

**Discussion**

Based on recent studies, hepatic necrosis in Pacific herring sampled from oiled sites in 1989 was probably a result of viral hemorrhagic septicemia (VHS), and exposure to *Exxon Valdez* oil may have induced viral expression in subclinical carrier fish. The North American strain of VHSV was first isolated from Pacific herring in Prince William Sound in association with severe population decline in 1993, four years after the spill (Meyers et al. 1994). More detailed study in 1994 revealed that VHSV isolation was significantly related to hepatic necrosis (Marty et al. 1998), and this association was confirmed in 38 VHSV-positive fish sampled from Prince William Sound in 1997 (G.D. Marty, unpublished data). The gross lesions in Pacific herring sampled from oiled sites in 1989--petechiation of the vent and maxillary process (Moles et al. 1993)--are consistent with VHS in juvenile, specific pathogen free, Pacific herring exposed to VHSV in the laboratory (Kocan et al. 1997). Finally, exposure of adult Pacific herring of unknown VHSV status to crude oil in the laboratory resulted in expression of VHSV in a dose-dependent manner, and hepatic necrosis was one of the most severe lesions in exposed individuals (M.G. Carls, unpublished data).

Hydrocarbon analysis of Pacific herring tissues in 1989 and 1990 provided weak but significant evidence that fish had been exposed to *Exxon Valdez* oil. The signature of tissue hydrocarbons in Pacific herring from Prince William Sound did not match *Exxon Valdez* oil; however, fish rapidly metabolize petroleum hydrocarbons (Collier and Varanasi 1991; Thomas et al. 1997), and tissue hydrocarbon levels may not reflect actual exposure.

Naphthalenes are the most likely PAH to be accumulated by Pacific herring exposed to oil-contaminated seawater because among the PAH in *Exxon Valdez* oil, naphthalenes are the most abundant (Short et al. 1996) and they dissolve most readily (Short and Heintz 1997). As evidence, naphthalenes were the most abundant PAH measured in surface seawater along the trajectory of the spill in Prince William Sound during the period when fish were collected in 1989 (Short and Harris 1996). The finding of naphthalenes as the only PAH above method detection limits in tissues is, therefore, expected at the sufficiently low concentrations of less-weathered *Exxon Valdez* oil in seawater. This has been confirmed by evidence from laboratory exposures (M.G. Carls, personal communication). However, some of the naphthalenes in Pacific herring from oiled areas in 1989 may be due to spurious naphthalene from unknown sources, as indicated by the presence of naphthalenes in samples collected from reference areas in 1989 and from all areas in 1990. Alternatively, movements of adult herring in the weeks and months after the spill are not known, and fish collected from reference sites may have been exposed to oil during their migrations. Unexplained elevations in naphthalene concentrations have occurred elsewhere in samples collected for Natural Resource Damage Assessment (J.W. Short, U.S. National Marine Fisheries Service, Auke Bay, Alaska; personal communication). Corroborative evidence that Pacific herring were exposed to *Exxon Valdez* oil includes contaminated or possibly contaminated bile in two of two herring examined in 1989 (Haynes et al. 1995).

Although oil-related differences in histopathology scores were statistically significant in samples collected in October 1990, these difference may have resulted more from differences in age between the two samples than to exposure histories. Macrophage

aggregates were significantly greater in fish from the previously oiled site, and increased numbers of macrophage aggregates have been associated with exposure to oil (Haensly et al. 1982) and other toxicants (Wolke 1992). However, scores for macrophage aggregates in Pacific herring from Prince William Sound in 1994 were highly variable (Elston et al. 1997) and strongly related to increased age (Marty et al. 1998). The highly significant correlation of age and increased scores for macrophage aggregates was confirmed in Pacific herring from Sitka Sound, Alaska, in 1995 and 1996, and the trend continues in ongoing semiannual studies of Pacific herring in Prince William Sound through 1997 ($n \approx$ 250/site/year; G.D. Marty, unpublished data).

Prevalence of parasites changed over time, but the relation of these changes to potential oil exposure are not known. It is highly unlikely that the oil spill affected parasite prevalence or scores other than *Anisakis simplex* in 1989. As supporting evidence, neither prevalence nor severity of *Goussia clupearum* or *Ichthyophonus hoferi* were significantly altered by a 16-d oil exposure in the laboratory (M.G. Carls, unpublished data). The relatively small size of each sample limits our ability to extrapolate sample prevalence to population trends. However, prevalences of most major parasites in 1990 samples were within the range found in Pacific herring from Prince William Sound in more extensive annual surveys from 1994 through 1997, although the prevalence of *Ichthyophonus hoferi* in 1990 was significantly lower than in years after 1993 (Marty et al. 1998, G.D. Marty, unpublished data). In 1991, prevalences of *Goussia clupearum* and *Ichthyophonus hoferi* were unusually low compared with other years in both Prince William Sound and Sitka Sound. By comparison, 1991 prevalence of *Ortholinea orientalis* was unusually high, particularly in the October sample. Study continues to determine the prevalence of various

parasites in Prince William Sound Pacific herring, and future results from year classes unaffected by the oil spill may provide valuable information for interpreting the significance of trends observed from 1989 through 1991.

In summary, adult Pacific herring in Prince William Sound were likely exposed to *Exxon Valdez* oil in April 1989, but fish no longer had significant evidence of exposure in 1990. Because Pacific herring nearing spawning condition in early spring are in a state of physiological stress, the added stress of oil exposure in 1989 could reasonably have led to expression of VHSV in these fish. Stress-induced expression of VHSV in Prince William Sound Pacific herring probably occurred in April of 1989. VHSV has been isolated from Pacific herring throughout the Pacific Northwest (Meyers and Winton 1995), and preliminary evidence indicates that about 10% of Pacific herring carry VHSV in subclinical form (R.M. Kocan, University of Washington, Seattle, WA; personal communication). The potential for development of VHS in carrier-fish subjected to additional stress makes Pacific herring unusually sensitive to effects of crude oil if exposure occurs in the weeks before spawning. However, we have no evidence that a VHSV outbreak resulted in a significant decline in population biomass in 1989. The population biomass was at near record levels in 1989 and remained fairly stable until it collapsed in 1993 . Lesion prevalences in fish sampled in 1990 or 1991 were essentially within normal limits, and there was no direct evidence to link the population collapse in 1993 to the 1989 oil spill (Meyers et al. 1994; Elston et al. 1997; Marty et al. 1998). This study was limited by relatively small sample size, particularly in 1989, as well as the large gap between the April 1989 and October 1990 samples. Also, the potential role of VHSV in Pacific herring was unknown before 1993 and, therefore, was not initially considered as a significant variable.

Based on these findings, the immediate response to large toxicant spills in the future should include adequate sample size and consideration of the potential interaction of multiple stressors in exposed individuals.

**Acknowledgments**

We thank Neil Willits for statistical analysis and Wendy Widmann for histotechnology. Mark Carls and Jeff Short contributed to the hydrocarbon analysis and reviewed the manuscript. The research described in this paper was supported by the *Exxon Valdez* Oil Spill Trustee Council through contracts with the Alaska Department of Fish and Game. However, the findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the Trustee Council.

**References**

Brown, E.D., Baker, T.T., Hose, J.E., Kocan, R.M., Marty, G.D., McGurk, M.D., Norcross, B.L., and Short, J. 1996. Injury to the early life history stages of Pacific herring in Prince William Sound after the *Exxon Valdez* Oil Spill. Am. Fish. Soc. Symp. 18: 448-462.

Collier, T.K., and Varanasi, U. 1991. Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in English sole (Parophrys vetulus) exposed to environmental contaminants. Arch. Environ. Contam. Toxicol. 20: 462-473.

Elston, R.A., Drum, A.S., Pearson, W.H., and Parker, K. 1997. Health and condition of

Pacific herring *Clupea pallasi* from Prince William Sound, Alaska, 1994. Dis. Aquat. Org. 31: 109-126.

Haensly, W.E., Neff, J.M., Sharp, J.R., Morris, A.C., Bedgood, M.F., and Boem, P.D. 1982. Histopathology of *Pleuronectes platessa* L. from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the Amoco Cadiz crude oil spill. J. Fish. Dis. 5: 365-391.

Hawkes, J.W. 1977. The effects of petroleum hydrocarbon exposure on the structure of fish tissues. *In* Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, Proceedings. *Edited by* D.A. Wolfe. Pergamon Press, New York. pp. 115-128.

Haynes, E., Rutecki, T., Murphy, M., and Urban, D. 1995. Impacts of the *Exxon Valdez* oil spill on bottomfish and shellfish in Prince William Sound. Fish/Shellfish Study 18 Final Report. *Exxon Valdez* Oil Spill. State/Federal Natural Resource Damage Assessment Final Report. 82 pp.

Hyatt, A.D., Hine, P.M., Jones, J.B., Whittington, R.J., Kearns, C., Wise, T.G., Crane, M.S., and Williams, L.M. 1997. Epizootic mortality in the pilchard *Sardinops sagax neopilchardus* in Australia and New Zealand in 1995. II. Identification of a herpesvirus within the gill epithelium. Dis. Aquat. Org. 28: 17-29.

Johnson, R.A., and Wichern, D.W. 1992. Applied multivariate statistical analysis. 3rd ed. Prentice Hall, Englewood Cliffs, N.J.

Khan, R.A. 1990. Parasitism in marine fish after chronic exposure to petroleum hydrocarbons in the laboratory and to the Exxon Valdez oil spill. Bull. Environ. Contam. Toxicol. 44: 759-763.

Khan, R.A. 1995. Histopathology in winter flounder, *Pleuronectes americanus*, following

chronic exposure to crude oil. Bull. Environ. Contam. Toxicol. 54: 297-301.

Kocan, R., Bradley, M., Elder, N., Meyers, T., Batts, W., and Winton, J. 1997. The North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory reared Pacific herring. J. Aquat. Anim. Health 9: 279-290.

Kocan, R.M., Marty, G.D., Okihiro, M.S., Brown, E.D., and Baker, T.T. 1996. Reproductive success and histopathology of individual Prince William Sound herring 3 years after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. 53: 2388-2393.

Malins, D.C. 1982. Alterations in the cellular and subcellular structure of marine teleosts and invertebrates exposed to petroleum in the laboratory and field: A critical review. Can. J. Fish. Aquat. Sci. 39: 877-889.

Marty, G.D., Freiberg, E.F., Meyers, T.R., Wilcock, J., Farver, T.B., and Hinton, D.E. 1998. Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring *Clupea pallasi* spawning in Prince William Sound, Alaska, USA. Dis. Aquat. Org. 32: 15-40.

Marty, G.D., Short, J.W., Dambach, D.M., Willits, N.H., Heintz, R.A., Rice, S.D., Stegeman, J.J., and Hinton, D.E. 1997. Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome-P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. Can. J. Zool. 75: 989-1007.

Meyers, T.R., Short, S., Lipson, K., Batts, W.N., Winton, J.R., Wilcock, J., and Brown, E. 1994. Association of viral hemorrhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince

William Sound and Kodiak Island, Alaska, USA. Dis. Aquat. Org. 19: 27-37.

Meyers, T.R., and Winton, J.R. 1995. Viral hemorrhagic septicemia virus in North America. Ann. Rev. Fish Dis. 5: 3-24.

Moles, A.D., Rice, S.D., and Okihiro, M.S. 1993. Herring parasite and tissue alterations following the *Exxon Valdez* oil spill. 1993 International Oil Spill Conference (Prevention, Preparedness, Response). United States Coast Guard, American Petroleum Institute, and U.S. Environmental Protection Agency, Tampa, Florida. pp. 325-328.

Payne, J.F., Kiceniuk, J.W., Squires, W.R., and Fletcher, G.L. 1978. Pathological changes in a marine fish after a 6-month exposure to petroleum. J. Fish. Res. Bd. Can. 35: 665-667.

Short, J.W., and Harris, P.M. 1996. Petroleum hydrocarbons in caged mussels deployed in Prince William Sound after the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. 18: 29-39.

Short, J.W., and Heintz, R.A. 1997. Identification of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the Northwestern Gulf of Alaska based on a PAH weathering model. Environ. Sci. Technol. 31: 2375-2384.

Short, J.W., Jackson, T.J., Larsen, M.L., and Wade, T.L. 1996. Analytical methods used for the analyses of hydrocarbons in crude oil, tissues, sediments and seawater collected for the natural resources damage assessment of the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. 18: 140-148.

Sindermann, C.J. 1990. Principal Diseases of Marine Fish and Shellfish, Vol. I. 2nd ed. Academic Press, New York.

Solangi, M.A., and Overstreet, R.M. 1982. Histopathological changes in two estuarine

fishes, *Menidia beryllina* (Cope) and *Trinectes maculatus* (Bloch and Schneider), exposed to crude oil and its water soluble fractions. J. Fish Dis. 5: 13-35.

Thomas, R.E., Carls, M.G., Rice, S.D., and Shagrun, L. 1997. Mixed function oxidase induction in pre- and post-spawn herring (*Clupea pallasi*) by petroleum hydrocarbons. Comp. Biochem. Physiol. 116C: 141-147.

Winer, B.J., Brown, D.R., and Michels, K.M. 1991. Statistical principles in experimental design. 3rd ed. McGraw-Hill, New York.

Wolke, R.E. 1992. Piscine macrophage aggregates: a review. Ann. Rev. Fish Dis. 2: 91-108.

Table 1. Sampling schedule for histopathology and hydrocarbon analysis of adult Pacific herring from Prince William Sound, Alaska, after the March 1989 *Exxon Valdez* oil spill. Designation of sites as oiled or unoiled was based on visual evidence of shoreline oil and the presence of polynuclear aromatic hydrocarbons characteristic of *Exxon Valdez* oil in intertidal mussels (Brown et al. 1996).

| Date | Site | Exposure | Histopathology sample size | Hydrocarbon analysis sample size |
|---|---|---|---|---|
| April 13, 1989 | Fairmount Bay | reference | 10 | 3 |
| April 12, 1989 | Galena Bay | reference | 10 | 3 |
| April 11, 1989 | Naked Island | oiled | 10 | 3 |
| April 14, 1989 | Rocky Bay | oiled | 10 | 3 |
| April 7, 1990 | Galena Bay | reference | ND[a] | 3 |
| April 11, 1990 | Wells Bay | reference | ND | 3 |
| April 10, 1990 | Naked Island | oiled | ND | 3 |
| April 9, 1990 | Port Chalmers | oiled | ND | 3 |
| October 6, 1990 | Knowles Head | reference | 50 | ND |
| October 10, 1990 | Green Island | oiled | 50 | ND |
| April 6, 1991 | Galena Bay | reference | 20 | ND |
| April 7, 1991 | Granite Bay | reference | 20 | ND |
| April 6, 1991 | Montague Island | oiled | 20 | ND |
| October 12, 1991 | Green Island | oiled | 49 | ND |
| Oct. 15-17, 1996 | Green Island & Zaikof Bay | ND | 159 | ND |

[a]ND = no data

Note: Location of most sites is mapped by Brown et al. 1996; others include Galena Bay (latitude 60° 57'; longitude 146° 42'), Granite Bay (latitude 60° 24'; longitude 147° 57'), and Wells Bay (latitude 60° 57'; longitude 147° 30'). In 1990, tissues for histopathology were from females only; histopathology samples in 1989, 1991, and 1996 were obtained at random with respect to gender.

Table. 2. Mean lesion scores in Pacific herring adults captured in April 1989, from oiled and reference sites in Prince William Sound, Alaska. Lesions were scored as none (0), mild (1), moderate (2), or severe (3).

| | Fairmount Bay (reference) | Galena Bay (reference) | Naked Island (oiled) | Rocky Bay (oiled) |
|---|---|---|---|---|
| Liver lesions | | | | |
| Hepatocellular glycogen depletion | 3.0 | 3.0 | 3.0 | 3.0 |
| Hepatic lipidosis | 0.9 | 1.0 | 0.4 | 0.6 |
| Coccidiosis (*Goussia clupearum*) | 0.5 | 0.9 | 0.9 | 0.9 |
| *Ichthyophonus hoferi* | 0.0 | 0.2 | 0.1 | 0.1 |
| Pigmented macrophage aggregates | 1.9 | 1.5 | 2.2 | 1.8 |
| Focal necrosis | 0.0 | 0.0 | 0.5 | 0.3 |
| Single cell necrosis | 0.3 | 0.1 | 0.5 | 0.5 |
| Hepatocellular megalocytosis | 0.8 | 1.1 | 0.7 | 1.0 |

| | Fairmount Bay (reference) | Galena Bay (reference) | Naked Island (oiled) | Rocky Bay (oiled) |
|---|---|---|---|---|
| Spleen lesions | | | | |
| Autolysis | 2.0 | 2.0 | 2.3 | 2.4 |
| Artifact | 1.0 | 1.0 | 1.0 | 1.0 |
| Congestion | 1.2 | 0.8 | 0.8 | 0.9 |
| Pigmented macrophage aggregates | 2.5 | 2.6 | 2.3 | 2.5 |
| Granulomatous inflammation | 0.0 | 0.0 | 0.0 | 0.0 |
| *Ichthyophonus hoferi* | 0.0 | 0.4 | 0.0 | 0.0 |
| Ellipsoid hypertrophy | 0.7 | 1.8 | 2.5 | 1.8 |
| focal arteriolar intimal hypertrophy | 0.2 | 0.4 | 0.8 | 0.1 |

Table 3. Mean age, weight, length, and lesion scores in Pacific herring adults captured in 1990 and 1991 from oiled and reference sites in Prince William Sound, Alaska. Lesions were scored as none (0), mild (1), moderate (2), or severe (3).

| | October 1990 | | April 1991 | | | October 1991 |
|---|---|---|---|---|---|---|
| | Green Island (oiled) | Knowles Head (reference) | Montague Island (oiled) | Galena Bay (reference) | Granite Bay (reference) | Green Island (oiled) |
| Measurement | | | | | | |
| Age (years) | 5.6 | 2.3 | 7.2 | 7.4 | 7.4 | ND[a] |
| Standard length (mm) | 202.1 | 171.5 | 221.9 | 222.5 | 221.6 | ND |
| Body weight (g) | 133.1 | 68.9 | 146.9 | 157.4 | 143.3 | ND |
| Liver lesions (mean score) | | | | | | |
| Hepatocellular glycogen depletion | 2.0 | 1.9 | 3.0 | 3.0 | 3.0 | 2.3 |
| Hepatic lipidosis | 0.6 | 0.6 | 1.1 | 0.9 | 1.2 | 0.5 |
| Coccidiosis (*Goussia clupearum*) | 0.8 | 1.0 | 0.7 | 1.0 | 0.8 | 0.5 |
| *Ichthyophonus hoferi* | 0.1 | 0.3 | 0.0 | 0.0 | 0.3 | 0.0 |
| Pigmented macrophage aggregates | 1.3 | 1.0 | 2.0 | 2.1 | 1.8 | 0.9 |

| | October 1990 | | April 1991 | | | October 1991 |
|---|---|---|---|---|---|---|
| | Green Island (oiled) | Knowles Head (reference) | Montague Island (oiled) | Galena Bay (reference) | Granite Bay (reference) | Green Island (oiled) |
| Focal necrosis | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Single cell necrosis | 1.1 | 0.4 | 0.1 | 0.0 | 0.0 | 0.7 |
| Hepatocellular megalocytosis | 1.3 | 1.3 | 1.0 | 1.1 | 0.8 | 0.7 |
| Kidney lesions (mean score) | | | | | | |
| Pigmented macrophage aggregates | 1.5 | 1.0 | 2.1 | 2.2 | 1.8 | 1.0 |
| Granulomatous inflammation | 0.04 | 0.1 | 0.2 | 0.3 | 0.1 | 0.04 |
| Interstitial hematopoietic cells | 1.1 | 1.0 | 1.1 | 1.2 | 1.2 | 1.0 |
| Congestion | 0.9 | 0.7 | 0.3 | 0.2 | 0.2 | 1.2 |
| *Ichthyophonus hoferi* | 0.2 | 0.2 | 0.0 | 0.0 | 0.3 | 0.0 |
| Mineralization | 0.02 | 0.02 | 0.0 | 0.0 | 0.1 | 0.1 |
| Tubular epithelial vacuolation | 0.1 | 0.1 | 0.3 | 0.2 | 0.1 | 0.2 |
| Intraductal myxosporean | 0.0 | 0.0 | 0.2 | 0.1 | 0.1 | 0.0 |

| | October 1990 | | April 1991 | | | October 1991 |
|---|---|---|---|---|---|---|
| | Green Island (oiled) | Knowles Head (reference) | Montague Island (oiled) | Galena Bay (reference) | Granite Bay (reference) | Green Island (oiled) |
| *Ortholinea orientalis* | 0.2 | 0.2 | 0.5 | 0.4 | 0.2 | 0.5 |
| Spleen lesions (mean score) | | | | | | |
| Autolysis | 0.2 | 0.1 | 0.1 | 0.0 | 0.0 | 0.2 |
| Artifact | 1.0 | 1.0 | 1.0 | 1.1 | 1.0 | 1.0 |
| Congestion | 1.3 | 1.0 | 0.7 | 0.5 | 0.3 | 1.0 |
| Pigmented macrophage aggregates | 1.6 | 1.1 | 2.3 | 2.7 | 2.7 | 1.0 |
| Granulomatous inflammation | 0.1 | 0.0 | 0.0 | 0.1 | 0.2 | 0.04 |
| *Ichthyophonus hoferi* | 0.2 | 0.2 | 0.0 | 0.0 | 0.1 | 0.02 |
| Ellipsoid hypertrophy | 1.0 | 0.7 | 1.5 | 1.4 | 1.3 | 1.0 |
| focal arteriolar intimal hypertrophy | 0.1 | 0.1 | 0.5 | 0.6 | 0.6 | 0.3 |

[a]ND = no data

Table 4. Semiquantitative scores for pigmented macrophage aggregates in the trunk kidney of Pacific herring randomly sampled from Prince William Sound, Alaska, after the March 1989, *Exxon Valdez* oil spill. Pigmented macrophage aggregates were scored as none (0), mild (1), moderate (2), or abundant (3). The 1988 year class was 2 years old in 1990 and 8 years old in 1996.

| | October 1990 | | | | | | October 1996 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Green Island | | | Knowles Head | | | Green Island and Zaikof Bay | | |
| Age | n | mean | SE | n | mean | SE | n | mean | SE |
| 2 | 0 | NC[a] | NC | 42 | 0.95 | 0.08 | 66 | 1.15 | 0.06 |
| 3 | 2 | 1 | 0 | 1 | 2 | NC | 31 | 1.19 | 0.10 |
| 4 | 2 | 1.5 | 0.5 | 3 | 1 | 0 | 29 | 1.24 | 0.08 |
| 5 | 13 | 1.31 | 0.13 | 2 | 1.5 | 0.5 | 5 | 1.4 | 0.24 |
| 6 | 19 | 1.63 | 0.11 | 0 | NC | NC | 6 | 1.67 | 0.21 |
| 7 | 4 | 1.75 | 0.25 | 0 | NC | NC | 9 | 2 | 0.24 |
| 8 | 0 | NC | NC | 0 | NC | NC | 10 | 2.1 | 0.18 |
| 10 | 0 | NC | NC | 0 | NC | NC | 1 | 3 | NC |
| 12 | 0 | NC | NC | 0 | NC | NC | 2 | 3 | 0 |
| All | 40 | | | 48 | | | 159 | | |

[a]NC = not able to calculate

Table 5. Combined sample prevalence (%) of major parasites in Pacific herring sampled from Prince William Sound, Alaska.

| Sample date | *Goussia clupearum* | *Ichthyophonus hoferi* | renal intraductal myxosporean (*Sphaerospora* sp.?) | *Ortholinea orientalis* |
|---|---|---|---|---|
| April 1989 | 63 | 13 | NE[a] | NE |
| October 1990 | 60 | 15 | 0.0 | 12 |
| April 1991 | 54 | 3.4 | 2.4 | 20 |
| October 1991 | 40 | 2.1 | 8.2 | 31 |

[a]NE = kidney was not examined

Table 6. *Hydrocarbon concentrations (ng hydrocarbon/g tissue) in Pacific herring sampled from oiled and reference sites in April, 1989. For all determinations, n=6 except for gonads from oiled sites, where n = 3.*

| | | Viscera | | Gonad | |
|---|---|---|---|---|---|
| Class | | reference | oiled | reference | oiled |
| total aromatics | mean | 92.9 | 155.3 | 17.0 | 41.9 |
| | SE | 16.3 | 42.6 | 2.2 | 16.9 |
| naphthalenes | mean | 89.1 | 143.9 | 17.0 | 38.0 |
| | SE | 16.9 | 37.9 | 2.2 | 18.5 |
| fluorenes | mean | 0.0 | 1.5 | 0.0 | 0.0 |
| | SE | 0.0 | 1.5 | 0.0 | 0.0 |
| dibenzothiophenes | mean | 0.0 | 0.0 | 0.0 | 0.0 |
| | SE | 0.0 | 0.0 | 0.0 | 0.0 |
| phenanthrenes | mean | 0.0 | 3.6 | 0.0 | 0.0 |
| | SE | 0.0 | 2.3 | 0.0 | 0.0 |
| chrysenes | mean | 0.0 | 0.0 | 0.0 | 0.0 |
| | SE | 0.0 | 0.0 | 0.0 | 0.0 |

Table 7. Hydrocarbon concentrations (ng hydrocarbon/g tissue) in Pacific herring sampled from oiled and reference sites in April, 1990. N=6 for all oiled sites, and n = 4 for all reference sites. Concentrations of fluorenes, dibenzothiophenes, phenanthrenes, and chrysenes were less than method detection limits.

| | | Viscera | | Ovary | | Testes | | Stomach contents | | muscle | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Class | | reference | oiled | reference | oiled | reference | oiled | reference | oiled | reference | oiled |
| aromatics | mean | 22.6 | 6.1 | 11.0 | 0.7 | 0.0 | 0.0 | 19.0 | 4.0 | 6.9 | 5.5 |
| | SE | 4.3 | 4.1 | 6.1 | 0.7 | 0.0 | 0.0 | 11.4 | 6.8 | 4.0 | 2.6 |
| naphthalenes | mean | 20.9 | 6.1 | 11.0 | 0.7 | 0.0 | 0.0 | 19.0 | 4.0 | 6.9 | 5.5 |
| | SE | 3.9 | 4.1 | 6.1 | 0.7 | 0.0 | 0.0 | 11.4 | 6.8 | 4.0 | 2.6 |

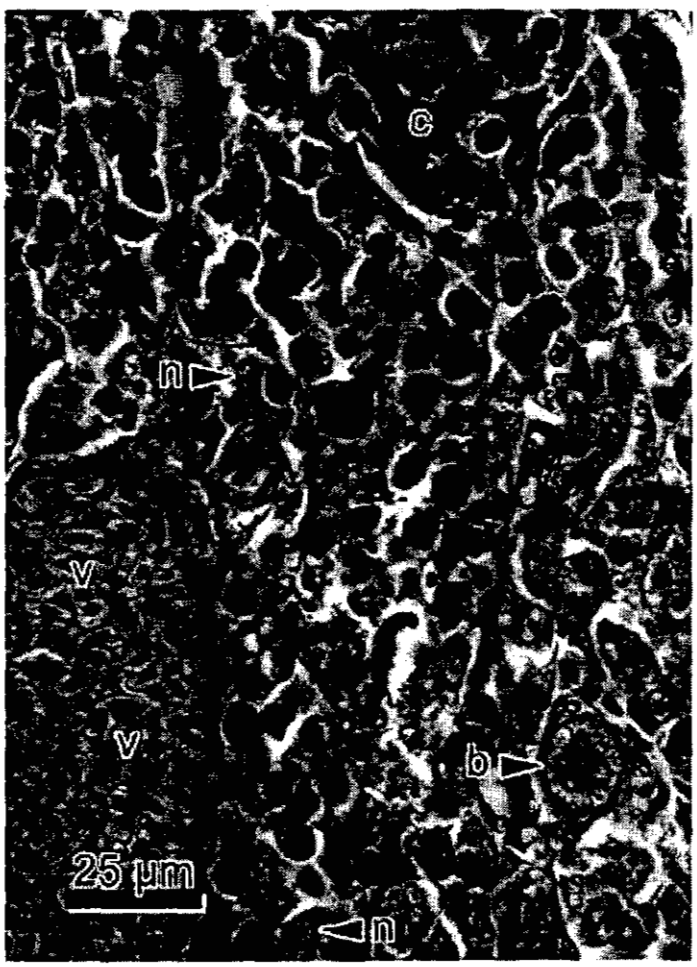



Figure 1. Liver of a female Pacific herring captured near an oiled site (Naked Island) on April 11, 1989. Viable hepatocytes have normal nuclei (n), whereas necrotic hepatocytes have coagulated cytoplasm (c) which often is contracted into an area about the same size as nuclei. A blood vessel (v) and bile duct (b) are normal.

Chapter 9.

# Effect of the Exxon Valdez oil spill on in situ survival of Pacific herring (Clupea pallasi) eggs

Evelyn D. Brown[1]
Institute of Marine Science, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Fairbanks, AK 99775-7220, USA

Edward M. Debevec
Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK 99775-6100, USA

[1]Author to whom reprint requests should be addressed
April 1996

Brown, E.D. and E. M. Debevec. 1996. Effect of the Exxon Valdez oil spill on in situ survival of Pacific herring (Clupea pallasi) eggs. Can. J. Fish. Aquat. Sci. 00: 0000-0000.

The Exxon Valdez oil spill of March 24, 1989, coincided with spawning of Pacific herring in Prince William Sound, Alaska. In situ survival of herring eggs just prior to hatch in 1989-1991 was assessed at oiled and non-oiled areas using a statistical (ANCOVA) model. Significantly lower egg survivals were measured in oiled areas than in unoiled areas in 1989. The hydrocarbon exposure data explained the decrease in survival at the +0.30 m and -9.14 m depths after accounting for the effect of egg density. A marginal oil effect at +1.52 m depth was observed in 1989 and 1991, but was confounded with wave exposure, probably due to desiccation and wave impacts. In 1989, surface oil was driven by wind and waves and in 1991, more storms resulting in higher winds occurred explaining the confounding effects of waves and oil during those two years. Direct exposure of eggs to oil was the most likely route of oiling. Although oil affected egg survival in 1989, the rates observed fall within the natural range of egg survivals reported in the literature

## Introduction

On March 24, 1989, the oil tanker Exxon Valdez struck Bligh Reef and spilled 42 million L of Prudhoe Bay crude oil into Prince William Sound, Alaska. Approximately 10% of the oil floated out of the sound, 40 to 45% was beached in the sound, and the remaining 45 to 50% evaporated or was broken down by biophysical processes (Wolfe et al. 1996). Approximately 634 linear km of shoreline was contaminated by oil (Gundlach et al. 1990). This was the largest spill to date in US coastal waters, and it coincided with the spawning period of Pacific herring in the sound. The length of oiled shoreline overlapped about half of the length of shoreline on which herring spawning was recorded (Brown and Short, in press). This coincidence suggested that the spill may have injured the local herring stock.

Shortly after Pacific herring eggs are deposited in the intertidal and subtidal zone (Haegele et al. 1981; Haegele and Schweigert 1985), many are removed by predation (Palsson 1984; Haegele and Schweigert 1989, 1991; Bishop, M., United States Forest Service, P.O. Box 1460, Cordova, Alaska, 99574-1460, unpublished data) and wave scouring (Hay and Miller 1982; Palsson 1984; Rooper 1996). Of the eggs not physically removed, many perish due to dessication and fungal infections (Steinfeld 1971; Palsson 1984; Purcell et al. 1990). It is the survival of the remaining in situ embryos that determines the potential number of larvae hatching from a particular spawning bed.

Previous research on the effects of the Exxon Valdez oil on the early life history of Pacific herring has concentrated on the larval life stage (McGurk and Brown, in press; Hose et al., in press; Kocan et al., in press; Norcross et al., in press). This article extends the study of oil spill impacts to the embryonic stage reporting in situ egg survivals measured directly from SCUBA surveys of live-dead egg ratios in herring spawn in the years 1989, 1990 and 1991. Analysis of covariance (ANCOVA) was used to quantify the effects on survival of oil exposure, site placement, depth, number of days to hatch, duration of spawning and their interactions. These survivals were interpolated over the period measurements were made to provide estimates of survival-to-hatch. The survival-to hatch observed in this study was compared to results from similar studies.

## Materials and Methods

Locations of herring spawning were mapped by personnel from the Alaska Department of Fish and Game during aerial surveys in 1989, 1990 and 1991 (Fig. 1). Visual observations of the extent of the oil slick were used to classify spawning areas as either oiled or non-oiled (control). Three areas were selected for study: (1) Fairmont Bay within the North Shore spawning area was classified as non-oiled; (2) the Naked Island archipelago, including Bass Harbor, Outside Bay, Cabin Bay, and Storey Island, was classified as oiled; and (3) Rocky Bay on the northern tip of Montague Island was classified as oiled. Within the three areas sampling transects were established following three criteria: (1) spawn had to contain only two to three egg layers to reduce mortality of eggs from asphyxiation and to aid in accurate counts of egg numbers; (2) eggs had to be distributed over a wide range of depths (+1.52 m to -9.14 m) to test for effects of

depth; and (3) mussels (Mytilus edulus) had to be present near transects so as to use the hydrocarbon concentrations in mussel tissue as an index of oil exposure of herring eggs (Short and Harris 1996; Brown and Short, in press).

Transects were established perpendicular to shore following a compass course set by divers. Sampling stations were set at depths of 1.52 m, 0.30 m, 0.00 m, -1.52 m, -4.57 m, and -9.14 m relative to Mean Lower Low Water (MLLW), the definition of low tide for all hydrographic maps published by the US National Ocean Service. Depths were initially based on feet rather than meters, thus producing fractional depths in meters. Depths were located with a diver's depth gauge and were corrected for tide stage. Each transect was sampled every four days, which resulted in three to six SCUBA dives along each transect between the time of spawning and the time of hatching, assuming a 24 d incubation period at an average water temperature of 7°C (Alderdice and Velsen 1971). Three samples of herring spawn were collected at each depth on each dive.

Samples of eggs were placed in pre-labeled mesh bags and kept moist in a cooler until they could be counted. All counts of live and dead eggs were made with a binocular microscope within 4 h of collection. Live eggs were clear and the embryo was visible in late-stage eggs, moribund eggs were tinged with white, and dead eggs were completely white and opaque. Moribund eggs were classified as dead. Eggs were recorded as unfertilized when no developing embryo was observed inside eggs. Counting ceased after three replicates of 100 eggs each had been tallied from each station, day and depth.

In 1989, 24 transects were established between April 9 and 19, when herring eggs were 1 to 2 wk old: five transects in non-oiled areas and 19 transects in oiled areas (Fig. 2). In 1990, 9 transects were established: three transects in non-oiled areas and six in oiled areas. In 1991, 13 transects were established: eight in non-oiled areas and five in oiled areas. Whenever possible, transects were established in the same areas in all three years, but this was not always possible because herring did not spawn in the same locations in all years resulting in an unbalanced survey design. Sampling efforts during each year were constrained by available time and money and by the extent of the spawn in oiled versus unoiled locations.

In 1989 and 1990, mussels were collected for hydrocarbon analyses. Samples of mussels were collected on the first day of sampling at each transect.. Three samples of mussels (= 6 samples/transect) were collected at the lowest depth at which mussels occurred (approximately 1.65 m) below MLLW. Brown and Short (in press) and Short and Harris (1996) describe the methods of hydrocarbon analysis; concentrations of total aromatic hydrocarbons are expressed as ppb (ng $g^{-1}$ wet tissue mass).

Analysis of in situ Egg Survival

A nested mixed-model analysis of covariance (ANCOVA) was used to test for significant differences in in situ survival of herring eggs between oiled and non-oiled areas while accounting for the number of days between the mean date of spawning and the date of sampling, and the duration of spawning (used as an indicator of egg density). The form of the model was

(1) $$\arcsin(s_{ijkl})^{0.5} = \mu + \tau_i + \delta_{j(i)} + \upsilon_k + (\tau\upsilon)_{ik} + \beta_{1(k)}X_1 + \beta_{2(k)}X_2 + \beta_{3(k)}X_1X_2 + \varepsilon_{ijkl},$$

where $s_{ijkl}$ = in situ egg survival (ratio of live/dead eggs) for the $i^{th}$ treatment, $j^{th}$ transect, $k^{th}$ depth, $l^{th}$ observation;

$\mu$ = overall mean;

$\tau_i$ = fixed effect of treatment $i$, $i = 1,2$;

$\delta_{j(i)}$ = random effect of transect $j$ within treatment $i$, $j = 1,2, \ldots t_i$;

$\upsilon_k$ = fixed effect of depth $k$, $k = 1,2, \ldots ,6$ in 1989 and $k = 1,2, \ldots ,5$ in 1990 and 1991;

$(\tau\upsilon)_{jk}$ = treatment by depth interaction;

$X_1$ = time since spawning (days);

$\beta_{1(k)}$ = regression coefficient for $X_1$ at depth $k$;

$X_2$ = duration of spawning (days);

$\beta_{2(k)}$ = regression coefficient for $X_2$ at depth $k$;

$X_1X_2$ = product of $X_1$ and $X_2$;

$\beta_{3(k)}$ = regression coefficient for $X_1X_2$ at depth $k$;

$\varepsilon_{ijkl}$ = random error term, distributed $N(0,\sigma^2)$.

Data from each year were fit to equation (1) separately using the PROC MIXED procedure in the statistical library SAS (SAS 1987). Terms with a significance test result of less than 0.05 were considered to be significant. With time since *first?* spawning and duration of spawning set at their respective mean values, least-squares means were calculated for the treatment by depth interaction term along with 95% confidence limits that were then back-transformed to the original scale. Least-squares means are needed to estimate marginal means for an unbalanced design. Estimates of a treatment effect and significance tests were also constructed at each depth.

A second ANCOVA model was fit for 1989 using the concentration of aromatic hydrocarbons in mussel tissues collected at each transect as a continuous measure of oil exposure. $\text{Log}_e$ -transformation of hydrocarbon concentration was necessary to meet the assumptions of normality and constant variance. The model used was

*Note: change subscripts to match equation 1*

(2) $$\arcsin(s_{ijk})^{0.5} = \mu + \delta_i + \upsilon_j + \beta_{1(j)}X_1 + \beta_{2(j)}X_2 + \beta_{3(j)}\log(X_3) + \varepsilon_{ijk},$$

where $s_{ijk}$ = in situ egg survival (ratio of live/dead eggs) for the $j^{th}$ transect, $k^{th}$ depth, $l^{th}$ observation;

$\mu$ = overall mean;

$\delta_i$ = random effect of transect $j$, $j = 1,2, \ldots 23$;

$\upsilon_j$ = fixed effect of depth $k$, $k = 1,2, \ldots ,6$ in 1989 and $k = 1,2, \ldots ,5$ in 1990 and 1991;

$X_1$ = time since spawning (days);
$\beta_{1(j)}$ = regression coefficient for $X_1$ at depth $k$;
$X_2$ = duration of spawning (days);
$\beta_{2(j)}$ = regression coefficient for $X_2$ at depth $k$;
$X_3$ = aromatic hydrocarbon concentration parts-per-billion (PPB);
$\beta_{3(j)}$ = regression coefficient for $X_3$ at depth $k$;
$\varepsilon_{ijk}$ = random error term, distributed $N(0,\sigma^2)$.

Contrasts were again constructed to test for an effect of aromatic hydrocarbons on survival at each depth. Average concentrations of aromatic hydrocarbons were calculated for oiled and non-oiled sites with the $\log_{10}$ of the ratio being used as the contrast coefficient for that term.

**Results**

Embryos from all sites were fertilized at rates at or above 98.0%. Therefore, there was no impact of oil on fertilization of herring embryos.

Using Equation (1), there were significant differences in <u>in situ</u> egg survival between oiled and non-oiled areas in 1989 and 1991, with a oil treatment effect in 1989 ($p<0.0001$), no significant treatment or treatment interactions in 1990, and an interaction of oil treatment with depth in 1991 ($p<0.0001$) (Table 1). In, 1989 oil treatment effects were found at depths of 0.30, 0.00, and -9.14 m ($p<0.015$), while in 1991 a treatment effect was found only at 1.52 m ($p<0.0001$) (Table 2). Mean egg survival at oiled sites was consistently lower than mean egg survival at control sites in 1989 (Figure 3). There was also an egg density (spawning duration) *effect (positive or negatively affecting survival; wasnt this an interaction effect??)* on egg survival in 1989 ($p=0.0003$).

In 1989, mussels from control sites had an average aromatic hydrocarbon concentration of 49 PPB, while those from oiled sites had an average of 753 PPB. The natural log of the ratio of unoiled over oiled mussel tissue concentraion was -2.73, which was used in the contrast between oiled and control sites. Using Equation (2), the significant effect of aromatic hydrocarbons in 1989 ($p=0.028$) (Table 3) indicated that oil exposure depressed survival of eggs, but only at depths of 0.30 and -9.14 m ($p=0.0013$ and 0.044, respectively) (Tables 4). Egg density or duration of spawning was again highly significant in 1989 as with the analysis using Equation (1) ($p=0.0003$) (*again signigicant in what way*???).

**Discussion**

Previous laboratory based research has demonstrated that the eggs of herring and other marine fish are injured by exposure to hydrocarbons either by direct exposure to oil-contaminated water (Lindén 1978; Rice 1985; Rice et al. 1987a), or by exposure of adult female herring to oil prior to spawning (Struhsaker 1977; Rice et al. 1987b).

To measure the impact of the oil spill on free-swimming wild herring larvae in the nearshore areas of the sound in 1989, McGurk and Brown (in press) measured the rates of growth of larvae captured within 1 km of the shore, and they estimated egg-larva survivals and mortality rates. They found no significant differences in growth of larvae between oiled and non-oiled areas, but they found significantly higher egg-larva mortality rates in oiled areas than in non-oiled areas. Oil-related differences in the cytogenetic and histopathology in newly hatched larvae immediately offshore of spawning beds were highly significant (Marty et al. this issue). Larval herring exposed to crude oil accumulated fluid in the abdominal cavity (ascites) and pericardial swelling, were shorter and had less gastrointestinal food than unexposed larvae which could ultimately affect growth and survival (Marty et al. this issue). To measure the impact of the oil spill on older herring larvae captured in offshore areas of the sound, Norcross et al. (in press) measured growth and morphological and cytological condition of larvae captured at sites greater than 1 km from the shores of the sound. They measured one of the lowest growth rates ever recorded for a natural population of Pacific herring larvae, and they found that larvae most likely originating from oiled spawning beds (based on a trajectory predicted by ocean circulation in the sound) had significantly higher levels of cytogenetic damage than larvae that originated from non-oiled beds. All three studies concluded that exposure to oil, either at or nearby the spawning beds or in the oil slick, may have reduced survival of herring larvae and may have contributed to the poor recruitment of the 1989 year class in 1992 and beyond.

This study offers partial support for the hypothesis that exposure of herring eggs to *Exxon Valdez* oil reduced the survival potential of herring larvae. Significantly lower in situ egg survivals were measured in oiled areas than in non-oiled areas in 1989 (Figure 3). Using hydrocarbon data from 1989, the effect appeared to be due to oil exposure at depths of +0.30 and -9.14 m (Tables 3 and 4). A marginal effect at +1.52 m may have been due to wave or air exposure which was confounded with treatment. The significant oil effect in 1991 may have also been due to wave or air exposure since it occurred at the +1.52 m depth. Wind and waves, as well as egg loss, were more pronounced in 1991 compared to 1990 (Biggs and Baker 1993), which may explain why a treatment effect was not observed at the +1.52 m depth in 1990. Our findings of increased mortalities, especially at the more exposed +1.52 m depths, is in agreement with other researchers who identify the upper intertidal zone as a dangerous one for Pacific herring eggs because of the risk of desiccation during low tides (Galkina 1971; Steinfeld 1971; Jones 1972). The distribution of oil in the surface waters of Prince William Sound was affected by winds and tended to impact beaches exposed to surface currents and winds to a greater degree (Royer et al. 1990; Venkatesh 1990; Galt et al. 1991). This would explain the confounding effects of wave or air exposure and treatment in 1989.

Previous studies identified water temperature, depth and egg density as natural factors that affect survival of herring eggs. However, temperature was unlikely to have played a role because laboratory incubation experiments reported by Alderice and Velsen (1971) showed that total hatch of Pacific herring is over 90% at water temperatures of 2-10°C. That temperature range included the natural temperatures in the sound from 1989-1991. In 1989, egg density

(measured using the proxy variable of duration of spawn) was highly signigicant with an interaction with days in 1989, but not in either of the two following years (Tables 1 and 2). The significance of egg density is relavant in 1989 because once accounted for, the evidence of a real oil effect is stronger (Tables 3 and 4). In other studies, density related egg mortality has only been observed with extremely high densities (Runnstrom 1941; Messiah and Rosenthal 1989), probably due to asphyxia (Galkina 1971).

Most studies reporting oil impacts on embryos up until hatch cite physiological and morphological effects rather than outright mortality (Struhsaker et al. 1974; Eldridge et al. 1978; Smith and Cameron, 1980; Serigstad et al. 1988; Mironov et al. 1993). In out study, fertilization of eggs was not affected by oil which is in agreement with other studies (Carls and Rice 1988). Stress in eggs from exposure to oil generally manifests during or after hatch (Kühnhold 1977; Linden 1978; Rice et al. 1987b; Carls and Rice 1988). The hatching period for this study was documented in a laboratory incubation study (M.D. McGurk, Triton Environmental, Richmond, B.C., unpublished data) and post-hatch effects were document by researchers working closely with us (Hose et al. this issue; Marty et al. this issue; Norcross et al. this issue).

The levels oil exposure of eggs in 1989 was high enough to cause a number of sublethal effects that would eventually lead to the death of the newly hatched larvae. The strongest effect due to oil exposure measured in the laboratory was acceleration of embryo development which caused early hatch (Kocan et al. this issue). This is a common response of fish embryos to stress caused by exposure to oil (Linden 1978; Westernhagen 1988; Kocan et al. this issue). There was a significant increase in the rates of morphological and cytogenetic abnormalities in newly hatched larvae from oiled sites versus unoiled sites (Hose et al. this issue). These larvae had been removed from our study sites as eggs and incubated until hatch in the laboratory. Similar findings of abnormal larvae occurred in the Baltic Sea following a spill of crude oil (Urho 1991). It is unlikely that larvae with significant abnormalities survived to enter the plankton population in 1989 (Hose et al. this issue; Kocan et al. this issue; Marty et al. this issue).

Indirect exposure of eggs through exposure of maturing adult female herring, rather than direct exposure of eggs at oiled sites, was a potential cause of the treatment effects observed in 1989. Although the uptake of oil in the ova or prespawning adult herring is significant, even at low levels (0.6 ppm over a 12 d period), impacts on spawning and survival of eggs from those females are not observed at those levels (Rice et al. 1987a, 1987b). In another study, adult female herring exposed to very low levels of benzene for 48 h exhibited aberrant swimming behavior and spawned prematurely (Struhsaker 1977). Neither aberrant swimming behavior or premature spawning was observed in adult herring in the sound in 1989 (Brown et al. this issue) and it is unlikely that concentrations of oil in the waters of Prince William Sound (Short and Harris 1996) was high enough to observe those affects. We, therefore believe that direct exposure of the eggs to oil in 1989 was the most likely route for oil injury observed.

Despite differences in survival found at oiled and unoiled sites, the range of egg survival rates measured in this study in situ were similar to ranges reported for medium and low densities of natural uncontaminated Pacific herring spawn (Taylor 1971; Hourston et al. 1984), Atlantic herring spawn (Johannessen 1986), and Baltic herring spawn (Aneer and Nellbring 1982; Braum 1985). Our egg survival rates were generally higher than rates reported by Kocan et al. (this

issue): 58.8-68.4%, who independently incubated eggs in the laboratory after the embryos had undergone a period of in situ egg incubation. Also, our estimate of average egg mortality rates of 0.40 to 4.5 $\%\cdot d^{-1}$ is in good agreement with most studies of natural herring spawn which show that mortality due to causes other than predation and wave scouring is usually less than 10 $\%\cdot d^{-1}$ (Hart and Tester 1934; Runnstrom 1941; Cleaver and Franett 1946; Baxter 1971; Hempel and Hempel 1971; Haegele at al. 1981; Palsson 1984; Johannessen 1986; Klinkhardt 1989; Rajasilta et al. 1989). We therefore conclude, that although oil exposure affected egg survival in Prince William Sound in 1989, it was a small effect and within the ranges of egg survival rates found naturally. The oil impacts on hatch and the larvae surviving hatch from egg incubated at our treatment sights were of greater significance (Brown et al. 1996; Brown and Hose, this issue; Hose et al., this issue; Kocan et al. this issue; Norcross et al. this issue; Marty et al. this issue).

## Acknowledgments

The 1989-1991 in situ egg mortality study was funded by the Exxon Valdez Trustee Council and supported through the Alaska Department of Fish and Game. We would like to thank T. Baker, L. Brannian, H. Geiger and J. Wilcock for their support and contribution to the survey design, statistical analysis and review of this project.

## References

Alderdice, D. F., and F. P. J. Velsen. 1971. Some effects of salinity and temperature on early development of Pacific herring (Clupea pallasi). J. Fish. Res. Board Can. 28: 1545-1562.

Aneer, G., and S. Nellbring. 1982. A SCUBA-diving investigation of Baltic herring (Clupea harengus membras L.) spawning grounds in the Askö-Landsort area, northern Baltic proper. J. Fish Biol. 21: 433-442.

Baxter, I. G. 1971. Development rates and mortalities in Clyde herring eggs. Rapp. P.-v. Réun. Cons.int. Explor. Mer. 160: 27-29.

Biggs, E.D. and T.T. Baker. 1993. Studies on Pacific herring Clupea pallasi spawning in Prince William Sound following the 1989 Exxon Valdez Oil Spill, 1989-1992. Annual Report for Fish/Shellfish Study No. 11 to the EVOS Trustee Council, Anchorage, AK. 129 pp.

Braum, E. 1985. Experiments on the respiration and in situ development of Baltic herring (Clupea harengus L.) eggs. Int. Revue ges. Hydrobiol. 70 (1): 87-100.

Brown, E. D., B. L. Norcross, and J. W. Short. This issue. Conditions affecting the distribution of oil from the Exxon Valdez spill and exposure of Pacific herring, Clupea pallasi, in Prince William Sound. Can. J. Fish. Aquat. Sci. 00: 000-000.

Brown, E. D., T.T. Baker, J.E. Hose, R.M. Kocan, G.D. Marty, M.D. McGurk, B.L. Norcross, and J.F. Short. 1996. Injury to the early life history stages of Pacific herring in Prince William Sound after the Exxon Valdez oil spill. American Fisheries Society Symposium No. 18: 000-000.

Carls, M. G. and S. D. Rice. 1988. Sensitivity differences between eggs and larvae of walleye pollock (Theragra chalcogramma) to hydrocarbons. Mar. Environ. Res. 26: 285-297.

Cleaver, F. C., and D. M. Franett. 1946. The predation by sea birds upon the eggs of the Pacific herring (Clupea pallasi) at Holmes Harbor during 1945. Washington State Dept. Fish. Biol. Rep. 46B.

Eldridge, M.B., T. Echeverria, and S. Korn. 1978. Fate of $^{14}$C-benzene in eggs and larvae of Pacific herring (Clupea harengus pallasi). J. Fish. Res. Board Can. 35: 861-865.

Galkina, L. A. 1971. Survival of spawn of the Pacific herring (Clupea harengus pallasi Val.) related to the abundance of the spawning stock. Rapp. R.-v. Réun. Cons. Int. Explor. Mer. 160: 30-33.

Galt, J. A., W. J. Lehr, and D. L. Payton. 1991. Fate and transport of the Exxon Valdez oil spill. Environ. Sci. Tech. 25: 202-209.

Gundlach, E. R., J. Bauer, R. Bayliss, S. Provant, and M. Kendziorek. 1990. Response to the Exxon Valdez oil spill by the Alaska Department of Environmental Conservation, p. 471-478. *In* M. L. Spaulding and M. Reed (eds.) Oil Spills Management and Legislative Implications, Proceedings of the Conference. American Society of Civil Engineers, New York.

Haegele, C. W., R. D. Humphreys, and A. S. Hourston. 1981. Distribution of eggs by depth and vegetation type in Pacific herring (Clupea harengus pallasi) spawnings in Southern British Columbia. Can. J. Fish. Aquat. Sci. 38: 381-386.

Hart, J. L., and A. L. Tester. 1934. Quantitative studies on herring spawning. Trans. Am. Fish. Soc. 64: 307-313.

Hempel, I., and G. Hempel. 1971. An estimate of mortality in eggs of North Sea herring (Clupea harengus L.). Rapp. R.-v. Réun. Cons. Int. Explor. Mer. 160: 24-26.

Hose, J. E., M. D. McGurk, G. D. Marty, D. E. Hinton, E. D. Brown, and T. T. Baker. In prep. Sublethal affects of the Exxon Valdez oil spill on herring embryos and larvae: morphologic, cytogenetic and histopathological assessments, 1989-1991. Can. J. Fish. Aquat. Sci. 00: 000-000.

Hourston, A. S., H. Rosenthal, and H. von Westernhagen. 1984. Viable hatch from eggs of Pacific herring (Clupea harengus pallasi) deposited at different intensities on a variety of substrates. Can. Tech. Rep. Fish. Aquat. Sci. No. 1274.

Johannessen, A. 1986. Recruitment studies of herring (Clupea harengus L.) in Lindaspollene, western Norway. FiskDir. Skr. Ser. HavUnders. 18: 139-240.

Jones, B. C. 1972. Effect of intertidal exposure on survival and embryonic development of Pacific herring spawn. J. Fish. Res. B. Can. 29: 1119-1124.

Klinkhardt, M. B. 1989. The occurrence of abnormal herring embryos (Clupea harengus) on a spawning-ground in the inner coastal waters of the German Democratic Republic. Rapp. R.-v. Réun. Cons. Int. Explor. Mer. 190: 178-182.

Kocan, R. M., J. E. Hose, E. D. Brown, and T. T. Baker. In prep. Herring embryo (Clupea pallasi) sensitivity to Prudhoe Bay petroleum hydrocarbons: laboratory evaluation and in situ exposure at oiled and unoiled sites in Prince William Sound. Can. J. Fish. Aquat. Sci. 00: 000-000.

Lindén, O. 1978. Biological effects of oil on early development of the Baltic herring Clupea harengus membras. Mar. Biol. 45: 273-283.

Marty, G. D., D. E. Hinton, J. E. Hose, M. D. McGurk, and E. D. Brown. This issue. Histopathology and cytogenetics of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the Exxon Valdez Oil Spill. Can. J. Fish. Aquat. Sci. 00: 000-000.

McGurk, M. D., and E. D. Brown. In press. Egg-larva mortality of Pacific herring in Prince William Sound, Alaska, after the Exxon Valdez oil spill. Can. J. Fish. Aquat. Sci. 00: 000-000.

Messiah, S. N. and H. Rosenthal. 1989. Mass mortality of herring eggs on spawning beds on and near Fisherman's Bank, Gulf of St. Lawrence, Canada. Aquat. Living Resour. 2: 1-8.

Mironov, O. G., A. D. Gordina, I.I. Rudneva, I.P. Murav'eva, and T.L. Gavenauskaite. 1993. Effect of heavy petroleum fractions on developing eggs of the blenny, Blennius sanguinolentus. J. Ichthyol. 33(1): 149-152.

Norcross, B.L., M. Frandsen, J.E. Hose, and E.D. Brown. This issue. Larval herring distribution, abundance, and cellular analysis in Prince William Sound, Alaska during 1989 following the Exxon Valdez oil spill. Can. J. Fish. Aq. Sci. 00: 000-000.

Palsson, W. A. 1984. Egg mortality upon natural and artificial substrata within Washington State spawning grounds of Pacific herring (Clupea harengus pallasi). M.Sc. Thesis, University of Washington, Seattle, WA.

Rajasilta, M. J., J. Eklund, J. Kaaria, and K. Ranta-aho. 1989. The deposition and mortality of eggs of the Baltic herring, Clupea harengus membras L., on different substrates in the south-west archipelago of Finland. J. Fish. Biol. 34: 417-427.

Rice, S. D. 1985. Effects of oil on fish, p. 157-182. In F. R. Englehardt (ed.) Petroleum Effects in the Arctic Environment. First edition. Elsevier Applied Science Publishers, New York, NY.

Rice, S. D., M. M. Babcock, C. C. Brodersen, J. A. Gharrett, and S. Korn. 1987a. Uptake and depuration of aromatic hydrocarbons by reproductively ripe Pacific herring and the subsequent effect of residues on egg hatching and survival. p. 139-154. In W. B. Vernberg et al. [eds.] Pollution Physiology of Estuarine Organisms. University of South Carolina Press, Columbia, SC.

Rice, S. D., M. M. Babcock, C. C. Brodersen, M. Carls, J.A. Gharrett, S. Korn, A. Moles, and J. W. Short. 1987b. Lethal and sublethal effects of the water soluble fraction of Cook Inlet crude oil on Pacific herring (Clupea harengus pallasi) reproduction. NOAA Technical Memorandum NMFS F/NWC-111, Auke Bay, AK.

Royer, T. C., J. A. Vermersch, T. J. Weingartner, H. J. Niebauer, and R. D. Muench. 1990. Ocean circulation influencing the Exxon Valdez oil spill. Oceanography 3(2): 3-10.

Runnstrom, S. 1941. Quantitative investigations on herring spawning and its yearly fluctuations at the west coast of Norway. FiskDir. Skr. Ser. HavUnders. 6.

SAS 1987. SAS/STAT Guide for personal computers, version 6 edition. SAS Institute, Cary, NC.

Serigstad, B., I. Sværen, and L. Føyn. 1988. The effects of oil-base drilling mud and cruide oil on demersal fish eggs. International Council for the Exploration of the Sea, Report C.M. 1988/ E:19 from the Marine Environmental Quality Committee. 17 p.

Short, J. F., and P. M. Harris. 1996. Petroleum hydrocarbons in caged mussels deployed in Prince William Sound, Alaska, after the Exxon Valdez Oil Spill. American Fisheries Society Symposium No. 00: 000-000.

Smith, R. L. and J. A. Cameron. 1980. Ultrastructural effects of crude oil on early life stages of Pacific herring. Trans. Am. Fish. Soc. 109: 224-228.

Steinfeld, J. D. 1971. Distribution of Pacific herring spawn in Yaquina Bay, Oregon, and observations on mortality through hatching. M.Sc. Thesis, Oregon State University, Oregon.

Struhsaker, J. W., M. B. Eldridge, and T. Echeverria. 1974. Effects of benzene (a water soluble component of crude oil) on eggs and larvae of Pacific herring and northern anchovy, p. 253-284. In F. J. Vernberg and W. B. Vernberg [eds.] Pollution and Physiology of Marine Organisms, Academic Press, New York.

Struhsaker, J. W. 1977. Effects of benzene (a toxic component of petroleum) on spawning Pacific herring (Clupea harengus pallasi). Fish. Bull., US 75: 43-49.

Taylor, F. H. C. 1971. Variation in hatching success in Pacific herring (Clupea pallasi) eggs with water depth, temperature, salinity and egg mass thickness. Rapp. R.-v. Réun. Cons. Int. Explor. Mer. 160: 33-41.

Urho, L. 1991. Impact of an oil spill on herring stock, p. 569-582. In Proceedings of the International Herring Symposium, Anchorage, Alaska, October 23-25, 1990. Alaska Sea Grant Report 91-01.

Venkatesh, S. 1990. Model simulations of the drift and spread of the Exxon Valdez oil spill. Atmosphere-Ocean 28: 90-105.

Westernhagen, H. von. 1988. Sublethal effects of pollutants on fish eggs and larvae. Fish Physiology XI(A): 253-356.

Wolfe, D. A., M. M. Krahn, E. Casillas, S. Sol, T. A. Thompson, J. Lunz, and K. J. Scott. 1996. Toxicity of intertidal and subtidal sediments contaminated by the Exxon Valdez Oil Spill. American Fisheries Society Symposium No. 00: 000-000.

Table 1. Analysis of covariance (equation (1)) of in situ herring egg survival in Prince William Sound, Alaska, for 1989-1991.

| | 1989 | | | | 1990 | | | | 1991 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | NDF | DDF | F | p | NDF | DDF | F | p | NDF | DDF | F | p |
| Oil | 1 | 20 | 25.75 | 0.0001 | 1 | 6 | 0.30 | 0.6059 | 1 | 10 | 4.37 | 0.0630 |
| Depth | 5 | 196 | 4.38 | 0.0008 | 4 | 369 | 0.50 | 0.7361 | 4 | 282 | 1.20 | 0.3111 |
| Depth*Oil | 5 | 196 | 1.62 | 0.1558 | 4 | 369 | 1.62 | 0.1686 | 4 | 282 | 7.81 | 0.0001 |
| Days(Depth) | 6 | 196 | 2.61 | 0.0187 | 5 | 369 | 4.00 | 0.0015 | 5 | 282 | 0.46 | 0.8088 |
| Duration(Depth) | 6 | 196 | 4.44 | 0.0003 | 5 | 369 | 0.40 | 0.8470 | 5 | 282 | 0.23 | 0.9514 |
| Day*Dur(Depth) | 6 | 196 | 2.74 | 0.0141 | 5 | 369 | 1.13 | 0.3447 | 5 | 282 | 1.01 | 0.4134 |

Table 2. Test of $H_0$: $S_{control}=S_{oiled}$ for 1989-1991 with equation (1). Estimates of treatment effects are $S_{control}-S_{oiled}$ so that positive estimates indicate survival at control sites greater than at oiled sites.

| | 1989 | | 1990 | | 1991 | |
|---|---|---|---|---|---|---|
| Depth | Estimate | p | Estimate | p | Estimate | p |
| +1.52 | 0.0703 | 0.2175 | -0.0501 | 0.6718 | 0.1901 | 0.0001 |
| +0.30 | 0.1960 | 0.0004 | 0.0914 | 0.2032 | 0.0002 | 0.9957 |
| 0.00 | 0.1011 | 0.0046 | 0.1106 | 0.1244 | 0.0199 | 0.4796 |
| -1.52 | 0.0413 | 0.2405 | -0.0344 | 0.6247 | 0.0203 | 0.4708 |
| -4.57 | 0.0501 | 0.1620 | 0.0454 | 0.5158 | -0.0470 | 0.2096 |
| -9.14 | 0.1484 | 0.0138 | | | | |

Table 3. Analysis of covariance (equation (2)) of in situ herring egg survival in Prince William Sound, Alaska, for 1989.

| Source | NDF | DDF | F | p |
|---|---|---|---|---|
| Depth | 5 | 201 | 3.35 | 0.0063 |
| Days(Depth) | 6 | 201 | 5.96 | 0.0001 |
| Duration(Depth) | 6 | 201 | 4.45 | 0.0003 |
| lAHC(Depth) | 6 | 201 | 2.42 | 0.0281 |

Table 4: Test of $H_0$: $S_{control}=S_{oiled}$ for 1989 with equation (2). Estimates of treatment effects are $S_{control}-S_{oiled}$ so that positive estimates indicate survival at control sites greater than at oiled sites.

| Depth | Estimate | p |
|---|---|---|
| +1.52 | -0.0007 | 0.9859 |
| +0.30 | 0.1675 | 0.0013 |
| 0.00 | 0.0400 | 0.2134 |
| -1.52 | 0.0147 | 0.6433 |
| -4.57 | 0.0198 | 0.5436 |
| -9.14 | 0.1119 | 0.0438 |

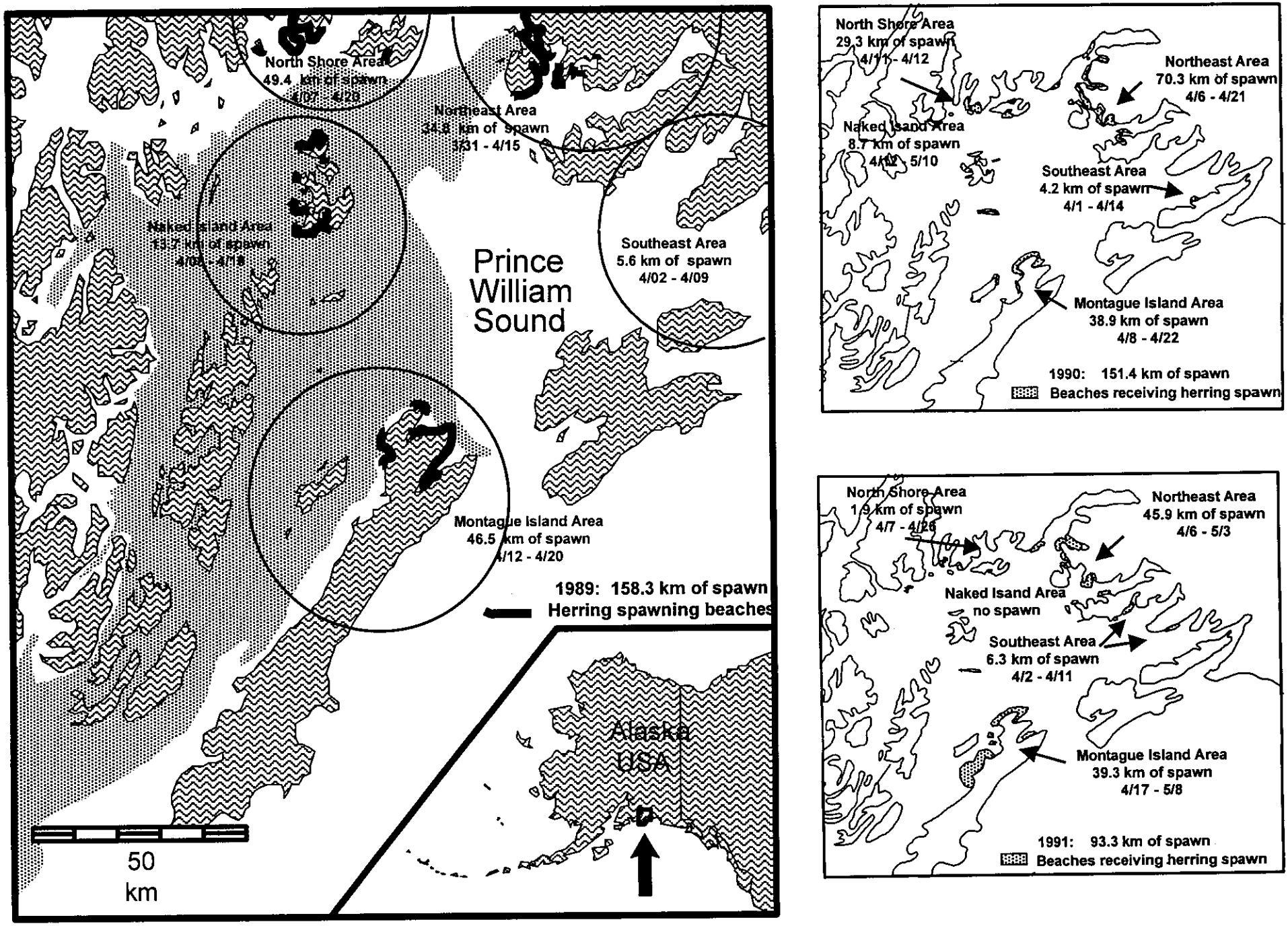



Figure 1. Composite map of the oil spill trajectory (lightly shaded area; Galt et al. 1991), herring spawning areas in 1989, 1990 and 1991 (dark shaded areas adjacent to shorelines). Ranges of spawning dates and kilometers of shoreline receiving spawn are listed for each of the five areas (Southeast, Northeast, North Shore, Naked Island, and Montague Island).

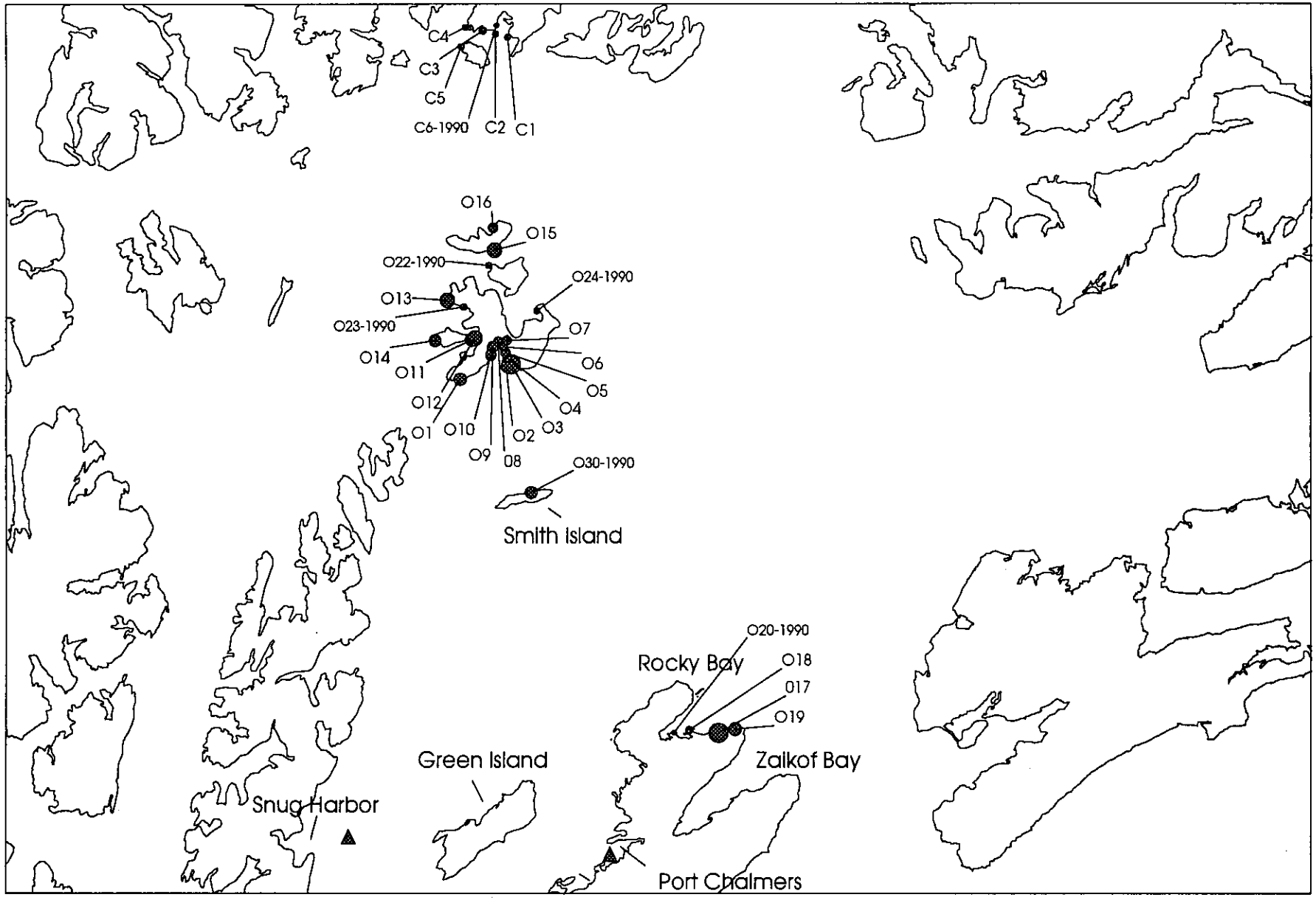



Figure 2. Location of egg survival study sites in Prince William Sound, Alaska, 1989-1991. Sites beginning with "C" are reference and "O" oiled.

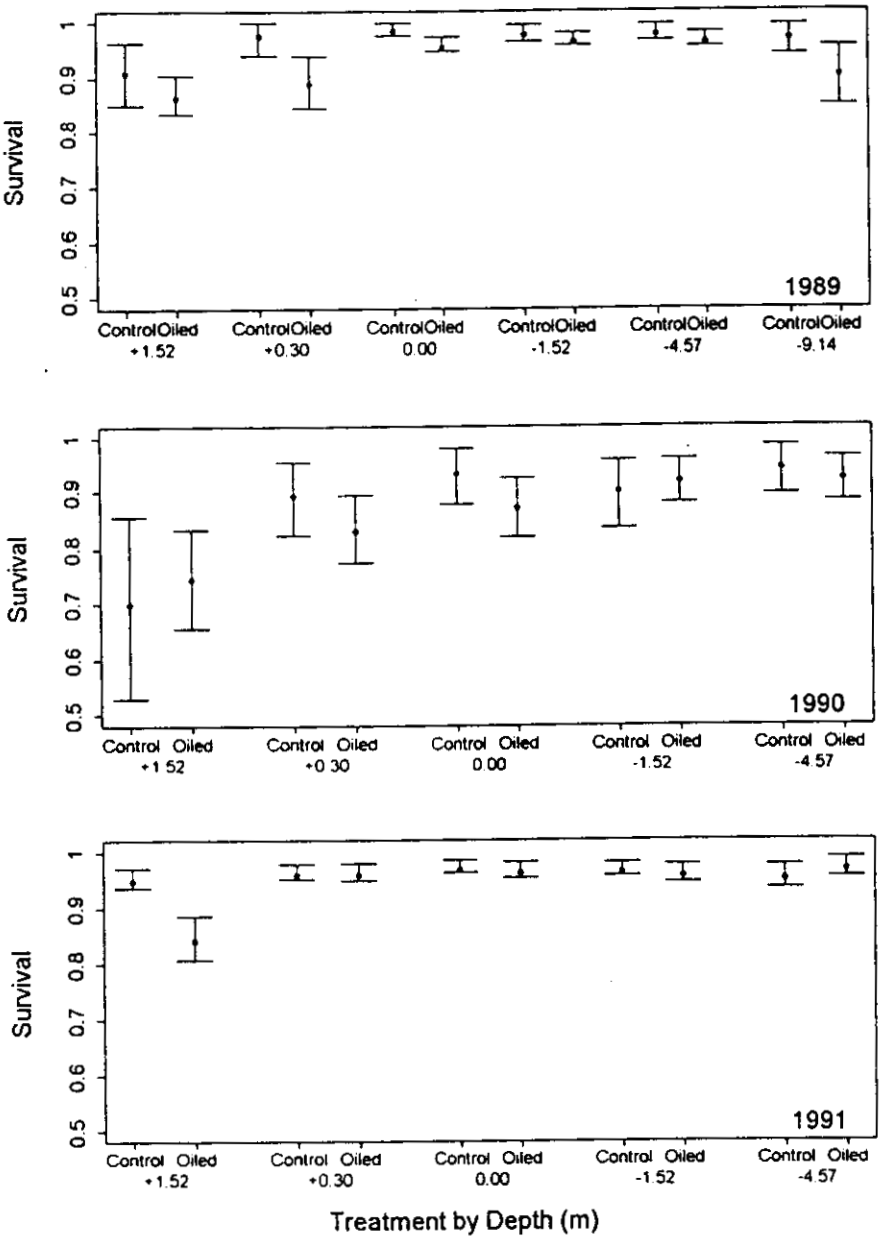



Figure 3. Least-squares means estimates of herring egg survival for treatment by depth, in Prince William Sound, Alaska, 1989-1991.

American Fisheries Society Symposium 18:448–462, 1996


# Injury to the Early Life History Stages of Pacific Herring in Prince William Sound after the *Exxon Valdez* Oil Spill

EVELYN D. BROWN
*Alaska Department of Fish and Game, Division of Commercial Fisheries, Research, and Development*
*Box 669, Cordova, Alaska 99574, USA*

TIMOTHY T. BAKER
*Alaska Department of Fish and Game, Division of Commercial Fisheries, Research, and Development*
*333 Raspberry Road, Anchorage, Alaska 99518, USA*

JO ELLEN HOSE
*Occidental College, Department of Biology, 1600 Campus Road, Los Angeles, California 90041, USA*

RICHARD M. KOCAN
*University of Washington, School of Fisheries, Fisheries Research Institute, Seattle, Washington 98195, USA*

GARY D. MARTY[1]
*University of California, Department of Medicine, School of Veterinary Science, Davis, California 95616, USA*

MICHAEL D. MCGURK
*Triton Environmental Consultants, Ltd.*
*120-13511 Commerce Parkway, Richmond, British Columbia, Canada V6V 2L1*

BRENDA L. NORCROSS
*University of Alaska, School of Fisheries and Ocean Science, Institute of Marine Science*
*Fairbanks, Alaska 99775, USA*

JEFF SHORT
*National Marine Fisheries Service, Alaska Fisheries Science Center*
*11305 Glacier Highway, Juneau, Alaska 99801, USA*

*Abstract.*—The *Exxon Valdez* oil spill occurred a few weeks before Pacific herring *Clupea pallasi* spawned in Prince William Sound. About half of the egg biomass was deposited within the oil trajectory, and an estimated 40 to 50% sustained oil exposure during early development. The resulting 1989 year-class displayed sublethal effects in newly hatched larvae, primarily premature hatch, low weights, reduced growth, and increased morphologic and genetic abnormalities. Genetic endpoints, especially anaphase aberration rates, were highly correlated with site-specific *Exxon Valdez* oil concentrations. Responses were specific and sensitive to this oil exposure. In newly hatched larvae, anaphase aberration rates were elevated at oiled sites, and in pelagic larvae genetic damage was greatest near oiled areas of southwestern Prince William Sound. Genetic damage in larvae from oiled areas progressively decreased during the 6-week study, but site-specific measures of instantaneous mortality suggest that a significant reduction (52.3%) in larval production occurred in 1989. Although approximately equal egg biomass was deposited in non-oiled and oiled areas, we estimated that oiled areas produced only $0.016 \times 10^9$ pelagic larvae compared with $11.82 \times 10^9$ non-oiled areas. Despite the estimated substantial decrease in larval production, reduced abundance in the 1989-year class recruiting as 4-year-old adults in 1993 could not be estimated because natural processes affecting recruitment are poorly understood; however, the 1989 year-class was a minority of the 1993 spawning population, one of the smallest cohorts observed in Prince William Sound, and it returned to spawn with an adult herring population reduced by approximately 75%, apparently because of a widespread epizootic.

The *Exxon Valdez* oil spill on 24 March 1989 occurred only a few days before the annual migration of Pacific herring *Clupea pallasi* into nearshore spawning areas of Prince William Sound. Because of temporal and spatial movements of the oil, the potential exposure of herring to oil was high. The herring migration follows a northeasterly pattern from the Montague–Green Island staging areas to-

[1]Present address: University of California, Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, Davis, California 95616, USA.

ward Bligh Island (Alaska Department of Fish and Game, unpublished data); the oil trajectory moved in the opposite direction from Bligh Island southwestward through Montague Strait (Royer et al. 1990; Figure 1). Of the 43.9 million liters of *Exxon Valdez* oil spilled, only about 10% was carried out of Prince William Sound as floating oil, whereas an estimated 40–45% was beached within the sound (Wolfe et al., in press). An unknown proportion of *Exxon Valdez* oil was dispersed as particulates throughout the water column to a depth of at least 25 m. Particulate oil was bioavailable to organisms throughout the summer in 1989 after the spill and to a lesser degree in 1990, after beach-cleaning operations (Short and Harris 1996a, this volume). The spill occurred at the beginning of the spring–summer zooplankton bloom in Prince William Sound. Ingestion of oil-contaminated food can be a significant route for oil exposure, and copepods, one of the major prey items for juvenile herring (Cooney 1995), significantly bioaccumulate petroleum hydrocarbons (Conover 1971; Capuzzo 1987). Because of the potential for oil contamination, commercial herring fisheries were closed in 1989.

In 1989 Pacific herring deposited their eggs on Prince William Sound beaches in early to mid-April, and the eggs hatched in early May. Larval herring remained in nearshore areas until late spring; in early summer they were transported to the western areas of the sound and into Montague Strait (Norcross and Frandsen 1996, this volume). Because of the rapid dispersion of particulate *Exxon Valdez* oil throughout the subsurface waters within the oil trajectory and its persistence near oiled beaches into summer (Short and Harris 1996a), exposure potential existed for spawning adults, eggs, and larvae for at least the first few months after the spill. The early life stages, being the most sensitive to the toxic effects of oil (Capuzzo 1987), were therefore emphasized in Natural Resource Damage Assessment studies.

The objective of the herring damage assessment study was to determine the effects of the *Exxon Valdez* oil spill on Prince William Sound herring. Using both long-term (prespill) fisheries baseline data and damage assessment studies and also targeting critical processes such as growth and reproduction when possible, we attempted to link organismal-level effects with population effects. This article summarizes and synthesizes the results of damage assessment studies of the early life history stages of Pacific herring and describes damage to embryos and larvae. Studies on embryos, juveniles, and adults are continuing, and their results, along with more detailed information on oil spill injury to all of the life stages of herring, will be described in future publications (Hose et al., in press; Kocan et al., in press; McGurk and Brown, in press; B. L. Norcross, unpublished).

## Overview of Damage Assessment Study Methods

### *Determination of the Oil Trajectory*

Information on the geographic extent of the oil trajectory was combined from several sources, including overflight maps produced by the National Oceanic and Atmospheric Administration (Galt et al. 1991), Alaska Department of Environmental Conservation, and Alaska Department of Fish and Game aerial surveyors; shoreline oiling estimates from the Alaska Department of Natural Resources and visual surveys during sample collection; and satellite images of the oil trajectory (Dean et al. 1990). The comprehensive oil-trajectory map was used to determine potential exposure of herring to *Exxon Valdez* oil (Brown 1995; Figure 1). The presence of oil at specific adult, egg, and larval collection sites was verified using *Exxon Valdez* oil measurements in caged or native mussels (Short and Harris 1996a).

### *Exxon Valdez Oil Analysis*

Concurrent with sampling of herring eggs, native bay mussels (*Mytilus trossulus*) were collected at −1 and −2 m mean lower low water (MLLW) adjacent to egg sampling sites in 1989 and at 0 and −1 m MLLW in 1990 (Figure 2). Mussel tissue was analyzed for 44 polycyclic aromatic hydrocarbons derived from *Exxon Valdez* oil (EVO-PAH) with gas chromatography (Short and Harris 1996a, 1996b, this volume). Method detection limits ranged from 0.020 to 0.040 μg/L for individual analytes and were based on 10-g samples. A limited number of herring egg and adult tissues were analyzed for EVO-PAH, but samples were considerably less than 10 g. These samples were not used to estimate oil exposure because of unacceptably high EVO-PAH detection limits; levels of oil known to cause sublethal effects would not be detected.

### *Embryo–Larval Studies*

There are five herring spawning areas in Prince William Sound (North Shore, Northeast, Southeast, Naked Island, and Montague Island areas; Figure 1). From 1989 to 1991, egg mortality at a series of non-oiled and oiled sites throughout the sound's

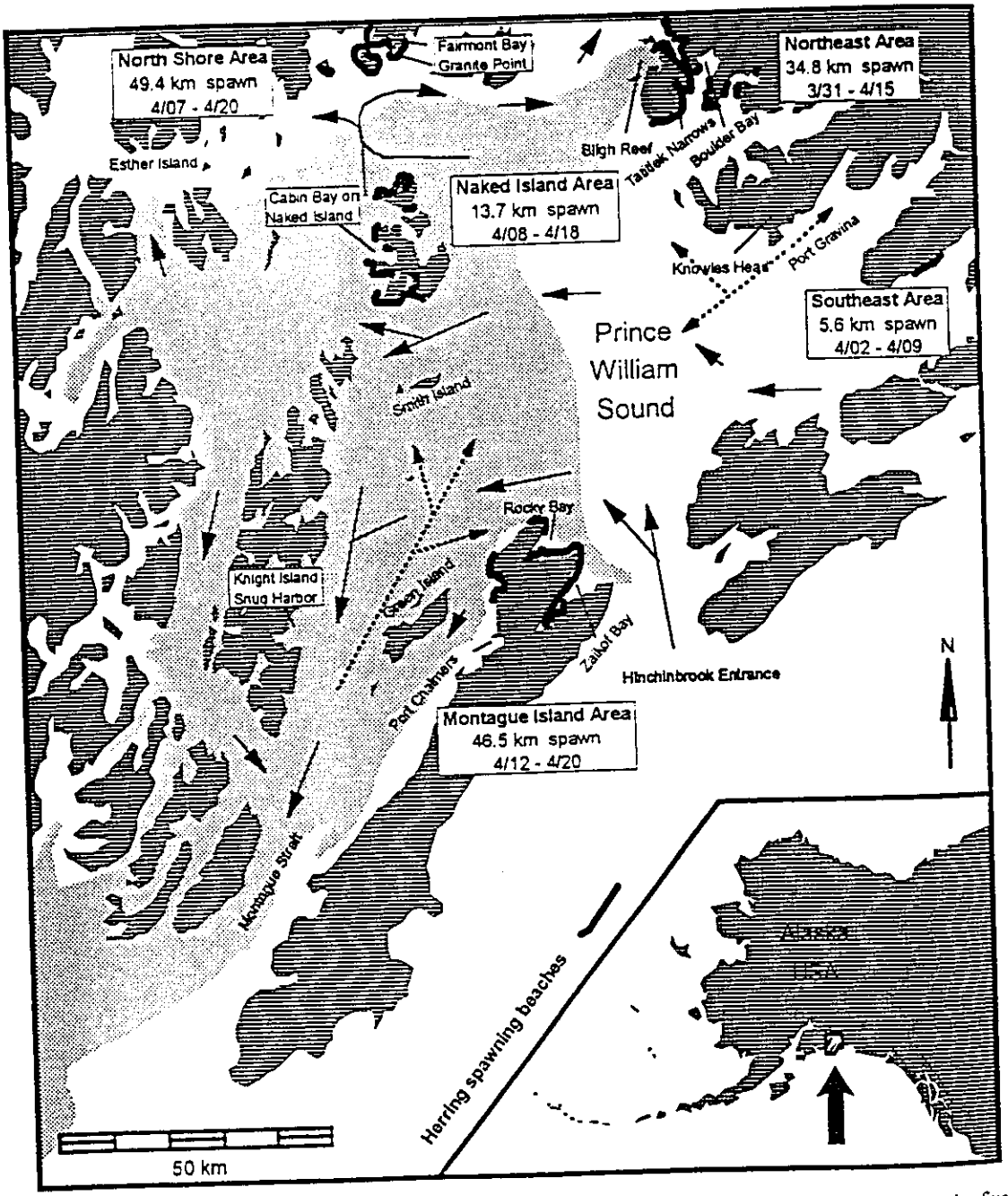
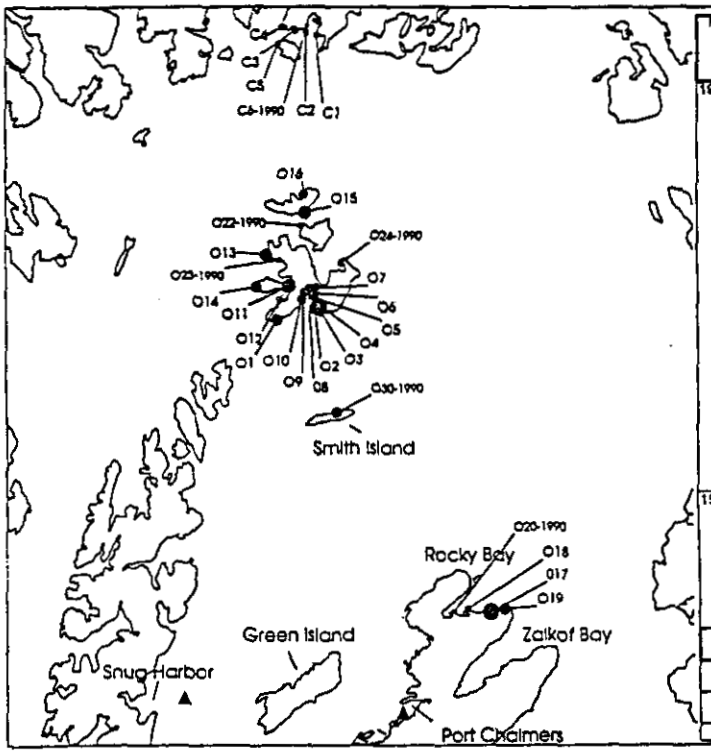



FIGURE 1.—The composite oil trajectory (light shaded area) of the *Exxon Valdez* oil spill (Galt et al. 1991), the five major Pacific herring spawning areas (circles), and the beaches receiving herring spawn in 1989 (heavy black lines), in Prince William Sound. Solid arrows indicate the general ocean circulation pattern in the sound. Dotted arrows indicate the migration of adult herring from over wintering grounds to spawning beaches.




| Mussel Tissue Hydrocarbon Samples (Circles) | Site No. | Mean Oil Concentration (ppb) | Standard Deviation |
|---|---|---|---|
| 1989 Study Sites | C1 | 18.69 | 15.71 |
| | C2 | 45.80 | 26.47 |
| | C3 | 79.77 | 38.91 |
| | C4 | 51.97 | 8.15 |
| | C5 | 53.33 | 43.46 |
| | O1 | 649.77 | 107.93 |
| | O2 | 388.16 | 123.98 |
| | O3 | 2,503.74 | 1,048.52 |
| | O4 | 216.73 | 74.47 |
| | O5 | 292.58 | 25.57 |
| | O6 | 158.69 | 27.66 |
| | O7 | 181.57 | 49.52 |
| | O8 | 198.18 | 31.47 |
| | O9 | 452.22 | 143.75 |
| | O10 | 453.58 | 39.62 |
| | O11 | 779.01 | 79.26 |
| | O12 | 1,142.21 | 113.29 |
| | O13 | 1,028.98 | 84.17 |
| | O14 | 716.97 | 171.67 |
| | O15 | 1,205.95 | 116.83 |
| | O16 | 374.07 | 18.11 |
| | O17 | 2,261.87 | 213.78 |
| | O18 | 97.52 | 14.26 |
| | O19 | 831.28 | 41.18 |
| 1990 Study Sites | C2 | 0.00 | 0.00 |
| | C3 | 3.73 | 5.28 |
| | C6 | 2.17 | 3.06 |
| | O20 | 7.08 | 7.85 |
| | O22 | 48.38 | 36.26 |
| | O23 | 26.08 | 22.94 |
| | O24 | 21.72 | 21.23 |
| | O30 | 581.74 | 143.33 |

| Bile Samples (Triangles) | Location | Naphthalene (ppb) | Phenanthrene (ppb) |
|---|---|---|---|
| 1989 | Snug Harbor | 140,000.00 | 28,000.00 |
| 1990 | Port Chalmers | 153,000.00 | 24,000.00 |
| Reference | No Oil | 22,000.00 | 2,000.00 |

FIGURE 2.—Sites where Pacific herring eggs were collected for density measurements and in situ and laboratory measurements of egg mortality, Prince William Sound, 1989 and 1990. Sites beginning with C were non-oiled (control) stations, those beginning with O were oiled stations. Shaded circles denote the concentrations in parts per billion (ppb, μg/L) of polycyclic aromatic hydrocarbons derived from *Exxon Valdez* oil in native bay mussels adjacent to egg collection sites; larger circles denote higher concentrations of *Exxon Valdez* oil. Most sites were sampled in 1989; 1990 sites are so labeled.

spawning areas was annually monitored in situ (Egg Deposition Study) (Biggs and Baker 1993; Brown 1995) and by returning subsamples to the laboratory for controlled incubations (Egg Incubation Study) (McGurk 1990b, 1991a, 1991b, 1993) (Figure 2). In 1990 a site in southeast Alaska, Sitka Sound, was also evaluated in the Egg Incubation Study (McGurk 1991a). The Egg Deposition Study yielded site-specific information on spawn timing and rates of egg density, biomass, and in situ mortality, which in 1990 and 1991 was corrected for egg loss (Biggs and Baker 1993). The Egg Incubation Study provided measurements of egg mortality, hatching success and dynamics, and length and weight of newly hatched larvae.

Subsamples of newly hatched larvae from the Egg Incubation Study were evaluated for sublethal morphologic, genetic, and histopathologic damage (Hose et al., in press). Morphologic damage was assessed using graduated severity indices (GSI); each skeletal, craniofacial, and/or finfold defect was scored and summarized for each individual assessed. Higher GSI scores indicated more severe malformations. Genetic damage was measured in pectoral fins by determining the percentage of anaphase–telophase mitotic configurations with chromosome or chromatid breaks (Kocan et al. 1982). Larvae were evaluated for histopathologic damage using standard processing procedures (Hose et al., in press). A laboratory experiment evaluated relationships between exposure to an oil–water dispersion of *Exxon Valdez* oil and hatching dynamics, embryonic stage sensitivity, embryo–larval survival, morphologic defects, and genetic damage (Kocan et al., in press).

In 1989 two studies measured the abundance and distribution of herring larvae nearshore (McGurk 1990a) and offshore (Norcross and Frandsen 1996; Norcross, unpublished). Planktonic larvae between hatch and the start of exogenous feeding were sam-

pled in May and June near oiled and non-oiled spawning beds using bongo nets (McGurk 1990a). Site-specific abundances of newly hatched larvae were compared with egg abundances to estimate instantaneous mortality rates (McGurk and Brown, in press). Surviving pelagic larvae drifting offshore but within Prince William Sound were sampled using a Tucker trawl over a 3-month period (Norcross and Frandsen 1996; Norcross, unpublished). In both studies (nearshore and offshore trawl surveys), growth was measured using length–frequency analysis. Subsamples of larvae collected in May and June were evaluated for morphologic, genetic, and histopathologic damage as described above (Hose et al., in press).

*Abundance Estimation*

Fishery management assessments and estimates of the abundance of spawned eggs were augmented after the *Exxon Valdez* oil spill. In 1984 and 1988–1992, the total number of eggs deposited in Prince William Sound was estimated by egg deposition surveys; 1989–1991 estimates of egg deposition used a correction for eggs that were consumed or dislodged from the study area before assessment (egg loss). Methods for egg deposition surveys, estimates of the abundance of the spawning population, adult age composition sampling, and size at age sampling are described in detail by Biggs and Baker (1993).

## Overview of Damage Assessment Study Results

*Exposure*

Hydrocarbon chemistry results from mussel tissue, rather than direct measurements in herring eggs, were a more reliable and useful index of site-specific oil exposure of herring embryos. Because of low sample volumes, *Exxon Valdez* oil analysis of herring eggs yielded unacceptably high detection limits, and *Exxon Valdez* oil was detected in eggs from only Cabin Bay on Naked Island. The analyte profile was identical to that of whole oil, which was observed adhering to the egg surface. Using the surrogate measurement of *Exxon Valdez* oil concentrations in adjacent native mussels, oil was detected in most spawning sites sampled within the oil trajectory (Hose et al., in press). In 1989 mean ($\pm$ SE) concentrations of EVO-PAH in mussels within the oil trajectory were significantly higher than at non-oiled areas (753 $\pm$ 22 $\mu$g/kg versus 49 $\pm$ 8 $\mu$g/kg, respectively; the majority of the PAH detected at non-oiled sites was naphthalene). Tarballs and sheens were visually observed at two of the Rocky Bay sites on Montague Island, two of the Bass Harbor sites, Naked Island sites located within oil booms, and sites near Cabin Bay on Naked Island. The variability of mussel *Exxon Valdez* oil concentrations at the 1989 herring sites was similar to the variability observed from other studies sampling within herring spawning areas (Short and Babcock 1996, this volume); therefore, the concentrations measured at our study sites appear representative of the entire 1989 spawning area.

Because egg deposition was not uniform, site-specific egg biomass measurements were used to calculate the proportion of eggs exposed to *Exxon Valdez* oil. Fifty-three percent of the total egg biomass was within the oil trajectory. Of the total eggs deposited, 35% were in the Montague Island area and 18% were in the Naked Island area. Measurements of EVO-PAH in native and caged mussels confirmed the "oiled" designation of all mussel study sites within the oil trajectory (Short and Babcock 1996; Short and Harris 1996a). Although oil was present along the outside of both Rocky and Zaikof bays on Montague Island (Figure 1), *Exxon Valdez* oil was documented in mussels only at Rocky Bay (Figure 2; most northeastern embayment); no mussels were collected in Zaikof Bay (immediately southeast of Rocky Bay), which contained approximately 13% of the total 1989 egg biomass (E. D. Brown, unpublished results). We therefore estimate that between 40 and 50% of the herring eggs were exposed to *Exxon Valdez* oil in 1989.

In 1990 EVO-PAH was not detected in herring spawn sites, except for Smith Island (Figure 2, site O30–1990). This site was sampled only in 1990 and its eggs represented less than 10% of the total deposited in Prince William Sound.

*Effects on Embryos*

Mortality was measured in situ at non-oiled and oiled sites until hatching. Contamination with EVO-PAH did not significantly affect mortality during embryonic incubation (Brown 1995). Eggs collected from oiled sites (particularly eggs from upper depths) and hatched in the laboratory exhibited lower survival than those from non-oiled areas, but the difference was not statistically significant (McGurk 1990b).

*Effects on Newly Hatched Larvae*

Site-specific differences in survival became more pronounced after hatching. Mean instantaneous rates of in situ egg-to-larva mortality were significantly lower at the non-oiled areas (0.187/d at

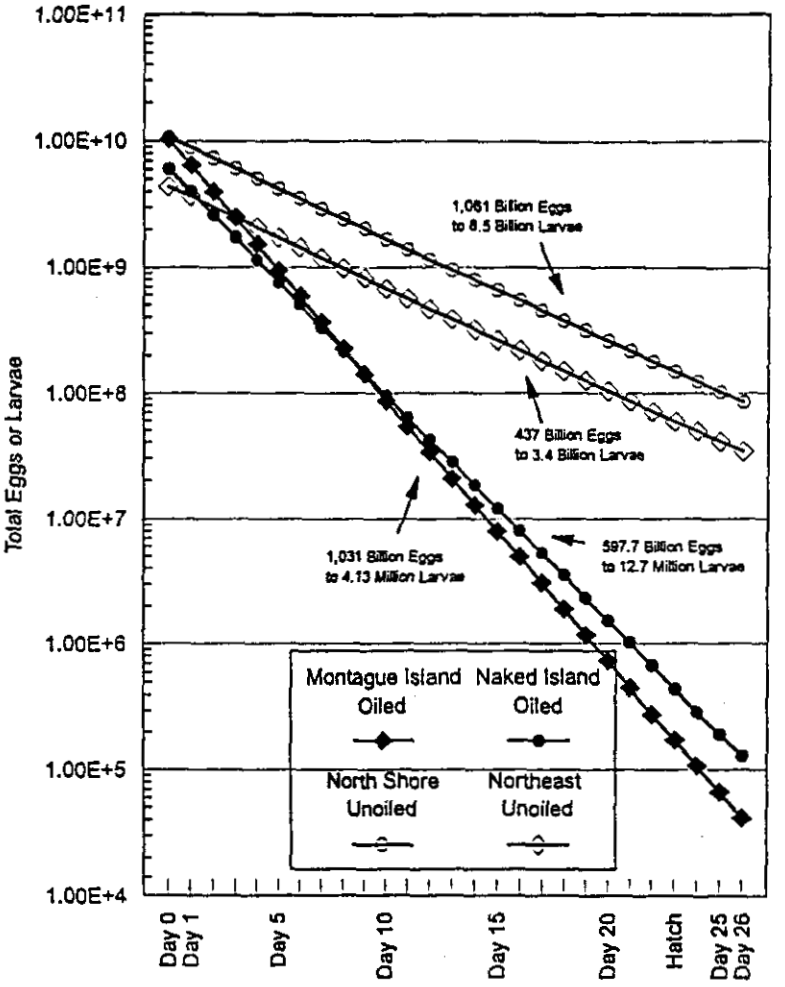



FIGURE 3.—Mortality of Pacific herring from *embryo to posthatch* (day 26 from fertilization), calculated as site-specific instantaneous mortality in the absence of any other estimates of larval survival that are not confounded with dispersal of larvae away from the beds, in two oiled and two non-oiled spawning sites, 1989 (McGurk and Brown, in press). The starting points for the curves are estimates of the actual numbers of eggs (logarithmic scale) deposited in each of the four major spawning areas.

Northeast and 0.186/d at North Shore) than at oiled areas (0.414/d at Naked Island and 0.477/d at Montague Island) (McGurk and Brown, in press); however, because of differences in beach characteristics, egg distributions, and larval dispersions between oiled and non-oiled areas, differences in egg–larval mortality estimates are not conclusive evidence of an oil effect. Site-specific instantaneous mortality rates, in the absence of any other estimates of larval survival that are not confounded with dispersal of larvae away from the egg beds, indicated that non-oiled areas produced more than 200 times more free-swimming larvae per billion eggs deposited 3 d after hatch than did the oiled areas (calculated by multiplying the initial abundance of eggs in each area by the mortality rate; Figure 3). The estimate of larval production surviving to the pelagic stage was 11.82 billion ($11.82 \times 10^9$) from the Northeast and North Shore non-oiled areas compared to 16.82 million from the Montague Island and Naked Island areas. When mortality rates of each of the non-oiled areas were applied to each of the two oiled areas, larval production expected in the absence of oil was estimated. Expected larval production in the oiled areas was estimated to be 12.98 billion larvae, with a difference between expected

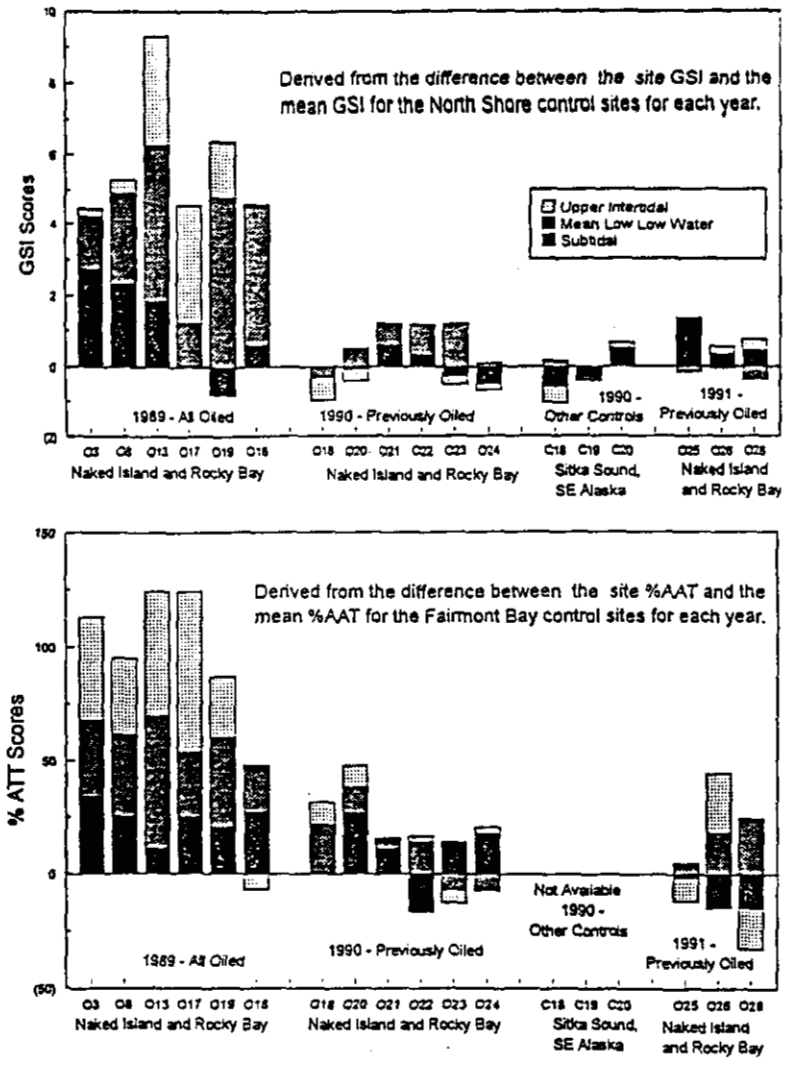



FIGURE 4.—Changes in graduated severity index (GSI) scores (top) and percentage anaphase aberrations (% AAT; bottom) for Pacific herring at the oiled sites and non-oiled sites in Galena Bay and Sitka Sound, southeast Alaska, relative to those indices in Fairmont Bay in the non-oiled North Shore area. The magnitude of the bars is calculated by subtracting mean scores by depth (upper intertidal, mean lower low water [0 m], and subtidal) at the Fairmont Bay sites from the mean scores for each site in the comparison.

and actual production of 12.96 billion larvae. This difference represents a 99.87% loss of potential larval production in oiled areas and a 52.3% loss overall. Posthatch larval weights and hatch timing were different between non-oiled and oiled areas in 1989 ($P < 0.01$). The strongest effect of oil was premature hatching. The presence of oil caused significant ($P < 0.01$) decreases in the 50% hatch date and in the fraction of larvae hatched in late stages of development. Hatching occurred sooner in the upper depths than in the lower depths and in oiled eggs than in non-oiled eggs. After corrections for age, posthatch larvae from oiled areas were 0.1 mm longer but weighed 4 $\mu$g less than larvae from non-oiled areas ($P < 0.01$) (McGurk 1990b). These measurements yield a condition index (weight/length$^3$) that is 7% less for oiled larvae.

Ratings of morphologic defects (graduated severity indices, GSI) were significantly higher at Naked Island and Rocky Bay on Montague Island than at Fairmont Bay in the non-oiled North Shore area, indicating more severe defects at the oiled sites

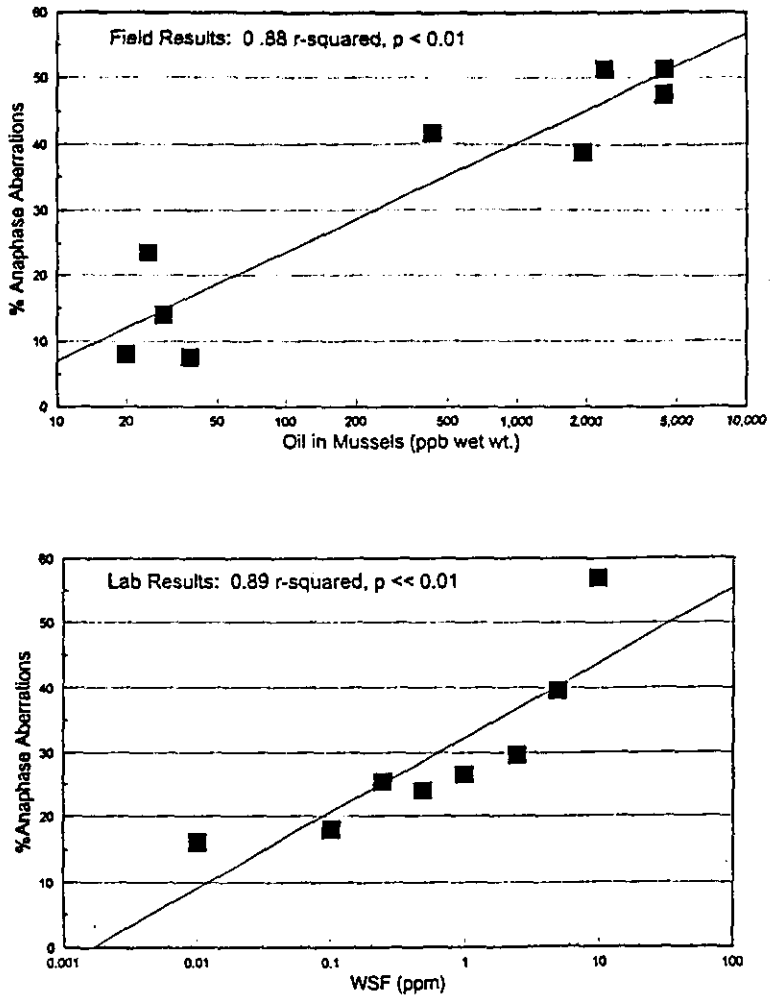



FIGURE 5.—Anaphase aberration rate in cells from posthatch larval Pacific herring as a measure of response to exposure level of *Exxon Valdez* oil (EVO) in the field (site-specific log-transformed EVO concentrations in mussels) and in the laboratory (log-transformed concentrations of high-molecular-weight, water-soluble fraction (WSF) of EVO in oil–water dispersions).

($P < 0.01$) (Figure 4, top). These abnormalities included skeletal, otic, or finfold defects and absent or abnormal lower jaws. Graduated severity index scores were significantly correlated ($P < 0.05$) with the log-transformed EVO-PAH concentrations present in adjacent mussels (Hose et al., *in press*). In 1989 GSI scores at all sites were higher than in successive years. Suboptimal laboratory incubation conditions present in 1989 were subsequently improved but would not have affected the magnitude of the difference observed between oiled and non-oiled treatments. In 1990 and 1991 GSI scores were generally similar between non-oiled and previously oiled areas. In 1990 a second non-oiled area, Sitka Sound in southeast Alaska, was evaluated as a control and yielded scores similar to those from North Shore. Relative differences in GSI scores at non-oiled areas in 1990 and 1991 were much less than differences between oiled and non-oiled stations in 1989. No histopathologic lesions attributable to *Exxon Valdez* oil exposure were observed in the 1990–1991 larvae.

Newly hatched larvae were also examined for genetic damage and analyzed with the same baseline approach as was used for assessing morphologic defects (Figure 4, bottom). At Fairmont Bay in the

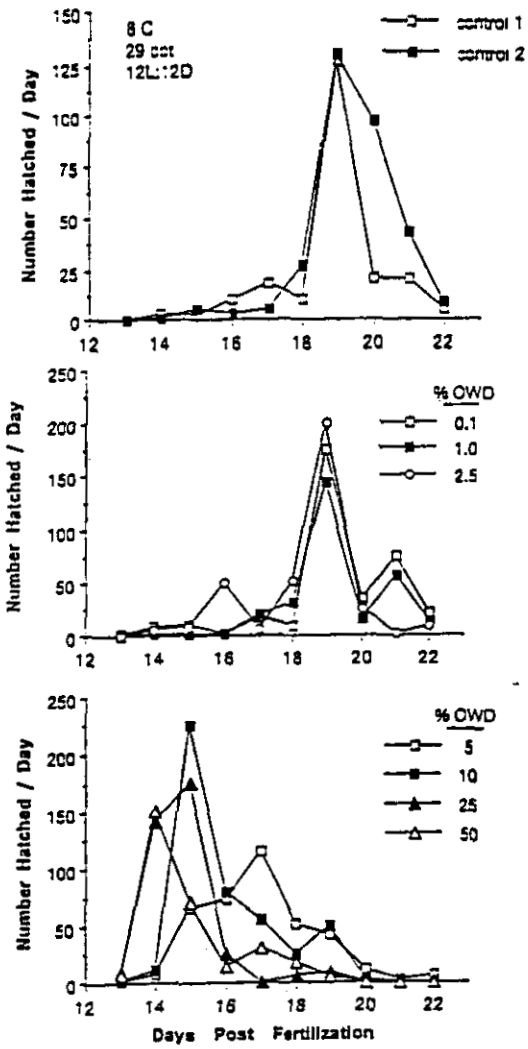



FIGURE 6.—Hatching dynamics of embryos of Prince William Sound Pacific herring exposed from fertilization to hatching to oil–water dispersions (OWD) measured by percent water-soluble fraction of Prudhoe Bay crude oil from the *Exxon Valdez* (Kocan et al., in press).

North Shore area, the anaphase aberration rate averaged 10% in 1989; this was not different from the 1990 mean of 18% but was significantly less than the 1991 value of 22% ($P < 0.05$). The 1989 to 1991 values suggest an upward trend in the baseline aberration rate; 1991 also had high in situ egg mortality rates (Brown 1995). In 1989, anaphase aberration rates were significantly elevated at all oiled sites ($P < 0.05$). Site-specific EVO-PAH concentrations were significantly correlated with the anaphase aberration rate ($r^2 = 0.88$, $P < 0.01$ and

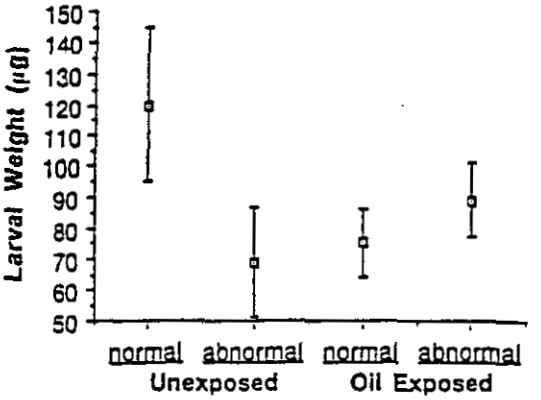



FIGURE 7.—Mean weight of newly hatched larvae of Pacific herring exposed continuously to oil–water dispersions as embryos (Kocan et al., in press).

Mantel's test, $P < 0.001$). After 1989 there were no genetic effects attributable to residual oil. In 1990 the anaphase aberration rate at Rocky Bay on Montague Island was higher than the North Shore rate, but the difference was much less than the 1989 difference.

Exposure of herring embryos in the laboratory to the water-soluble fraction of *Exxon Valdez* oil produced morphologic abnormalities, genetic damage, and other responses in newly hatched larvae identical to larval responses at oiled sites in 1989 (Kocan et al., in press) (Figure 5). The percentage of anaphase aberrations was a highly sensitive indicator of oil exposure (Figure 5). Premature hatch was well documented in exposures exceeding 5% oil–

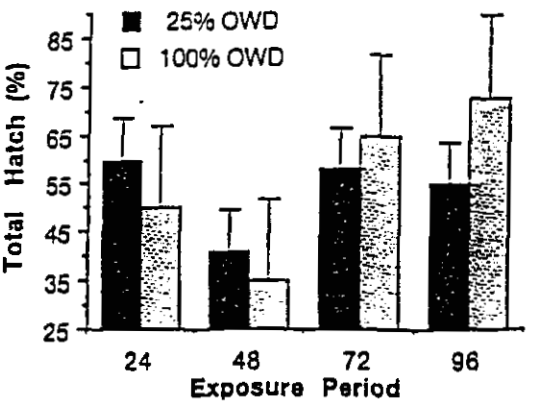



FIGURE 8.—Embryo development stage sensitivity for Pacific herring measured as percent of total hatch after exposure to the water-soluble fraction of *Exxon Valdez* oil–water dispersions (Kocan et al., in press).

water dispersion (OWD) or 0.48 mg/L of high-molecular-weight petroleum hydrocarbons (HMW; Figure 6). Larval weights were greatly reduced in abnormal larvae whether exposed to oil or not, but weights were also reduced in morphologically normal larvae exposed to oil (Figure 7). Exposure to 24-h pulses of OWD at different embryonic stages demonstrated that the earliest developmental stages (24–48 h postfertilization) were the most susceptible to toxicity (Figure 8). Differences in analytical techniques for *Exxon Valdez* oil preclude comparisons between effects levels in field and laboratory studies.

*Effects on Pelagic Larvae*

Two studies of growth in pelagic larvae were conducted in 1989, one evaluating herring larvae captured 1–5 km from spawning beds and the second assessing larvae farther offshore. Constant growth rates of nearshore larvae ranged from 0.10 to 0.17 mm/d among stations, and no difference was detected between oiled and non-oiled stations (McGurk and Brown, in press). Measurements of larvae captured at offshore stations yielded a growth rate of 0.15 mm/d (Norcross, unpublished), which is similar to the rate measured nearshore. More than 85% of larvae offshore were captured within the oil trajectory (Norcross and Frandsen 1996). In May, 61% of the larvae were collected just north of Naked Island (247 larvae/100 $m^3$) and 27% off Knight Island (108 larvae/100 $m^3$). By June, 87% of the larvae were captured in Montague Strait (69–733 larvae/100 $m^3$). Few larvae were collected in July.

Morphologic defects were evaluated in larvae collected near their spawning beaches and farther offshore in May and June 1989. Soon after hatching in May, larvae near non-oiled beaches (North Shore area and Tatitlek Narrows, Northeast area) had GSI scores significantly lower than those from oiled sites (equivalent of 0–16% abnormal). At Bass Harbor on Naked Island, 72% of the larvae were abnormal, and at Montague Island, where many premature larvae were collected, 80% were abnormal. In late May, larvae from the two non-oiled areas still had low GSI scores relative to those from the oiled stations (48–52% abnormal). In contrast, Norcross (unpublished) found that although 89% of the larvae captured offshore throughout Prince William Sound were severely deformed in May, only 24% were severely deformed in June. In both months, morphologic defects were significantly more severe among larvae from areas within the oil trajectory.

Pelagic larvae were also evaluated for genetic damage (J. E. Hose, unpublished data). In early May, anaphase aberration rates were low (6–8%) near non-oiled beaches of North Shore and Tatitlek Narrows. Anaphase aberration rates were significantly higher in nearshore larvae from Bass Harbor (33%) and Montague Island (28%) and in offshore larvae north of Naked Island (35%). In late May to early June, aberration rates of 2- to 4-week-old larvae decreased off oiled beaches. Although lower than earlier in May, the aberration rates of Bass Harbor larvae (21%) and Montague Island larvae (14%) were still significantly elevated over rates from the non-oiled areas near Fairmont Bay, Tatitlek Narrows, and Esther Island (8%). Values for larvae collected offshore in Hinchinbrook Entrance (16%) in late May to early June were similar to values for Montague Island (14%), where these larvae are thought to originate. During this same period, the aberration rate north of Naked Island declined from 35 to 22%. Three offshore sites within the western part of the oil trajectory also had elevated aberration rates (21 and 26%). No histopathological lesions attributable to *Exxon Valdez* oil exposure were observed in pelagic larvae (G. D. Marty, unpublished data).

## Discussion

The *Exxon Valdez* oil spill occurred shortly before the most toxicologically sensitive period of embryo–larval development for Pacific herring (Capuzzo 1987; Kocan et al., in press), and a large proportion of the eggs deposited were exposed. In addition, 30–50% of the eggs were distributed near the surface (E. D. Brown, unpublished data), where exposure to the toxic microlayer (Kocan et al. 1987) of oil was likely during tidal cycles. Using site-specific egg biomass measurements, approximately 53% of total herring eggs deposited were within the oil trajectory. Although only a small portion of the spawn was visibly contaminated with crude oil, measurements of EVO-PAH in native mussels adjacent to our study sites demonstrated that most spawning areas within the oil trajectory were exposed to *Exxon Valdez* oil; only one area within the trajectory, Zaikof Bay, was not analyzed for this oil. The mean EVO-PAH concentration in mussels from oiled areas was 753 ppb wet weight (SE, 22 ppb); this estimate is similar to the 700 ppb (SE, 25 ppb) mean concentration measured at oiled sites in April in a more comprehensive study of Prince William

Sound (Short and Babcock 1996). Our estimate that between 40 and 50% of the total 1989 eggs deposited were exposed to *Exxon Valdez* oil is much greater than that estimated by Pearson et al. (1995), who found visible oil only on 7% of the shoreline with herring spawn. From recent studies (Short and Babcock 1996; Short and Harris 1996a; Wolfe et al., in press), it is apparent that much of the spilled oil was not visible because major portions dispersed into the water column or were subsequently dispersed from heavily oiled beaches to remote locations.

It would have been preferable to relate embryo–larval responses to actual oil concentrations in herring eggs rather than to the surrogate mussel measurement; however, the analytical sensitivity of the chemical data for the herring egg samples was severely compromised by the small quantity of tissue analyzed and the correspondingly high hydrocarbon detection limits. Thus, the presence of *Exxon Valdez* oil hydrocarbons in herring egg samples from Cabin Bay is strong evidence for contamination at this area, but the presence of *Exxon Valdez* oil cannot be ruled out in the remaining samples that did not show detectable EVO-PAH. In a corresponding 1989 study, Pearson et al. (1995) found *Exxon Valdez* oil concentrations in herring egg samples elevated above non-oiled areas only at Cabin Bay. Pearson et al. (1995) did not find any significant correlations between biological responses and oil exposure in 1989 or 1990 except for a positive association between PAH concentrations in eggs-on-kelp and developed eggs in the mid intertidal zone (0 to −2.4 m MLLW) for 1989. Their measurement of *Exxon Valdez* oil in herring eggs-on-kelp is biased toward underestimating the oil concentrations because kelp does not accumulate petroleum hydrocarbons (NRC 1985) and the proportion of kelp in their sample was not described.

Sublethal effects in posthatch and pelagic larval herring were substantial and measurable throughout the oil trajectory during their entire pelagic period. Morphologic defects and genetic damage were strongly associated with ambient *Exxon Valdez* oil concentrations in mussels ($P < 0.001$ to $P < 0.05$). The morphologic defects, premature hatch, and low larval weights observed in *Exxon Valdez* oil-exposed embryo–larval herring are not specific to oil exposure because they are generalized responses to stress during early development (von Westernhagen 1988; Purcell et al. 1990). Pearson et al. (1985) stated that oil toxicity in embryo–larval herring is manifest primarily as morphologic defects. This response has been consistently replicated in field studies of other oil spills using herring and other fish species (see Urho 1990) and in numerous laboratory exposures to crude oil (Lindén 1975; Lonning 1977; Lindén 1978), oil fractions (Smith and Cameron 1979; Carls and Rice 1989) and to individual PAH components of petroleum (Struhsaker et al. 1974; Hose et al. 1984; Stene and Lonning 1984). In the egg incubation experiment, the baseline scores for defect severity were higher in 1989 than in subsequent years; the cause has not been fully resolved. The magnitude of the difference between oiled and non-oiled areas was much greater than temporal or spatial variations among non-oiled areas (Figure 4). Statistical relationships between *Exxon Valdez* oil indices and morphologic defects were stronger with increasing molecular weight of the EVO-PAH, an association with substantial experimental evidence (Black et al. 1983).

Other *Exxon Valdez* oil-induced effects, such as premature hatch and reduced larval weights observed in both the laboratory and the field, would be expected to reduce posthatching survival. Smaller larvae with less-developed jaws would feed less efficiently and be more susceptible to predation (von Westernhagen 1988). McGurk and Brown (in press) found that instantaneous mortality rates between egg and early larval periods were two to three times higher at the oiled stations in Prince William Sound than at the non-oiled stations; however, physical differences between beaches at the two groups confounded interpretation of the data. These results are similar to those from studies with pink salmon that documented persistently lower survival of young from oiled streams until 1992 (Bue et al. 1996, this volume).

The response with the most specificity to crude oil contamination is genetic damage, as measured by the anaphase aberration rate. This endpoint is highly sensitive to mutagenic compounds (Kocan et al. 1982) (Figure 5), some of which are present in crude oils (Dipple 1982; Flesher and Meyers 1991), and to oil–water dispersion of *Exxon Valdez* oil in laboratory exposure (Kocan et al., in press). Significant responses in cells of marine organisms are typically observed in the low (ppb) range for organic mutagens (Kocan et al. 1982; Anderson et al. 1994). Anaphase aberration rates in newly hatched herring larvae were strongly correlated with EVO-PAH concentrations in adjacent mussels (Figure 5); aberration rates were also correlated with every measured PAH except naphthalene, which is nonmutagenic. In pelagic larvae, genetic damage was greatest near oiled areas of southwestern Prince William Sound and was undetectable at open water

sites in Montague Strait and Hinchinbrook Entrance. This pattern is consistent with observations that oiled beaches served as persistent reservoirs of oil and that oil concentrations in open water were low (Short and Harris 1996a). Anaphase aberration rates progressively decreased at Naked and Montague islands during the 6-week period from hatching to metamorphosis. The decline in aberration rates could have resulted from mortality of severely damaged larvae, mixture of larvae from oiled and non-oiled areas, and reduced toxicity of the free-floating, weathered oil.

The 1989–1991 aberration rates in larvae from non-oiled areas suggest an upward trend in the baseline aberration rate. Rather than being caused by persistent oil, based on the analysis of in situ mussel tissue, the increase in 1991 may be related to the severe climatic conditions, unusually cold water temperatures, and enhanced mortality that year. In addition, the upward trend in the baseline aberration rate could have been effected by residual genetic damage in the returning adults exposed to oil. Whereas the adults may have spawned in non-oiled areas, they may have traversed oiled areas during the summer of 1989 and beyond in the normal course of seasonal movement; there are no data to support this theory of heritable genetic damage in the returning 1990 and 1991 adults.

That we can potentially track oil toxicity in a planktonic organism by monitoring genotoxic effects over space and time is noteworthy. In addition, the correlations suggest that genetic damage may be detected in the absence of observable deformities. Genetic measurements, although rarely performed in these situations, are a recommended component of oil spill assessments (NRC 1985). In the single oil spill in which genetic measurements of fish embryos were used (the *Argo Merchant* spill), genetic damage was observed near floating oil sheens and decreased with distance from the oil (Longwell 1977). Measurements of genetic damage would be expected to be specific for mutagenic events; however, the influences of natural environmental extremes (temperature, oxygen, etc.) and mortality have not been systematically investigated. Although in general both the anaphase aberration and malformation endpoints were generally correlated in larval herring and were of similar sensitivity in laboratory exposures to *Exxon Valdez* oil (Kocan et al., in press), genetic damage can be considered a more specific response to oil exposure.

The consequences of genetic damage during early development are not predictable with any certainty but probably include developmental malformations and decreased survival (Cross et al. 1987). The demonstration of genetic damage in somatic cells of herring larvae, however transitory, raises the possibility of germ-cell damage that could lead to decreased reproductive potential. Reduced growth and immune responsiveness result from generalized metabolic alterations caused by oil exposure (Capuzzo 1987).

We observed extremely low growth rates in nearshore herring larvae in May and in offshore larvae through June 1989. Other researchers have measured growth rates in larval Alaskan herring at much higher ranges: 0.30 mm/d (McGurk et al. 1993), 0.18–0.29 mm/d (McGurk 1984; Norcross, unpublished), and 0.31–1.48 mm/d for Alaskan herring rearing in Norway (Wespestad and Moksness 1990). Many of the lowest reported rates, 0.19–0.22 mm/d, resulted from starvation experiments (McGurk 1984). There was no evidence of reduced prey fields that would have affected growth in 1989 (Celewycz and Wertheimer 1996, this volume; McGurk and Brown, in press), and water temperatures were within normal ranges for Prince William Sound. The distribution of herring larvae in spring 1989 is consistent with potential exposure to dispersed oil from oiled beaches and by ingestion of oiled prey. Particulate oil is thought to have been bioavailable throughout the oil trajectory into June (Short and Harris 1996a).

Although estimates of the geographic extent and magnitude of damage are difficult to develop because of the complexities of the Prince William Sound ecosystem, we estimate that more than 40% of the 1989 year-class was affected by *Exxon Valdez* oil at toxic levels. The high correlation of increased chromosome aberrations at anaphase in larvae collected in oiled areas provides additional proof of exposure. Although we could not predict the exact proportion of herring exposed to *Exxon Valdez* oil concentrations sufficient to cause subsequent mortality, we could compare differences between expected and actual larval production using instantaneous mortality rates measured at control sites as baseline rates. Using the 1989 Prince William Sound estimates, the difference between expected (24.80 billion) versus actual (11.84 billion) larval production represents a 52.3% decrease in the sound; there was a 99.9% drop in larval production (12.98 billion expected versus 16.82 million actual) in the oiled areas alone. A decrease in abundance of this magnitude may be observable depending on the strength of the year-class. The 1989 year-class was a minority of the 1993 spawning population, one of the smallest cohorts observed in Prince William

Sound (Funk 1993), and returned to spawn with an adult herring population reduced by approximately 75% apparently because of the occurrence of a widespread epizootic (Meyers et al. 1994; Marty, unpublished data); however, natural variability and density-dependent effects cannot be ruled out as the cause of the small year-class and disease. There is a high degree of natural variability in herring recruitment; although environmental factors seem to cause a significant portion of that variability, the mechanisms involved remain elusive (Collie 1990). We currently do not predict recruitment because the processes driving it are poorly understood. Current studies on juvenile herring and their ecosystem (Cooney 1995) and on disease and reproductive impairment in adult herring in Prince William Sound may reveal enough information about herring recruitment to revisit herring damage assessment results and better estimate the effect of the *Exxon Valdez* oil spill on this Prince William Sound herring population.

## Acknowledgments

We thank the many researchers who contributed to the implementation of this project or to preparation of the manuscript: National Marine Fisheries Service personnel (S. Rice, A. Moles, M. Carls, S. Korn, C. Broderson, J. Hamilton, and T. Rutecki), University of Alaska faculty (M. Stekoll, L. Haldorson, J. Collie, and T. Cooney), Canadian Department of Fisheries and Oceans biologists (M. Stocker, J. Schweigert, C. Haegele, D. Hay, and R. Tanasichuk), Alaska Department of Fish and Game (ADFG) scientists (J. Brady, B. Bechtol, D. Crawford, W. Donaldson, F. Funk, H. Geiger, D. Jones, S. Morstad, S. Sharr, K. Schultz, J. Wilcock, S. Fried, and L. Brannian), ADFG technicians (D. Coyer, S. Walker, T. McNeil, B. Haley, D. Norman, M. Miller, D. Nees, K. Becker, E. Wolfe, and S. Morgan). This article is contribution PP-115 of ADFG, Commercial Fisheries Management and Development Division, Juneau.

## References

Anderson, S. L., J. Knezovich, and J. E. Hose. 1994. Genotoxic and developmental effects in sea urchins are sensitive indicators of effects of gentoxic chemicals. Environmental Toxicology and Chemistry 13: 1033–1041.

Biggs, E. D., and T. T. Baker. 1993. Studies on Pacific herring *Clupea pallasi* spawning in Prince William Sound following the 1989 *Exxon Valdez* oil spill, 1989–1992. Draft report for Natural Resource Damage Assessment Fish/Shellfish Study 11, *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

Black, J. A., W. J. Birge, A. G. Westerman, and P. C. Francis. 1983. Comparative toxicology of aromatic hydrocarbons. Fundamental and Applied Toxicology 3:353–358.

Brown, E. D. 1995. Injury to Prince William Sound following the *Exxon Valdez* oil spill: final report for Natural Resource Damage Assessment Fish/Shellfish Study 11. *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

Bue, B. G., S. Sharr, S. D. Moffitt, and A. K. Craig. 1996. Effects of the *Exxon Valdez* oil spill on pink salmon embryos and preemergent fry. American Fisheries Society Symposium 18:619–627.

Capuzzo, J. M. 1987. Biological effects of petroleum hydrocarbons: assessments from experimental results. Pages 343–410 *in* D. F. Boesch and N. N. Rabalais, editors. Long-term environmental effects of offshore oil and gas development. Elsevier Applied Science Publishers, London.

Carls, M. G., and S. D. Rice. 1989. Abnormal development and growth reductions of pollock *Theragra chalcogramma* embryos exposed to water-soluble fractions of oil. U.S. National Marine Fisheries Service Fishery Bulletin 88:29–37.

Celewycz, A. G., and A. C. Wertheimer. 1996. Prey availability to juvenile salmon after the *Exxon Valdez* oil spill. American Fisheries Society Symposium 18:564–577.

Collie, J. S. 1990. Herring population dynamics and management in Sitka Sound, Alaska. Pages 463–476 *in* Proceedings, international herring symposium. Alaska Sea Grant College Program AK-SG-91-01, Fairbanks.

Conover, R. J. 1971. Some relations between zooplankton and bunker C oil in Chedabucto Bay following the wreck of the tanker *Arrow*. Journal of the Fisheries Research Board of Canada 28:1327–1330.

Cooney, R. T. 1995. Sound ecosystem assessment (SEA)—an integrated science plan for the restoration of injured species in Prince William Sound. Draft final report to the *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

Cross, J. N., and six coauthors. 1987. Contaminant concentrations and toxicity of sea-surface microlayer near Los Angeles, California. Marine Environmental Research 23:307–323.

Dean, K. G., W. J. Stringer, J. E. Groves, K. Ahlnas, and T. C. Royer. 1990. The *Exxon Valdez* oil spill: satellite analyses. Pages 492–502 *in* Proceedings, conference on oil spills, management and legislative implications. American Society of Civil Engineers, New York.

Dipple, A. 1982. Polynuclear aromatic carcinogens. Pages 246–265 *in* M. Cooke, A. J. Dennis, and G. L. Fisher, editors. Polynuclear aromatic hydrocarbons: physical and biological chemistry. Springer-Verlag, New York.

Flesher, J. W., and S. R. Meyers. 1991. Rules of molecular geometry for predicting carcinogenic activity of unsubstituted polynuclear aromatic hydrocarbons. Teratogenesis, Carcinogenesis and Mutagenesis 11: 41–54.

Funk, F. 1993. Forecast of the Pacific herring biomass in Prince William Sound, Alaska, 1993. Alaska Department of Fish and Game Regional Information Report 5J93, Juneau.

Galt, J. A., W. J. Lehr, and D. L. Payton. 1991. Fate and transport of the *Exxon Valdez* oil spill. Environmental Science and Technology 25:202–209.

Hose, J. E., J. B. Hannah, H. W. Puffer, and M. L. Landolt. 1984. Histologic and skeletal abnormalities in benzo[*a*]pyrene-treated rainbow trout alevins. Archives of Environmental Contamination and Toxicology 13:675–684.

Hose, J. E., M. D. McGurk, G. D. Marty, D. E. Hinton, E. D. Biggs, and T. T. Baker. In press. Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphologic, cytogenetic, and histopathological assessments, 1989–1991. Canadian Journal of Fisheries and Aquatic Sciences.

Kocan, R. M., J. E. Hose, E. D. Biggs, and T. T. Baker. In press. Laboratory evaluation of herring (*Clupea pallasi*) embryo sensitivity to Prudhoe Bay petroleum hydrocarbons, and in situ exposure of embryos at oiled and unoiled sites in Prince William Sound. Canadian Journal of Fisheries and Aquatic Sciences.

Kocan, R. M., M. L. Landolt, and K. M. Sabo. 1982. Anaphase aberrations: a measure of genotoxicity in mutagen-treated fish cells. Environmental Mutagenesis 4:181–189.

Kocan, R. M., H. V. Westernhagen, M. L. Landolt, and G. Furstenberg. 1987. Toxicity of sea-surface microlayer: effects of hexane extract on Baltic herring (*Clupea harengus*) and Atlantic cod (*Gadus morhua*) embryos. Marine Environmental Research 23:291–305.

Lindén, O. 1975. Acute effects of oil and oil/dispersant mixture on larvae of Baltic herring. Ambio 4(3):130–133.

Lindén, O. 1978. Biological effects of oil on early development of the Baltic herring *Clupea harengus membras*. Marine Biology 45:273–283.

Longwell, A. C. 1977. A genetic look at fish eggs and oil. Oceanus 20:46–48.

Lonning, S. 1977. The effects of crude ekofisk oil and oil products on marine fish larvae. Astarte 10:37–48.

McGurk, M. D. 1984. Ring deposition in the otoliths of larval Pacific herring, *Clupea harengus pallasi*. U.S. National Marine Fisheries Service Fishery Bulletin 82:113–120.

McGurk, M. D. 1990a. Early life history of Pacific herring: Prince William Sound herring larvae survey. Final report to NOAA (National Oceanic and Atmospheric Administration) NOS (National Ocean Service), 50ABNC-7-00141, Anchorage, Alaska.

McGurk, M. D. 1990b. Early life history of Pacific herring: 1989 Prince William Sound herring egg incubation experiment. Final report and appendices to NOAA (National Oceanic and Atmospheric Administration) NOS (National Ocean Service), 50ABNC-7-00141, Anchorage, Alaska.

McGurk, M. D. 1991a. Viable hatch of Pacific herring eggs from Prince William Sound and Sitka Sound, Alaska, in 1990. Final report to Alaska Department of Fish and Game, IHP-90-055, Cordova.

McGurk, M. D. 1991b. Viable hatch of Pacific herring eggs from Prince William Sound and Sitka Sound, Alaska, in 1991. Final report to Alaska Department of Fish and Game, IHP-91-042, Cordova.

McGurk, M. D. 1993. Revised appendices of Prince William Sound herring egg incubation experiments: 1989, 1990 and 1991. Final report to Alaska Department of Fish and Game, IHP-93-011, Cordova.

McGurk, M. D., and E. D. Brown. In press. Egg-larval mortality of Pacific herring in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. Canadian Journal of Fisheries and Aquatic Sciences.

McGurk, M. D., A. J. Paul, K. O. Coyle, D. A. Ziemann, and L. J. Haldorson. 1993. Relationship between prey concentration and growth, condition, and mortality of Pacific herring, *Clupea pallasi*, larvae in an Alaskan subarctic embayment. Canadian Journal of Fisheries and Aquatic Sciences 50:163–180.

Meyers, T. R., and six coauthors. 1994. Epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA associated with the isolation of North American viral hemorrhagic septicemia virus (VHSV). Diseases of Aquatic Organisms 19:27–37.

Norcross, B. L., and M. Frandsen. 1996. Distribution and abundance of larval fishes in Prince William Sound, Alaska, during 1989 after the *Exxon Valdez* oil spill. American Fisheries Society Symposium 18:463–486.

NRC (National Research Council). 1985. Oil in the sea—inputs, fates and effects. National Academy Press, Washington, DC.

Pearson, W. H., E. Moksness, and J. R. Skalski. 1995. A field and laboratory assessment of oil spill effects on survival and reproduction of Pacific herring following the *Exxon Valdez* spill. Pages 626–661 *in* P. G. Wells, J. N. Butler, and J. S. Hughes, editors. *Exxon Valdez* oil spill: fate and effects in Alaskan waters. American Society for Testing and Materials Special Technical Publication 1219, Philadelphia.

Pearson, W. H., D. L. Woddruff, 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 (2311205196) to API (American Petroleum Institute), Environmental Affairs Department, Washington, DC.

Purcell, J. E., D. Grosse, and J. J. Glover. 1990. Mass abundances of abnormal Pacific herring larvae at a spawning ground in British Columbia. Transactions of the American Fisheries Society 119:463–469.

Royer, T. C., J. A. Vermersch, T. J. Weingartner, H. J. Niebauer, and R. D. Muench. 1990. Ocean circulation influencing the *Exxon Valdez* oil spill. Oceanography 3:3–10.

Short, J. W., and M. M. Babcock. 1996. Prespill and postspill concentrations of hydrocarbons in mussels and sediments in Prince William Sound. American Fisheries Society Symposium 18:149–166.

Short, J. W., and P. M. Harris. 1996a. Petroleum hydrocarbons in caged mussels deployed in Prince William

Sound after the *Exxon Valdez* oil spill. American Fisheries Society Symposium 18:29–39.

Short, J. W., and P. M. Harris. 1996b. Chemical sampling and analysis of petroleum hydrocarbons in the near-surface seawater of Prince William Sound after the *Exxon Valdez* oil spill. American Fisheries Society Symposium 18:17–28.

Smith, R. L., and J. A. Cameron. 1979. Effect of water soluble fraction of Prudhoe Bay crude oil on embryonic development of Pacific herring. Transactions of the American Fisheries Society 108:70–75.

Stene, A., and S. Lonning. 1984. Effects of 2-methylnaphthalene on eggs and larvae of six marine species. Sarsia 69:199–203.

Struhsaker, J. W., M. B. Eldridge, and T. Echeverria. 1974. Effects of benzene (a water soluble component of crude oil) on eggs and larvae of Pacific herring and northern anchovy. Pages 253–284 *in* F. J. Vernber and W. B. Vernberg, editors. Pollution and physiology of marine organisms. Academic Press, New York.

Urho, L. 1990. Impact of an oil spill on herring stock. Pages 569–582 *in* Proceedings, international herring symposium. Alaska Sea Grant College Program AK-SG-91-01, Fairbanks.

von Westernhagen, H. 1988. Sublethal effects of pollutants on fish eggs and larvae. Pages 253–346 *in* W. S. Hoar and D. J. Randall, editors. Fish physiology, volume XIA. Academic Press, New York.

Wespestad, V. G., and E. Moksness. 1990. Observations on growth and survival during the early life history of Pacific herring *Clupea pallasi* from Bristol Bay, Alaska, in a marine mesocosm. U.S. National Marine Fisheries Service Fishery Bulletin 88:191–200.

Wolfe, D. A., and eleven coauthors. In press. Fate of the oil spilled from the T/V *Exxon Valdez* in Prince William Sound, Alaska. Environmental Science and Technology.