

**EARLY LIFE HISTORY OF PACIFIC HERRING:  
1989 PRINCE WILLIAM SOUND HERRING EGG  
INCUBATION EXPERIMENT**

**by**

**Michael McGurk, David Warburton,  
Terry Parker, and Mark Litke**

**Triton Environmental Consultants Ltd.  
#120 -13511 Commerce Parkway  
Richmond, B. C., Canada  
V6V 2L1**

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## ACKNOWLEDGMENTS

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## ABSTRACT

In March, 1989, oil spilled from the tanker Exxon Valdez, washed onto Pacific herring, Clupea pallasii haren gus, spawning beaches in Prince William Sound, Alaska. The purpose of this study was to measure the viable hatch of herring eggs spawned on oiled and non-oiled beaches of the Sound. Over 180 samples of live eggs were taken from five control transects outside of the contaminated area and 18 transects within the contaminated area. They were flown to the Vancouver Aquarium and incubated to hatch.

Fifty nine percent (SD = 0.18) of the 180 samples of eggs survived incubation and 84% (SD = 0.12) of the newly-hatched larvae were viable, giving a mean viable hatch of 50% (SD = 0.17). This is within the range of survival and viability reported in the scientific literature for natural herring spawn not contaminated by hydrocarbons.

Univariate statistics showed that oil had no significant ( $P > 0.05$ ) effect on survival of eggs or on viability of larvae, but that it had a significantly ( $P = 0.033$ ) negative effect on percent viable hatch, on the age of 50% hatch ( $P < 0.001$ ), and on the frequency of the latest stage of development at hatch ( $P = 0.007$ ). Multivariate statistics showed that the effect of oil on the vector of biological variables was significant ( $0.02 < P < 0.05$ ). One of reasons for a weak relationship between oil and the biology of herring embryos is that the presence/absence index of oil contamination is an imprecise index of actual exposure to oil. The oil effect was also partly masked by environmental factors associated with depth that exerted a strong control over survival, age at hatch, and stage of development at hatch.

Eggs died at a constant rate of 3%-d-1 over the incubation period for both oiled and control samples. The ratio of live eggs to total eggs was significantly ( $P < 0.01$ ) lower in oiled eggs than in control eggs and in shallow depth classes compared to deep depth classes, but this was solely a reflection of the strong influence of the schedule of hatching and not a result of differences in survival. The ratio of live to total eggs at the beginning of the experiment was an imprecise index of egg survival.

The fraction of larvae that were viable, as defined by the absence of gross morphological abnormalities, did not vary significantly with oil treatment or depth.

Only one of the six major types of deformity exhibited a significant correlation with oil treatment; missing or deformed jaws were significantly ( $0.001 < P < 0.01$ ) more frequent in oiled samples than in control samples. However, since jaw deformities were only the third most common deformity, they did not affect the overall relationship between viability, oil treatment and depth.

The strongest effect of oil was an acceleration of embryo development. The presence of oil caused significant ( $P < 0.01$ ) decreases in the date of 50% hatch and in the fraction of larvae hatched in late stages of development. Early hatched larvae tended to be shorter, heavier, to carry a larger yolk sac and to be less developed than late hatched larvae. After corrections for the effects of age, small but significant ( $P < 0.01$ ) differences in size were found between viable larvae from oiled and control groups and between depth classes. Oiled larvae were 0.1 mm longer and 4  $\mu\text{g}$  lighter in weight than control larvae, so their condition ( $WL^{-3}$ ) was 7% lower than control larvae. Length decreased with depth, and weight increased with depth, so condition increased at a rate of about  $1\% \cdot \text{ft}^{-1}$ . These results suggest that oil may have stimulated metabolism and development of larvae. Water temperature during early incubation on the spawning grounds may also have played a confounding role; water temperature at control sites were always several degrees higher than temperatures at oiled sites.

Artificial variables were created from the matrix of data using factor analysis. Factor 3 was found to contain all of the information that was correlated with the presence of oil. In the absence of any other information on oil exposure, this factor was used to rank the 1989 herring spawning grounds in Prince William Sound by relative oil impact.

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\*The appendices are too large to include in this volume. Readers desiring the appendices may obtain them from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

## 1. INTRODUCTION

On March 24, 1989, the oil tanker Exxon Valdez struck Bligh Reef and spilled 250,000 bbl of Prudhoe Bay crude oil onto the surface of Prince William Sound, Alaska. Several weeks later, Triton Environmental Consultants Ltd. was hired by the U.S. National Oceanic and Atmospheric Administration (NOAA) to assess the impact of this spill on the viable hatch of Pacific herring, Clupea harengus pallasii, eggs laid on beaches in the Sound. This is the final report of those investigations. Appendices to this report are contained in a separate volume.

The study was designed by Triton in cooperation with the Alaska Department of Fish and Game (ADF&G). ADF&G conducted a SCUBA survey of the proportion of live to dead herring eggs at 19 transects in the Sound as part of their research on the effects of the spill on herring in Prince William Sound. This survey could not measure the actual hatching success of these eggs or the viability of newly-hatched larvae, so an incubation experiment was designed to extend monitoring into the late embryo and early larval stages. Triton conducted this experiment. Its objectives were to measure egg survival, larval viability, and the mean length, weight and fitness of newly-hatched larvae. These variables were compared between oiled and non-oiled samples.

Triton also conducted a companion study in 1989- a survey of growth, mortality and dispersal of wild herring larvae in the Sound. The results of that study are reported by McGurk et al. (1990).

## 2. MATERIALS AND METHODS

### 2.1 Study Sites

In 1989 herring spawned at four major sites in Prince William Sound: the Northeast area centered on Tatitlek Narrows, the North area centered on Fairmount Bay, the Naked Island archipelago, and the northern end of Montague Island (Fig. 1). Oil from the Exxon Valdez drifted southwest from Bligh Reef through the Naked Island archipelago, and along the eastern and western shores of Knight Island and around the western shore of Montague Island (Fig. 2). Therefore, beaches on Naked and

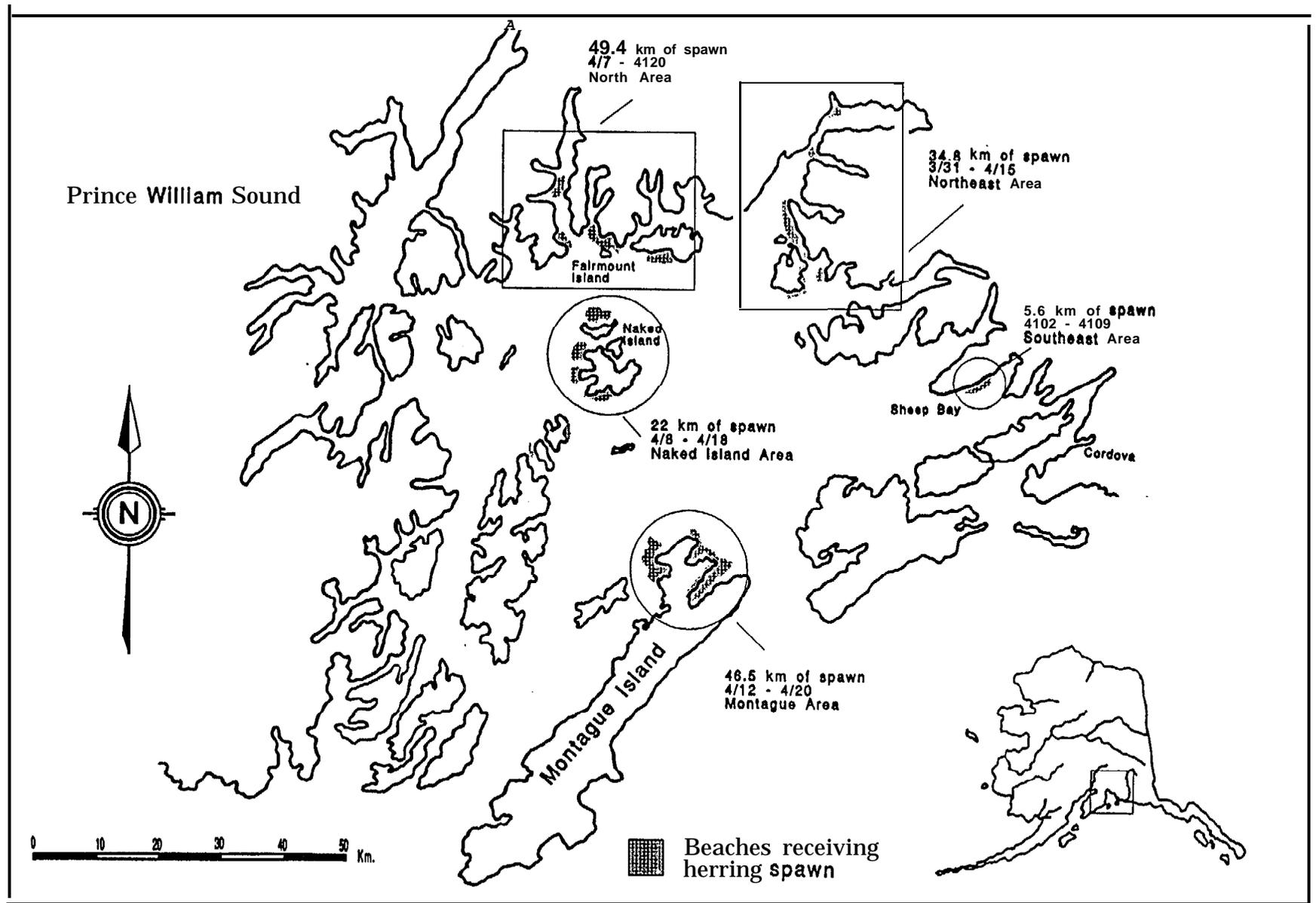


Figure 1. Map of Prince William Sound showing the four major herring spawning areas and the locations where eggs were collected for the incubation experiment.

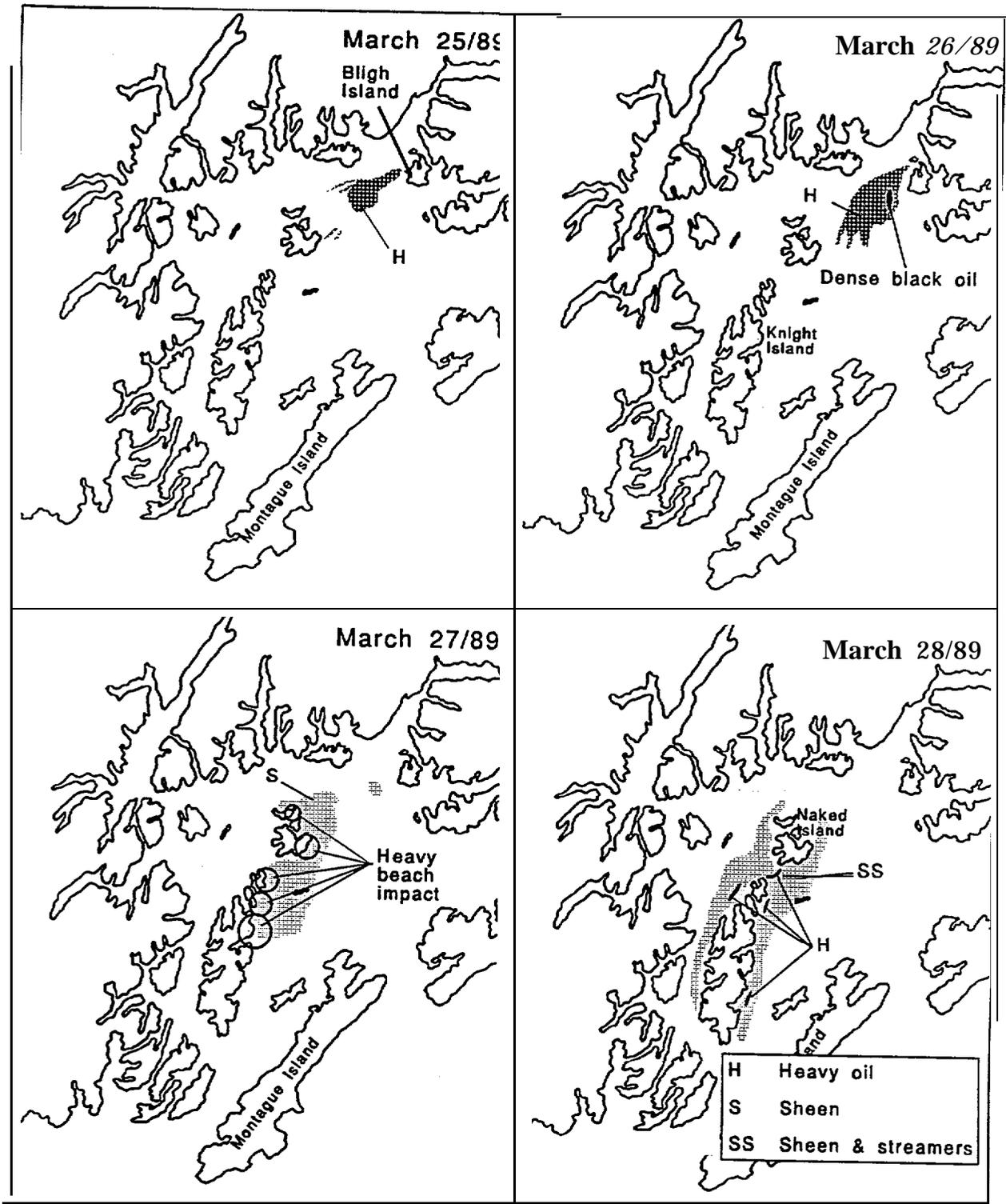


Figure 2. Seven maps of Prince William Sound showing the trajectory of oil spilled from the Exxon Valdez from March 25 to April 4, 1989.

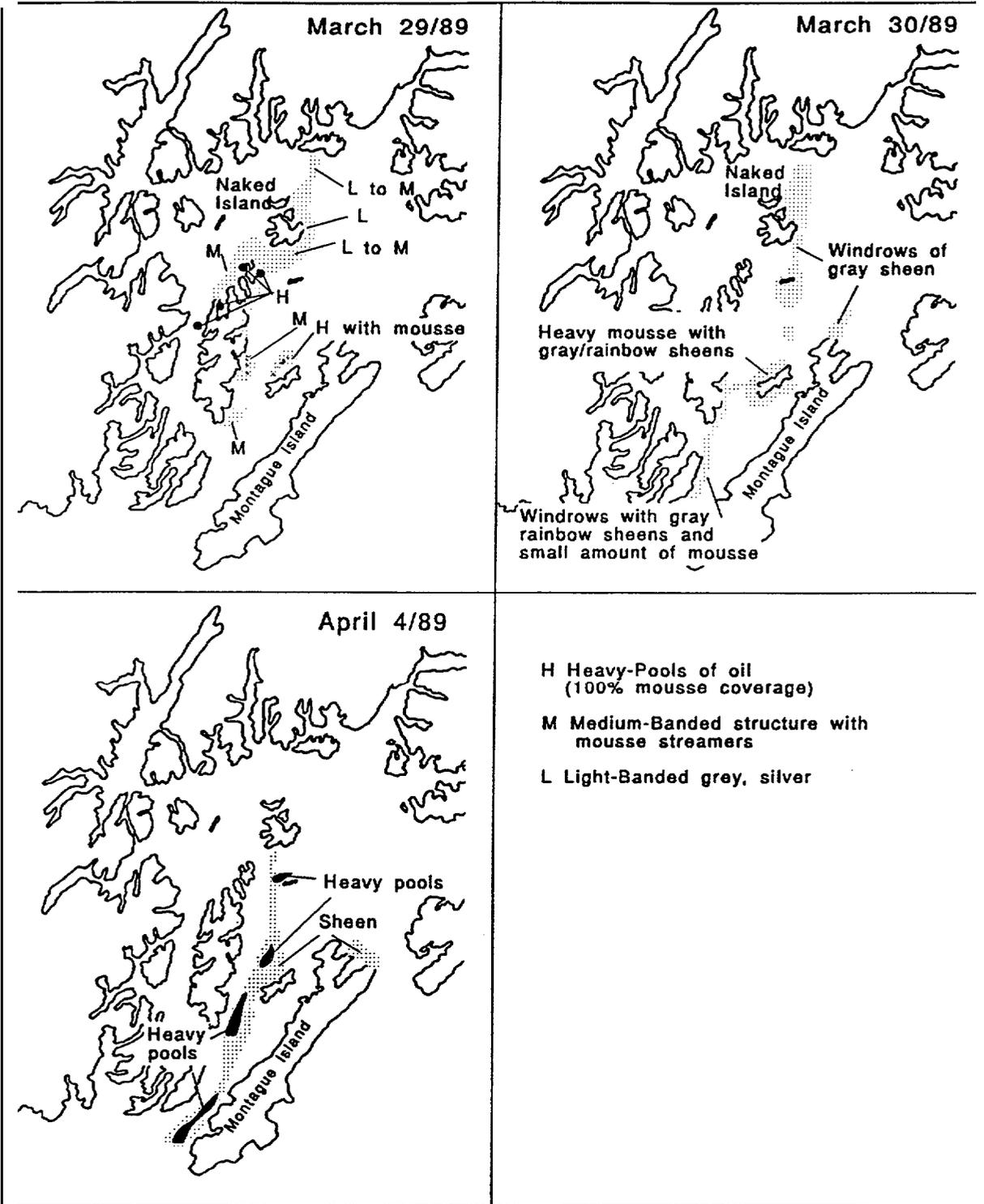


Figure 2. Seven maps of Prince William Sound showing the trajectory of oil spilled from the Exxon Valdez from March 25 to April 4, 1989.

Storey Islands and on the northern tip of Montague Island, were exposed to oil, but beaches on Fairmount Island and Tatitlek Narrows were never contaminated.

One control (non-oil-contaminated) spawning site and five potentially oil-contaminated spawning sites were chosen by ADFG biologists (Table 1). The control site was Fairmount Bay, and the five contaminated sites were: Bass Harbor, Outside Bay, and Cabin Bay on Naked Island; Storey Island north of Naked Island; and Rocky Bay on Montague Island.

It must be noted at this point that although we use the terms such as “oiled and “oil-contaminated” in this report, we lack information on the actual concentrations of hydrocarbons. Consequently this report is really a comparison between batches of eggs that may have been exposed to oil (treatments) and batches that were apparently not exposed to oil (controls). However, the word “treatment” is just as misleading as “oiled” because it implies a planned exposure of known concentration. In the absence of any satisfactory label, we continue to use words such as “oiled” and “contaminated.

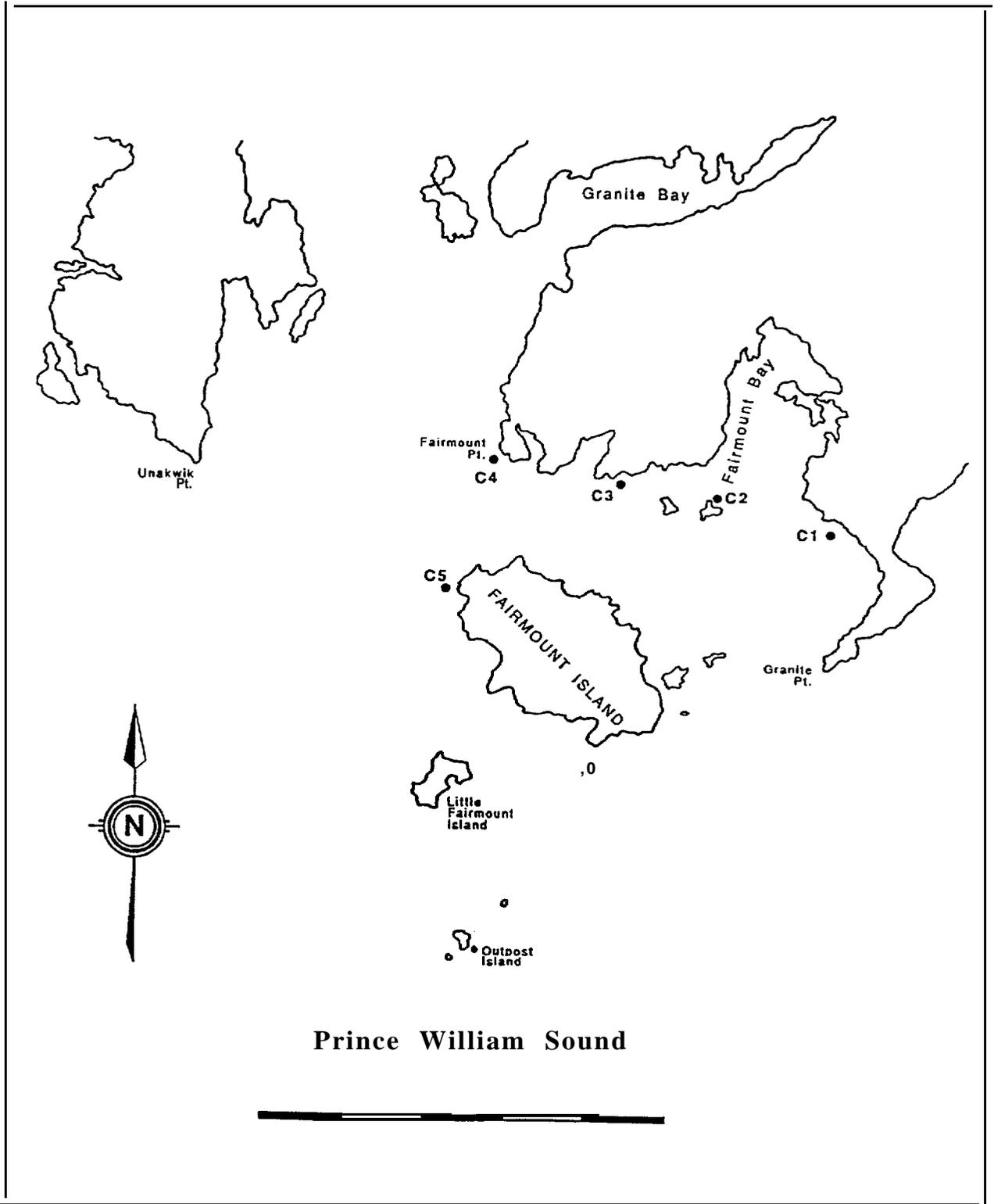
At each of these beaches SCUBA divers established between one and five transects perpendicular to the shoreline (Fig. 3). Transects were only established on spawn of medium density (two to three egg layers) so that all of the eggs collected for incubation in the laboratory came from spawn of the same egg density. Each transect was defined by a weighted rope that was anchored at its upper end by a stake that was marked by a bright red sign. Divers swam down the rope from the upper tide line to the lower edge of the zone of vegetation. At depths of 5, 0, -5 and -15 ft from mean low water (measured with depth gauges and tide tables) they laid a 0.1 m<sup>2</sup> frame on the substrate and took three separate handfuls of herring spawn from within the frame. Each handful of eggs was placed in its own porous paper bag, labelled according to date, beach, transect, depth, replicate number and sample number, and brought to the surface where it was immediately stored between layers of sea ice in an insulated chest. The chests were flown to Cordova, Alaska, and then to Seattle, Washington, where they were driven to the Vancouver Public Aquarium. The entire trip from the spawning beach to the Aquarium took less than 12 h. Over a 10 d period in late April, six coolers containing 180 samples were sent to the Aquarium.

Table 1. Study sites for collection of herring eggs.

Transect number	Location	Description:		Date of 1st survey	Water temp. (C)	Site notes/description:	oil observations	Spawn dates
		lat.	long.					
c1	Fairmount Bay	6052.91	14722.90	21-Apr-89	6.3	Spawn on ribbon/fucus, low tide; exposed to North	No oil	April 11-13
C2	Fairmount Isl. Area	6053.01	14724.16	21-Apr-89	7.2	Spawn, low; exposed to South	No oil	April 11-13
C3	Fairmount Oyster F.	6052.45	14723.32	21-Apr-89	7.2	Spawn, low slack; exposed from East	No oil	April 11-13
C4	Fairmount Oyster W.	6052.60	14724.58	21-Apr-89	7.2	Spawn on LBK, low slack; westerly exposure	No oil	April 12-15
C5	Fairmount Island W.	6051.80	14724.98	21-Apr-89	6.1	Spawn on mixed kelp; westerly exposure	No oil	April 12-15
01	So. Naked Island	6037.15	14727.62	22-Apr-89	5.0	Spawn on mix LBK southerly exposure	No oil evidence	April 13
02	Inside Bass, Naked	6037.73	14723.15	22-Apr-89	7.2	Mixed LBK, reds, hair; SW exposure	No oil evidence	April 12-13, 15
03	Bass Harbour Anch1	6037.85	14722.72	22-Apr-89	5.0	Heavy spawn under oil/boom; inside boom, protect	Heavy-med. oil	April 12-13, 15
04	Bass Harbour Anch2	6038.13	14723.04	22-Apr-89	5.0	Spawn inside oil boom; SW exposure	None visible	April 13, 15, 17-1
05	E. Bass Harbour	6038.39	14723.28	22-Apr-89	5.0	1015 hrs.; SW exposure, semi-protected	No oil evidence	April 13, 15, 17-1
06	NE Bass Harbour	6038.65	14723.43	23-Apr-89	5.0	Spawn on fucus/LBK; SW exposure/protected	No oil evidence	April 12-13
07	N Bass Harbour	6038.95	14723.10	23-Apr-89	5.0		No oil evidence	April 9, 11-13
08	NW Bass Harbour	6036.93	14723.99	23-Apr-89	5.0	Spawn on fucus/reds/LBK; SE exposure/protected	No oil evidence	April 9, 11-13
09	W Bass Harbour 1	6038.66	14724.49	23-Apr-89	5.0	Spawn on fucus/reds; S exposure/protected	No oil evidence	April 11-13
010	W Bass Harbour 2	6038.27	14724.68	23-Apr-89	5.0	Spawn on fucus/reds; SE exposure	No oil evidence	April 11, 13
011	N Outside Bay	6038.69	14726.50	24-Apr-89	5.0	Fucus/reds/LBK; W exposure/protected	No oil evidence	April 15
012	E Outside Bay	6039.06	14726.23	24-Apr-89	5.0	Spawn high in intertidal zone; W exposure	No oil evidence	April 15
013	NW Naked Island	6040.84	14728.87	24-Apr-89	5.0	Near rocky point.; W exposure	No oil evidence	April 13
014	W Naked Island	6038.95	14730.05	24-Apr-89	5.0		No oil evidence	April 15
015	So. Storey Island	6042.90	14724.26	26-Apr-89	5.0	Spawn on fucus/eelgrass; S exposure/protected	Tar balls/Lt. Sheens	April 12-13
016	No. Storey Island	6044.04	14724.95	26-Apr-89	5.0	Spawn on mixed kelp; N exposure	Spots of tar/beach	April 11-13
017	Rocky Bay	6019.32	14759.38	29-Apr-89	5.0	Spawn on LBK; visible pools of oil	Visible oil pools	April 13-15, 17-18
018	Rocky Bay	6019.43	14702.46	30-Apr-89	5.0	Spawn on mixed kelp tar on beach	Tar balls on beach	April 14-15, 17-18
019	Rocky Bay	6020.72	14701.22	30-Apr-89	5.0	Spawn on mixed kelp windrows of loose eggs on	Strong smell of oil	April 14-15, 17-18

## Notes

1. LBK = long brown kelp; ribbon , red , Fucus and hair are all categories of kelp.



**Figure 3A.** Map of the Fairmount Island area in Prince William Sound showing control transects C1 to C5 where eggs were collected. See Fig. 1 for location of Fairmount Island.

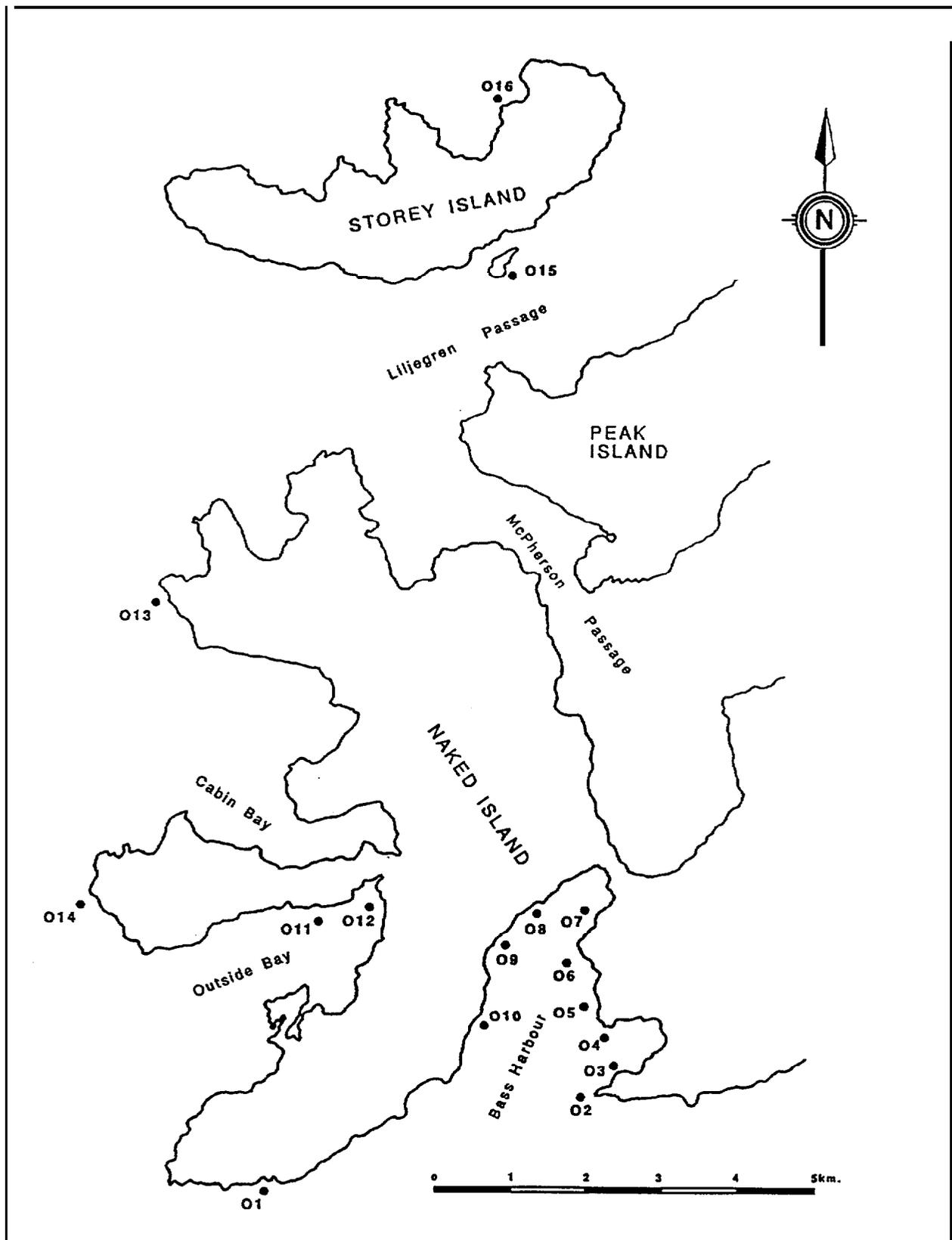


Figure 3B. Map of the Naked Island archipelago in Prince William Sound showing oiled transects 01 to 016 where eggs were collected. See Fig. 1 for location of Naked Island archipelago.

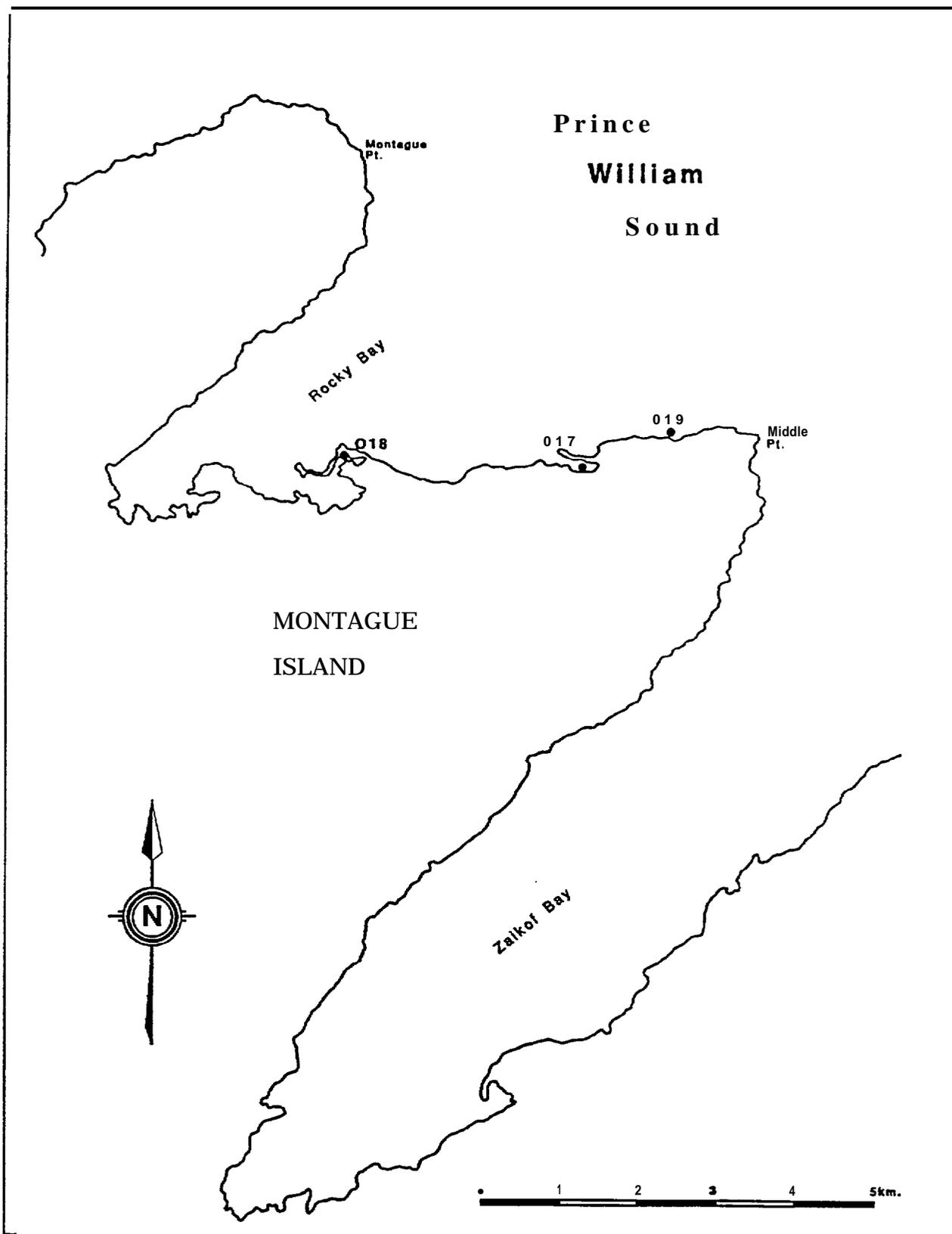


Figure 3C. Map of Rocky Bay on Montague Island in Prince William Sound showing oiled transects 017 to 019 where eggs were collected. See Fig. 1 for location of Montague Island.

## 2.2 Incubation

The incubation experiment *was* run “blind” by assigning each sample a new randomly-chosen code number which was marked on the incubation bottle. The list that cross-referenced the ADF&G sample number and the new code number was locked away until the end of the data collection period.

The samples contained too many eggs to be counted easily when they first arrived in the laboratory, so all samples were cut down to about 300 eggs each. All counts of live eggs and larvae began after the date of cut-down.

Twelve tubs each capable of holding 16 bottles were available in the laboratory (Fig. 4). As the samples arrived they were scattered among the tubs so that each contained a mixture of different beaches, transects and depths. This prevented confounding the results of the experiment with any possible “tub” effect caused by the location of the tubs in relation to each other and to sources of light and vibration in the laboratory.

Each oval tub had sides 64 x 64 cm long and a depth of 43 cm. A constant flow of freshwater into the tubs cooled the incubation bottles. The depth of freshwater was maintained at 10 cm by an elevated drainpipe in the center of each tub. Each day at 1000, 1300 and 1500 h local time three tubs were chosen at random and the water temperatures in the three tubs were measured. Thus, nine temperature measurements were taken each day.

Incubation bottles rested on the floor of the tubs (Fig. 5). They were 15 cm high with a volume of 1 L. They were filled with seawater taken from the recirculating seawater system of the Aquarium. The seawater in each bottle was replaced daily with fresh seawater. The seawater in the Aquarium’s recirculating system was pumped into a reservoir from a depth of 12 m in Burrard Inlet, which is several hundred meters from the Aquarium. The salinity of the reservoir water was recorded every morning by the engineering staff of the Aquarium.

Each sample of herring eggs was contained inside a cone of Vexar mesh (Fig. 6). An airstone was attached to the bottom of each mesh cone with insulated copper wire. The exposed ends of the wire were sealed with inert silicone gel. The stream of air

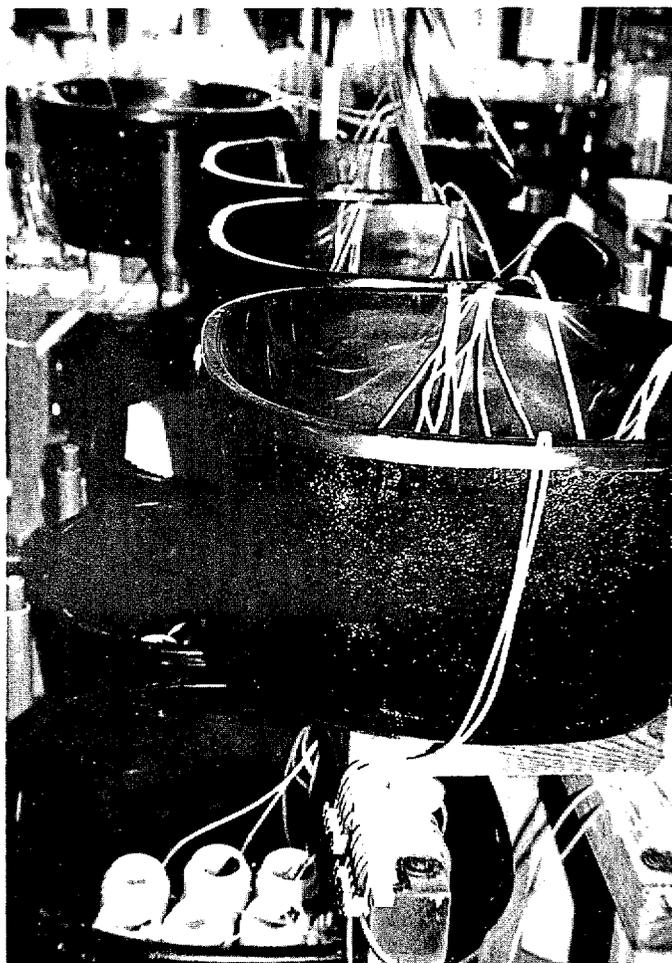


Figure 4. Incubation tubs in the larval fish laboratory of the Vancouver Public Aquarium.



Figure 5. Incubation bottles with air hoses.

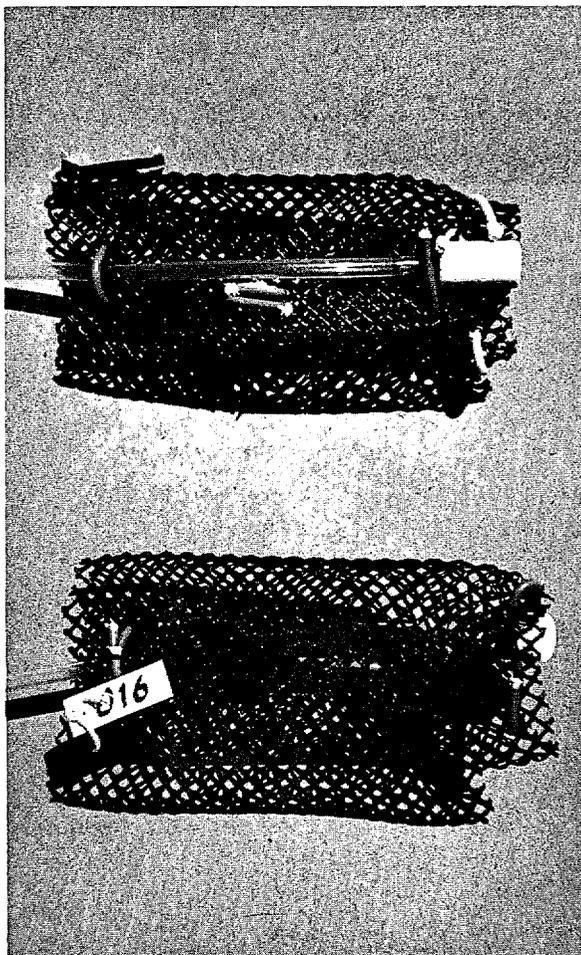


Figure 6. The mesh cone with attached airstone in an open position and enclosing a sample of spawn. The tag shows Triton sample number 16.

bubbles from an airstone travelled the length of the mesh cone and provided continuous aeration of the egg mass.

### 2.3 Data Collection

Each sample bottle was examined every 1 to 2 d during the experiment. The seawater in each bottle was emptied into a glass dish and all newly-hatched larvae were captured with a pipette, anesthetized in a solution of MS222, and then preserved in a solution of 3.3% formalin and 13.4 ppt seawater. Then, the mesh cone was removed from the bottle and the numbers of live and dead eggs in the sample were counted with a dissecting microscope. Live eggs were clear and the embryo was visible in late-stage eggs; moribund eggs were tinged with white, and dead eggs were completely opaque. Fig. 7 shows three live eggs. Counting took approximately 5 to 10 min, after which the egg mass was placed back in its mesh cone, and the bottle was filled with fresh seawater and placed back in its tub.

The incubation period was over when all samples contained only egg shells, dead eggs and vegetation. At this time, the technicians began to record data from the preserved larvae. The larvae from each bottle on each date were sorted into normal and deformed groups and the number of fish in each group was counted. The developmental stage of each normal larva was recorded using Doyle's (1977) morphological staging system for the development of Atlantic herring, Clupea harengus harengus, larvae.

After the larvae were examined for deformities, a sub-sample of 10 normal fish was chosen at random, the length of each fish was measured and then it was assigned a developmental stage. The length and height of the yolk sac was measured and the larva was then rinsed in freshwater, dried at 60°C for 24 h, and stored in a dessicator until it was weighed to the nearest  $\mu\text{g}$  with an electrobalance.

### 2.4 Data Analysis

Mean age of eggs was used as an index of their state of development. Age was calculated as the number of days elapsed from the midpoint of the range of dates of spawning as recorded by ADF&G aerial surveys in 1989 and shown in Table L The

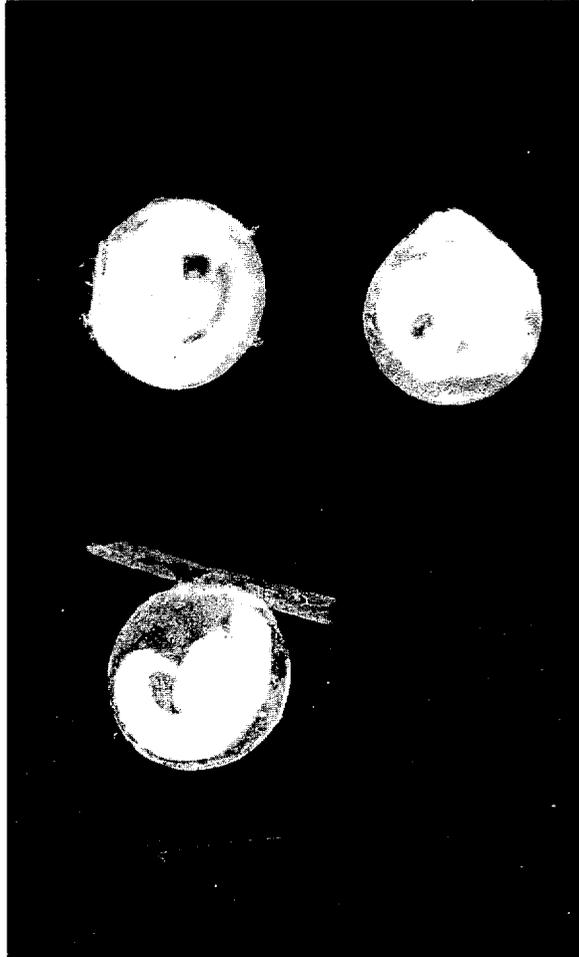


Figure 7. Three live herring eggs showing late-stage eyed embryos.

duration of spawning at a site varied from 2 to 5 d with a mean of 2 (SD = 2, n = 24) d.

Two kinds of indices of oil contamination were examined. The simplest was a division of the samples into oiled and non-oiled; Fairmount Island samples were non-oiled and all others were oiled. An attempt was also made to divide the oiled class into subclasses based on observations made by the SCUBA divers at the time the eggs were collected. These observations are recorded in Table 1. Very Light Oil was assigned to eggs from transects O-3 to O-14 from Naked Island because no oil was seen by the divers, but oil was known from aerial surveys to have been in the area before herring spawned. Light Oil was assigned to transects O-1, O-2, O-15 and O-16 because small amounts of oil were seen by divers. Medium oil was assigned to transects O-17, O-18 and O-19 in Rocky Bay because significant quantities of oil, including oil pools and tar pools, were seen by the divers. However, this second index was not used in the final analysis of this report because we found that population parameters, e.g. survival, hatching schedule etc., did not vary with treatment level, indicating that the four subclasses did not correspond to real differences in oil exposure.

The number of eggs surviving to age  $t$ ,  $s(t)$ , was

$$(1) s(t) = [E_l(t) + \sum_{t_0}^t N(t)]/E(t_0)$$

where  $E_l(t)$  = number of live eggs at age  $t$ ,  $N(t)$  = cumulative number of larvae at age  $t$ , and  $E(t_0)$  = the total number of eggs (live and dead) in the incubation bottle at the age of cut-down,  $t_0$ . Egg and larval numbers are shown in Appendix B. Survival is synonymous with other terms that have been used in the scientific literature such as percent hatching and hatching success. It is the opposite of terms such as pre-hatching mortality.

The fraction of live eggs at age  $t$ ,  $f(t)$ , was

$$(2) f(t) = E_l(t)/[E_l(t) + E_d(t)]$$

where  $E_d(t)$  = number of dead eggs at age  $t$ .

The cumulative fraction of hatched larvae at age  $t$ ,  $h(t)$ , was

$$(3) h(t) = \frac{\sum_{t=0}^t N(t)}{N(t_n)}$$

where  $N(t_n)$  = the total number of larvae hatched at the end of the incubation period.

The change in  $s(t)$ ,  $f(t)$  and  $h(t)$  with age was analysed using the “accelerated time to failure” model of Chambers and Leggett (1989). This is a Weibull distribution modified to include auxiliary variables. For example,

$$(4a) f(t) = \exp\left[-\left(\frac{t}{a}\right)^y \exp(b_1z + b_2x + b_3xz)\right]$$

where  $f(t)$  = fraction of live eggs at age  $t$  (d),  $y$  = scale parameter of Weibull distribution,  $a$  = location parameter of the Weibull distribution,  $z$  = depth (ft) from which eggs were collected,  $x$  = dummy variable with a value of 1 for control eggs and 0 for oiled eggs, and  $b_1$ ,  $b_2$  and  $b_3$  = coefficients for depth, oil treatment, and their interaction, respectively. Age was shifted for  $s(t)$  and  $h(t)$  using a threshold of 15 d, the minimum age for all observations. This improved the fits of the models to these data. Age was not shifted for  $f(t)$  because it reduced the fit of the model. The models were fit to the data after double in-transformation~ e.g.

$$(4b) \ln\{-\ln[s(t)]\} = -y\ln(a) + y\ln(t-15) + b_1z + b_2x + b_3zx$$

$$(4c) \ln\{-\ln[f(t)]\} = -y\ln(a) + y\ln(t) + b_1z + b_2x + b_3zx$$

with stepwise multiple regression. Only variables whose coefficients were significant at the 0.05 level were retained.  $h$ -transformation meant that all extreme values of a response variable, i.e. 0 and 1, were removed from the data before analysis.

This report uses a great number of fractions. In the biometrical literature fractions are often normalized with the  $\arcsin(P)^{0.5}$  transformation before entering analysis of variance (Sokal and Rohlf 1981), but fractions were not transformed in this

report because normalization was most often achieved using a Weibull distribution. For those fractions that were not normalized with a Weibull function extensive preliminary analysis showed that none of the findings were changed by arcsin transformation. Therefore, for the sake of clarity we present all fractions without arcsin transformation.

Herring larvae lose length and weight upon fixation in formalin. We adjusted the post-preservation lengths and weights for fixation shrinkage in order to make our results comparable to those of live herring larvae. This was only possible because the relationships between fixation shrinkage of herring larvae and the concentration of formalin and seawater in the preservative have been extensively investigated by Hay (1982, 1984).

Hay (1982: Fig. 5) showed that 2 wk old herring larvae preserved for 10 din 10% formalin and 27 ppt seawater shrank by an amount equal to  $0.564 + 0.016L$ , where  $L$  = live length (mm). This means that percent shrinkage is equal to  $1.6 + 56.4/L$ . This equation was corrected for the differences in formalin concentration and salinity between his results and the present incubation experiment (3.3% formalin and 13.4 ppt salinity) using Hay's (1982) multiple regression of percent shrinkage on salinity, formalin concentration and temperature. This equation predicted that our preservative would produce only 77.9% of the shrinkage produced by 10% formalin and 27 ppt salinity, so percent shrinkage was  $0.779(1.6 + 56.4/L)$  or  $1.2 + 43.9/L$ . Therefore, we corrected preserved lengths to live lengths using the rearranged equation

$$(5) L = 0.444 + 1.012X$$

where  $X$  = preserved length (mm).

Hay (1984) reported that the mean percent loss in dry weight of yolk sac herring larvae preserved in 4% formalin decreased from -36.2% in freshwater (0 ppt) to -21.3% at a salinity of 15 ppt, which implies an extra 1.0% increase in fixed dry weight for every 1 ppt increase in salinity. Therefore, at an average salinity of 13.4 ppt, the weight loss was calculated to be -22.9%, i.e.  $-36.2\% + 1.0\%/ppt \times 13.4ppt$ , and live weight was equal to fixed weight/(1-0.229) or fixed weight  $\times 1.297$ .

Yolk sac volume was calculated from the equation for an ellipsoid (Hourston et al. 1984)

$$(6) \quad V = \frac{4}{3} \pi \left( \frac{L_y}{2} \right)^2 \left( \frac{H}{2} \right)$$

where V = volume (mm<sup>3</sup>), L<sub>y</sub> = length (mm) of yolk sac, and H = height (mm) of yolk sac. Neither L<sub>y</sub> or H were corrected for fixation shrinkage.

Condition of larvae was calculated as

$$(7) \quad CF = W/L^3$$

where CF = condition factor (μg·mm<sup>-3</sup>), W = live dry weight (μg) and L = live length (mm).

### 3. RESULTS

#### 3.1 Incubation Temperature and Salinity

Temperature of the incubation tubs rose from 8.0°C over the first three days of May to a mean of 9.2°C on May 16 (Fig. 8, Appendix A). The trend was not linear with time, so polynomial regression was used to describe the trend. Dummy variables for the three times of the day at which temperatures were taken were included in the regression model in order to determine if temperatures varied during a day as well as between days. The model that explained the most variance (r<sup>2</sup> = 0.55, n = 54) with all-significant parameters (P < 0.05) was

$$(8) \quad T = -78.29 + 1.276D - 4.466 \times 10^{-3} D^2 + 0.2056g$$

(SE)	(25.91)	(0.394)	(1.498 × 10 <sup>-3</sup> )	(0.0937)
(P)	(0.004)	(0.002)	(0.003)	(0.003)

where T = temperature (°C), D = Julian date, and g = a dummy variable with a value of 1 for 1000 h and zero for the other two times of day. This model shows that the temperature of the incubation water was 0.2°C lower in the morning than it was in the afternoon at all dates (Fig. 8). It suggests that water temperature followed a

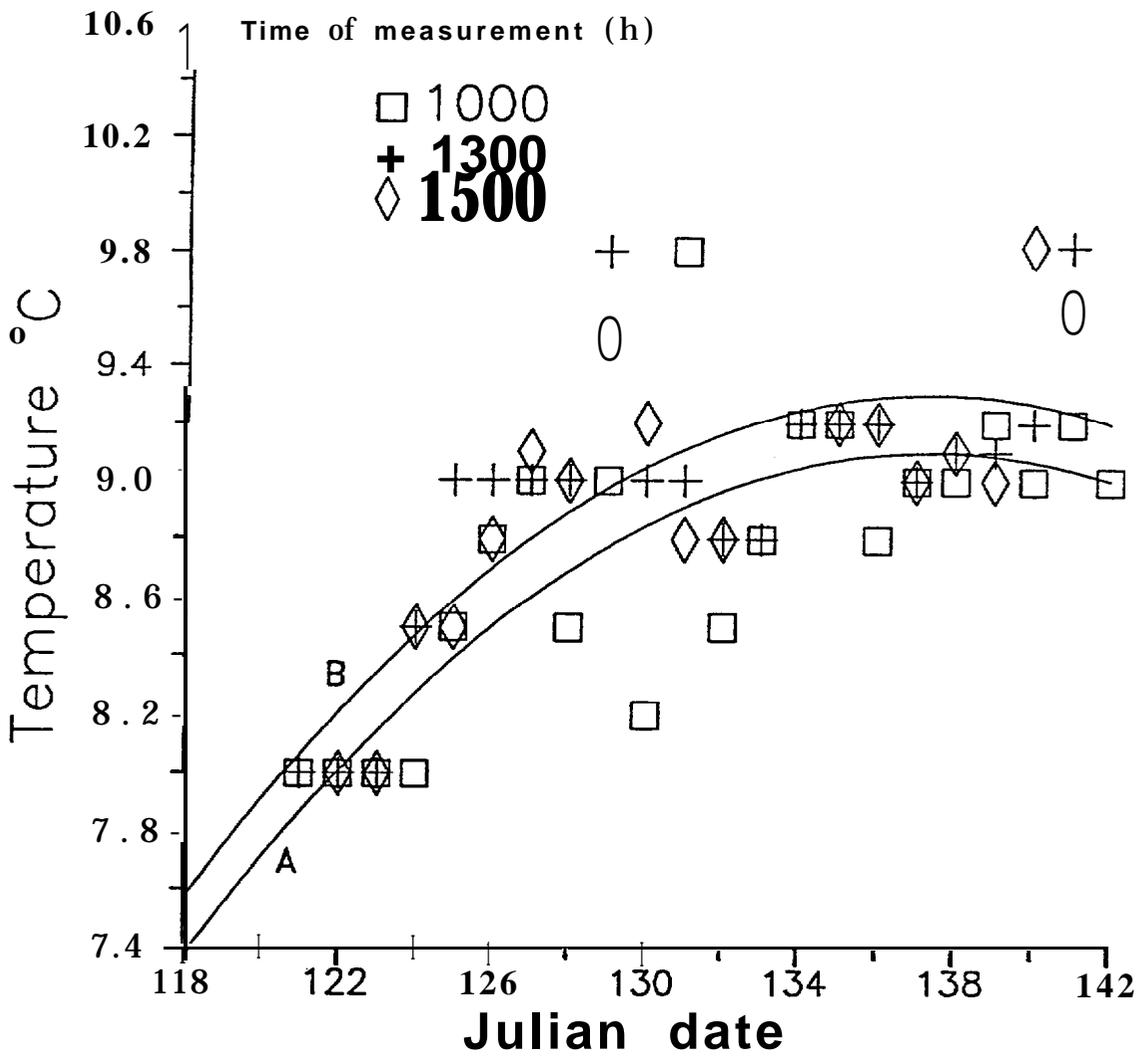


Figure 8. Temperature of incubation water, showing that water was 0.2°C cooler at 1000 h than at 1300 and 1500 h. Curve A describes temperature at 1000h and curve B describes temperature at 1300 and 1500 h. Both curves were calculated from equation (8).

daily cycle: low at night rising in the morning to a maximum in the afternoon, and then falling to an overnight low.

Fig. 9 shows that the salinity of the seawater system varied cyclically with date about a mean of 26.8 ppt (SD = 0.6, n = 24). A periodogram showed that the average cycle time was 4 d. The cause of the cycle is not known; it was not caused by the pumping of fresh seawater into the Aquarium's reservoir because at least 300 gal were pumped into it every half hour (John Rawle, Vancouver Public Aquarium, Vancouver, B.C., pers. comm.).

### 3.2 Egg Survival

#### Total Survival

The fraction of eggs that survived incubation and hatched larvae ranged from 0.071 to 0.999 with a grand mean of 0.592 (SD = 0.177, n= 180) (Table 2). A two-way analysis of variance (ANOVA) showed that survival varied significantly ( $P < 0.001$ ) with depth, but not with oil treatment ( $P > 0.05$ ) or with the interaction of depth and oil treatment ( $P > 0.05$ ). Multiple regression showed that the greatest amount of variance in survival ( $r^2 = 0.10$ , n = 180) was explained by a quadratic regression on depth

(9)	<u>variable</u>	<u>coefficient</u>	<u>SE</u>	<u>P</u>
	constant	0 . 6 0 5 8	0.0148	<0.0001
	depth	-0.0159	0.0037	<0.0001
	depth <sup>2</sup>	-0.0013	0.0003	<0.0001

The predicted survival is shown in Fig. 10A. It was maximal near a depth of 5 ft.

#### Age Trajectory of Survival

In contrast to the results for total survival, a Weibull model showed that survival at age,  $s(t)$ , decreased at a constant rate of about  $3\% \cdot d^{-1}$  in all depth-treatment cells and there was no significant effect of depth or oil treatment. The parameters of the model are shown in Table 3 and the predicted  $s(t)$  is shown in Fig. 11. Examination of Fig. 11 shows that this model overestimates  $s(t)$  for the oiled/5 ft class. This

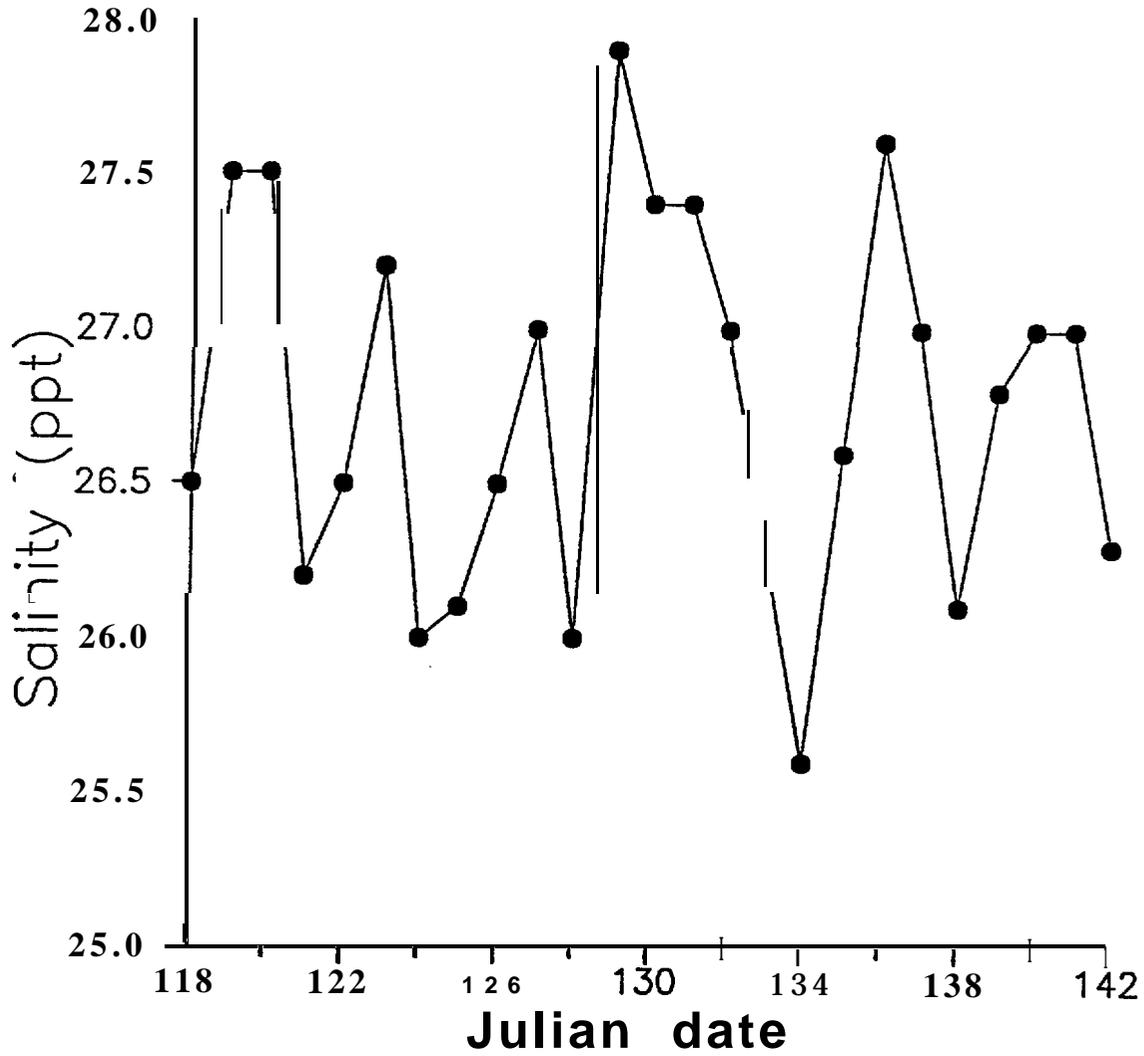


Figure 9. Salinity of Vancouver Public Aquarium seawater system, showing a periodicity of about 1 cycle every four days.

Table 2. Summary statistics of herring egg samples.

Location	ADFG sample number	Depth (ft)	Rep. no.	TRITON fraction et hatch		Age (d)		fraction of larvae viable	fraction hatch	Fraction of larvae In developmental stages				Fraction of larvae In deformity classes						Yom sac volume (mm <sup>3</sup> )		Weight (ug)		Length (mm)		Condition (ug mm <sup>-3</sup> )				
				number	survived	50%	95%			pre-1a	1a	1b	1c	normal	spine	yolk	Jaw	stubby	head	caudal	mean	SD	mean	SD	mean	SD	mean	SD	n	
				no.	to hatch																									
Fairmount	C-01	5	3	53	0.652	22.7	26.1	0.76s	0.514	0.070	0.197	0.197	0.535	0.789	0.103	0.028	0.000	0.000	0.000	0.000	0.000	0.184	0.130	168	29	8.3	0.6	0.361	0.0SS	22
Fairmount	C-01	5	1	101	0.577	23.6	27.9	0.667	0.500	0.030	0.056	0.165	0.730	0.867	0.060	0.047	0.026	0.000	0.000	0.000	0.152	0.157	144	58	8.8	0.6	0.227	0.12247		
Fairmount	C-01	5	2	2	0.45s	23.7	26A	0.475	0.218	0.000	0.079	0.0s6	0.835	0.475	0.072	0.424	0.022	0.007	0.000	0.000	0.182	0.151	16432		6.3	1.1	0.324	0.17639		
Fairmount	C-01	0	3	172	0.672	21.5	25.2	0s40	0.631	0.004	0.014	0.363	0.619	0.940	0.053	0.000	0.000	0.007	0.000	0.000	0.129	0.122	13073		9.2	0.9	0.182	0.12261		
Fairmount	C-01	0	1	7s	0.740	22.0	25.4	0.941	0.697	0.018	0.0s5	0.194	0.703	0.941	0.059	0.000	0.000	0.000	0.000	0.144	0.101	146	56	8.8	0.6	0.213	0.03925			
Fairmount	C-01	0	2	143	0.697	21.9	25.4	0.770	0.537	0.135	0.216	0.000	0.649	0.770	0.122	0.106	0.000	0.000	0.000	0.164	0.117	16726		6.3	1.0	0.312	0.12525			
Fairmount	C-01	-5	3	128	0.456	23.3	25.6	0.626	0.266	0.007	0.241	0.641	0.110	0.628	0.366	0.000	0.007	0.000	0.000	0.230	0.117	147	63	6.5	0.9	0.264	0.14131			
Fairmount	C-01	-s	1	107	0.964	23.7	27.0	0.952	0.936	0.003	0.0s2	0.339	0.606	0.952	0.035	0.003	0.010	0.000	0.000	0.219	0.236	1ss	40	8.9	0.6	0.271	0.0S4	46		
Fairmount	C-01	-5	2	91	0.475	22.7	25.1	0.914	0.434	0.000	0.000	0.691	0.30s	0.914	0.049	0.037	0.000	0.000	0.000	0.204	0.127	16945		8.3	0.9	0.302	0.13220			
Fairmount	C-02	0	1	56	0.232	24.6	27.0	0.706	0.164	0.000	0.125	0.000	0.875	0.706	0.083	0.167	0.042	0.030	0.000	0.143	0.136	16527		9.0	0.9	0.246	0.094	20		
Fairmount	C-02	0	3	36	0.702	23.1	25.3	0.921	0.653	0.000	0.261	0.366	0.331	0.921	0.039	0.000	0.039	0.000	0.000	0.156	0.112	172	29	9.1	0.6	0.236	0.06226			
Fairmount	C-02	0	2	151	0.716	23.4	26.9	0.896	0.643	0.000	0.056	0.086	0.624	0.656	0.096	0.000	0.008	0.000	0.000	0.160	0.113	16327		9.3	0.7	0.206	0.04532			
Fairmount	C-02	-5	2	141	0.779	20.9	24.6	0.946	0.737	0.046	0.206	0.285	0.462	0.946	0.008	0.046	0.000	0.000	0.000	0.160	0.143	17029		9.0	1.0	0.245	0.06344			
Fairmount	C-02	-5	1	119	0.379	19.6	24.9	0.722	0.274	0.000	0.000	0.583	0.417	0.722	0.056	0.222	0.000	0.000	0.000	0.194	0.165	17036		8.8	1.0	0.260	0.06620			
Fairmount	C-62	-5	3	159	0.750	23.6	27.0	0.662	0.662	0.005	0.077	0.436	0.462	0.882	0.046	0.000	0.072	0.000	0.003	0.000	0.160	0.119	162	30	8.9	1.0	0.244	0.10747		
Fairmount	C-02	-1s	1	125	0.709	24.1	26.8	0.910	0.645	0.017	0.371	0.461	0.152	0.910	0.062	0.028	0.000	0.000	0.000	0.221	0.143	16344		8.6	1.1	0.287	0.149	3S		
Fairmount	C-02	-1s	3	19	0.551	23.3	25.6	0.845	0.465	0.019	0.022	0.369	0.563	0.645	0.066	0.067	0.000	0.000	0.000	0.221	0.149	185	41	8.7	1.2	0.307	0.14342			
Fairmount	C-02	-15	2	168	0.446	27.4	25.5	0.771	0.344	0.000	0.170	0.277	0.553	0.771	0.104	0.021	0.104	0.000	0.000	0.121	0.118	86	79	9.3	1.0	0.125	0.12526			
Fairmount	C-03	0	1	23	0.606	24.1	25.9	0.617	0.49s	0.021	0.162	0.260	0.549	0.817	0.028	0.155	0.000	0.000	0.000	0.204	0.172	172	30	8.6	1.1	0.302	0.13936			
Fairmount	C-03	0	2	37	0.596	23.6	25.9	0.893	0.532	0.012	0.054	0.292	0.643	0.693	0.071	0.036	0.000	0.000	0.000	0.075	0.062	182	30	9.3	0.7	0.227	0.05027			
Fairmount	C-03	0	3	142	0.703	24.6	27.1	0.756	0.532	0.036	0.021	0.036	0.907	0.756	0.197	0.047	0.000	0.000	0.000	0.056	0.109	17619		9.5	1.1	0.220	0.12621			
Fairmount	C-03	-s	3	74	0.570	22.9	26.4	0.846	0.463	0.013	0.152	0.641	0.194	0.646	0.135	0.004	0.000	0.013	0.000	0.162	0.126	16720		6.7	1.0	0.284	0.142	2S		
Fairmount	C-03	-5	1	62	0.629	25.2	27.7	0.768	0.483	0.000	0.185	0.026	0.786	0.766	0.113	0.073	0.000	0.04s	0.000	0.146	0.170	174	26	9.0	1.1	0.264	0.11841			
Fairmount	C-03	-5	2	24	0.545	24.3	26.2	0.895	0.466	0.023	0.196	0.163	0.616	0.695	0.058	0.012	0.035	0.000	0.000	0.169	0.147	16540		6.1	1.3	0.361	0.19329			
Fairmount	C-03	-15	1	152	0.683	24.9	28.7	0.795	0.543	0.000	0.041	0.009	0.951	0.7s5	0.052	0.115	0.000	0.006	0.000	0.105	0.145	17236		9.1	0.8	0.245	0.13931			
Fairmount	C-03	-15	2	41	0.560	24.1	25.9	0.62S	0.464	0.006	0.172	0.033	0.787	0.626	0.066	0.057	0.049	0.000	0.000	0.120	0.141	161	23	9.0	0.8	0.225	0.057243			
Fairmount	C-03	-15	3	96	0.653	24.3	25.0	0.920	0.501	0.037	0.102	0.364	0.497	0.920	0.046	0.000	0.021	0.000	0.011	0.000	0.174	0.175	1s0	30	8.9	1.1	0.297	0.23032		
Fairmount	C-04	5	2	25	0.591	21.9	24.6	0.637	0.495	0.012	0.105	0.174	0.709	0.637	0.128	0.035	0.000	0.000	0.000	0.146	0.140	16424		9.0	1.0	0.236	0.09351			
Fairmount	C-04	5	1	127	0.440	22.9	25.1	0.900	0.396	0.054	0.146	0.146	0.654	0.900	0.077	0.023	0.000	0.000	0.000	0.122	0.130	171	26	8.9	1.2	0.270	0.13736			
Fairmount	C-04	5	3	100	0.673	24.4	26.9	0.656	0.577	0.026	0.147	0.053	0.774	0.656	0.042	0.068	0.026	0.000	0.005	0.000	0.190	0.1S9	161	31	8.5	1.0	0.295	0.16333		
Fairmount	C-04	0	2	35	0.617	22.2	23.9	0.888	0.725	0.009	0.047	0.440	0.504	0.6s6	0.069	0.034	0.004	0.004	0.000	0.097	0.092	16024		6.9	1.0	0.246	0.12729			
Fairmount	C-04	0	1	15s	0.512	23.4	25.8	0.s46	0.331	0.000	0.037	0.402	0.561	0.s46	0.146	0.000	0.061	0.146	0.000	0.09s	0.080	13429		6.9	0.8	0.224	0.0S3	22		
Fairmount	C-04	0	3	6	0.966	23.3	24.7	0.8%	0.662	0.000	0.025	0.331	0.S44	0.895	0.047	0.025	0.025	0.000	0.007	0.000	0.157	0.154	16S	32	8.7	1.0	0.2ss	0.15030		
Fairmount	C-04	-5	1	176	0.813	22.7	26.2	0.890	0.723	0.026	0.141	0.247	0.588	0.890	0.0s4	0.022	0.004	0.000	0.000	0.166	0.159	17326		9.0	1.1	0.266	0.141	34		
Fairmount	C-04	-s	2	9s	0.633	23.4	24.9	0.963	0.610	0.023	0.217	0.097	0.664	0.963	0.026	0.000	0.000	0.009	0.000	0.221	0.177	177	32	6.4	0.6	0.300	0.05317			
Fairmount	C-04	-5	3	III	0.331	22.6	25.8	0.925	0.306	0.050	0.425	0.075	0.450	0.925	0.025	0.017	0.017	0.006	0.006	0.172	0.170	17226		6.5	0.9	0.304	0.15025			
Fairmount	C-05	5	2	95	0.644	22.9	24.9	0.937	0.791	0.000	0.050	0.213	0.73S	0.937	0.042	0.000	0.021	0.000	0.000	0.145	0.144	19469		9.2	0.6	0.256	0.14532			

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Table 2. Summary statistics of herring egg samples. (Continued)

Location	ADFG sample number	Depth (ft)	Rep. no.	Sample number	Age (d) fraction at hatch				Fraction of larvae in developmental stages				Fraction of larvae in deformity classes						Yolk ssc volume (mm <sup>3</sup> )		Weight (µg)		Length (mm)		Condition (µg mm <sup>-3</sup> )				
					50%		95%		fraction viable	fraction hatch	pre-1a	1a	1b	1c	normal	spine	yolk	Jaw	stubby	head	caudal	mean	SD	mean	SD	mean	SD	mean	SD
					fraction	fraction	fraction	fraction																					
unt	C-05	5	1	57	0.663	24.5	2S.8	0.635	0.570	0.000	0.025	0.063	0.642	0.63S	0.046	0.039	0.0S7	0.014	0.000	0.000	0.116	0.125	171	25	9.11.0	0.251	0.11735		
	C-05	5	3	3	0.429	22.8	24.9	0.743	0.320	0.006	0.019	0.146	0.626	0.746	0.036	0.210	0.000	0.006	0.000	0.000	0.139	0.166	16140		6.S	1.5	0.303	0.175	35
Fairmount	C-0S	0	3	40	0.626	22.6	24.5	0.925	0.57s	0.010	0.219	0.294	0.47S	0.925	0.020	0.045	0.005	0.005	0.000	0.000	0.154	0.120	1s4	31	8.7	0.6	0.263	0.10317	
Fairmount	C-05	0	2	106	0.602	22.5	24.7	0.856	0.s16	0.010	0.169	0.610	0.210	0.65S	0.144	0.000	0.000	0.000	0.000	0.191	0.061	17323		6.6	1.0	0.220	0.109	21	
Fairmount	C-05	0	1	69	0.601	23.4	26.4	0.695	0.717	0.000	0.037	0.419	0.544	0.695	0.024	0.076	0.000	0.003	0.000	0.000	0.057	0.076	15620		9.4	0.6	0.190	0.039	41
Fairmount	C-0S	-5	3	167	0.72s	24.2	26.0	0.922	0.6s9	0.024	0.034	0.244	0.69S	0.922	0.049	0.020	0.005	0.005	0.000	0.000	0.172	0.125	157	27	6.4	0.6	0.260	0.123	39
Fairmount	C-0S	-5	2	92	0.740	24.0	26.0	0.856	0.633	0.054	0.0S4	0.041	0.651	0.856	0.066	0.050	0.018	0.000	0.009	0.000	0.157	0.127	179	3a	6.8	1.0	0.284	0.11840	
Fairmount	C-05	-5	1	7	0.553	23.0	24.4	0.92s	0.s14	0.102	0.027	0.26S	0.52S	0.929	0.033	0.027	0.000	0.011	0.000	0.000	0.154	0.146	178	32	6.9	1.2	0.29S	0.16213	
Bass Harbour	O-01	0	3	155	0.759	23.6	25.8	0.660	0.660	0.009	0.04S	0.046	0.69S	0.660	0.046	0.000	0.026	0.046	0.000	0.000	0.107	0.116	15927		6.6	1.0	0.247	0.094	21
Bass Harbour	O-01	0	1	137	0.504	21.7	24.3	0.617	0.412	0.007	0.922	0.052	0.020	0.817	0.170	0.000	0.013	0.000	0.000	0.000	0.265	0.106	19036		8.5	0.5	0.30s	0.060	19
Bass Harbour	O-01	0	2	66	0.860	23.6	26.?	0.650	0.s59	0.007	0.035	0.203	0.7s5	0.650	0.154	0.084	0.077	0.035	0.000	0.000	0.057	0.068	150	2s	6.6	1.0	0.226	0.063	36
Bass Harbour	O-01	-s	1	106	0.651	23.6	25.6	0.694	0.562	0.035	0.276	0.323	0.364	0.694	0.030	0.066	0.005	0.00s	0.000	0.000	0.145	0.128	13265		6.7	1.0	0.224	0.15342	
Bass Harbour	O-01	-s	2	175	0.714	22.3	23.7	0.651	0.607	0.004	0.245	0.56S	0.166	0.s51	0.116	0.033	0.000	0.000	0.000	0.000	0.236	0.061	17663		6.4	1.0	0.321	0.16124	
Bass Harbour	O-01	-s	3	90	0.622	24.4	26.0	0.792	0.496	0.036	0.158	0.077	0.727	0.792	0.060	0.036	0.109	0.000	0.000	0.000	0.126	0.115	13563		9.2	0.7	0.163	0.12333	
Base Harbour	O-01	-15	2	61	0.653	26.1	26.0	0.712	0.465	0.000	0.174	0.256	0.571	0.712	0.119	0.091	0.076	0.000	0.000	0.000	0.109	0.127	146	53	9.0	0.8	0.211	0.10425	
Bass Harbour	O-01	-15	1	39	0.716	22.9	24.9	0.66s	0.635	0.000	0.063	0.445	0.492	0.s65	0.063	0.031	0.021	0.000	0.000	0.000	0.105	0.095	156	2s	9.1	0.6	0.217	0.07123	
6ese Harbour	O-01	-15	3	124	0.527	23.0	26.0	0.927	0.469	0.006	0.165	0.628	0.201	0.927	0.049	0.016	0.006	0.000	0.000	0.000	0.165	0.097	15969		6.3	0.6	0.227	0.14122	
Bass Harbour	O-02	0	3	1	0.624	22.2	24.9	0.961	0.613	0.031	0.012	0.360	0.596	0.961	0.019	0.000	0.000	0.000	0.000	0.000	0.126	0.125	171	27	9.2	0.6	0.231	0.07413	
Bass Harbour	O-02	0	1	99	0.707	21.9	24.0	0.946	0.669	0.012	0.09s	0.506	0.366	0.946	0.046	0.000	0.000	0.000	0.006	0.000	0.134	0.090	16635		8.8	1.2	0.269	0.11737	
Bass Harbour	O-02	0	2	18	0.522	22.0	25.0	0.923	0.462	0.010	0.550	0.196	0.244	0.923	0.036	0.03S	0.000	0.000	0.000	0.000	0.137	0.128	16331		6.4	1.1	0.314	0.239	29
Bass Harbour	O-02	-5	3	134	0.606	23.7	26.4	0.901	0.54s	0.000	0.227	0.250	0.523	0.w1	0.029	0.035	0.029	0.006	0.000	0.000	0.132	0.121	17937		9.3	0.6	0.233	0.06222	
Base Harbour	O-02	-5	1	83	0.307	22.7	24.7	0.966	0.297	0.000	0.067	0.404	0.526	0.966	0.000	0.034	0.000	0.000	0.000	0.000	0.12s	0.145	16720		9.1	0.5	0.225	0.04721	
Bass Harbour	O-02	-5	2	34	0.774	22.5	24.6	0.959	0.742	0.000	0.311	0.265	0.425	0.959	0.016	0.018	0.005	0.000	0.000	0.000	0.1s0	0.125	16327		6.7	1.0	0.275	0.15723	
Bass Harbour	O-02	-15	3	118	0.416	22.9	24.0	0.912	0.360	0.007	0.161	0.467	0.365	0.912	0.06\$	0.000	0.000	0.000	0.000	0.000	0.119	0.115	16526		6.6	0.9	0.275	0.120	31
Bass Harbour	O-02	-15	1	17	0.535	22.S	24.2	0.666	0.474	0.027	0.141	0.611	0.222	0.666	0.070	0.005	0.03s	0.000	0.000	0.000	0.141	0.104	177	31	9.0	0.7	0.250	0.049	30
Bass Harbour	O-02	-15	2	4	0.416	22.9	25.0	0.664	0.360	0.000	0.197	0.197	0.606	0.664	0.03s	0.063	0.015	0.000	0.000	0.000	0.139	0.066	171	32	9.3	0.6	0.219	0.06412	
Bass Harbour	O-03	5	3	49	0.227	22.9	24.6	0.346	0.076	0.015	0.015	0.830	0.139	0.346	0.077	0.194	0.367	0.015	0.000	0.000	0.175	0.106	15722		9.4	0.9	0.196	0.06316	
Bass Harbour	O-03	5	1	103	0.757	22.6	26.1	0.69S	0.6s0	0.005	0.063	0.359	0.573	0.696	0.034	0.010	0.056	0.000	0.000	0.000	0.134	0.119	171	22	6.9	1.0	0.26S	0.10%	47
Base Harbour	O'03	5	2	5	0.321	22.4	23.9	0.966	0.317	0.000	0.244	0.244	0.512	0.2ss	0.000	0.012	0.000	0.000	0.000	0.000	0.227	0.026	171	2s	6.1	0.2	0.324	0.031	2
Bass Harbour	a-03	0	2	164	0.9ss	23.7	26.1	0.712	0.681	0.00s	0.090	0.062	0.833	0.712	0.103	0.013	0.066	0.066	0.000	0.000	0.103	0.119	15928		6.9	1.0	0.24S	0.14932	
Base Harbour	O-03	0	1	14	0.708	22.7	24.0	0.904	0.640	0.026	0.262	0.566	0.144	0.904	0.037	0.026	0.033	0.003	0.000	0.000	0.153	0.102	163	2S	6.8	1.0	0.259	0.10224	
Base Harbour	O-03	0	3	104	0.646	22.6	25.4	0.691	0.446	0.000	0.206	0.562	0.2343	0.691	0.096	0.064	0.122	0.000	0.000	0.000	0.202	0.130	184	50	6.9	0.9	0.266	0.06332	
Bass Harbour	O'03	-5	2	139	0.169	22.7	24.0	0.861	0.163	0.000	0.669	0.111	0.000	0.661	0.111	0.000	0.026	0.000	0.000	0.000	0.266	0.145	15535		7.6	0.7	0.332	0.07612	
Oase Harbour	O-03	-s	1	55	0.517	23.6	24.9	0.839	0.434	0.000	0.000	0.33s	0.661	0.639	0.107	0.000	0.054	0.000	0.000	0.000	0.132	0.123	16324		8.6	0.9	0.274	0.101	10
Base Harbour	O'03	-s	3	120	0.579	23.6	25.9	0.676	0.50s	0.011	0.361	0.161	0.467	0.676	0.067	0.050	0.000	0.006	0.000	0.000	0.142	0.119	16946		6.7	0.9	0.303	0.10536	
Bass Harbour	O-04	0	2	169	0.624	21.3	23.7	0.93s	0.565	0.000	0.000	0.375	0.625	0.936	0.00s	0.054	0.000	0.000	0.000	0.000	0.216	0.137	16427		6.6	1.0	0.272	0.069	1S
Bass Harbour	O-04	0	3	71	0.516	19.6	23.9	0.s63	0.446	0.000	0.130	0.242	0.627	0.663	0.037	0.012	0.062	0.025	0.000	0.000	0.136	0.131	16731		6.6	0.9	0.276	0.090	37

Table 2. Summary statistics 01 herring egg samples. (Continued)

Location	ADFG sample number	Depth (ft)	Rep. no.	TRITON sample number	Age (d)		Fraction of larvae in developmental stages				Fraction of larvae in deformity classes						Yolk sac volume (mm <sup>3</sup> )		Weight (µg)		Length (mm)		Condition (µg mm <sup>-3</sup> )						
					fraction et hatch	fraction of larvae viable	fraction viable	pre-1a	1a	1b	1c	normal	spine	yolk	jaw	stubby	head	caudal	mean	SD	mean	SD	mean	SD	msn	SD	n		
					50%	25%	viable	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	hatch	
Ssss Harbour	O-04	0	1	153	0.536	20.6	21.8	0.969	0.520	0.006	0.492	0.375	0.125	0.09	0.016	0.000	0.008	0.006	0.000	0.000	0.000	0.251	0.176	156	25	8.2	0.9	0.342	0.20712
Bass Herb our	O-04	-5	2	73	0.699	21.2	24.?	0.923	0.s45	0.011	0.116	0.394	0.479	0.923	0.046	0.011	0.007	0.014	0.000	0.000	0.155	0.134	166	29	9.2	0.8	0.228	0.0s1 so	
6sss Herb our	O-04	-5	1	32	0.166	21.5	23.7	0.750	0.124	0.083	0.0S3	0.167	0.657	0.750	0.063	0.063	0.0S3	0.000	0.000	0.000	0.205	0.139	17825		9.0	1.7	0.334	0.315 9	
6sss Harbour	O-04	-5	3	171	0.993	22.6	24.6	0.763	0.756	0.024	0.185	0.329	0.462	0.763	0.088	0.141	0.006	0.000	0.000	0.000	0.147	0.133	16427		9.0	0.7	0.238	0.094 45	
6.sss Harbour	0'04	-1s	2	66	0.505	20.6	23.5	0.823	0.416	0.015	0.2S2	0.492	0.231	0.823	0.106	0.04S	0.000	0.023	0.000	0.000	0.216	0.126	17936		6.4	0.6	0.312	0.09532	
Bass Harbour	0'04	-1s	3	121	0.268	20.6	23.5	1.000	0.266	0.035	0.612	0.212	0.141	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.283	0.106	202 36		8.0	0.6	0.417	0.14212	
6sss HerbOur	0-04	-15	1	149	0.571	21.5	24.4	0.7s4	0.453	0.019	0.071	0.781	0.129	0.7S4	0.129	0.039	0.013	0.026	0.000	0.000	0.177	0.111	1s4 33		6.7	0.9	0.264	0.094 27	
8sss Harbour	0-0S	0	2	162	0.562	25.0	28.6	0.532	0.22s	0.000	0.029	0.072	0.899	0.532	0.180	0.2S6	0.000	0.000	0.000	0.000	0.051	0.066	1s0 30		8.9	0.9	0.244	0.119 30	
6SSS Harbour	O-08	0	3	147	0.260	23.4	26.1	0.770	0.216	0.016	0.197	0.016	0.770	0.770	0.000	0.230	0.000	0.000	0.000	0.000	0.0s3	0.0s3	16334		9.3	1.2	0.240	0.06516	
6ss.s Harbour	O-08	0	1	64	0.4s3	24.5	27.3	0.944	0.456	0.019	0.316	0.159	0.505	0.944	0.000	0.026	0.026	0.000	0.000	0.000	0.126	0.133	16724		8.4	0.9	0.293	0.100 25	
6sss Harbour	0-es	-5	1	176	0.s65	23.7	27.1	0.835	0.739	0.000	0.041	0.147	0.612	0.835	0.04S	0.063	0.016	0.016	0.000	0.000	0.155	0.123	15S 27		6.6	0.8	0.241	0.0S6 33	
6sss Harbour	O-08	-5	2	130	0.328	24.4	26.0	0.672	0.2ss	0.000	0.032	0.234	0.734	0.872	0.043	0.085	0.000	0.000	0.000	0.000	0.052	0.052	180 27		9.4	0.7	0.216	0.03712	
6sss Harbour	0-0s	-5	3	114	0.449	23.6	25.2	0.696	0.403	0.000	0.256	0.570	0.172	0.896	0.031	0.070	0.000	0.000	0.000	0.000	0.107	0.054	15534		8.6	0.9	0.249	0.05217	
6SSS Harbour	0-0S	.15	3	13	0.476	24.2	35.9	0.946	0.453	0.000	0.030	0.530	0.440	0.S46	0.030	0.000	0.022	0.000	0.000	0.000	0.109	0.072	173 29		9.3	0.6	0.222	0.07022	
Bass Harbour	O-08	-15	1	33	0.621	23.8	25.9	0.961	0.597	0.006	0.112	0.691	0.191	0.961	0.022	0.017	0.000	0.000	0.000	0.000	0.116	0.143	16522		9.1	0.9	0.231	0.06820	
Bass Harbour	0-06	-15	2	47	0.666	24.7	26.1	0.973	0.646	0.000	0.000	0.331	0.669	0.973	0.007	0.007	0.000	0.014	0.000	0.000	0.143	0.116	151 33		6.6	1.1	0.231	0.07123	
6SSS Harbour	0-10	0	1	16	0.017	23.9	25.3	0.908	0.742	0.010	0.117	0.3s0	0.483	0.908	0.041	0.000	0.051	0.000	0.000	0.000	0.155	0.114	176 21		9.1	0.9	0.252	0.16027	
Bass Harbour	0-10	0	3	115	0.776	24.2	25.9	0.623	0.639	0.036	0.031	0.531	0.401	0.S23	0.057	0.000	0.042	0.078	0.000	0.000	0.179	0.145	171 30		8.7	0.6	0.275	0.093 32	
6sss Harbour	0-10	0	2	65	0.569	25.4	27.9	0.896	0.528	0.024	0.104	0.152	0.720	0.896	0.0343	0.055	0.016	0.000	0.000	0.000	0.064	0.099	15224		6.7	1.0	0.244	0.15S 22	
Sess Harbour	0-10	-5	2	45	0.833	23.6	26.0	0.666	0.73s	0.004	0.042	0.034	0.920	0.S43S	0.051	0.059	0.004	0.000	0.000	0.000	0.066	0.073	16323		9.3	0.6	0.20S	0.06723	
Bass Harbour	O-10	-5	1	117	0.467	24.4	27.5	0.641	0.393	0.006	0.006	0.409	0.560	0.841	0.091	0.066	0.0013	0.000	0.000	0.000	0.125	0.105	16935		6.7	0.6	0.262	0.013921	
6sss Harbour	0-10	-5	3	30	0.467	24.1	25.2	0.s07	0.424	0.023	0.302	0.395	0.279	0.907	0.047	0.000	0.047	0.000	0.000	0.000	0.197	0.093	166 27		6.3	0.9	0.302	0.10221	
Bass Harbour	O-10	-15	1	so	0.357	24.1	2S.2	0.932	0.333	0.000	0.352	0.466	0.182	0.932	0.045	0.023	0.000	0.000	0.000	0.000	0.220	0.159	17223		6.7	0.9	0.271	0.06517	
8sss Harbour	0-10	-15	3	163	0.5s0	24.2	27.9	0.789	0.442	0.012	0.090	0.269	0.608	0.789	0.169	0.042	0.000	0.000	0.000	0.000	0.130	0.147	171 33		6.7	0.6	0.277	0.12524	
6sss Harbour	0-10	-15	2	132	0.527	24.0	25.6	0.s41	0.443	0.000	0.000	0.000	1.000	0.841	0.057	0.102	0.000	0.000	0.000	0.000	0.161	0.296	171 24		8.5	0.6	0.2S4	0.091 3	
Outside Gey	0-11	5	2	10	0.477	21.7	24.1	0.ss3	0.421	0.0S2	0.520	0.123	0.27S	0.8S3	0.029	0.056	0.010	0.000	0.012	0.000	0.213	0.139	17329		6.6	0.7	0.2S6	Owl 22	
Outside Bay	0-11	5	3	140	0.269	21.9	25.1	0.825	0.23s	0.025	0.113	0.113	0.750	0.625	0.075	0.000	0.100	0.000	0.000	0.000	0.117	0.105	147 37		8.9	0.8	0.222	0.0S4 29	
Outside Bay	0-11	5	1	158	0.223	21.9	25.7	0.632	0.141	0.000	0.1s4	0.303	0.513	0.632	0.132	0.000	0.171	0.06S	0.000	0.000	0.139	0.102	16s 36		6.6	1.0	0.269	0.15332	
Outside Bay	O-11	0	2	180	0.770	21.1	25.1	0.932	0.718	0.000	0.112	0.671	0.217	0.932	0.007	0.041	0.020	0.000	0.000	0.000	0.16S	0.161	15S 32		6.6	0.9	0.236	0.0s2 2s	
Outside Bay	0-11	0	3	09	0.826	21.7	23.6	0.ss1	0.711	0.025	0.206	0.155	0.613	0.SS1	0.034	0.025	0.0S0	0.000	0.000	0.000	0.107	0.141	16321		6.7	1.0	0.2SS	0.10837	
Outside Bay	O-11	0	1	160	0.642	21.6	24.3	0.52s	0.267	0.059	0.157	0.4S1	0.324	0.529	0.235	0.000	0.225	0.010	0.000	0.000	0.160	0.125	161 25		6.6	1.2	0.259	0.10s 2s	
Outside Bay	O-11	-s	1	59	0.5437	23.4	2s.4	0.82S	0.52s	0.000	0.011	0.268	0.721	0.699	0.067	0.034	0.000	0.000	0.000	0.000	0.114	0.10S	1ss 3s		9.1	0.7	0.231	0.0S2 34	
Outside Bay	0.11	-s	3	4s	0.s40	22.3	23.6	0.69S	0.755	0.000	0.061	0.220	0.719	0.696	0.054	0.020	0.027	0.000	0.000	0.000	0.165	0.133	1s6 2s		8.7	1.1	0.221	0.166 30	
Outside Sey	O-11	-6	2	123	0.s0S	20.7	23.S	0.883	0.714	0.021	0.117	0.473	0.369	0.ss3	0.117	0.000	0.000	0.000	0.000	0.000	0.158	0.120	1S6 26		9.3	0.8	0.244	0.07227	
Outside Sey	O-12	5	1	a	0.425	21.5	23.3	0.942	0.400	0.000	0.2s1	0.609	0.130	0.942	0.05S	0.000	0.000	0.000	0.000	0.000	0.157	0.103	15521		8.3	0.6	0.276	0.0S5 9	
Outside Say	0-12	5	2	105	0.395	22.0	23.7	0.943	0.372	0.000	0.(s30	0.023	0.977	0.243	0.023	0.034	0.000	0.000	0.000	0.000	0.027	0.036	15940		9.6	0.5	0.171	0.042 8	
Outside Bay	D-12	5	3	122	0.ss7	20.6	23.6	0.7ss	0.42S	0.000	0.000	0.121	0.879	0.766	0.071	0.152	0.010	0.000	0.000	0.000	0.021	0.034	15s 20		9.3	0.7	0.199	0.04013	

Table 2. Summary statistics of herring egg samples. (Continued)

Location	ADFG		TRITON		Age (d)		fraction fraction		Fraction of larvae In				Fraction of larvae In				Yolk sac		Weight (ug)	Length (mm)		Condition (ug mm <sup>-3</sup> )					
	sample number	Depth (ft)	Rep. no.	sample number	traction survived to hatch	at hatch 50% 95%	larvae viable	fraction hatch	developmental stages				deformity classes				volume (mm <sup>3</sup> )	mean		SD	mean	SD	mean	SD			
	number	(ft)	no.	number	survived to hatch	50% 95%	viable	hatch	pre-1a	1a	1b	1c	normal	spine	yolk	Jew	stubby	head	caudal	mean	SD	mean	SD	mean	SD	n	
Outside Bay	0-12	0	3	22	0.628	21.6 23.7	0.892	0.560	0.005	0.065	0.2S1	0.649	0.892	0.03S	0.054	0.011	0.005	0.000	0.000	0.116	0.069	163	3S	9.4	0.7	0.2240	0.05029
Outside Bay	0-12	0	2	170	0.999	22.7 25.0	0.914	0.913	0.000	0.079	0.036	0.ss3	0.914	0.036	0.041	0.036	0.000	0.000	0.000	0.115	0.133	161	24	9.3	1.0	0.214	0.07260
Outside Bay	0-12	0	1	54	0.521	21.9 25.2	0.633	0.330	0.023	0.055	0.227	0.695	0.633	0.031	0.160	0.156	0.000	0.000	0.000	0.102	0.118	161	19	9.0	0.9	0.233	0.06119
Outside Bay	0-12	-5	1	51	0.634	23.7 25.6	0.ss6	0.436	0.007	0.007	0.220	0.766	0.633	0.135	0.021	0.106	0.050	0.000	0.000	0.100	0.114	165	14	9.4	0.9	0.213	0.07436
outside Bay	0-12	-5	2	89	0.4s9	23.1 25.5	0.s2s	0.3s7	0.013	0.052	0.090	0.645	0.s26	0.103	0.013	0.05s	0.000	0.000	0.000	0.136	0.166	175	34	9.1	0.8	0.2400	0.06222
outside Bay	0-12	-5	3	11	0.49s	21.8 25.4	0.s17	0.306	0.023	0.509	0.223	0.24S	0.617	0.120	0.000	0.211	0.000	0.000	0.051	0.243	0.193	167	26	8.4	1.1	0.32S	0.21216
Cabin Bay	0-13	0	3	50	0.s46	22.6 2S.S	0.722	0.466	0.014	0.024	0.189	0.774	0.722	0.036	0.231	0.000	0.009	0.000	0.000	0.123	0.121	164	29	6.9	1.1	0.25S	0.13S26
Cabin Bay	0-13	0	1	173	0.s44	21.7 25.9	0.s6s	0.4s3	0.005	0.005	0.051	0.939	0.ss6	0.020	0.091	0.000	0.000	0.000	0.000	0.104	0.112	162	25	6.6	1.0	0.2S1	0.12722
Cabin Bay	0-13	0	2	82	0.3s3	22.5 24.6	0.35s	0.129	0.000	0.036	0.3s5	0.577	0.35s	0.036	0.000	0.000	0.60s	0.000	0.000	0.105	0.0s5	179	37	6.6	0.5	0.2630	0.06313
Cabin Bay	0-13	-s	2	128	0.606	22.7 24.1	0.s63	0.5s4	0.000	0.042	0.232	0.726	0.963	0.032	0.005	0.000	0.000	0.000	0.000	0.147	0.133	159	27	S.7	0.6	0.246	0.0S216
Cabin Bay	0-13	-s	3	110	0.613	23.2 24.9	0.950	0.772	0.016	0.3S4	0.443	0.157	0.950	0.019	0.025	0.006	0.000	0.000	0.000	0.107	0.127	177	33	9.0	0.6	0.247	0.07320
Cabin Say	0-13	-5	1	9	0.ss3	22.5 24.2	0.ss0	0.601	0.005	0.036	0.70s	0.246	0.S60	0.020	0.024	0.024	0.052	0.000	0.000	0.191	0.127	172	27	9.0	0.7	0.245	0.06429
Cabin Bay	0-13	-15	3	136	0.534	23.4 26.4	0.915	0.469	0.000	0.169	0.515	0.315	0.91s	0.069	0.000	0.000	0.008	0.00S	0.000	0.157	0.112	153	20	8.8	0.6	0.235	0.07623
Cabin Bay	0-13	-15	1	75	0.625	25.3 27.5	0.817	0.511	0.000	0.310	0.155	0.535	0.617	0.117	0.019	0.047	0.000	0.000	0.000	0.10s	0.0s6	162	29	9.0	0.7	0.234	0.07432
Cabin Bay	0-13	-15	2	94	0.652	23.6 25.0	0.sss	0.570	0.000	0.050	0.362	0.5s6	0.686	0.050	0.000	0.064	0.000	0.000	0.000	0.126	0.167	178	26	9.2	0.6	0.237	0.06112
Outside Bay	0-14	5	3	20	0.547	21.7 24.1	0.730	0.399	0.054	0.054	0.473	0.419	0.730	0.014	0.243	0.014	0.000	0.000	0.000	0.150	0.128	172	34	9.0	0.7	0.241	0.03116
Outside Bay	0-14	5	1	76	0.357	22.9 25.9	0.434	0.1s5	0.053	0.053	0.115	0.779	0.434	0.071	0.3s9	0.106	0.000	0.000	0.000	0.076	0.129	150	24	9.1	0.7	0.202	0.04612
Outside Bay	0-14	5	2	93	0.325	21.4 22.9	0.963	0.314	0.037	0.201	0.075	0.ss7	0.963	0.037	0.000	0.000	0.000	0.000	0.000	0.107	0.117	171	21	9.1	0.6	0.234	0.07710
Outside Say	0-14	0	2	11	0.728	21.5 23.0	0.ss2	0.642	0.007	0.170	0.399	0.424	0.6s2	0.045	0.0S6	0.007	0.000	0.000	0.030	0.159	0.097	176	34	6.9	0.9	0.238	0.03039
Outside Bay	0-14	0	1	3s	0.670	20.6 23.5	0.ss5	0.593	0.024	0.06S	0.310	0.592	0.665	0.077	0.018	0.016	0.000	0.003	0.000	0.121	0.117	161	19	6.8	1.0	0.252	0.09626
Outside Bay	0-14	0	3	21	0.4S6	21.5 22.9	0.900	0.437	0.071	0.157	0.3S5	0.386	0.900	0.029	0.000	0.071	0.000	0.000	0.000	0.179	0.150	177	27	6.7	0.9	0.2S2	0.0S2 12
Outside Bay	0-14	-5	2	111	0.574	21.7 24.0	0.033	0.476	0.033	0.0s0	0.117	0.800	0.633	0.000	0.167	0.000	0.000	0.000	0.000	0.121	0.101	1S7	36	9.1	0.6	0.225	0.07614
Outside Bay	0-14	-5	3	26	0.709	21.5 22.7	0.610	0.432	0.000	0.09S	0.S0S	0.096	0.610	0.159	0.14S	0.000	0.065	0.000	0.000	0.193	0.114	155	27	6.4	0.7	0.262	0.05020
Outside Bay	0-14	-5	1	145	0.s19	20.0 24.4	0.037	0.510	0.000	0.0s0	0.754	0.1s7	0.637	0.131	0.012	0.020	0.000	0.000	0.000	0.115	0.100	156	23	8.9	0.5	0.225	0.06025
Story Island	0-15	0	3	164	0.732	23.9 26.3	0.923	0.676	0.009	0.063	0.1s0	0.74s	0.923	0.06S	0.005	0.000	0.000	0.005	0.000	0.074	0.069	161	28	6.7	1.2	0.2790	0.19519
Story Island	0-15	0	2	161	0.573	24.6 25.9	0.772	0.442	0.000	0.047	0.035	0.916	0.772	0.222	0.006	0.000	0.000	0.000	0.099	0.1s6	163	34	9.1	0.s	0.24s	0.07014	
Story Island	0-15	0	1	7s	0.740	24.0 2S.9	0.961	0.711	0.000	0.227	0.367	0.406	0.961	0.027	0.000	0.000	0.004	0.000	0.000	0.103	0.067	164	22	9.0	0.6	0.228	0.04531
Story Island	0-15	-5	2	146	0.377	23.7 2S.2	0.ss9	0.327	0.000	0.000	0.440	0.560	0.s69	0.063	0.04s	0.000	0.000	0.000	0.063	0.090	16s	25	9.7	0.4	0.163	0.03221	
Story Island	0-15	-5	3	44	0.769	23.5 25.9	0.959	0.72s	0.000	0.004	0.6439	0.2-97	0.959	0.030	0.000	0.006	0.004	0.000	0.000	0.121	0.066	167	33	9.0	0.7	0.230	0.0623s
Story Island	0-15	-5	1	177	0.631	22.8 27.5	0.639	0.530	0.000	0.046	0.s0s	0.345	0.s39	0.029	0.115	0.000	0.017	0.000	0.000	0.102	0.0s7	153	41	9.3	0.8	0.191	0.04720
Story Island	0-15	-15	3	174	0.733	23.3 2S.3	0.659	0.630	0.000	0.110	0.SS7	0.224	0.659	0.055	0.039	0.031	0.016	0.000	0.000	0.120	0.114	153	2s	6.S	1.0	0.2770	0.13432
Story Island	0-15	-15	2	66	0.357	25.s 27.7	0.933	0.333	0.000	0.050	0.317	0.633	0.933	0.060	0.000	0.017	0.000	0.(s30	0.000	0.103	0.068	167	23	9.1	0.4	0.222	0.04117
Story Island	0-1s	-15	1	157	0.691	2s.3 2s.9	0.7s3	0.541	0.00s	0.015	0.030	0.s49	0.763	0.131	0.0SS	0.000	0.000	0.000	0.000	0.037	0.04s	151	27	9.5	1.1	0.193	0.0S526
Story Island	0-16	0	1	97	0.357	22.7 23.9	1.000	0.357	0.000	0.187	0.421	0.393	1.000	0.000	0.000	0.600	0.000	0.000	0.000	0.104	0.068	16s	55	S.7	0.6	0.276	0.15217
Story Island	0-16	0	3	112	0.732	24.1 26.3	0.952	0.696	0.000	0.253	0.211	0.536	0.952	0.030	0.018	0.000	0.000	0.000	0.000	0.189	0.124	156	19	6.9	1.2	0.240	0.0S418
Story Island	0-16	0	2	133	0.4s2	23.4 24.9	0.941	0.435	0.000	0.206	0.235	0.559	0.941	0.020	0.039	0.000	0.000	0.000	0.000	0.142	0.133	1S3	25	6.7	1.1	0.270	0.11630
Story Island	0-1s	-s	3	129	0.ss5	23.2 24.9	0.s50	0.497	0.023	0.000	0.429	0.549	0.650	0.090	0.060	0.000	0.000	0.030	0.000	0.144	0.151	172	17	s.?	0.7	0.2760	0.10017

Table 2. Summary statistics of herring egg samples. (Continued)

Location	ADFG		TRITON Rep. no.	Age (d) fraction at hatch		Fraction of larvae in developmental stages				Fraction of larvae in deformity classes							Yolk sac volume (mm <sup>3</sup> )		Weight (ug)		Length (mm)		Condition (ug mm <sup>-3</sup> )					
	sample number	Depth (ft)		sample number	survived to hatch	50%	95%	fraction of larvae viable	*action hatch	pre-1a	1a	1b	1c	normal	spine	yolk	Jew	stubby	head	caudal	mean	SD	mean	SD	mean	SD	mesn	SD
Story Island	O-16	-s	1	144	0.626	24.7	26.8	0.945	0.781	0.005	0.26S	0.055	0.67S	0.945	0.005	0.045	0.005	0.000	0.000	0.000	0.096	0.092	15427	6.7	1.0	0.267	0.16535	
Story Island	0-16	-s	2	43	0.76S	22.9	26.0	0.931	0.712	0.025	0.262	0.327	0.3S6	0.931	0.054	0.010	0.005	0.000	0.000	0.000	0.124	0.095	16227	8.6	0.6	0.269	0.091	37
Story Island	O-16	-15	3	63	0.293	24.5	26.9	0.902	0.2s4	0.030	0.171	0.000	0.829	0.902	0.098	0.000	0.000	0.000	0.000	0.056	0.046	15119	8.3	0.6	0.269	0.060	8	
Story Island	0-16	-15	1	26	0.711	23.5	25.1	0.666	0.631	0.000	0.123	0.037	0.840	0.66S	0.059	0.053	0.000	0.030	0.000	0.000	0.111	0.107	15721	8.2	1.2	0.331	0.21022	
Story Island	O-16	-15	2	12	0.559	23.6	25.5	0.879	0.491	0.000	0.2S4	0.395	0.321	0.679	0.026	0.000	0.095	0.000	0.000	0.000	0.231	0.167	16430	8.6	0.9	0.263	0.10525	
Rocky 6Sy	0-17	5	2	42	0.368	21.7	22.8	0.750	0.291	0.12S	0.750	0.125	0.000	0.750	0.250	0.000	0.000	0.000	0.000	0.319	0.060	16459	6.2	0.3	0.310	0.136	6	
Rocky Bay	O-17	s	3	27	0.071	21.?	23.0	1.000	0.071	0.156	0.375	0.469	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.262	0.065	17121	6.6	0.9	0.283	0.07611		
Rocky 6Sy	0-17	s	1	150	0.194	22.2	24.6	0.646	0.164	0.000	0.022	0.217	0.761	0.846	0.043	0.022	0.065	0.000	0.022	0.000	0.063	0.069	14925	9.4	0.9	0.187	0.051	17
Rocky Bay	0-17	o	1	116	0.612	23.3	26.8	0.665	0.s29	0.000	0.032	0.173	0.795	0.665	0.055	0.032	0.038	0.000	0.000	0.000	0.069	0.103	15521	9.2	0.9	0.217	0.115	2S
Rocky Bay	O-17	o	3	64	0.517	21.7	23.1	0.784	0.405	0.000	0.020	0.944	0.036	0.784	0.208	0.000	0.000	0.000	0.000	0.112	0.065	15926	8.5	0.6	0.260	0.05714		
Rocky Bay	0-17	0	2	135	0.s42	22.4	27.3	0.704	0.592	0.004	0.363	0.263	0.345	0.704	0.056	0.040	0.199	0.000	0.000	0.000	0.217	0.152	15925	8.8	0.9	0.25S	0.12662	
Rocky Bay	0-17	-s	3	102	0.690	24.0	26.9	0.661	0.594	Oats	0.174	0.409	0.40s	0.661	0.054	0.066	0.019	0.000	0.000	0.000	0.140	0.146	16635	9.0	0.9	0.236	0.081	46
Rocky 6Sy	O-17	-5	2	67	0.63S	23.1	26.7	0.628	0.694	0.007	0.232	0.360	0.401	0.828	0.088	0.020	0.064	0.000	0.000	0.000	0.177	0.124	16631	6.9	0.7	0.242	0.05735	
Rocky Bay	O-17	-s	1	32	0.759	22.s	25.7	0.670	0.660	0.063	0.133	0.451	0.351	0.670	0.091	0.006	0.026	0.006	0.000	0.000	0.163	0.126	16932	9.0	0.9	0.251	0.15734	
Rocky 6Sy	O-18	5	1	50	0.194	23.0	25.5	0.766	0.149	0.109	0.453	0.109	0.328	0.766	0.125	0.000	0.109	0.000	0.000	0.000	0.207	0.200	16420	9.0	1.4	0.285	0.251	10
Rocky 6Sy	O-18	5	3	166	0.616	22.7	26.8	0.031	0.512	0.000	0.257	0.1S3	0.590	0.631	0.082	0.000	0.067	0.000	0.000	0.000	0.147	0.1s2	15525	9.0	0.8	0.226	0.06228	
Rocky Bay	0-18	5	2	81	0.610	23.S	26.0	0.646	0.517	0.000	0.022	0.641	0.267	0.848	0.106	0.009	0.037	0.000	0.000	0.000	0.172	0.105	17419	9.4	0.6	0.215	0.03822	
Rocky Bay	O-18	0	1	15	0.609	21.6	23.0	0.916	0.5s9	0.026	0.s44	0.372	0.051	0.918	0.056	0.000	0.026	0.000	0.000	0.000	0.276	0.103	19029	8.6	0.6	0.300	0.06618	
Rocky say	O-18	0	3	131	0.401	22.9	24.9	0.643	0.257	0.004	0.076	0.576	0.344	0.643	0.183	0.058	0.116	0.000	0.000	0.000	0.162	0.131	16325	8.9	1.0	0.251	0.12033	
Rocky Bay	0-18	0	2	85	0.465	22.4	26.1	0.662	0.316	0.000	0.2s5	0.394	0.341	0.662	0.176	0.029	0.112	0.000	0.000	0.000	0.196	0.171	17036	9.0	0.9	0.250	0.12036	
Rocky 6Sy	0-18	-5	2	46	0.856	23.1	24.4	0.506	0.433	0.016	0.136	0.758	0.070	0.506	0.054	0.328	0.105	0.006	0.000	0.000	0.197	0.146	17326	9.2	0.7	0.234	0.07546	
Rocky Bay	O-18	-5	1	26	0.577	23.0	25.1	0.794	0.4s6	0.042	0.079	0.228	0.651	0.794	0.122	0.000	0.037	0.037	0.011	0.000	0.116	0.064	16022	8.9	0.7	0.236	0.06622	
Rocky 1fSy	O-18	-5	3	56	0.597	23.2	25.7	0.665	0.516	0.027	0.311	0.264	0.378	0.865	0.066	0.000	0.045	0.005	0.000	0.000	0.250	0.165	16626	8.9	1.1	0.263	0.11840	
Rocky Bay	0-19	5	1	165	0.509	22.3	26.2	0.799	0.407	0.024	0.541	0.110	0.325	0.799	0.0S6	0.029	0.066	0.000	0.000	0.000	0.177	0.156	16327	9.1	1.0	0.242	0.11055	
Rocky Bay	O-19	5	3	148	0.597	21.4	24.8	0.660	0.s25	0.032	0.259	0.285	0.424	0.660	0.070	0.000	0.051	0.000	0.000	0.000	0.322	0.194	17531	6.2	1.3	0.372	0.19529	
Rocky Bay	0-19	5	2	87	0.662	22.S	25.5	0.654	0.433	0.046	0.097	0.430	0.437	0.654	0.166	0.060	0.060	0.000	0.000	0.000	0.140	0.162	17330	6.9	0.7	0.259	0.10440	
Rocky Bay	0-19	0	1	113	0.541	22.8	24.5	0.766	0.414	0.0250	0.436	0.063	0.456	0.766	0.036	0.025	0.171	0.000	0.000	0.000	0.178	0.163	15726	8.4	1.0	0.301	0.22120	
Rocky 6Sy	0-19	0	3	17s	0.797	22.6	25.3	0.793	0.632	0.000	0.10S	0.327	0.S66	0.793	0.125	0.000	0.062	0.000	0.000	0.000	0.113	0.106	15717	9.3	0.8	0.202	0.05634	
Rocky 6Sy	0-19	0	2	31	0.629	22.2	24.5	0.7ss	0.477	0.004	0.542	0.263	0.192	0.756	0.096	0.042	0.100	0.004	0.000	0.000	0.139	0.145	1602s	9.3	0.6	0.208	0.067	34
Rocky Bay	O-19	-5	1	72	0.s0s	23.6	26.3	0.645	0.660	0.0060	0.149	0.055	0.790	0.845	0.049	0.003	0.103	0.000	0.000	0.000	0.171	0.161	1s630	8.9	0.9	0.242	0.11652	
Rocky 6Sy	0-19	-5	3	66	0.635	24.2	26.4	0.769	0.602	0.000	0.036	0.402	0.560	0.789	0.045	0.023	0.143	0.000	0.000	0.000	0.096	0.067	16124	9.3	0.7	0.205	0.03733	
Rocky Bay	O-19	-s	2	136	0.s7s	22.9	25.6	0.sss	0.402	0.004	0.157	0.s56	0.153	0.699	0.061	0.000	0.220	0.000	0.000	0.000	0.176	0.132	16942	6.9	0.8	0.247	0.084	36

Notes:

- ADFG - Alaska Department of Fish and Game; TRITON - Triton Environmental Consultants Ltd.
- C - control station; O -011 station.

**Table 3. Parameter values of the modified Weibull models describing survival at age, the fraction of eggs at age that were alive, and the cumulative fraction of hatched larvae at age.**

<u>Parameter</u>	<u>coefficient</u>	<u>SE</u>	<u>P</u>	<u>r<sup>2</sup></u>	<u>n</u>
<b>Survival [s(t)]</b>					
	<b>32.62</b>	<b>2.41</b>	<b>&lt;0.0001</b>	<b>0.10</b>	<b>819</b>
	<b>0.8991</b>	<b>0.0955</b>	<b>&lt;0.0001</b>		
<b>Fraction of live eggs [f(t)]</b>					
<b>a</b>	<b>30.49</b>	<b>1.44</b>	<b>&lt;0.0001</b>	<b>0.34</b>	<b>730</b>
<b>γ</b>	<b>6.4666</b>	<b>0.3353</b>	<b>&lt;0.0001</b>		
<b>b1</b>	<b>0.0281</b>	<b>0.0072</b>	<b>0.0001</b>		
<b>b</b>	<b>2</b>	<b>-0.3098</b>	<b>0.1059</b>	<b>0.0035</b>	
<b>Cumulative fraction hatched [h(t)]</b>					
<b>a</b>	<b>8.63</b>	<b>0.16</b>	<b>&lt;0.0001</b>	<b>0.70</b>	<b>1265</b>
<b>γ</b>	<b>4.8357</b>	<b>0.0903</b>	<b>&lt;0.0001</b>		
<b>b1</b>	<b>0.0487</b>	<b>0.0055</b>	<b>&lt;0.0001</b>		
<b>b2</b>	<b>-0.3191</b>	<b>0.0762</b>	<b>&lt;0.0001</b>		
<b>b3</b>	<b>-0.0362</b>	<b>0.0113</b>	<b>0.0015</b>		

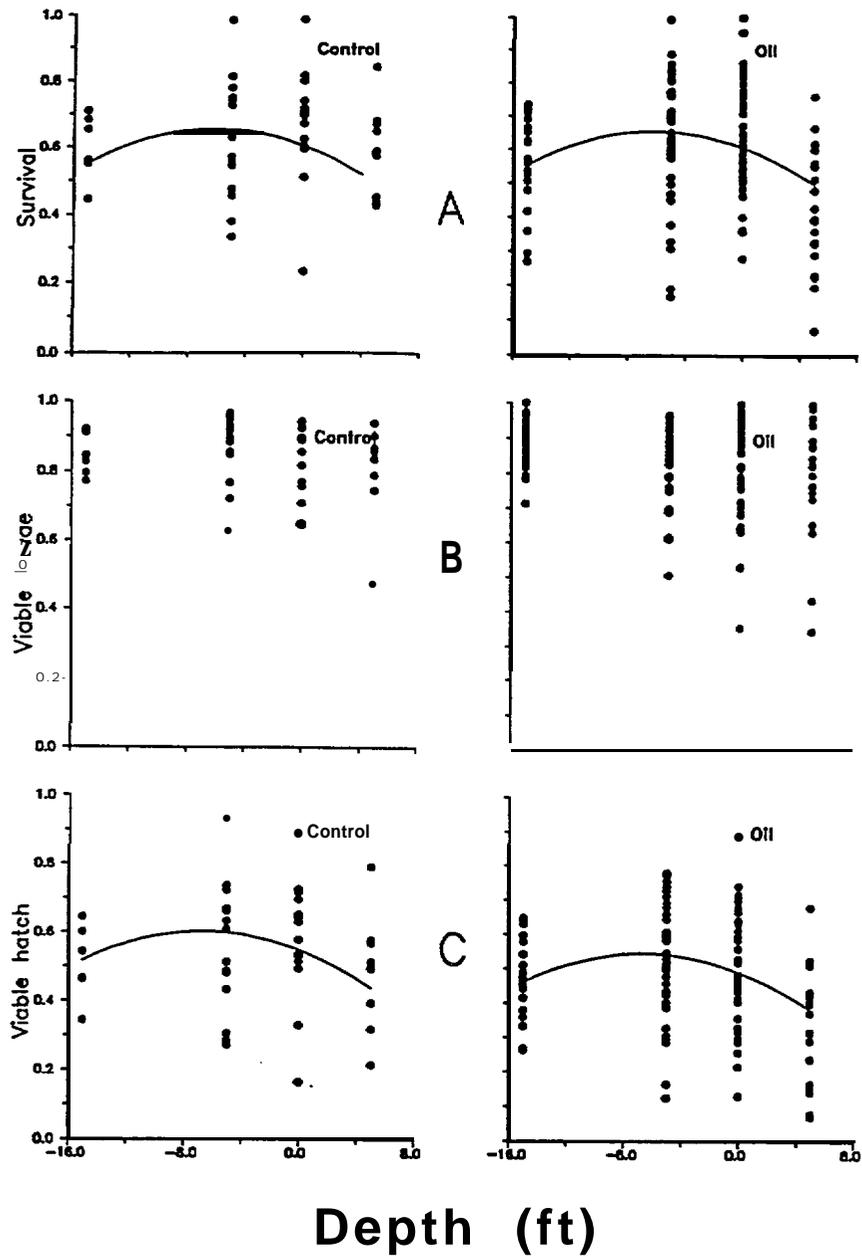


Figure 10. A Fraction of control and oiled herring eggs at four depth classes that survived to hatch larvae. Solid line is survival predicted from equation (9). B. Fraction of larvae that were viable. C. Fraction of eggs that hatched viable larvae. Solid line is viable hatch predicted from equation (10).

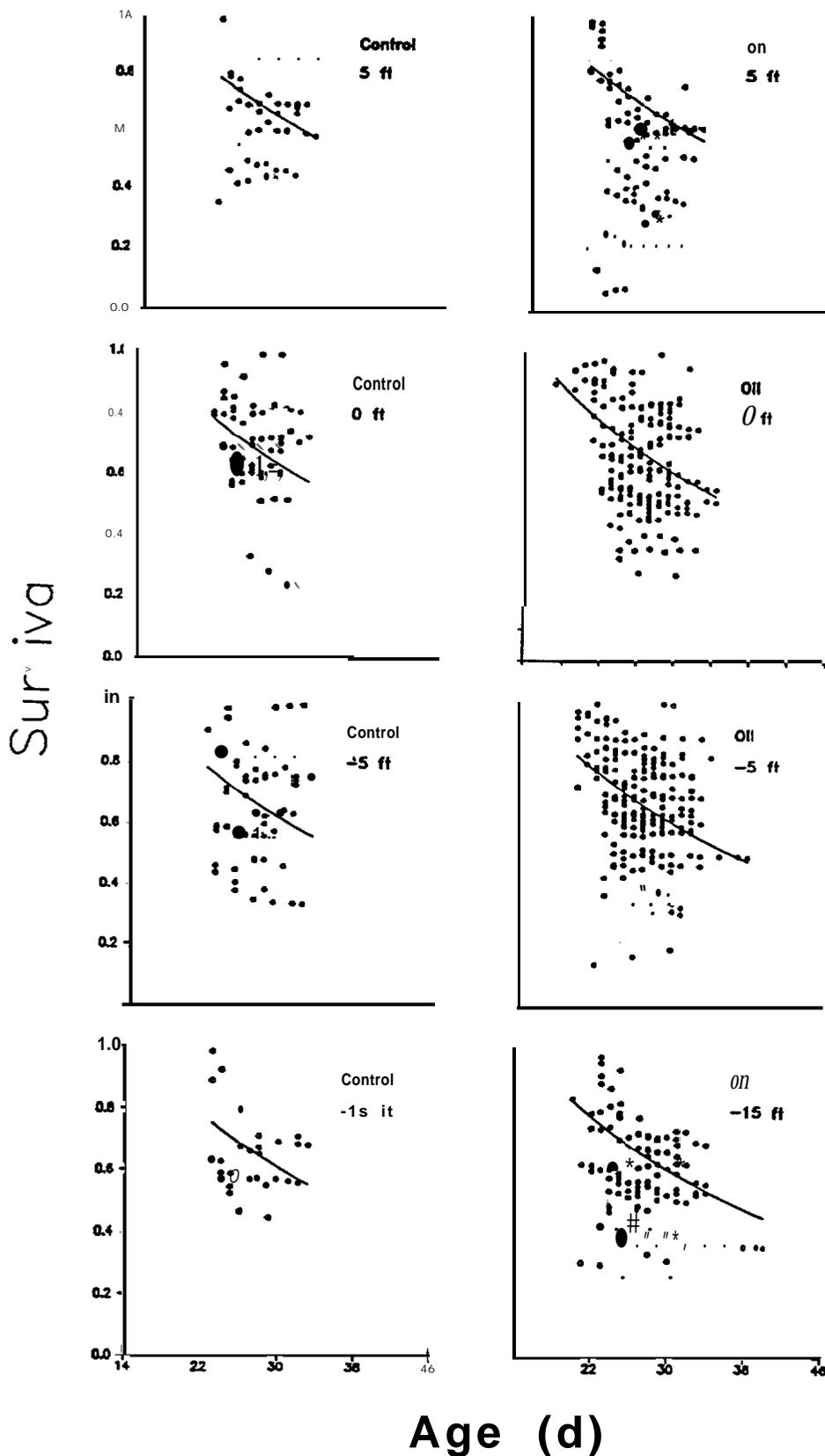


Figure 11. Age trajectory of survival of control and oiled herring eggs from four depths. Solid line is predicted survival from a Weibull model. See Table 3 and text for details.

suggests the presence of a depth effect and perhaps an oil effect, but there is too much variability in  $s(t)$  to detect it statistically.

### 303 Fraction of Live Eggs

The fraction of eggs that were live was constant or declined slowly until age 22 and then it decreased rapidly to zero by an age of about 32 d (Fig. 12). The sudden decline after age 22 was due to the onset of hatching during this period the eggs that remained unhatched were predominantly dead or dying. A modified Weibull model was fit to these data; its parameters are shown in Table 3 and its predicted  $f(t)$  is plotted in Fig. 12. The model showed that both depth and oil treatment were significant auxiliary factors;  $f(t)$  increased with increasing depth and was higher in the control group than in the oiled group. This is a reflection of the hatching schedule and not egg survival; eggs hatched earlier in shallow water than in deep water and they hatched earlier in oiled eggs than in control eggs. This subject is examined in greater detail in section 3.4 of this report.

#### Fraction of Live Eggs as an Index of Total Survival

If the fraction of live eggs during the hatching period is an unreliable indicator of survival, then perhaps the fraction of live eggs observed before the beginning of hatching may be an index of total survival. This hypothesis was the rationale for ADF&G's survey of live/dead herring egg ratios in Prince William Sound in 1989. We tested it by regressing survival on  $f(t)$  for the 180 replicate samples shown in Table 2. Fig. 13 shows that there was a significant correlation between  $s(t)$  and  $f(t)$ , but that the best-fitting regression only explained 17% of the variance in  $s(t)$ . In other words, ratios of live to total eggs are not good predictors of survival at any age; they can only estimate the approximate fate of an egg mass, i.e. whether survival will be greater or less than about 0.4.

### 3.4 Hatching Schedule

The average age of the eggs at collection ranged from 11 to 17 d with a mean of 14 d (SD =2, n = 21), which meant that the eggs began to hatch several days after they arrived in the laboratory. The mean age at which 50% of the larvae had hatched was 22.9 d (SD = 1.2, n = 180) and the mean age at which 95% of the larvae had

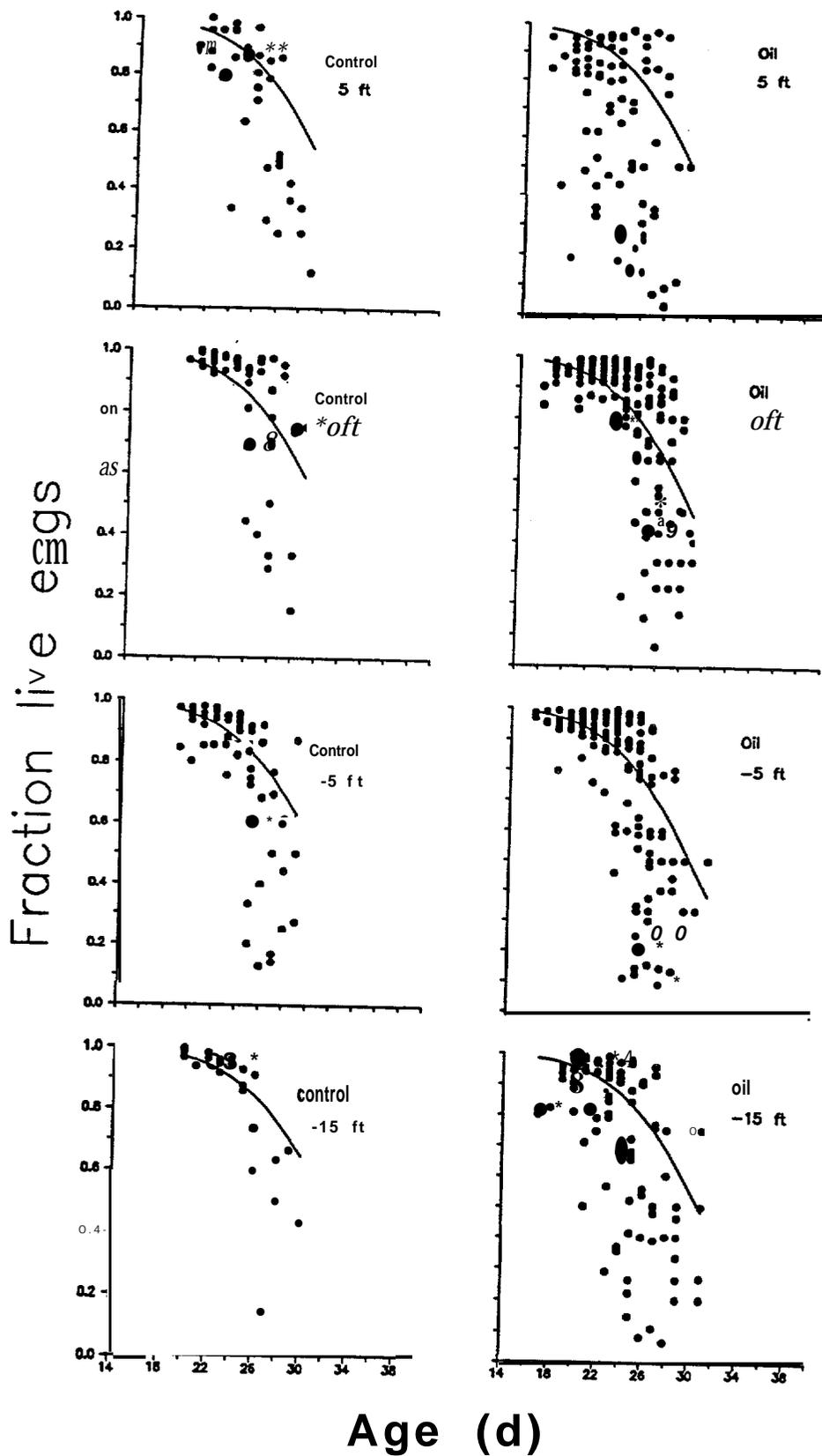


Figure 12. Fraction of herring eggs that were alive at age. Solid line is a modified Weibull model incorporating age, depth and oil treatment. See Table 3 and text for details.

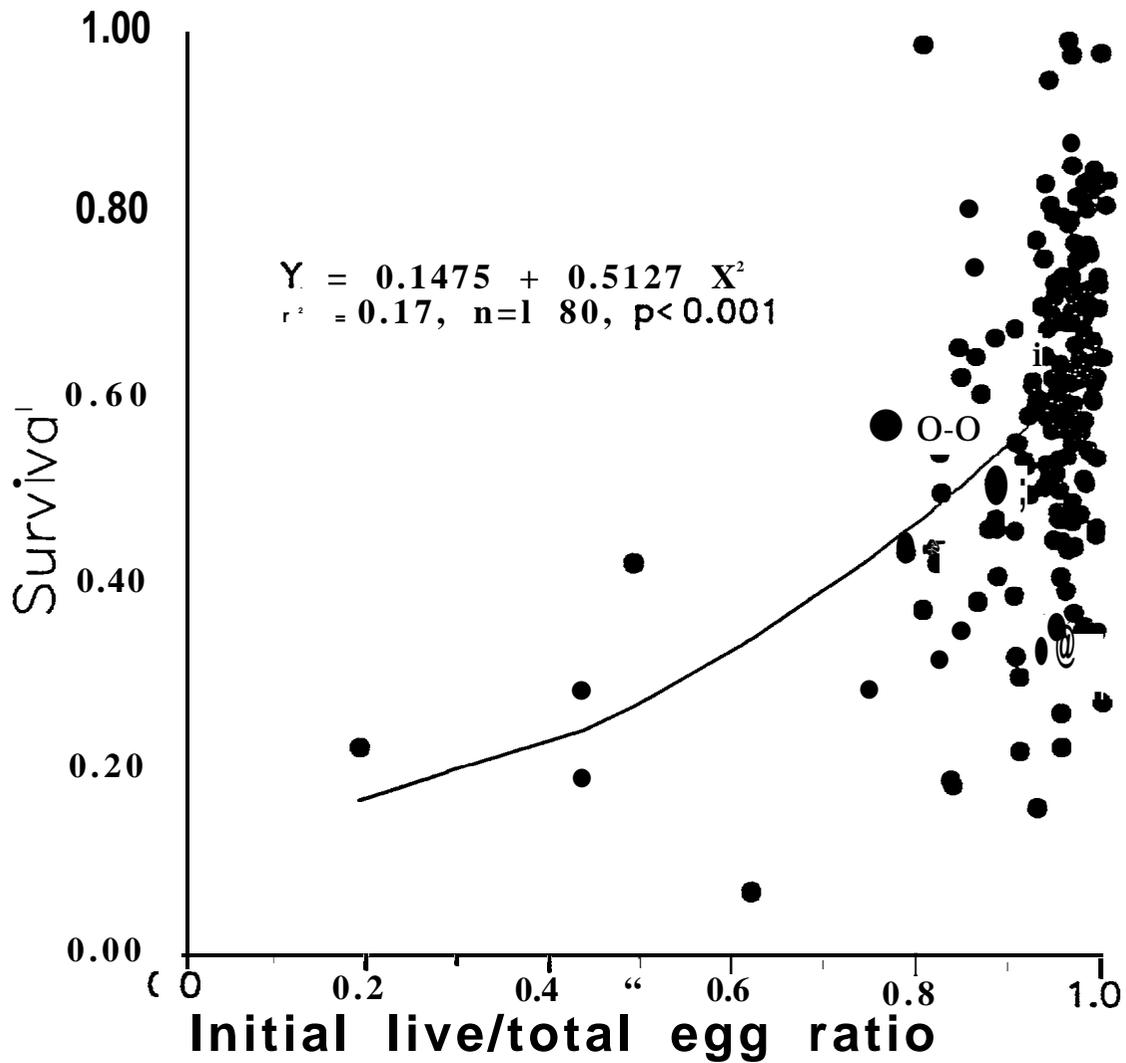


Figure 13. Survival as a function of the fraction of live eggs measured at the age of cut-down before hatching began. The solid line is a quadratic regression of survival on the fraction of live eggs. It shows that the fraction of live eggs is a poor predictor of survival.

hatched was 25.4 d (SD = 1.3, n = 180) (Table 2). Most hatching was completed by age 31 d, although at least one sample continued to produce larvae until age 35 (Fig. 14).

A modified Weibull model showed that the cumulative fraction of hatched larvae,  $h(t)$ , was significantly affected by depth, oil treatment, and the interaction of depth and oil treatment (Table 3 and Fig. 14). Hatching occurred sooner in the upper depths than the lower depths, and it occurred sooner in oiled eggs than in non-oiled eggs. The interaction of these variables reduced the effect of depth for the control samples, but it increased the difference in hatching schedules for the oiled samples. For example, 50% of the larvae in the oiled/5 ft cell had hatched by age 22.5 d, but only 20% of the larvae in the oiled/-15 ft cell had hatched at the same age.

### 3.5 Viable Larvae

There were six kinds of gross morphological deformity (Appendix C). Table 2 shows the total fraction of larvae in each of the six deformity classes for all 180 samples. They were, in order of decreasing frequency kinked or coiled spines (mean = 0.071, SD = 0.055); deformities of the yolk sac including no yolk sac, an anomalously small yolk sac, and a double yolk sac (mean = 0.046, SD = 0.070); missing or deformed jaw (mean = 0.034, SD = 0.053); a short stubby body (mean = 0.009, SD = 0.048); deformations of the head (mean = 0.001, SD = 0.003); and incomplete development of the caudal region of the body (mean = 0.001, SD = 0.004). Fig. 15 shows two examples of coiled spines; many of these fish were still alive and swimming in spirals when they were collected from the bottles, so the deformity was not the result of pre-preservation rigor mortis or of post-preservation shrinkage. Absence of a yolk sac was the most common deformation of the yolk sac; they were clearly distinguishable from larvae whose yolk had been ripped off by rough handling because no remnant of a yolk sac membrane or of its insertion in the ventral surface of the body was visible. Fig. 16 shows two examples of jaw deformity note that the lower jaw is not long enough to extend to the tip of the snout as it does in normal larvae, e.g. 1c larvae in Fig. 19. Fig. 17 shows two larvae with misshapen heads.

Cumulative fraction hatched

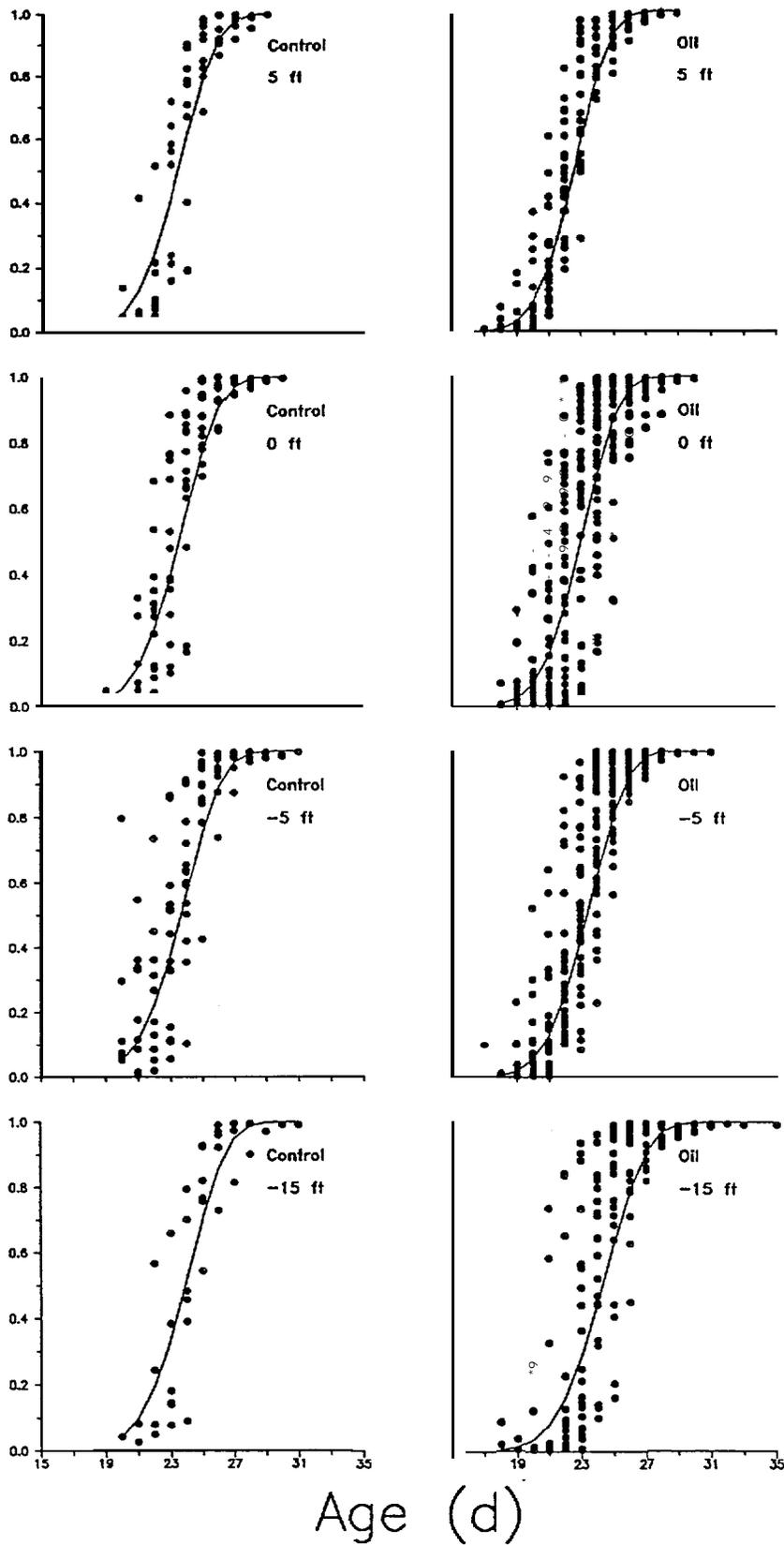


Figure 14. Hatching schedules of herring eggs from control and oiled samples at four depths. Solid line is the predicted cumulative fraction of hatched larvae from a modified Weibull model. Table 3 and text for

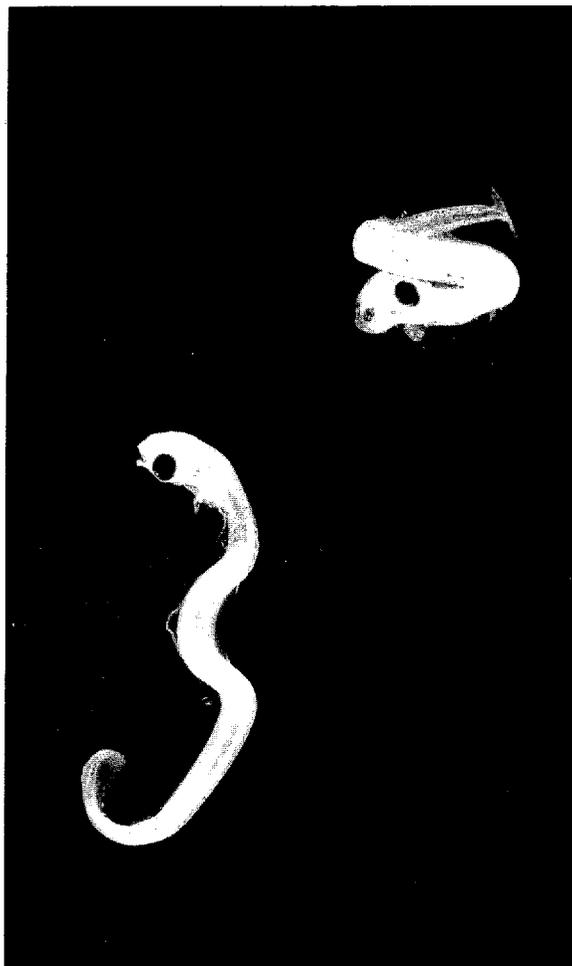


Figure 15. Two larvae with coiled spine deformity.



Figure 16. Two larvae with lower jaw deformity. Note that the lower jaw does not extend to the tip of the snout as it does in the normal larvae shown in Fig. 19.



Figure 17. Two larvae with deformed heads.

### Fraction of Viable Larvae

Table 2 also shows the fraction of larvae that did not exhibit any of these deformities; it was the fraction of larvae that were viable and it ranged from 0.346 to 1.000 with a mean of 0.838 (SD = 0.117). A two-way ANOVA showed that there were no significant effects of depth, oil treatment, or their interaction on the fraction of viable larvae. This is shown graphically in Fig. 10B.

### Fraction of Viable Hatch

The fraction of hatch that was viable is the product of egg survival and the fraction of viable larvae (Table 2). It ranged from 0.071 to 0.882 with a mean of 0.500 (SD = 0.169). A two-way ANOVA showed that the fraction of viable hatch varied significantly ( $P < 0.0001$ ) with depth and with oil treatment ( $P = 0.033$ ), but not with the interaction of these two factors. Multiple regression showed that the most variance in viable hatch ( $r^2 = 0.12$ ,  $n = 180$ ) was explained by a quadratic regression on depth

(lo)	<u>variable</u>	<u>coefficient</u>	<u>SE</u>	<u>P</u>
	constant	0.4920	0.0158	<0.0001
	x	0.0577	0.0275	0.0374
	depth	-0.0159	0.0036	<0.0001
	depth <sup>2</sup>	-0.0012	0.0003	<0.0001

where  $x =$  a dummy variable with a value of 1 for control eggs and 0 for oiled eggs. The fit of this model is shown in Fig. 10C.

### Deformity Classes

A two-way ANOVA showed that only the jaw deformity varied significantly ( $0.001 < P < 0.01$ ) with oil treatment. There was no significant variation with depth or with the interaction of depth and oil treatment. Comparisons of means showed that the means were significantly ( $0.01 < P < 0.05$ ) higher in the O and 5 ft depth classes of the control group than in the O and 5 ft classes of the treatment groups. There were no differences between the -5 and -15 ft classes.

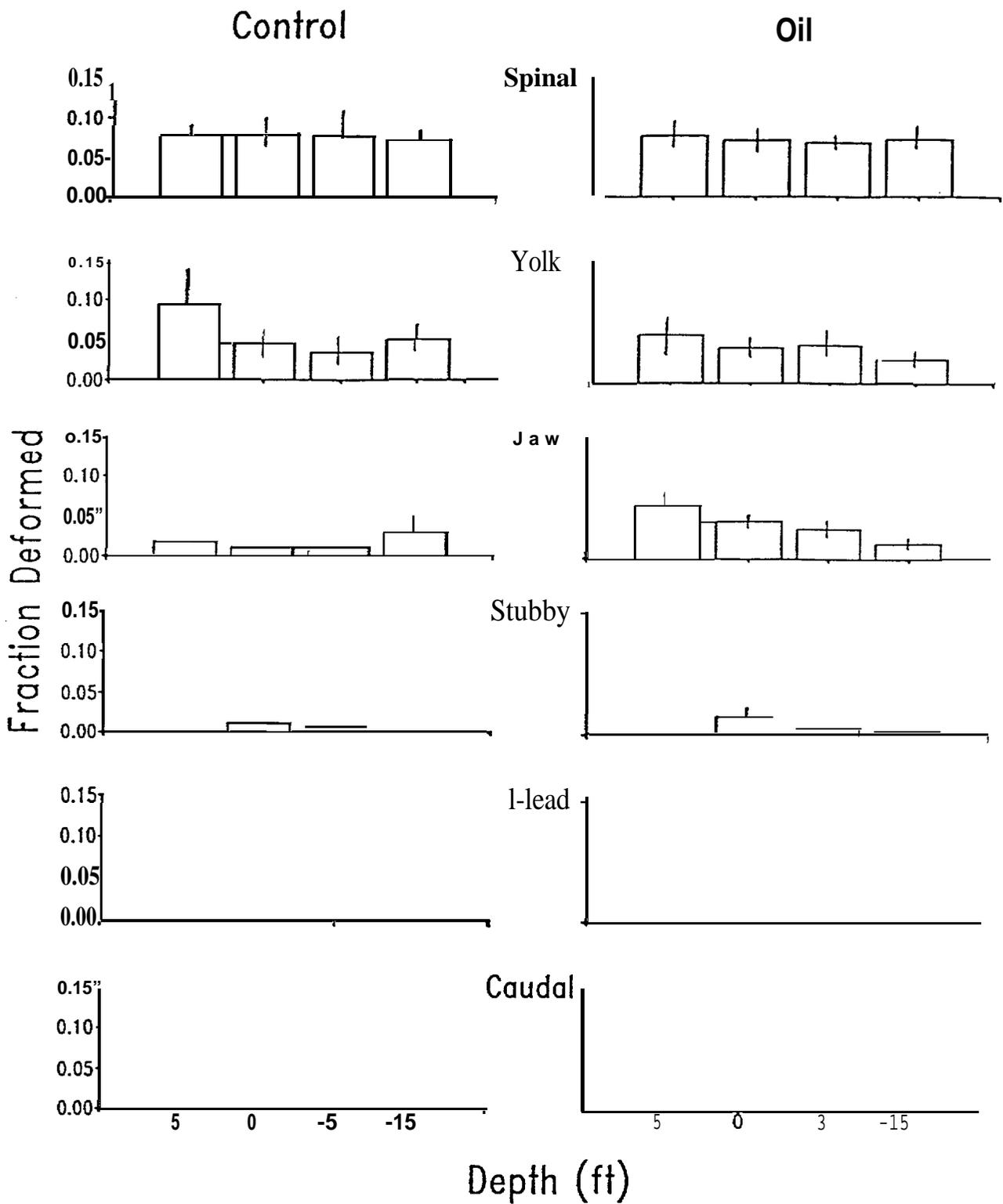


Figure 18. Mean fractions ( $\pm 1$  SE) of larvae in deformity classes for control and oiled groups. Stars indicate the statistical significance of differences between control and treatment means of the same depth class: \* $0.01 < P < 0.05$ , \*\* $0.001 < P < 0.01$ , \*\*\* $P < 0.001$ .

### 3.6 Developmental Stage

Fig. 19 shows the four developmental stages of newly-hatched herring larvae based on Doyle's (1977) staging system: pre-1a, 1a, 1b and 1c. Pre-1a was not described by Doyle (1977), but was invented by us in order to account for larvae that were much less developed than the 1a class. None of the pre-1a larvae were found to be alive when the incubation bottles were opened.

Mean fractions of larvae in the four stages of development are shown in Fig. 20. The data was first analysed using two-way ANOVAs; significant results were found only in the two extreme stages: pre-1a and 1c. The mean fraction of larvae classified as pre-1a varied significantly ( $P = 0.001$ ) with depth, but not with oil treatment or with the interaction of depth and oil treatment. Comparison of means showed that this depth effect was due to a significantly higher fraction of pre-1a larvae in the oiled/5 ft class than in the oiled/-15 ft class. The mean fraction of 1c larvae varied significantly ( $P = 0.007$ ) with oil treatment, but not with depth or the interaction of depth and oil treatment. Comparison of means showed that this oil effect was due to the fact that the mean fraction of 1c larvae in the control/5 ft class was significantly higher than all four depth classes in the oiled group.

The data was also analysed by comparing control and treatment means of the same depth classes. Only one of the 16 comparisons was significant - the fraction of 1c larvae in the control/5 ft group was significantly ( $0.001 < P < 0.01$ ) greater than the fraction of 1c larvae in the oiled/5 ft group. This difference is marked in Fig. 20.

### 3.7 Size and Condition of Larvae

Mean lengths, dry weights, yolk sac volumes and condition factors for each of the 180 samples are shown in Table 2. They are plotted against age for each of the eight combinations of oil treatment and depth in Figs. 21 to 24. Examination of these plots shows that size and condition varied with age. Therefore, comparisons were made between treatment/depth cells using age as a covariate.



Figure 19. Four stages of newly hatched herring larvae ranked right to left in order of increasing size and development: pre-la, la, lb, and lc.

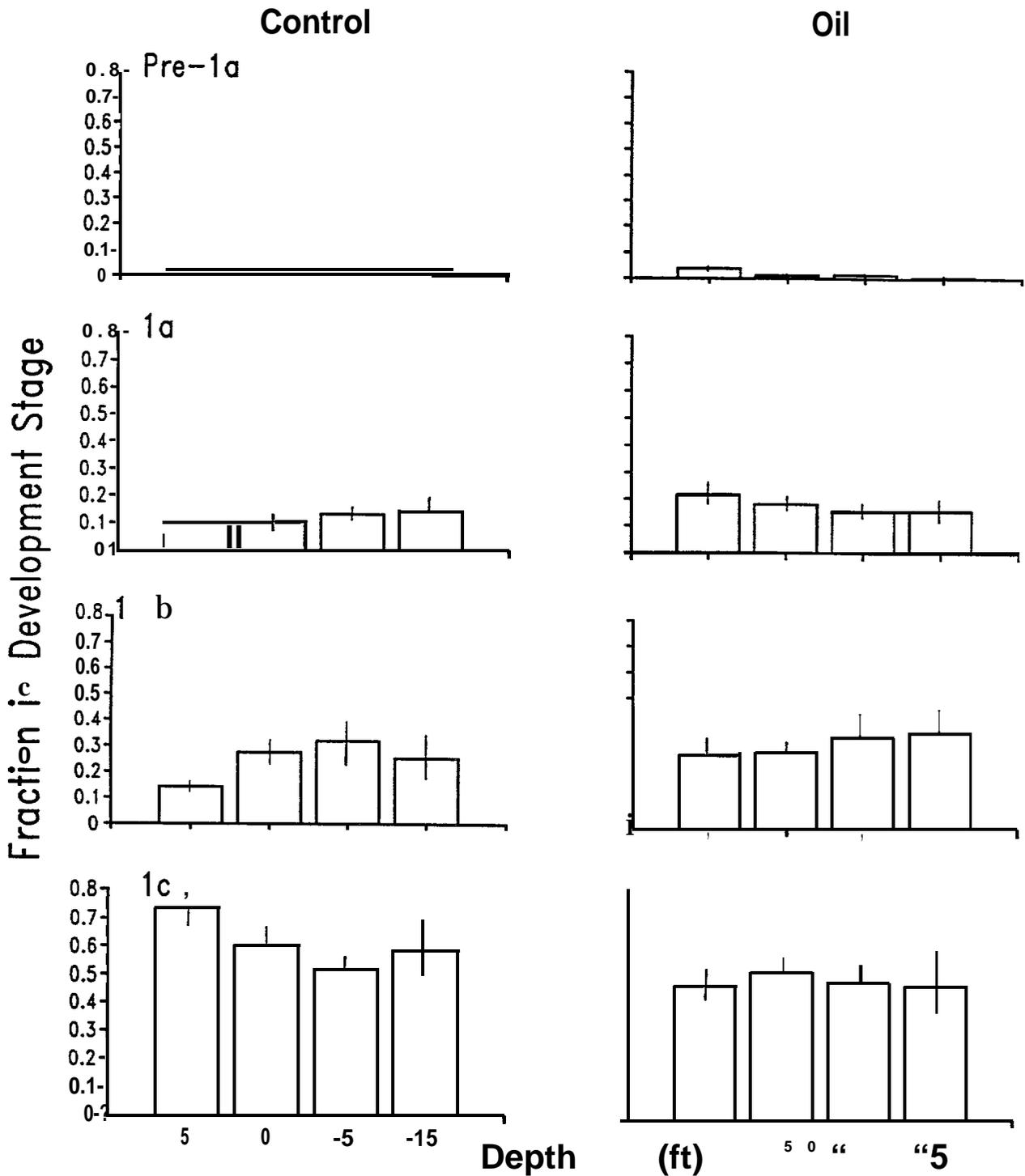


Figure 20. Mean fractions ( $\pm 1$  SE) of larvae in the four stages of development. Stars indicate the statistical significance of differences between control and treatment means of the same depth classes: \* $0.01 < P < 0.05$ , \*\* $0.001 < P < 0.01$ , \*\*\*  $P < 0.001$ .

### 3.7.1 Length

Fig. 21 shows that length rose from 6-8 mm at ages of 16-20 d to 9-10 mm at ages of 21-27 d, and then it fell in late-hatching larvae greater than 27 d old. The initial increase in length with age was due to growth in the egg by unhatched larvae. The decrease in length of larvae that hatched at an older age may have been due to the delayed hatching of non-viable larvae.

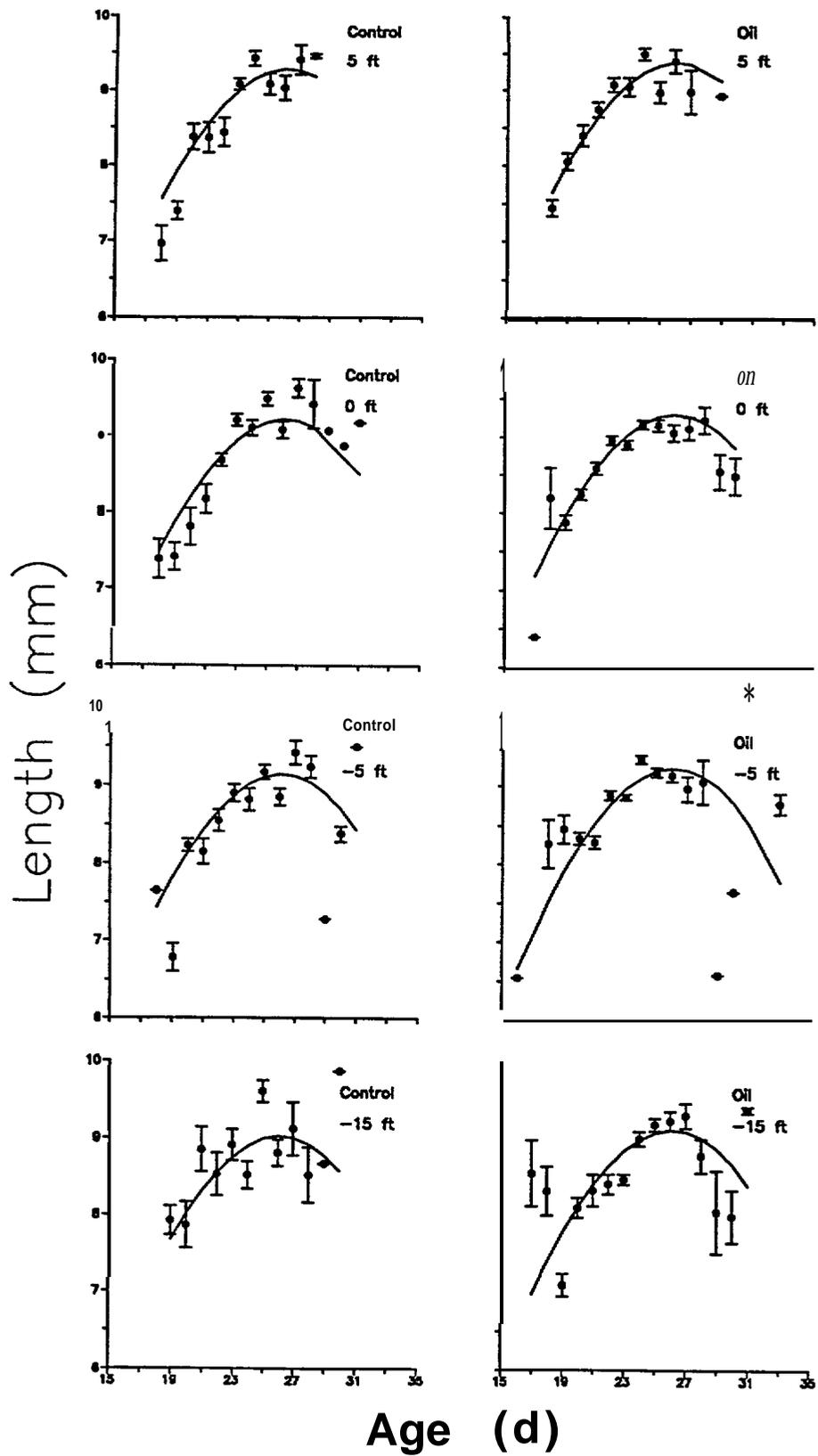
Preliminary trials showed that the trend of length with age was best described with a polynomial of the third degree. The multiple regression equation that explained the maximum amount of variance in length ( $r^2 = 0.168$ ,  $n = 4820$ ) with all-significant parameters was

(11)	<u>variable</u>	<u>coefficient</u>	<u>SE</u>	<u>P</u>
	constant	-3.8229	0.6375	<0.0001
	age	0.7530	0.0411	<0.0001
	age <sup>3</sup>	-3.67x10 <sup>-4</sup>	2.5x10 <sup>-5</sup>	<0.0001
	x	-0.0990	0.0273	0.0003
	depth	0.0122	0.0021	<0.0001

where  $x =$  a dummy variable with a value of 1 for control sites and 0 for oiled sites. This equation is plotted in Fig. 21; it shows that length decreased with depth at a rate of  $0.01 \text{ mm}\cdot\text{ft}^{-1}$ , that it was approximately 0.1 mm lower in the control sites than in the treatment sites, and that there was no interaction of depth and treatment.

### 3.7.2 Weight

Fig. 22 shows that dry weight of newly-hatched herring larvae decreased linearly with age due to the expenditure of yolk by metabolism. The multiple regression equation that explained the most variance ( $r^2 = 0.076$ ,  $n = 4820$ ) with all-significant coefficients was



**Figure 21.** Mean length ( $\pm 1$  SE) of larvae at age of hatching showing larger length in oiled than in control transects and decreasing length with increasing depth. Solid line is length predicted from equation (11).

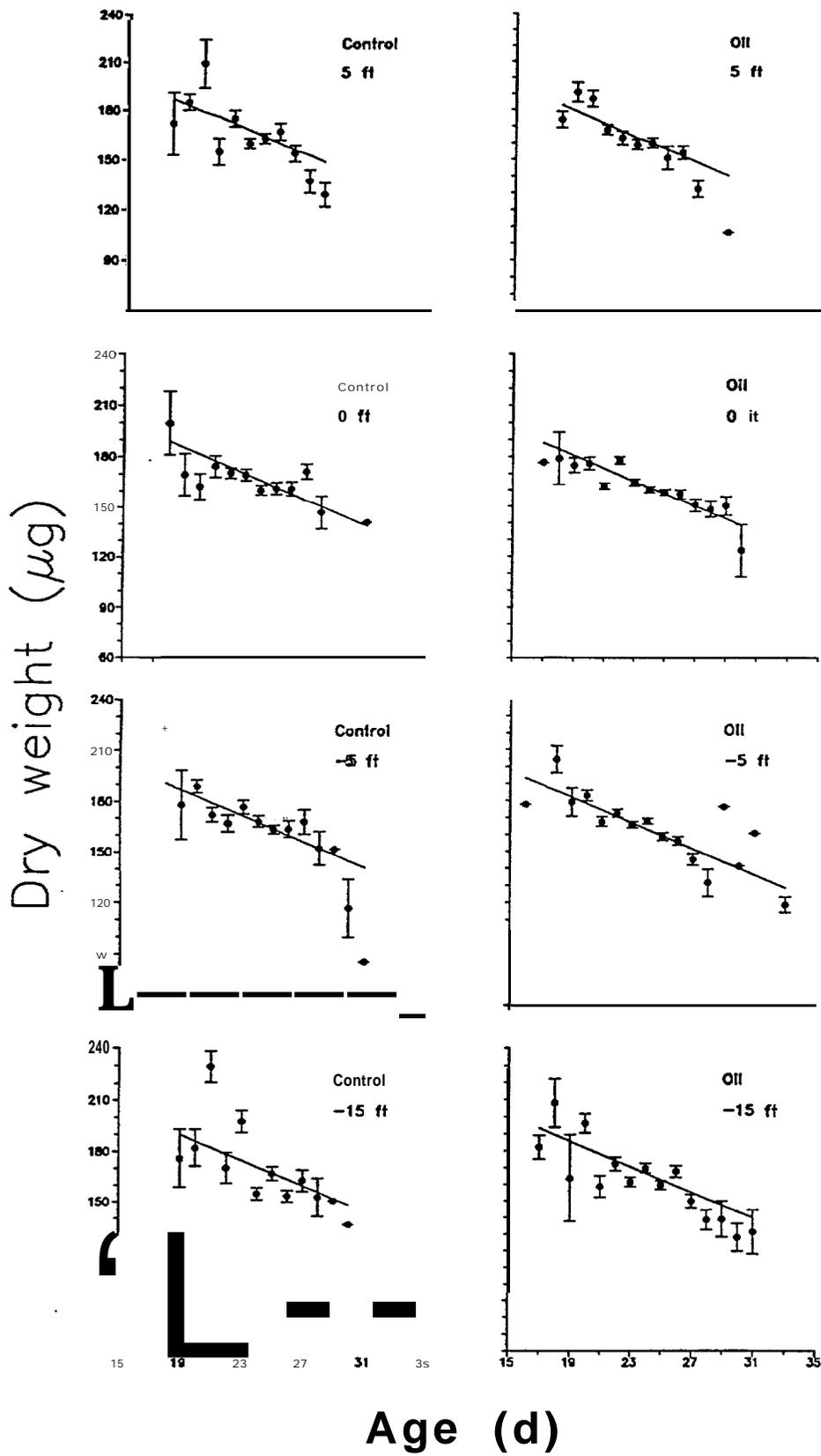


Figure 22. Mean dry weight ( $\pm 1$  SE) of larvae showing linear decrease in weight with age at hatching. Solid line is weight predicted by equation (12).

(12)	<u>variable</u>	<u>coefficient</u>	<u>SE</u>	<u>P</u>
	constant	253.1	4.5	<0.0001
	age	-3.828	0.196	<0.0001
	x	4.510	0.945	<0.0001
	depth	-0.3395	0.0727	<0.0001

This equation is plotted in Fig. 22; it shows that weight decreased at a constant rate of  $3.8 \mu\text{g}\cdot\text{d}^{-1}$ , that control larvae were  $4 \mu\text{g}$  heavier at all ages than oil treated larvae, that larvae increased in weight with increasing depth at a rate of  $0.3 \mu\text{g}\cdot\text{ft}^{-1}$  (= a difference of  $6.7 \mu\text{g}$  between depths of +5 and -15 ft), and that there was no interaction of depth and oil treatment.

### 3.7.3 Condition Factor

Condition decreased exponentially with age, as was expected from the nonlinear growth of length with age (Fig. 23). Preliminary trials showed that this decrease was best described as a simple exponential decay of condition with age, rather than by a polynomial of age. The multiple regression ( $r^2 = 0.216$ ,  $n = 4820$ ) of  $\ln(\text{condition})$  on age and auxiliary variables was

(13)	<u>variable</u>	<u>coefficient</u>	<u>SE</u>	<u>P</u>
	constant	0.3043	0.0489	<0.0001
	age	-0.0765	0.0021	<0.0001
	x	0.0677	0.0102	<0.0001
	depth	-0.0062	0.0008	<0.0001

This equation shows that condition decreased at an average instantaneous rate of  $7.7\%\cdot\text{d}^{-1}$ , that it was 7% higher in control transects than in oiled transects, that it increased with decreasing depth at a rate of  $1 \%\cdot\text{ft}^{-1}$ , and that there was no interaction of depth and oil treatment.

### 3.7.4 Yolk Sac Volume

Yolk sac volume also decreased with age, but the decrease was best described with a polynomial of age ( $r^2 = 0.451$ ,  $n = 4820$ ) rather than exponential decay, i.e.

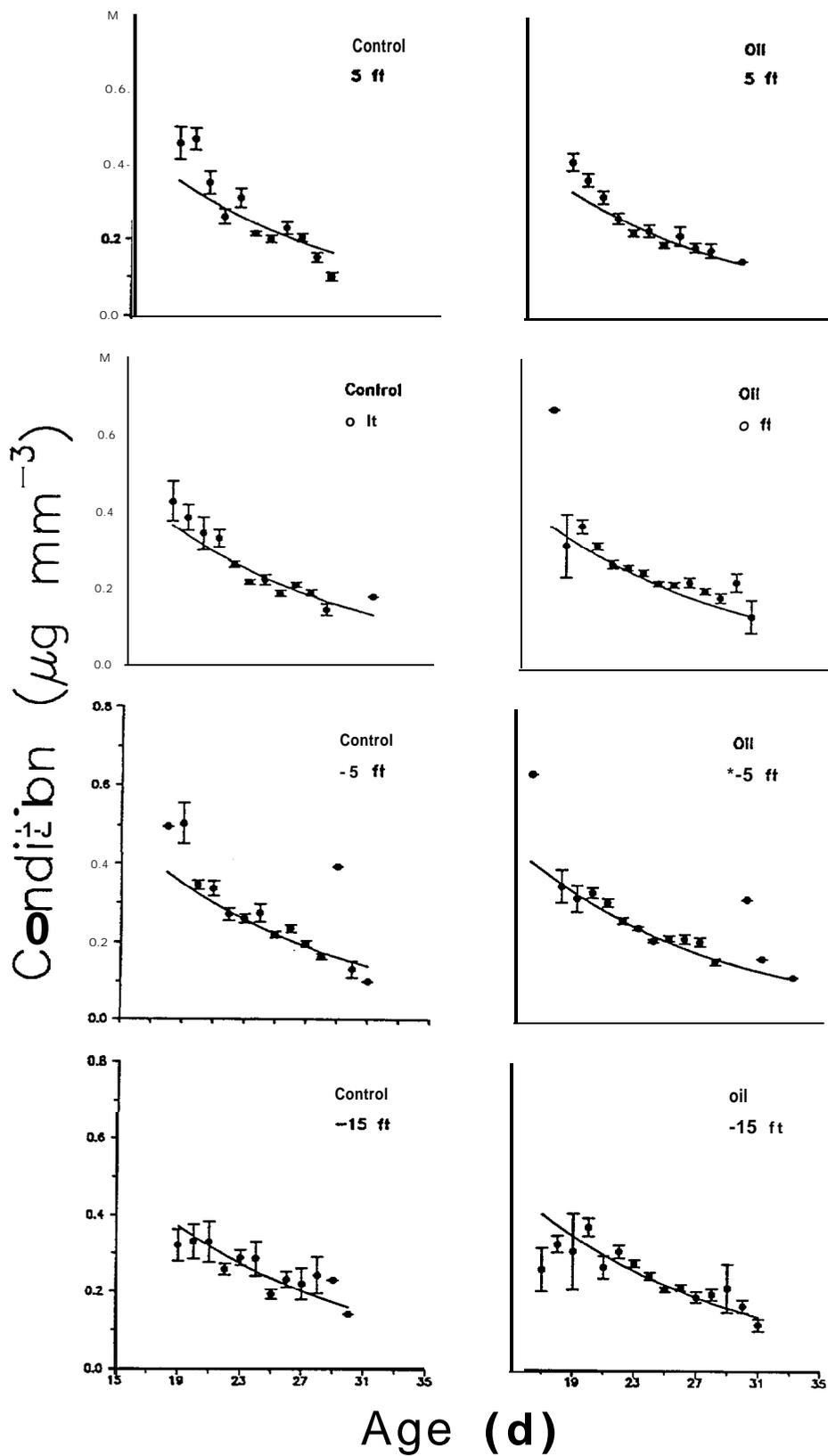


Figure 23. Mean condition ( $\pm 1$  SE) of larvae showing exponential decrease in condition with age at hatching. Solid line is condition predicted by equation (13).

(14)	<u>variable</u>	<u>coefficient</u>	<u>SE</u>	<u>P</u>
	constant	2.1430	0.0750	<0.0001
	age	-0.1101	0.0048	<0.0001
	age <sup>3</sup>	4.27x10 <sup>-5</sup>	2.9x10 <sup>-6</sup>	<0.0001
	x	0.0149	0.0035	< 0.0001
	depth	-0.0021	0.0003	<0.0001
	x*depth	-0.0017	0.0005	0.0011

Fig. 24 shows the fit of this equation to the mean yolk sac volumes.

### 3.8 Multivariate Analyses

In sections 3.1 to 3.7 of this report we examined the data on a variable by variable basis; in this section we examine the data as a single object using multivariate statistics. The reason for this is that the variables are all manifestations of a single phenomenon - the effects on growth and development of herring embryos of concentrations of hydrocarbons. By treating the data as a single matrix, we account for interactions between variables that cannot be accounted for by univariate analyses.

#### 3.8.1 Correlation Matrix

The first step of multivariate analysis was to examine the correlation matrix of the variables derived from the means of the 180 samples shown in Table 2. Examination of large matrices that included such variables as the fraction of larvae in all four developmental stages and the fraction of larvae in all six classes of abnormalities showed that almost all of the statistically significant correlations were retained by a matrix containing only nine variables: survival, age at 50% hatch, fraction of larvae in development stage 1c, fraction of larvae that were viable, yolk sac volume, dry weight, length, oil treatment (1 for control and 0 for oiled), and depth. This reduced variable set was used in all subsequent analysis.

Table 4 shows that the highest correlations occurred between size, the fraction of larvae in stage 1c, and age at 50% hatch. As expected, late hatch was associated with an increased fraction of larvae in stage 1c, longer length, and smaller yolk sac volumes and early hatch was associated with decreased fraction of larvae in stage 1c,

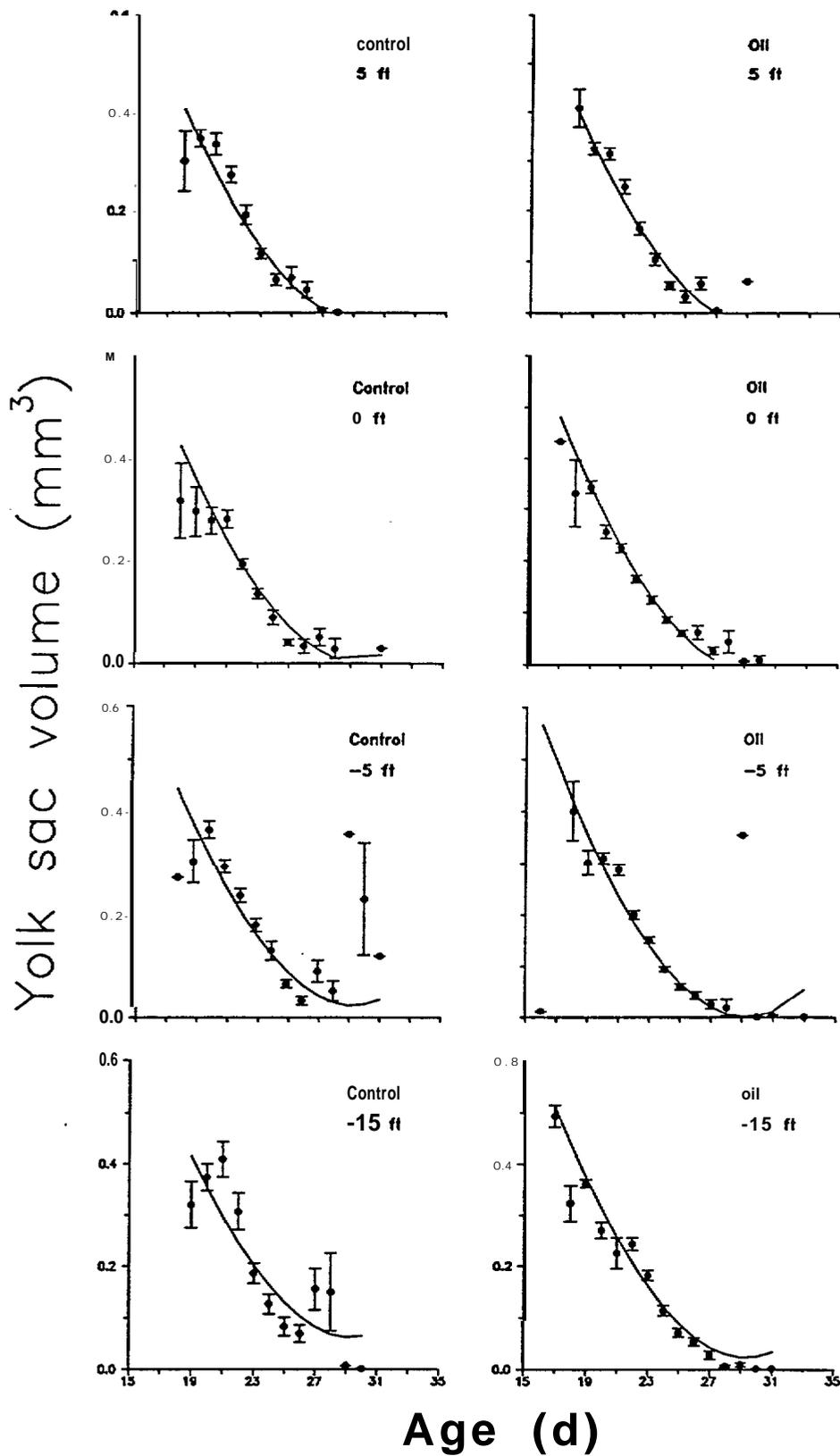


Figure 24. Mean yolk sac volume ( $\pm 1$  SE) of larvae showing decrease in volume with age at hatching. Solid line is yolk sac volume predicted by equation (14).

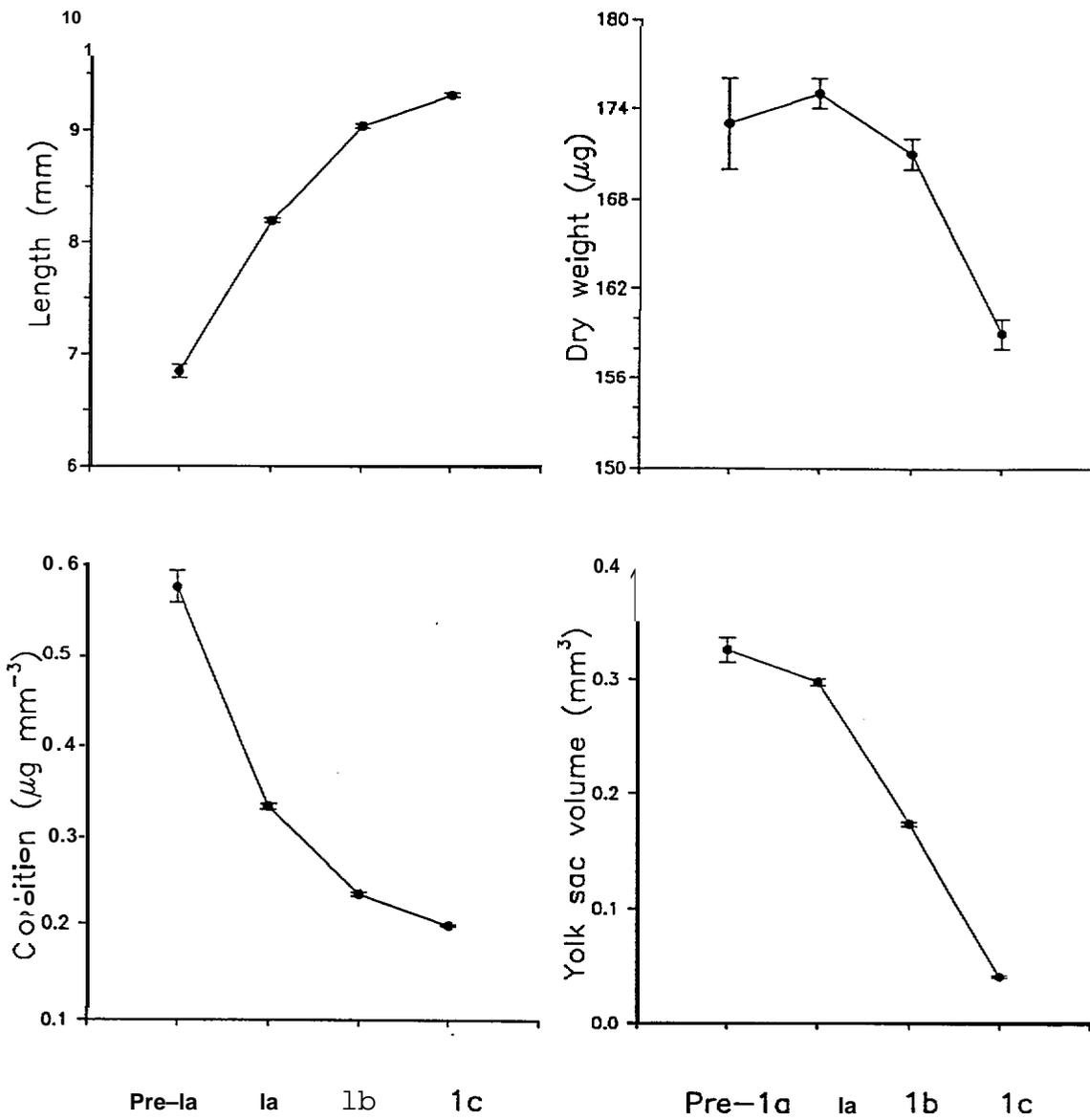


Figure 25. Mean ( $\pm 1$  SE) length, weight, condition and yolk sac volume of herring larvae classified into development stages.

Table 4. Correlation matrix of variables describing herring embryo survival, hatching schedule, development, viability and size with oil treatment and depth.

	survival	age50	1c	normal	ysvol	weight	length	oil	depth
survival	1.00								
age50	0.14	1.00							
1c	0.13	0.36 *	1.00						
normal	0.15 *	-0.01	-0.03	1.00					
ysvol	-0.12	-0.31 *	-0.58 **	0.05	1.00				
weight	-0.02	-0.08	-0.12	0.12	0.28 **	1.00			
length	0.15 *	0.15 *	0.33 **	-0.08	-0.58 **	-0.05	1.00		
oil	0.12	0.13	0.20 *	0.04	0.07	0.00	-0.06	1.00	
depth	-0.06	-0.30 *	0.08	-0.19 *	0.01	0.03	0.04	0.06	1.00

Notes:

- survival = fraction of eggs surviving to hatch;  
 age50 = age (d) at 50% hatch;  
 1 c = fraction of larvae hatching at development stage 1 c;  
 normal = fraction of larvae with no morphological abnormalities  
 ysvol = yolk sac volume (mm<sup>3</sup>);  
 weight = dry weight (ug) of larva;  
 length = length of larva (mm);  
 oil = 1 for control transect and 0 for oiled transects;  
 depth = depth (-15 to 15 ft) at which eggs were spawned.

shorter length and larger yolk sac volumes. Weight was positively correlated with yolk sac volume, but not with length, development stage or age at hatch. There were no correlations between survival and size or survival and development stage, but there was a weak positive correlation with the fraction of viable larvae. The only significant correlation between these variables and oil treatment was a weak positive correlation between the fraction of larvae in development stage 1c and the absence of oil. Both age at 50% hatch and the fraction of viable larvae were negatively correlated with depth, i.e. both decreased as depth increased from -15 ft to 5 ft.

### **3.8.2 Multivariate ANOVA**

The extension of univariate ANOVA to the case of multiple dependent variables is called multivariate ANOVA or MANOVA. In this procedure the single dependent variable specified in an ANOVA is replaced by a vector of dependent variables. The seven biological variables listed in Table 4: fractional survival, age at 50% hatch, fraction in stage 1c, fraction viable larvae, yolk sac volume, larval weight and larval length, were the dependent variables and oil treatment and depth were the factors. The WOVA reflected the pattern of correlations seen in Table 4 by showing that a highly significant ( $n = 180$ ,  $P = 0.004$ ) variation in the vector was due to depth and a barely significant ( $P = 0.026$ ) variation was due to oil treatment. The oil x depth interaction was not significant ( $P = 0.164$ ).

These results are similar to those from ANOVAs reported earlier in this report: both oil treatment and depth are responsible for significant changes in the suite of variables that characterize herring embryo survival, viability, development, and size, but the effect of depth is often more significant than the effect of oil. This result is due, in part, to our present lack of knowledge concerning the exact degree of exposure to oil within the treated samples of eggs.

### **3.8.3 Factor Analysis**

The correlation matrix showed that there were significant relationships between biological variables, and the MANOVA showed that the vector of biological variables varied significantly with oil treatment and depth. The next step in analysis was to identify and describe the processes that underlie the observed variation in the biological variables. The procedure is called factor analysis; it identifies the major

axes of variation by converting a set of observed variables into a set of artificial variables or factors. Unlike the raw variables, the factors are completely uncorrelated with each other, so the information contained in one factor will not be duplicated in another. This makes the biological interpretation of the data much more clear.

Before extracting the factors the raw variables were standardized by subtracting their mean and dividing by their standard deviation, i.e.

$$(15) Z_{ij} = (X_{ij} - \bar{X}_i) / s_i$$

where  $z_{ij}$  = case  $j$  of standardized variable  $i$ ,  $x_{ij}$  = case  $j$  of raw variable  $i$ ,  $\bar{X}_i$  = mean of raw variable  $i$  (i.e. the grand mean of the 180 sample means), and  $s_i$  = standard deviation of variable  $i$ .

Table 5 shows the eigenvalues and the percent of variance explained by each of the factors extracted from these standardized variables. Only the first four factors are examined further because they were the only factors with eigenvalues greater than one and because all other factors each explained only 3-10% of the variance in the sample means. Together, factors 1 to 4 explained 66.1% of the variance in the standardized means.

The loadings of these factors are shown in Table 6, after varimax rotation, which was used to make the loadings more easily interpretable. The loadings are coefficients whose sign and magnitude indicate the contribution of each standardized variable to the factor. Based on these loadings we interpreted factor 1 as an index of the development of the larvae, i.e. as a contrast between small yolk sac volumes and long larval lengths versus large yolk sac volumes and short lengths. We interpreted factor 2 as the effect of depth on age of 50% hatching and larval viability, i.e. high ages of 50% hatch and high viability in deep water compared to low ages of 50% hatch and low viability in shallow water. We interpreted factor 3 as the effect of oil on stage of development, age of 50% hatch, larval length, and egg survival. We interpreted factor 4 as a contrast between depth and survival, viability and weight, i.e. high survival and viability in deep water and low survival and viability in shallow water.

**Table 5. Eigenvalues and percent of variance explained by the nine factors extracted from the nine standardized biological variables.**

<u>Factor</u>	<u>Eigenvalue</u>	<u>Percent of variance</u>	<u>Cumulative percent of variance</u>
1	2.3306	25.9	25.9
2	1.3979	15.5	41.9
3	1.1607	12.9	54.3
4	1.0563	11.7	66.1
5	0.9142	10.2	76.2
6	0.7976	8.9	85.1
7	0.6148	6.8	91.9
8	0.4549	5.1	97.0
9	0.2731	3.0	100.0

**Table 6. Loadings on factors 1 to 4 after varimax rotation. Variable names are explained in Table 4.**

<u>Variable</u>	<u>Rotated factor</u>			
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
oil	-0.077	0.024	0.904	0.067
depth	0.125	0.852	0.187	-0.144
survival	0.287	-0.086	0.238	0.556
age50	0.326	-0.661	0.317	-0.055
1c	0.704	-0.080	0.412	-0.061
normal	-0.083	-0.240	-0.037	0.713
ysvol	-0.876	0.155	0.042	0.135
weight	-0.197	0.280	-0.057	0.578
length	0.807	0.097	-0.179	0.089

In summary, factor analysis reveals three conclusions that were suggested by the correlation matrix of Table 4. First, the greatest contrast in the data set is the inverse relationship between larval size (especially yolk sac volume) and stage of development at hatch. Second, depth has two impacts on the biology of herring embryos: the age of 50% hatch decreases with decreasing depth, and the fraction of surviving eggs and viable larvae decreases with decreasing depth. Third, the effect of oil treatment is also twofold: the most important effect is an acceleration of development, which causes earlier hatch and increases the frequency of early development stages at hatch; the second effect is a decrease in survival of eggs.

This analysis identified factor 3 as a possible index of oil treatment. This is illustrated by Fig. 26, which shows that factor 3 is the only one of the four factors to exhibit differences between control and oiled samples. Factor 3 can now be used to rank the samples according to the degree of “oil impact”. Table 7 shows the ranking of the 180 samples according to their values of factor 3. If one assumes that there is a direct relationship between exposure to hydrocarbon concentrations and ‘viability’ of herring embryos, then this ranking is actually a prediction of the rank order of hydrocarbon concentrations to which the eggs were exposed. This prediction can be tested if data on the hydrocarbon concentration of samples of herring eggs ever becomes available.

#### 4. DISCUSSION

This study shows that there was a weak, but statistically significant, effect of oil from the Exxon Valdez spill on the biology of herring eggs laid on beaches in central and southern Prince William Sound. There are two possible reasons for the weak statistical link: the spawning beaches were not contaminated by high concentrations of hydrocarbons, and we lacked a satisfactory measurement of the amount of hydrocarbons to which each egg sample was exposed and of the duration of its exposure to hydrocarbons. It is almost certain that some of the eggs from the oiled class were exposed to low concentrations of hydrocarbons, as is suggested by the accelerated hatching. However, considering the large volume of oil that was spilled and the large number of beaches that were fouled by oil, we believe that the second reason was also a major cause of the weak relationship.

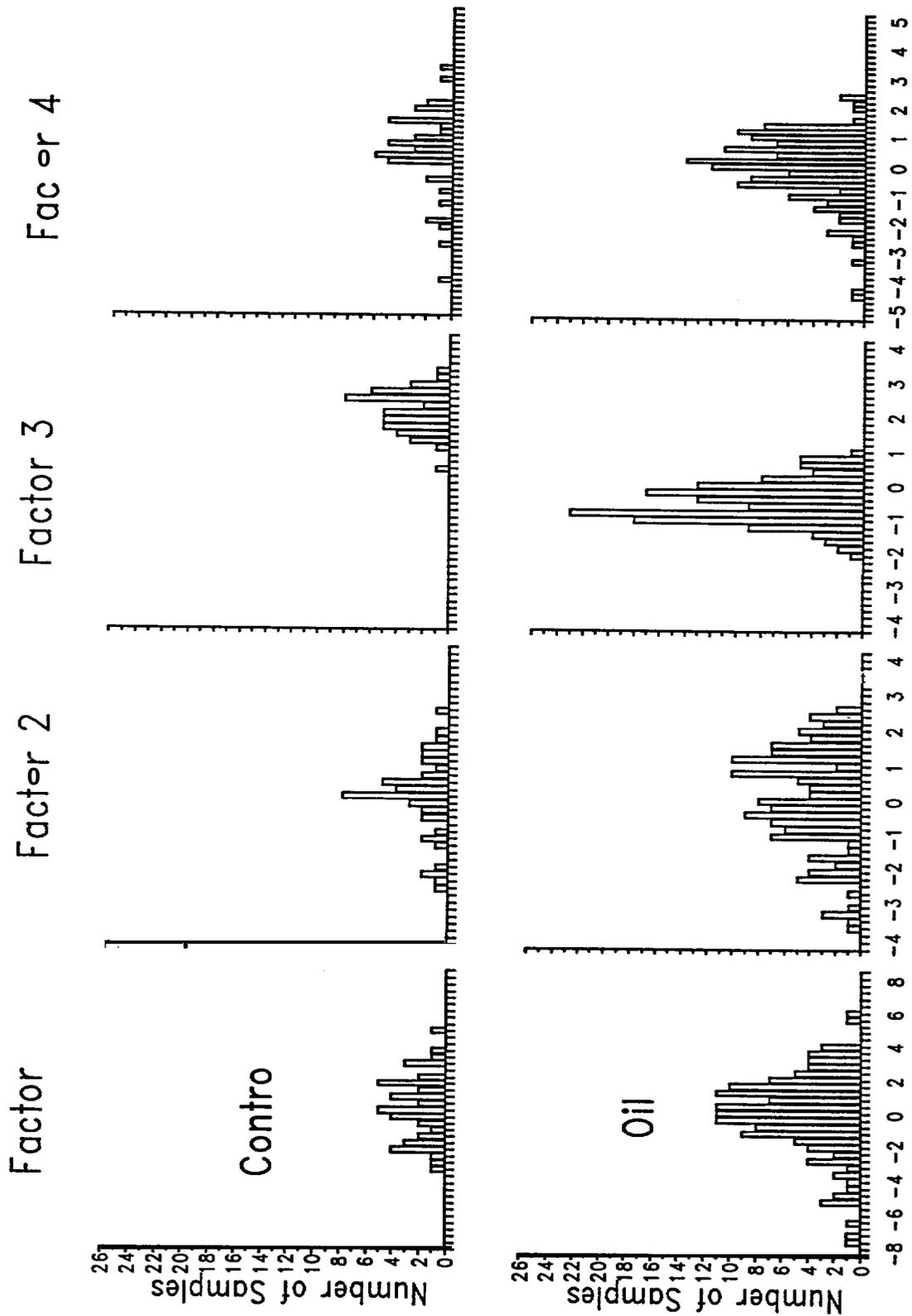


Figure 26. Frequency distributions of control and oiled samples against factors 1 to 4, showing that only factor 3 is an index of oil contamination.

Table 7. Rank of herring egg samples based on factor 3, in order of decreasing factor value.

Location	ADFG no.	Depth (ft)	Rep, no.	Triton no.	Factor 3	
					value	rank
Fairmount	C-01	5	1	101	2.494	8
Fairmount	C-01	5	2	2	2.885	2
Fairmount	C-01	5	3	53	2.270	15
Fairmount	C-01	0	2	143	2.166	21
Fairmount	C-01	0	3	172	1.525	34
Fairmount	C-01	0	1	78	2.292	14
Fairmount	C-01	-5	1	107	2.359	13
Fairmount	C-01	-5	2	91	1.340	37
Fairmount	C-01	-5	3	126	1.226	40
Fairmount	C-02	0	1	58	2.214	19
Fairmount	C-02	0	2	151	2.218	18
Fairmount	C-02	0	3	36	1.404	36
Fairmount	C-02	-5	3	159	1.905	23
Fairmount	C-02	-5	1	119	0.317	59
Fairmount	C-02	-5	2	141	1.058	43
Fairmount	C-02	-15	1	125	1.306	38
Fairmount	C-02	-15	3	19	1.468	35
Fairmount	C-02	-15	2	168	0.841	44
Fairmount	C-03	0	3	142	2.486	10
Fairmount	C-03	0	2	37	1.695	30
Fairmount	C-03	0	1	23	2.260	16
Fairmount	C-03	-5	2	24	2.488	9
Fairmount	C-03	-5	1	62	2.573	7
Fairmount	C-03	-5	3	74	1.064	42
Fairmount	C-03	-15	3	98	1.563	32
Fairmount	C-03	-15	1	152	2.440	11
Fairmount	C-03	-15	2	41	1.909	22
Fairmount	C-04	5	1	127	1.797	28
Fairmount	C-04	5	3	100	3.040	1
Fairmount	C-04	5	2	25	1.818	25
Fairmount	C-04	0	1	156	1.860	24
Fairmount	C-04	0	2	35	1.751	29
Fairmount	C-04	0	3	6	2.615	5
Fairmount	C-04	-5	2	96	2.224	17
Fairmount	C-04	-5	1	176	1.798	27
Fairmount	C-04	-5	3	70	1.184	41
Fairmount	C-05	5	1	57	2,754	3

Table 7. Rank of herring egg samples based on factor 3, in order of decreasing factor value. (Continued)

Location	ADFG no.	Depth (ft)	Rep. no.	Factor 3		
				T r i t o n no.	value	rank
Fairmount	C-OS	5	2	95	2.182	20
Fairmount	C-OS	5	3	3	2.397	12
Fairmount	C-05	0	3	40	1.678	31
Fairmount	C-OS	0	2	108	1.249	39
Fairmount	C-05	0	1	69	1.800	26
Bass Harbor	0-01	0	3	155	0.714	47
Bass Harbor	0-01	0	1	137	-1.475	173
Bass Harbor	0-01	0	2	68	0.709	48
Bass Harbor	0-01	-5	1	106	-0.303	88
Bass Harbor	0-01	-5	2	175	-0.838	126
Bass Harbor	0-01	-5	3	90	0.180	62
Bass Harbor	0-01	-15	1	39	-0.873	127
Bass Harbor	0-01	-15	2	61	0.194	61
Bass Harbor	0-01	-15	3	124	-1.080	151
Bass Harbor	0-02	0	1	99	-0.743	119
Bass Harbor	0-02	0	2	18	-0.940	135
Bass Harbor	0-02	0	3	1	-0.706	118
Bass Harbor	0-02	-5	3	134	-0.672	116
Bass Harbor	0-02	-5	1	83	-1.171	161
Bass Harbor	0-02	-5	2	34	-0.479	103
Bass Harbor	0-02	-15	3	118	-1.235	162
Bass Harbor	0-02	-15	2	4	-1.250	164
Bass Harbor	0-02	-15	1	17	-1.677	175
Bass Harbor	0-03	5	1	103	-0.033	71
Bass Harbor	0-03	5	2	5	-0.308	89
Bass Harbor	0-03	5	3	49	-1.480	174
Bass Harbor	0-03	0	1	14	-0.904	131
Bass Harbor	0-03	0	3	104	-0.921	132
Bass Harbor	0-03	0	2	154	0.836	45
Bass Harbor	0-03	-5	3	120	-0.498	107
Bass Harbor	0-03	-5	1	55	-0.067	73
Bass Harbor	0-03	-5	2	139	-1.239	163
Bass Harbor	0-04	0	3	71	-0.988	142
Bass Harbor	0-04	0	1	153	-1.309	166
Bass Harbor	0-04	0	2	169	-0.408	100
Bass Harbor	0-04	-5	1	32	-1.309	167
Bass Harbor	0-04	-5	2	73	-1.142	156

Table 7. Rank of herring egg samples based on factor 3, in order of decreasing factor value. (Continued)

Location	ADFG no.	Depth (ft)	Rep. no.	Triton no.	Factor 3	
					— value	— rank
Bass Harbor	0-04	-5	3	171	-0.193	81
Bass Harbor	0-04	-15	2	88	-1.776	177
Bass Harbor	0-04	-15	3	121	-2.125	180
Bass Harbor	0-04	-15	1	149	-1.752	176
Bass Harbor	0-08	0	1	64	0.064	65
Bass Harbor	0-08	0	2	162	0.771	46
Bass Harbor	0-08	0	3	147	-0.639	114
Bass Harbor	0-08	-5	2	130	-0.611	112
Bass Harbor	0-08	-5	3	114	-0.994	143
Bass Harbor	0-08	-5	1	178	0.637	50
Bass Harbor	0-08	-15	3	13	-1.152	158
Bass Harbor	0-08	-15	1	33	-1.313	168
Bass Harbor	0-08	-15	2	47	0.045	66
Bass Harbor	0-10	0	1	16	-0.106	74
Bass Harbor	0-10	0	3	115	0.107	64
Bass Harbor	0-10	0	2	65	0.693	49
Bass Harbor	0-10	-5	2	45	0.379	57
Bass Harbor	0-10	-5	1	117	-0.151	76
Bass Harbor	0-10	-5	3	30	-0.451	102
Bass Harbor	0-10	-15	3	163	-0.332	93
Bass Harbor	0-10	-15	1	80	-1.320	169
Bass Harbor	0-10	-15	2	132	0.369	58
Outside Bay	0-11	5	2	10	-1.102	154
Outside Bay	0-11	5	3	140	-0.495	105
Outside Bay	0-11	5	1	158	-0.830	124
Outside Bay	0-11	0	3	109	-0.181	79
Outside Bay	0-11	0	2	180	-1.048	147
Outside Bay	0-11	0	1	160	-0.926	133
Outside Bay	0-11	-5	1	59	-0.269	85
Outside Bay	0-11	-5	2	123	-1.418	172
Outside Bay	0-11	-5	3	46	0.043	67
Outside Bay	0-12	5	1	8	-1.145	157
Outside Bay	0-12	5	2	105	-0.635	113
Outside Bay	0-12	5	3	122	-0.549	110
Outside Bay	0-12	0	1	54	-0.497	106
Outside Bay	0-12	0	2	170	0.428	55
Outside Bay	0-12	0	3	22	-0.933	134

Table 7. Rank of herring egg samples based on factor 3, in order of decreasing factor value. (Continued)

Location	ADFG no.	Depth (ft)	Rep. no.	Triton no.	Factor 3	
					— value	— rank
Outside Bay	0-12	-5	3	77	-1.005	144
Outside Bay	0-12	-5	1	51	-0.184	80
Outside Bay	0-12	-5	2	89	-0.294	86
Cabin Bay	0-13	0	1	173	-0.066	72
Cabin Bay	0-13	0	3	60	0.019	68
Cabin Bay	0-13	0	2	82	-0.500	108
Cabin Bay	0-13	-5	3	110	-0.974	139
Cabin Bay	0-13	-5	1	9	-1.079	150
Cabin Bay	0-13	-5	2	128	-0.156	77
Cabin Bay	0-13	-15	2	94	-0.783	120
Cabin Bay	0-13	-15	1	75	-0.230	82
Cabin Bay	0-13	-15	3	136	-1.062	148
Outside Bay	0-14	5	3	20	-0.880	129
Outside Bay	0-14	5	1	76	-0.136	75
Outside Bay	0-14	5	2	93	-0.969	138
Outside Bay	0-14	0	3	21	-1.081	152
Outside Bay	0-14	0	2	11	-0.810	122
Outside Bay	0-14	0	1	38	-0.684	117
Outside Bay	0-14	-5	1	145	-1.851	178
Outside Bay	0-14	-5	3	28	-1.031	146
Outside Bay	0-14	-5	2	111	-0.591	111
Story Island	0-15	0	3	164	0.472	52
Story Island	0-15	0	1	79	-0.255	84
Story Island	0-15	0	2	161	0.445	53
Story Island	0-15	-5	1	177	-1.063	149
Story Island	0-15	-5	3	44	-0.530	109
Story Island	0-15	-5	2	146	-1.124	155
Story Island	0-15	-15	3	174	-0.803	121
Story Island	0-15	-15	1	157	0.266	60
Story Island	0-15	-15	2	66	-0.489	104
Story Island	0-16	0	3	112	0.168	63
Story Island	0-16	0	1	97	-0.975	140
Story Island	0-16	0	2	133	-0.295	87
Story Island	0-16	-5	2	43	-0.401	99
Story Island	0-16	-5	3	129	-0.379	96
Story Island	0-16	-5	1	144	0.582	51
Story Island	0-16	-15	2	12	-0.948	137

Table 7. Rank of herring egg samples based on factor 3, in order of decreasing factor value. (Continued)

Location	ADFG no.	Depth (ft)	Rep. no.	Triton no.	Factor 3	
					— value	— rank
Story Island	0-16	-15	1	26	0.392	56
Story Island	0-16	-15	3	63	-0.005	69
Rocky Bay	0-17	5	3	27	-1.978	179
Rocky Bay	0-17	5	2	42	-1.162	160
Rocky Bay	0-17	5	1	150	-0.874	128
Rocky Bay	0-17	0	2	135	-0.321	91
Rocky Bay	0-17	0	3	84	-1.406	171
Rocky Bay	0-17	0	1	116	-0.019	70
Rocky Bay	0-17	-5	2	67	-0.380	97
Rocky Bay	0-17	-5	3	102	-0.414	101
Rocky Bay	0-17	-5	1	52	-0.812	123
Rocky Bay	0-18	5	2	81	-0.836	125
Rocky Bay	0-18	5	3	166	-0.180	78
Rocky Bay	0-18	5	1	50	-1.082	153
Rocky Bay	0-18	0	1	15	-1.380	170
Rocky Bay	0-18	0	3	131	-0.897	130
Rocky Bay	0-18	0	2	85	-1.023	145
Rocky Bay	0-18	-5	1	29	-0.326	92
Rocky Bay	0-18	-5	3	56	-0.645	115
Rocky Bay	0-18	-5	2	48	-0.977	141
Rocky Bay	0-19	5	2	87	-0.394	98
Rocky Bay	0-19	5	3	148	-0.310	90
Rocky Bay	0-19	5	1	165"	-0.944	136
Rocky Bay	0-19	0	1	113	-0.246	83
Rocky Bay	0-19	0	2	31	-1.296	165
Rocky Bay	0-19	0	3	179	-0.357	95
Rocky Bay	0-19	-5	3	86	-0.343	94
Rocky Bay	0-19	-5	2	138	-1.162	159
Rocky Bay	0-19	-5	1	72	0.432	54

It is highly probable that the concentration of oil and the duration of oil exposure varied widely within small geographic areas due to differences in the topography of the shoreline, in the strength of local wind and wave events, and in the tide levels at the time that the front of the oil slick first encountered shore. The variability of exposure within the so-called “oiled” group is shown by the ranking of samples by factor 3. Table 7 shows that within Bass Harbor the rankings ranged from 45 to 180 and within Rocky Bay they ranged from 54 to 179, but the control sites in Fairmount Bay had a much narrower range of ranking: 2 to 59. (Other “oiled” sites had a similarly wide range of rankings.) This indicates that exposure to oil varied substantially within the oiled areas. A correct assessment of the actual impact of the Exxon Valdez spill requires a much more precise index of oil contamination than the simple presence/absence index used in this study.

One consequence of using a simple presence/absence index of oil treatment was that it was difficult to disentangle the effects of oil from the effects of independent environmental factors (e.g. temperature or exposure) that were related with depth. This difficulty was compounded by the fact that these factors varied non-linearly with depth; they were greatest for shallow and deep water and least for the mid-range of depths.

### Survival

The survivals reported in this study (range = 7.1 to 99.9%, mean = 59.2%, SD = 17.7) are very similar to those that have been reported by other authors for medium and low densities of natural herring spawn incubated in environments free of predators and chemical contamination. Hourston et al. (1984) incubated natural spawn of Pacific herring collected in the Strait of Georgia, British Columbia, in laboratory tanks. They reported that percent hatching was highly variable, ranging from 16 to 100%, and that it tended to decrease with increasing egg density, probably due to asphyxiation of eggs inside clumps. Mean hatch was 30% for cases of heavy intensity, defined as  $>78 \text{ eggs}\cdot\text{cm}^{-2}$ , 71% for cases of light to medium intensity, i.e.  $<78 \text{ eggs}\cdot\text{cm}^{-2}$ , and 54% for all cases combined. Similar results were reported by Johannessen (1986) for natural Atlantic herring, Clupea harengus harengus, spawn collected from western Norway: percent hatching ranged from 17.2

to 84.4 and decreased from 50.5 for light egg densities ( $\leq 25 \text{ eggs}\cdot\text{cm}^{-2}$ ) to 27.7 for heavy egg densities ( $50\text{-}100 \text{ eggs}\cdot\text{cm}^{-2}$ ) for a grand mean of 42.8%.

We did not find any evidence of a significant effect of oil on survival or hatching success, but survival is usually less sensitive to pollutants than percent viability, according to von Westernhagen's (1988) review of the sublethal effects of pollutants on fish eggs and larvae. The only other study that compared survival of natural herring spawn from oil-contaminated and pristine areas found significant reductions in survival in the contaminated area eight months after the oil spill. Aneer and Nellbring (1982) compared hatching success of Baltic herring, Clupea harengus membras, eggs collected from sites in the northern Baltic sea. They collected spawn of low to medium density in June-July, 1978, from pristine areas and from a neighboring area contaminated by the 'Tsesis' oil spill of October, 1977, and incubated them in laboratory containers. Percent hatch ranged from 0.0 to 94, and was significantly ( $P < 0.01$ ) higher in the uncontaminated area (mean = 58.5%, SD = 39.0,  $n = 43$ ) than in the contaminated area (mean = 34.8%, SD = 23.7,  $n = 51$ ). We suspect that a similar significant relationship between survival of herring eggs and degree of oil contamination may exist in the data reported in this study, but that the relationship may be obscured by the wide range of actual oil contaminations within the oil treatment group.

Another factor that obscured the putative oil-survival relationship is the dome-shaped relationship between percent survival and depth. This phenomenon has not been previously reported in the scientific literature, apparently because this is the first study that examined percent hatch and percent viable hatch of herring spawn over the majority of its depth range. Previous studies examined portions of the range. However, its existence is supported by at least two studies on survival of Pacific herring eggs. Jones (1972) incubated artificially spawned herring eggs in incubators that simulated tidal exposure and showed that "prehatching mortality" increased linearly with number of hours of exposure to air; from 13% in unexposed eggs to 31% in eggs that were exposed to air for 8 hours twice daily. Taylor (1971) incubated artificially spawned herring eggs on ropes at various depths and showed that percent survival decreased linearly with depth regardless of egg density. Combining these two results indicates that percent survival should be low in the upper intertidal zone and low near the lowest depths at which spawn is laid and maximal at some intermediate depth. Our study supports these predictions by

showing that survival and the fraction of viable hatch was low at both depth extremes and maximal at a depth of about -5 ft from the lower low watermark.

### Live/Dead Egg Ratios

Our finding that herring eggs died at a rate of about  $3\% \cdot d^{-1}$  is in good agreement with most studies that have examined the mortality of natural herring spawn. It is generally accepted that mortality of herring eggs due to causes other than predation is low, i.e. less than  $10\% \cdot d^{-1}$ . Baxter (1971) and Hempel and Hempel (1971) reported that an average of 95.8910 and 96.1 to 94.3% of North Sea and Clyde Sea herring eggs, respectively, were alive. Haegele et al. (1981) reported that they rarely ever saw natural Pacific herring spawn with less than 90% live eggs. The very high mortality rates that have been reported for herring spawn (up to 90% - see review by Pallson 1984) are due almost entirely to predation by birds, fishes and invertebrates.

One of the consequences of the low rate of non-predation mortality is that the ratio of live eggs to total eggs measured at only one age is not an accurate index of the viability of herring embryos. In this study the ratios of most samples fell within a narrow range of 0.80-0.99, and only a few exhibited extraordinarily low live/total egg ratios. Herring eggs apparently do not exhibit morbidity or non-viability until relatively late in their development, at an age when larvae have begun to hatch. During the hatching period the live/total egg ratio is dominated by the schedule of hatching. If the number of hatched larvae is not known, then the survival dynamics of the egg mass are not known. Thus, knowledge of the live/dead egg ratio is not enough in itself to reliably and accurately predict total egg survival.

This conclusion may be subject to change if a more accurate index of oil contamination becomes available in the future. If it does, then the analysis should be repeated in order to test the usefulness of the live/dead egg ratio.

### Hatching Schedule

The significant decrease in age at 50% hatching that was observed in the oiled treatment is in agreement with results reported by studies that examined the effect of low concentrations of the water soluble fraction (WSF) of petroleum

hydrocarbons on developing eggs of fish. It is in contradiction to studies that used high concentrations of WSF hydrocarbons. von Westernhagen's (1988) review of this subject indicates that most authors report delayed hatch of larvae after treatment with petroleum hydrocarbons, primarily because they used high concentrations of WSF. For example, Linden (1978) reported delayed hatch of Baltic herring larvae exposed to  $54 \text{ mg}\cdot\text{L}^{-1}$  of the WSF of light fuel oil. Struhsaker et al. (1974) reported similar results for Pacific herring exposed to pulses of benzene at concentrations of  $40\text{-}45 \text{ mg}\cdot\text{L}^{-1}$ . Other authors cited by von Westernhagen (1988) report similar results for other species of fish. The most likely reason for delayed hatch is that the embryos are narcotized by high concentrations of WSF hydrocarbons. However, at least two authors have reported premature hatching of fish embryos after treatment with low concentrations of WSF hydrocarbons (Ernst et al. 1977: *Fundulus grandis*; Leung and Bulkley 1979: *Oryzias latipes*). The mechanism is considered to be stimulation of the hatching mechanism by oil components.

It is not unusual for a pollutant to shorten or lengthen incubation depending on its concentration. In fact, von Westernhagen (1988) reports that most pollutants, especially metals, appear to stimulate early hatch. The results of this study suggest that most of the oiled egg samples from Prince William Sound were exposed to low concentrations of hydrocarbons. This prediction should be tested by reanalyzing the data on cumulative fraction of hatching with a more precise index of hydrocarbon concentration.

The significant increase in age at 50% hatch with increasing depth was almost certainly a response to a decrease in water temperature with depth. It must be remembered that the eggs had already incubated on their spawning grounds for approximately 14 d before they were collected. This was sufficient time for significant differences in stage of development to have been established between eggs from different depths.

What are the consequences of early hatching to survival of herring larvae? This question is difficult to answer with certainty because most fisheries scientists believe that survival of fish larvae in the sea is determined mainly by the presence or absence of predators (Bailey and Houde 1989), so there is a strong and unpredictable environmental component to this problem. For the sake of argument, we will assume that predation pressure on herring larvae was the same at all sites

regardless of their level of oil contamination. It is well known that mortality rates of natural populations of marine fish larvae decrease exponentially with size, e.g. Bailey and Houde (1989: Fig. 1), so it is possible that small difference in larval size may have led to larger differences in total survival over the larval stage. In order to test the null hypothesis of identical larval survival between oiled and non-oiled sites we refer to the mortality rates measured from wild herring larvae collected in Prince William Sound in May-June, 1989. McGurk et al. (1990) reported that the mortality rates for the single largest cohort found at each of four sites, Tatitlek Narrows, Fairmount Island, Bass Harbor and Rocky Bay, were all a constant 0.25 d<sup>-1</sup>, and there were no significant ( $P > 0.05$ ) differences associated with site. Therefore, the null hypothesis is supported; we conclude that the relative frequency of early and late stage larvae did not lead to detectable differences in population survival.

### Viability of Larvae

The average viability of herring larvae measured in this study, 84%, is very close to that found by other authors. For example, Hourston et al. (1984) reported that the viability of Pacific herring larvae was usually high (over 80% in 89% of their samples) and not related to the type of spawning substrate, the intensity of spawning, or whether the eggs were naturally spawned or artificially spawned.

The rank order of morphological deformities reported in this study is also similar to that reported in the scientific literature for other sub-species of herring and other species of fish. The most conspicuous deformity is usually associated with curvature of the spine, followed by abnormal development of the head, jaw and eye and irregular development of the yolk (von Westernhagen 1988). These deformities are not a specific response to pollutants, but are common in all eggs of all fishes. They are the equivalent of spontaneous abortions in mammals and may be caused by natural stressors as well as unnatural stressors. It is commonly assumed that all deformed larvae die soon after hatch either because they cannot feed or because they cannot evade predators. This assumption is supported by the fact that not a single deformed larvae was ever observed in the survey of wild herring of Prince William Sound in May-June, 1989 (McGurk et al. 1990).

Although bent spines are the most common abnormality observed in herring embryos exposed to hydrocarbons (Linden 1878, Smith and Cameron 1979,

Struhsaker et al. 1984), we did not find any significant differences between our control and treatment classes in the fraction of spinal deformities. This may be due to the non-specificity of spinal deformities - other stressors could have produced enough variability in its frequency to obscure a relationship with the presence/absence of oil. Only a more accurate index of oil contamination will allow a test of the hypothesis of a positive relationship between the frequency of spinal deformity and oil treatment.

Unlike spinal deformities, there was a highly significant increase in jaw deformities in larvae from oiled eggs. A response of jaw development to hydrocarbons has also been reported by previous controlled experiments. For example, Struhsaker et al. (1984) reported jaw anomalies in Pacific herring larvae exposed as eggs to 4.8-45 mg benzene-L<sup>-1</sup>, and Smith and Cameron (1979) reported a high incidence of jaw deformities in Pacific herring larvae that had been exposed at an age of 6 d to concentrations of 1 mg·L<sup>-1</sup> of the WSF of Prudhoe Bay crude oil for only 48 h. Linden (1978) reported similar deformities in Baltic herring embryos exposed to 59 mg hydrocarbons·L<sup>-1</sup>.

This study does not deal with sublethal effects of exposure to hydrocarbons that are not expressed as morphological deformities. Other investigators were contracted for this purpose.

### Viable Hatch

The fact that the product of survival and viability is 6% lower in oiled eggs than in control eggs [see equation (10) and Fig. 10C] supports the idea that there are both a survival-oil relationship and a viability-oil relationship hidden in the data set.

### Size and Condition of Larvae

In general, pollutant stressors such as petroleum hydrocarbons tend to produce fish larvae with reduced length (von Westernhagen 1988). These premature larvae are heavier than untreated larvae because they carry a larger yolk sac, and so they also have a higher condition. The results of this study are the exact opposite: on average after correction for age and depth, larvae from oiled samples were 0.1 mm longer, 4  $\mu$ g lighter, had 7% lower condition and had a 1% larger yolk sac than larvae from

control samples. Although these differences were statistically significant, they were probably too minor, e.g. a 1.2% increase in mean length and a 2.4% decrease in mean weight, to have had any effect on subsequent larval survival. These results suggest that oil may have had the effect of stimulating the hatch of larger larvae, but it is difficult to reconcile that conclusion with a significantly reduced age at 5097 hatch.

We suggest that one reason for the apparently anomalous results was a confounding of the oil effect with a temperature effect. Oil from the Exxon Valdez spill contaminated beaches in the central and southern parts of Prince William Sound, but not beaches in the north of the Sound. This pattern coincides with a geographic trend of low water temperatures in central and southern Prince William Sound and higher temperatures in the north. Table 1 shows that surface water temperatures at the control transects in and near Fairmount Bay were 1.1 to 2.2°C higher in late April than those at oiled transects of Naked Island and Montague Island. A similar pattern of higher May-June temperatures in the north of the Sound was reported by McGurk et al. (1990). The cause of the temperature differences is the inflow of cold oceanic water into the Sound through Hinchinbrook Entrance; sites close to the Entrance are always colder than sites far from the Entrance.

At present, there is no way of incorporating the effect of temperature into the general linear models of size and condition [equations (11) to (14)] because we do not possess any records of temperature for the incubation period before April 21, 1989, and because the temperature records for the period April 21- May 2, 1989, do not contain any information on temperature at depth. It may be possible to remove the temperature effect by examining size and condition of larvae within smaller geographic areas, such as the Naked Island archipelago, where temperature would be expected to vary much less than within larger geographic units. However, this analysis requires an index of oil contamination that varies within the oil-treatment group.

### Ranking of Samples with Factor 3

We encourage future investigators to test our prediction of the rank order of oil contamination of herring eggs.

## **5. RECOMMENDATIONS**

### **Reanalyse Data using Hydrocarbon Concentrations**

As stated several times in this report, the major drawback of this study was our reliance on a simple presence/absence index of oil exposure. This was unavoidable because we had no other information at the time of writing this report. We are aware that samples of herring eggs were taken by ADF&G from the transects used in this study and frozen, and that these samples have been or will be analysed for hydrocarbon concentration. Therefore, we recommend that the data set presented in this report and in the accompanying appendices be reanalysed with the hydrocarbon concentrations whenever their measurement is completed. At the very least, the hydrocarbon concentrations should be compared with the values of factor 3 in order to test our predictions of the rank order of oil impact between samples and transects.

### **Replicate Egg Incubation Experiment in 1990**

Some residual oil is still contained within the gravel of spawning beaches in Prince William Sound. It may affect the survival and viability of herring embryos spawned in the spring of 1990. Therefore, we recommend that the herring egg incubation experiment be replicated in 1990. We suggest that biochemical indices of growth and condition should be employed, as well as morphological indices, because biochemical indices have a clearly defined methodology, they are more precise in measurement, and they may lead to a more biologically meaningful assessment of the capacities of the larvae. Specifically, we recommend the use of RNA-DNA ratios of newly-hatched herring larvae as an index of their instantaneous growth rates. We strongly recommend the use of Clemmessen's (1988) method of measuring RNA and DNA concentrations because it is more accurate and more precise than all other methods previously reported in the scientific literature. McGurk et al. (1990) describe a comparison of methods for measuring nucleic acid concentrations that identifies Clemmessen's (1988) method as superior to all others. We also recommend the use of mixed-function oxygenase (MFO) enzymes as an index of exposure to hydrocarbons (Payne et al. 1987).

## 6. REFERENCES CITED

- Aneer, G., and S. Nellbring. 1982. A SCUBA-diving investigation of Baltic herring (Clupea harengus membras L.) spawning grounds in the Asko-Landsort area, northern Baltic proper. *J. Fish Biol.* 21:433-442.
- Bailey, K M., and E. D. Houde. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. *Adv. Mar. Biol.* 25:1-83.
- Baxter, I. G. 1971. Development rates and mortalities in Clyde herring eggs. *Rapp. P.-v. Réun. Cons. int. Explor. Mer* 160:27-29.
- Chambers, R. C., and W. C. Leggett. 1989. Event analysis applied to timing in marine fish ontogeny. *Can. J. Fish. Aquat. Sci.* 46:1633-1641.
- Clemmessen, C. M. 1988. A RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larvae. *Meeresforsch.* 32:134-143.
- Doyle, M. J. 1977. A morphological staging system for the larval development of the herring, Clupea harengus L. *J. mar. biol. Ass. U.K.* 57:859-867.
- Ernst, V. V., J. M. Neff, and J. W. Anderson, 1977. The effect of water-soluble fractions of No. 2 fuel oil on the early development of the estuarine fish, Fundulus grandis Baird and Girard. *Environ. Pollut.* 14:25-36.
- Haegle, C. W., R. D. Humphreys, and A. S. Hourston. 1981. Distribution of eggs by depth and vegetation type in Pacific herring (Clupea harengus pallasii) spawnings in southern British Columbia. *Can. J. Fish. Aquat. Sci.* 38:381-386.
- Hay, D. E. 1982. Fixation shrinkage of herring larvae: effects of salinity, formalin concentration, and other factors. *Can. J. Fish. Aquat. Sci.* 39:1138-1143.
- Hay, D. E. 1984. Weight loss and change of condition factor during fixation of Pacific herring, Clupea harengus pallasii, eggs and larvae. *Fish Biol.* 25:421-433.

Hempel, I., and G. Hempel. 1971. An estimate of mortality in eggs of North Sea herring (Clupea harengus L.). Rapp. P.-v. Réun. Cons. int. Explor. Mer 160:24-26.

Hourston, A. S., H. Rosenthal, and H. von Westernhagen. 1984. Viable hatch from eggs of Pacific herring (Clupea harengus pallasii) deposited at different intensities on a variety of substrates. Can. Tech. Rep. Fish. Aquat. Sci. 1274: 19p.

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. Fish. Res. Board Can. 29:1119-1124.

Leung, T. S., and R. V. Bulkley. 1979. Effects of petroleum hydrocarbons on length of incubation and hatching success in the Japanese medaka. Bull. Environ. Contain. Toxicol. 23:236-243.

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

McGurk, M. D., D. Warburton, and V. Komori. 1990. Early life history of Pacific herring: Prince William Sound herring larvae survey. Contractor's report prepared for U.S. National Oceanic and Atmospheric Administration, OMA/OAD, Anchorage, Alaska, by Triton Environmental Consultants Ltd., Burnaby, B.C., Canada.

Palsson, W. A. 1984. Egg mortality upon natural and artificial substrata within Washington State spawning grounds of Pacific herring (Clupea harengus pallasii). M.Sc. Thesis, University of Washington, Seattle, WA, U.S.A. 191p.

Payne, J. F., L. L. Fancey, A. D. Rahimtula, and E. L. Porter. 1987. Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. Comp. Biochem. Physiol. 86C: 233-245.

Rice, S. D., R. E. Thomas, and J. W. Short. 1977. Effect of petroleum hydrocarbons on breathing and coughing rates and hydrocarbon uptake-depuration in pink salmon

fry. p. 259-277. In F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg [eds.]. *Physiological responses of marine biota to pollution*. Academic Press, New York.

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

Smyth, H. F., Jr. 1967. Sufficient challenge. *Food Cosmet. Toxicol.* 5:51-58.

Sokal, R. R., and F. J. Rohlf. 1981. *Biometry*. W. H. Freeman and Company, New York, N.Y.

Struhsaker, J. W., M. B. Eldridge, and T. Echeverria. 1974. Effect of benzene (a water soluble component of crude oil) in eggs and larvae of Pacific herring and northern anchovy. p. 253-284 In Vemberg, F. J., and W. B. Vemberg [eds.] *Pollution and physiology of marine organisms*. Academic Press, New York.

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

Westernhagen, H. von. 1988. Sublethal effects of pollutants on fish eggs and larvae. *Fish Physiology XI(A)*: 253-346.