Exxon Valdez Oil Spill
Restoration Project Annual Report

Herring Spawn Deposition and Reproductive Impairment

Restoration Project 94166-1
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: This project was initiated in 1989 as State/Federal Natural Resources Damage Assessment Fish/Shellfish Study Number 11 under the title Injury to Prince William Sound Herring. Annual reports were issued in 1990 and 1991 and a number of contractor reports were submitted detailing individual research components. Project funding was continued in 1992, but was discontinued in 1993 and the project went into close out. A final report for research conducted from 1989 through 1992 was submitted in December 1994 (Brown, E.D. 1995. Injury to Prince William Sound herring following the *Exxon Valdez* oil spill). This final report was comprised of 8 chapters representing accepted or submitted journal articles covering most of the research topics investigated by this project. Due to an unanticipated decline in the abundance of spawning adults during 1993, stock assessment and genetic damage studies were reinitiated as Project 94166-1. This report covers the stock assessment component of that project for spawn deposition biomass estimates and egg loss studies. This project was continued in FY95 as project 95166 and is recommended to continue with refinements to improve accuracy and efficiency until significant recruitment to pre-spill population levels occurs.

Abstract: Underwater diver surveys of deposited eggs were used to estimate the 1994 adult spawning population of Pacific herring *Clupea pallasi* in Prince William Sound. The stratified random sampling design employed diver estimates of egg numbers within a systematically placed 0.1^2 m quadrat along transects randomly selected from all areas of spawn identified during aerial surveys. Diver estimates of egg numbers were corrected for systematic bias using an inverse prediction procedure that compared diver egg counts and gravimetrically determined laboratory egg counts for the same samples. Egg loss rates due to physical removal from spawning beds were investigated, but analyses were not yet completed and diver estimates of total egg numbers were corrected using an assumed value of 10% eggs lost prior to surveys. The estimated spawning biomass of herring was calculated to be 15,485 tonnes with a 95% confidence interval ranging from 9,025 tonnes to 21,945 tonnes.

Key Words: *Clupea pallasi*, *Exxon Valdez* oil spill, herring, Prince William Sound, spawn deposition surveys, spawning biomass, stock assessment.

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INTRODUCTION

The biomass of spawning adult Pacific herring *Clupea pallasi* in Prince William Sound (PWS) during 1994 was estimated to be 15,485.2 tonnes using underwater diver surveys of deposited eggs. This measure of abundance is necessary for monitoring recovery of the injured herring population, including recovery to population levels sufficient for sustainable commercial harvest. In addition, this project collected information about natural losses of deposited eggs which will be used to improve spawner biomass estimates and to provide early life history abundance and survival information to improve understanding of the ecological importance of herring in the PWS ecosystem. Herring provide important forage for many species including some species severely injured by the *Exxon Valdez* oil spill. Predator species include humpbacked whales, seals, sea lions, gulls, sea ducks, shorebirds, halibut, salmon, rockfish, and other fish. In addition to their ecological value, herring are a major commercial resource in PWS. From 1969 to 1993, the average annual combined ex-vessel value of five commercial PWS herring fisheries was $8.3 million. In addition, several thousand pounds of herring and herring spawn on kelp are harvested annually for subsistence purposes and form an important part of the local native culture of Chenega and Tatitlek.

Relation to Other Oil Spill Studies

The *Exxon Valdez* oil spill coincided with the spring migration of herring to spawning grounds and adult herring swam through oiled waters on their way to nearshore staging areas. Studies of oil spill injuries to herring were initiated in 1989 and research continued through 1992 with contributions from both state general funds and the Trustee Council (Brown 1995). Significant histopathological damage was measured in adults collected in oiled areas in both 1989 and 1990 confirming exposure of the fish to toxins. Oiling of spawning areas caused elevated levels of physical and genetic abnormalities in newly hatched larvae and reduced hatching success of the embryos. Additionally, most of the PWS herring summer rearing and feeding areas were oiled in 1989, based on the oil trajectory and historic fisheries records since 1914 (Reid 1971).

Mortality of young herring was significantly greater in oiled areas in 1989 and 1990, and sublethal effects were measurable in larvae and adults in 1989 and 1990. Persistent sheening and suspended oil-sediment droplets leaching from beaches and cleaning operations in 1989 and 1990 continued to expose adult and juvenile herring to oil. Laboratory exposures of pre-spawning adult herring to oil showed high concentrations of oil in ovarian tissue. Laboratory studies measuring the effect of known doses of oil on newly hatched larvae linked estimated doses of oil measured in PWS and injuries observed in field samples. In addition, measurements of oil in tissues from mussels collected at PWS beaches were significantly

correlated to indices of injury in herring larvae from spawning beds adjacent to mussel collection sites, and were most correlated with genetic injury endpoints.

Although herring survival varies tremendously under normal conditions, abundance for the 1989 year class is extremely low and results to date strongly implicate the spill as a major cause. One hypothesis is that injury to germ tissue caused by exposure to oil would result in non-viable embryos and larvae. A pilot experiment to measure the ability of herring from this age class to produce viable offspring was conducted in 1992 and hatching success of eggs collected from fish spawning in previously oiled areas was less than half that of eggs collected from fish spawning in pristine areas. Additionally, there were approximately twice as many abnormal larvae from fish spawning in previously oiled areas. Information from this pilot study was used to formulate a study design for the reproductive impairment component of project 94166, which will be reported under a separate cover by NOAA Auke Bay Lab.

In 1993, the total observed spawning population was less than one third of preseason predictions and the average sizes of herring in each age class were some of the smallest on record. The total commercial harvest for that year was one of the lowest on record. Pathology studies from the spring of 1993 implicated viral hemorrhagic septicemia (VHS) as a potential source of mortality and stress (Meyers et al. 1994). Investigations of the incidence and effects of diseases occurring in PWS herring were continued in 1994. Spawn deposition surveys were not conducted in that year, and an acoustic survey was conducted near Green and Montague Islands to obtain an updated estimate of the population size following the apparent high mortality of the previous winter.

OBJECTIVES

1. Estimate the biomass of spawning herring in PWS using SCUBA diving spawn deposition survey techniques such that the estimate is within $\pm$ 25% of the true value 95% of the time.

2. Quantify egg loss rates (the proportion of eggs removed through time) from spawning areas due to wave action, predation, desiccation, or fungal infections between the time of egg deposition (spawning) and the time of hatching. Quantify egg loss by habitat type and egg density.

3. Incorporate egg loss and egg survival estimates with results from previous studies and revise the models as necessary.

Information was also gathered for the following objectives not formally included in the 1994 work plan and which will be addressed at conclusion of the egg loss study component of this project:

4. Define herring spawning habitat types by similarities in temperature, salinity, depth, gradient, substrate, vegetation, and exposure to wave action. Characterize and map habitat utilized for spawning. Estimate the abundance and distribution of adult herring and eggs by habitat type. Test a model of the relationship of spawn timing, spawner density and abundance to egg distribution and density.

5. Incorporate egg loss and survival data with physical oceanographic and meteorological data to formulate and test a model of the relationship of meteorological conditions to wave height and egg desiccation.

6. Test a model of the relationship between predation, wave action, desiccation, fungal infections, habitat type, and egg density.

7. Test a model relating sound-wide embryo survival to habitat type, egg density, and meteorological conditions.

METHODS

Spawn Deposition Survey and Biomass Estimation

Biomass estimation based on spawn deposition surveys consisted of three major components: (1) a spawn deposition survey; (2) age-weight-length (AWL), sex ratio, and fecundity sampling; and (3) egg loss determination.

Spawn Deposition Survey Design:-- Spawn deposition surveys were conducted to obtain biomass estimates within ± 25% of the true biomass 95% of the time. Survey design was described in detail by Biggs and Funk (1988) and followed the two-stage sampling design of similar surveys in British Columbia (Schwiegert et al. 1985) and Southeast Alaska (Blankenbeckler and Larson 1982, 1987). Surveys consisted of random sampling for the first stage (transects) and systematic sampling for the second stage (quadrats within transects). Surveys were stratified by area to account for geographic differences and the potential for discrete herring stocks. Areas surveyed included Southeast, Northeast, and Montague Island (Figure 1).

Mean egg densities along each transect were combined to estimate average egg density by summary area. Spawning bed width along each of the transects was used to estimate average spawning bed width by summary area. Average width, average density, and total spawning bed shoreline length (judged from aerial surveys) were used to estimate total number of eggs deposited in each summary area. Average fecundity and sex ratio obtained from AWL sampling, and estimates of total number of eggs deposited from diver surveys were used to calculate herring population numbers and biomass. Confidence intervals were calculated assuming a normal distribution of total egg estimates.

Spawn Deposition Survey Sampling Procedure:-- The general location of spawning activity was determined from visible milt observed during aerial surveys (Figure 1). Spawning activity was summarized on maps showing spawning locations and the dates on which milt was observed. Linear distances of shorelines over which herring spawned were estimated directly by aerial surveyors and were later measured from hand drawn aerial survey maps. Hand drawn maps were transcribed to computerized maps and linear distance estimated by the software was compared to surveyor estimates. Aerial observations were corrected using direct observations of eggs at the time of dive surveys.

Mapped shorelines containing herring spawn were divided into the shortest resolvable segments on the map scale (approximately 0.18 km) to aid in locating transects (Figures 2 and 3; Table 1). The total number of potential transects were calculated from the total of all shoreline where spawning was observed. A minimum sampling goal of 0.035 % of all potential transects within the spawning area was set to meet specified accuracy and precision based on variances obtained during 1984, and 1988 to 1992 surveys. Shoreline segments were assigned random numbers and the desired number of transects were randomly selected from among all possible shoreline segments. Each segment selected was assigned a sequential transect number and charted on waterproof field maps. Approximate locations for each transect were obtained from these field maps and exact locations were fixed as the dive skiff approached the shore before bottom profiles, bottom vegetation, or herring spawn became visible from the skiff. Typically, the skiff driver would choose an easily recognizable shoreline feature within the targeted shoreline segment as a reference point (e.g. a tree, rock, or cliff located above the high tide line) to locate the transect. The sampling transect extended seaward along a compass course perpendicular to shore from this fixed reference point.

Diving operations began several days after spawning ceased to allow water turbidity due to milt to decrease and for the large numbers of sea lions usually present near spawning herring to disperse. Two three-person dive teams consisted of a lead diver counting eggs (typically the person most experienced at this survey task), a second diver recording data, and a third diver on the surface serving as a dive tender. Diving and tending duties were rotated daily.

The number of herring eggs occurring within a standardized sampling quadrat was estimated at regular intervals along the length of the transect. The sampling quadrat consisted of a 0.1 m^2 frame constructed of PVC pipe with a depth gauge and compass attached. Location for the first quadrat placement along the transect was haphazardly selected within the first 5 meters of spawn. Succeeding quadrat placements were systematically spaced every 5 meters along the compass course until the apparent end of the spawn. At each quadrat placement, the lead diver estimated the number of eggs in units of thousands (K) within the quadrat and communicated the numbers through hand signals to the second diver. Number of eggs, vegetation type, percent vegetation cover, substrate, and depth were recorded by the second diver in pencil on water-proof plastic paper data forms attached to a clipboard. Divers verified the end of the spawn by swimming at least an additional 20 m past the end of the spawn until a steep drop-off was encountered or vegetation was no longer present.

Biomass Estimation:-- Analysis of the spawn deposition survey data was similar to methods used in 1988 (Biggs and Funk 1988), and 1989-1992 (Brown 1995). The biomass estimator was

$$B=TB', \tag{1}$$

where

B = estimated spawning biomass in tonnes,
T = estimated total number of eggs (billions) deposited in an area, and
B' = estimated tonnes of spawning biomass required to produce one billion eggs.

Estimates for T and B' were derived from separate sampling programs and were independent. The estimated variance for the product of the independent random variables T and B' was calculated according to Goodman (1960)

$$Var(B)=T^2Var(B')+B^2Var(T)-Var(T)Var(B'), \tag{2}$$

where

Var(B') = an unbiased estimate of the variance of B', and
Var(T) = an unbiased estimate of the variance of T.

Total Number of Eggs (T):-- The total number of eggs deposited in an area was estimated from a two-stage sampling design using random sampling at the primary stage and systematic sampling at the secondary stage, similar to the design described by Schwiegert et al. (1985). To compute variances based on systematic second stage samples, it was assumed that eggs were randomly distributed in spawning beds with respect to the 0.1 m^2 sampling unit. While this assumption was not examined, in practice the variance component contributed by the second sampling stage is much smaller than that contributed by the first stage and violation of this assumption has little effect on the overall variance. The total number of eggs (T), in billions, in an area was estimated as

$$T=N\hat{y}10^{-6}/(1-R), \tag{3}$$

where

L = the shoreline length of the spawn-containing stratum in meters,
N = $L/0.1^{0.5}$ = the total number of possible transects,
$0.1^{0.5}$ = 0.3162 m = width of transect strip,

$\hat{y}$ = average estimated total number of eggs (thousands) per transect,
10^{-6} = conversion from thousands to billions of eggs, and
R = estimated proportion of eggs disappearing from the study area from the time of spawning to the time of the survey.

Average total number of eggs per transect (in thousands) was estimated as the mean of the total eggs (in thousands) for each transect using

$$\hat{y}=\frac{\sum_{i=1}^{n}\hat{y}_i}{n}, \tag{4}$$

where

$$\hat{y}_i=M_i\bar{y}_i, \tag{5}$$

and

n = number of transects actually sampled,
i = transect number,
M_i = $w_i/0.1^{0.5}$ = number of possible quadrats in transect i,
w_i spawn patch width in meters measured as the distance along the transect between the first quadrat containing eggs and the last quadrat containing eggs, and
$\bar{y}_i$ = average quadrat egg count in transect i (in thousands of eggs).

Average quadrat egg count within a transect, $\bar{y}_i$, was computed as

$$\bar{y}_i=\frac{\sum_{j=1}^{m_i}y_{ij}}{m_i}, \tag{6}$$

where

j = quadrat number within transect i,
m_i = number of quadrats actually sampled in transect i, and
y_{ij} = adjusted diver-estimated egg count (in thousands of eggs) from the diver calibration model for quadrat j in transect i.

The variance of T, ignoring the unknown variability in R, was similar to that given by Cochran (1963) for three stage sampling with primary units of equal size. In this case the

expression was modified because the primary units (transects) did not contain equal numbers of secondary units (quadrats), and the variance term for the third stage comes from the regression model used in the diver calibration samples. Therefore the estimated variance of T, conditioned on R, was

$$Var(T)=\frac{[N^2(10^{-6})^2[\frac{(1-f_1)}{n}s_1^2+\frac{f_1(1-f_2)}{n}s_2^2+\frac{f_1f_2}{n}s_3^2]]}{\sum_{i=1}^{n} m_i \quad \sum_{i=1}^{n} m_i}, \tag{7}$$
$$(1-R)^2$$

where

$$s_1^2=\frac{\sum_{i=1}^{n}(\hat{y}_i-\hat{y})^2}{n-1} = \tag{8}$$

variance among transects,

$$s_2^2=\sum_{i=1}^{n} M_i^2 \sum_{j=1}^{m_i} \frac{(y_{ij}-\bar{y}_i)^2}{n(m_i-1)} = \tag{9}$$

variance among quadrats,

$$s_3^2=\sum_{i=1}^{n}\sum_{j=1}^{m_i} Var(y_{ij}) = \tag{10}$$

sum of the variances of the individual predicted quadrat egg counts from the diver calibration model,

$$f_1 = \frac{n}{N} = \tag{11}$$

proportion of possible transects sampled, and

$$f_2 = \frac{m_i}{M_i} = \tag{12}$$

proportion of quadrats sampled within transects (same for all transects).

Diver Calibration Sample Collection:-- Spawn deposition survey methods for estimating spawning biomass utilize diver estimates of the number of eggs deposited within a systematically placed 0.1 m^2 quadrat. It is possible or even likely that estimates of egg abundance vary considerably from the true abundance. A portion of that variability can be attributed to systematic effects which can be accounted for in a calibration model. Estimates of the effects of vegetation type and diver bias on egg counts were used to adjust the original counts, resulting in more accurate estimates of egg abundance.

Diver calibration samples were collected concurrently with spawn deposition surveys throughout the field season. Calibration samples were stratified by diver, vegetation type within four broad categories, and by egg density over three broad categories. Both divers independently estimated the number of eggs on removable vegetation in each calibration quadrat. All egg-containing vegetation within the quadrat was removed and placed in numbered mesh bags. The number of loose and attached eggs left after removal were estimated by the lead diver and recorded. Based on accuracy estimated for previous survey results, an approximate sample goal of 80 calibration samples was set for each diver who had less than three years survey participation and 40 for each calibrated diver who had participated in calibration sampling for three or more years of surveys. Calibration samples for each diver were to be taken equally from each of four vegetation categories: eelgrass (EEL), fucus (FUC), large brown kelp (LBK), and hair kelp (HRK); and equally from each of three ranges of egg densities: low (0-20,000), medium (20,000-80,000), and high (>80,000) within each vegetation category. Aboard the dive vessel, calibration samples were arranged within a sampling quadrat placed on the deck and all divers estimated the number of eggs within the quadrat to increase the number of calibration samples available for each diver and to simulate estimates conducted at low tide. Calibration samples were preserved in Gilson's solution and labelled as described by Becker and Biggs (1992). The actual number of eggs present in each calibration sample was later approximated gravimetrically in the laboratory using procedures also described in Becker and Biggs (1992).

Diver Calibration Modelling:-- Initial analysis of the 1994 spawn deposition diver calibration data was performed by David Evans, ADF&G, Anchorage, and is summarized

here. More detailed information describing the motivation, methods, and results of his analysis are presented in his original paper as Appendix A.

The data set used in the analysis was a subset of the full data set such that a fully factorial structure was obtained. The subsample included data for five divers who made observations on all four vegetation types (eelgrass, hair kelp, fucus, and large brown kelp) in each of four years (1990-1992 and 1994). Each quadrat estimate used in the calibration sample had a single vegetation type and year associated with it. The number of observations in each vegetation/year cell ranged from 3 to 59. In some instances, the egg abundance for a quadrat was estimated by more than one diver. These multiple observations on a single quadrat were therefore not independent and required special consideration in the analysis. Evans referred to this as a repeated measures element in the data.

The analysis was performed as a two-step process. For the first step, analysis of variance supplemented with graphical displays was used to determine which categories could be combined in the calibration model. The second step was to pool the appropriate categories and estimate parameters in the calibration model, again using an analysis of variance.

Scatterplots of diver count versus lab count for all factor combinations, indicated a tendency for increased variability in diver estimates with increasing density of eggs implying that the error in a diver's estimate was better expressed as a proportion of the true egg abundance rather than as a fixed number. This suggested that diver count could be modeled as a lognormal random variable so that assumptions of normality and constant variance could be met by using the log of diver count as a transformed random variable in subsequent analyses. Lab count was generally log transformed as well to obtain a linear relationship between diver counts and lab counts.

To account for multiple diver estimates on a single quadrat, it was necessary to identify quadrats within a year and vegetation type such that every unique combination of year, vegetation type, and replicate number would specify a unique quadrat. A split-plot analysis of variance was then used to test for significant effects while accounting for the repeated measures nature of the observations. Two separate error terms are required in a split-plot analysis because different factors typically have different experimental units. For this completely randomized split-plot design, the experimental unit for year and vegetation was a quadrat (whole-plot level) and the corresponding error term was an estimate of the variation between quadrats of the same vegetation type within each year. Different treatments (divers) were applied to each quadrat, but rather than partitioning the quadrat among the divers, each diver was applied to the entire quadrat. Therefore, the experimental unit for diver is also an individual quadrat (split-plot level) and an estimate of the variation between divers within a vegetation type and year was the appropriate error term.

An analysis of variance using a split-plot structure was run to determine which categories could be combined, thus reducing the number of diver-vegetation combinations in the prediction model and gaining precision in the estimates. It was assumed for this analysis that

all treatments (year, vegetation, and diver) were applied randomly with year and vegetation applied at the whole plot level and diver applied at the sub-plot level. The error term for testing year, vegetation, and year*vegetation interaction effects were the sum of replicate, year*replicate, vegetation*replicate, and year*vegetation*replicate. The usual error term would be correct for testing effects containing diver and for the lab count covariate. For main and interaction effects which were significant, plots of the least-squares estimates were inspected for insights into differences between categories.

Results from the analysis were used to pool categories that were statistically the same. After pooling, another analysis of variance was run and estimates were obtained for the model parameters. After taking logs of the lab and diver counts, the calibration model has the form

$$E[ldc_{ijk}] = \mu + Y_i + D_j + Vs_k + \beta \cdot llc_{ijk}, \tag{13}$$

where Y_i = effect of the i^{th} year,
D_j = effect of the j^{th} diver,
V_k = effect of the k^{th} vegetation type,
ldc_{ijk} = log of the count by the j^{th} diver on the k^{th} vegetation type in the i^{th} year,
llc_{ijk} = log of the lab count corresponding to ldc_{ijk},
β = regression coefficient of log(*diver count*) on log(*lab count*),

and where only main effects are shown for simplicity. Note that the exact form of this model depended on the pooling of the previous section. For example, if all years were pooled, then that term was no longer needed in the model. Each category as defined by the pooling was simplified to the form

$$E[ldc_{ijk}] = k_{ijk} + \beta \cdot llc_{ijk}, \tag{14}$$

where $k_{ijk} = \mu + Y_i + D_j + V_k$.

Equations of this form were generated for each category as defined by the pooling.

In practice, we used an inverse prediction procedure to estimate a lab count from an observed diver count. For a given diver count (Y_0) in the i^{th} year by the j^{th} diver on the k^{th} vegetation type, we used the equation

$$\hat{E}[\log(X_{ijk})]_{Y_0} = \frac{1}{\beta}[\log(Y_0) - k_{ijk}] \qquad (15)$$

to obtain a point estimate of the regressor variable, $\log(X_{ijk})_{Yo}$, where X_{ijk} is the corresponding lab count. This was then back-transformed to the original scale, which produces a biased estimate of $E[X_{ijk}]$, but an unbiased estimate of the median and the final form of the calibration equation for category *ijk* becomes

$$(\hat{X}_{ijk})_{Y_0} = e^{\frac{1}{\beta}[\log(Y_0) - k_{ijk}]}. \qquad (16)$$

Variances for the adjusted egg counts were obtained through a bootstrap procedure.

Spawning Biomass per Billion Eggs (B'):-- AWL, sex ratio, and fecundity data were used to estimate the relative relationship between spawning biomass and egg deposition. The relationship between fecundity and female weight was used to calculate total numbers of eggs deposited and tonnes of herring spawners. The tonnes of spawning biomass required to produce one billion eggs (B') was estimated as

$$B' = \frac{\bar{W}S}{F(\bar{W}_f)} 10^3, \qquad (17)$$

where

$\bar{W}$ = estimated average weight in grams of all herring (male and female) in the spawning population in an area,

S = estimated ratio of total spawning biomass (male and female) to female spawning biomass,

$F(\bar{W}_f)$ = estimated fecundity at the average weight of females in the spawning population in an area, in numbers of eggs, and

10^3 = conversion factor $= \frac{10^{-6}}{10^{-9}} =$ grams to tonnes / eggs to billions

Because average weight, sex ratio and fecundity were all estimated from the same herring samples, the estimates were not independent. The variance of B' was approximately:

$$
\begin{aligned}
Var(B') = (10^3)^2 \Big(\Big[\frac{S}{F(\bar{W}_f)} \Big]^2 Var(\bar{W}) \\
+ \Big[\frac{\bar{W}}{F(\bar{W}_f)} \Big]^2 Var(S) \\
+ \Big[\frac{\bar{W}S}{F(\bar{W}_f)^2} \Big]^2 Var(F(\bar{W}_f)) \\
+ 2Cov(\bar{W},S) \Big[\frac{S}{F(\bar{W}_f)} \Big] \Big[\frac{\bar{W}}{F(\bar{W}_f)} \Big] \\
- 2Cov[\bar{W},F(\bar{W}_f)] \Big[\frac{S}{F(\bar{W}_f)} \Big] \Big[\frac{\bar{W}S}{F(F(\bar{W}_f))^2} \Big] \\
- 2Cov[S,F(\bar{W}_f)] \Big[\frac{\bar{W}}{F(\bar{W}_f)} \Big] \Big[\frac{\bar{W}S}{F(\bar{W}_f)^2} \Big] \Big).
\end{aligned}
\tag{18}
$$

Because S was estimated from pooled or single AWL samples (depending on availability of fish), it was not possible to estimate the covariance terms containing S, $Cov(\bar{W},S)$ and $Cov[S,F(\bar{W}_f)]$. Because the term involving $Cov[\bar{W},F(\bar{W}_f)]$ has been shown to be very small in previous analyses and probably contributes little to Var(B'), these covariance terms were not included in the estimate of Var(B').

Herring Age, Weight, Length, Sex, and Fecundity

Biological samples were collected for age and sex composition, calculation of average weight and length, and estimation of fecundity. Most samples were aptured by volunteer commercial seine vessels or vessels under short term contrac as part of an existing ADF&G test fishing sampling program. Sampling generally occurred soon after concentrations of herring appeared in nearshore areas becoming accessible to purse seines and continued periodically throughout the spawning migration. Because aerial surveys did not indicate substantial accumulations of herring in areas other than Montague Island in 1994, all samples were collected in that area. Age and sex composition and average herring size were calculated using only AWL samples collected near the peak of spawning as determined from aerial survey sightings of milt and herring schools.

AWL sampling was stratified by date and locality for test fishing catches in spawn deposition summary areas. Sample size for each stratum was set to simultaneously estimate proportions by age when sampling from a multinomial population (Thompson 1987). The goal was to select the smallest sample size for a random sample from a multinomial population such that the probability would be at least 1-α (precision = 0.05) that all the estimated proportions were simultaneously within 5% (accuracy = 0.05) of the true population age proportions. A sample size of 450 herring per stratum was selected to ensure that this level of precision and accuracy would be obtained for any number of age classes and proportions when less than 5% of the collected scales were unreadable. Herring AWL sampling procedures are described in greater detail by Baker et al. (1991) and followed standard protocols outlined in project operational manuals (Wilcock *In press*).

Fecundity samples were subsampled from female herring in AWL samples and were stratified by fish length. Egg and gonad weights were measured and used to calculate average fecundity at the average female weight ($F(\bar{W}_f)$) from expression (17). Fecundity sampling goals were set such that fecundity estimates would contribute no more than 1% to the confidence interval width of the biomass estimate. It was determined that a sample size of 150 to 200 herring pooled across areas would be sufficient to maintain the coefficient of variation below 2.0%. To collect females across the range of all possible sizes, sample goals were 20 to 30 females within each 10 mm length category from 181 to 250 mm standard length, and 20 to 30 females 180 mm or smaller. The female gonad weight was the weight of the ovaries removed from each female.

Mean Weight and Sex Ratio:-- Average weight and sex ratio was estimated as a weighted average of estimates from each sampled locality based on observed aerial survey biomass at each locality. Because biological samples were collected only at Montague Island and because spawning observed in other areas was limited, AWL samples from Montague Island were used to estimate mean weight and sex ratio for all spawn deposition summary areas.

Sex ratio, S, was calculated as the ratio of the number of herring of both sexes in AWL samples to the number of females. The binomial distribution is applicable to estimating the proportion, p, of females in AWL samples, where S = 1/p. The variance of S is

$$Var(S)=\frac{S^2(S-1)}{n}, \qquad (19)$$

where n is the number of fish in the AWL sample.

Fecundity for Biomass Estimates: Average fecundity for PWS was estimated from a fecundity-weight relationship as $F(\bar{W}_f)$, and used in equation 17 to estimate biomass from spawn deposition. The variance of estimated average fecundities was approximated by the variance of predicted means from the fecundity-weight linear regression (Draper and Smith 1981)

$$Var[F(\bar{W}_f)] = s^2[\frac{1}{n} + \frac{1}{q} + \frac{(\bar{W}_f - \bar{WF})^2}{\sum (W_i - \bar{WF})^2}], \quad (20)$$

where

s^2	=	the residual mean square from the fecundity-weight linear regression,
$\bar{W}_f$	=	the average weight of female fish in the spawning population,
$\bar{WF}$	=	the average weight of females in the fecundity sample,
W_i	=	the weights of individual females in the fecundity sample,
n	=	the total number of females in the fecundity sample from each area, and
q	=	the total number of females in the representative AWL sample or pooled samples from the corresponding area.

A linear relationships between female body weight and fecundity was used because Hourston et al. (1981) found that female body weight at spawning explained 70% of the variation in fecundity among individuals, but length and age only explained another 2% of the variation.

Egg Loss Study

The proportion of eggs lost through physical removal and the mortality rate of remaining eggs was investigated to improve diver survey biomass estimates and to improve understanding of the mechanisms controlling early life history survival. The total number of eggs estimated from diver surveys (term T, equation 1) was corrected for eggs lost between the time of herring spawning and diver surveys as term R in equation 3. In prior spawn deposition studies for PWS, an assumed constant egg loss rate of 10% was used to correct spawn deposition estimates based on values recommended in the literature (Haegele et al. 1981, Blankenbeckler and Larson 1982). This estimated loss was based on the assumption that surveys were generally conducted 5-6 days after spawning. Egg loss was studied during spawn deposition surveys of PWS in 1990 and 1991 to more accurately quantify loss rates (Brown 1995). These studies indicated that egg loss varied substantially over time and between sites and suggested that using a constant rate of 10% may be inappropriate in some instances. These studies also suggested that spawning habitat may play a key role in determining egg loss rates, but the study design did not include collection of data to relate egg loss to habitat type, environmental conditions, or predation. The 1994 study modifications included measurements of 1) slope, substrate, and vegetation to describe habitat characteristics; and 2) temperature and salinity to describe environmental conditions. In addition, information was collected about bird predators in collaboration with EVOS Project 95320Q, Avian Predation on Herring Spawn. A Reimbursable Services Agreement (RSA) was initiated with the University of Alaska to investigate the factors important for estimating egg loss using the results from previous studies and the 1994 study. They also began investigating the modelling of egg loss to eventually construct an embryo survival model. A

progress report for this work is included as Appendix B. More detailed descriptions of their analytical methods and results for egg loss studies will be included in their final report, anticipated for late FY96 or early FY97.

Egg Loss Sampling Procedure.--Ten transects were established in 1994 on Montague Island to study egg loss (Figure 2; Table 2). Transect locations were chosen to represent typical spawning beach habitat characteristics within the spawn deposition summary area and to cover the range of potential exposures to wave action during incubation. Similar to spawn deposition transects, egg loss transects were established perpendicular to shore following a compass course. Three sampling stations were located along each transect line at depths within the range of usual herring spawn (+1.65 m to -9.90 m). Sampling stations were set at (1) 1.0 m above MLLW, (2) 1.0 m below MLLW, and (3) 3.0 m below MLLW based on information from previous egg loss and egg distribution studies. Station depths for some transects were adjusted accc ding to actual deposition of eggs. Depth at each station was initially determined using SCUBA diver depth gauges and later corrected for tide level. During transect establishment, beach gradient, substrate, and vegetation present at the site were recorded.

A grid of 5 x 2 permanent 0.1 m^2 quadrats was placed along transect lines at each depth station. Grids were generally oriented perpendicular to the transect and parallel to the shoreline, but actual placement was adjusted to conform to bottom contour, occurrence of spawn, and to represent vegetation typical of the site at that depth.

To collect information on egg loss due to predation and wave action, predator exclusion frames were placed at each of the three depth stations along each transect line. Exclusion devices were constructed from steel shrimp trap frames approximately 1 m^3 in volume and enclosed in mesh. Placement at each depth included: (1) one frame covered with small mesh intended to retain all eggs lost from wave action and to exclude large predators, (2) one frame covered with mesh large enough to exclude avian predators, but which would allow physical egg removal by wave action, and (3) a control plot marked by steel spikes, but without frames or mesh.

Transects were generally visited every three to four days. During each site visit, divers estimated egg density within each of five 0.1 m^2 quadrats along the bottom row of the fixed quadrat grid and the top row was reserved in case of destruction of any quadrats in the bottom row. Divers also collected eggs and vegetation within a separate 0.1 m^2 quadrat haphazardly placed near the egg loss grid for calibration samples. Diver calibration samples were preserved and processed in the same manner as those collected for spawn deposition surveys. During each site visit, measurements were made of air temperature, water temperature, and salinity. In addition, precipitation, tide height, wind speed and direction were noted.

To investigate the range of temperatures to which incubating eggs would be exposed, mechanical temperature recorders were installed at two egg loss sites. However, recorders were not activated properly and only temperatures measured during site visits were collected.

An additional sample containing over 200 eggs, was haphazardly selected from vegetation adjacent to the frames during each visit and depth. For each such sample, live/dead ratios were estimated and the eggs were examined for signs of desiccation or other signs of morbidity. Subsamples of live embryos were also collected just prior to hatch and preserved for later evaluation of morphological abnormalities and cytogenetics. Subsequent funding for processing of these samples was not included in the FY95 work plan.

Near the mid-point date of the incubation period, a sample of potential herring egg predators within an approximately 1 m^2 patch of spawning area adjacent to each egg loss transect was collected for species identification. Eggs and vegetation collected for this sample were preserved in Gilson's solution and all vertebrate and invertebrate animals were frozen. Frozen samples were submitted to nearshore researchers at the University of Alaska Fairbanks for identification.

RESULTS

Biomass Estimation

The total biomass of herring spawning naturally in PWS during 1994 was estimated to be 15,485 tonnes from spawn deposition diver surveys (Table 3). The variance of this estimated total was high, and the 95% confidence limits ranged from 9,025 tonnes to 24,190 tonnes (Table 4). Most of the estimated biomass spawned in the Montague Island summary area (15,478 tonnes), but small biomasses of spawning herring were calculated for the Southeastern (5 tonnes) and Northeastern (2 tonnes) summary areas (Figure 1). The proportion of spawning that occurred at Montague Island was similar using spawn deposition survey estimates of biomass (99.96%) to the proportion calculated using sightings of milt along shorelines (95.96%). The biomass of spawning herring calculated from spawn deposition surveys was somewhat lower than was the peak biomass estimated from aerial surveys for all summary areas (Table 3).

Diver Calibration Modelling:-- Analysis of variance results (Table 5) indicated significant effects of vegetation, diver, and year*diver interaction ($p<0.005$ in all cases) and was reflected by inspection of plots of least-square means (Appendix A). During 1990 and 1991, one diver tended to estimate substantially lower than he did in 1992 and 1994 when he was more consistent with three other divers over all four years. A fifth diver seemed to have a similar trend of consistency with the other divers in 1992 and 1994, but was higher in 1990 and 1991. These patterns among divers were consistent over vegetation types. Because they appeared to be most similar, calibration results for the three most consistent divers from 1990

through 1994 and the two less consistent divers from 1992 and 1994 were pooled over these years.

Fucus seemed to be different from the other vegetation types in 1991 and large brown kelp seemed to be different from the others in 1994. After discussions with the divers involved, it was decided to keep each of these separate with fucus pooled over 1990, 1992, and 1994 and only the 1994 data for large brown kelp used.

The second analysis of variance run with the pooled categories provided parameter estimates that were converted to inverse prediction formulas as described above. The resulting adjustments for the 1994 spawn deposition data are

All divers, eelgrass or hairkelp:

$$Adjusted\ eggcount = e^{\frac{1}{0.943}[\log(DiverEstimate)+0.0795]}, \quad (21)$$

All divers, fucus:

$$Adjusted\ egg\ count = e^{\frac{1}{0.943}[\log(DiverEstimate)+0.3520]}, \quad (22)$$

All divers, large brown kelp:

$$Adjusted\ egg\ count = e^{\frac{1}{0.943}[\log(DiverEstimate)+0.3840]}. \quad (23)$$

Herring Age, Weight, Length, Sex, and Fecundity

Age and sex composition and the average size for samples collected at each locality were collected as part of ongoing ADF&G fishery management activities and will be published separately in regular Commercial Fisheries Management and Development Division reporting series (personal communication, D. Sharp, Alaska Department of Fish and Game, Cordova; unpublished data, J. Wilcock, Alaska Department of Fish and Game, Cordova). The average size at age of all sampled herring and the estimated contribution by age to the 1994 PWS herring biomass in tonnes and in number of fish estimated from spawn deposition surveys are presented in Table 4. As expected from preseason forecasts (Funk 1995), the total biomass consisted largely of age-6 herring from the 1988 year class (63.5% contribution by weight and 63.9% by number of fish). The abundance of herring from the 1989 year class continued to be low and composed only 2.6% of the total biomass by number of fish. Abundance of ages 3 and 4 was also relatively low (1.0% and 11.5% by number of fish) and did not appear to indicate substantial survival success for these recruiting younger year classes.

The average weight of all sampled herring was 126.0 g and the average length was 215 mm. Average weights for all age classes were somewhat lower than average in 1994 and were similar to average weights observed in 1993 (Funk 1995).

Sex ratio of all samples collected in 1994 was 2.27 and the average weight for all females was 128.9 g (Table 3). Regression results for the weight to fecundity relationship are presented in Figure 4. Fecundity of female herring at the average female weight was calculated to be 21,881 eggs/female (Table 3) and was similar to fecundity at age estimated for previous years.

Egg Loss Study

Sites for 10 egg loss transects established on Montague Island during 1994 (Figure 2; Table 3) were chosen to represent a range of habitat characteristics over which herring spawn occurred. All sites were visited at least eight times during incubation. Exposures varied from very protected shoreline near the head of Rocky Bay at site 2, to extremely exposed rocky oceanic shoreline at site 6 on Montague Point. Rocky substrates were most frequent (7) at egg loss sites reflecting the selection of this substrate by spawning herring, but sand or mud bottoms occurred at 5 of the installed sites.

Avian predation exclusion frames were installed at all sites, but a number of frames were dislodged by wave action over the course of incubation, particularly the frames enclosed in small mesh. It was also found that algal and detrital build-up was severe on the small mesh frames and that loose eggs tended to drift into the frame from outside the enclosure and accumulate. Because of these shortcomings, small mesh enclosures were not felt to accurately represent egg loss and were dropped from the analysis. Large mesh frames were less frequently dislodged, and data from these frames will be included in the egg loss completion report. More detailed discussion of avian predator methods and results is included in the annual reports for project 95320Q. Estimated total consumption of eggs was not possible for the 1994 study. Sampling design modifications based on 1994 field sampling were included in the 1995 project descriptions for these studies to permit estimation of total avian predation.

Preliminary analysis of egg loss data collected for 1994 was conducted under a reimbursable services agreement with the University of Alaska (Appendix B). They graphically examined 1994 egg loss results in conjunction with re-examination of previous egg loss results to identify factors important for studying and modelling egg loss. Their findings were used to refine sampling design for work in 1995 to permit completion of this study component. More detailed descriptions of their methods and results will be included in the final report for that project component.

DISCUSSION

Preliminary estimates from the 1994 spawn deposition surveys were incorporated into age structured assessment (ASA) models to project the returning run biomass in 1995 as part of ongoing Department stock assessment and management functions (Funk 1995). ASA modelling generally incorporates other stock abundance estimates including aerial surveys of peak biomass of herring schools and kilometers of visible milt, estimated biomass from fall acoustic surveys, and information about age structure and average fish size to calculate projected returns. During the years of high abundance for herring (1988-1992), spawn deposition surveys provided abundance estimates that varied considerably from these other indicators of population size and spawn deposition estimates were accorded minimal weighting in ASA modelling. In general, differences between spawn deposition survey estimates and other stock assessment methods in 1994 were not as great as in these prior years. Biomass estimation based on spawn deposition surveys in 1994 were somewhat lower than biomass estimates based on aerial surveys of peak abundance, although it is generally felt that aerial surveys typically tend to underestimate abundance because not all fish schools or milt releases are visible to surveyors. Herring biomass estimated from acoustic surveys near Montague Island during the fall of 1993 was approximately 20,000 tonnes (DeCino et al. 1995) and an acoustic survey conducted in the same area during the fall of 1994 provided a biomass estimate of 8,969 tonnes (personal communication, G.Thomas, Prince William Sound Science Center, Cordova, AK). Funk (personal communication, F. Funk, Alaska Department of Fish and Game, Juneau) explored various weightings for various stock assessment methods for the 1995 projection. He concluded that age composition and spawn deposition estimates provided the most useful inputs for projecting 1995 abundance and tuned the model exclusively to those indicators for that year.

Accurately estimating the magnitude of herring populations is made difficult because they are a highly mobile species and exhibit large changes in distribution and abundance over a wide range of spatial and temporal scales. Spring spawning migrations provide perhaps the best opportunity to estimate abundance because herring are more aggregated and more visible than at other times of the year. Acoustics and other spectral technologies (e.g. LIDAR, CASI) could provide accurate and cost effective means of quantifying herring abundance, but these methods are limited in the amount of area that can be surveyed and occurrence of herring beyond areas surveyed is difficult to reconcile. Species verification of the quantified targets is also required.

Spawn deposition surveys are designed to estimate spawning abundance for all observed spawning herring, but accuracy for the method is constrained on several points. It is assumed that all fully recruited age classes spawn annually after recruitment and that all spawning is observed. The extent of incomplete participation in spawning is not known, but surveyors attempt to minimize the occurrence of unobserved spawning through frequent surveys. Two other important factors which can affect the accuracy of spawn deposition estimates are egg loss and calibration of divers. Although estimates of egg loss were not yet possible for the 1994 analysis, this information should become available upon completion of the egg loss

study component and previous estimates of biomass can be adjusted using revised loss rates. Revised biomass estimates will continue to provide information useful to fine tuning of ASA population models. Formulation and application of diver calibration models was investigated for this study, and a logical alternative was chosen from among the various possible approaches. Of all terms included in biomass calculations from spawn deposition surveys, diver calibration models may have the greatest potential for affecting population abundance estimates. Investigation of diver calibration models should continue as an integral part of project operations. Because these and others constraints to the accuracy of spawn deposition surveys cannot be cost effectively eliminated, other potential methods of herring stock assessment should continue to be studied in conjunction with spawn surveys. In particular, acoustic surveys during herring spawning migrations may have the potential for estimating spring biomass at lower cost and take advantage of the aggregative behavior of herring at this time of year.

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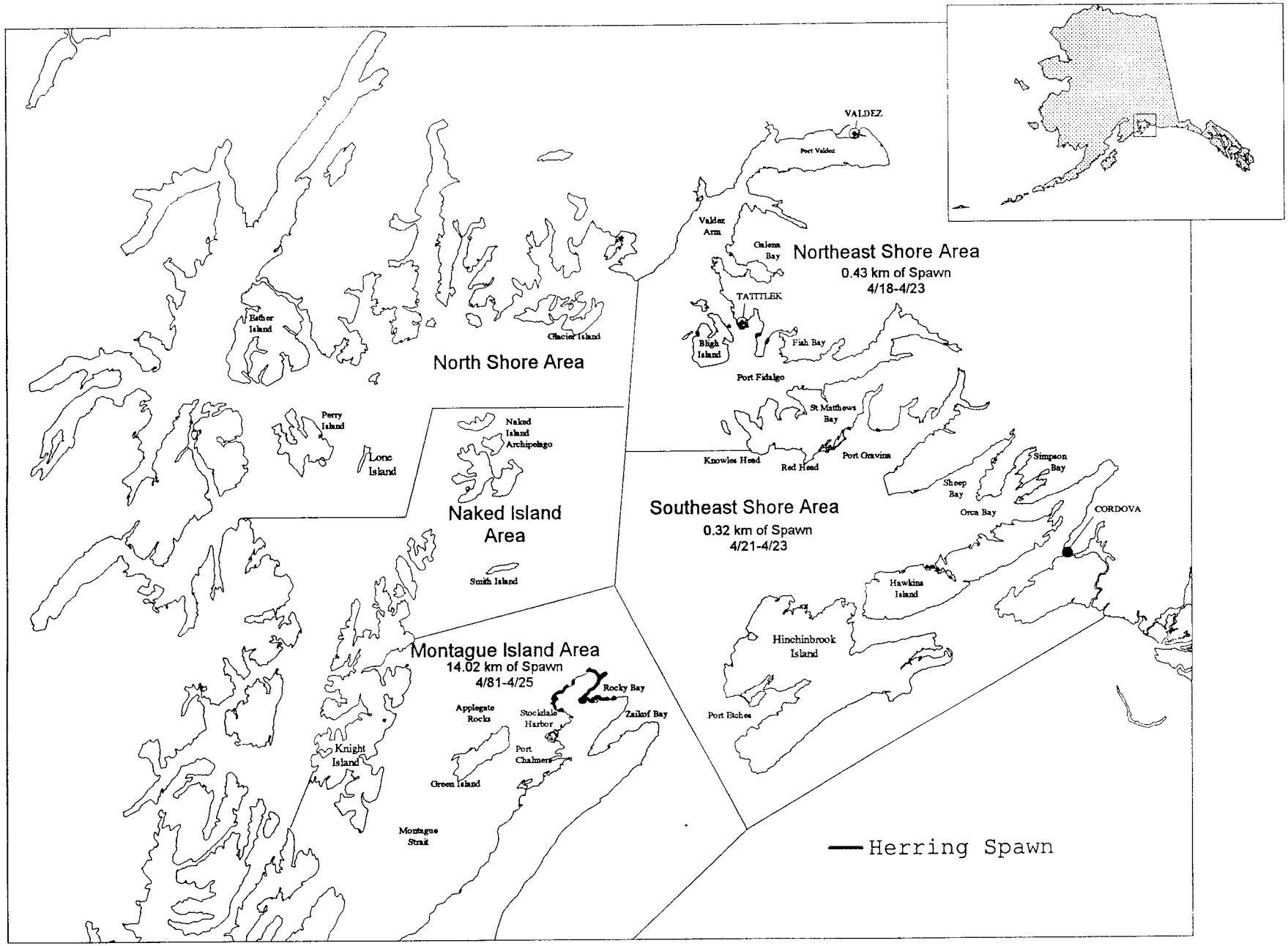


Figure 1. Location of spawning herring and kilometers of shoreline observed during aerial surveys in Prince William Sound, Alaska, 1994.

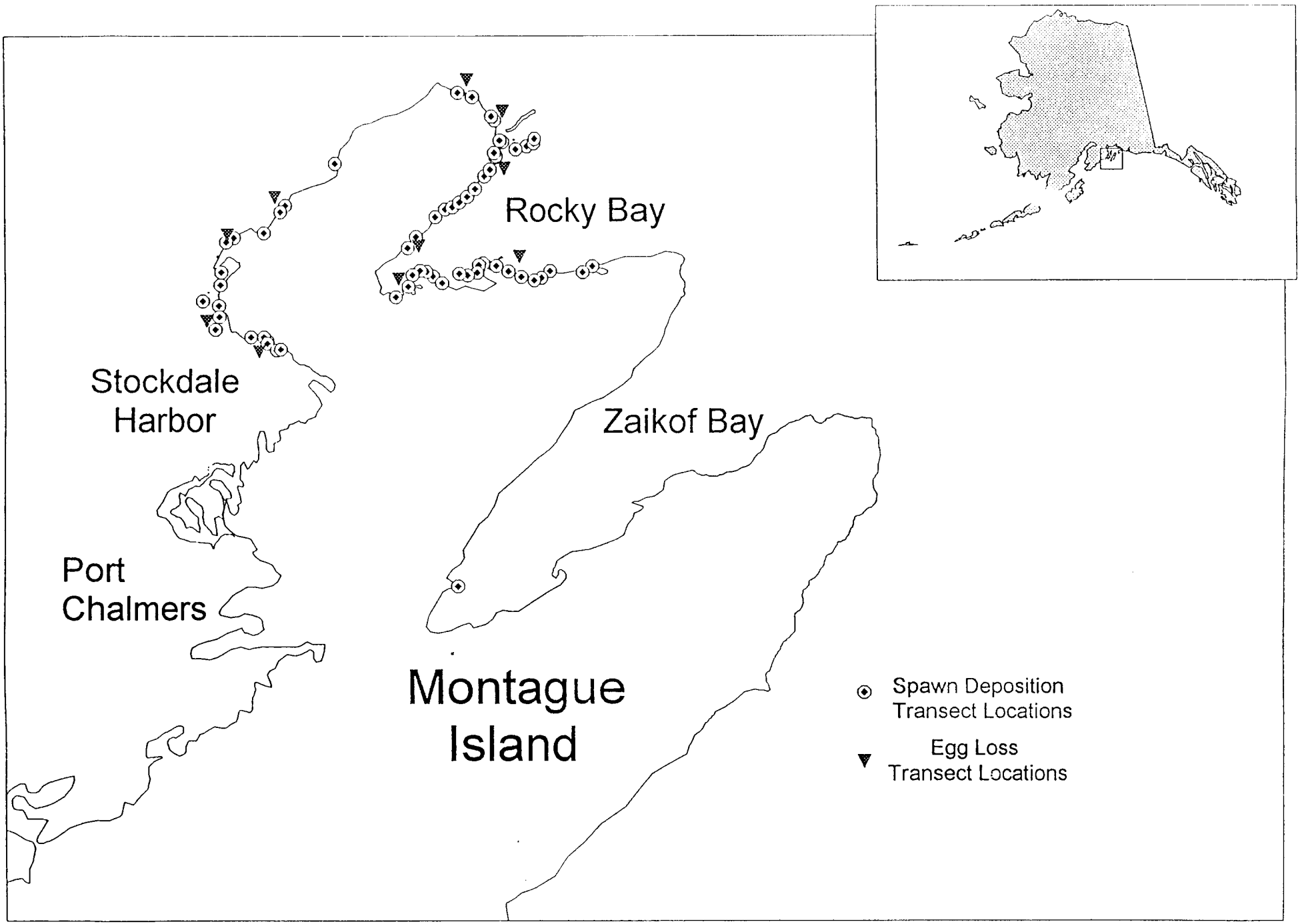


Figure 2. Spwan deposition and egg loss transect locations in the Montague Island summary area, Prince William Sound, Alaska, 1994.

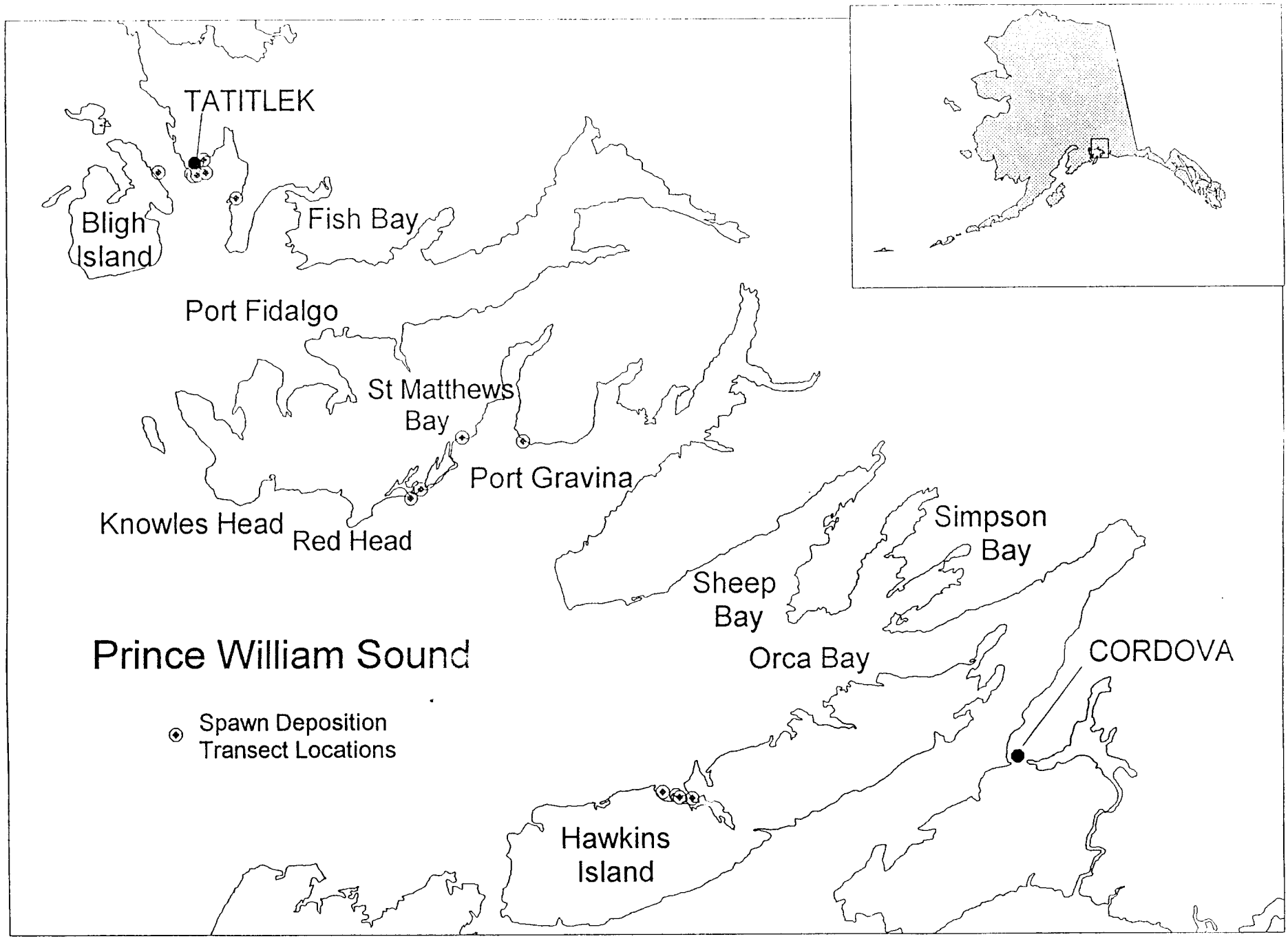
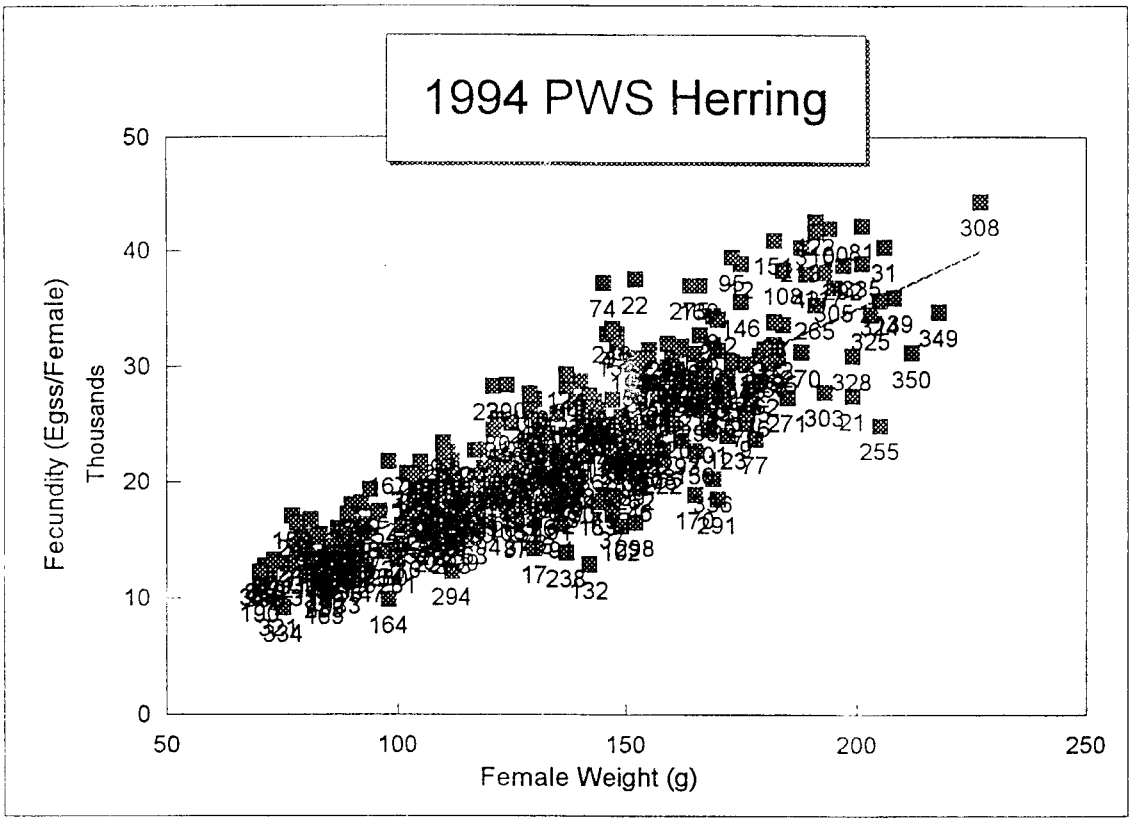


Figure 3. Spawn Deposition transects in the Southeastern and Northeastern summary areas of Prince William Sound, Alaska, 1994.



Number of Observations	340
Degrees of Freedom	338
Slope of Regression	184.44
Standard Error.	6.29
Intercept of Linear Regression	-1893.24
Standard Error of Y Estimate	3857.40
R Squared	0.718

Figure 4. Regression of Pacific herring female weight and number of eggs per female from samples collected at Montague Island, Prince William Sound, Alaska, 1994.

Table 1. Location and survey date of herring spawn deposition transects, Prince William Sound, Alaska, 1994.

Summary Area	Transect Location	Transect Number	Date Surveyed
Montague Island	Zaikof Bay	13	5/14/95
	Rocky Bay	14	4/28/95
	Rocky Bay	15	4/28/95
	Rocky Bay	16	4/29/95
	Rocky Bay	17	4/29/95
	Rocky Bay	18	4/28/95
	Rocky Bay	19	4/28/95
	Rocky Bay	20	4/29/95
	Rocky Bay	21	4/29/95
	Rocky Bay	22	4/29/95
	Rocky Bay	23	4/29/95
	Rocky Bay	24	4/29/95
	Rocky Bay	25	4/29/95
	Rocky Bay	26	4/30/95
	Rocky Bay	27	4/30/95
	Rocky Bay	28	5/1/95
	Rocky Bay	29	5/1/95
	Rocky Bay	30	5/4/95
	Rocky Bay	31	4/30/95
	Rocky Bay	32	4/30/95
	Rocky Bay	33	4/30/95
	Rocky Bay	34	5/1/95
	Rocky Bay	35	5/1/95
	Rocky Bay	36	5/1/95
	Rocky Bay	37	5/4/95
	Rocky Bay	38	5/11/95
	Rocky Bay	39	5/11/95
	Rocky Bay	40	5/11/95
	Rocky Bay	41	5/11/95
	Rocky Bay	42	5/7/95
	Rocky Bay	43	4/30/95
	Rocky Bay	44	5/12/95
	Rocky Bay	45	5/13/95
	Rocky Bay	46	5/13/95
	Rocky Bay	48	5/13/95
	Rocky Bay	49	5/13/95
	Rocky Bay	50	5/13/95
	Rocky Bay	51	5/3/95
	Rocky Bay	52	5/3/95

Summary Area	Transect Location	Transect Number	Date Surveyed
Montague Island (continued)	Rocky Bay Reef	53	5/13/95
	Montague Point	54	5/14/95
	Montague Point	55	5/7/95
	Montague Point	56	5/8/95
	Stockdale Harbor	57	5/6/95
	Montague Point	58	5/4/95
	Montague Point	59	5/4/95
	Montague Point	60	5/4/95
	Graveyard Point	61	5/2/95
	Graveyard Point	62	5/2/95
	Graveyard Point	63	5/2/95
	Graveyard Point	64	5/6/95
	Graveyard Point	65	5/6/95
	Graveyard Point	66	5/6/95
	Graveyard Point	67	5/6/95
	Graveyard Point	68	5/6/95
	Stockdale Harbor	69	5/6/95
	Stockdale Harbor	70	5/6/95
	Stockdale Harbor	71	5/6/95
	Stockdale Harbor	72	5/6/95
	Stockdale Harbor	73	5/6/95
Southeast	Canoe Passage	74	5/10/95
	Canoe Passage	75	5/10/95
	Canoe Passage	76	5/10/95
	Canoe Passage	77	5/10/95
	St. Mathews	78	5/10/95
	St. Mathews	79	5/10/95
	Hell's Hole	80	5/9/95
	Hell's Hole	81	5/9/95
Northeast	Landlocked Bay	1	4/20/95
	Landlocked Bay	2	4/20/95
	Landlocked Bay	3	4/20/95
	Tatitlek Narrows	82	5/10/95
	Boulder Bay	83	5/9/95
	Boulder Bay	84	5/9/95
	Tatitlek Narrows	85	5/9/95
	Boulder Bay	86	5/9/95
	Boulder Bay	87	5/9/95
	Boulder Bay	88	5/9/95

Table 2. Location and spawn dates for herring egg loss study transects at Montague Island, Prince William Sound, Alaska, 1994.

Transect No.	Location	Date Installed	Date Removed	Number of Site Visits	Spawning		Eggs Eyed at -1m Depth	Hatch at -1m Depth	Substrate	Site Gradient (%)		Exposure	COMMENTS
					Begin	End				Intertidal	Subtidal		
1	Rocky Bay-Inner	04/21	05/19	10	04/21	04/23	05/03	05/14	Rocky/boulders& gravel	7	4	SE facing; semi-exposed shoreline	Installed +1m first and - sites after spawn ended 4/26; thermographs installed .
2	Head of Rocky Bay	04/25	05/19	9	04/20	04/21	05/08	05/19	Mud on rock outcropping	9	16.7	NW facing; protected inner bay	Installed +1m first and - sites after spawn on 4/26/94; thermographs installed. Nearly 100% mortality.
3	Rocky Bay-Middle	04/25	05/19	11	04/20	04/24	05/04	05/14	Sand and Mud	1.1	2	N facing; semi-protected shoreline	Bird predation heavy here during set-up-some egg loss;upper station@0MLLW
4	Inside Rocky Bay	04/22	05/17	8	04/20	04/21	05/03	05/14	Rocky/Large Boulders	5	13	SE facing; moderately exposed shoreline	Lots of loss eggs in IT during set-up; +1m installed; lower depths by 4/26/94
5	Montague Reef	04/22	05/19	9	04/20	04/21	05/03	05/15	Solid Rock/sand between	3.3	2.7	NE facing; exposed oceanic site	Heavy spawn here; installed -1m on 4/25 and re-established the +1 site.
6	Montague Point	04/24	05/19	9	04/20	04/22	05/03	05/15	Solid Rock	6.5	1.8	NE facing; most extreme exposure	Put in +1m at +1.5 ft level; put in - station 4/25; lost upper site and predator exclsion frames
7	N Graveyard Pt	04/23	05/19	9	04/20	04/22	05/04	05/12	Rocky w/Large Boulders	5.6	5.1	NW facing; exposed oceanic site	Upper station @+2 ft level; no spawn @+1m; lower stations in 4/24/94
8	N. Graveyard Beach	04/23	05/19	8	04/19	04/21	05/02	05/15	Boulders	5.1	1.4	W facing; semi-exposed/sheltered site	Put in upper depth at +2 ft; no spawn @+1m; lower depths in on 4/26/94
9	Graveyard Pt.	04/23	05/19	9	04/19	04/21	05/02	05/15	Rocky w/sand fill & blds.	2.5	4.4	NW facing; exposed rocky outcropping	Put in all three depths at once; some bird predation and loss prior to installation.
10	Stockdale Harbor	04/24	05/19	9	04/22	04/24	05/02	05/12	Sand and Mud	4.5	3.8	S facing; sheltered bay	Active spawn here during install; upper at +0.25 ft and lower depths in 4/26/94

Table 3. Calculation of spawning herring biomass by project summary area from spawn deposition surveys and comparison with aerial surveys of fish schools and visible milt, Prince William Sound, Alaska, 1994.

Calculation		Symbol	Summary Area			
			Southeast	Northeast	Montague	Total
Area of Spawn	Kilometers of Visible Milt		0.43 [1]	0.51 [1]	22.57 [2]	25.51
	Number of Transects Possible	*(N)*	1,374	1,629	71,373	74,375
	Number of Transects Sampled	*(n)*	8	10	60	78
	Proportion of Transects Sampled	*(f1)*	0.582%	0.614%	0.084%	0.105%
	Quadrats Sampled in Spawn Patches	*(sum of mi)*	30	45	1,162	1,237
	Proportion of Quadrats Sampled	*(f2)*	6.325%	6.325%	6.325%	6.325%
	Avg. Width of Spawn Patch (m)		18.8	22.5	96.8	79.3
	Total Area of Spawn Patches (km^2)		0.01	0.01	2.18	2.20
Deposited Eggs	Average Eggs/Transect (1,000's)	*(y hat)*	238	92	14,914	11,508
	Proportion of Eggs Lost Before Survey	*(R)*	10%	10%	10%	10%
	Total Eggs in Area (billions)	*(T)*	0.363	0.167	1,183	1,183
Eggs per Female (AWL Sampling)	Avg. Herring Weight in AWL Samples (g)	*(W)*	126.0	126.0	126.0	126
	Number of Fish in AWL Sample		2,812	2,812	2,812	
	Number of Females in AWL Sample		1,292	1,292	1292	
	Sex Ratio	*(S)*	2.27	2.27	2.27	2.27
	Average Weight of Females (g)	*(Wf)*	128.9	128.9	128.9	129
	Fecundity of Female at Average Weight	*F(Wf)*	21,881	21,881	21,881	21,881
	Slope of Fecundity Regression		184.44	184.44	184.44	
	Intercept of Fecundity Regression		-1893.24	-1893.24	-1893.24	
Herring Biomass	**Tonnes per Billion Eggs**	*(B')*	13.09	13.09	13.09	13.09
	Estimated Biomass (tonnes)	*(B)*	4.7	2.2	15,478.3	15,485.2
	Peak Aerial Survey Biomass (tonnes)		299.4	99.7	17,418.3	17,817.4
Distribution	Percent of Kilometers of Visible Milt		1.70%	2.02%	88.44%	100.00%
	Percent of Estimated Biomass		0.03%	0.01%	99.96%	100.00%

[1] Sum of aerial surveyor estimates of the length of visible spawn.

[2] Sum of line segment lengths using hand drawn aerial surveyor shoreline observations redrawn in computer mapping software (MapInfo).

Table 4. Variance of calculations of spawning herring biomass from spawn deposition surveys by project summary area, Prince William Sound, Alaska, 1994.

Calculation		Symbol	Summary Area			
			Southeast	Northeast	Montague	Total
Egg Counts	Among Transect Variance	$(s1^2)$	1.46E+08	1.45E+08	4.71E+08	
	Within Transect Variance	$(s2^2)$	5.65E+05	4.70E+04	2.11E+09	
	Sum of Variance of Ind. Pred. Obs.	$(s3^2)$	1.82E+02	8.75E+01	1.37E+05	
	Variance of Estimated Total Eggs	*Var(T)*	42	47	49,305	49,394
AWL Sampling	Variance of Average Herring Weight	*Var(W)*	1.560E+02	1.560E+02	1.560E+02	1.560E+02
	Variance of Sex Ratio	*Var(S)*	2.338E-03	2.338E-03	2.338E-03	2.338E-03
	MSE from Fecundity Regression		1.488E+07	1.488E+07	1.49E+07	1.49E+07
	Mean Weight in Fecundity Sample		134.3	134.3	134.3	134.3
	Sum of x^2 in Fecundity Regression		6.51E+06	6.51E+06	6.51E+06	6.51E+06
	Number of Fish in Fecundity Sample		340	340	340	340
	Variance of Est. Avg. Fecundity	*Var(F(Wf))*	56,434	56,434	56,434	169,302
	Covariance of Avg. Wt., Fecundity	*Cov(W,F)*				
Biomass	**Variance of Tonnes per Billion Eggs**	*Var(B')*	1.78	1.78	1.78	5.34
	Variance of Estimated Biomass (tonnes)	*Var(B)*	7.15E+03	7.99E+03	1.08E+07	1.09E+07
	Standard Error of B		85	89	3,294	3,296
	Coefficient of Variation of B		1781%	4084%	21%	21%
	95% Confidence Interval of B					
	Interval Width as +/- % of B		3491%	8005%	42%	42%
	Lower Bound (tonnes)		(161)	(173)	9,023	9,025
	Upper Bound (tonnes)		170	177	21,934	21,945

Table 5. Analysis of variance for split plot analysis of diver calibration samples.

Dependent variable: Log(*Diver Estimate*)

Source	Degrees of Freedom	SS	MS	F	P
Model	554	1438	2.59	21.6	<0.0001
Error	590	71.9	0.12		
Corrected Total	1144	1510			
R-squared	0.95				

Source	Degrees of Freedom	TI	MS	F	P
Y	2	34.9	17.5		
V	3	71	23.8		
Y*V	6	24.9	4.2		
D	5	14.1	2.8		
Y*D	8	19.8	2.2		
V*D	15	11.1	0.74		
Y*V*D	22	24.9	0.99		
LBCNT	1	892	892	7325	<0.0001
R	82	63.4			
Y*R	84	65.9			
V*R	201	139.7			
Y*V*R	121	76.7			

Source	Degrees of Freedom	TIII	MS	F	P
Y	2	0.18	0.09	0.12[a]	>0.05
V	3	9.7	3.2	4.52[a]	0.0039
Y*V	6	4.7	0.79	1.11[a]	0.36
D	5	2.4	0.48	3.94	0.0016
Y*D	8	3.1	0.39	3.2	0.0013
V*D	15	1.8	0.12	1	0.45
Y*V*D	22	2.9	0.14	1.1	0.33
LBCNT[b]	1	0.03			
R	82	43.2			
Y*R	84	57.9			
V*R	201	125.5			
Y*V*R	121	76.7			

[a] Uses M. plot error term=(R+Y*R+V*R+Y*V*R Type I SS)/Sum(R+Y*R+V*R+Y*V)DF

[b] Should be tested from Type I SS table above.

Table 6. Estimated mean weight and length and contributions of each age and year class to to the herring biomass estimated from spawn deposition surveys in Prince William Sound, Alaska, 1994.

Year Class	Age Class	Number Sampled	Mean Weight (g)	Mean Standard Length (mm)	Biomass by Age Class			
					Weight (tonnes)	Percent by Weight	Number of Fish (x 1,000)	Percent by Number
1993	1	0			0.0	0.0	0.0	0.0
1992	2	7	34	147	3.5	0.0	105.3	0.1
1991	3	99	70	182	84.7	0.5	1,207.6	1.0
1990	4	537	88	194	1,234.4	8.0	14,062.4	11.5
1989	5	84	110	209	351.1	2.3	3,181.9	2.6
1988	6	1573	125	215	9,837.4	63.5	78,437.0	63.9
1987	7	32	132	219	230.6	1.5	1,742.3	1.4
1986	8	63	155	231	542.9	3.5	3,502.7	2.9
1985	9	237	153	231	1,898.5	12.3	12,420.6	10.1
1984	10	173	160	232	1,257.0	8.1	7,876.6	6.4
1983	11	3	155	230	12.5	0.1	80.8	0.1
1982	12	4	186	244	32.6	0.2	174.8	0.1
1981	13+	0			0.0	0.0	0.0	0.0
Total		2,812	126	215	15,485.2	100.0	122,791.8	100.0

APPENDIX A.: DIVER CALIBRATIONS, 1994 SPAWN DEPOSITION SURVEY

DIVER CALIBRATIONS: 1994 SPAWN DEPOSITION SURVEY

by

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Preliminary Data manipulations

Minor manipulations of the original calibration data were performed prior to analysis. These are outlined in detail in Appendix A. More significant data manipulations comprised selection of observations for which the reliability code was 2 or less, deletion of all data prior to 1990, and all data not pertaining to the 1994 spawn deposition divers (EB,BH,BB,MM,KB). The remaining dataset constituted a more completely crossed set than that containing all years and all divers, enabling diver and year comparisons to be made more easily (fewer missing cells, which can prevent calculation of certain least-square means). While the above would lead to some loss of precision in the event that there are no differences between 1994 divers and others, the unlikeliness of this scenario in the first place, and the simplifications gained in the analysis are believed to justify the data selection. Table 1 illustrates the distribution of data points for all divers, vegetation types and years, and describes the data manipulation more fully.

Scatter plots of diver estimates vs lab counts for which the reliability code was 2 or less were made for each combination of year (1990 through 1994),vegetation (eelgrass, hair kelp, fucus and LBK) and diver (1994 divers:EB, BH,BB,MM,KB,JW). They are presented in Appendix H.

Analysis

Error structure and general model form

The analytical methods used to answer questions relating to, for example, whether a diver over or under-estimates to the same degree on fucus as on eelgrass, must account for the way diver estimates become more variable with increasing size of the estimated population (see Appendix H, Figure H8, diver MM for a good example). One way to deal with this is to assume that the estimates are realizations of lognormal random variables, which are inherently positive and for which the variance is related to the mean. Specifically, if Y is lognormal(a,b), such that the probability density is given by :

$$f(y) = \frac{1}{y\sqrt{2\pi}b} e^{\frac{-(Log_e(y)-a)^2}{2b^2}}, \qquad (1)$$

then E(Y) is $e^{(a+\frac{b^2}{2})}$ and the V(Y) is $e^{2a+2b^2}-e^{2a+b^2}$.

		DIVER																
		TM	FF	EB	DN	DJ	HG	SM	BH	BB	KB	MM	JW	SMT	SMG	SW	DCCC	DNEC
YR	VEG																	
88	EEL	1	1	6	6	.	.	.	.	.	.	.	.	.	.	.	.	.
	HAIR	.		7	5	.	.	.	.	.	.	.	.	.	.	.	.	.
	FUCUS	.	2	4	3	.	.	.	.	.	.	.	.	.	.	.	.	.
	LBK	1	.	7	8	.	.	.	.	.	.	.	.	.	.	.	.	.
89	EEL	.	.	19	24	3	.	.	.	.	.	.	.	.	.	.	.	.
	HAIR	.	.	19	22	6	.	.	.	.	.	.	.	.	.	.	.	.
	FUCUS	.	.	7	6	.	.	.	.	.	.	.	.	.	.	.	.	.
	LBK	.	1	25	22	3	.	.	.	.	.	.	.	.	.	.	.	.
90	EEL	.		20	28	.	7	.	18	21	13	3	.	19	16	18	16	2
	HAIR	.	.	21	18	.	7	.	18	5	18	25	.	19	54	54	20	25
	FUCUS	.	.	17	14	.	8	.	18	4	59	10	.	21	12	11	58	11
	LBK	.	.	33	28	.	16	.	25	9	38	27	.	18	80	81	39	26
91	EEL	.	.	8	6	.	2	.	21	14	15	5	.	6	7	7	17	.
	HAIR	.	.	5	5	.	3	.	36	28	28	30	.	5	6	28	14	.
	FUCUS	.	.	6	1	.	4	.	12	11	12	24	.	17	9	19	18	.
	LBK	.	.	7	7	.	5	.	25	17	19	30	.	3	8	28	20	.
92	EEL	.	.	33	.	.	.	.	29	27	32	21	23	.	.	.	.	.
	HAIR	.	.	25	.	.	.	.	32	30	28	31	28	.	.	.	.	.
	FUCUS	.	.	21	.	.	.	.	20	30	16	26	10	.	.	.	.	.
	LBK	.	.	22	.	.	.	.	25	26	21	23	14	.	.	.	.	.
94	EEL	.	.	17	.	.	.	.	5	5	14	6	.	.	.	.	.	.
	HAIR	.	.	7	.	.	.	1	4	4	9	8	.	.	.	.	.	.
	FUCUS	.	.	9	.	.	.	.	10	9	8	8	6	.	.	.	.	.
	LBK	.	.	12	.	.	.	2	11	12	13	14	8	.	.	.	.	.

Table 1 Distribution of calibration data. Entries are numbers of observations in each category. Shaded areas depict data used.

With a model of the form (a main effects model is presented for the sake of simplicity):

$$Y_{ijkl} = e^{\mu + Yr_i + D_j + V_k + \beta \log(X_{ijkl}) + \epsilon_{ijkl}} \qquad (2)$$

(or equivalently, $Y_{ijkl} = \beta'_{ijk} \; X_{ijkl}^{\beta} \; \epsilon'_{ijkl}$) (3))

where

Yr_i	=	effect of ith year
D_j	=	effect of jth diver
V_k	=	effect of kth vegetation type
β	=	regression coefficient of $Log(Y_{ijkl})$ on $Log(X_{ijkl})$, and the
ϵ_{ijkl}	~	$N(0, I\sigma^2)$

the Y_{ijkl} are lognormal$(\mu + Yr_i + D_j + V_k + \beta Log(X_{ijkl}), \sigma)$, and account is taken of the observed relationship between mean and variance and of the fact that diver estimates are innately positive.

Taking $\log_e$ of both sides of Eq.2 we have

$$Log(Y_{ijkl}) = \mu + Y_i + D_j + V_k + \beta \log(X_{ijkl}) + \epsilon_{ijkl} \qquad (4)$$

and the model is now one for which the expected value of a normal random variable ($\text{Log}(Y_{ijkl})$), with constant variance independent of the mean, is a linear function of the parameters. It lends itself to a classical analysis of variance, in which normal theory tests are valid.

It is noted that the dependent variable in Equation 4 is taken to be Log(Diver Estimate), as opposed to Log(Lab Count), which has been used in the past. Inherent in this action is the assumption that laboratory counts are made without variation (and by implication, that the abilities of different technicians to count eggs is equal: see Appendix B for appropriate test), while diver estimates are permitted to suffer from measurement error. In other words, the model allows for the expectation that a diver would estimate differently in a reexamination of a quadrat, but does not allow a lab counter to recount a sample differently. Bernard (1984), however, found the variability of diver observations from one quadrat to another within a vegetation type to be lower than that of the corresponding lab counts. Such an argument may have contributed to previous decisions to use Log(Diver Estimate) as the dependent variable. An alternative explanation given by Bernard (1984) was that a diver may harbour some unwarranted knowledge of the mean egg density within a transect, and that as the diver moves from quadrat to quadrat, the estimate is unwittingly adjusted towards that mean. This would

lead to under-representation of the sampling variation component in the overall variability of diver estimates, with the result that the comparison of the overall variabilities of lab counts and diver estimates is made unfairly. In such a situation, it is foreseeable that the variability of a repeated diver estimate within a quadrat is underestimated. With this explanation the variability of a diver estimate within the same quadrat could be significant while that of the corresponding lab count could be negligible. In addition, the development of the laboratory counting technique was predicated on the determination of an unbiased and precise measure of the number of eggs in a sample. Significant variability would severely undermine the purpose of the calibration exercise, and it is difficult to envision the calibration process functioning if the lab counts were more variable than the diver estimates. An independent test to compare the two sources of variability is presented in Appendix C, using data associated with the current study. The variability in laboratory counts is significantly smaller than that of a diver estimate (of the magnitude of 2%). Does this suggest that the 'unwarranted knowledge' hypothesis of Bernard (1984) is true? To examine this more closely, overall variabilities (*i.e.* including sampling and repeated measurement variabilities) of diver estimates and lab counts should be compared for this study. This was not conducted.

Specific Model Components

The largest model fitted was one which allowed for all interactions between year, vegetation and diver:

$$Log(Y_{ijkl}) = \mu + Yr_i + D_j + V_k + YrV_{ik} + YrD_{ij} + DV_{jk} + YrDV_{ijk} + \beta \log(X_{ijkl}) + \epsilon_{ijkl} \qquad (5)$$

The slope of the regression of $\text{Log}(Y_{ijkl})$ on $\text{Log}(X_{ijkl})$, β, is not permitted to change with year, vegetation or diver. The intercept is allowed to vary with the different factors, however. On the original scale (Equation 3), β'_{ijk} is allowed to vary, while only one value for β is fitted.

Associated with each diver estimate is a year, a vegetation and a diver variable. To change values of year or vegetation, one has to look at a different quadrat. The diver variable, on the other hand, can change within a quadrat, as different divers report different interpretations of the density of the same group of eggs. The diver estimates within a quadrat are therefore repeated measures on that quadrat. The significance of this arrangement is that two error terms are required to test the different components of the model. One is needed to test those components involving the year and vegetation factors (main plots) while the other is required to test those involving the diver factor (sub plot). There are a number of ways of dealing with this type of structure, and the method used here

is that of a split-plot analysis. The data were analyzed as though originating from a split-plot arrangement of 'treatments' in a completely randomized experimental design. In order to do this, however, a replicate variable had to be assigned to each observation in the dataset so that within a year/vegetation combination, each quadrat was associated with a unique replicate number, while counts within a quadrat received the same replicate value. The assignment of a replicate variable is described in Appendix A.

The correct error term for tests of main plot factors (year/vegetation components) derives from a 'replicate-within-treatment' entry in the ANOVA table. Here 'treatments' consist of all the year-vegetation combinations, so that for four years and four vegetation types, for example, there would be 16 such treatments. To derive the replicate-within treatment component in SAS, when the treatments are formed from a crossed arrangement of year and vegetation, the components (sums of squares) representing replicates, replicates*vegetation, replicates*year and replicates*vegetation *year entries are summed. For example, in the main plot section of the ANOVA table, for 3 years, 4 vegetation types, 10 replicates:

Source	df		Source	df
Yr	2	}		
V	3	}---------->	Treatments	11
Yr*V	6	}	Rep(Treatment)	108
Rep	9	]		
Rep*Yr	18	]----108-- -->	Represents the replicate-within-treatment component,	
Rep*V	27	]	and is the appropriate error term for main plot factors	
Rep*Yr*V	54	]		

To isolate the replication-within-treatment main-plot error term, the model depicted in Equation 5 was fitted together with the components involving replicates listed in the left hand ANOVA table above. These components were fitted last in the model, and the last four Type I sums of squares (sequential sums of squares) given by SAS were summed and divided by the sum of the associated degrees of freedom to yield an estimate of the appropriate main-plot error. The full ANOVA table consisted of the entries:

Source
Yr
V
Yr*V
D
D*Yr
D*V
D*Yr*V
Log(Lab Count)
Rep

Rep*Yr
Rep*V
Rep*Yr*V

The components Yr, V and the Yr*V interaction were then tested against the main-plot error term just described. The remaining entries, except for the covariate Log(Lab Count), were tested using Type III partial sums of squares and the model error given by SAS. Although the Log(Lab Count) regression component is almost surely to be significant, a test on this should be made with the Type III sum of squares and the error term derived from an analysis in which components involving the replicate factor are omitted.

Once significance of the various model interactions had been determined, plots of least square means were made to allow a clearer visualization of the effects. Least square means are estimates adjusted for any lack of balance in the distribution of numbers of observations over the different factors involved (year, vegetation, diver), and also for the covariate, Log(Lab Count). An examination of the plots, together with knowledge of the statistical significance of the interactions involved, and with input from biologists, enabled decisions to be made regarding the validity of pooling different years, vegetation types or divers. The greater the degree to which the data can be pooled, the more precise are the estimates of the different parameters deemed necessary in the calibration, and of course, the simpler the final adjustment model. Once it had been decided which factors could be legitimately pooled, appropriate data manipulations were made in SAS and parameter estimates obtained from PROC GLM.

Prediction

The final fitted model described the variation in Log(Diver Estimate) as a function of some combination of year,vegetation type, diver and Log(Lab Count). The calibration problem at hand is an inverse prediction problem, in that predictions of the regressor variable Log(Lab Count) will be made given new diver estimates. Given a new diver estimate, ***Yo,*** and the fitted model, described in Equation 5, a point estimate of the regressor variable, $Log(X_{ijk})_{Yo}$, is:

$$\hat{Log}(X_{ijk})_{Yo} = \frac{Log(Yo) - (\hat{\mu} + \hat{Yr}_i + \hat{D}_j + \hat{V}_k + \hat{YrV}_{ik} + \hat{YrD}_{ij} + \hat{DV}_{jk} + \hat{YrVD}_{ijk})}{\hat{\beta}} \quad (6)$$

Transformation to original scale.

In previous calibrations, when Log(Lab Count) was taken as the dependent variable, predictions were straightforward, and corrections for the bias introduced by exponentiating

the predicted values $\hat{Log}(Lab\ Count)$ were made (although there is some question about the exact nature of the bias correction used previously-see Appendix D).

In the current work, Log(Diver Estimate) represents the dependent variable, and exponentiation of $\hat{Log}(X_{ijk})_{Yo}$, from fitting the model described in Equation 5, provides an estimate on the original scale, $(\hat{X}_{ijk})_{Yo}$, with units of number of eggs:

$$(\hat{X}_{ijk})_{Yo} = e^{\hat{Log}(X_{ijk})_{Yo}} \tag{7}$$

The random variable $\hat{Log}(X_{ijk})_{Yo}$ is a ratio of two normal random variates. While the ratio of two standard normal variates is distributed as a Cauchy random variable, this helps little in the determination of the distribution of $e^{\hat{Log}(X_{ijk})_{Yo}}$, which is likely complex. Without this knowledge, the bias in the back-transformation is not knowable. No bias correction was therefore made in the current study. If one was to derive a reason to use Log(Lab Count) as a dependent variable, as practiced in the past, the ability to adjust bias may be it. A simulation could be performed to assess this problem. None has been carried out yet, however.

While an expression for the variance of an inversely predicted quantity is available for the single-predictor case (Clupea Neter, Wasserman and Kutner, 1990:p 175; Draper and Smith, 1981:p 49), no derivations for the multiple predictor case could be found after a fairly extensive search. Attempts to derive a suitable expression from first principles failed. The variance of $(\hat{X}_{ijk})_{Yo}$ was ultimately determined by the bootstrap technique. Details are presented in Appendix G.

Results

The split-plot analysis, used to account for the repeated measures nature of the diver estimates, was a very computer intensive exercise, such that there were limitations on the size of the data set that could be handled. The consequence was that after inclusion of the calibration data from the egg mortality study (see Appendix A), only the 1991 through 1994 data could be used in a single analysis to assess the three and two-way interactions. No evidence of a three-way interaction between year, vegetation and diver was found ($p=0.33$, using split-plot error term). Neither was there evidence of a vegetation by diver or of a year by vegetation interaction ($p=0.45$, split-plot error term and $p=0.36$, main-plot error term, respectively). A significant year by diver interaction was found, however ($p=0.0013$, using split-plot error term) and a significant main vegetation effect ($p=0.0039$, using main plot error term). The analysis of variance table is presented in Appendix E. A residual plot is depicted in Appendix J.

Since there were many degrees of freedom associated with each interaction, and because only a partial data set could be examined in any one analysis, a more visual assessment of the interactions was considered prudent. This was achieved using plots of least-square means obtained from an analysis in which the repeated measures nature of the diver estimates is ignored. While such an analysis yielded inappropriate error terms for the testing of hypotheses, visual comparisons of the least-square means obtained should be meaningful. Such least square means are not only corrected for the other classification variables present in the model, but also for the regression variable, Log(Lab Count). Thus, unlike the case for raw means, where a comparison of two divers, for example, could not only contain other classification effects, but could be made at different values of Log(Lab Count), a comparison of least square means would contain no other effects, and would be made at the same value of Log(Lab Count). In this way, a more meaningful comparison is obtained. Plots of the two-way interactions are presented in Figures I1 through I3 (Appendix I).

The significant interaction between year and diver detected in the analysis is evident in Figure I1. During 1990 and 1991, diver KB tended to estimate substantially lower than he did in 1992 and 1994, when he estimated similarly to the 1990 through 1994 efforts of EB,BH, and BB , which were among themselves similar. An almost identical situation appears to exist for diver MM, except that he estimated higher in 1990 and 1991. These patterns were consistent over the vegetation types (lack of a three-way interaction). Divers EB,BH,BB from 1990 through 1994 and divers KB and MM from 1992 through 1994 thus appeared to estimate similarly, and it was considered valid to pool these divers over these years.

A plot of the vegetation by diver least square means (Figure I2) displays little evidence of interaction, and the general form of the curves are horizontal, suggesting, as did Figure I1 that there is little difference between divers. There does appear, however, to be a vertical separation of the vegetation curves, reflecting the significant ($p=0.0039$) main vegetation effect reported above.

While the main analysis found no interaction of year and vegetation, the plot described in Figure I3 shows an outlying point for fucus in 1991, and that LBK in 1994 was somewhat lower than the remaining vegetation types. It is possible for ANOVA-type assessments of multiple degree of freedom interactions to miss this type of observation due to the diluting effect of other insignificant interaction contrasts. From input from divers, and from the observation from Figure I2 that fucus appeared to differ from other vegetation types, it was apparent that fucus should be isolated in the analysis, but that the data should only be pooled over 1990, 1992 and 1994. Likewise, LBK was isolated in the analysis so that predictions made for LBK were only based upon 1994 data.

The analysis is presented in Appendix F. The resulting adjustments for the 1994 spawn deposition data are thus:

All divers (EB,BB,BH,KB,MM)
Eelgrass or Hairkelp

$$Adjusted\ egg\ count = e^{\frac{(Log(DiverEstimate) + 0.0795)}{0.943}} \quad (8)$$

All divers (EB,BB,BH,KB,MM)
Fucus

$$Adjusted\ egg\ count = e^{\frac{(Log(DiverEstimate) + 0.352)}{0.943}} \quad (9)$$

All divers (EB,BB,BH,KB,MM)
LBK

$$Adjusted\ egg\ count = e^{\frac{(Log(DiverEstimate) + 0.384)}{0.943}} \quad (10)$$

Issues for consideration for future surveys and analyses and other ad hoc comments

1) As far as sample size (number of transects and number of quadrats per transect) is concerned, it is difficult to say much about it when the egg estimate is but one component of the biomass estimate. The impetus to increase sample size for the spawn deposition survey will depend upon the variability of the fecundity estimate. I have only dealt with diver adjustments, and in a fairly insular manner at that, so that I haven't compared the variabilities of the spawn and fecundity estimates, and how they affect the final biomass estimate. Neither have I taken a look at the transect to transect versus quadrat to quadrat variability , which would be required to assess the efficiency of the partitioning of resources within the spawn deposition survey.

2) The analysis methods described above attempted to take into account the repeated measures nature of the diver estimates by using a split-plot analogy. No tests of the (rather) complex assumptions required for the validity of the split-plot analysis were conducted (sphericity of variance-covariance matrix). In addition, in the diver calibration analysis, no account was taken of the transect structure of the data, and it could be argued that vegetation comparisons are likely to be made more precisely when conducted within a transect, in the same way diver comparisons are likely to be made more precisely when conducted within a quadrat.

3) A fair amount of effort was required to determine which diver estimates were associated with which quadrat (see gyrations within Appendix A). This was ultimately determined by bag number, when it was noticed that the recording of station number was terminated in 1994. If, in the future, it was determined that the split plot analysis is the appropriate analysis, it would make life easier if the diver estimates made within a quadrat were easily recognized.

4) Collection of diver estimates made repeatedly on the same quadrat would allow an independent check upon the estimated split-plot variance obtained from the analysis of variance table.

5) Using the diver estimate as the dependent variable meant that the bootstrap technique had to be used in order to determine the variance of the inversely predicted lab count. It also meant that corrections for bias introduced by exponentiation of the predicted logarithm of the diver estimate were not made. It is conceded that the above are drawbacks to the inverse prediction method. On the other hand, the bias corrections outlined in Appendix D, for the case where Log(Lab Count) is used as the dependent variable are not free from bias themselves. This, in conjunction with the more statistically defensible action of using Log(Diver Estimate) as the dependent variable leads me to consider the inverse prediction method preferable.

6) Some concern has previously been expressed over the problem of prediction of lab counts from diver estimates which lie beyond the range of the calibration data, *i.e.* the problem of extrapolation. With the exception of one observation for fucus, the 1994 spawn data fall within the bounds of the calibration data, and extrapolation does not appear to be a problem (Figure 1).

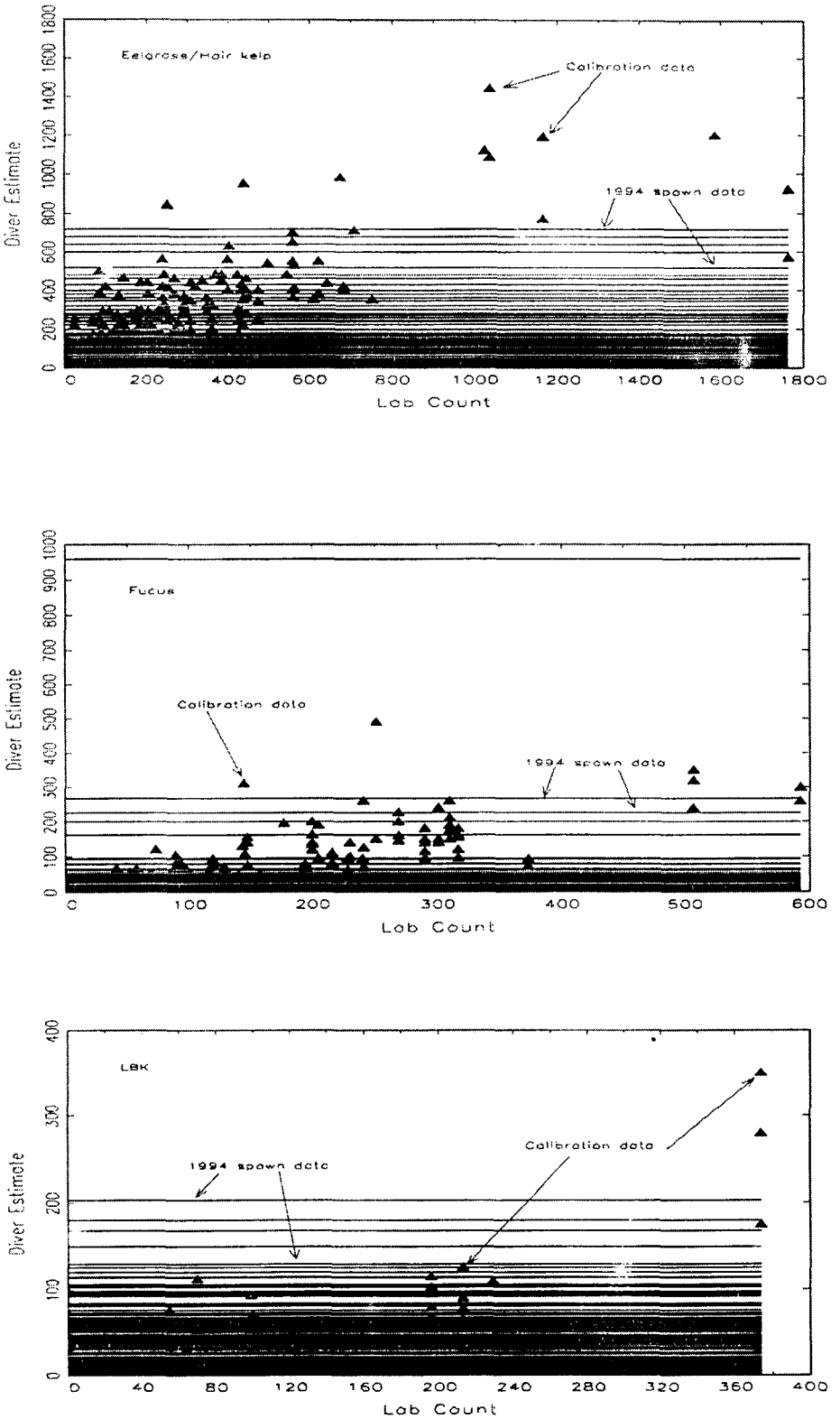

Figure 1 Range of 1994 spawn deposition data vs that of calibration data

References

Beauchamp,J. and J.Olsen. 1973. Corrections for bias in regression estimates after logarithmic transformation. Ecology 54: 1403-1407.

Bernard, D. 1984. Variance of herring biomass and seeing-eye divers:w/corrections. Intradepartmental memorandum to Dennis Blankenbeckler.

Biggs, E. and Baker, T. 1993. Studies on Pacific herring *Clupea pallasi* spawning in Prince William Sound following the 1989 *Exxon Valdez* oil spill. 1989-1992. Natural Resource Damage Assessment Fish/Shellfish Study, Number 11.

Goodman, L. 1963. On the exact variance of products. Journal of the American Statistical Association 55: 708-713.

Original data file: **caldata.wk4**

This contained 1984, 1988,1989,1990,1991,1992 and 1994 calibration data.

The file **calan.wk4** was formed from **caldata.wk4**. The following was performed in the transition:

1) In 'station' variable, 'Quad' was changed to '6000'
'APE' was changed to '7000'
"Pred' was changed to '8000'

2) A 'Tot Eggs' column was formed which was the sum of 'Estimate' and 'Eggsleft'

3) 1984 data were deleted.

4) Some lines were deleted due to lack of lab count entry (e.g., 88,50,3,lbk,4,40,0,1,.....; 89,147,4,fu,3,2,0.5,5,..;92,46,999,des,2,260,0,8,..; 94,31,quad,hr,2,900,0,9,....;)

5) Bagnumber character entries, were assumed meaningful and were changed to numeric entries (eg SB->-1 at 94,3,APE,rh/eg,2,435,0,513;)

6) Bagnumber variable assigned to instances where none exists (e.g., 94,10,pred,eel,1,45,0,^,eb;)

New egg mortality data became available from '90 and '91. After assigning bag numbers by grouping identical lab counts (consistently occurred in twos), transect numbers (using negative values to avoid using similar values to those which exist for '90 and '91 spawn deposition data) and appropriate blank columns, the egg mortality data were concatenated to **calan.wk4.**

A text file, **calan.prn** was produced from **calan.wk4** and imported into SAS, where the data were checked for certain irregularities, specifically the existence of more than one estimate and lab count within a date/location/transect/veg-type/bagnumber/diver combination:

```
options ps=60;data one; infile "c:\herring\divcal\calan.prn" missover ;
 input yr tran stat 24-26 vcode est bn div$ recnt 71 labc larv 89 rel x ;
 if recnt=1  then delete; run; proc sort; by yr vcode tran bn div est;run;
 proc freq data=one; by yr vcode tran bn div; tables labc  /noprint out=two; run;
 data three; set two; if percent<100; run; proc print data=three; run;
```

Some duplicate records were found (e.g., 92,76,999,2,93,KB, 200, 203; 92,998,18,4,18

MM, 65, 178), some records were missing a recount entry (e.g., 91,34,3,fu,3,47,80,85,HG, ^,273; 91,55,904,fu,3,18,0,82,SM,^,67), and some records indicated that the same diver gave more than one estimate for a quadrat (e.g.,four records starting with 91,910,902,hrk,2, 75,0,21,KB).

To correctly assign a replicate variable (see below), it was necessary to amend bag numbers in instances where, within a transect, a station number changed while a bag number did not, *i.e.*. those instances where a bag number is reused within a transect. The file **temp.asc** was first formed in SAS:

```
options ps=60; data one; infile "c:\herring\divcal\calan.prn" missover ;
input yr tran stat 24-26 vcode est bn div$ recnt 71 labc larv 89 rel x ; if recnt=1  then
delete;run;proc sort out=two; by yr tran bn stat;run;
data three;set two;keep yr tran bn stat; file "c:\herring\divcal\temp.asc"; put yr tran bn stat;
run;
```

A GAUSS program was then used to detect where a bag number had been reused within a transect:

```
new;load x[2937,4]=c:\herring\divcal\temp.asc ;@yr tr bn st @
x=sortmc(x,1|2|4|3);x=missrv(x,-1.5);e=x./=-1.5;x=selif(x,e);i=1;y=0;
do while i<rows(x);if sumc((x[i+1,1:2].==x[i,1:2])')==2 and ((x[i+1,4]-x[i,4])/=0 and
(x[i+1,3]-x[i,3])==0);i;wait;y=y+1;endif;i=i+1endo;end;
```

In the few instances where this occurred, a value of 0.5 was added to the bagnumber (e.g., five observations beginning with 92,13,ag,4,65,0,29,eb,...).

The diver code SM was recoded according to:

'94	:	no change to SM
'90	:	change to SMT for spawn deposition data change to SMG for egg mortality data
'91	:	change to SMT for spawn deposition data change to SMG for egg mortality data

In order to account for the repeated measures nature of the diver counts, the data were analyzed in the context of a split-plot arrangement of 'treatments' in a completely randomized experimental design. In order to do this, a replicate variable was needed, which identified those quadrats which represented a certain year/vegetation combination. The only reliable way of determining if a series of counts were from the same quadrat (by different divers) was by determining whether the count arised on the same date and location, was from the same transect number, was of the same vegetation type and was associated with the same bag number. **Calan.prn** was first manipulated with a SAS program which deleted observations for which the recnt variable was 1.0 (recounts were made to ascertain reproducibility of laboratory egg counts between counters), recoded divers to a numeric value, and formed

calan.out:

```
options ps=60;data one;infile "c:\herring\divcal\calan.prn" missover;
input yr tran stat 24-26 v est bn  div$ recnt 71 labc larv 89 rel x ;
if recnt=1 then delete;if div="TM" then div=1;if div="FF" then div=2;if div="EB" then
div=3;
if div="DN" then div=4;if div="DJ" then div=5;if div="HG" then div=6;if div="SM"
then div=7;if div="BH" then div=8;if div="BB" then div=9;
if div="KB" then div=10;if div="MM" then div=11;if div="JW" then div=12;
if div="SMT" then div=13;if div="SMG" then div=14;if div="SW" then div=15;
if div="DC" then div=16;if div="DNE" then div=17;
file "c:\herring\divcal\calan.out";put yr v tran  bn div rel est labc;run;
```

A GAUSS dataset **calan.dat** was formed from **calan.out** using the ATOG utility in GAUSS, and a command file:

```
input c:\herring\divcal\calan.out;output calan;invar yr v tran bn div rel est labc ;
```

A GAUSS program then operated upon **calan.dat** to add the replicate variable, and produced **calan.asc**:

```
new;open s=calan.dat; @ yr=1 v=2 tr=3 bn=4 div=5 rel=6 est=7 labc=8 @
x=readr(s,rowsf(s)); y=seekr(s,1);
x=sortmc(x,1|2|3|4|5);i=2;p=1;r=zeros(rows(x),1);r[1]=1;do while i<=rows(x);
 if x[i,1:4]==x[i-1,1:4]; r[i-1:i]=p|p;elseif x[i,1:2]==x[i-1,1:2];p=p+1; r[i]=p;else;
p=1;r[i]=p;endif;
i=i+1;endo;x=x~r;screen off;outwidth 256;output file=calan.asc reset;x;end;
```

Calan.asc then became the functional data set upon which the statistical analysis was effected.

Appendix B: Tests of Ho:No difference between lab counters' abilities

For 1991 and 1992 recounts of sample bags were performed, whereby different laboratory technicians counted the same calibration sample. This provided a means to test the assumption that there are no differences between technicians with respect to their ability to determine numbers of eggs.

A file **lc1.txt** was formed from the original **caldata.wk4** file (containing all calibration data). Only data associated with 1991 and 1992 were transferred, and only those recounts involving two technicians (due to ease of subsequent data manipulations). There were only 3 to 4 instances where there were three or more technicians per sample. Also, only complete records were used (eg instances where some or all of the bag numbers were missing were removed). The SAS code used for the data management and the analysis is shown below. The results are presented in Table B1.

Technician Pair	ABS(Mean Difference (K))	Number of reps	*P* value for Ho:No difference
JG/DA	4.3	17	0.24
JG/SB	19.3	3	0.29
JG/EB	6.0	3	0.33
EB/DA	44.7	4	0.14
DB/KC	4.0	3	0.40
JG/KC	21.0	2	0.78

Table B1. Test of Ho:No difference in lab counters' egg-counting abilities.

It should be noted that five of the six instances above rely on small samples sizes, with the consequence that the tests are likely not very powerful. From the data available, there is no evidence that laboratory technicians' counting abilities are different. Whether the technicians are biased in their counts is another question. It cannot be answered with current data since there is no measure of absolute egg number with which to compare the technicians' estimates.

The following SAS code manipulates the data from **lc..txt** to yield tests of the equality of the

egg-counting abilities of various pairs of laboratory technicians.

```
options ps=60;data one;infile 'c:\herring\divcal\lc1.txt';
input year tran stat veg$ vcode est eggl bag div$ rec labc larv rel id$;
if id="JG" or id="jg" then id=1;if id="DA" or id="da" then id=2;if id="SB" or
id="sb" then id=3;if id="TM" or id="tm" then id=4;if id="EB" or id="eb" then
id=5;if id="ALL" or id="all" then id=6;if id="KC" or id="kc" then id=7;if id="DB"
or id="db" then id=8;if id="CR" or id="cr" then id=9;if id="MM" or id="mm" then
id=10;if id="JA" or id="ja" then id=11;if id="KB" or id="kb" then id=12;if id="BH"
or id="bh" then id=13;if id="JW" or id="jw" then id=14;
drop veg vcode est eggl larv rel;run;proc sort data=one out=two;;by year tran stat bag div
rec id;run;title 'data two';proc print data=two;run;data fix;set two;ob=_n_;if ob=1 then
delete;crec=rec;keep crec;run;data new;merge fix two;if crec=. then delete;if rec=0 and
crec=0 then delete;id1=year*100000+tran*1000+bag;lag2id1=lag2(id1);if id1=lag2id1
then delete;drop id1 lag2id1 crec;ob=_n_;run;title 'data new';proc print data=new;run;data
odd;set new;mod=mod(ob,2);
if mod ne 0;drop mod;rename labc=labcodd;rename id=idodd;run;data even;set new;
mod=mod(ob,2);if mod eq 0;drop mod;rename labc=labceven;rename id=ideven;run; data
diff;merge odd even;diff=labcodd-labceven;trt=idodd*100+ideven;if trt=102 or trt=201
then code=1;if trt=201 then diff=-diff;if trt=103 or trt=301 then code=2;if trt=301 then
diff=-diff;if trt=105 or trt=501 then code=3;if trt=501 then diff=-diff;if trt=305 or
trt=503 then code=4;if trt=503 then diff=-diff;if trt=205 or trt=502 then code=5;if
trt=502 then diff=-diff;if trt=708 or trt=807 then code=6;if trt=807 then diff=-diff;if
trt=107 or trt=701 then code=7;if trt=701 then diff=-diff;if trt=110 or trt=1001 then
code=8;if trt=1001 then diff=-diff;if trt=112 or trt=1201 then code=9;if trt=1201 then
diff=-diff;if trt=111 or trt=1101 then code=10;if trt=1101 then diff=-diff;if trt=109 or
trt=901 then code=11;if trt=901 then
diff=-diff;if trt=711 or trt=1107 then code=12;if trt=1107 then diff=-diff;run;title 'data
diff';proc print;run;proc sort;by code;run;proc means mean t prt;var diff;by code;run;
```

Appendix C: A test of equality of the variance of a diver estimate vs that of a lab egg count

Some analyses have shown that laboratory counts are a more variable quantity than diver estimates (Bernard, 1984). This has lent some justification to the practice of using the former as the dependent variable, and the latter as the independent variable in calibration regressions, in spite of the fact that a calibration based upon variable 'actual' counts has to be considered compromised to some degree.

An estimate of the variability of a diver estimate (within a quadrat) from an analysis of data from 1988 through 1994 was compared to an estimate of variability of a lab counter, derived from 1994 data. The former estimate (with 607 degrees of freedom) was taken from the Error line in an ANOVA table produced by PROC GLM (SAS). The model fitted had Log(Diver Estimate) as the dependent variable, and was one of full interaction together with isolation of the main-plot error term, so that the residual error reflects the variability of a diver estimate within a quadrat. The estimate of the variability of the laboratory count was derived from a considerably smaller study, and one involving only one technician, who scored twelve different bags (each bag representing the eggs collected by a diver from a quadrat) each five times in a blind study. Using bag number as a class variable, a pooled variance estimate with 48 degrees of freedom of Log(lab count) was obtained from the error line in an analysis of variance table produced from PROC ANOVA (SAS). An $F_{607/48}$ statistic was calculated:

$$F_{607/48} = \frac{0.132}{0.0027} = 48.9 \; : \; p < 0.0001$$

The variation of a laboratory count is significantly smaller than that of a diver estimate and is in the region of 2% of the latter. One note of caution is that in contrast to the estimate of variability of a diver estimate, the laboratory estimate is based on a relatively small study, with only one technician.

Appendix D: Some questions about previous bias corrections for predicted lab egg counts

Introduction

Attempts to replicate some of the expressions used for bias correction in previous studies (e.g., pages 18,19 from Biggs and Baker (1993)) failed. They are described here for lack of a more suitable place to put them. The results have no relevance to the current analysis because the order of dependent and regressor variables is reversed. Their relevance, if in fact they are correct, lies in the accuracy of previous predictions, and future calibrations where, for whatever reason, the dependent variable is taken as laboratory egg count.

The model

Consider the model:

$$Y_{ij} = e^{\mu + a_i + \beta Log(X_{ij}) + \epsilon_{ij}} \qquad \text{(D1)}$$

where

Y_{ij}	=	jth laboratory egg count for ith level of factor a,
X_{ij}	=	jth diver estimate for ith level of factor a,
ϵ_{ij}	=	jth error for ith level of factor a $\sim$Normal$(0,\sigma^2)$, and
a_i	=	contribution from ith level of factor a (two levels)

The choice of model was determined by considerations of a) error structure, b) the inherently positive nature of egg counts and c) practicalities of analysis. A distinct observable relationship between the mean and variance of the laboratory egg counts exists. Implied by the assumption that the ϵ_{ij} are normally distributed, is the fact that the Y_{ij} are lognormally distributed. Since the variance of a lognormal random variable is dependent upon its mean, in a manner similar to that which is observed in the data, the choice of the normal errors seems appropriate. The error structure chosen also implies that the laboratory egg counts must be positive and the linear nature of the parameters in the exponent of the model permits general linear model theory to be invoked once logarithms of both sides of the equation have been taken.

Fitting the model:

$$\hat{Log}(Y)_{X_i o} = \hat{\mu} + \hat{a}_i + \hat{\beta} Log(X_i o) \qquad \text{(D2)}$$

where

$X_i o$ is a new diver observation for level i of factor a and
$\hat{Log}(Y)_{X_i o} \sim N(\mu + a_i + \beta Log(X_i o), \sigma'^2)$.

The distribution of $\hat{Log}(Y)_{X_i o}$ derives from the fact that if the ϵ_{ij} in Equation D1 are $N(0,\sigma^2)$, then the least squares estimators of the parameters in Equation D2 are normally distributed and are also uniformly minimum variance unbiased estimators. The variance σ'^2 is defined:

$$\sigma'^2 = V(\hat{Log}(Y)_{X_i o}) = \sigma^2 \mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i o} \quad \text{(D3)}$$

where $\mathbf{X}'_{X_i o} = [1 \; A_i \; Log(X_i o)]$ and $\mathbf{X}$ = (ixj) x 3 design matrix.

Predicted egg count

The predicted laboratory egg count for a new diver estimate, $X_i o$ is given by:

$$\hat{Y}_{X_i o} = e^{\hat{Log}(Y)_{X_i o}} = e^{\hat{\mu} + \hat{a}_i + \hat{\beta} Log(X_i o)} \quad \text{(D4)}$$

Bias of the predicted egg count

The distribution of $\hat{Y}_{X_i o}$ is lognormal with

$$E(\hat{Y}_{X_i o}) = e^{\mu + a_i + \beta Log(X_i o) + \frac{\sigma^2}{2}\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i o}} \quad \text{(D5)}$$

and thus the bias in the predicted value given by Equation D4 is:

$$Bias = E(\hat{Y}_{X_i o}) - E(Y_{X_i o}) \quad \text{(D6)}$$

so that:

$$Bias = e^{\mu + a_i + \beta Log(X_i o)}\left[e^{\frac{\sigma^2}{2}\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i o}} - e^{\frac{\sigma^2}{2}}\right] \quad \text{(D7)}$$

Bias Correction

Multiplying $\hat{Y}_{i,Xo}$ by the expression $e^{\frac{\sigma^2}{2}\left[1-\mathbf{X}'_{X_{i0}}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i0}}\right]}$ corrects for the bias : $E(\hat{Y}_{X_{i0}})$ x $e^{\frac{\sigma^2}{2}\left[1-\mathbf{X}'_{X_{i0}}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i0}}\right]} = e^{\mu+a_i+\beta Log(X_i o)+\frac{\sigma^2}{2}} = E(Y_{X_{i0}})$.

Since σ^2 is unknown, s^2 from the error line in the regression may be substituted into the above bias correction factor. The bias-corrected predicted laboratory egg count, $\hat{Y}'_{X_{i0}}$ is then:

$$\hat{Y}'_{i,Xo} = e^{\hat{\mu}+\hat{a}_i+\beta Log(X_i o)} e^{\frac{s^2}{2}\left[1-\mathbf{X}'_{X_{i0}}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i0}}\right]} \qquad \text{(D8)}$$

Although s^2 is an unbiased estimator of σ^2, the correction term in Equation D8 is not necessarily unbiased. The correction does, however, reproduce the results of Beauchamp and Olsen (1973) to a close approximation. A simulation also suggests that the correction is a good one. Using available calibration data for a particular diver, vegetation type and year, a regression of Log(Lab Count) on Log(Diver Estimate was effected and some ball-park values for the intercept, slope and residual mean square obtained. For each of 5000 instances, a data set was generated consisting of a regular sequence of diver counts and a corresponding set of lab counts generated from lognormal distributions described by the chosen parameter values. For each data set, a regression of Log(Lab Count) upon Log(Diver Estimate) was performed, and a back-transformed (and therefore biased) prediction of Lab Count made for a specified diver estimate. The bias in the predicted value was calculated for each case and a mean value over all 5000 instances ultimately calculated. This value was then compared to the bias derived in Equation D7. With respect to the bias correction described in Equation D8, each prediction was corrected with the derived adjustment and a corrected bias calculated. The mean of the 5000 bias-corrected predictions was then calculated. The results of the simulation are depicted in Figure D1.

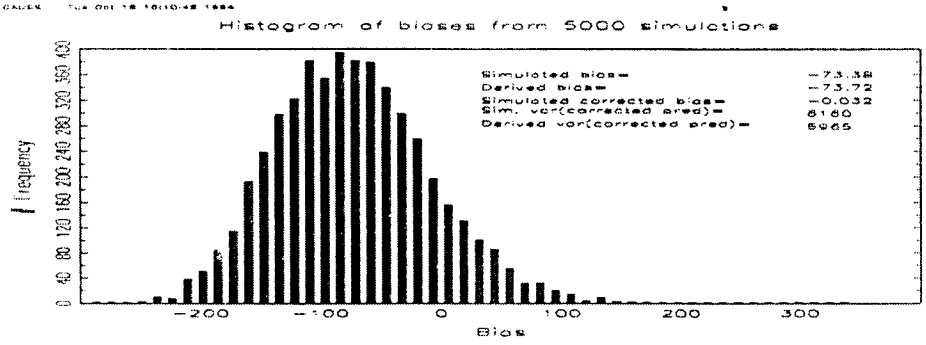

Figure D1 Simulation of bias and bias correction

The simulation was performed in GAUSS. The code is as follows:

```
new;a=1.5;b=0.77;sig=sqrt(0.27);    @ From preliminary analysis of some cal. data: @
xp=500;  @ Predict at div. est of 500@ xo=1|ln(xp);
n=20; @ reg. done with 20 obs. @
xde=seqa(50,50,n);   @ diver counts @x=ones(n,1)~ln(xde);
xpx=xo'*inv(x'x)*xo;
mu=a+b*ln(xde);  @ mean of normal :log(lab egg counts)
@sim=5000;mat=0~0~0;p=1;
do while p<=sim;i=1;rvmat=zeros(n,1);do while i<=n;
rvmat[i]=lognorm(mu[i],sig,1);  @ generate lognrmal rv's @i=i+1;endo;screen off;
{a1,b1,c1,d1,e1,f1,g1,h1,i1,j1,k1}=ols(0,ln(rvmat),ln(xde)); @ reg fitting@
prxo=exp(c1[1]+c1[2]*ln(xp));  @ predicted lab count @
expa=exp(a+b*ln(xp)+sig^2/2);      @ actual lab count according to model @
bias=prxo-expa; @ bias od predicted value (xo) @cf=exp(g1^2/2*(1-xpx));
corrpr=prxo*cf; @ bias-corrected predicted value @
cbias=corrpr-expa;  @ bias of corrected value @mat=mat|(bias~cbias~corrpr);p=p+1;
endo;screen on;mat=mat[2:rows(mat),1:3];bias=meanc(mat);
sd=stdc(mat[.,3]); @ simulated sd of corrected predicted value @
"Simulated bias = ";;bias[1];"Simulated Corrected bias = ";;bias[2];
derbs=exp(a+b*ln(xp))*(exp(sig^2/2*xpx)-exp(sig^2/2));"Derived bias +";;derbs;
"Simulated variance of corrected predicted value  = ";;sd^2;
p=exp(a+b*ln(xp));q=exp(sig^2/2*(1-xpx));r=(1-xpx)^2;s=exp(sig^2*xpx);t=exp(sig^2*
xpx)-1;
dervar=(p^2*q*r*sig^4)/(2*(n-1))+q*p^2*s*t+(q*r*sig^4)/(2*(n 1))*p^2*s*t;
"Derived variance of bias-corrected value=";;dervar;library pgraph;graphset;_pqgedit=1;
xlabel("Bias");title("Histogram of biases from 1000 simulations");hist(mat[.,1],50);end;
```

Approximate variance of bias-corrected predicted value

Required is an approximate variance of the bias-corrected predicted egg count, *i.e.*:

$$\hat{V}(Y_{X_{i}o}) = \hat{V}(e^{\hat{\mu}+\hat{a}_i+\hat{\beta}Log(X_{i}o)} e^{\frac{s^2}{2}\left[1-\mathbf{x}'_{\mathbf{x}_{i}o}(\mathbf{x}'\mathbf{x}^{-1})\mathbf{x}_{\mathbf{x}_{i}o}\right]}) \qquad \text{(D9)}$$

By virtue of the fact that s^2 and $\{\hat{\mu}, \hat{a}_i, \beta\}$ are independent,

$$\hat{V}(\hat{Y}'_{X_i o}) = \left[e^{\hat{\mu}+\hat{a}_i+\beta Log(X_i o)}\right]^2 \hat{V}(e^{\frac{s^2}{2}\left[1-\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i o}\right]}) + e^{s^2\left[1-\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i o}\right]}\hat{V}\left[e^{\hat{\mu}+\hat{a}_i+\beta Log(X_i o)}\right]$$

$$-\hat{V}(e^{\frac{s^2}{2}\left[1-\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i o}\right]})\ \hat{V}\left[e^{\hat{\mu}+\hat{a}_i+\beta Log(X_i o)}\right] \qquad \text{(D10)}$$

after Goodman (1963).

The delta method, incorporating the estimator: $\hat{V}(s^2) = \frac{2*s^4}{(n-1)}$ (by virtue of the fact that $\frac{(n-1)s^2}{\sigma^2} \sim \chi^2_{n-1}$) was used to approximate the first variance estimate on the right hand side of Equation D10 as:

$$\frac{s^2}{2}\left[1-\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i o}\right]) = e^{\frac{s^2}{2}\left[1-\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i o}\right]\frac{\left[1-\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i}\right.}{2(n-1)}} \qquad \text{(D11)}$$

The second variance referred to on the right hand side of Equation D10 is that of the lognormal random variable, $e^{\hat{\mu}+\hat{a}_i+\beta Log(X_i o)}$. Recall that the least square estimators of μ, a_i, and β obtained from fitting Equation D2 are normally distributed and unbiased (given ϵ_{ij} in Equation D1 are normally distributed), and thus the random variable : $(\hat{\mu}+\hat{a}_i+\beta Log(X_i o)) \sim N\left(\mu+a_i+\beta Log(X_i o),\ \sigma^2\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i o}\right)$. An approximate variance is then:

$$+\hat{a}+\beta Log(Xo)) = e^{2\left[\hat{\mu}+\hat{a}_i+\beta Log(Xo)\right]+s^2\left[\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i o}\right]}\left[e^{s^2\left[\mathbf{X}'_{X_i o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_i}\right.}\right. \qquad \text{(D12)}$$

All the components of Equation D10 have now been explicitly expr'ssed, and an approximate variance of the predicted lab egg count is calculable. The final expression is:

$$\cdot_{X_{i}o}) = \left[e^{\hat{\mu}+\hat{a}_i+\hat{\beta}Log(X_i o)}\right]^2 e^{\frac{s^2}{2}\left[1-\mathbf{X}'_{X_{i}o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i}o}\right]}\frac{\left[1-\mathbf{X}'_{X_{i}o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i}o}\right]^2 s}{2(n-1)} \qquad \text{(D13)}$$

$$e^{s^2\left[1-\mathbf{X}'_{X_{i}o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i}o}\right]} e^{2\left[\hat{\mu}+\hat{a}_i+\beta Log(X_i o)\right]+s^2\mathbf{X}'_{X_{i}o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i}o}}\left[e^{s^2\mathbf{X}'_{X_{i}o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i}o}}-1\right] -$$

$$e^{\frac{s^2}{2}\left[1-\mathbf{X}'_{X_{i}o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i}o}\right]}\frac{\left[1-\mathbf{X}'_{X_{i}o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i}o}\right]^2 s^4}{2(n-1)} e^{2\left[\hat{\mu}+\hat{a}_i+\beta Log(X_i o)\right]+s^2\mathbf{X}'_{X_{i}o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i}o}}\left[e^{s^2\mathbf{X}'_{X_{i}o}(\mathbf{X}'\mathbf{X}^{-1})\mathbf{X}_{X_{i}o}}-1\right]$$

While the straight substitutions of σ^2 by s^2 and of μ, a_i, and β by the LS estimates and the use of the delta approximation probably leaves the variance estimate biased, a simulation suggest it is not a bad estimator (see Figure D1). The simulation was performed in GAUSS. The code is specified above.

Appendix E: Analysis of variance table for split-plot analysis

Dependent variable: Log(Diver Estimate)

Source	DF	SS	MS	F	P
Model	554	1438	2.59	21.6	<0.0001
Error	590	71.9	0.12		
Corrected Total	1144	1510			

R-Square:0.95

Source	DF	TI	MS	F	P
Y	2	34.9	17.5		
V	3	71	23.8		
Y*V	6	24.9	4.2		
D	5	14.1	2.8		
Y*D	9	19.8	2.2		
V*D	15	11.1	0.74		
Y*V*D	25	24.9	0.99		
LBCNT	1	892	892	7325	<0.0001
R	82	63.4			
Y*R	84	65.9			
V*R	201	139.7			
Y*V*R	121	76.7			

Source	DF	TIII	MS	F	P
Y	2	0.18	0.09	0.12*	>0.05
V	3	9.7	3.2	4.52*	0.0039
Y*V	6	4.7	0.79	1.11*	0.36
D	5	2.4	0.48	0.68	>0.05
Y*D	8	3.1	0.39	3.2	0.0013
V*D	15	1.8	0.12	1.0	0.45
Y*V*D	22	2.9	0.14	1.1	0.33
LBCNT+	1	0.03			
R	82	43.2			
Y*R	84	57.9			
V*R	201	125.5			
Y*V*R	121	76.7			

* Uses M. plot error term=(R+Y*R+V*R+Y*V*R Type I SS)/Sum(R+Y*R+V*R+Y*V) DF

+ Should be tested from Type I SS table above.

The relevant pooling of divers and vegetation types was accomplished by assignment of a two-level diver variable, which distinguished the divers KB and MM during 1990 and 1991, so that their contributions during these years could be avoided. Similarly, a five-level vegetation code was formed, level 1 labelling eelgrass and hairkelp, level two labelling fucus over 1990, 1992 and 1994 , level three labelling fucus for 1991 (data avoided), level four labelling LBK before 1994 (data avoided), and level five labelling LBK for 1994. The following SAS code was used to generate the final adjustment model:

```
data one;infile "c:\herring\divcal\calan.asc" ; input yr v tran bn d rel est labc rep;
if rel>2 then delete; if d=3 or d=8 or d=9 or d=10 or d=11;
if yr>89; if d=3 or d=8 or d=9 then d=1; if (d=10 or d=11) and yr<92 then d=2;
if (d=10 or d=11) and yr>91 then d=1; if (v=1 or v=2) then v=1; if v=3 and yr ne 91
then v=2;
if v=3 and yr=91 then v=3; if v=4 and yr<94 then v=4;
if v=4 and yr=94 then v=5; lest=log(est); llc=log(labc); drop tran bn rel rep ; run;
proc glm ; classes d v ; model lest= d v d*v llc /solution ; run;
```

The following parameter estimates were obtained:

Parameter	Estimate
Intercept	-0.469
D1	0.0847
D2	0
V1	0.379
V2	-0.417
V3	-0.099
V4	0.243
V5	0
D1V1	-0.746
D1V2	0.449
D1V3	-0.351
D1V4	0
D1V5	0
D2V1	0
D2V2	0
D2V3	0
D2V4	0
LLC	0.943

Appendix G: Bootstrap estimation of the variance of $(\hat{X}_{ijk})_{Yo}$

A vector of bootstrap residuals, ϵ^*_{ijkl}, was obtained by resampling with replacement from the original vector of residuals associated with the final fitted model. A vector of bootstrap Log(Diver Estimates), $Log(Y^*_{ijkl})$, were then obtained according to:

$$Log(Y^*_{ijkl}) = \hat{Log}(Y_{ijkl}) + \epsilon^*_{ijkl}$$

The final model was then refitted with the bootstrap observations so formed, and inverse predictions made for all observations in the data set using the bootstrap fitted model.

The above was repeated 1000 times. The variance of an inversely predicted value was estimated from the sample variance of the vector of 1000 bootstrapped inverse predictions.

The bootstrap was conducted in GAUSS. The code follows:

```
/* Bootstrap estimate of variances of adjusted diver estimates-
recall that inverse predictions used, with more than one predictor */
new;
dataloop calboot.dat out; @ Calibration data containing diver,veg,lest,llc@
code div  with 1 for d==1,0 for d==2;
code v1   with 1 for v==1,0 for v/=1;@v=1:orig vcode=1 or 2     @
code v2   with 1 for v==2,0 for v/=2;@v=2:orig vcode=3,yr /= 91 @
code v3   with 1 for v==3,0 for v/=3;@v=3:orig vcode=3,yr=91    @
code v4   with 1 for v==4,0 for v/=4;@v=4:orig vcode=4,yr<94    @
                          @Only need 4 vars to code for    @
                          @5 levels. v=5 in SAS:orig vcode=@
                          @4,yr=94 and==v1,v2,v3,v4=0.    @
make divv1=div*v1;make divv2=div*v2;make divv3=div*v3;drop d v;endata;
open s1=out;x=readr(s1,rowsf(s1));@lest,llc,div,v1,v2,v3,v4,divv1,divv2,divv3@
x=x[.,1]~x[.,3:10]~x[.,2]; @lest,div,v1,v2,v3,v4,vdivv1,divv2,divv3,llc@
y=x[.,1];      @lest:dependent var.@
x=x[.,2:10];   @Indep. vars.@
open s2="calbtsp.dat";sp=readr(s2,rowsf(s2)); @ 1994 spawn data: vc,llest @
                  @ vc=1 if orig vcode=1 or 2 @
                  @ vc=2 if orig vcode=3      @
                  @ vc=3 if orig vcode=4      @
__output=0;_olsres=1;
{a1,b1,c1,d1,e1,f1,g1,h1,i1,j1,k1}=ols(0,y,x);res=j1;
n=4;                  @#bootstrap samples@
bs=zeros(n,10);       @Matrix to hold bootstrap par. estimates @
```

```
h=1;do while h<=n;
ebs=sampwr(res,rows(res)); @Obtain random sample from res vector @
ybs=y+ebs;                 @ Create bs set of llest @
{a2,b2,c2,d2,e2,f2,g2,h2,i2,j2,k2}=ols(0,ybs,x); @ Fit bs observations @
bs[h,.]=c2'; @mu,div,v1,v2,v3,v4,divv1,divv2,divv3,beta @
h=h+1;endo;
vadj=zeros(rows(sp),1); @Create vector to hold variances(adj) @
i=1;do while i<=rows(sp);  @Cycle through 1994 spawn dep data @
if sp[i,1]==1;                    @ If vc code=1 @
fn fadj(l2,m2)=(l2-(m2[1]+m2[2]+m2[3]+m2[7]))/m2[10];
elseif sp[i,1]==2;                @ If vc code=2 @
fn fadj(l2,m2)=(l2-(m2[1]+m2[2]+m2[4]+m2[8]))/m2[10];else;
fn fadj(l2,m2)=(l2-(m2[1]+m2[2]))/m2[10];        @ If vcode=3   @endif;
mat=zeros(n,1);        @ Vector to hold ith set of bs adjustments @
j=1;do while j<=n;         @ Cycle through bs matrix of par estimates @
b=bs[j,.];  @mu,div,v1,v2,v3,v4,divv1,divv2,divv3,beta @
adj=fadj(sp[i,2],b);
mat[j]=adj;                       @ Enter jth adjustment @j=j+1;endo;
mat=exp(mat);              @ Convert back to original scale @
vadj[i]=(stdc(mat))^2;      @ For ith line in sp enter bs var. est@
i=i+1;                    @ Move to next line in sp @endo;
spnew=sp~vadj;              @ Merge spawn data with bs. vars @
output file=calboot.out reset;screen off;spnew;screen on;closeall;end;
```

Calboot.dat was obtained from **calan.asc** (described in Appendix A) and has the following columns: diver, vegetation Log(Diver Estimate) and Log(Lab Count). The following SAS code was used to create **calboot.asc** (which was later converted to the GAUSS data set **calboot.dat/dht** with the ATOG command):

```
options ps=60;options ls=80;
filename bill "c:\herring\divcal\calboot.asc"; data one;
infile "c:\herring\divcal\calan.asc" ; input yr v tran bn d rel est labc rep;
if rel>2 then delete; if d=3 or d=8 or d=9 or d=10 or d=11; if yr>89;
if d=3 or d=8 or d=9  then d=1; if (d=10 or d=11) and yr<92 then d=2;
if (d=10 or d=11) and yr>91 then d=1; if (v=1 or v=2) then v=1;
if v=3 and yr ne 91 then v=2; if v=3 and yr=91 then v=3;
if v=4 and yr<94 then v=4; if v=4 and yr=94 then v=5;
lest=log(est); llc=log(labc); drop tran bn rel  rep ; run;data two;set one;file bill;
put d v lest llc;run;
```

The ATOG commands:
input c:\herring\divcal**calboot.asc;**output **calboot;**invar d v lest llc;

Appendix H: Scatter plots of diver estimates vs lab count for 1994 spawn deposition divers for all vegetation types over the years 1990, 1991, 1992 and 1994

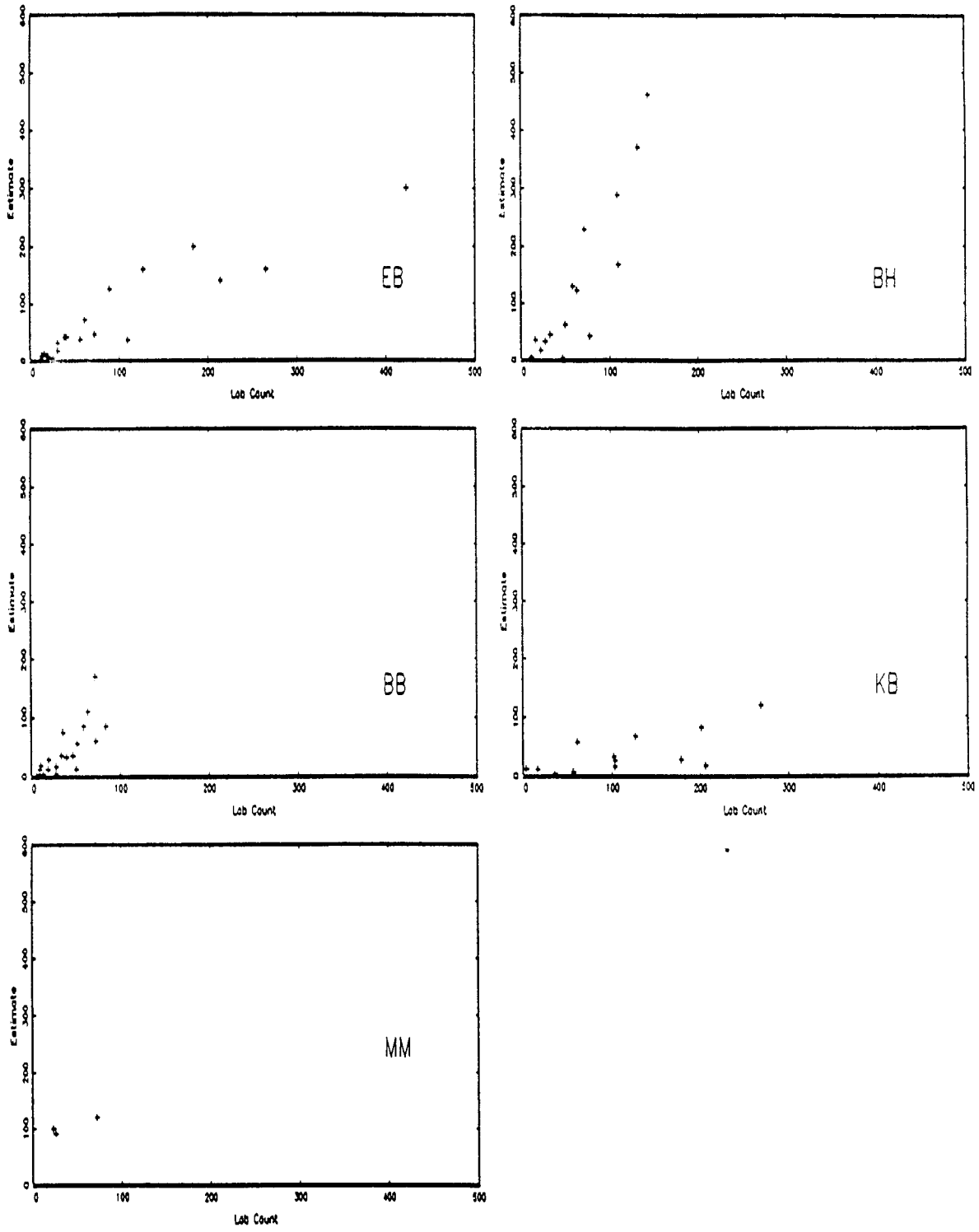

Figure H1. 1990, eelgrass, divers, EB,BH,BB,KB,MM

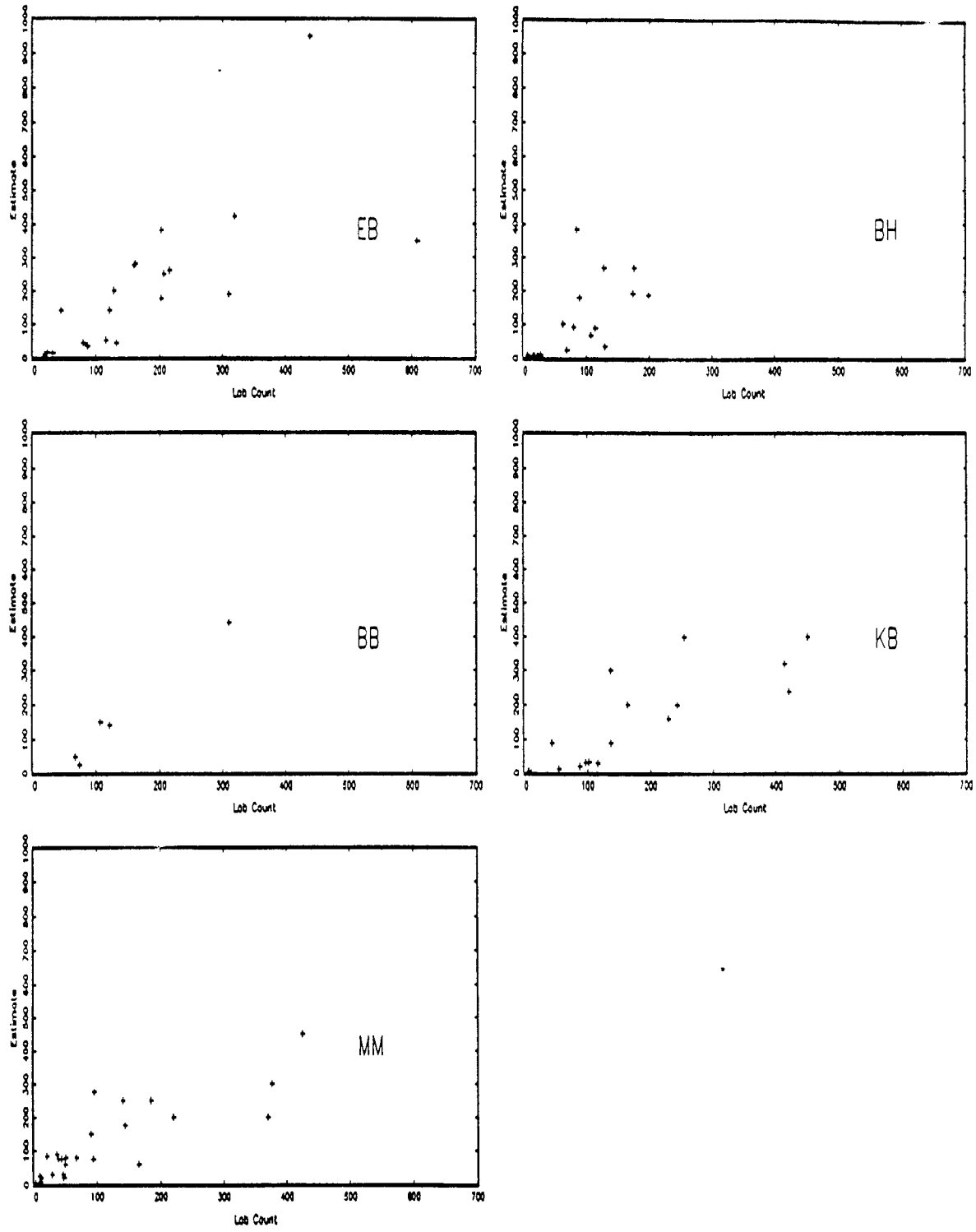

Figure H2. 1990, hair kelp, divers EB,BH,BB,KB,MM.

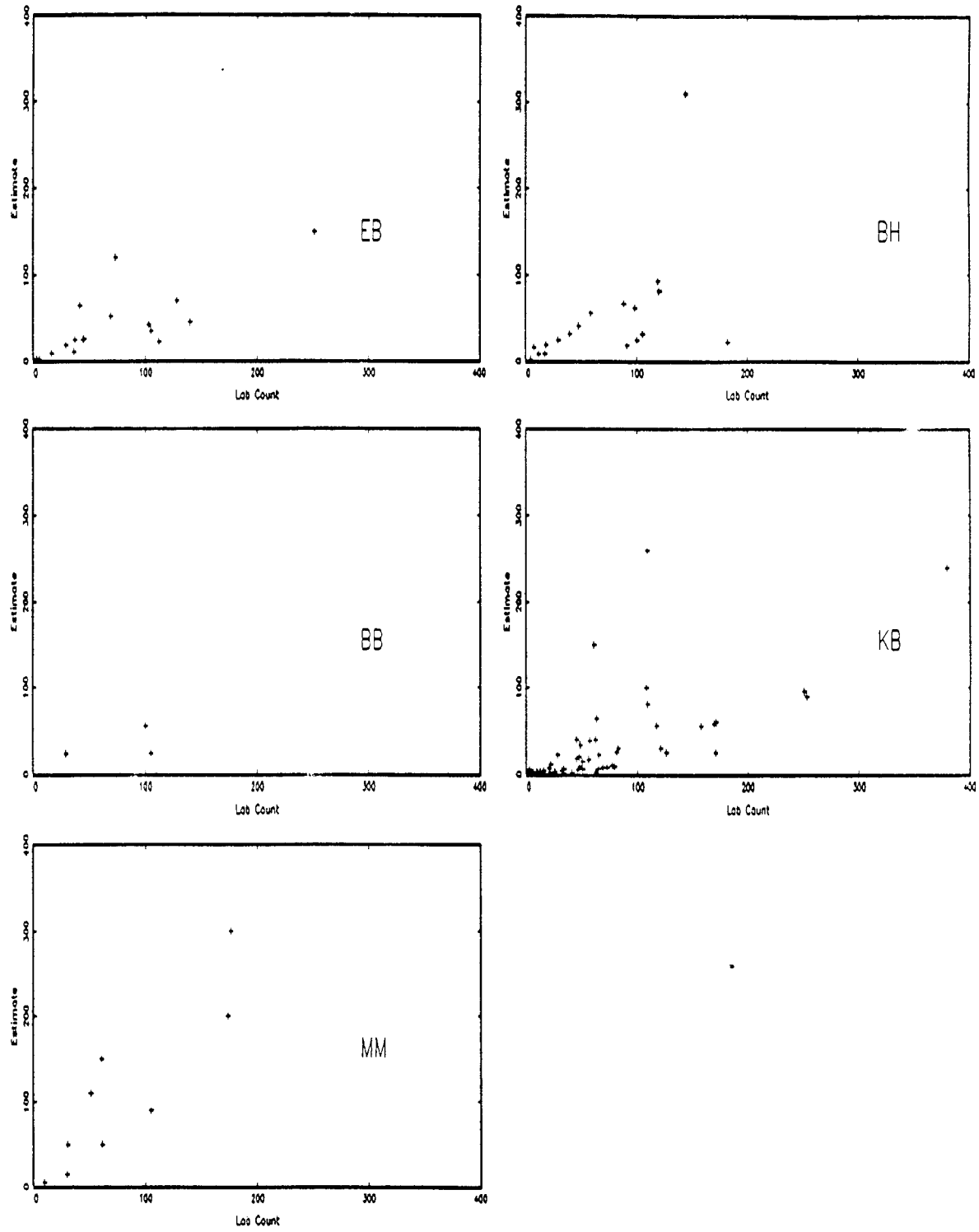

Figure H3. 1990,fucus, divers EB,BH,BB,KB,MM,

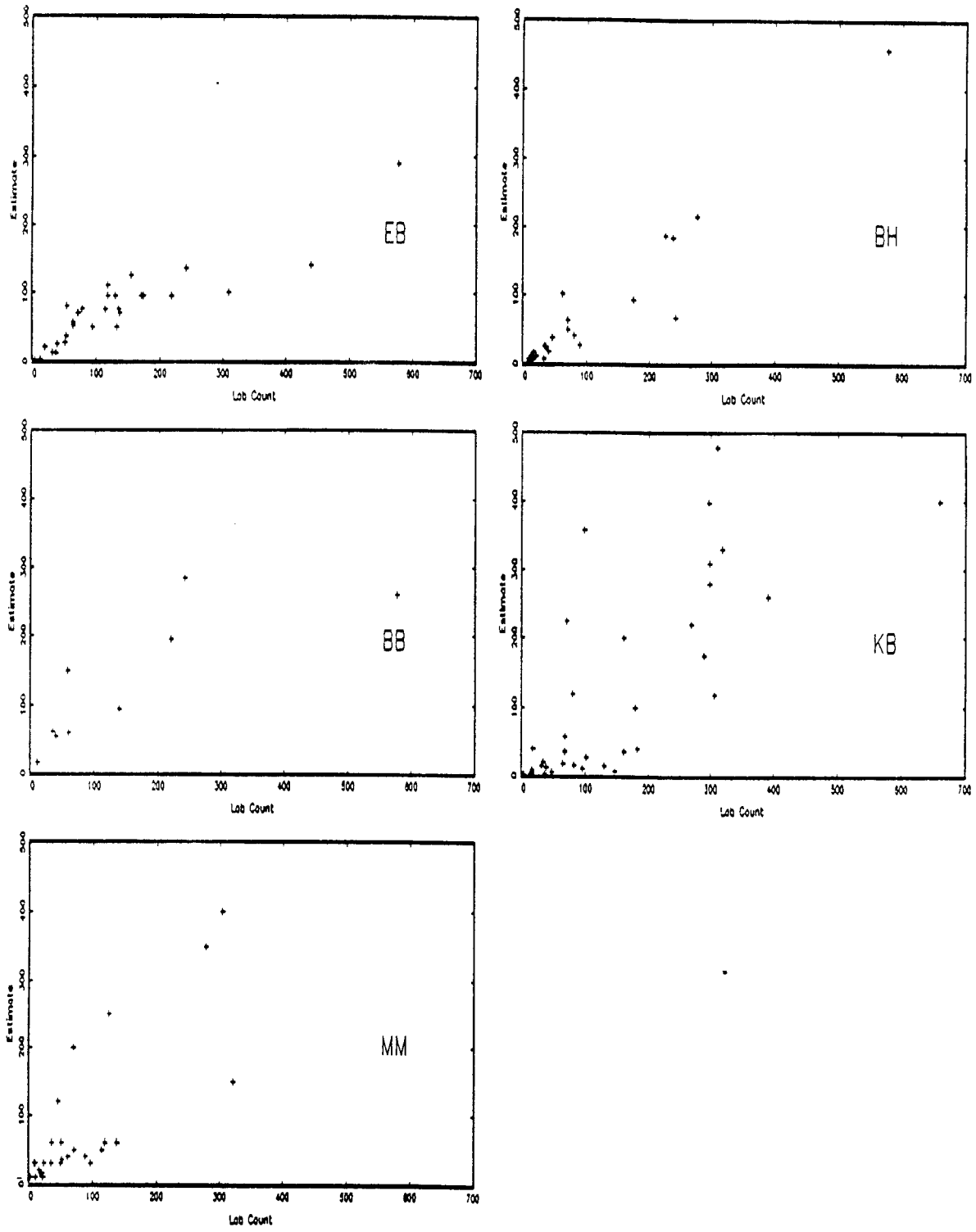

Figure H4. 1990, LBK, divers EB,BH,BB,KB,MM.

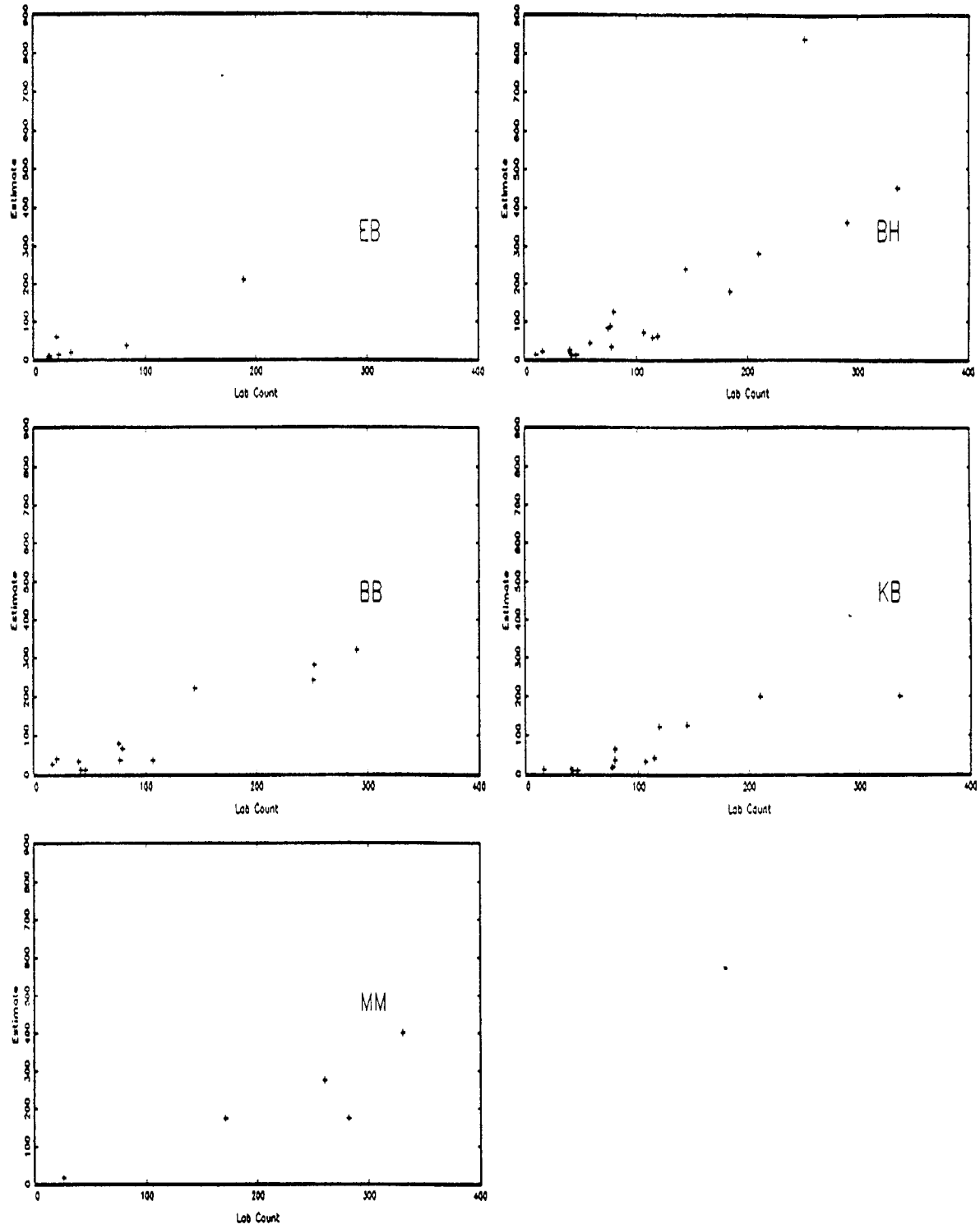

Figure H5. 1991, eelgrass, divers EB,BH,BB,KB,MM

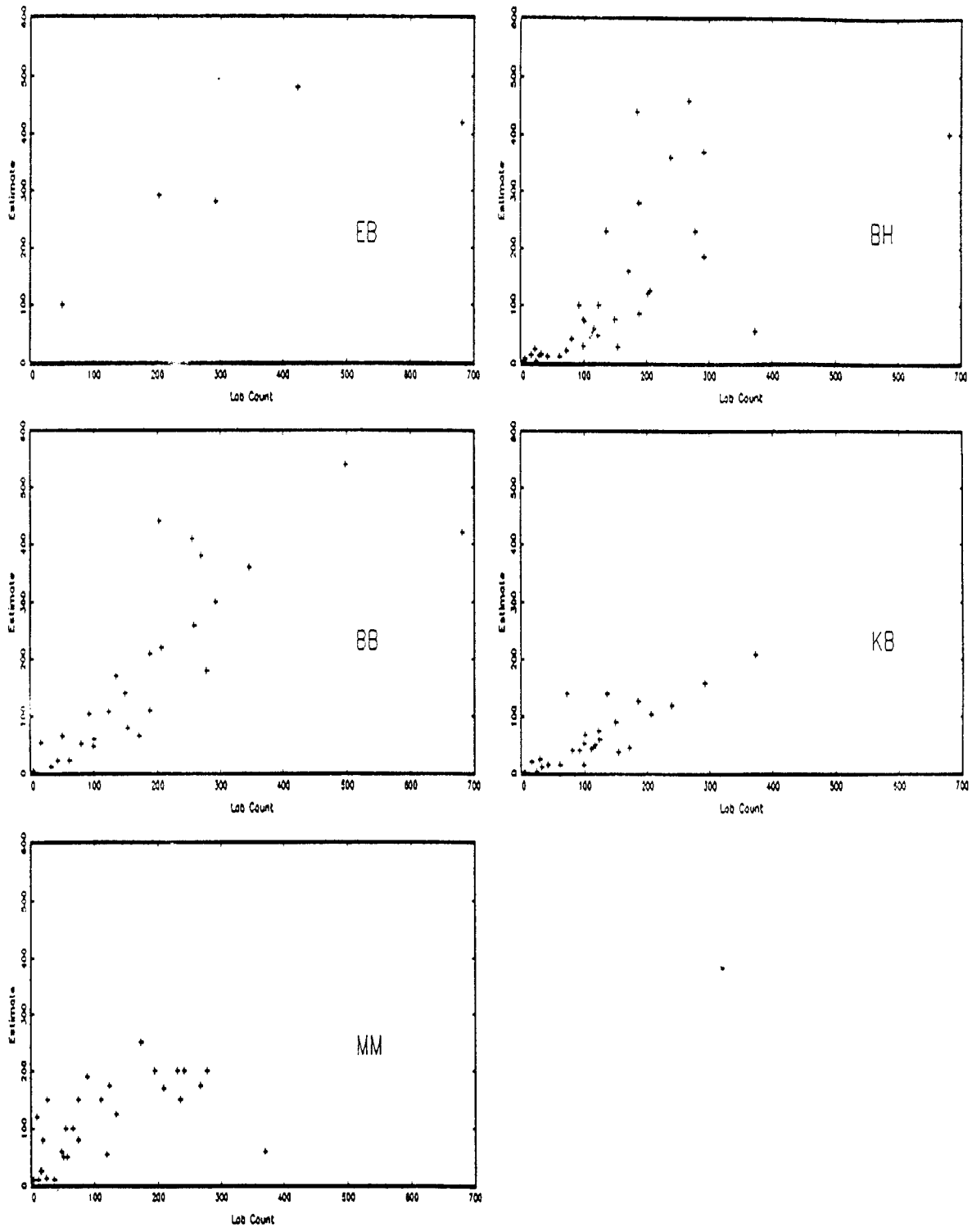

Figure H6. 1991, hair kelp, divers EB,BH,BB,KB,MM

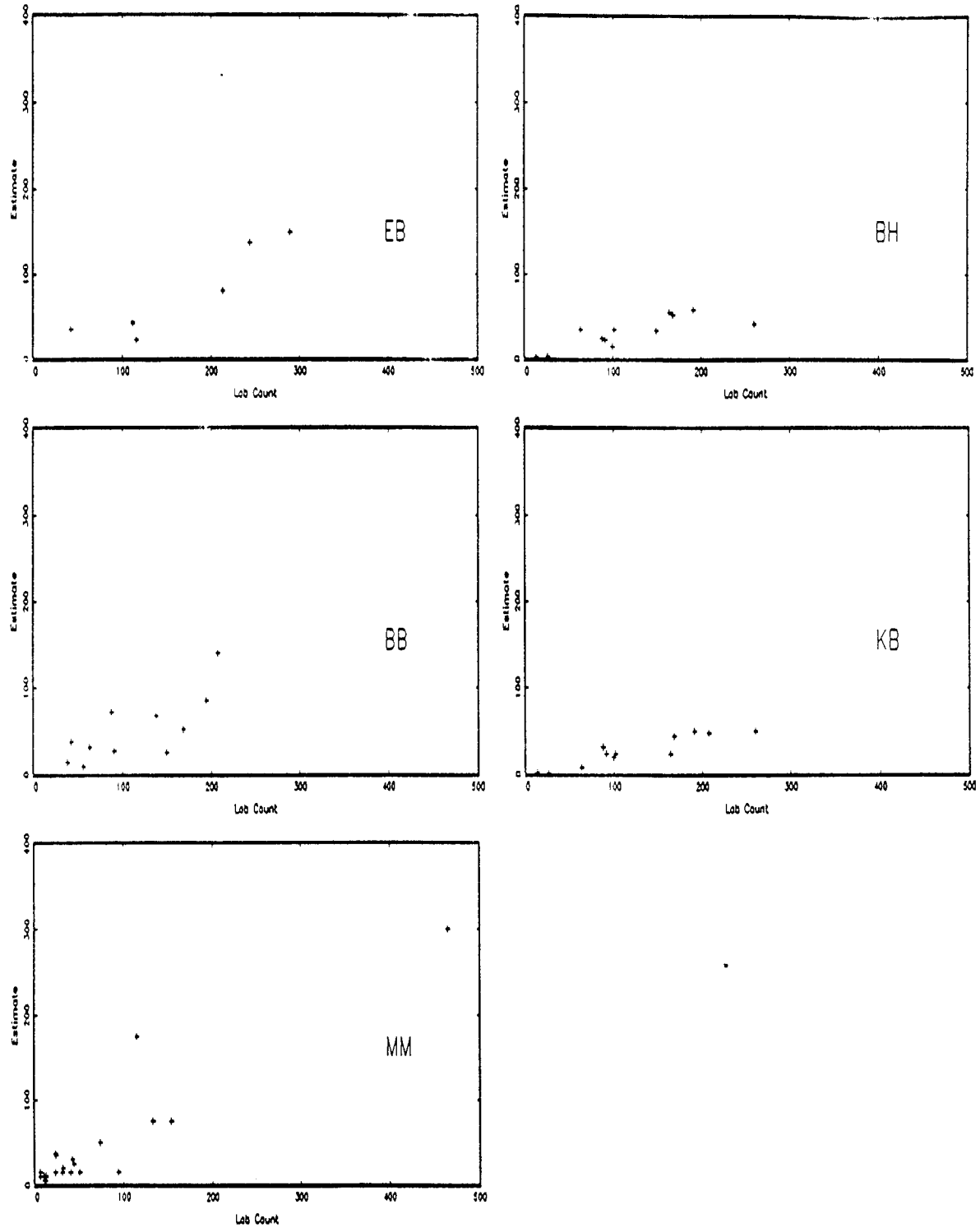

Figure H7. 1991, fucus, divers EB,BH,BB,KB,MM

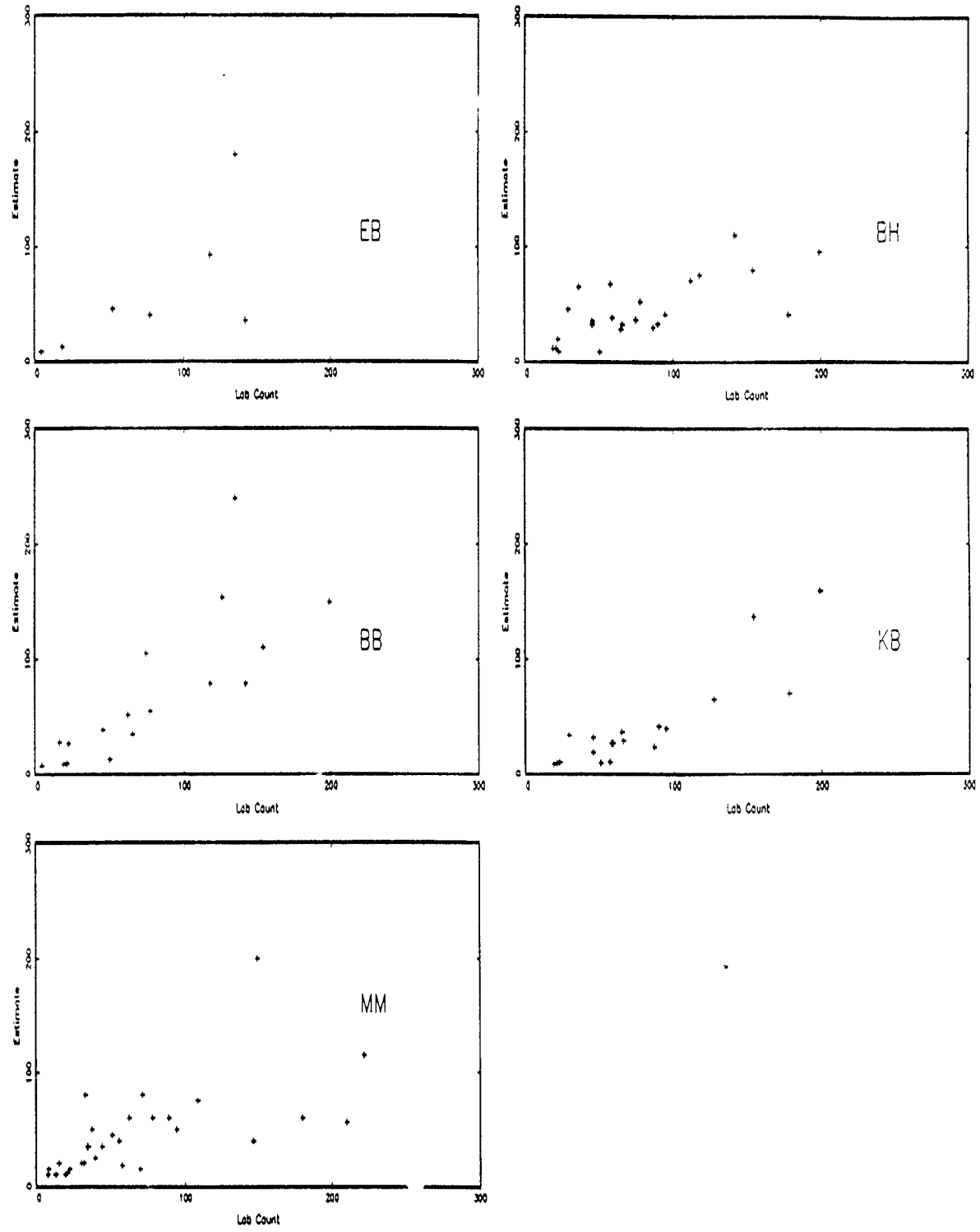

Figure H8. 1991, LBK, divers EB,BH,BB,KB,MM.

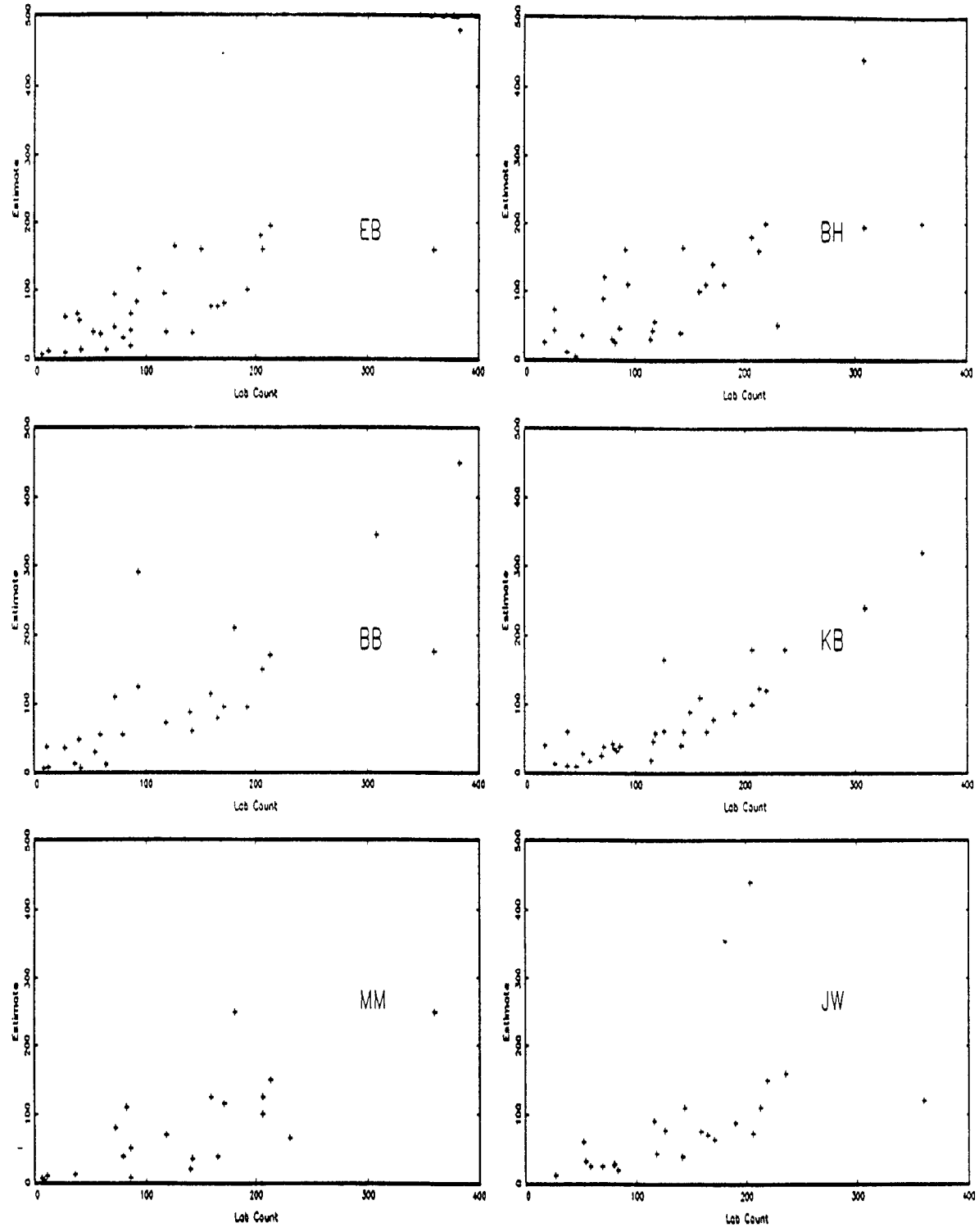

Figure H9. 1992, eelgrass, divers EB,BH,BB,KB,MM,JW

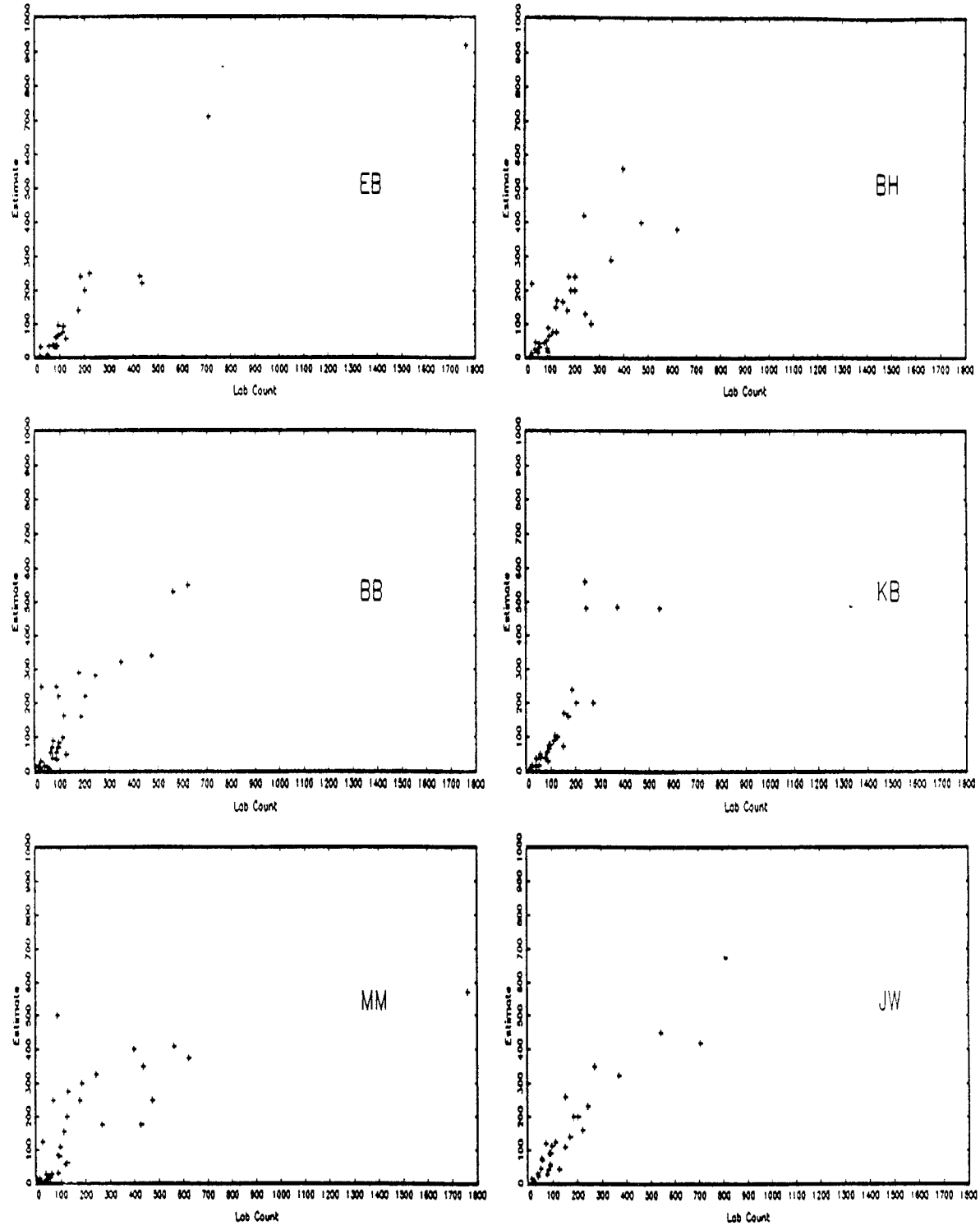

Figure H10. 1992, hair kelp, divers EB,BH,BB,KB,MM,JW

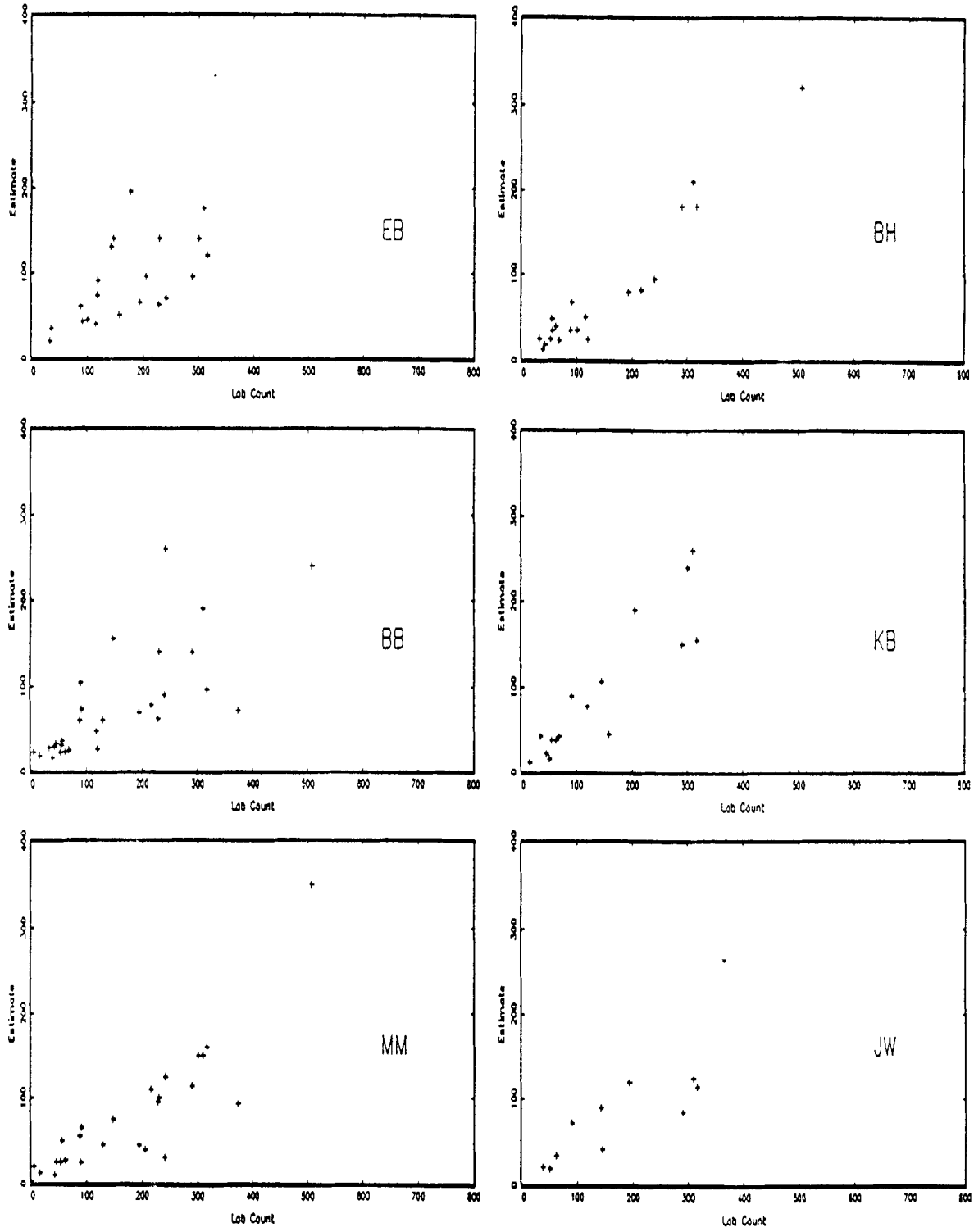

Figure H11. 1992,fucus, divers EB,BH,BB,KB,MM,JW

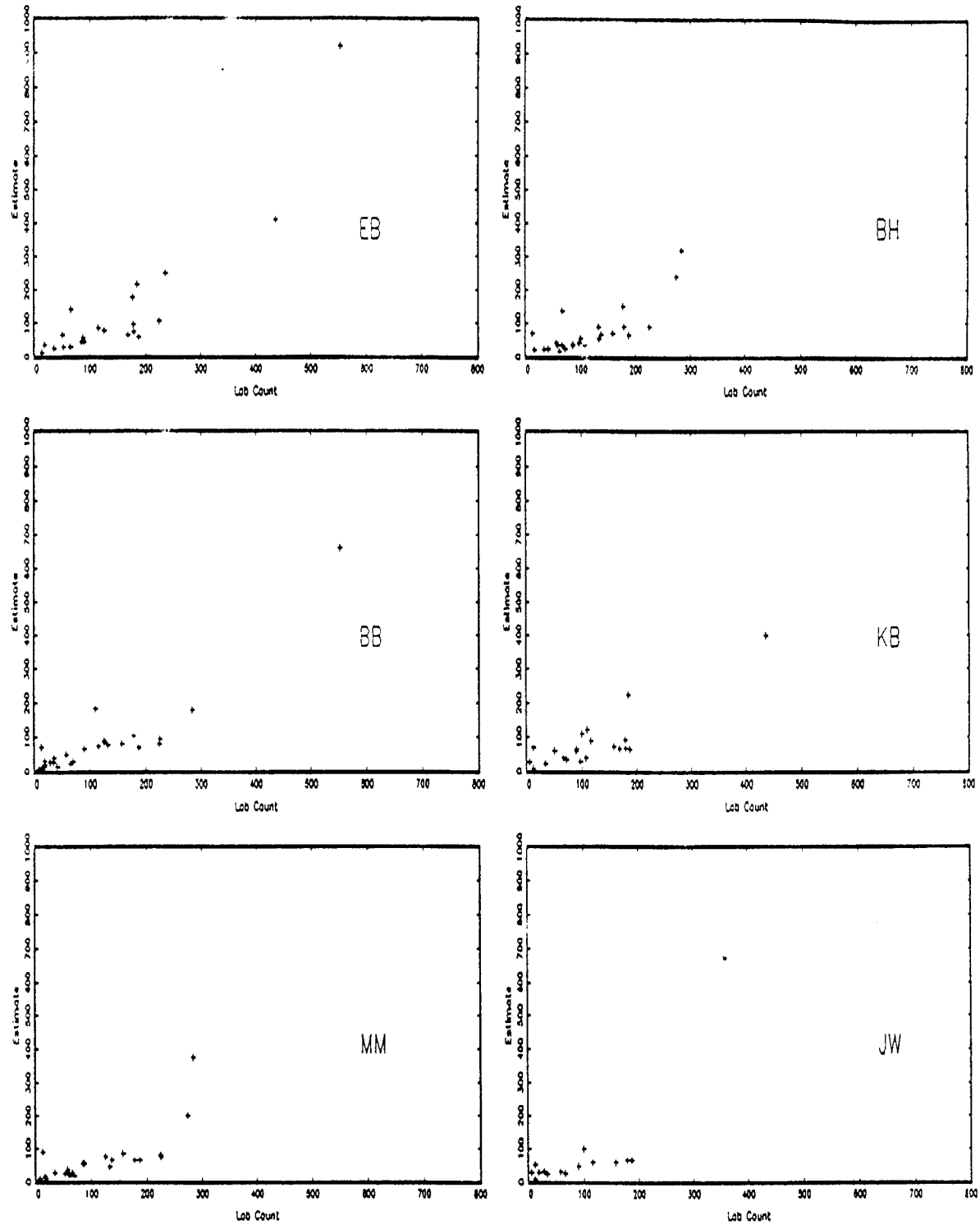

Figure H12. 1992, LBK, divers EB,BH,BB,KB,MM,JW

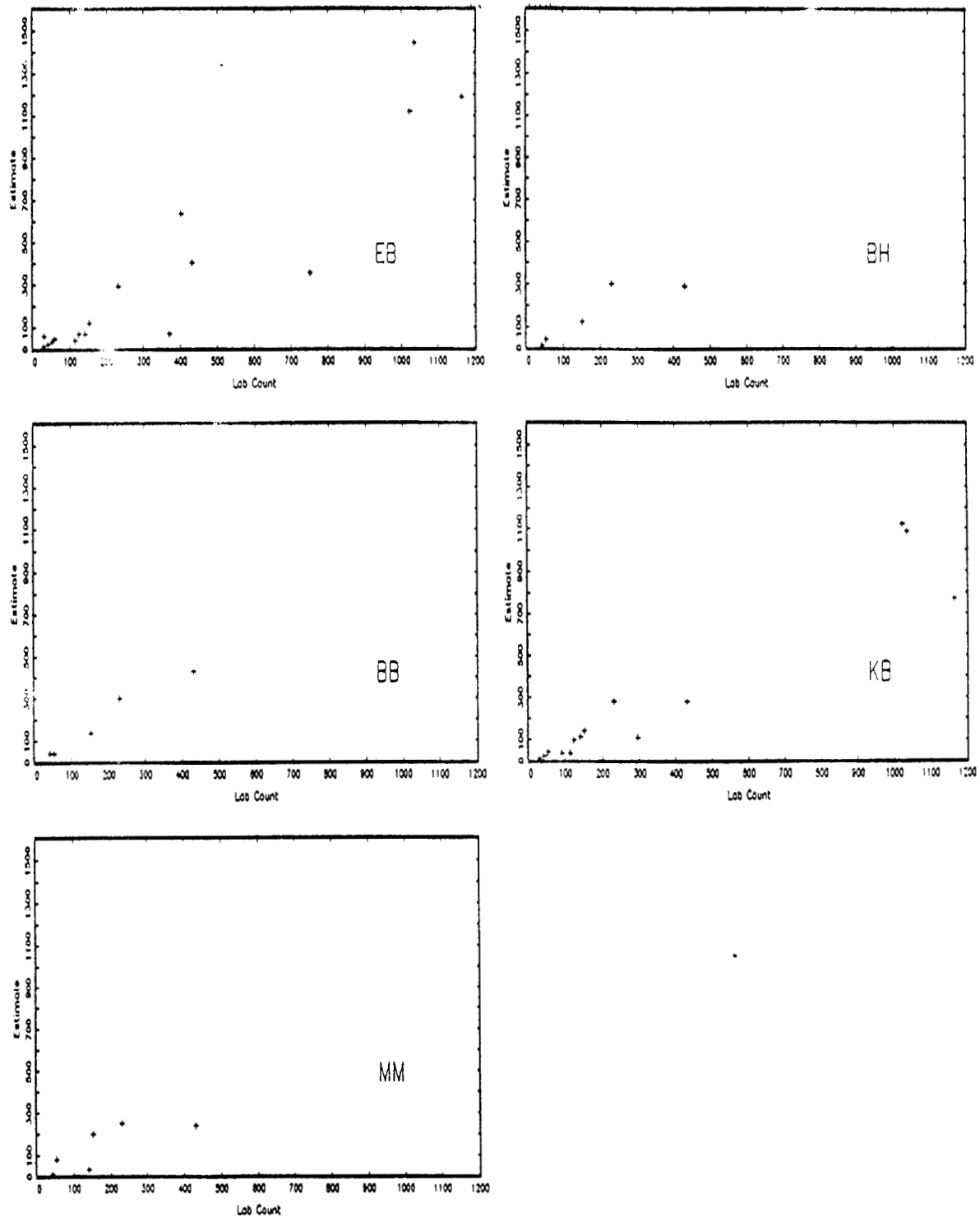

Figure H13. 1994, eelgrass, divers Eb, BH, BB, KB, MM

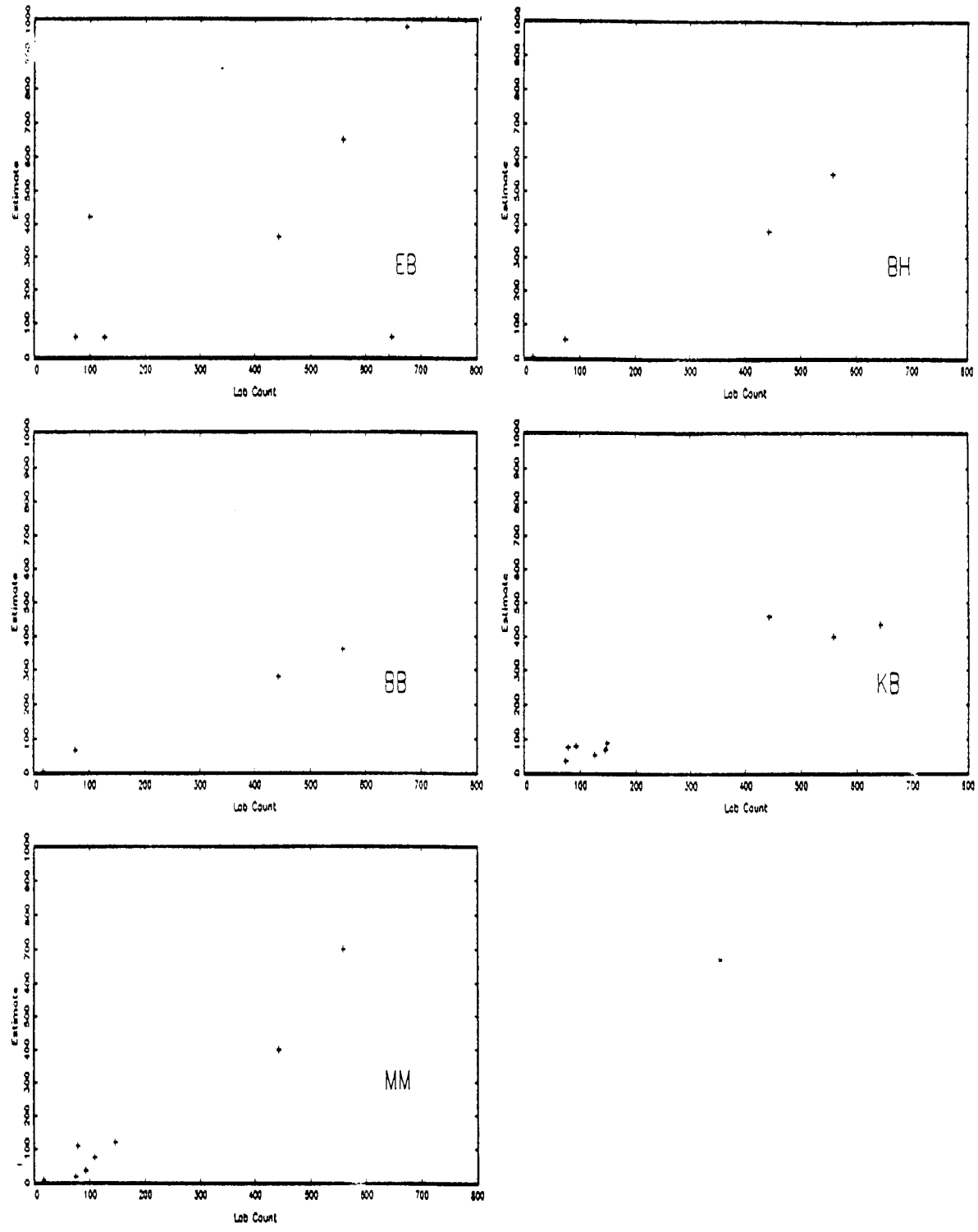

Figure H14. 1994, hair kelp, divers EB, BH, BB, KB, MM

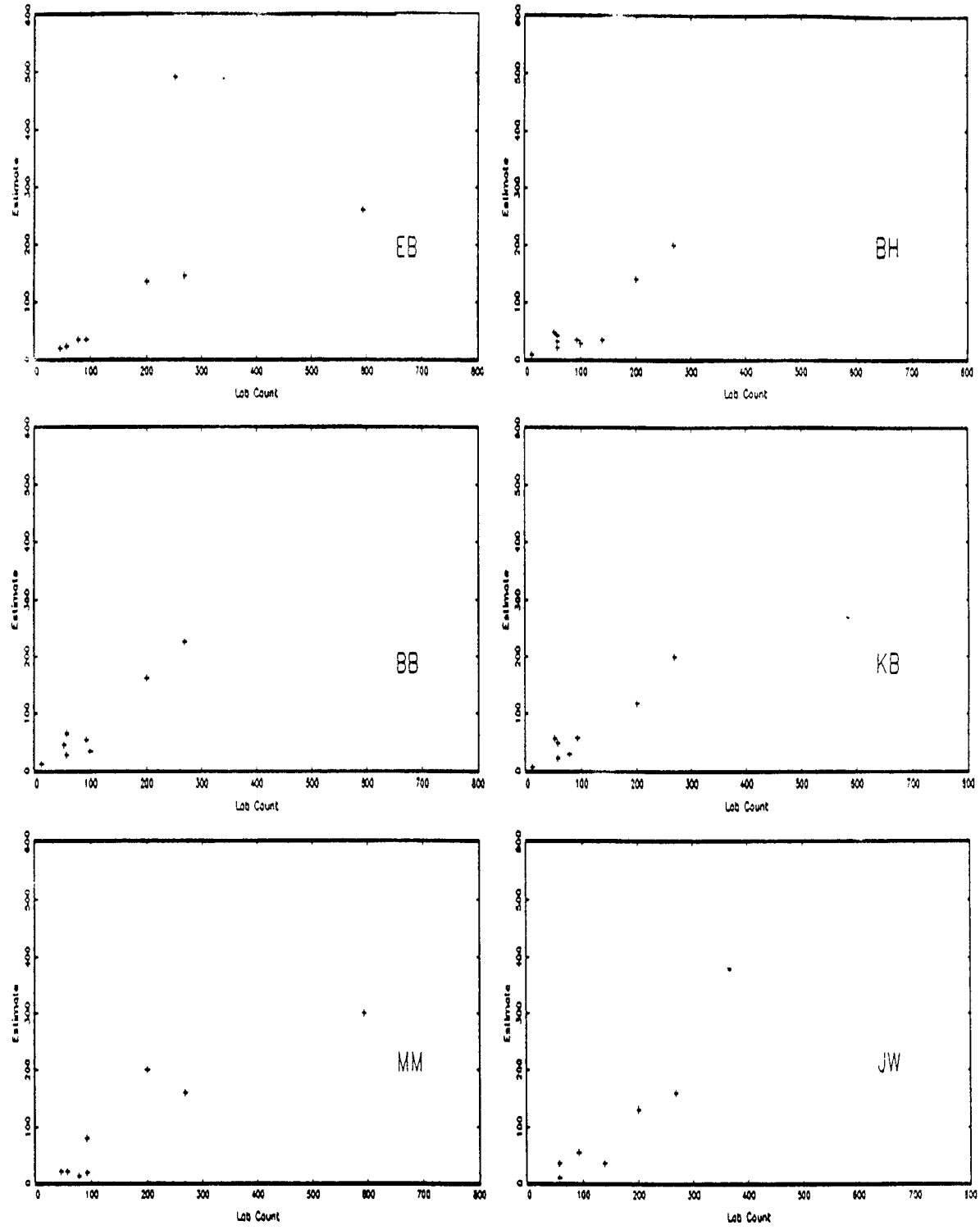

Figure H15. 1994, fucus, divers EB,BH,BB,KB,MM,JW

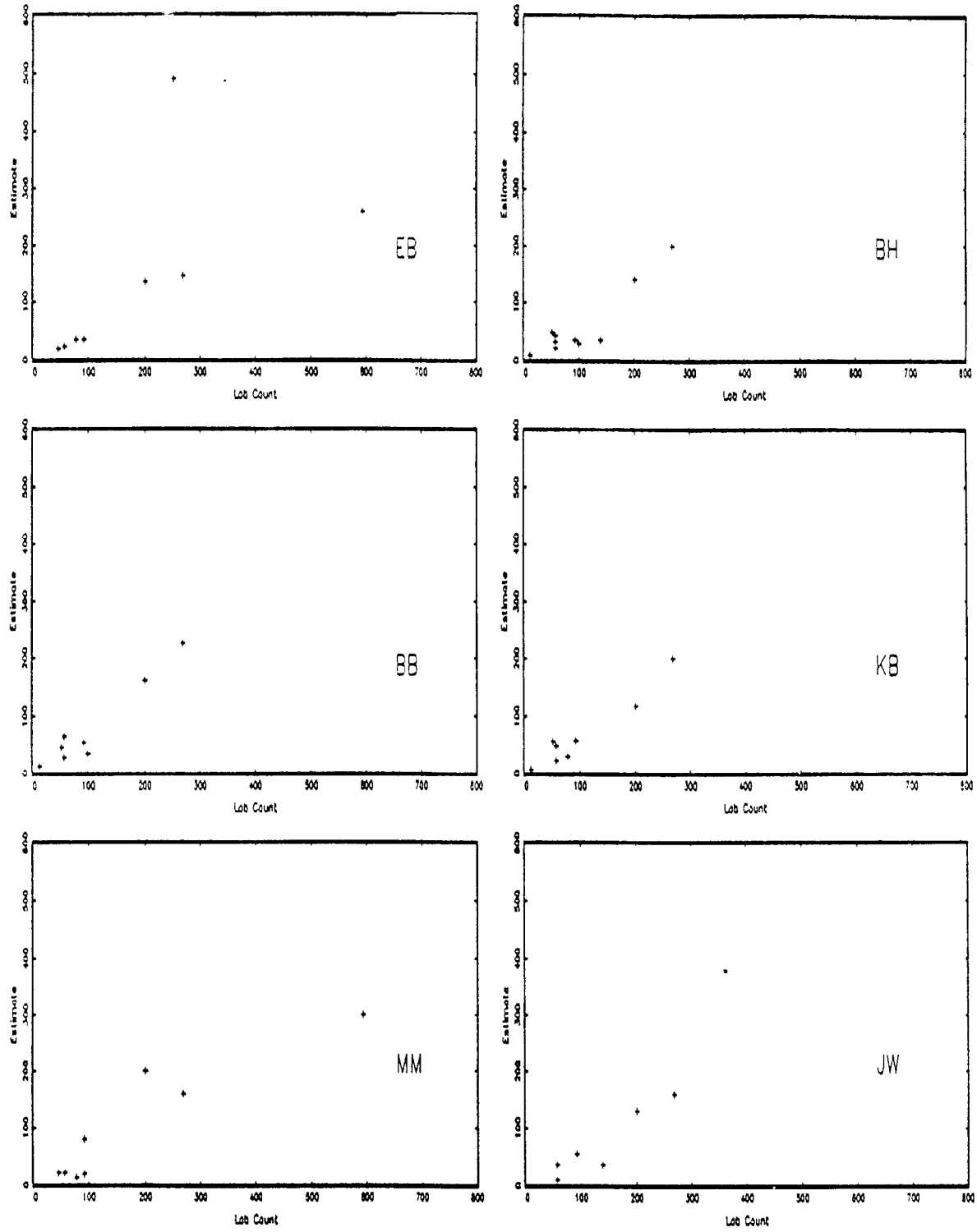

Figure H16. 1994, LBK, divers EB,BH,BB,KB,MM,JW

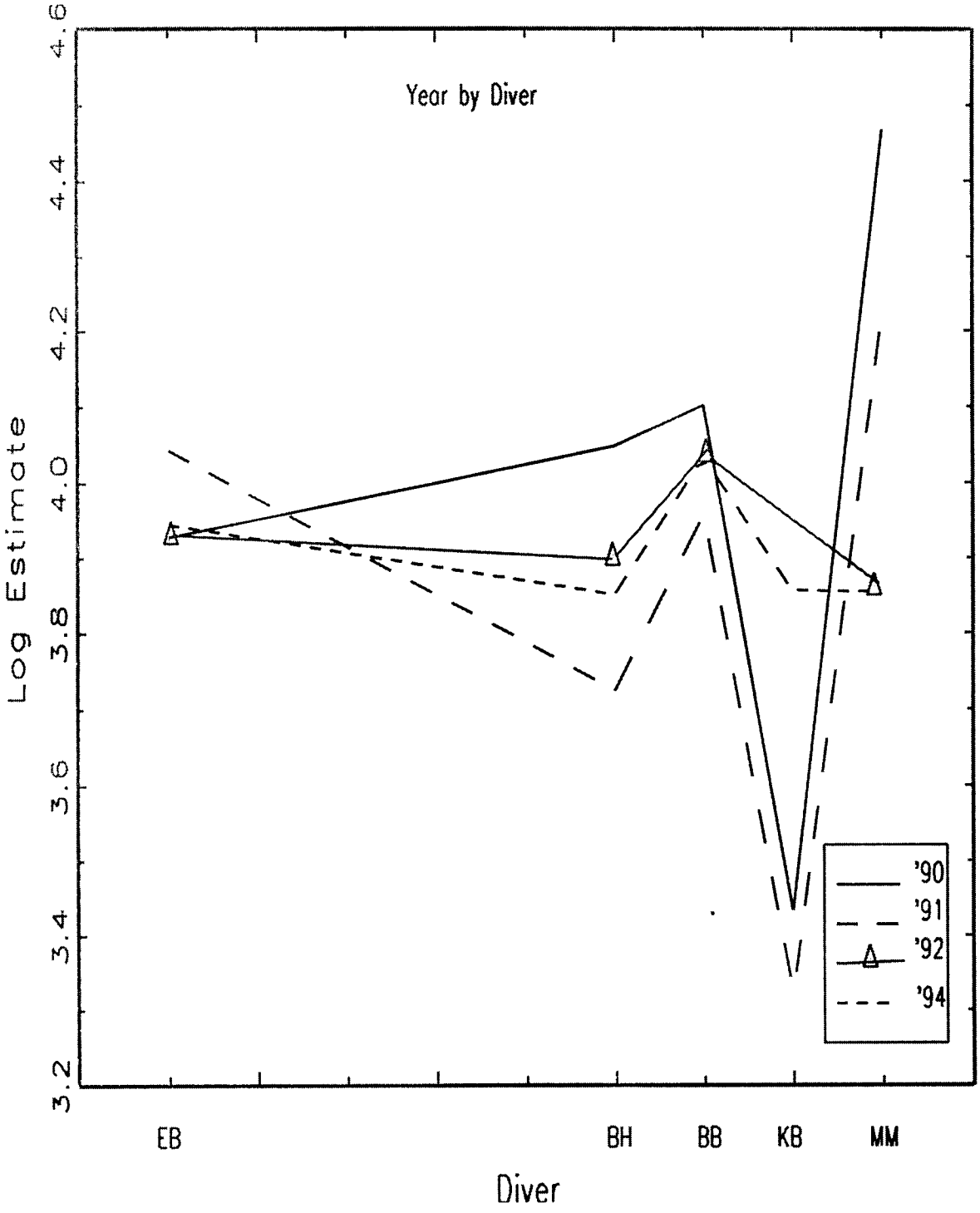

Figure I1 Year by diver interaction. Plot of least square means.

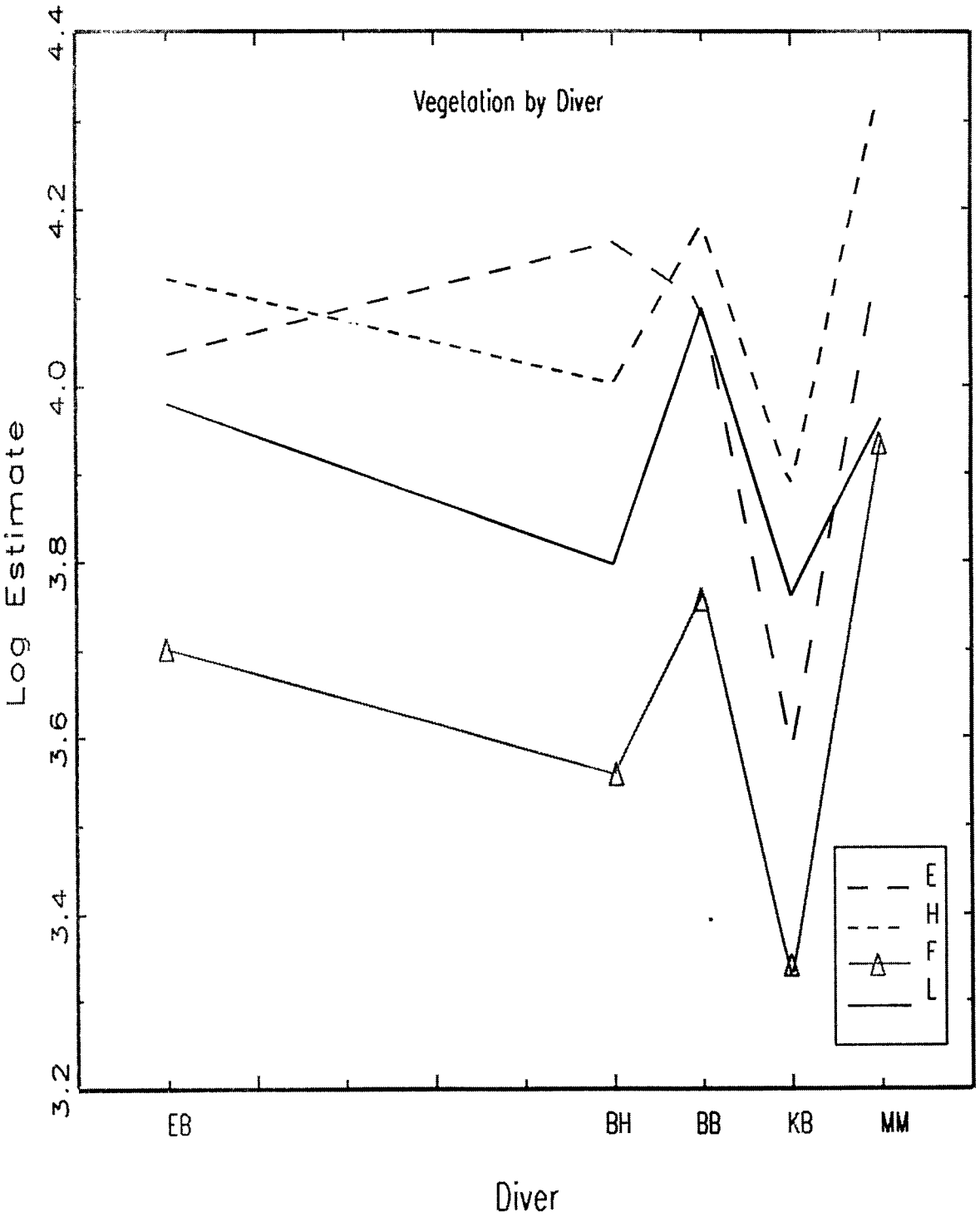

Figure I2 Vegetation by diver interaction. Plot of least square means.

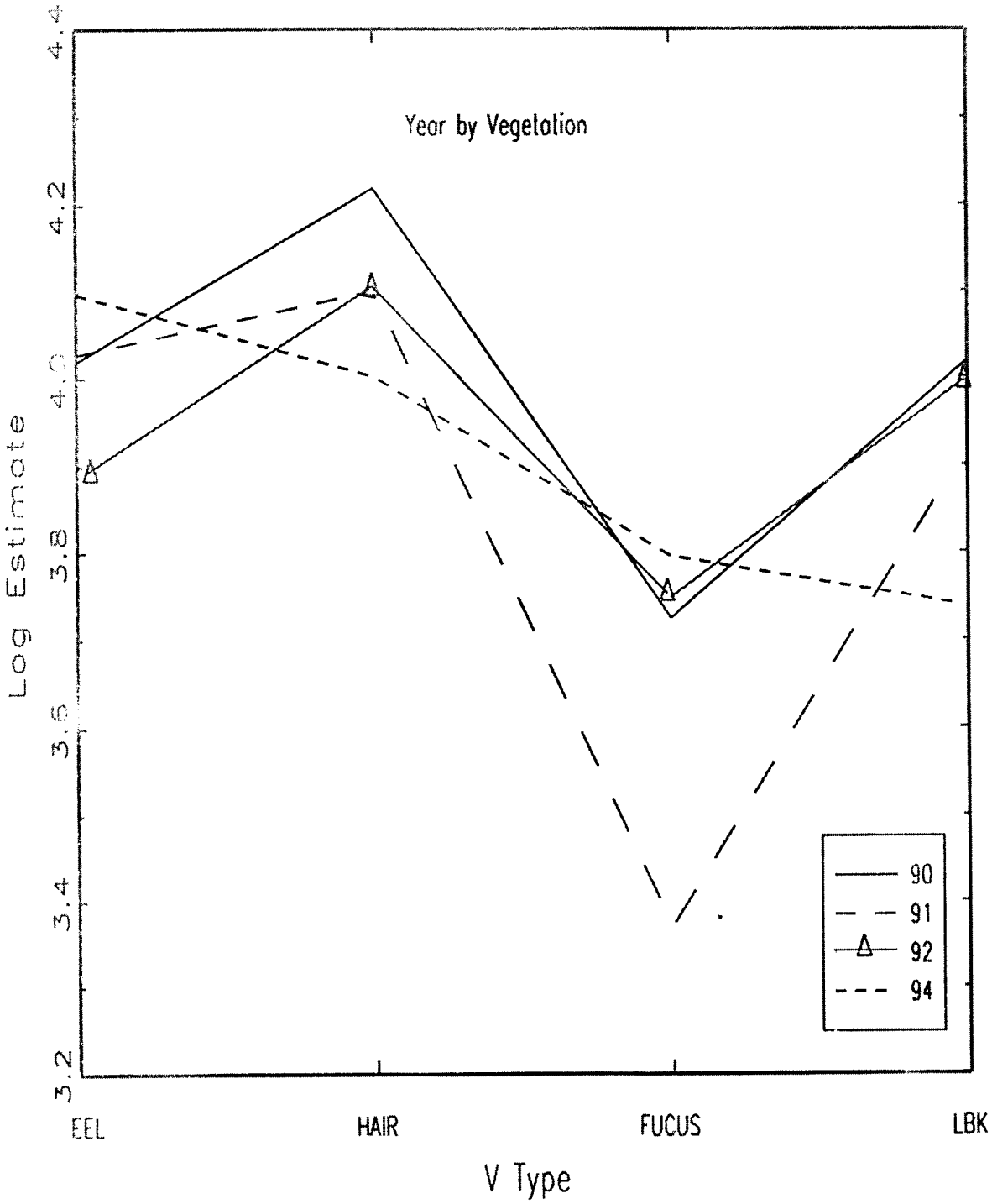

Figure I3 Year by vegetation interaction. Plot of least square means.

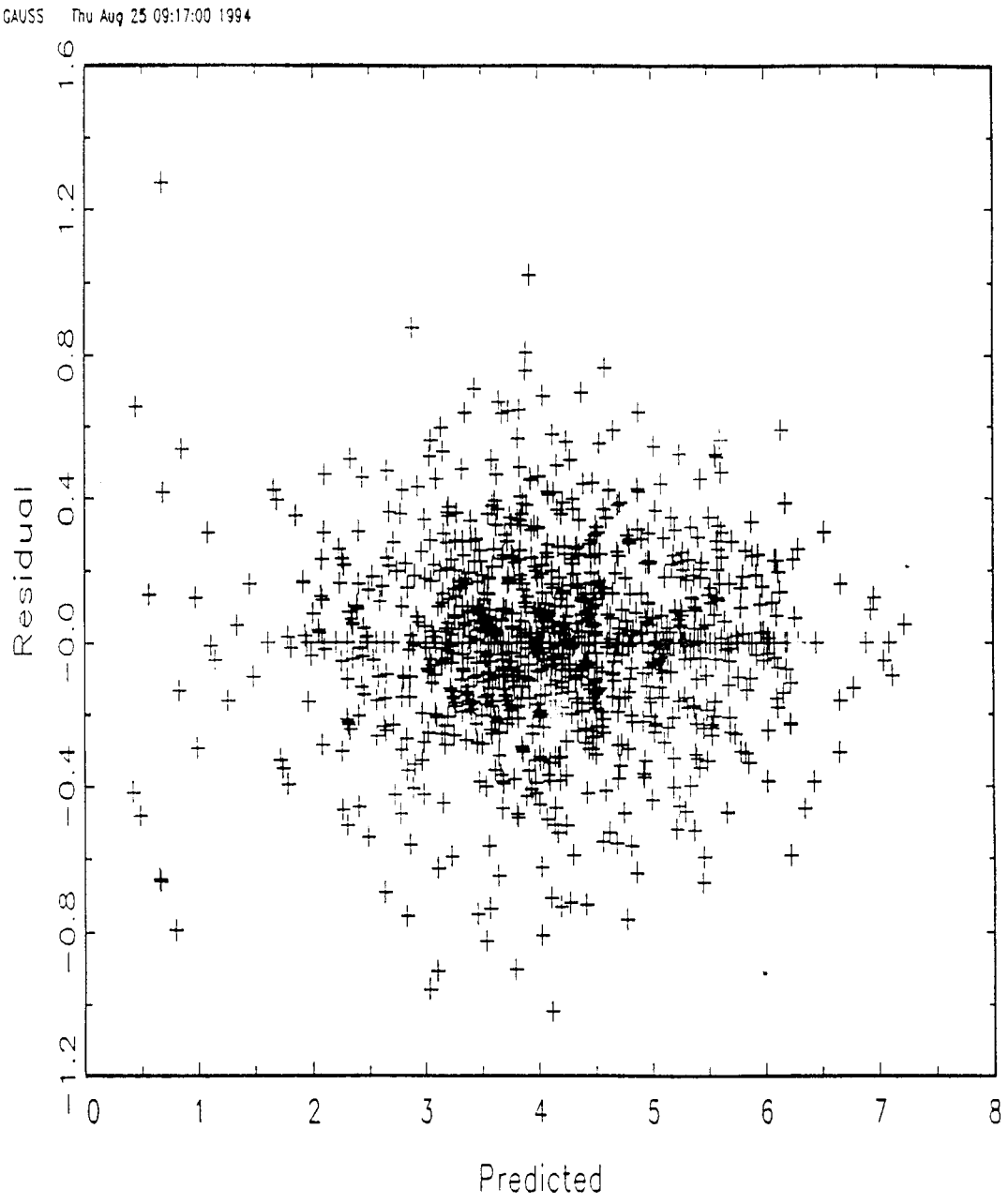

Figure J1 Plot of residuals from split-plot analysis.

APPENDIX B: FACTORS AFFECTING EGG LOSS

JCSFOS 95-04

FACTORS AFFECTING EGG LOSS OF PRINCE WILLIAM SOUND HERRING AND RECOMMENDATIONS FOR 1995 FIELD RESEARCH

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Commercial Fisheries Management and Development Division
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June 12, 1995

INTRODUCTION

The reasons for the failure of the Prince William Sound herring (*Clupea pallasi*) fishery in 1993 and 1994 are not well understood at the present time. If herring recruitment is dependent on the number of eggs produced by the spawning stock, then a combination of physical and biological processes may be involved. Physical processes (Royer 1986) may be involved, because they affect fish stocks through effects on growth and mortality at all stages of life, including the egg stage. Herring recruitment in particular show strong relationships with the environment (e.g. Zebdi 1991, Wespestad 1991). Other potential physical variables that may induce inter-annual variability in egg loss and survival include habitat and substrate variables (e.g. exposure to waves, exposure to air, depth, substrate type). Biological interactions may also be involved, in that bird species (glaucous-winged gulls, diving ducks), invertebrates (crabs, seastars) and fish species (e.g. salmonids, flatfishes, sculpins) are found in the nearshore zone and known to be predators of herring eggs and juveniles. Other predators such as fish species also affect the abundance of herring eggs and juveniles. Finally, the *Exxon Valdez* oil spill of 1989 may also be affecting herring recruitment with potential effects on adult and juvenile health, egg viability, and genetic composition.

Ongoing studies are addressing some of these processes through field studies of herring spawning, egg removal and egg mortality. The Alaska Department of Fish and Game has been analyzing factors affecting the survival of Pacific herring eggs in Prince William Sound since the occurrence of the *Exxon Valdez* Oil Spill. Mapping and enumeration of spawn deposition using aerial and dive surveys dates back to 1972 (Funk 1993). The FY94 project 94166 'Herring Spawn Deposition and Reproductive Impairment" to the *Exxon Valdez* Trustee Council (Wilcock and Brown 1994) describes the studies that are ongoing at the present time, which includes a study to analyze factors affecting egg loss and mortality.

Biometrics and modeling assistance was contracted to principal investigators Haldorson and Quinn of the Juneau Center, School of Fisheries and Ocean Sciences (SFOS), University of Alaska Fairbanks (UAF) in late 1994 for a period of 3 to 4 man-months through an RSA. Chris Rooper was hired as a graduate research assistant to conduct the analysis of egg loss as part of his master's thesis project. For FY95 and beyond, we proposed to continue the study on egg loss and to initiate a new study on relationship of recruitment to biological and environmental variables. This work falls under the Herring Natal Habitats project #95166 under the SEA Plan. The ultimate goal of this project is to build a sound-wide embryo survival model including factors such as habitat type, egg density, predation, and meteorological conditions.

Here we report our work only on the egg loss study. This report contains our preliminary results from the analysis of factors affecting egg loss of Prince William Sound Herring. We first review earlier work done on this problem in the context of the effect of the oil spill on egg lossand mortality. We then summarize the information obtained on habitat variables and describe data acquisition and preparation, describe graphical and statistical analyses of egg loss in relation to habitat variables, and finally discuss the results and provide research recommendations for further field work.

BACKGROUND

Studies of egg loss for herring in Prince William Sound were conducted in 1990, 1991, and 1994. The focus of the 1990 and 1991 studies was to study the effects of oil on egg loss. The lack of sufficient spawning biomass in 1993 and 1994 led to total fishery closures, and renewed interest in egg loss. Studies in 1990 and 1991 did not include collection of data to relate egg loss to habitat, environmental conditions, or predation. The 1994 study collected some information regarding these factors, but the primary research effort will be in 1995. In these studies, the major auxiliary variable used in analyses was depth, although vegetation type was used to estimate calibration factors for different divers.

Methods and results from the 1990 and 1991 studies are found in Biggs-Brown and Baker (1993). Analyses of covariance were conducted with egg abundance as the dependent variable, transects and depth as factors, and days as the covariate, along with several interaction terms; all main effects and interactions were statistically significant. The covariate term included the interaction between depth and transect, which permitted egg loss rate to vary for each combination of depth and transect. The egg loss model explained about 70% of the variability in the data, and most of the explained variability was explained by transect-related parameters. The authors speculated that oil itself was probably not involved in the differences in egg loss, because very little was present at that time. It turned out that transects in previously-oiled areas were in more exposed locations. From this observation, the authors suggested that wave or tidal action is the most important factor in egg loss in Prince William Sound.

One of the critical uses of egg loss information in assessment of the Prince William Sound herring population is to annually estimate spawning biomass from estimates of the number of eggs spawned. Because the survey occurs some days after spawning, some loss of eggs occurs, which requires a correction factor. In the past a correction factor of 10% similar to values found in the literature has been used. However, recent research on herring in Prince William Sound and British Columbia (Biggs-Brown and Baker 1993, Schweigert, pers. comm.) suggests that egg loss is variable across years and across sites and higher than previously thought (Wilcock and Brown 1994). Biggs-Brown and Baker (1993) estimated egg loss in Prince William Sound for the first time and determined a correction factor range of about 10 to 15% for 1990-91 data. The total loss of eggs from the beginning of spawning until hatching ranged from 50% to 91%.

Studies of egg mortality for herring in Prince William Sound were conducted in 1989, 1990, and 1991; results are reported in Baker and Biggs (1993). Again, the focus of these studies was to determine the effect of oil on egg mortality. The major auxiliary variable used was depth. The survival model explained only about 25 to 44% of the variability in the data, and most of the explained variability was due to transect and depth factors. While significant differences between oiled and unoiled areas occurred, the authors found that estimates of egg survival at the time of hatching did not support the conclusion that oil had a direct impact on eggs, for survival of eggs in oiled areas in 1989 (the year of the oil spill) was higher than in unoiled areas. However, there may have been effects on adult females, because survival in oiled areas dropped considerably in oiled areas between 1989 and 1990 and didn't change in unoiled areas. Furthermore, the effect was ameliorated in 1991.

In our analyses, we revisit the analyses of Baker and Biggs-Brown and attempt to explain the variability between transects by other factors: wave exposure, substrate and air exposure. Because transects represent specific locations, the use of transects as a factor does not provide understanding of the possible mechanisms which affect egg loss rates. However once data are analyzed by mechanistic factors, care must be taken to examine which factor levels have data present. Hence, before undertaking statistical analyses, we first perform graphical analyses. Not only are graphical analyses useful for examination of first-order effects, but they are also useful for determining whether interaction terms can be included in statistical models. When various combinations of factor levels do not have data, then interaction terms cannot be included.

MATERIALS AND METHODS

Data Acquisition and Preparation

We traveled to Cordova in late July, 1994, obtained the summarized data from ADF&G, along with SAS files from earlier analyses (Biggs-Brown and Baker 1993, Baker and Biggs 1993), and discussed the analytical framework and future research endeavors. At that meeting, it was agreed that ADF&G and University personnel would work cooperatively to plan future research, including field studies.

Also, we evaluated the suitability of habitat and environmental variables that could be used in analyses. This led to several potential approaches, including classification of dive sites by an exposure dummy variable, the use of fetch as an explanatory variable (in cooperation with the Prince William Sound Science Center), plans for obtaining habitat and physical variables to obtain a habitat variable through cluster analysis (in cooperation with the Prince William Sound Science Center), discussion of avian predation variables (in cooperation with the U.S. Forest Service), area classification (for 1990 and 1991 data, where herring spawn was spread across the Sound), consideration of inter-annual differences in the analysis, the potential effect of egg density (which cannot be done until completion of a GIS system), and the effect of run timing and duration on egg loss (which cannot be done until a long time series has been collected).

Rooper and Evelyn Brown classified each site as a dichotomous exposure variable to wave action (exposed, protected). PWSSC apparently did not have time to look into fetch and there were too few variables for cluster analysis. Avian predation results from the U.S. Forest Service were not used in this preliminary analysis, as they are available only for 1994 data. Remaining available variables included depth, transect, substrate type, year, oiled/unoiled.

Individual transect locations and their classifications by oil, substrate type, year, and wave exposure are shown in Figure 1. Very few exposed transects occurred in 1990 and 1991, and all were on rocky substrate. Most transects were protected, and most were on rocky substrate. Oiled transects occurred in the south Sound; unoiled transects in the north. Thus, the presence of oil is confounded with north/south location; we report our results by presence of oil, but discuss the confounding issue of location and oil in the final section. We also note from inspection of Figure 1 that not all combinations of factor levels are present: all gravel and boulder transects were

protected, the boulder transect occurred only in 1990 and only in the oiled area, and so on. This lack of a balanced design makes comparison of factors more difficult. As the original experiments were not designed to account for habitat variables, it is not surprising that a balanced design did not result.

Another measure of exposure, in this case within the transect, was used to quantify the changes in egg loss over depth. This variable was total exposure to air, at each depth, throughout the incubation period. We used a tide program to calculate the amount of air exposure in hours. We distinguish the two types of exposure by referring to one as wave exposure and the other as air exposure. We use air exposure as an alternate explanatory variable in place of depth.

Statistical Methodology

Statistical Framework

The analysis of egg loss is predicated upon the assumption that the instantaneous rate of egg loss Z is constant over days. Reference day 0 is considered to be the beginning of the spawning period. If $N(t)$ is the number of eggs at reference day t and N_0 is the number of eggs at reference day 0, then

$$N(t) = N_0 e^{-Zt} e^{\varepsilon}$$

where ε is a random error term with mean 0 and constant variance. Taking the logarithm of this equation, one obtains

$$\ln N(t) = \ln N_0 \; - Zt \; + \; \varepsilon$$

showing that a linear regression of ln(egg abundance) versus days can be used to estimate $\ln N_0$ and Z from the y-intercept and slope respectively.

Graphical Analysis

The first part of our analysis was to use graphical techniques to view the data by various breakdowns using combinations of habitat variables. We constructed graphs of ln(egg abundance) versus days for these combinations and plotted the best-fitting linear regression line, the slope of which represents the rate of egg loss (Z).

The various breakdowns examined were:

1. Egg loss by year,
2. Egg loss by depth,
3. Egg loss by substrate type,
4. Egg loss by wave exposure, and
5. Egg loss by oiled/unoiled.

Graphical analysis of the egg loss rate Z was also performed using these same habitat variables. Both depth and air exposure were used as the independent variables in the graphs to determine which variable was a more useful explanatory variable.

Statistical Analysis

We first used the SAS statistical package to repeat the analyses of covariance of the egg loss and mortality data in Baker and Biggs (1993) to assure that our analyses were complementary. We then changed to SYSTAT for graphical and statistical analyses because of its ease of use. We blended the previous data with the new habitat data described above using Rbase and Excel; all data files are now in Excel.

We then conducted analyses to look at habitat as an explanatory variable. Our first dependent variable was the egg loss rate Z and independent variables were the habitat and year variables. We examined several factorial analysis of variance models with various interaction terms and sequentially removed terms that were not significant. Due to time constraints we did not perform analyses of covariance on the log(egg abundance) data, but we intend to later on.

RESULTS

To analyze the 1990 and 1991 data, linear regressions were fitted to the data points for each transect. Graphs in Appendix A show data from individual transects. The transects were separated by depth, giving a total of five possible regressions for each transect (one at each depth). The estimates of instantaneous rate of egg loss Z for each transect, summarized in Table 1, are highly variable. The Z values were also used as the dependent variable for factorial analyses to evaluate the significance of the various habitat breakdowns.

The 1994 data are represented graphically in Appendix A, but have not yet been statistically analyzed.

Graphical Results

Graphical analysis of the 1990 and 1991 ln(egg abundance) data is shown by transect and by combination of habitat variables. The regression lines inserted on the graphs show the instantaneous egg loss rates Z (slopes), as well as the ln(number of eggs at time of spawn) (y-intercepts).

Figure 2 shows ln(egg abundance) broken down by substrate type and depth. Since the factorial analysis below did not show significant inter-annual variation for these two years (1990 and 1991), data from both years were combined. Sufficient data for these graphs were available for protected transects only. The rocky substrate is represented by four transects, the boulder by one transect, and the gravel by three transects. The slopes appear to become less steep as depth increases, suggesting that egg loss rate decreases at lower depths.

Figure 3 complements Figure 2, showing the instantaneous egg loss rates (Z) versus air exposure and also versus depth for the same substrate breakdowns. Egg loss rates from individual transects at each depth were plotted by substrate type. The regressions corresponding to boulder and gravel substrates were not significant ($P>0.05$). The regression of instantaneous egg loss rate on total air exposure for the rocky transects was highly significant ($P<0.005$). This may be a reflection of the larger amount of data available for rocky transects. Slopes using air exposure were more negative in magnitude than slopes using depth, indicating that air exposure may be a more useful variable than depth.

Figures 4 and 5 summarize the graphical results of the oiled/unoiled breakdowns. Figure 3 shows ln(egg abundance) data from rocky, protected transects, both oiled and unoiled. Rocky protected transects were used in an attempt to eliminate any noise associated with substrate and/or wave exposure differences. The majority of transects from these two years were rocky (eleven out of fifteen), and all exposed transects were on rocky substrates. This breakdown enabled us to get the most data points for examination of other habitat variables. The unoiled data is represented by five transects, the oiled data by three. The ln(egg abundance) data for these transects are plotted against days from spawning. As in Figure 2, egg loss rate appears to decrease as depth increases.

Figure 5 shows the corresponding instantaneous egg loss rates (Z) versus air exposure and depth. The egg loss rates were used as dependent variables for regressions within each category, oiled or unoiled. Regressions from oiled transects were insignificant ($P>0.05$), while regressions from unoiled transects were found to be highly significant ($P<0.005$).

Using the same techniques as above, we examined egg loss by wave exposure. Figures 6 and 7 summarize the results of this analysis. Figure 6 shows ln(egg abundance) versus days from spawning by depth for transects determined to be either protected or exposed. The substrate variable was accounted for by choosing only those exposed and protected transects occurring within a rocky substrate. Again this combination was used to maximize the number of data points available for analysis. The exposed data is represented by three transects, the protected data by eight. As in Figures 2 and 4, egg loss rate appears to decrease with depth.

Figure 7 shows the corresponding instantaneous egg loss rates (Z) plotted against air exposure and depth. Regressions of these egg loss rates were significant for both exposed and protected transects ($P<0.05$).

Statistical Results

A factorial analysis was carried out on the combined 1990 and 1991 data using instantaneous egg loss rate Z as the dependent variable. This analysis attempted to measure the effects of the various habitat variables used in the graphical analysis and any interaction effects between the variables. First, we selected year, substrate, oiled/unoiled, wave exposure, and depth as independent variables. Due to missing data in some cells, this analysis could only be carried out on main effects (Table 2). Year and substrate type were found to be insignificant factors: year was

highly insignificant (P=0.62) and substrate type was also insignificant (P =0.110). The significant factors were oiled/unoiled, wave exposure, and depth (P<0.05).

If data are pooled over substrate, then a variety of two-way interactions can be analyzed for the remaining factors (Table 3). None of the interaction terms between habitat variables was significant in this analysis. These interaction terms were removed from the model one at a time, which resulted in only the individual habitat variables remaining in the model (Table 4).

Data from the rocky substrate were also sufficient to undertake a factorial analysis of Z as a function of habitat variables with interactions (Table 5). None of the interaction terms between habitat variables was significant in this analysis. These interaction terms were removed from the model one at a time, which resulted in only the individual habitat variables remaining in the model (Table 6).

Depth was the variable that had the highest statistical significance and that accounted for the most variability of egg loss rates (roughly 30%). It accounted for more variability than all others combined in two of the analyses (Tables 4 and 6). This suggests that depth was the most important factor affecting egg loss rates in 1990 and 1991. Other habitat variables of significance include wave exposure and oiled/unoiled condition.

An analysis of covariance was performed to determine the validity of air exposure as a substitute for depth. Figures 3, 5, and 7 show differences between using depth or air exposure as an independent variable. Table 7 summarizes the factorial analysis with air exposure, which is analogous to Table 2 with depth. Table 8 summarizes the statistical differences by comparing the contribution to sum of squares with either depth or air exposure included in the model. This analysis was performed with the entire data set and only data from rocky transects to examine the sensitivity of the results.

An F-test was performed to determine if the two models were significantly different. The conclusion was that the models with air exposure were not significantly different than those models using depth (P>0.50). This result was consistent for both data sets.

The benefit of using air exposure instead of depth is that it would reduce the number of parameters estimated (one rather than five) without significantly increasing variation. The total exposure to air for each depth at each transect is plotted in Figure 9. This shows the exponential increase of total air exposure as depth decreases.

Table 8 also presents the percent variability explained by the habitat models. The various models explained from 50-87% of the variability in egg loss. Air exposure models explained about 10% less of the total variability compared with depth models.

Table 9 summarizes the average instantaneous egg loss rates (Z) for the habitat factors found to be significant. Averages and corresponding standard errors of egg loss rates were calculated from Table 1 for all levels of the depth, wave exposure and oiled/unoiled factors. Average egg loss rate decreases with increasing depth. Average egg loss rate is higher for

protected transects than for exposed transects, which is a counterintuitive result. Average egg loss rate is higher for oiled (southerly) transects than for unoiled (northerly) transects.

DISCUSSION AND RECOMMENDATIONS

Reported egg loss rates and resultant survival through the egg stage of Pacific herring are highly variable, with survival as low as 1% (Outram 1958, Palsson 1984). Causes of egg loss have been identified as predation, desiccation, and wave action. Predators include birds, notably gulls, diving ducks, and shorebirds; marine invertebrates; and fish - although the latter have received little study. Bird predators have often been found to contribute most predatory mortality (Palsson 1984, Haegele 1993); however, on the west coast of Vancouver Island, Haegele and Schweigert (1989) found that invertebrates, birds and mammals (gray whales) consumed 13, 4 and 3 percent of herring eggs, respectively. Because substantial amounts of eggs are deposited in intertidal zones, desiccation and wave action are potential egg loss factors. Desiccation has been implicated as a cause of reduced survival in upper intertidal zones (Jones 1972), and wave action has been found to cause substantial egg loss (Hay and Miller 1982).

As depth of egg deposition increases, the egg loss rate apparently decreases (Baker and Biggs 1993). This pattern could be due to predation by non-diving birds, such as gulls; desiccation and wave action. Our results indicate that depth was the variable that accounted for most of the variation in egg loss. The significant results in our ANOVA procedures are clearly a function of increasing egg loss rates at higher depths (Figure 7). Biggs-Brown and Baker (1993) had suggested it would not be possible to obtain significant regressions of egg density on egg age in Prince William Sound. However, we did not find this to be the case. Our use of instantaneous egg loss rates as the dependent variable was based on the relatively high number of significant negative slopes among the regressions of egg abundance on time (Table 1). The slopes were significant in 41 of 66 regressions. Of the significant regressions, six had positive slope, and four of those six were at one transect in the rocky exposed habitat, suggesting that spawning occurred in that transect area after the initial survey. Wave exposure does not prove to be significant with the removal of this transect from the analyses. Therefore, if there were strong evidence for such spawning, those transects should probably be removed from analyses.

In our analyses, we found no significant interactions between factors, which means that main effects alone can be examined. One caveat to this conclusion is that the sampling design was not fully balanced, so that complete understanding of main effects and interactions of the factors in this study cannot be had. Our results indicate that depth within the intertidal zone is the most important variable affecting egg loss rates. The mortality factors that would be consistent with this pattern are predation by non-diving birds, desiccation, and wave action. Two of the processes, predation and desiccation, are probably a function of the amount of time the eggs are exposed. Consequently, we conclude that the variable of interest should be the amount of air exposure, rather than the depth (height above or below the 0 tide mark). The amount of air exposure is not a linear function of depth (Figure 9), and it will vary among years, depending on the time when eggs are present.

We investigated this variable by estimating the time of air exposure at the depths sampled using a tide program for 1990 and 1991. Air exposure explained almost the same amount of variation in egg loss rate as depth, but with four fewer parameters. The model with air exposure was not significantly different from the model with depth, suggesting that the air exposure model is more parsimonious. The regression graphs with air exposure as the independent variable indicate that the depths sampled were not appropriate to resolve the effect of air exposure. In subsequent programs, samples should be taken at depth intervals that cover a series of intervals on the air exposure scale.

Although depth was the most important variable in determining egg loss rates, two other variables we examined - oil and wave exposure - were significant or nearly so (Tables 2, 4, 6, 7).

The significance of wave exposure is intuitive, although our results are counterintuitive: average egg loss was higher for protected transects than for exposed ones. As wave exposure was classified after the fact and not directly measured, this result may simply be a result of some other lurking variable related to location (Figure 1). Nevertheless, future sampling should be designed to quantify the effects of wave exposure.

The significance of oil is more problematic. The confounding of north/south location and presence of oil means that these two factors cannot be separated with the data at hand. Five years after the oil spill, it is unlikely that oil could have a direct effect on egg loss. Furthermore, the spawning locations of herring have contracted to the areas around Montague Island and may not change much until the herring stock rebounds. Thus, location is probably not an important variable to be measured in the short-term.

Depth and wave exposure accounted for much of the variation in instantaneous egg loss rates, suggesting that an egg mortality model based on those variables might adequately describe egg loss in the Sound. However, if spawning expands in the next few years beyond Montague Island back to the normal range, our results suggest that egg loss may vary by north/south location or some other geographic classification.

The lack of significance of year as a factor in explaining egg loss rate is heartening. The lack of inter-annual variability in egg loss rate makes it possible to derive simple calibration factors for the survey. Nevertheless, inter-annual variability in spawning location and abundance is substantial and may be related to environmental, biological and ecological factors. Our study did not address these variables.

The clear pattern of increasing rates of egg loss at locations higher in the intertidal may be used as an indication of which processes are most important in the depth-related patterns of egg loss rates. For example, the higher egg loss rates at depths with more air exposure could be due to non-diving avian predators, such as gulls. However, those egg loss patterns should not be interpreted as an indication of the relative importance of various processes in egg loss; for example, these results do not indicate that gull predation is more important than predation by diving birds such as scoters. Total egg loss is a function of both egg loss rate and abundance. Our

analyses have focused on egg loss rates. Future analyses will include egg abundance with the goal of estimating total egg loss as a function of depth (or air exposure), wave exposure, and habitat.

Other factors previously considered could also explain variability in egg loss rates. Bird predation, fish predation, vegetation type, desiccation and fungal infection could affect egg loss and mortality. Consideration should be given to whether site specific information (transect*depth) can be collected during subsequent field seasons within time and cost constraints. We intend to extend this analysis to include bird predation, fish predation and vegetation type. These results will be presented in the final project report.

Recommendations for Field Research

Future studies on egg loss in Prince William Sound should be designed to enhance quantification of the processes involved in egg loss, and to allow formulation of a Sound-wide model of egg loss. Results from our initial studies suggest several ways in which future studies could facilitate completion of those objectives, including:

1) Depths sampled should be expanded to include a series of samples taken at intervals on the air exposure scale. Basically, this would entail adding several depths between the +1 and +5 depths sampled in previous studies.

2) The effects of wave exposure need to be quantified. We recommend that a balanced sampling design be developed to examine wave exposure at rocky habitat sites around Montague Island. This sampling program should incorporate the development of criteria for scoring wave exposure of study sites and some methods for confirming the classification of sites with actual measurements of wave action.

3) We recommend that some sampling be conducted to quantify the effects of subtidal predators on egg loss. Bird predation is being examined by a separate program, but no studies are being conducted on fish and invertebrate predators. On Vancouver Island, Haegele and Schweigert (1989) found that invertebrate predators removed about four times as many eggs as did bird predators; therefore, we suggest that some sampling be done to assess egg loss from fish and invertebrate predation.

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Table 1. Summary of transect regressions by habitat variable and depth. For each transect the slope, y-intercept, coefficient of determination R^2, and p-value for the significance of the slope are shown.

		Rocky Exposed Transects			Rocky Protected Transects								Gravel and Boulder Protected Transects			
depth	transect*	O22	O26	O28	C2	C6	C12	C15	C21	O18	O20	O25	C3	O23	O24	O21
5	slope	-0.074	-0.237	-0.016			-0.032		-0.114				0.001	-0.056	-0.038	
	intercept	3.228	2.525	2.429			2.258		3.062				1.741	2.711	2.432	
	R^2	0.425	0.814	0.098			0.753		0.828				0.000	0.469	0.328	
	p-value	0	0.098	0.060			0		0				0.887	0	0	
1	slope	-0.049	-0.007	0.013	-0.050	-0.022	-0.017	-0.024	-0.048	-0.112	-0.017	-0.065	-0.002	-0.003	-0.015	-0.078
	intercept	3.217	2.378	2.368	2.432	2.502	2.309	1.755	2.214	3.458	-0.633	2.895	2.292	1.749	1.194	3.319
	R^2	0.386	0.011	0.183	0.162	0.095	0.155	0.201	0.306	0.472	0.014	0.214	0.001	0.001	0.012	0.517
	p-value	0	0.545	0.010	0.005	0.012	0.057	0.024	0.021	0	0.802	0.020	0.785	0.830	0.437	0
0	slope	-0.046	-0.008	0.025	-0.009	-0.042	-0.009	-0.038	-0.043	-0.109	-0.048	-0.016	-0.018	-0.041	-0.053	-0.035
	intercept	3.200	2.368	2.072	1.680	2.907	2.722	2.358	2.396	3.410	0.344	2.011	2.631	1.500	2.157	2.661
	R^2	0.400	0.017	0.439	0.007	0.364	0.097	0.489	0.467	0.559	0.093	0.009	0.099	0.100	0.226	0.239
	p-value	0	0.457	0	0.563	0	0.131	0	0	0	0.084	0.660	0.011	0.015	0	0.001
-5	slope	-0.014	-0.002	0.013	0.003	0.005	-0.015	-0.016	-0.007	-0.066	-0.056	-0.011	-0.015	-0.007	-0.038	-0.063
	intercept	3.001	2.404	1.949	2.442	2.501	2.815	2.271	2.390	1.622	1.702	1.606	1.606	0.685	2.353	2.779
	R^2	0.117	0.001	0.076	0.009	0.025	0.240	0.267	0.017	0.201	0.193	0.017	0.036	0.004	0.281	0.304
	p-value	0.008	0.878	0.110	0.463	0.212	0.013	0.002	0.534	0.005	0.003	0.580	0.139	0.645	0	0
-15	slope	-0.017	0.025	0.006	0.011	0.009	-0.044	-0.022	-0.019		-0.023		-0.015	-0.065	-0.066	-0.052
	intercept	3.219	0.871	2.598	2.023	2.276	2.453	2.414	2.184		1.814		2.508	1.842	1.530	2.226
	R^2	0.189	0.170	0.019	0.028	0.060	0.380	0.274	0.121		0.100		0.069	0.262	0.205	0.252
	p-value	0	0.036	0.435	0.174	0.040	0.007	0.002	0.088		0.027		0.035	0.001	0.002	0.001

*C denotes unoiled transect
*O denotes oiled transect

Table 2. Results of factorial analysis of egg loss rate Z, as a function of habitat variables for combined data (1990-1991)

Analysis of Variance

Dependent Variable: Instantaneous Egg Loss Rate, Z

Multiple R: 0.565 Squared Multiple R: 0.319

Source	Sum of Squares	DF	Mean Square	F-Ratio	P
Year	0.000	1	0.000	0.248	0.620
Substrate	0.006	2	0.003	2.298	0.110
Oiled/Unoiled	0.009	1	0.009	6.983	0.011
Wave Exposure	0.006	1	0.006	5.016	0.029
Depth	0.021	5	0.004	3.424	0.009
Error	0.070	57	0.001		

Table 3. Results of factorial analysis with two way interactions of egg loss rate as a function of habitat variables excluding substrate, with combined data (1990-1991).

Analysis of Variance

Dependent Variable: Instantaneous Egg Loss Rate, Z

Multiple R: 0.697 Squared Multiple R: 0.486

Source	Sum of Squares	DF	Mean Square	F-Ratio	P
Year*Wave Exposure	0.000	1	0.000	0.379	0.541
Year*Oiled/Unoiled	0.001	1	0.001	0.827	0.368
Depth*Wave Exposure	0.002	4	0.001	0.512	0.727
Depth*Oiled/Unoiled	0.001	4	0.000	0.256	0.904
Depth*Year	0.008	4	0.002	1.603	0.191
Wave Exposure	0.002	1	0.002	1.423	0.239
Year	0.000	1	0.000	0.195	0.661
Oiled/Unoiled	0.005	1	0.005	3.944	0.053
Depth	0.013	4	0.003	2.851	0.035
Error	0.052	44	0.001		

Table 4. Results of factorial analysis of egg loss rate with two way interactions and the substrate variable removed. Combined data (1990-1991).

Analysis of Variance

Dependent Variable: Instantaneous Egg Loss Rate, Z

Multiple R: 0.499 Squared Multiple R: 0.249

Source	Sum of Squares	DF	Mean Square	F-Ratio	P
Year	0.000	1	0.000	0.047	0.830
Oiled/Unoiled	0.008	1	0.008	6.276	0.015
Wave Exposure	0.004	1	0.004	3.446	0.069
Depth	0.016	4	0.004	3.167	0.020
Error	0.076	58	0.001		

Table 5. Results of factorial analysis of egg loss rate with two way interactions. Data within rocky substrate only, both years combined (1990-1991).

Analysis of Variance

Dependent Variable: Instantaneous Egg Loss Rate, Z

Multiple R: 0.705 Squared Multiple R: 0.497

Source	Sum of Squares	DF	Mean Square	F-Ratio	P
Year*Depth	0.006	4	0.001	0.990	0.427
Depth*Wave Exposure	0.004	4	0.001	0.666	0.621
Wave Exposure	0.003	1	0.003	2.049	0.162
Year	0.000	1	0.000	0.083	0.775
Oiled/Unoiled	0.005	1	0.005	3.249	0.081
Depth	0.010	4	0.003	1.786	0.157
Error	0.045	31	0.001		

Table 6. Results of factorial analysis of egg loss rate excluding two way interactions. Data from rocky substrate only, both years combined (1990-1991).

Analysis of Variance

Dependent Variable: Instantaneous Egg Loss Rate, Z

Multiple R: .635 Squared Multiple R: 0.403

Source	Sum of Squares	DF	Mean Square	F-Ratio	P
Year	0.001	1	0.001	0.416	0.523
Oiled/Unoiled	0.006	1	0.006	4.442	0.042
Wave Exposure	0.007	1	0.007	4.801	0.034
Depth	0.030	4	0.008	5.574	0.001
Error	0.053	39	0.001		

Table 7. Results of factorial analysis of egg loss rate Z, as a function of habitat variables for combined data (1990-1991). Total air exposure used instead of depth (compare to table 2).

Analysis of Variance

Dependent Variable: Instantaneous Egg Loss Rate, Z

Multiple R: 0.525 Squared Multiple R: 0.276

Source	Sum of Squares	DF	Mean Square	F-Ratio	P
Year	0.000	1	0.000	0.039	0.844
Substrate	0.005	2	0.003	2.063	0.136
Oiled/Unoiled	0.009	1	0.009	7.557	0.009
Wave Exposure	0.006	1	0.006	5.117	0.006
Total air exposure	0.017	1	0.017	13.646	0.000
Error	0.074	61	0.001		

Table 8. Comparisons of sums of squares using depth or total air exposure as variables.

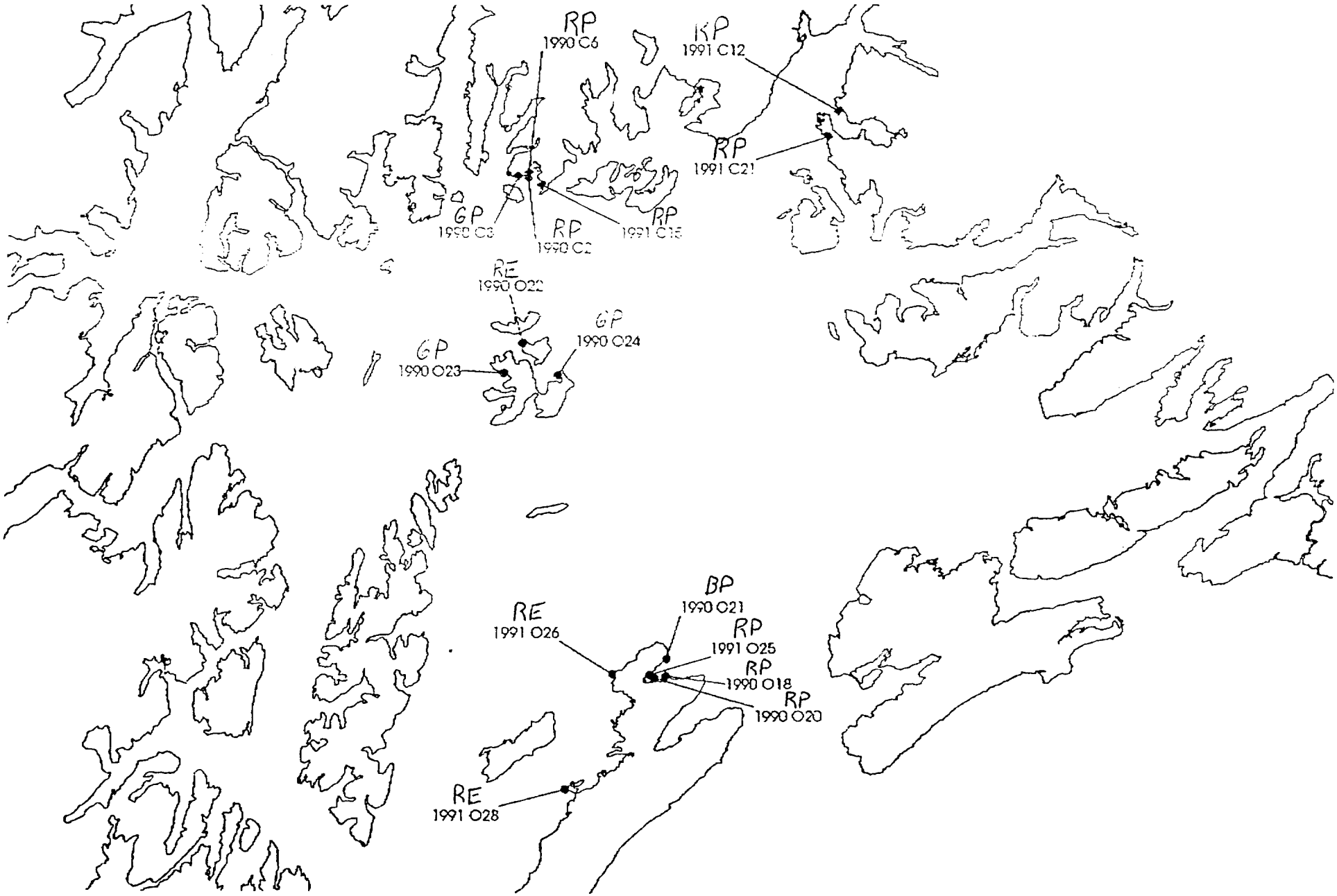
Sum of Squares

Source	Using depth/all data combined	Using total air exposure/ all data combined	Using depth/ rocky transects only	Using total air exposure/ rocky transects only
Oiled/Unoiled	0.009	0.009	0.008	0.008
Wave Exposure	0.006	0.006	0.008	0.009
Substrate	0.006	0.005		
Year	0.000	0.000		
Depth	0.021		0.031	
Total air exposure		0.017		0.027
Total	0.042	0.037	0.047	0.044
Error	0.070	0.074	0.054	0.058
% Variability Explained	60.0%	50.0%	87.0%	75.9%
F-test P-value	F=0.814 ns(P>0.50)		F=0.759 ns(P>0.50)	

Table 9. Mean instantaneous egg loss rates Z for all data combined and rocky substrate only, with standard errors.

		All Data Combined	Rocky Substrate Data Only
Wave Exposure	Exposed	**-0.026** 0.017	**-0.026** 0.017
	Protected	**-0.034** 0.004	**-0.033** 0.006
Oiled/Unoiled	Oiled	**-0.039** 0.007	-0.036 0.011
	Unoiled	**-0.022** 0.005	**-0.018** 0.006
Depth	5 ft	**-0.071** 0.027	-0.095 0.039
	1 ft	**-0.033** 0.009	**-0.036** 0.010
	0 ft	**-0.033** 0.008	**-0.031** 0.010
	-5 ft	**-0.019** 0.006	**-0.015** 0.007
	-15 ft	**-0.021** 0.008	**-0.008** 0.007

Figure 1. Prince William Sound transect locations for 1990 and 1991. Codes denote variable classifications for substrate and wave exposure.



*R denotes rocky substrate
*G denotes gravel substrate
*B denotes boulder substrate

**E denotes wave exposed
**P denotes wave protected

***O denotes oiled
***C denotes unoiled

Figure 2. Ln(egg abundance) by substrate type (combined 1990-1991 data, protected transects only), along with best fitting regression line. The negative of the slope is our best estimate of Z.

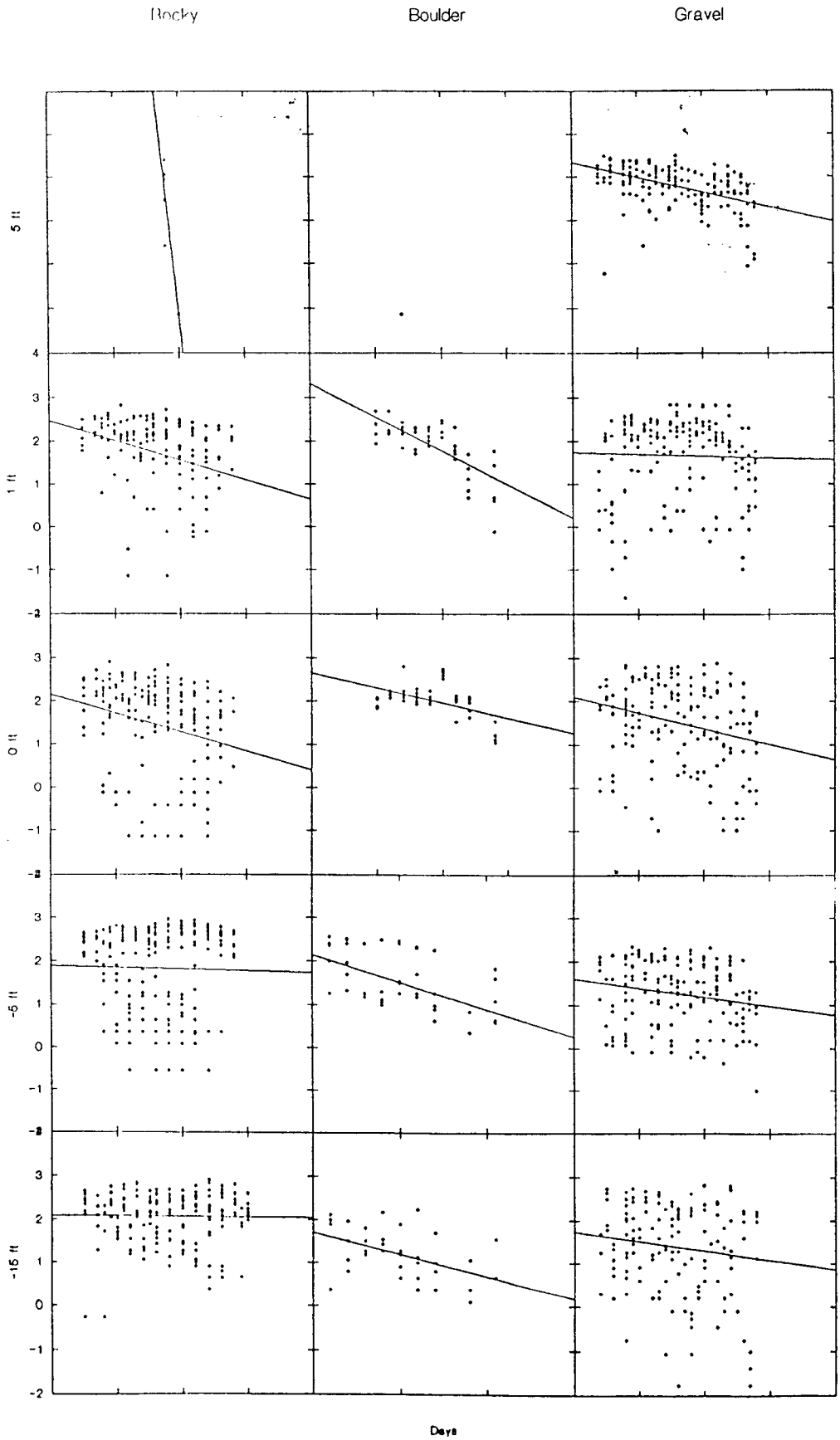
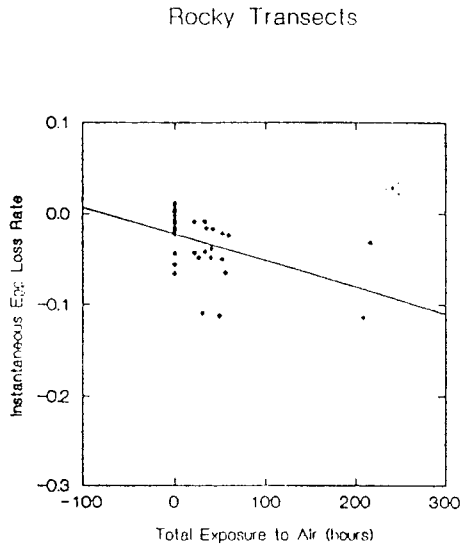
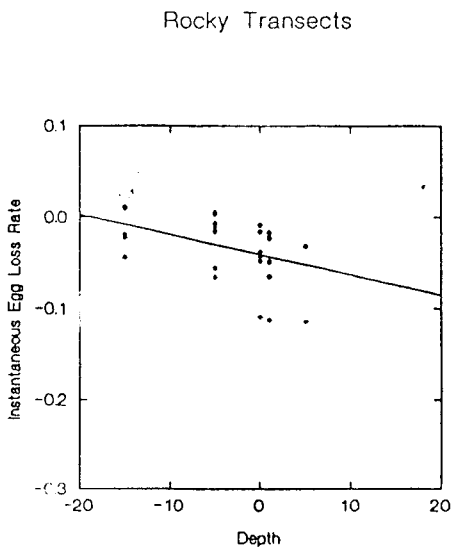
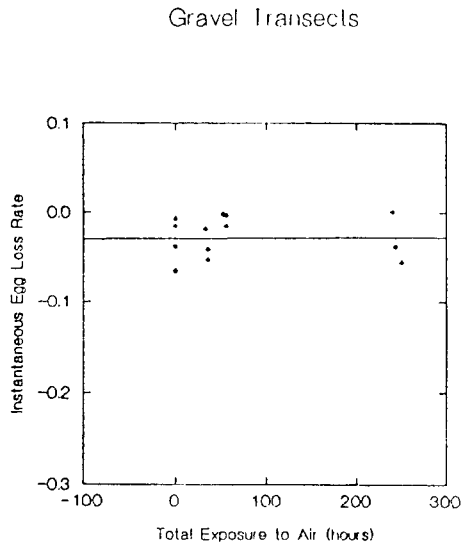
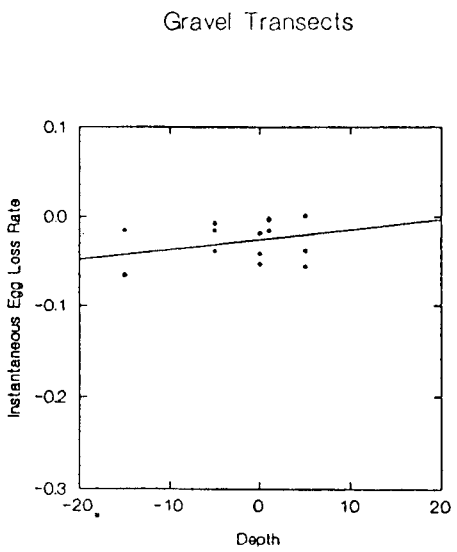


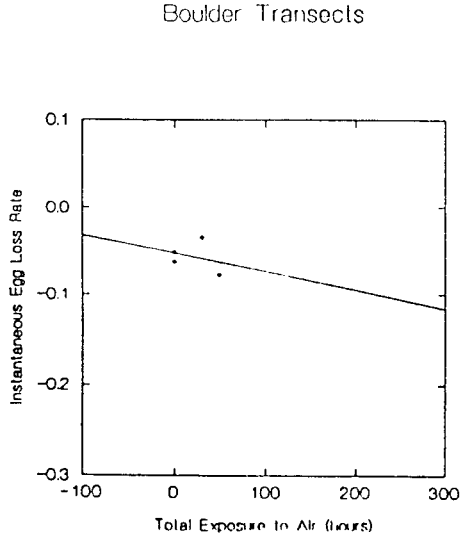
Figure 3. Instantaneous egg loss rates (Z) by substrate type. Data from protected transects, 1990-1991 combined.











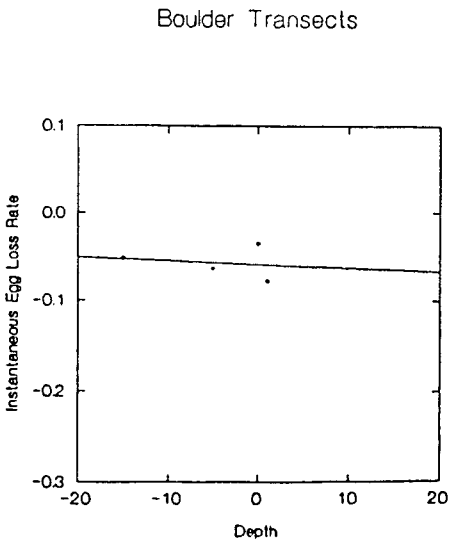


Figure 4. Ln(egg abundance) for oiled and unoiled transects (combined 1990-1991 rocky protected data) showing best fitting regression line. The negative of the slope is our estimate of Z.

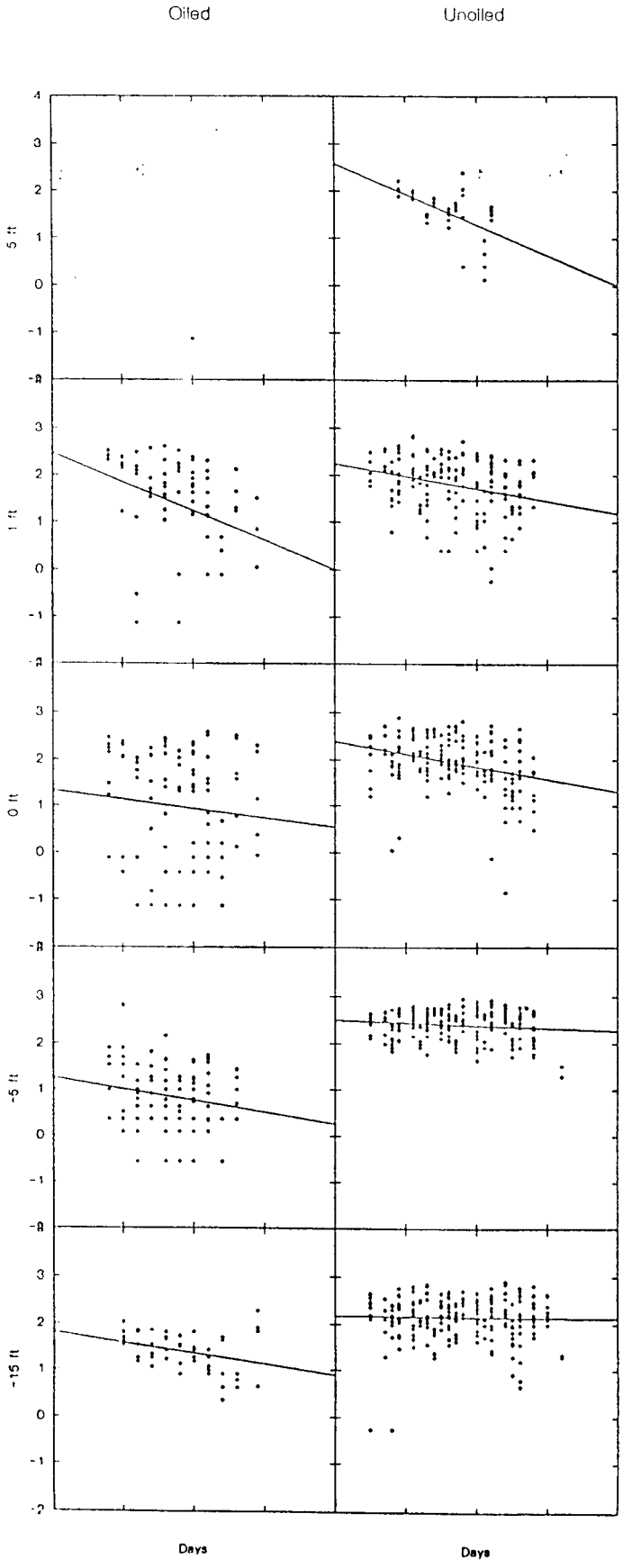


Figure 5. Instantaneous egg loss rates (Z) for oiled and unoiled transects. Data from rocky protected transects, 1990-1991 combined.

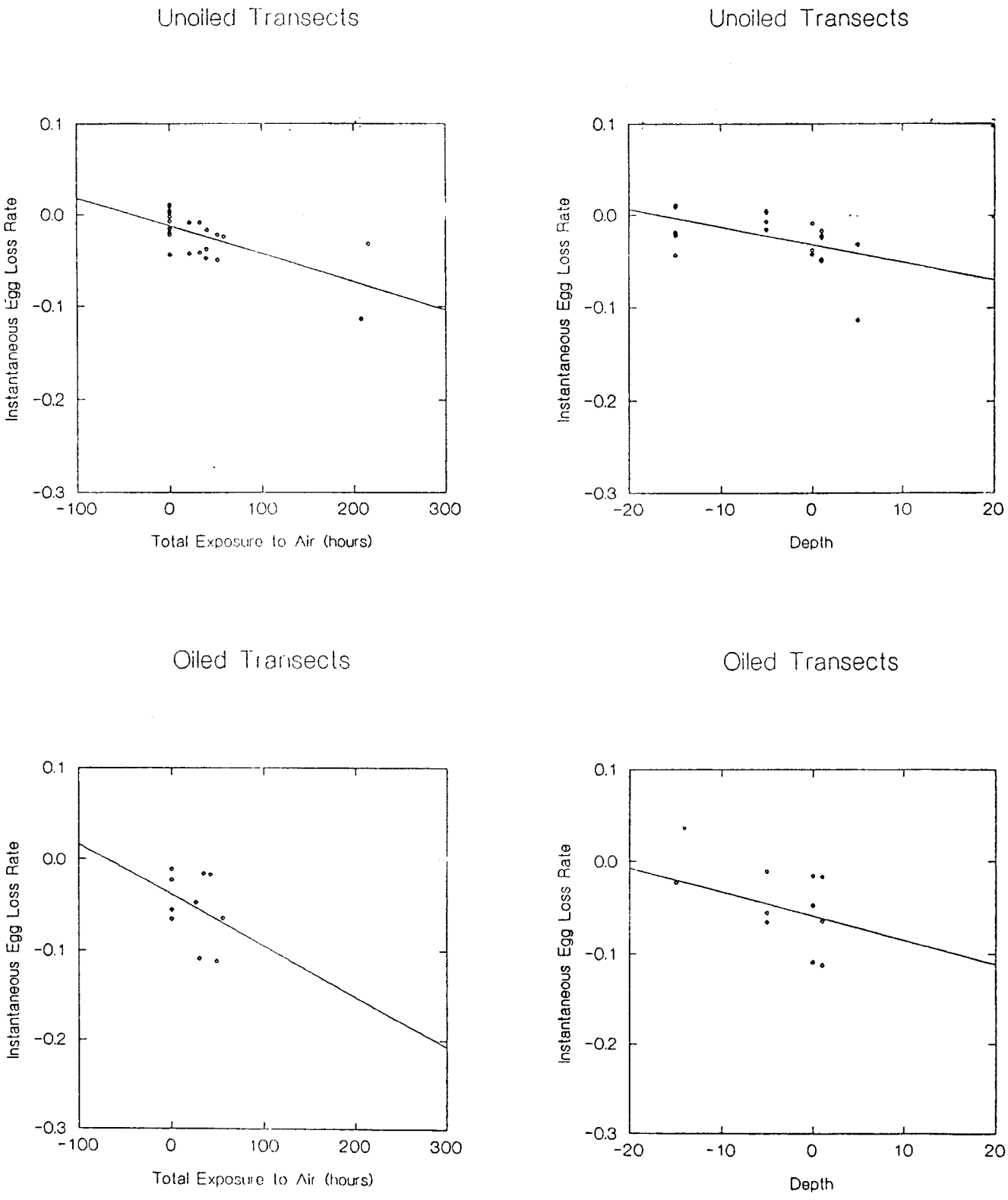


Figure 6. Ln(egg abundance) for exposed and protected transects (combined 1990-1991 rocky data) showing best fitting regression line. The negative of the slope is our estimate of Z.

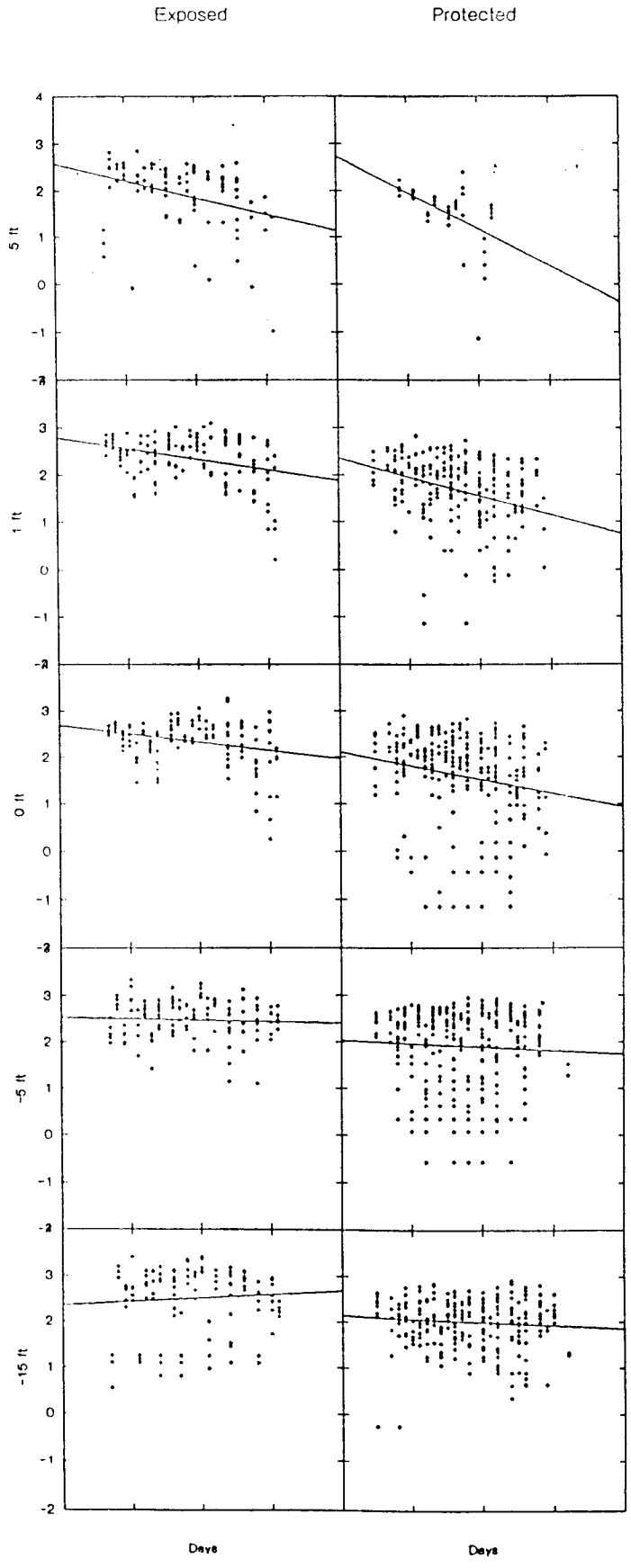
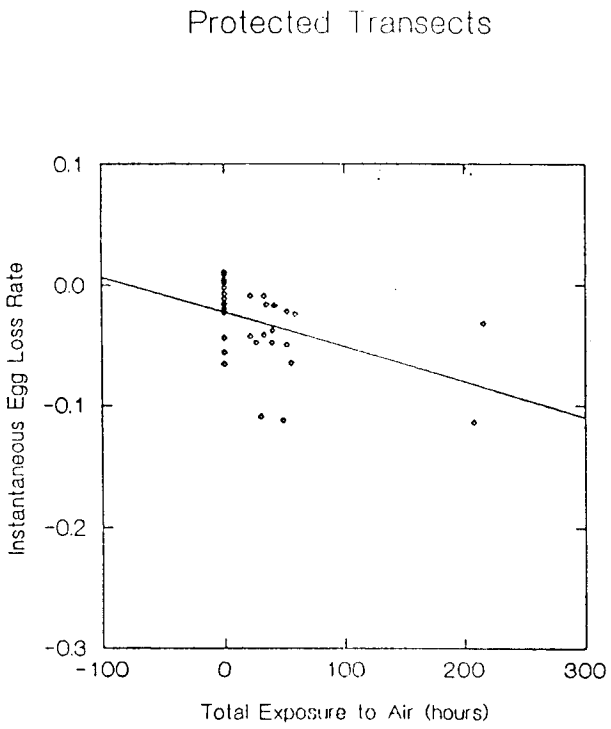
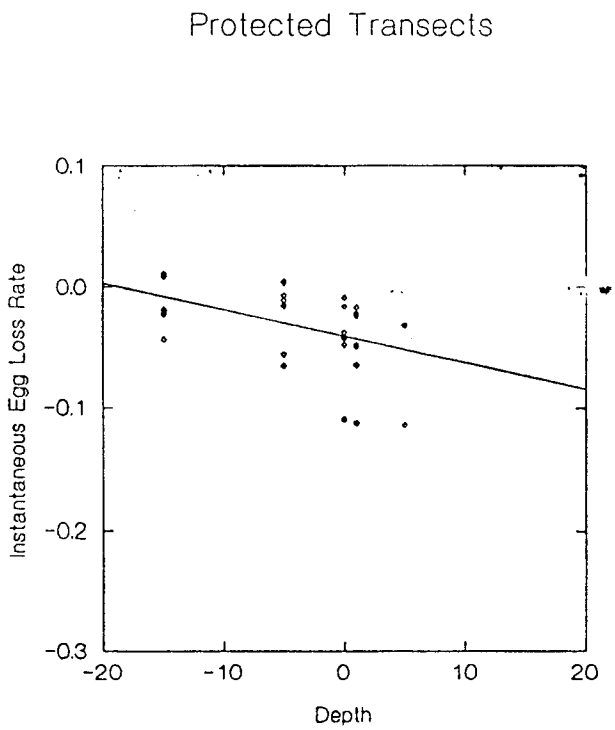
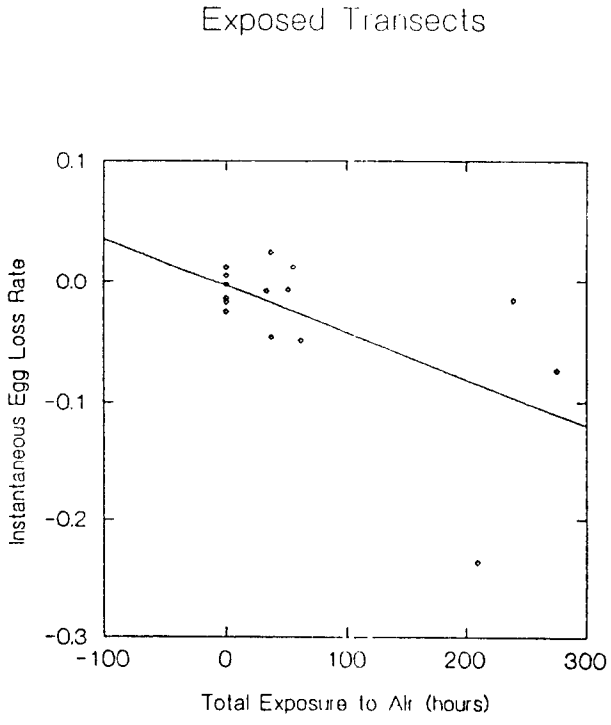


Figure 7. Instantaneous egg loss rates (Z) for exposed and protected transects. Data from rocky transects, 1990-1991 combined.







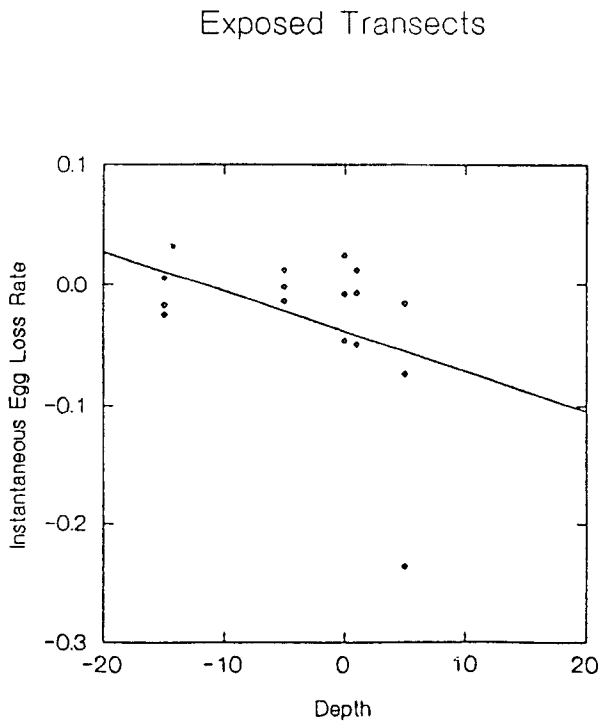


Figure 8. Mean instantaneous mortality rates of herring eggs in rocky protected habitats, error bars are one standard error, sample sizes are indicates on the graph.

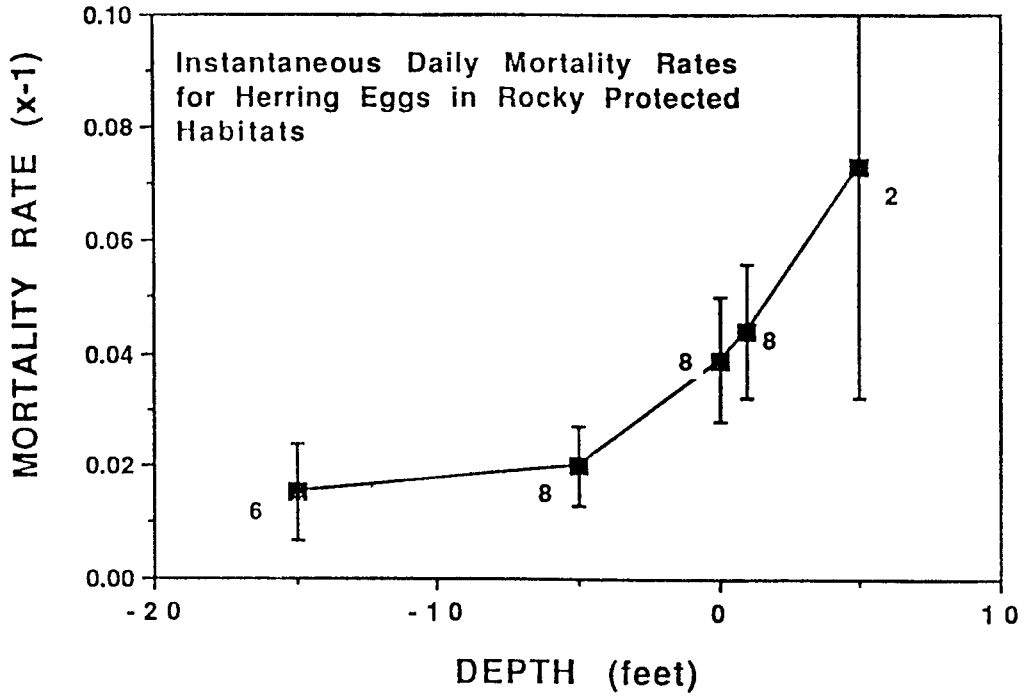
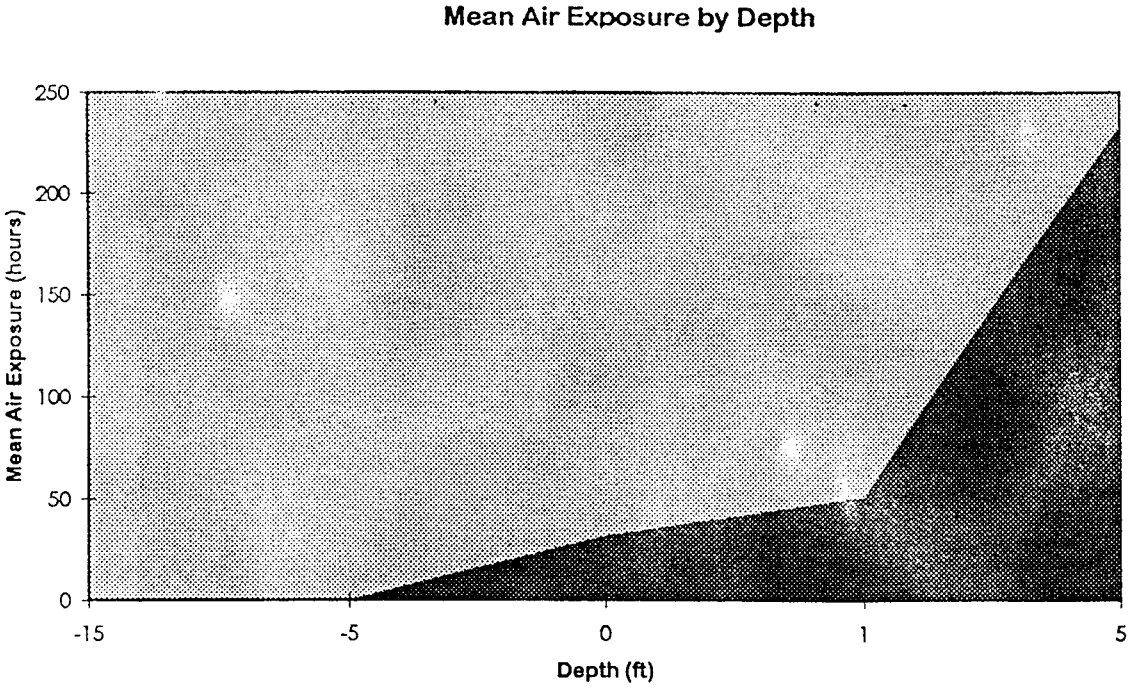


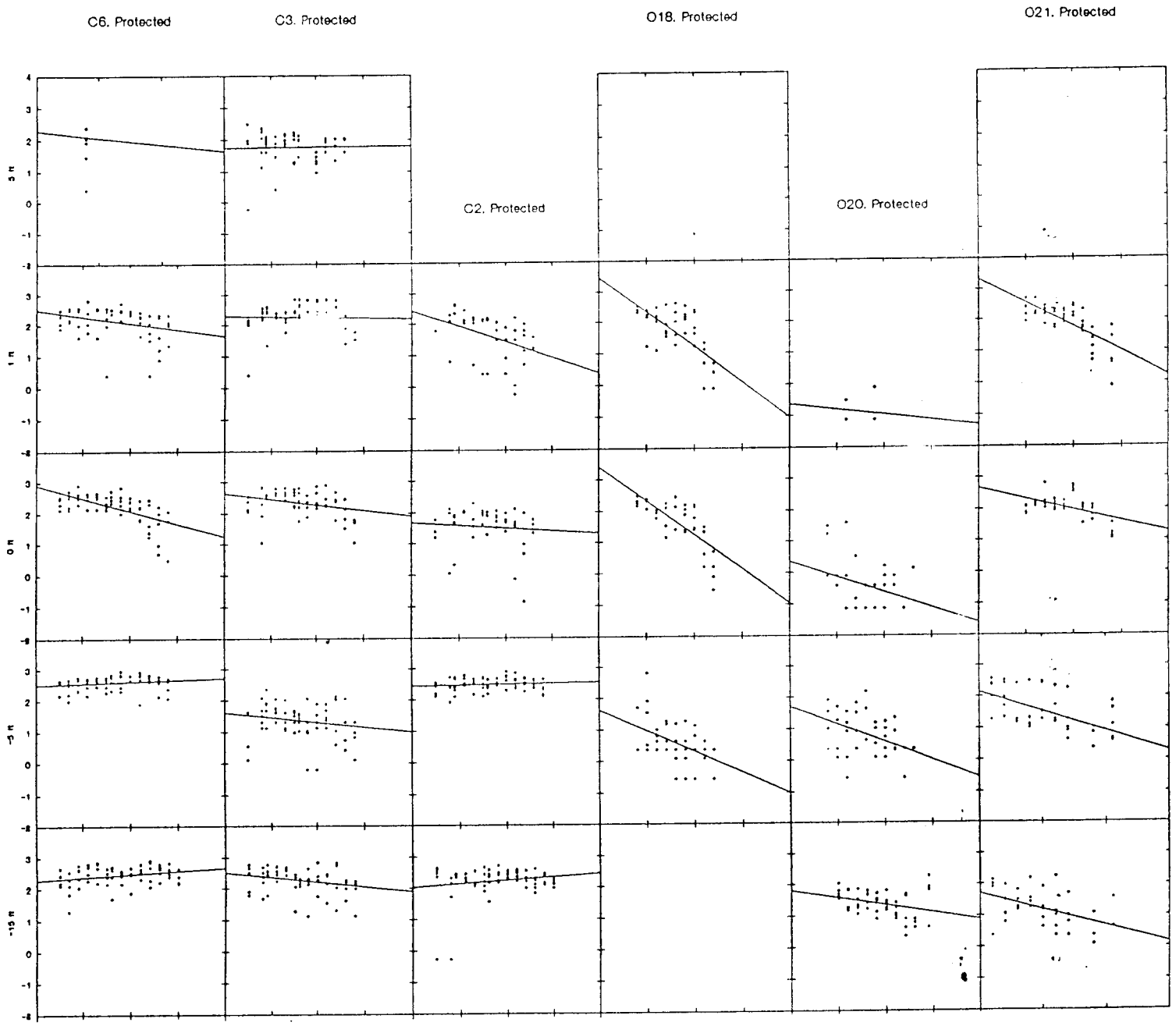
Figure 9. Mean time of air exposure by depth.



APPENDIX A

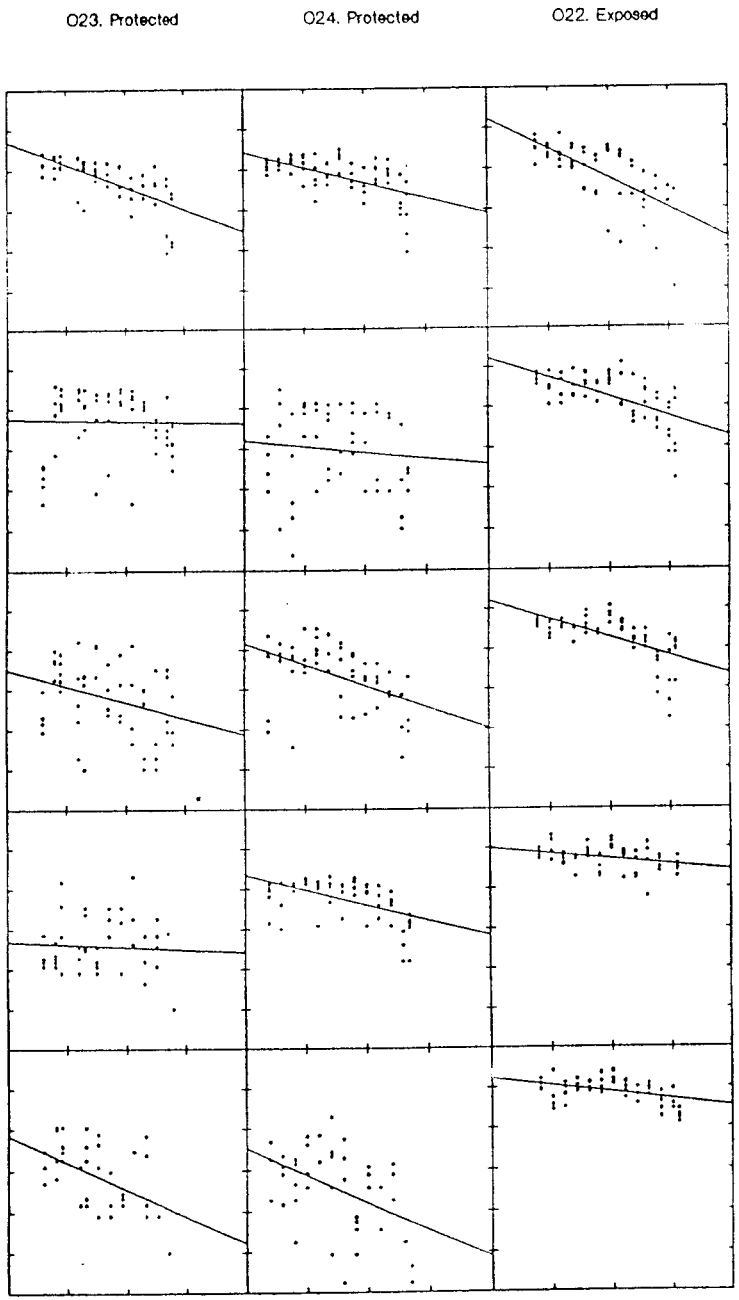
Appendix A.

Figure 1. Ln(egg abundance) for each transect by depth 1990, along with best fitting regression line, the negative of the slope is our estimate of Z.



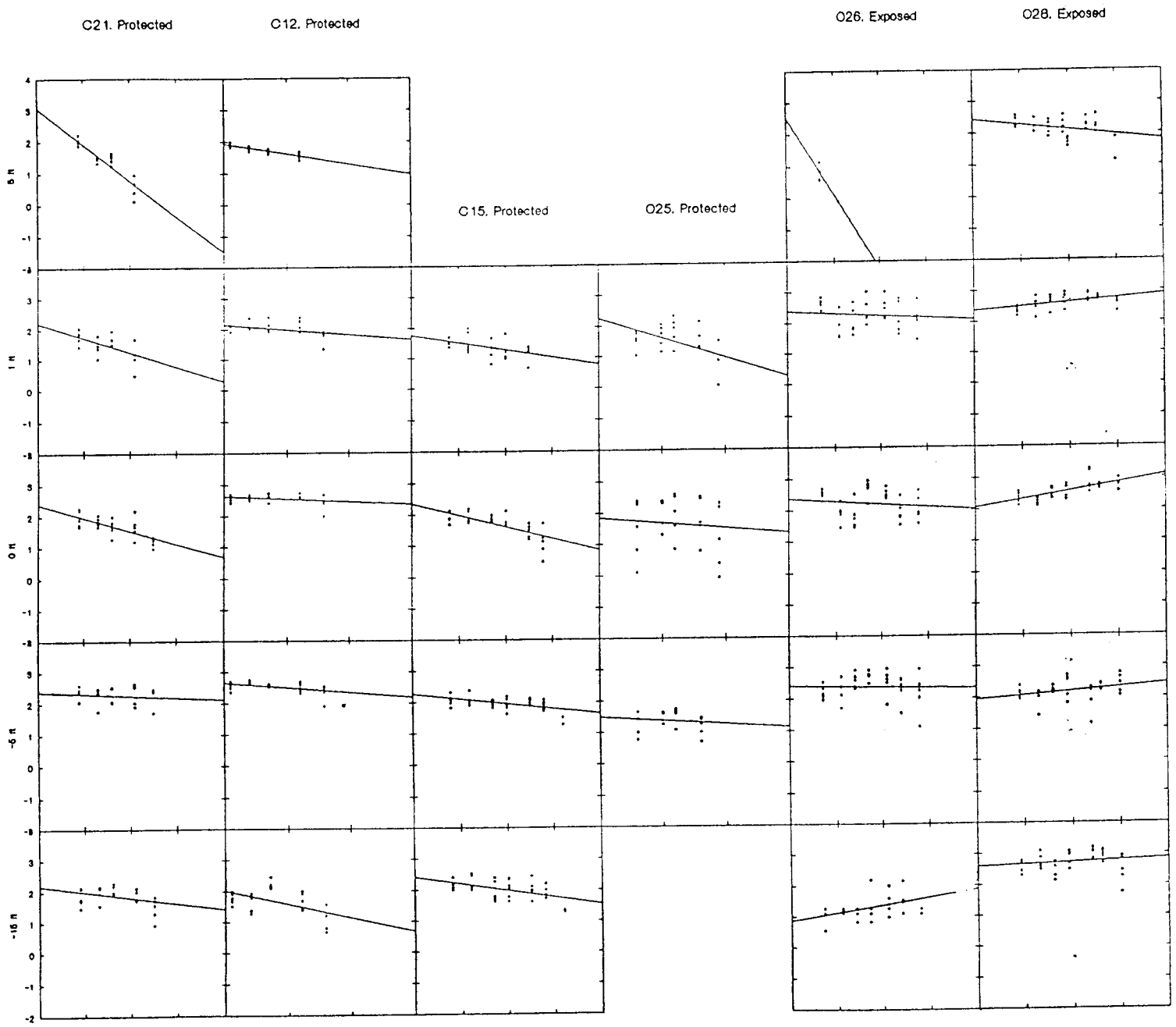
Appendix A.

Figure 1 (cont). Ln(egg abundance) for each transect by depth 1990, along with best fitting regression line, the negative of the slope is our estimate of Z.



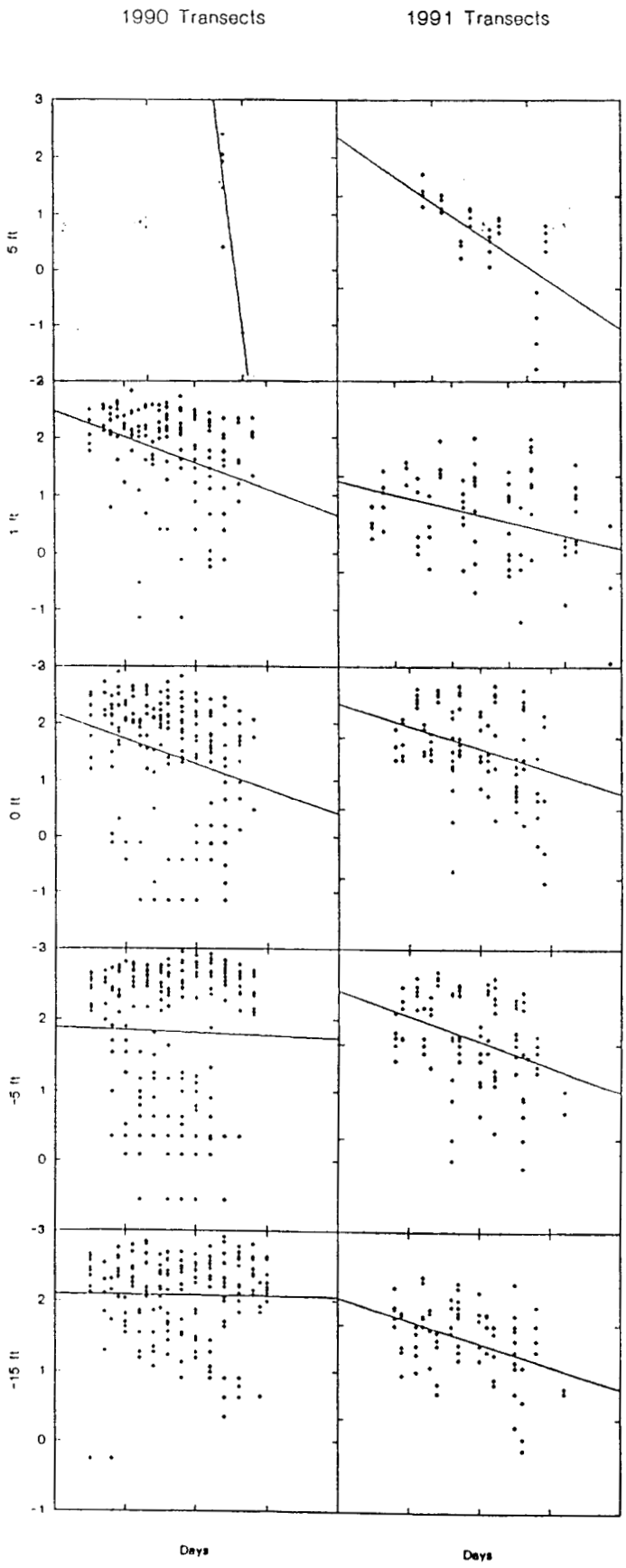
Appendix A.

Figure 2. Ln(egg abundance) for each transect by depth 1991, along with best fitting regression line, the negative of the slope is our estimate of Z.



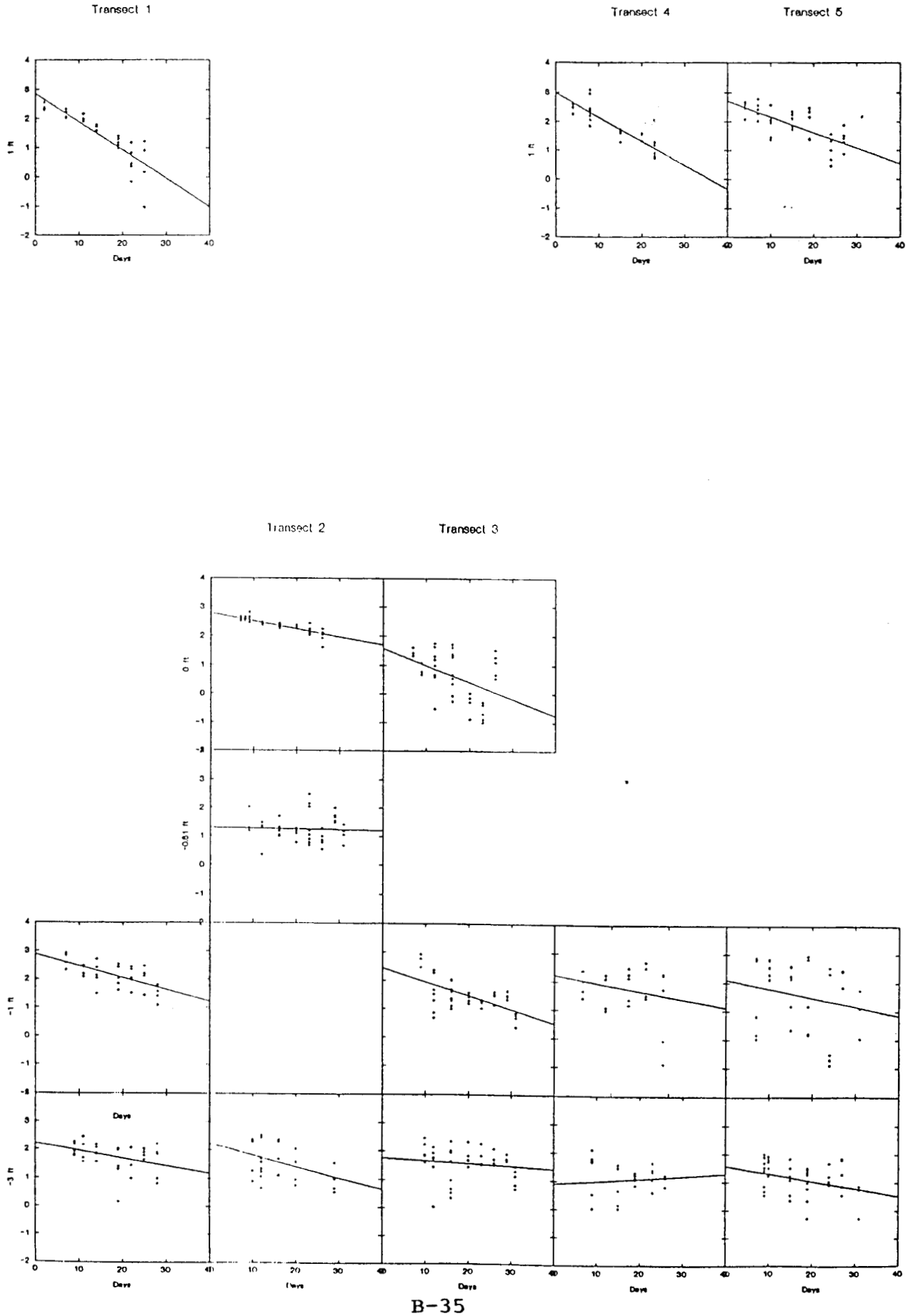
Appendix A.

Figure 3. Ln(egg abundance) by year by depth 1990-1991, along with best fitting regression line, the negative of the slope is our best estimate of Z.



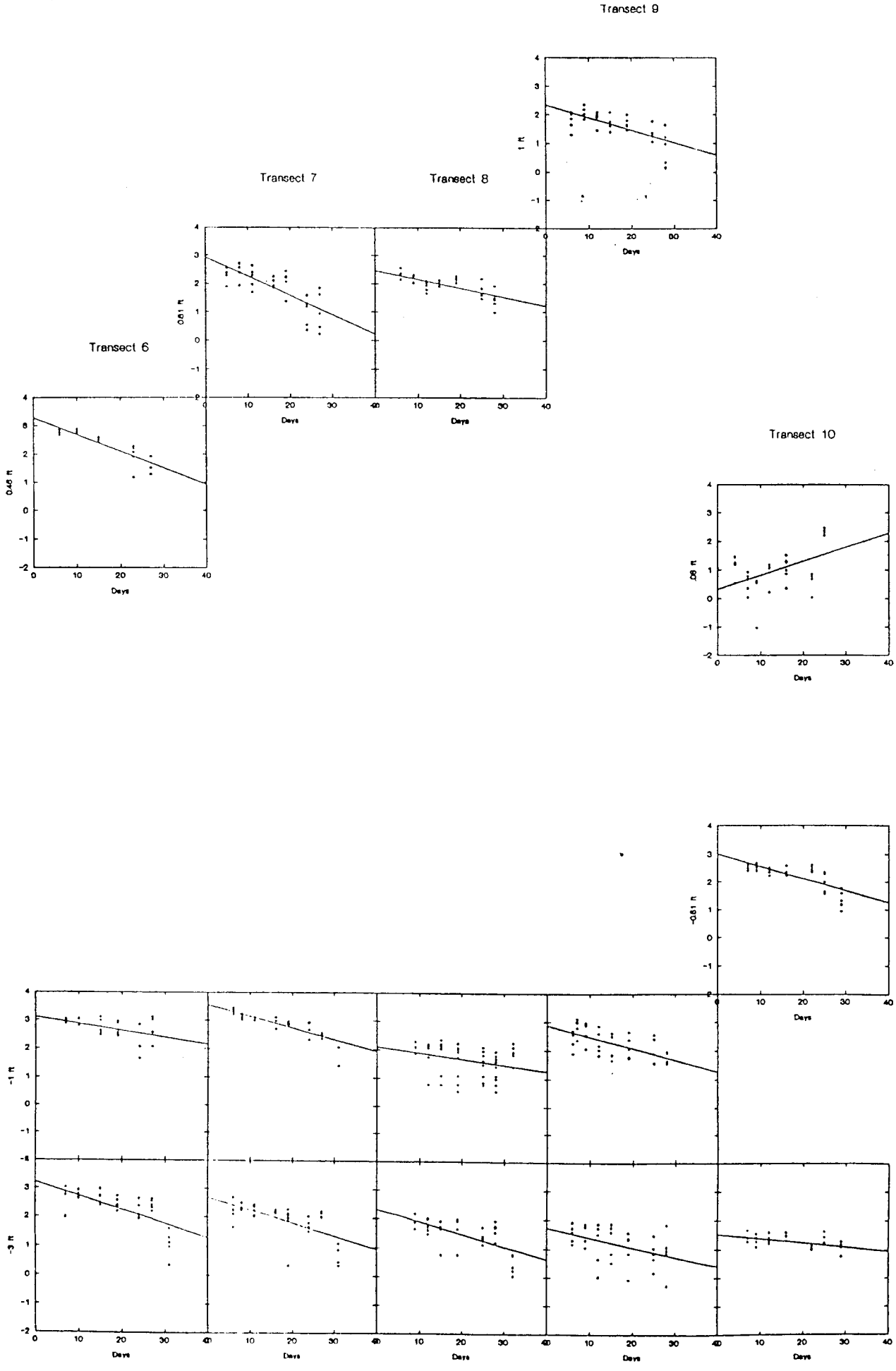
Appendix A.

Figure 4. Ln(egg abundance) 1994 data by transect, along with best fitting regression line, the negative of the slope is our best estimate of Z.



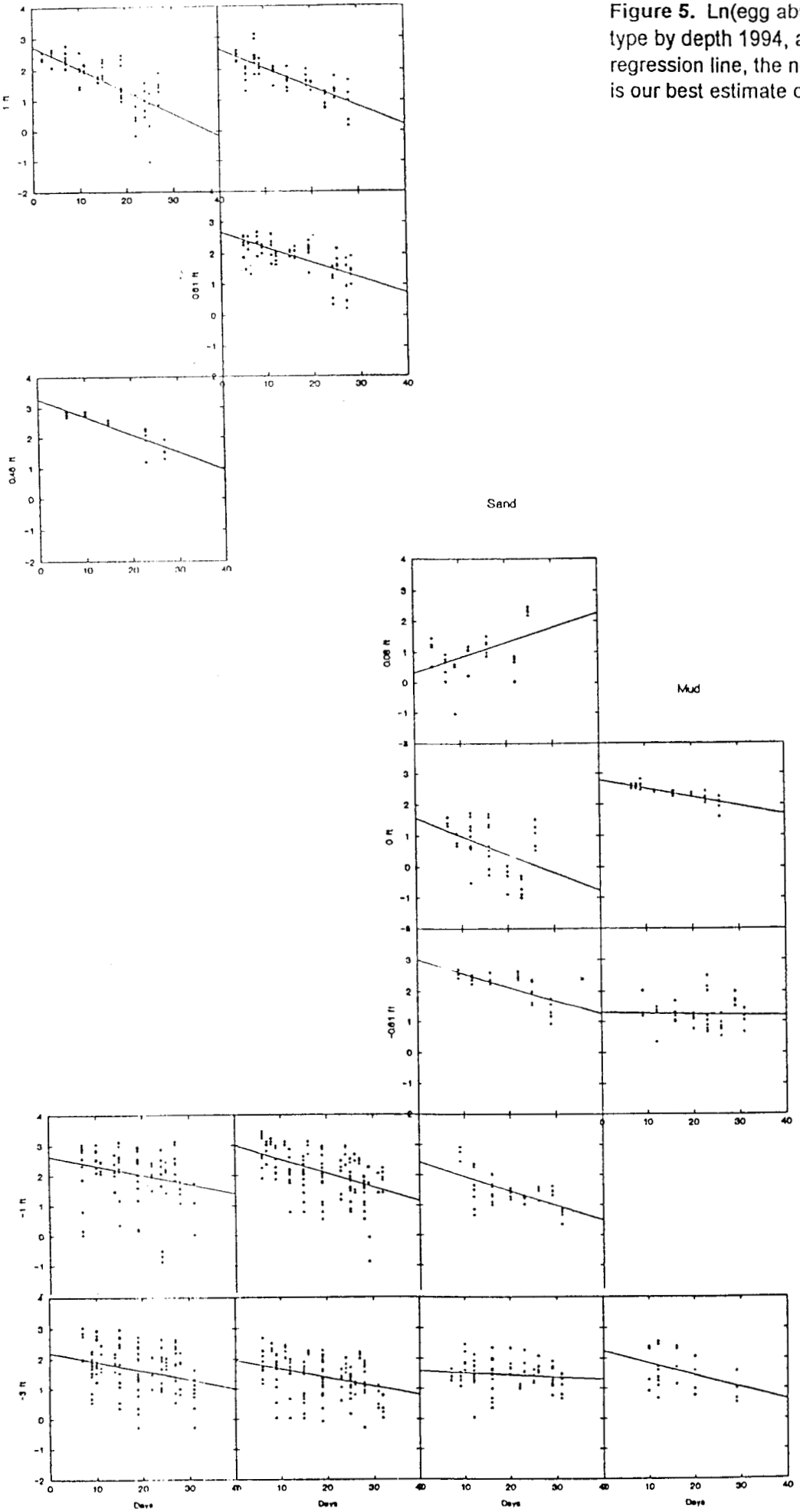
Appendix A.

Figure 4 (cont.). Ln(egg abundance) 1994 data by transect, along with best fitting regression line, the negative of the slope is our best estimate of Z.



Appendix A.

Figure 5. Ln(egg abundance) by substr type by depth 1994, along with best fittir regression line, the negative of the slop is our best estimate of Z.



Appendix A.

Figure 6. Ln(egg abundance) exposed and protected transects by depth 1994, along with best fitting regression line, the negative of the slope is our best estimate of Z.