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## APPLICATION OF AN EGG PRODUCTION METHOD TO ESTIMATE THE SPAWNING BIOMASS OF PACIFIC SARDINES OFF SOUTHERN CALIFORNIA IN 1986

By

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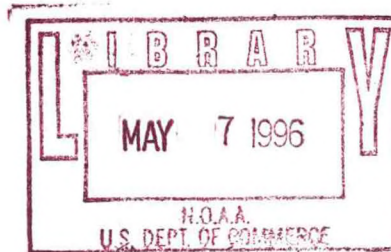
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## EDITOR'S NOTE

This paper was edited and published as an Administrative Report in 1996 so the work of the authors could be preserved and made available for reference in future studies. The original manuscript was submitted to the editorial committee for publication in 1988 CalCOFI Reports (Volume 29), but was not published at that time because the authors did not revise the text to respond to concerns of the reviewers. The manuscript has changed hands several times in the ten years since it was written because all of the authors have taken new jobs outside of the Marine Resources Division, California Department of Fish and Game (CDFG). Unfortunately, they are not available for further work on the manuscript. Consequently, no attempt has been made to reconcile the reviewer's substantive comments; only minor grammatical changes were made.

Comments from the reviewers have been included as an appendix in this document. Their original comments were unlabeled. Since the identities are unknown, it is not clear if reviewers 3 and 4 as described in the appendix are or are not the same person.

Original egg and adult data used in this study have been preserved and may be obtained in ASCII files by writing to the address on the title page.

Marci Yaremko  
CDFG



## ABSTRACT

An egg production method was used to estimate the spawning biomass of Pacific sardines (Sardinops sagax) off southern California in 1986. The egg production method estimates spawning biomass as the ratio of daily egg production to daily specific fecundity of adult fish. Sardine spawning biomass within the survey area was estimated at 7,659 metric tons (MT) (SE = 3,900 MT; CV = 0.51).

Adult sampling efforts resulted in 378 specimens, only one female of which exhibited hydrated ovaries. Batch fecundity determinations were made on migratory nucleus staged ovaries. Results using this method were not significantly different from those generated from ova diameter frequency distributions. All adult parameters except sex ratio differed markedly on a north - south basis, so biomass analyses were carried out separately for the two regions. Daily specific fecundity was determined to be 7.7 and 38.1 eggs / g biomass per day in the northern and southern survey regions, respectively. A total of 260 sardine eggs were collected in 59 of 330 CalVET samples. Estimates of daily egg production rate were 0.28 and 0.51 eggs / 0.05 m<sup>2</sup> in the northern and southern survey regions, respectively.

## INTRODUCTION

In 1974 a moratorium on fishing Pacific sardines (Sardinops sagax) was enacted by the California Legislature. That legislation also provided that a directed take be allowed if the spawning biomass recovered to a level of at least 18,144 MT. The California Department of Fish and Game (CDFG) was thus required to assess the magnitude of the spawning biomass annually. Initially, the Department utilized indirect indices to assess the sardine population, including aerial surveys, plankton surveys, and monitoring of incidental catches. In 1983 and 1984, substantial increase in these indices of sardine abundance signaled the start of recovery. In seeking a more quantitative method of evaluating the spawning biomass as it neared 18,144 MT, Wolf and Smith (1985) developed and applied an egg production area method. This method was derived from an egg production method developed at the Southwest Fisheries Center of the National Marine Fisheries Service (NMFS) to assess the spawning biomass of northern anchovy (Parker 1980). The egg production method estimates spawning biomass as the ratio of daily egg production to daily specific fecundity:

$$B = PA (kW / RFS) \quad (1)$$

where

B =	spawning biomass, MT
P =	daily egg production rate in number of eggs per day per 0.05m <sup>2</sup>
A =	area of survey in units of 0.05 m <sup>2</sup>
k =	conversion factor from grams to MT
W =	average weight of mature females in grams
R =	sex ratio; fraction of population that is female, by weight (g)
F =	batch fecundity in number of eggs
S =	fraction of mature females spawning per day

Parameter estimates are calculated as grand averages based on sample means and variances weighted according to sample size. For Wolf and Smith's (1985) area method, the equation is rearranged to solve for the spawning area of a specified target biomass:

$$A_1 = B_1 RFS / PkW \quad (2)$$

where

A <sub>1</sub> =	spawning area of target biomass in nautical miles <sup>2</sup> ,
B <sub>1</sub> =	target biomass, prespecified.



Wolf and Smith estimated adult reproductive parameters for sardines from historical sardine data, from other pelagic species, and from the relationship between anchovy and sardine parameters off Peru applied to anchovy and sardines off California. They determined that a useful estimate of spawning area for 18,144 MT of sardines off California was 500 n.mi.<sup>2</sup>. A plankton survey for sardine eggs in the Southern California Bight in 1985 found eggs representing a spawning area of about 670 n.mi.<sup>2</sup> (Wolf and Smith 1986). It was judged that the spawning biomass of sardines was a least 18,144 MT, and a directed fishery of 907 MT, the first since 1974, was opened on January 1, 1986. In 1986 the CDFG, in a cooperative effort with the NMFS, attempted a direct application of the egg production method to sardines. Although this technique is not considered extremely accurate for biomass levels of less than 100,000 MT (MacCall 1984), it presented a good opportunity to directly measure adult reproductive parameters for sardines. This knowledge would in turn strengthen the egg production area method. We adapted procedures of the anchovy egg production method (Lasker 1985) for use with sardines wherever possible. Those cases in which data were not available to substitute sardine data for anchovy are discussed in the following sections.

This report describes the 1986 egg production surveys for sardines and discusses the results and their application to refining models and future biomass surveys for sardines.

#### SURVEY DESIGN AND DESCRIPTION

Surveys for sardine eggs and adults were conducted separately, because available vessels were not large enough to accommodate all the required gear and personnel. The surveys were planned to coincide with the season of peak spawning. Collection of sardine eggs, larvae and young-of-the-year, and gonadal development indices of incidentally caught sardines, all indicated that spawning activity was greatest in the summer and fall of 1985 and 1986, so surveys were planned for August. This contrasts with historical times of peak spawning in April and May.

The objectives of the egg survey were to determine the spawning area for application in the area method, allowing evaluation of the biomass relative to 18,144 MT, and to determine daily egg production rate, P, for application in egg production method. The objective of the adult survey was to determine estimates of adult reproductive parameters for use in an egg production estimate of spawning biomass.

#### ADULT SURVEY

Two vessels were chartered, the commercial mid-water trawler Pacific States I, and the commercial purse seine vessel Lakeside,

as it was not clear how best to capture adult/or spawning sardines. The trawler was intended for fishing in deeper water than the purse seine. The adult survey took place from August 5 - 14, 1986. Search areas (Figure 1) were occupied from north to south, at the rate of about one area per day. Actual fishing approximated a judgement sampling scheme, in that information about sardine location and abundance (e.g., sonar, information from fisherman, sightings of fish) determined the sampling distribution. Sample design called for five male fish per sample, and 50 females per set, rather than the 25 females collected in northern anchovy surveys. This was because fewer positive sets were anticipated given the relatively small sardine biomass. Fish collected were measured (standard length), sex was determined, and reproductive maturity was visually estimated at sea. Ovaries were removed and preserved separately, as preliminary tests showed incomplete preservation of gonads when left in fish with body cavities slit open. Fish bodies were frozen for laboratory work.

The 11 positive sets on sardines were located off the coast of Ventura, off Santa Cruz Island, in Santa Monica Bay, and off the coast in the Newport Beach - Huntington Beach area (Figure 1). Over 95% of the fish were captured with the purse seine, and most fish captured were mixed with mackerel. Only 378 total sardines were collected, 321 of which were female and used in the analysis. Sample size ranged from one to 55 fish and five of the 11 samples contained the desired 50 females. The weighted sample mean and variance equations used to estimate reproductive parameters in the anchovy egg production method were thus appropriate for the sardine data set.

#### Average Female Weight, W

Body weight and ovary weight were determined by applying correction factors to frozen or preserved weights. Correction factors were determined from samples of fish collected following the cruise. Fish bodies lost an average of 5.5% of the fresh weight through freezing, and fish ovaries gained an average of 22.3% of the fresh weight through formalin preservation.

Average female weight differed significantly on a north - south basis ( $t = 0.01$ ,  $(1)$ ,  $321 = 2.337$ ;  $[t] = 58.64$ ). Because the egg production model requires homogeneity in parameter estimates throughout the survey area, biomass analyses were carried out separately for north and south regions. The frequency distribution of average female weight per set per region is depicted in Figure 2. The grand average and coefficient of variation for each region are listed in Table 1.

The distribution of positive sets in relation to surface temperature isotherms for the survey period are different for the two survey regions (Figure 3). Collections in the north appear



to have occurred in water masses of 17 °C and colder, while those in the south were made in waters of 20 °C and warmer.

Whole body weight (WB) was regressed on ovary-free body weight (FW), producing the regression equation:

$$WB = -7.8902 + 1.1118 (OFW), r^2 = 0.99 \quad (3)$$

This relationship was used to determine whole body weight for females with hydrated ovaries, because hydration significantly inflates body weight (Picquelle and Stauffer 1985).

### Sex Ratio, R

The frequency distribution of average female fraction by weight per sample for north and south regions is depicted in Figure 4. There were no apparent correlations between location or time and sex ratio. Grand average sex ratio and coefficient of variation are shown in Table 1.

### Batch Fecundity, F

Only two females with hydrated oocytes were collected during the survey, and one of these exhibited post-ovulatory follicles, so could not be used for batch fecundity determinations. Rarity of ripe female sardines is apparently not unusual. Clark (1934) reported that only 39 ripe fish had been observed in over 11 years of studying the California sardine fishery.

To determine batch fecundity, we applied the hydrated oocyte method of Hunter et al. (1985) to fish with ovaries in the stage immediately proceeding hydration, the migratory nucleus stage. This method has been applied successfully to estimation of batch fecundity in Pacific mackerel (*Scomber japonicus*, Dickerson unpubl. data). Oocytes with migratory nuclei, the largest size class on slides of egg smears, were easily distinguishable under a dissecting microscope. Batch fecundity determinations were made on a subsample of 44 females. Frequency distributions of ovary-free weight for the batch fecundity subsample and for the entire sample population were highly similar.

To test the validity of migratory nucleus oocyte counts as a measure of batch fecundity, we determined ova diameter frequency distributions for the same subsample. Hunter et al. (1985) recommend this method when hydrated females are not available. The number of oocytes in the largest mode was determined using the computer program NORMSEP (Abramson 1971). Two visual counts and two size frequency distributions were determined from the same egg slide for each fish. The mean counts within the largest size class were not significantly different for the two methods

(pooled  $t_{0.01, (2), 22} = 2.819$ ;  $[t] = 0.053$ ). The visual count method consistently produced more repeatable results among different readers as well as between replicate reads. Variability in counts using the ova diameter frequency methods were unavoidable due to measurement error, even between replicate reads. In addition, the change squashing or misalignment of the large axis of migratory nucleus oocytes often caused these to be classed in the smaller oocyte diameter mode.

The linear regression of batch fecundity (BF) on ovary-free weight (OFW) (Figure 5) produced the regression equation:

$$BF = -21003.99 + 495.67 (OFW), r^2 = 0.687 \quad (4)$$

This relationship was used to determine batch fecundity for all females. Average batch fecundities per set were larger in the northern region (Figure 6). Grand average batch fecundity and coefficient of variation are listed in Table 1.

#### Spawning Fraction, S

Spawning fraction for multiple-spawning fish is defined as the proportion of mature females which spawn on a given day. The timing of spawning can be determined by examining histological sections of ovaries for incidence of post-ovulatory follicles. Postovulatory follicle ageing criteria were adapted from methods used for the northern anchovy (Hunter and Macewicz 1985) and the Peruvian sardine (Goldberg et al. 1984). Post-ovulatory follicles can be reliably aged up to two days following spawning. Theoretically, the numbers of females in each spawning-day category should be approximately equal, and each measure could be used to estimate spawning fraction. However, fish behavior, gear type, and sample location are some of the factors which can cause biases in the collection of fish in different spawning states. It appears that hydrated, or day-0 (spawning on the night of capture), female northern anchovy are oversampled during trawl surveys (Picquelle and Stauffer 1985). Thus, the egg production programs determine spawning fraction with an adjustment to counter this bias. The number of day-0 females is replaced with the actual number of day-1 (spawned one day prior to capture) females sampled to calculate the total number of mature females in each set, and the proportion of day-1 spawners to total mature females determines the estimate of spawning fraction. Alheit (1985) also found a bias in sampling day-0 northern anchovy off Peru, and used an average of the frequencies of day-1 and day-2 spawners as an estimate of spawning fraction.

Approximately equal numbers of mature female sardines in each spawning state were collected: day-0 = 44, day-1 = 36, and day-2 = 43. The proportions of day-0, day-1 and day-2 spawners



per set per region were also similar (Figure 7). Because hydrated females were not effectively sampled, it appears that, unlike northern anchovy, sardines are not oversampled for day-0 spawners. Paired sample t-tests ( $t_{0.01, (2), 11} = 11 [t] < 0.07$ ) determined there were no significant differences between mean spawning fractions calculated from each of the spawning states. We therefore calculated spawning fraction as the average proportions of day-1 plus day-2 spawners per set, and as the average proportions of day-0, day-1 and day-2 spawners per set for comparison with the adjusted measure. Various estimates of spawning fraction and corresponding spawning frequencies for sardines are shown in Table 2. It appears that female sardines were spawning about once every 9 days during the survey period. The adjusted grand average spawning fraction and coefficient of variation calculated by the egg production programs (Table 1) values were used in biomass calculation.

The proportion of females in various stages of the day-0 spawning state were examined according to time of capture (Figure 8). Females with hydrated oocytes as well as day-0 post-ovulatory follicles were captured in the midst of spawning. The time period encompassing the capture of these fish, 2200 hrs - 0300 hrs, is an indication of the daily time of peak spawning. Thus, use of the anchovy egg production models based on a peak spawning time of 2200 hrs is acceptable for sardines. No ovaries exhibited 50% alpha atresia in yolked oocytes, indicating that the survey was conducted during the active spawning season.

#### EGG SURVEY

The egg survey was conducted aboard the occidental College research vessel Vantuna, between August 4 - 13, 1986. Three hundred and thirty stations were occupied, from the 10-fathom curve out to about 30 miles, from Point Conception to the U.S. - Mexico border (Figure 9). Stations were 4 n.mi. apart offshore and alongshore, so that each station represented 16 n.mi.<sup>2</sup> sea surface area. Plankton samples were collected at each station using a 150 um mesh CalVET net fished from 70 meters where depth allowed. Surface temperature was recorded at each station.

A total of 260 sardine eggs was collected at 59 stations (Figure 10). Positive stations were located off the coast of Ventura, off Santa Cruz and Santa Rosa Islands, within the Santa Monica Bay, and from the coast out to Santa Catalina Island in the San Pedro Basin. Occurrence of eggs seemed to correlate fairly well with the locations of positive sets for adults (Figure 1), except that sample for adults was not attempted within the San Pedro Channel from Santa Monica Bay out to Santa Catalina Island, where a large number of eggs were collected. The distribution of positive stations in 1986 is also fairly similar to that for 1985, when stations were 10 n.mi.<sup>2</sup> apart along shore (Figure 11).

The spawning area represented strictly by positive stations with embedded negative stations where these numbered two or less, (Figure 12) measured approximately 970 n.mi.<sup>2</sup>. This was about 45% greater than the spawning area within the 1985 survey area. In addition, because the 1986 observed spawning area was well in excess of the estimated critical area of 500 n.mi.<sup>2</sup> for a spawning biomass of 18,144 MT, a directed fishery of 907 MT was opened on January 1, 1987.

A total of 413 sardine larvae was collected at 114 stations (Figure 13), as compared to 24 larvae collected at 10 stations in 1985.

Figure 14 displays the frequency distribution of eggs collected at the range of survey temperatures. No eggs were collected below 15.5 °C. There appear to be two modes present in the distribution of sardine eggs: 16.5 - 16.9 °C and 20.5 - 21.5 °C. This temperature-related pattern of egg collection correlates well with the distribution of adult collections, suggesting these may be optimal temperature ranges for sardine spawning. At both temperatures the majority of eggs collected were in developmental states 6, 7 and 11.

#### Daily Production of Eggs, P

Eggs were staged by microscopic examination and then age was determined by a FORTRAN-77 computer program called Stageage.for (Hewitt, et al., 1984), based on temperature-developmental time relationships for anchovy. An egg mortality model was fit to the data with a weighted non-linear least squares regression, where each egg sample was weighted proportionally to the area of the station. Samples were also divided into strata, where stratum one consisted of positive egg stations and embedded stations with no eggs if the latter numbered two or less. All other stations were assigned to stratum zero. Strict definition of strata in this manner reduces variability in the estimate of P, and also allows more direct comparison of the results of the egg production method with the area method, where spawning area is calculated only from positive stations. The egg mortality model used was:

$$P^{jt} = P^i e^{-Zt} \quad (5)$$

where  $P^{jt}$  = number of eggs at age t at the jth station in stratum i,  
 $t$  = age in days from the time of spawning to the time of sampling at the jth station,  
 $z$  = instantaneous mortality rate per day,  
 $P^0$  = daily egg production rate in stratum 0; zero by definition, and  
 $P^1$  = daily egg production rate in stratum 1.



The resultant egg mortality curve is shown in Figure 15. The value of P obtained was within the range of estimated values of P used in the area method (equation 2). The instantaneous mortality rate from this regression was fixed and the regression rerun to determine P<sup>1</sup> for the north and south regions separately. Overall egg production rate in each region was then calculated from the relation:

$$P = (A^1/A) P^1 \quad (6)$$

where P = the total daily egg production rate,  
A<sup>1</sup> = the area of stratum 1, and  
A = the total survey area.

The values of P and A for each region are reported in Table 1.

#### BIOMASS ESTIMATE

The reproductive parameter estimates (Table 1) were used to calculate the spawning biomass within the survey area of each of the two regions, using equation 1. These values were 4,756 MT (c.v. = 0.792) in the north and 2,903 MT (c.v. = 0.349) in the south, for a combined biomass estimate of 7,659 MT (c.v. = 0.509). All parameter estimates except sex ratio were notably different between regions. In both regions, egg production rate and spawning fraction displayed the greatest coefficients of variation, while average female weight was the last variable.

#### DISCUSSION

The egg production estimate of the 1986 spawning biomass of Pacific sardines was 7,659 MT. This is in marked contrast to the biomass inferred using the area method ( $B \geq 18,144$  MT). The differences can be explained by examining the various estimates of sardine reproductive parameters (Table 3). In particular, the values measured in the 1986 egg production analysis should be compared to the estimated values used by Wolf and Smith (1985) in the area method. Estimated egg production rates used in the area method were at least 66% greater than either value obtained in the egg production survey. The average female weight used in the area method was at least 29% less than the lowest actual measurement. The estimated batch fecundity was also at least 62% less than either measure obtained in the egg production survey.

These differences between the estimated parameters and the first actual measurements indicate that a re-evaluation of the area relationship is necessary. Because this first attempt at measuring reproductive values consisted of very small numbers of samples and individuals with resultant high parameter variation,

it does not seem appropriate to substitute all new parameters into the area equation. However, measured values of average female weight and batch fecundity had very low coefficients of variation. In addition, the measured batch fecundities more closely resemble historical values (MacGregor 1957) than does the estimated value used in the area method (Table 3). So, if measured values for W and F are inserted into equation 2, using estimated values for the other parameters,  $A^1$  ranges between 177 and 4,721 n.mi.<sup>2</sup>, for the range of estimated egg production rates and spawning fractions (Table 4). Without making judgments about the true magnitude of P or S, the estimate of  $A^1$  for a spawning biomass of 18,144 MT is probably considerably higher than 500 n.mi.<sup>2</sup> as previously estimated, perhaps as high as 2,300 n.mi.<sup>2</sup>.

We acknowledge several limitations with our data set. First, the small sample size imparts high variability in any estimates of biomass or area. Second, knowledge of sardine spawning behavior in terms of peak spawn time or duration of spawning in a day, and of temperature and development relationships is incomplete. Examination of data from staged sardine eggs collected in CalCOFI cruises for the period 1951-1959 indicates that peak spawning may occur around 0000 hrs (Smith 1973) and continue until 0600 hrs (P. Smith pers. comm. 1987). This differs from the anchovy egg production model which uses a peak spawning time of 2200 hrs with a duration of four hrs. These differences indicate that sardine eggs aged according to the anchovy model in 1986 might actually be younger. This would result in a different egg mortality curve, with higher mortality rate and higher  $P^0$ . A greater egg production rate would in turn produce a lower estimate of  $A^1$  for a spawning biomass of 18,144 MT.

The 1986 egg production estimate was also limited in that spawning is known to have occurred outside of the survey area. Eggs were found at the outer edges of the survey area, and historical spawning grounds ranged out as far as Tanner and Cortez Banks, and well into Mexican waters. Young-of-the-year fish were observed in Monterey Bay and San Francisco Bay in 1986, indicating that spawning occurred north of Pt. Conception. Further, all sardines collected were mixed in schools of mackerel, and only a single hydrated female was captured. These facts may indicate that our gear and/or sampling strategies are not obtaining representative samples of spawning as well as non-spawning sardines. Egg production surveys being planned for 1987 and 1988 will increase the area covered and will be aimed at collecting larger numbers of mature sardines. Values will continue to be used to improve the egg production area technique.

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Table 1. Estimates of egg production parameters and spawning biomass by region for Pacific sardines off California in 1986.

(COEFFICIENTS OF VARIATION IN PARENTHESES)

PARAMETER	NORTH	SOUTH	TOTAL
Daily egg production, P (no. eggs/0/05 m <sup>2</sup> day)	0.276 (0.557)	0.513 (0.322)	
Area of region, A (km <sup>2</sup> )	6,615.5	10,774.1	17,389.6
Avg. female weight, W (g)	199.872 (0.032)	154.784 (0.047)	
Batch fecundity, F (no. eggs/batch/ mature female)	71,381.8 (0.049)	51,742.9 (0.086)	
Spawning fraction, S (proportion of mature females spawning per day)	0.0384 (0.467)	0.1886 (0.283)	
Sex ratio, R (female proportion of population by weight-g)	0.559 (0.1173)	0.603 (0.0519)	
Daily specific fecundity (no. eggs/g biomass/day)	7.681	38.065	
SPAWNING BIOMASS, B (MT)	4,756 (0.792)	2,903 (0.349)	7,659 (0.509)



Table 2. Estimates of spawning fraction and spawning frequency for Pacific sardines.

Method	Mean	Variance	Spawning Frequency (spawn every x days)
day-0, adjusted	0.1142	0.0018	8 - 9
day-0	0.1242	0.0291	8
day-1	0.1157	0.0399	8 - 9
day-2	0.0867	0.0120	11 - 12
day-1, day-2	0.1012	0.0250	9 - 10
day-0, day-1, day-2	0.1094	0.0257	9 - 10

Table 3. Comparison of sardine egg production parameter estimates.

SPECIES / LOCALITY	SOURCE	P	W	R	F	S
<u>Sardinops sagax</u> / southern California	CDFG 1986	NO. 0.28	199.87	0.56	71,381.8	0.038
		SO. 0.51	154.78	0.60	51,742.9	0.189
<u>Sardinops sagax</u> / southern California	Wolf & Smith 1985 (expected values for the Area method)	1.50 or 5.0	120.00	0.50	32,000.0	0.020
						0.150
<u>Sardinops sagax</u> / California	MacGregor 1957 Clark 1934				57,567.0	
					44,110.0	
<u>Sardinops sagax</u> / Magdalena Bay, Mexico	Torres-Villegas 1985		85.88	0.47	18,941.0	0.065
						0.059
<u>Sardinops sagax</u> / Gulf of CA, Mexico	Torres-Villegas et al. 1985		57.64	0.61	21,390.0	0.167
<u>Sardinops sagax</u> / Peru	Lo et al. 1985		160.00		54,567.0	
<u>Sardinops sagax</u> / northern Chile	Retamales et al. 1985		223.60		57,019.0	0.178



Table 4. Incorporation of measured values of average female weight, W, and batch fecundity, F for estimation of spawning area, A<sup>1</sup>.

B <sup>1</sup>	W	R	F	P	S	A <sup>1</sup>	
20,000	199.872	0.5	71,381.8	5.0	0.02	189	
					0.15	1,416	
				1.5	0.02	629	
	154.784			51,742.9	5.0	0.02	177
						0.15	1,326
					1.5	0.02	589
					0.15	4,419	

## FIGURE CAPTIONS

1. Searching and fishing areas and location of positive purse seine sets for 1986 adult sardine survey.
2. Frequency distribution of average female weight per set in each survey region.
3. Location of positive sets on sardines in each region and in relation to surface temperature isotherms.
4. Frequency distribution of average sex ratio per set in each survey region.
5. Linear regression of batch fecundity on ovary-free weight.
6. Frequency distribution of average batch fecundity per set in each survey region.
7. Frequency distribution of spawning fraction per set in each survey region based on day-0, day-1 and day-2 spawners.
8. Distribution of females in different day-0 ovarian categories according to time of day.
9. Sardine 1986 egg survey station plan.
10. Locations of positive egg stations in 1986.
11. Locations of positive egg stations in 1985.
12. The 1986 spawning area as defined by positive egg stations.
13. Locations of positive larval stations in 1986.
14. Frequency distribution of sardine eggs by temperature of collection in 1986.
15. 1986 egg mortality curve.



Figure 1

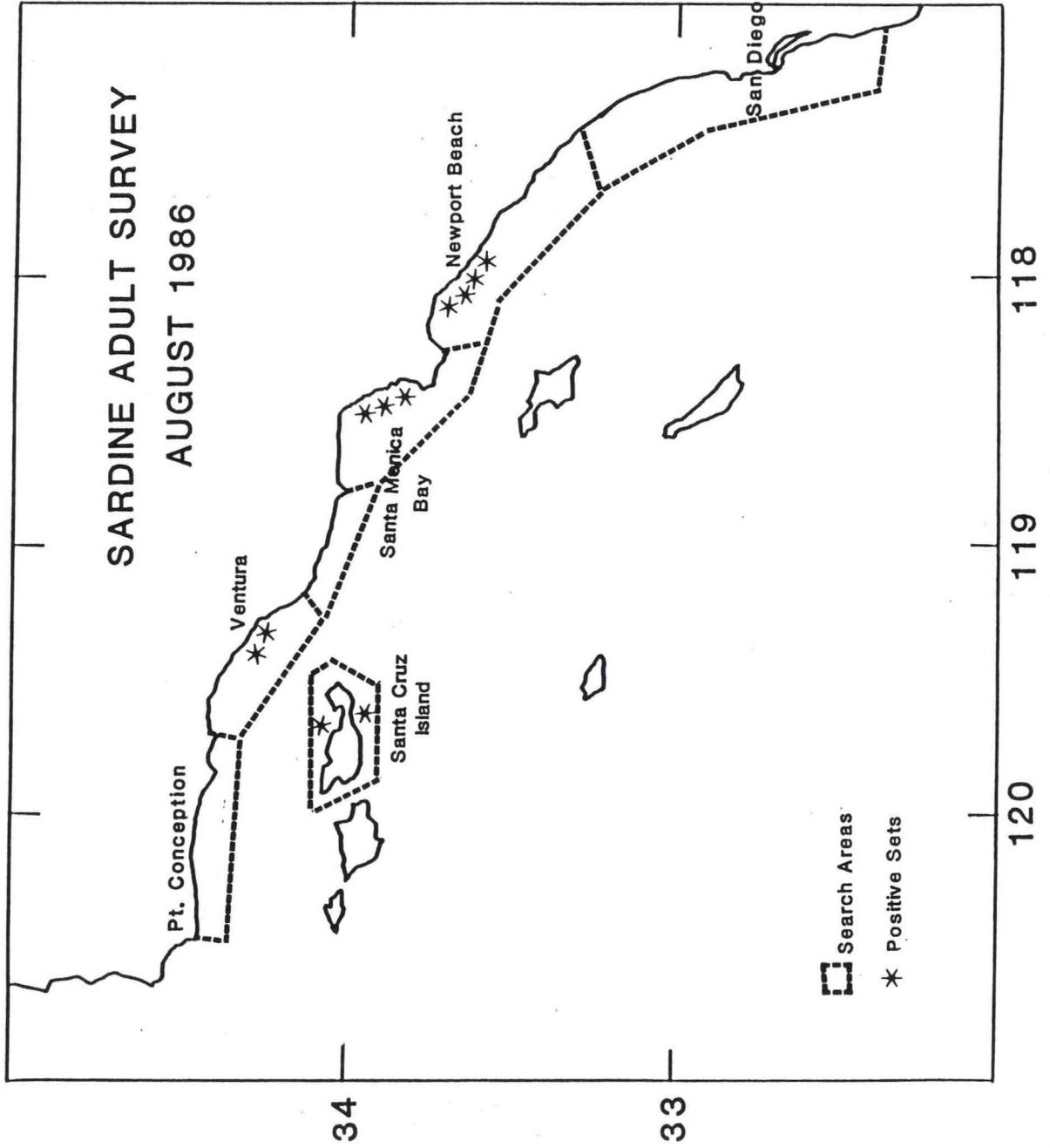


Figure 2

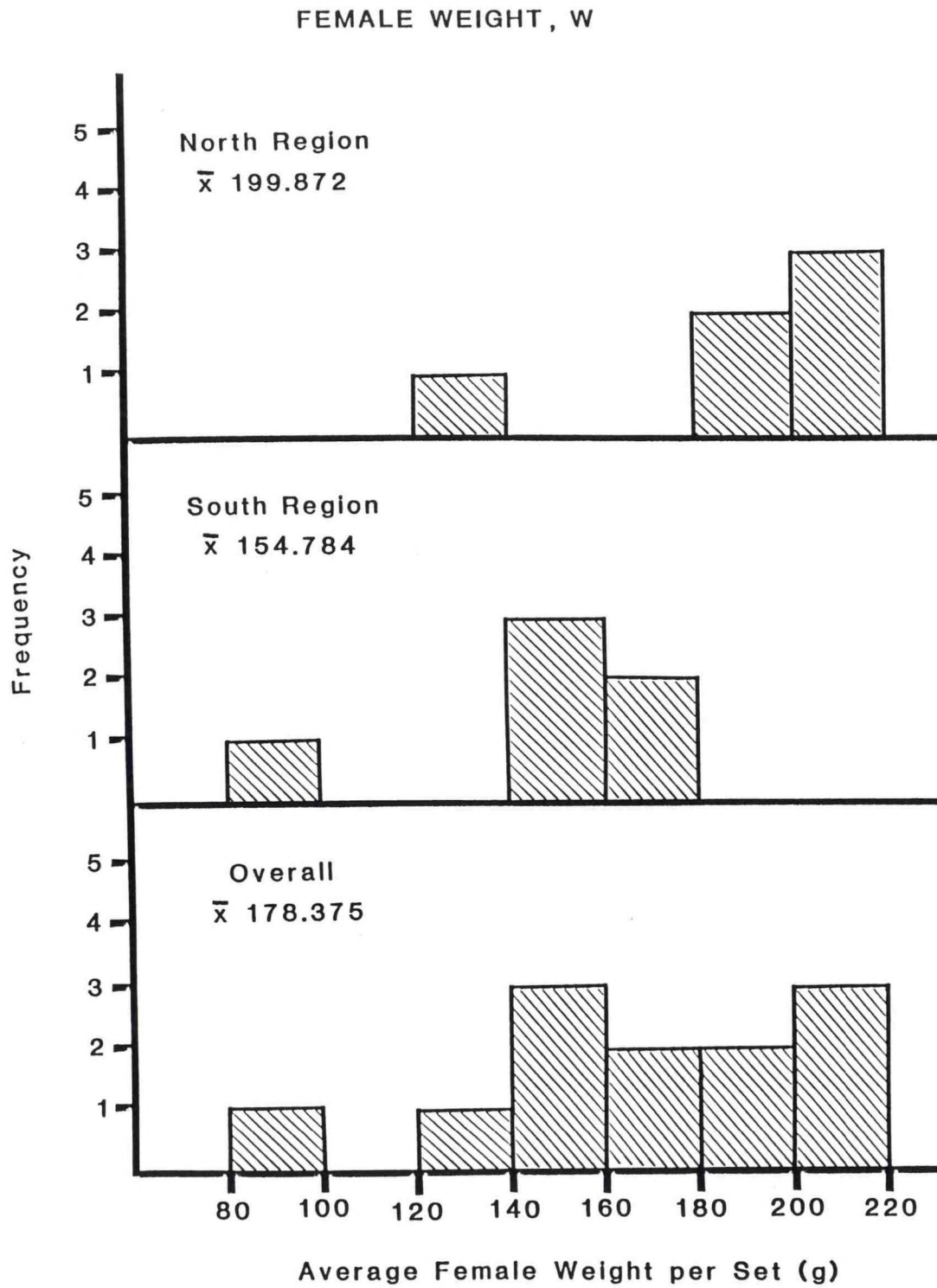




Figure 3

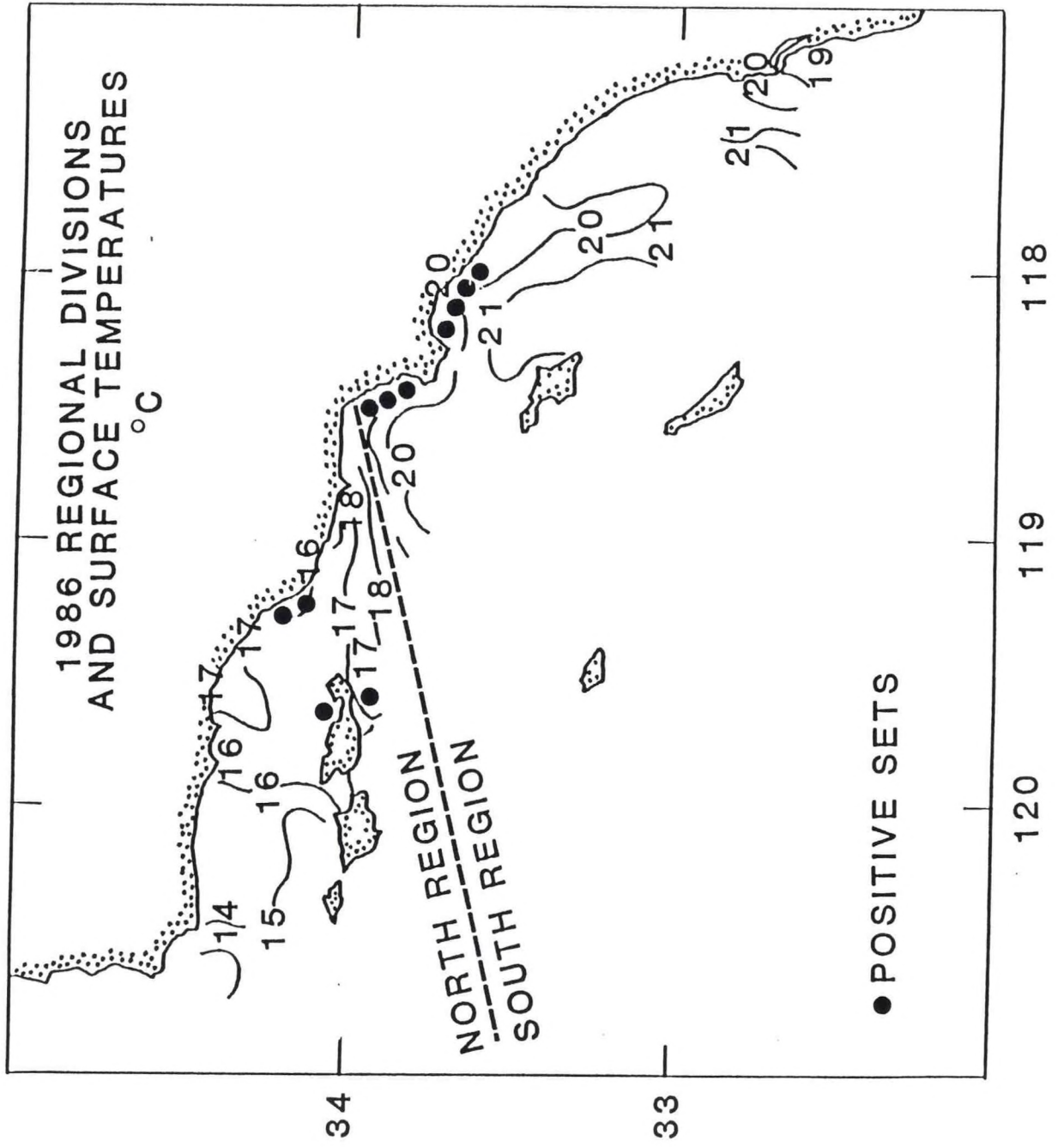


Figure 4

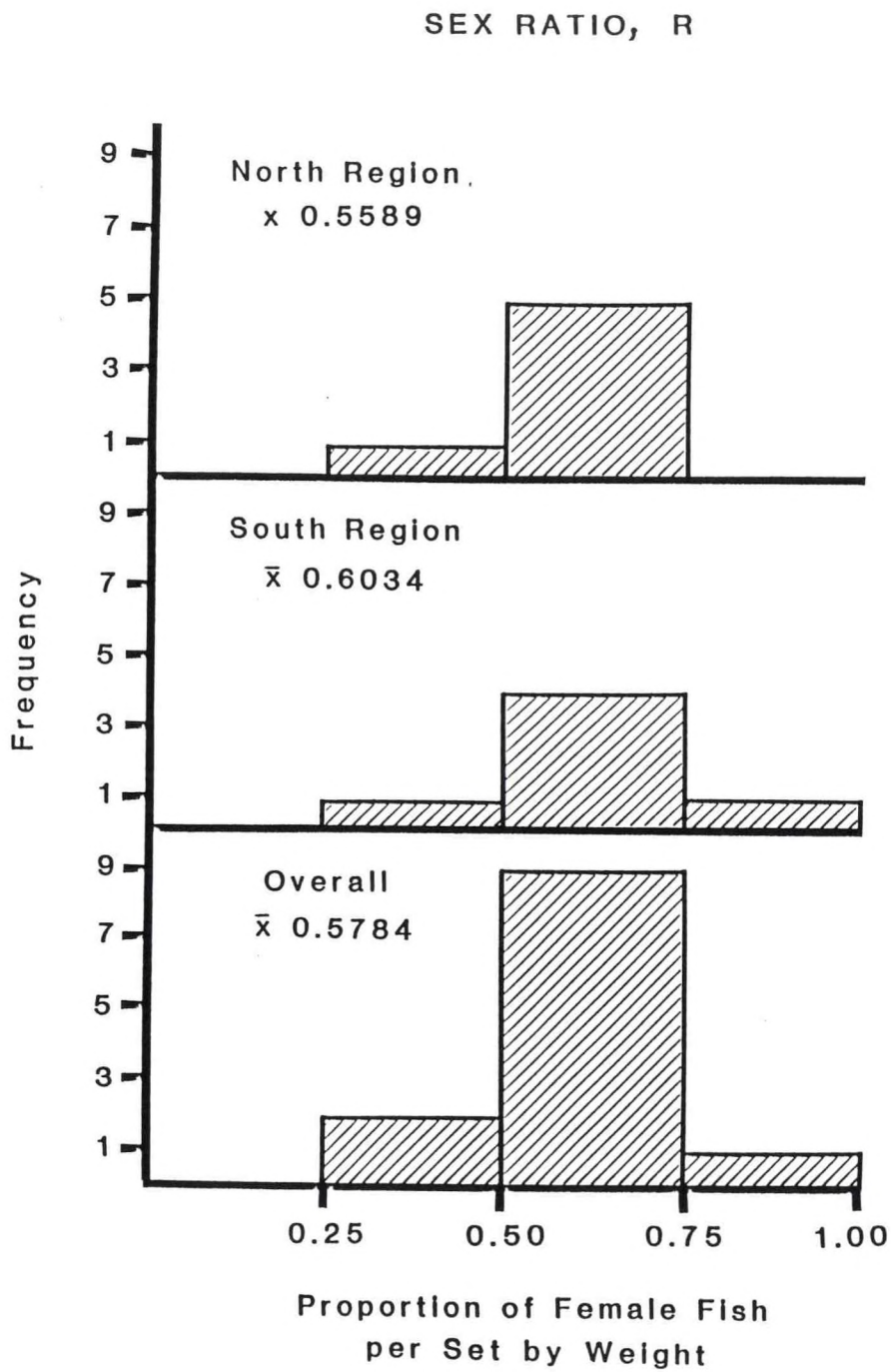




Figure 5

LINEAR REGRESSION OF BATCH FECUNDITY ON OVARY-FREE WEIGHT  
FIT TO 44 FEMALES (1 hydrated, 43 with migratory nuclei)

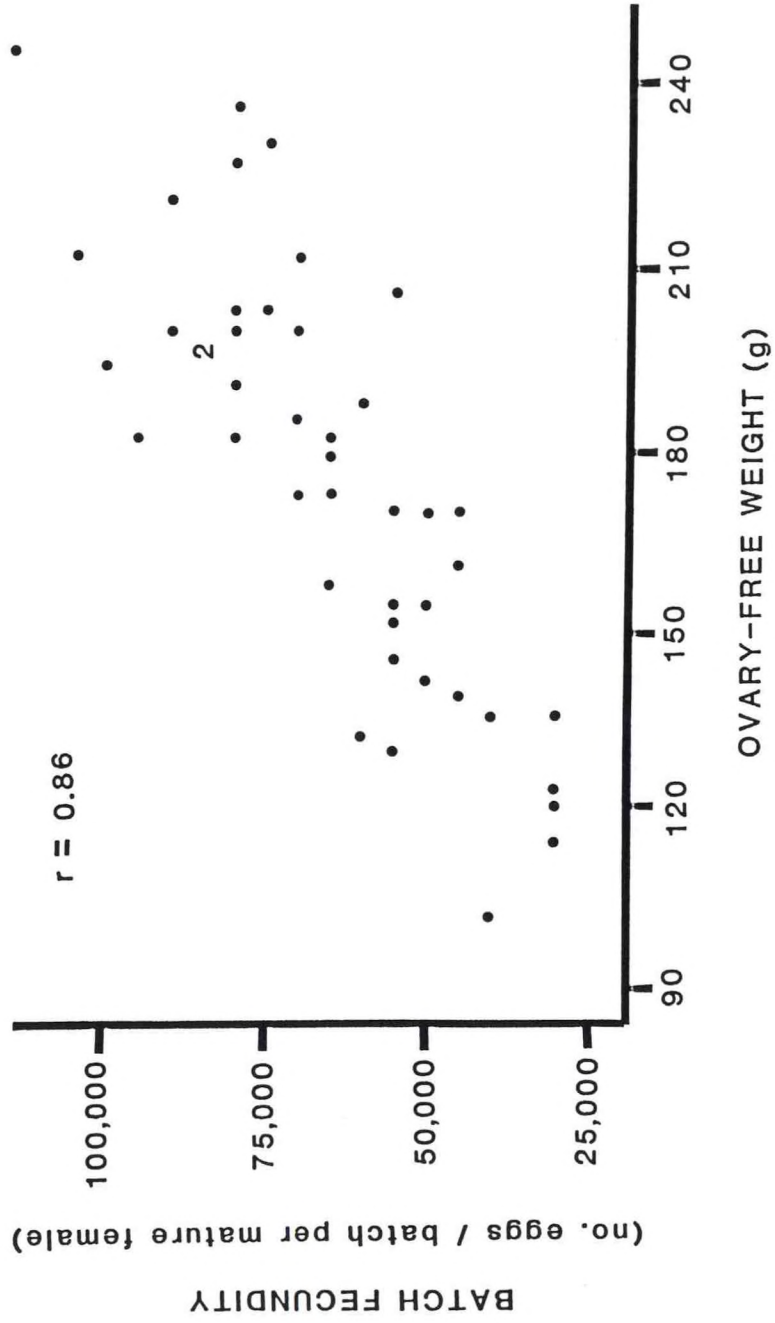
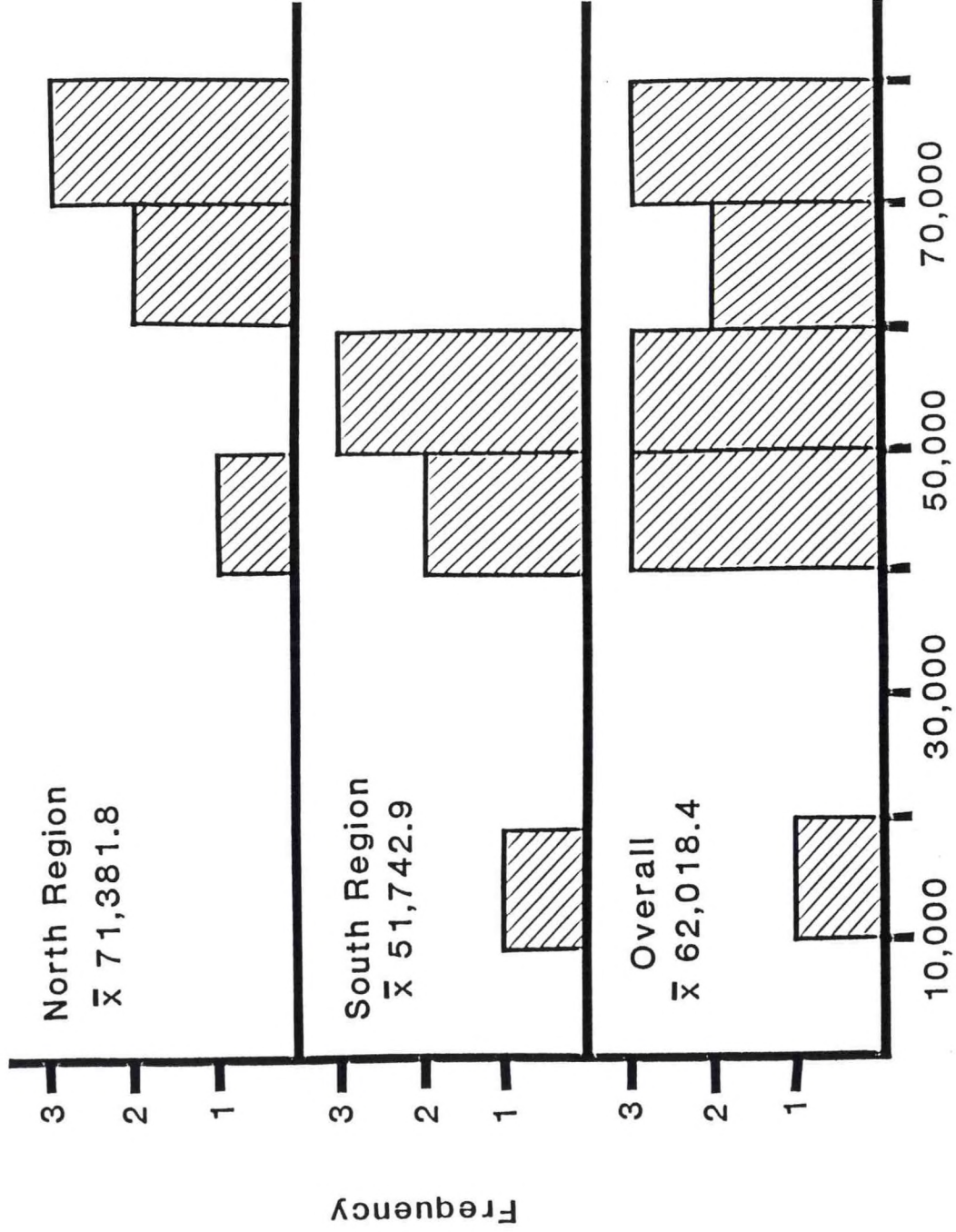


Figure 6

BATCH FECUNDITY, F



Average Batch Fecundity per Set  
(no. eggs / batch per mature female)

Figure 7

SPAWNING FRACTION, S

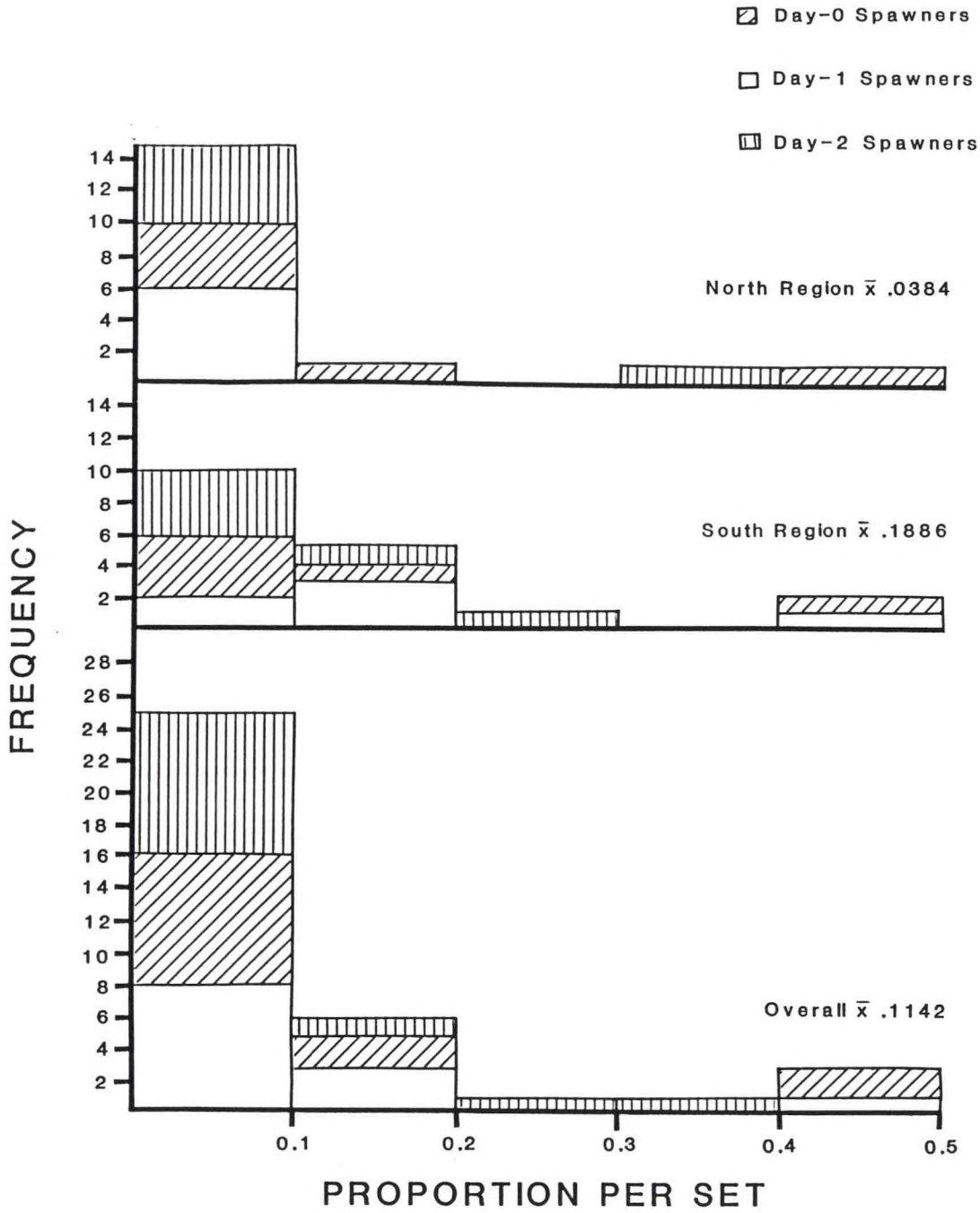




Figure 8

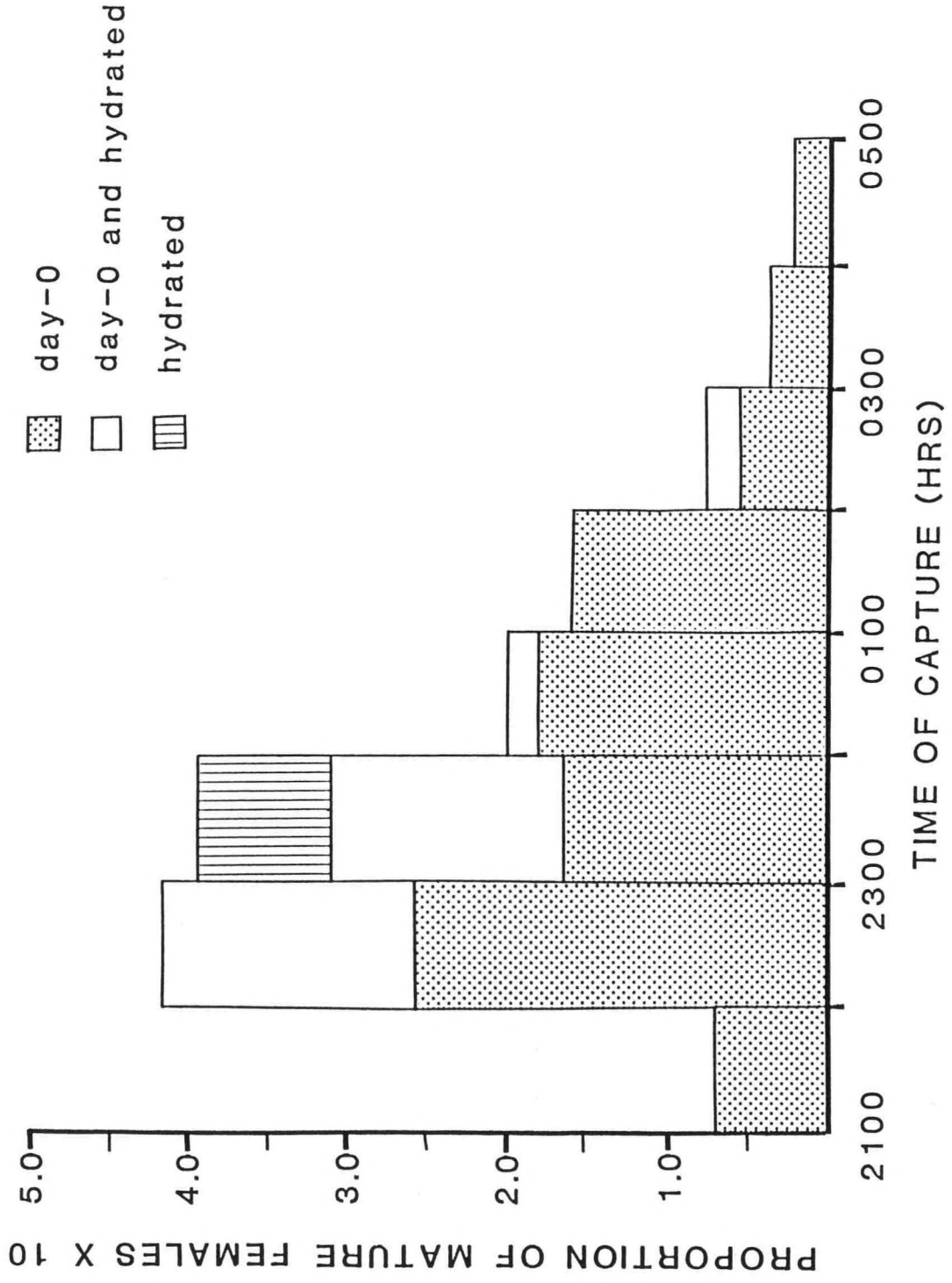


Figure 9

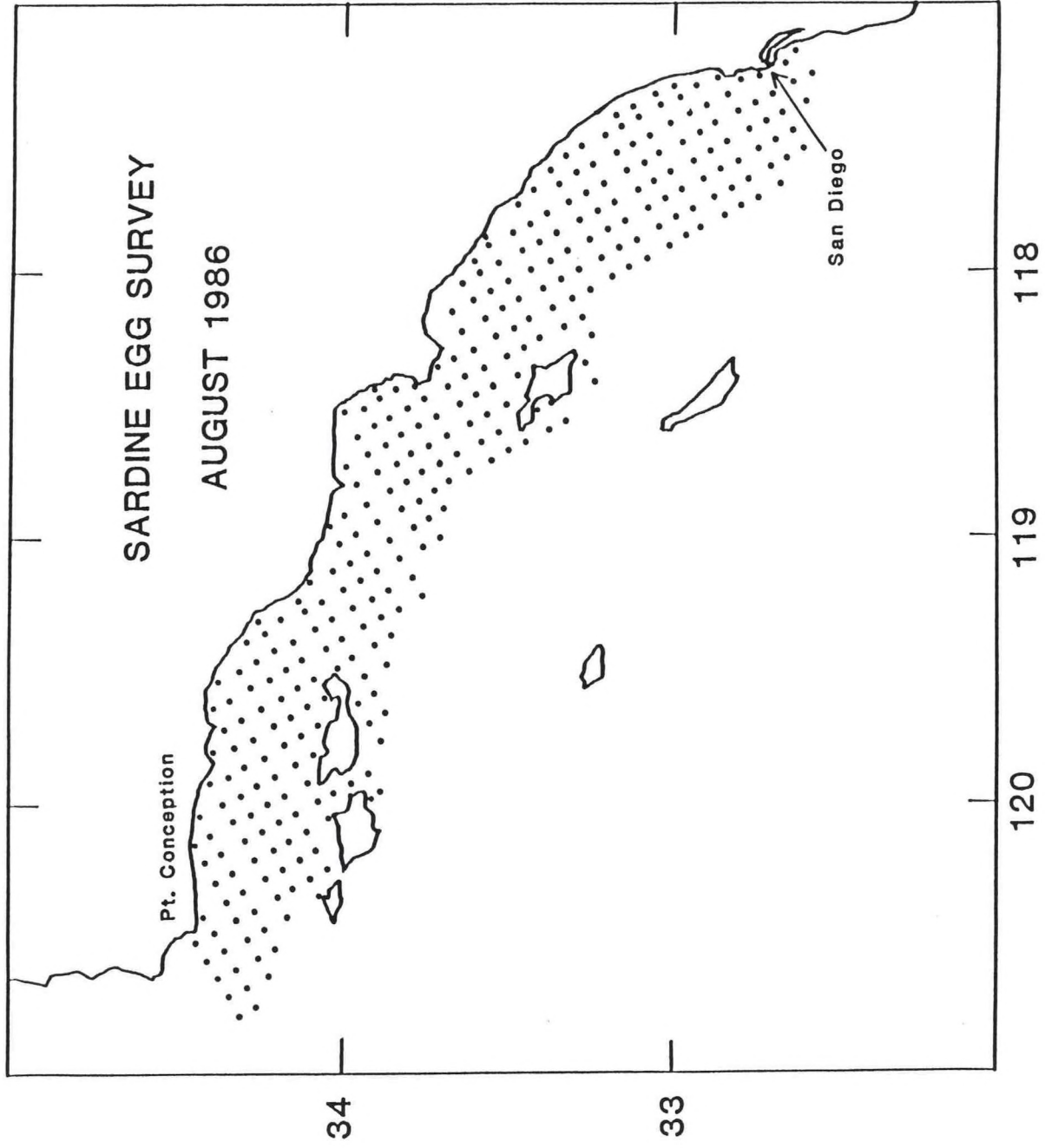


Figure 10

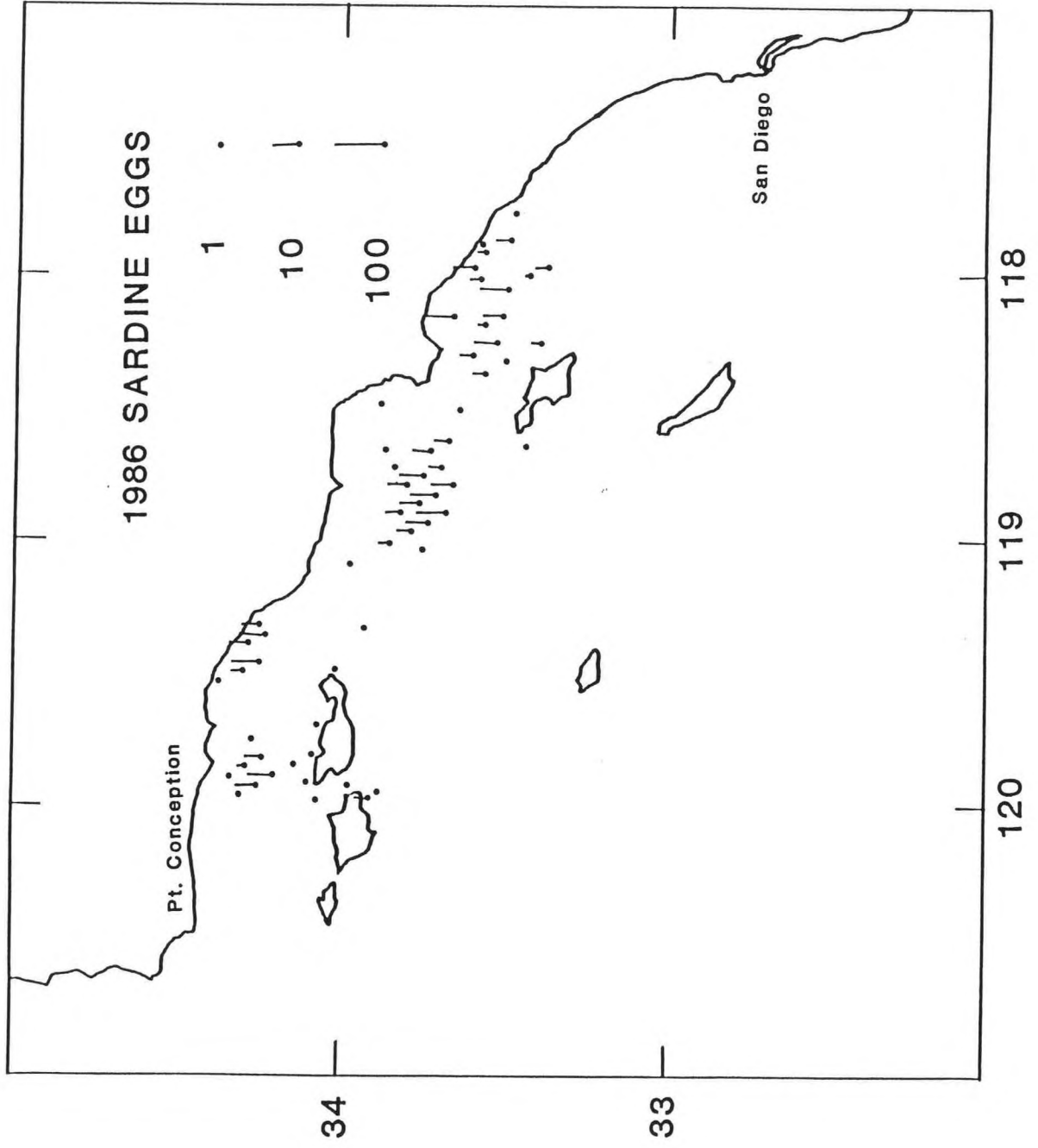




Figure 11

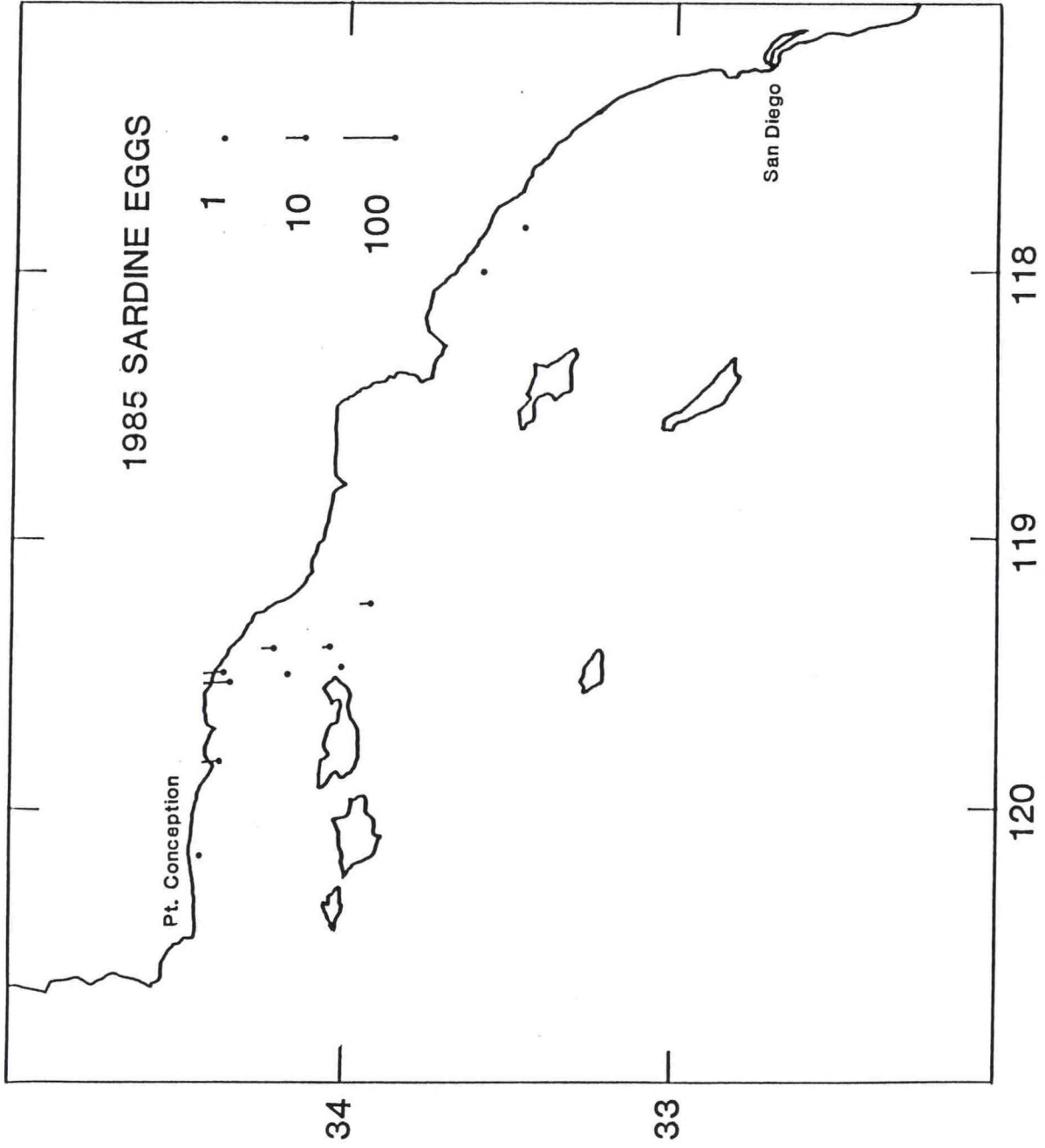


Figure 12

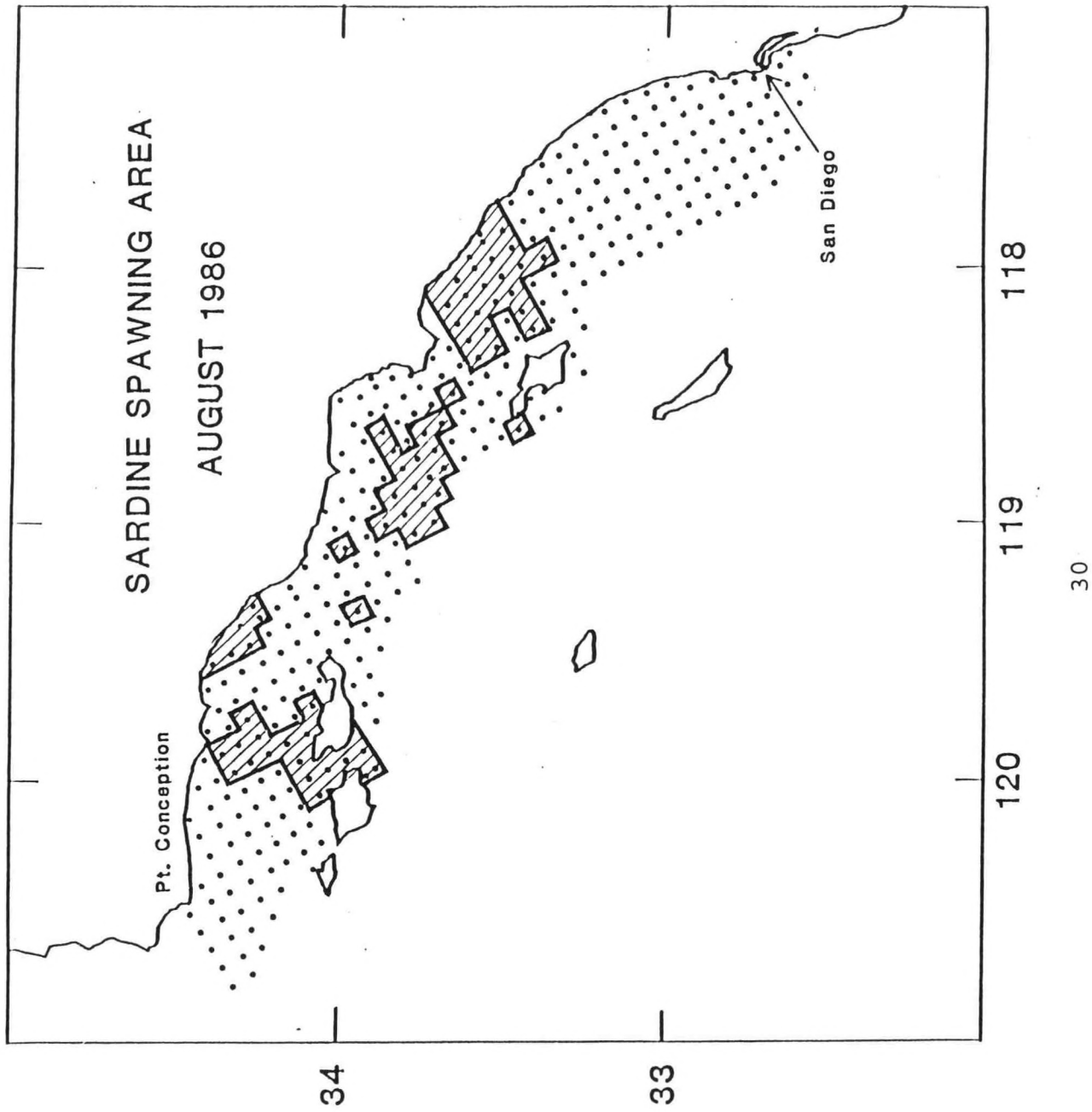


Figure 13

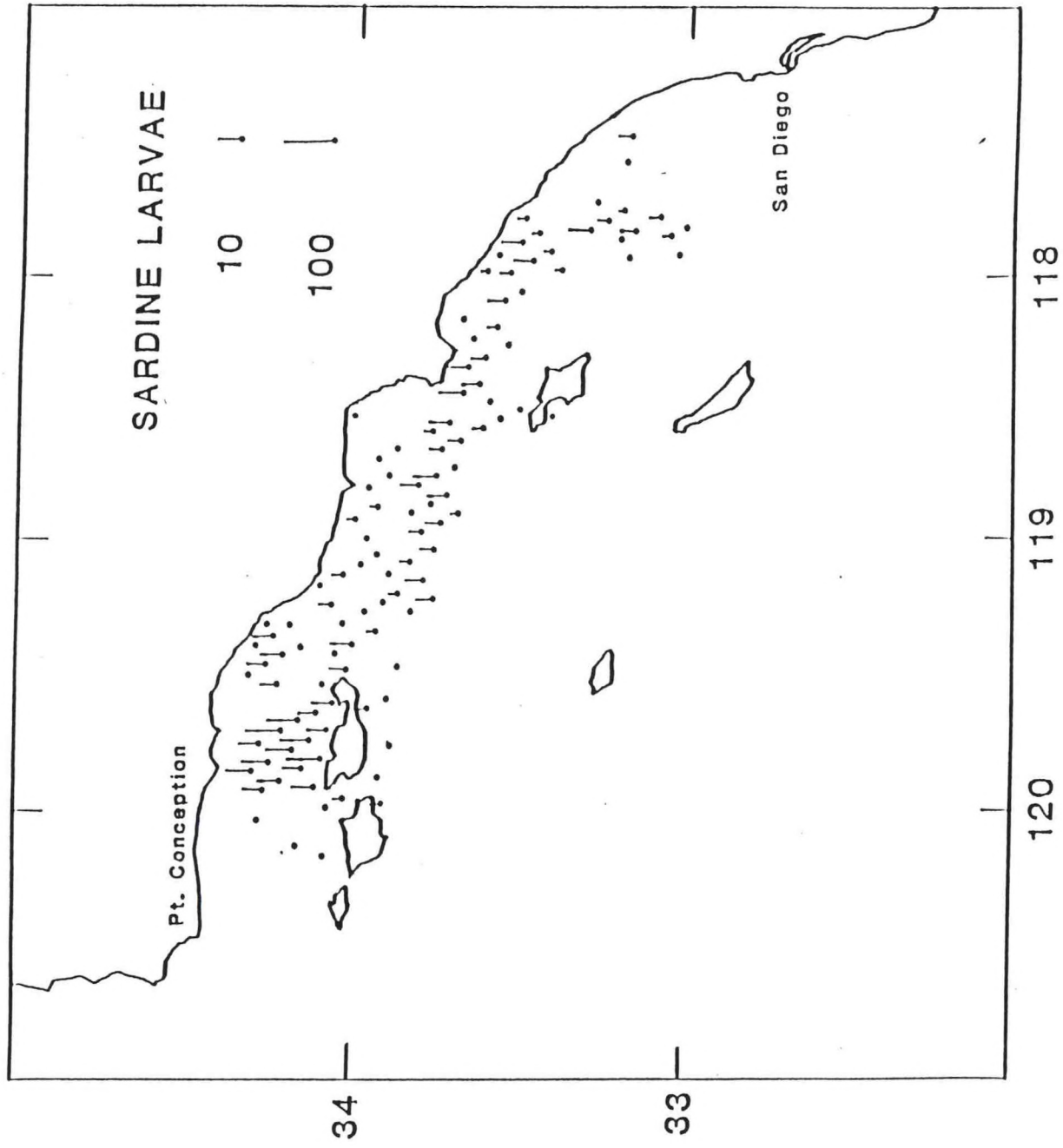




Figure 14

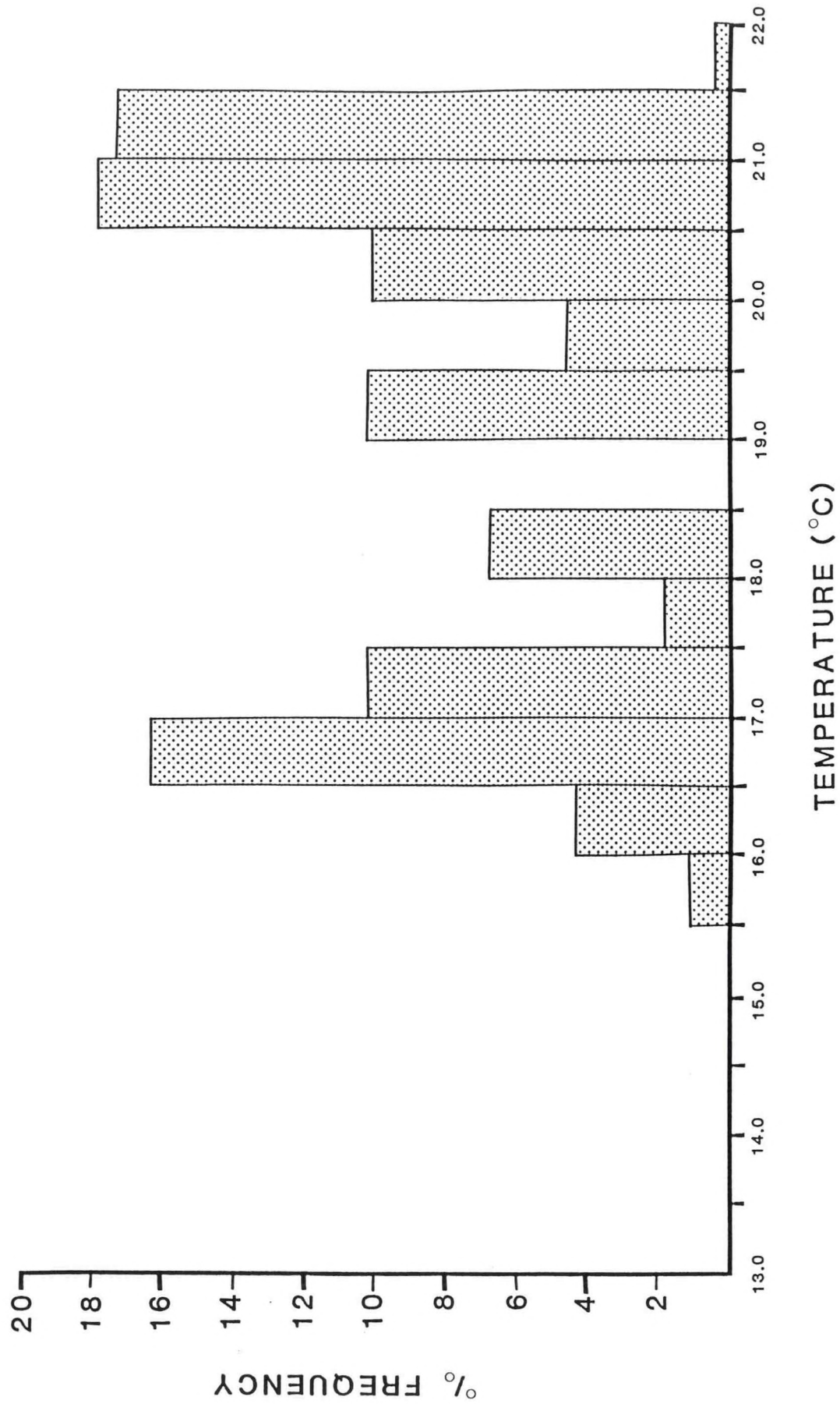
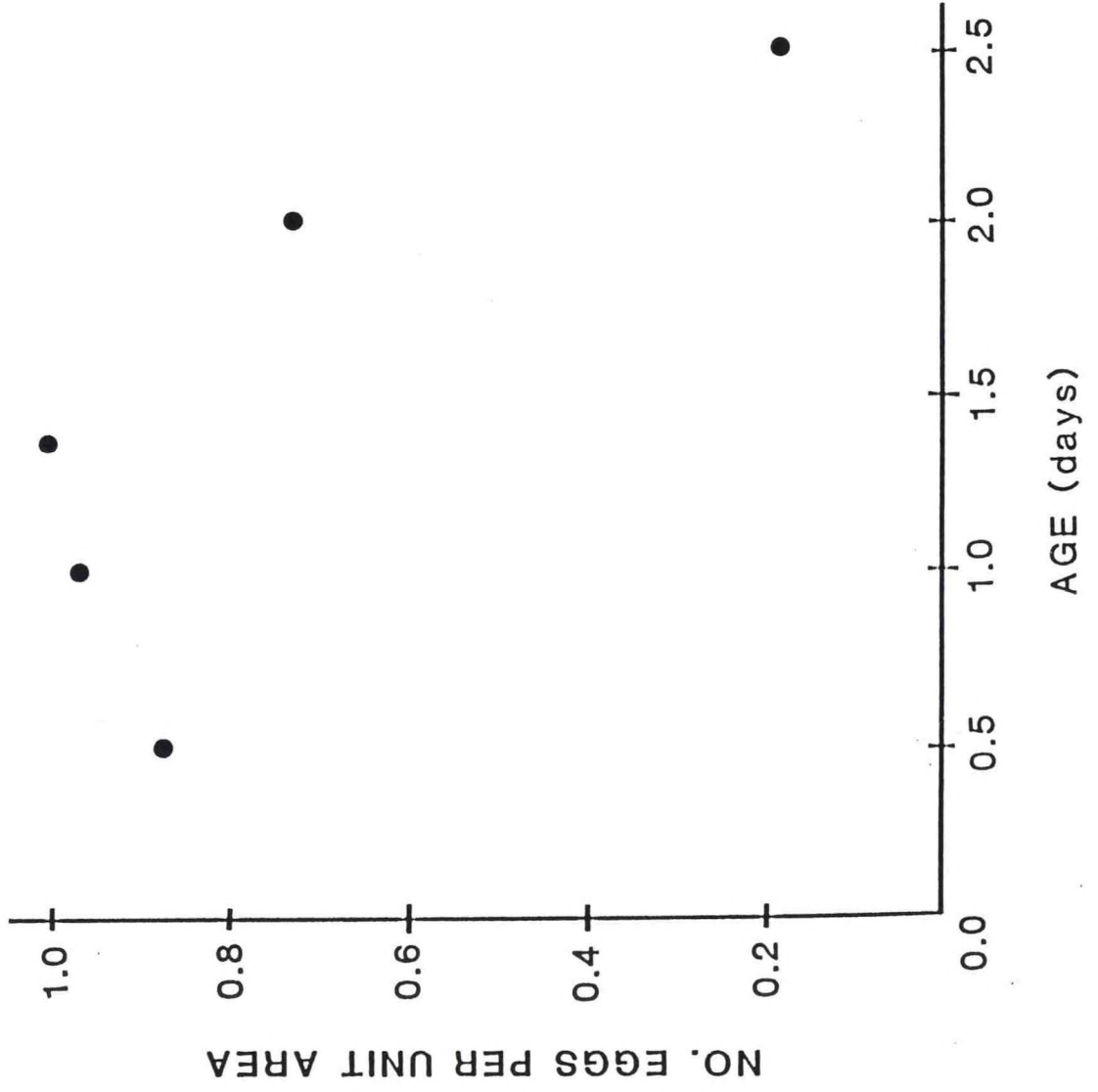


Figure 15

SARDINE EGG MORTALITY CURVE 1986

$$P=1.890 * \text{EXP}(-0.05 * \text{AGE})$$



## APPENDIX

### COMMENTS SUBMITTED BY REVIEWER # 1

In the second paragraph under "Batch Fecundity" on page 5, the text refers to unpublished data by Dickerson regarding batch fecundity in Pacific Mackerel. Is a manuscript in prep on this subject?

In the discussion section beginning on page 9 when comparing results from the EPM and the area method, is it possible to infer density dependent growth, as in mackerel?

Editor's note: Most corrections suggested by reviewer # 1 have been incorporated into the text as they were mainly grammatical in nature.

### COMMENTS SUBMITTED BY REVIEWER # 2

This paper is good, both in the biology and in the writing, and it should be published.

While the estimated biomass seems to be OK, I believe that the variances have been underestimated. There are two sources of this problem:

1) The post-cruise stratification was overdone. For example, the positive stratum includes three stations where a single egg was sampled, judging by Figure 10, but adjacent stations are placed in the negative stratum. A better post-cruise stratification would simply include in the positive stratum all stations between Pt. Dume and Santa Barbara. My reasoning is that a single egg could have appeared at nearly any of the stations in that region, but would have been much less likely to have been encountered in the north and south segments of the proposed negative stratum. I don't know how much additional variance would be added by this treatment, but it shouldn't be difficult to calculate.

2) The north-south stratification ignored covariance between the two strata; i.e. the calculation at the bottom of Table 1 assumes that the north and south estimates are entirely independent. Note that this error was also made in estimating the variances of previous stratified anchovy biomass estimates. Sokal and Rohlf (1982, Biometry p. 573) give the variance of the sum of two random variables as:



$$s^2(Y_1 + Y_2) = s_1^2 + s_2^2 + 2r_{12}s_1s_2$$

where  $r$  is the correlation coefficient. We don't know what the value of  $r$  is, but it is probably larger than zero and less than 1. So let's use those values as bounds. The lower bound is the value in the manuscript (s.d. = 3898 MT or CV = 0.509). The upper bound is s.d. = 4780 MT, or CV = 0.624, assuming strongly positive correlation.

Another possible source of error which should be discussed briefly is the effect of the suspected late timing of the survey with respect to the spawning season. In 1981, two anchovy biomass estimates were made, one in February and one in April. The April estimate seemed to be valid, and had a low variance, but nonetheless came out quite low compared to the earlier estimate. Our interpretation has been that the April estimate did reflect the spawning biomass of the segment that was still spawning, but that a large portion of the potentially spawning population had shut down by then. There is a reasonable possibility that this may have happened in the sardine estimate, but we will not be able to know until future surveys help us refine our knowledge of the sardine's spawning season and reproductive biology.

### COMMENTS SUBMITTED BY REVIEWER # 3

The manuscript should be published; however it needs a lot of work. I have written a large number of comments on the manuscript and will only discuss the major problems here.

The principal problems concern the Adult Survey section. There were apparently 11 positive sardine sets and in the middle of page 4 it is stated that they contained 1 to 55 fish and that five of the positive samples contained the desired 50 females. It is never stated how many females were included in the study, how many were in the northern area and southern areas, or how many were used in estimations of the various parameters. For some reason the authors decided to present their data as the means of the northern, southern and total samples; for example Figures 2,4, and 6. However they are inconsistent and for batch fecundity they don't split the data by northern and southern regions.

Why with 11 samples are there 12 samples in Figures 2,4, and 6? How did the authors calculate a proportion for the sample with only one fish? How many fish were there in the samples with less than the desired 50 females? Why weight the sample with one fish, or those with less than 50 fish equally to those with 50 fish? The whole concept of using proportions of individual samples is a problem

with only five complete samples. However; even if they had the correct number of samples (11 ?) and the samples were all the same size the data would have been more meaningful if the frequency of the fish, rather than the frequency of the sample means were used. There are too small a number of samples to have much resolution in figures utilizing the sample means.

The title of Figure 7 and the units in the figure are not compatible. I don't have the slightest idea of what this figure actually represents.

The title of Figure 8 is also very poorly written. But the real problem is that the figure shows the proportion of mature females which are in the three different day-0 stages by time of capture. Obviously the proportion of females in the three combined day-0 stages (i.e. day-0, day-0 plus hydrated, and hydrated) should not change by the time of night. Figure 8 shows that from 2200 to 2400 about 40 percent of the mature females were day-0's, from 2400 to 0200 about 18 percent were day-0's, and from 0200 to 0500 less than 10 percent were day-0's. If this is actually the case I feel that this entirely invalidates the use of day-0's as the time of night that the samples were taken would be the primary factor determining the spawning fraction. If you want a high biomass you sample early in the morning and if you want a low biomass you sample from 10 pm to midnight.

If Figure 8 is actually correct I suggest that the authors also plot the day-1's and day-2's by hour of the night to demonstrate that these stages are not biased by time of sampling. This is particularly important due to the small sample size and the fact that there was such a large difference in the spawning fractions in the northern and southern areas. In any case the spawning fraction problem must be cleaned up before the manuscript should be published.

#### COMMENTS SUBMITTED BY REVIEWER # 4

It is not clear whether the definition of W in equation 1 is WB or OFW. Based on further material presented on page 5, I am assuming  $W = OFW$ . In any case, the abbreviations used in equation 3 are conflicting with those in equation 1. Use W instead of WB if they are synonymous. Similarly, for equation 4 if batch fecundity (BF) is the same as F in equation 1, maintain consistency in variables.

The first paragraph on page 4 and references to Figure 2 contain discrepancies in the number of samples. The text states that five of the 11 samples contained the desired 50 females, while Figure 2 displays 12 sample means; six in the north and six in the south, none of which have the number of females defined. Once clarified, Figures 3, 4 and 6 must be revised to reflect the accurate changes in the north and south. Also, the sample with only one fish



should be annotated where displayed on the figures.

Page 4, paragraph 3: the significance of paired sample t-tests [t] are unclear. The same for page 5, last paragraph, where the number "22" is also undefined, as well as the number "11" in the same calculation at the top of page 7.

The last paragraph on page 6 defines the number of mature female sardines in each spawning state for day-0, 1 and 2, but does not provide a value for mature females beyond day-2. The description of the calculation of spawning fraction is ambiguous, and should be presented in a formula. As stated in the text, there is no "adjusted grand average spawning fraction" provided in Table 1. The "egg production programs" used to calculate the spawning fraction and CV should be mentioned by name and in the literature.

The statement in paragraph 3 on page 8 suggesting there may be optimal temperature ranges for sardine spawning is very speculative. It is suggested that this either be omitted or substantiated with the number and percentage of positive stations vs. temperature. The reader cannot tell from your data if the bimodal distribution occurs in the temperature of the stations.

In your discussion of calculation of P in equation 5, you state that an egg mortality model was generated by weighing each egg sample proportionally to the area of the station. But in the first paragraph under "Egg Survey" on page 7, the text says each station represents the same area.

In the "Daily Production of Eggs" section on page 8, the spawning area calculated using the area method only utilizes positive stations, but it is unclear whether embedded stations are included in the calculation.

You define Figure 5 as a linear regression, yet there is no regression line or parameters given. Your graph is a scatter plot. Figure 8 should utilize a true proportion or a percentage. Figure 13 should not be described as "spawning area as defined by positive egg stations" as the area also includes embedded negative stations. Figure 14 should include the frequency of stations by temperature, because according to Figure 3, there was very little water sampled between 18 and 19 degrees C.