

Sea Survey Design and Analysis for an Egg Production Method of Anchovy Biomass Assessment

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ABSTRACT

A sea survey of spawning habitat is described for the northern anchovy, *Engraulis mordax*. The central population occurs within a permanent gyral circulation off southern California and in the adjacent nearshore and main branches of the California Current. The geographic scope of spawning is maximal in winter and spring, and its extent appears to be a function of population biomass.

About 8 hours after the onset of spawning it becomes possible to obtain effective samples: adult data can be used for about a day and egg data for a little more than 2 days to estimate vital rates, such as egg production and mortality. The spawning process is so patchy and dynamic that it has not yet been possible to gather useful data on adults or eggs during the spawning interval.

The population egg production rate is determined by a survey using about 1,000 vertical plankton tows of 3.5 m³ between 70 m depth and the surface. The array of samples is intended to be representative and inclusive of the entire spawning area. Observations of the number of eggs per sample represent a contagious distribution (patchy), and the assumptions necessary for a "normal" or "log-normal" model are not met. The assumption of independence of sample parameters (mean and variance) is not supported with either model. The probability distribution most closely approximated is the "negative binomial." The parameters of that distribution change with the age of the egg.

The Southwest Fisheries Center Egg Production Method was initiated with much historical, geographic, and biological data obtained in the California Cooperative Oceanic Fisheries Investigations (CalCOFI). It should be possible to initiate an egg production method on other species in other regions without this vast time-series of data. Preliminary laboratory work, field surveys, and analyses are described for research at other temperatures and for the diagnosis of egg production for species which spawn at all times of day rather than nocturnally. The geographic limits of spawning should initially be described from oblique plankton tows filtering larger volumes of water (500-1,000 m³). Also, differences in spawning patch intensity may require observations of more than the 3.5 m³ which is adequate for the anchovy in this region.

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THE SPAWNING PROCESS

Spawning Area

The northern anchovy, *Engraulis mordax*, spawns within the regional eastern boundary current, the California Current system. Figure 1 is a series of overplots of all samples of anchovy eggs collected from 1951 to 1981. These serve to describe the maximum spatial distribution of spawning in each bimonthly period. Spawning occurs primarily nearshore of the main branch (Hickey 1979) of the California Current. The main branch of the California Current is 300 km off Washington and Oregon (lat. 45°N), 430 km off Cape Mendocino (lat. 39°N), 270 km off Point Conception (lat. 34°N), 240 km off northern Baja California (lat. 30°N), and 200 km off Cape San Lazaro (lat. 25°N).

The boundaries of the main branch of the California Current shift in position and are indistinct at every season. The 1950-78 average in a section normal to the coast at lat. 32°N reveals the three branches of the California Current postulated by Hickey (1979) (Lynn et al. 1982). The speeds of the surface currents at the cores of these three zones are given in Table 1. It may be inferred from Figure 1 that the nearshore zone of the California Current system is the most consistent spawning site of the northern anchovy and that virtually no spawning takes place in the outer zone or in the main branch at the season of heaviest southward flow.

Table 1.—Zones of the California Current.

Month	Outer		Main		Inner	
	u ¹	d ²	u	d	u	d
January	³ +3.5	600	- 6.9	400	3.5	100
April	-5.2	500	- 6.9	250	-5.2	50
July	-3.5	700	-12.0	250	6.9	100
October	-2.6	700	- 6.1	400	5.2	100

¹u is the current speed at the core in kilometers/day.

²d is the distance of the core from the coast at CalCOFI line 90 (see Lynn et al. 1982) in kilometers

³indicates Equatorward flow.

In addition to the large-scale features, there are local environmental events which appear to influence the pelagic spawning population. Temperature (Lasker et al. 1981; Fiedler 1983) and surface chlorophyll concentration estimated from analysis of satellite infrared images (Pelaez and Guan 1982; Fiedler 1983) appear to be important in fine-scale distributions. In the short period in which satellite image analysis has been possible, 0.2 mg/m³ appears to be a lower limit of chlorophyll in which anchovy spawning takes place (Fiedler 1983). There also appears to be some diminution of incidence of eggs at temperatures below 13.5°C (Fiedler 1983) although temperatures as low as 11°C are not lethal for anchovy eggs. It also appears that a certain amount of stability in terms of mixing (Lasker 1975; Bakun and Parrish 1982; Smith and Lasker 1978) and absence of offshore and southerly transport (Hewitt and Methot 1982; Power 1983) are favorable to the establishment and maintenance of spawning areas.

Population size also appears to control the spawning area (Ahlstrom 1965; MacCall 1983). There is anchovy spawning further offshore and north when the biomass is large, but the spawning area appears to contract toward the Los Angeles Bight when the biomass is smaller. A simple description of this (MacCall 1983) would be that a 100,000-ton spawning biomass would extend off-

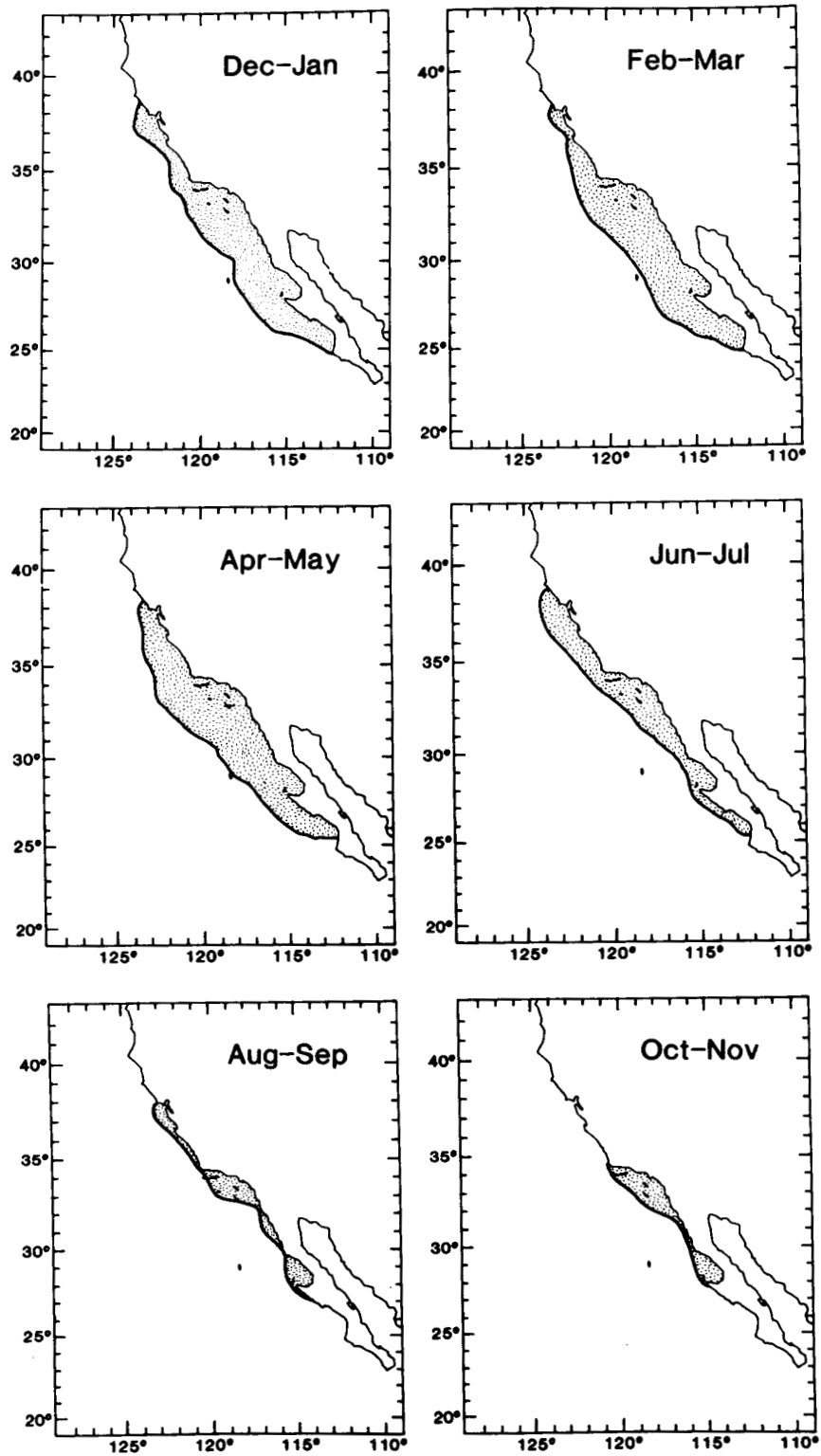


Figure 1.—Long-term spawning range of the northern anchovy as estimated from approximately 30,000 observations of anchovy eggs taken from a larger area between 1951 and 1983. For maps of all observations between 1951 and 1979 consult CalCOFI Atlases 9 and 28 (Kramer and Ahlstrom 1968; Hewitt 1980).

shore only 260 km, but a 1-million ton spawning biomass would extend offshore 360 km.

Spawning Season

Some anchovy spawning takes place at every time of the year, but winter and spring are times of the most active spawning.

THE SAMPLING PROCESS

Catchability and Vulnerability of Adults

Epipelagic schooling fish are difficult to sample quantitatively. At times the sample trawls have been placed based on sonar mapping the previous day. At other times the samples have been taken where newly spawned eggs were detected by preliminary examination of plankton samples. Uniform samples of all tows conducted for the purpose of estimating egg production represent the 7 to 40-m depth range. The upper limit is set by the minimum depth at which a mid-water trawl can be fished.

One aspect of the variation in catchability can be illustrated by examination of the variation in the sex ratio. If one considers the data on 362 observations for which sex ratio was determined between 1977 and 1982, there is an interesting distribution of sex ratios and standard deviations of sex ratio by time of day (Table 2; Hunter and Macewicz 1980). For comparison, the overall mean of sex ratios is 0.497 with a standard deviation of 0.233 and a standard error of the mean ratio of 0.012.

The dispersion of values, about 50% female, is wider than one would expect of a binomial sampling error distribution with 10-30 specimens, thus we believe that the phenomenon reflects actual biological features of the anchovy schooling and behavior pattern. In further support of this idea, the distribution about 50% is skewed to the side of underestimation of females and the bias arises from a peak time 2200-2359 which coincides with the maximum spawning activity as seen from the surveys of stage I and II eggs (see Moser and Ahlstrom 1985). The strength and prevalence of this spawning behavior are demonstrated by the fact that a 5% overall bias in sex ratio may be caused by only 10-15% of females and their attendant males.

We postulate that during the spawning act more males than gravid females are present, and this leads to temporal and spatial heterogeneity; also, the collection of samples with a trawl from a volume which is 15 m thick, 15 m wide, and 2,000 m long has considerable chance of mixing these proportions by transecting spawning and nonspawning clusters. Thus it is that the modal catch category, 43 of 362 samples, is at 50% female. The binomial sampling theorem for fish sample sizes of 10-30 fish would indicate that about 6% of the samples would yield <30% female and also 6% would yield >70% female. The actual observations are given in Table 3.

For the use made of the adult data at present, the sex ratio bias (see Hunter et al. 1985) is not thought to be of any great importance: the ratio of 1-d postovulatory gonads is used for the inverse of the daily spawning fraction, and the sex ratio is determined to be 50% because there is no weight differentiation by sex and the numeric ratio is likely to be 50% as well. In another section (Alheit 1985) we shall see that the Peruvian adult sampling system with purse seiner exhibits no bias, and we assume that the explanation lies with the evasion of the trawl or the depth distribution of the sexes. In

Table 2.—Percent females in trawl catches of northern anchovy at different times of day.

Time of day	No. of samples	% females	SD (%)
1800-1959	77	52.6	18.9
2000-2159	88	47.0	27.9
2200-2359	80	45.3	26.0
0000-0159	64	50.5	22.2
0200-0359	24	50.4	17.7
0400-0559	21	57.1	11.1
0600+	8	65.6	14.7

Table 3.—Actual and expected catches of females of northern anchovy at different times of day during spawning.

Mean time (h)	No. of samples	No. of samples expected at	
		<30% female	>70% female
1900	77	5	6
2100	88	5	26
2300	80	5	22
0100	64	4	9
0300	24	1	1
0500	21	1	0
0600+	8	1	0

Table 4.—Percent positive egg captures and mean number of northern anchovy per tow by time of day.

Time	Observations	Positive samples	% positive	Mean no.
1800-1959	142	5	3.5	0.120
2000-2159	135	26	19.3	0.919
2200-2359	147	48	32.7	3.265
0000-0159	152	33	21.7	3.303
0200-0359	151	22	14.6	0.464
0400-0559	132	8	6.1	0.462
0600-0759	137	6	4.4	0.153
0800-0959	129	1	0.8	0.008
1000-1159	129	5	3.9	0.039
1200-1359	133	2	1.5	0.023
1400-1559	128	2	1.6	0.016
1600-1759	151	1	0.7	0.007

general, the commercial fishery exhibits a sex ratio of the order of 60% female, and the sea survey has a ratio of 50% female (Mais 1974).

The inference that changes in catchability and vulnerability of the adults are controlled somewhat by the spawning act is strongly supported by the coincidence of spawning detected in the sea. Statistics are available from 3,936 observations between 1980 and 1983 of which 1,666 had 1 or more anchovy eggs. Prior to the first cellular division (Stage I eggs, see Moser and Ahlstrom 1985) there are small numbers of observations and these reflect the temporal distribution of spawning in much the same way as the adult sex ratio variance (Table 4).

Sampling Requirements for Egg Survey

The egg survey supplies two values to the spawning biomass estimate: The size of the spawning area and the daily production of eggs per unit area. When establishing standards for the survey of egg production, it is necessary to consider the inclusion of the entire spawning area, the representativeness of the samples within the area, and the sufficiency of the number of observations for the required precision.

There is an obvious bias associated with egg production outside the surveyed area. The product of the survey area and the egg production/unit area is unbiased even if the survey is much larger than the spawning area. That is, as the surveyed area outside the spawning area increases there is a corresponding decrease in the mean number of eggs produced/unit of survey area. However, a secondary objective of the Southwest Fisheries Center Egg Production Method is to determine the error distribution of each estimated parameter. The Central Limit Theorem is not valid in reduction of standard-error-of-the-mean value with increasing number of observations if those values are spatially coherent. If there was a broad contiguous and continuous area of observations with no eggs in the observations, the standard error would be correspondingly biased as an underestimate.

It is not necessary to know the nature of the underlying distribution of observations to use the Central Limit Theorem to estimate the error distribution of the mean. However, it may be useful to consider some probability-generating distributions in order to forecast what an adequate number of observations would be, given the sample variance and the objectives of the survey. As an example of the differences one might encounter, consider: If the eggs were distributed such that the observations produced a "Normal" distribution, then with each sample one would obtain an independent estimate of the population mean and the population variance; if the eggs were distributed such that the observations produced a "Poisson" distribution, then the estimated mean of the population would simultaneously produce an estimate of the variance of the population (equal to the mean); if the eggs were distributed with areas of high density and areas with no eggs at all such that the presence of one egg would predict the presence of other eggs in the sample so that a "Negative Binomial" distribution of observations obtains, one would need many independent observations to obtain a mean number of eggs/unit area with a normal distribution of standard error of the mean. Thus the expense of effort of estimation-per-unit precision is much lower with "Normal" and "Poisson" distributions than it would be with an underlying "Negative Binomial" distribution. To diagnose this, one needs to compare the variance and mean of several estimates. If the variance is independent of the mean, the distribution is "Normal"; if the variance is a power function of the mean, the distribution is probably "Negative Binomial," although there are other possibilities. For a first approximation, the "Negative Binomial" appears to be a useful working model for design of the egg production survey to determine the mean and variance of the egg production parameters.

The establishment of the "Negative Binomial" model of underlying distribution of observations does not support speculation as to the scale or origin of the patchiness, and thus no single set of samples can be used to interpret pattern.

For the purpose of determining sampling requirements for an egg survey, it is necessary only to show that the number of samples is adequate to describe the mean and standard error of the production of eggs per unit area. A second level of analysis and sampling is

necessary to jointly describe the scale and intensity of the patchiness and so allow speculation and research as to the processes which underlie the observed pattern. Knowledge of scale of the "patches" and intervening "spaces" and their shapes is necessary for further work.

We discuss the description of pattern below merely to emphasize what we would need to know to interpret processes such as:

1. The interannual variability in patch scale possibly caused by differences in fecundity attributable to such parameters as batch size, interval between spawnings, and age composition of spawners;
2. The geographic variability in the scale and intensity of predation;
3. The interaction of patch scales and numbers of anchovy eggs with patch scales and numbers of their principal predators, including adults within the school, adults and juveniles in other schools of the same species, and other noncannibalistic predators on anchovy eggs;
4. The relevance of interaction between the sampler size and the anchovy egg patch scale in order to transfer these sample designs to other anchovy populations or to other species; and
5. The comparison of vertical samples to older oblique samples, or the effects of taking samples of different size and shape on survey efficiency.

One reason that the standard sampling tactic (the vertical egg tow on a systematic grid) is not fully effective for advanced description of pattern is that observations are taken at one size scale and separated by a fixed distance. If we take the characteristics of the negative binomial—the mean, the coefficient of patchiness, " k ", and the percent of the area, $P(0)$, with no eggs—only the mean is a characteristic of the sampled population; k and $P(0)$ result from interactions between the scale of the sampler and the scale of the patches of eggs. For example, if the vertical sampler mouth opening were progressively increased from 0.05 m² to 10 m², we would expect the probability of "zero" observations to decrease. The decrease would depend on what fraction of the interpatch spaces were between 25 cm (the diameter of the smaller sampler) and 357 cm (the diameter of the larger sampler). For k the equation for the population is:

$$k_i = \frac{\mu_i^2}{\sigma_i^2 - \mu_i}$$

where k_i is the scale-dependent coefficient of patchiness, μ_i is the population mean of all possible areas for sample size " i ", and σ_i is the population variance for that set. If the true scale of anchovy egg pattern were approximately the same as the larger sampler, the sample variance would increase under the influence of larger observations and there would be a concomitant diminution of the variance from the effect of the larger sampler lying across more patch boundaries and thus integrating patch and space densities. If the true scale of the anchovy egg patches were smaller than the larger sampler, the variance would decrease because all observations would integrate some space densities. If the true scale of the anchovy egg patches were greatly larger than either sampler, the derived k 's would tend to be indistinguishable.

Lastly, if the true scale of the anchovy egg pattern is larger than the spatial interval between stations, the variance will be less because adjacent observations will be more similar than observations chosen at random.

There is evidence that several scales of pattern exist which probably originate from different processes. The smallest scale is imposed by the process of fertilization and is at the scale of a single

female or a small group of females. The next scale dimension is imposed by the general schooling habit. The largest scale within the subpopulation is called the "school group" and it may contain several thousand schools of juveniles and adults in varying degrees of spawning condition. For the purposes of this discussion we can label these scales, meters, hundreds of meters, and thousands of meters: the biogeographic boundaries of the entire interbreeding subpopulation (central subpopulation of the northern anchovy) is 400 km cross-shore and 1,000 km along the coast.

The larger scales are of practical concern for the egg production method using the Central Limit Theorem; namely, the effect of multiple observations forming a normal distribution of the standard error of the mean is diminished by coherence among adjacent observations. Preliminary analyses of replicate egg samples (Smith and Hewitt 1985) indicate that cross-shore observation transects with observations separated by only 500-1,000 m would be coherent, while observations in excess of 5,000 m apart are independent of the major persistent source of patchiness at the "school" scale, 100-1,000 m. School groups have not yet been positively identified by the egg production surveys, but they are obvious from aerial and wide-ranging sonar mapping surveys (Fiedler 1978; Smith 1978).

To summarize, sample design decisions for the Southwest Fisheries Center Egg Production Method of biomass assessment have been based on prior knowledge, e.g., 1) the anchovy's spawning season, 2) the spawning area, 3) size of the school groups, and 4) size of the schools.

The volume of one observation is 3.5 m^3 or $1/20 \text{ m}^2$ between 70 m and the surface. The distance between observations is 4 nmi in the cross-shore direction and 10 or 20 nmi in the alongshore plane. For the central subpopulation of the northern anchovy, the recommended sampling area is 200 mi cross-shore and 600 mi alongshore.

Random vs. Centric Systematic Area Sampling

The sample design for the egg production method does not use random sampling: the lines are fixed in cross-shore positions which conform to historical surveys of biological and oceanographic features which can be measured only with straight sections at or near right-angles to the coast. The danger with fixed transects is that characteristics, such as proximity to a canyon or upwelling site, the passage close to an island, or the aversion to stations in shipping lanes, all adversely affect the necessary assumption that all objects to be sampled have had an equal opportunity to appear in a sample. Another problem is the regular spacing of systematic samples: if the alongshore or cross-shore spacing coincides with any periodic element in the distribution of the organisms being sampled, then the possibility exists for bias through oversampling some phases of the spatial periodism. For example, if the number of organisms varied like a sine wave, it is possible that regularly placed samples will hit the peaks or troughs in the distribution, thereby over- or underestimating the population. In a strict sense, statistical limits cannot be established with sets of systematic samples: there is some evidence (Milne 1959) that for periodic differences to be important, they would have to be obvious. Milne (1959) stated ". . . with proper caution, one will not go very far wrong, if wrong at all, in treating the centric-systematic-area sample as if it were random."

INITIATING AN EGG PRODUCTION PROCEDURE

The Southwest Fisheries Center Egg Production Method was created using new principles of sampling and mortality analysis of anchovy eggs in the sea and residual gonadal tissues indicating recent ovulation. There existed a wealth of data on the geographic distribution of adults (Mais 1974) and the geographic and temporal distribution of anchovy spawn (Kramer and Ahlstrom 1968). It should not be necessary to repeat the CalCOFI effort at each site and for each species to develop egg production procedures for other species in other geographic areas. It is the purpose of this section to describe minimal initial steps for the egg production method.

Initiation of Field Sampling

Observations of larger larvae (5-10 mm) from high-volume oblique tows (ca. $1,000 \text{ m}^3$) would be superior to the CalVET (3.5 m^3) tows for establishing the regional boundaries and season optimum for the egg production method for a given species. For widespread pelagic species exhibiting multiple spawning, interstation distances of 40-120 nmi should suffice (74-222 km) to delimit spatially the spawning area. Monthly, bimonthly, or seasonal surveys should be sufficient.

Laboratory and Field Studies of Spawning Behavior

While it is conceivable that temperature-dependent rates of egg development can be derived from the analysis of field samples and ancillary depth and temperature data, it is recommended that laboratory-controlled temperature experiments be used to establish the temperature-dependent rate model. In particular, the extremes of temperature may be difficult to observe adequately in the field in any given year.

In the field, even when there is no main time of day for spawning, it is possible to determine the number of eggs spawned per day by:

$$N_t = N_0 \int_{-\infty}^t \exp[-Z(t-x)]f(x) dx$$

where N_t is the number of eggs in a particular stage and later stages at time t ; N_0 is the total number entering the series; $\exp[-Z(t-x)]$ is the probability that an egg at time x is still alive at time t ; Z is the constant age-specific death rate; and $f(x)$ is the frequency of the stage at time x (Manly 1974). The actual frequency function can be inserted in the equation. For example, a normal distribution of spawning time would yield the equation:

$$N_t = N_0 \exp(-Zt) \int_{-\infty}^{t-\mu/\sigma} (2\pi)^{-1/2} \exp(-1/2x^2) dx$$

One must, of course, have more than four samples to solve this equation (Manly 1974).

When regional and seasonal criteria for spawning have been established, there must be an intensive field study to find the sample distribution in time and space which will be sufficiently precise for the purposes of egg production estimate of spawning biomass. The conditions which must be noted from the data set are (after Southwood 1978): 1) Distribution of the time of spawning, 2) variability of survival rates, 3) stage-dependent sampling efficien-

cy, 4) duration of stages, and 5) probability distribution of eggs as a function of time and age.

Working assumptions for the central population of the northern anchovy at lat. 33°N in March are:

- 1) Spawning occurs between 1800 and 0200, essentially normally distributed with a midpoint of 2200 hours.
- 2) The survival rate is assumed to be constant between 0200 of the first day after spawning until hatching begins (normally 60 h).
- 3) All stages are sampled with equal efficiency, but the period when spawning is actually occurring or after hatching begins is excluded from survival stimulation.
- 4) Duration of egg stages is proportional to a single temperature (usually the surface temperature).
- 5) The probability distribution of eggs/unit surface area is completely characterized by the two parameters of the negative binomial distribution, the arithmetic mean and the dispersion parameter k , a function of the population mean and variance. These parameters are age-specific, the mean decreasing with age and k increasing with age.

The last assumption must be considered for the regression method of estimating the precision of the slope and intercept estimates, as there are, as yet, no formal solutions for regression equations under these conditions.

Staging Eggs in Other Species or in Tropical or Arctic Habitats

The anatomical description of the 11 stages of eggs is given for anchovy in Moser and Ahlstrom (1985). Also, an experimental definition of the mid-age of each stage and the conversion of stage, time-of-tow, and temperature information are described by Lo (1985). We here describe some approaches to fishes whose spawning behavior and definition of stages are dissimilar to the anchovy.

The possible number of discrete stages assigned to the continuum of embryonic development is ultimately determined by the presence of recognizable anatomical features. For example, although not used in this work, the 2-cell, 4-cell, and 8-cell stages of the embryonic development could be used to follow population features immediately after fertilization. For convenience the nominal hatching time of 60 h has been divided into 11 stages for a mean duration of about 6 h. If one were dealing with a tropical fish with a hatching time of 20 h, it would seem more reasonable to maintain the number of arbitrarily defined stages at 10 or so, rather than to reduce the number of stages to 3 of about 6-h length. Similarly in the Arctic situation, where hatching may take several weeks, one may need to pool several days' spawning to gain sufficient sample sizes to estimate mortality rate and egg production.

Were the stages equal in duration and spawned at an instant, one would easily see the progression of these stages with time. Since the period of spawning is approximately 1/4 d, and the duration of the stages is from 2 to 9 h, the system used here was originated for the sardine (Ahlstrom 1943) temperature-specific development rate and later applied to the anchovy (see below). Since these are both temperature-zone clupeoid fishes, the rationale for these stages is listed below for the purpose of using this technique for fishes in other latitudes or taxa.

While it has not been possible to obtain exact information on fertilization time or the exact timing of the transitions between stages, it is possible to combine quantitative laboratory and field data to make a best description of the major events in embryogenesis. This

description can then be used to design definitive work in the laboratory or field as needed for future studies on these or other fish. We base all of the arbitrary descriptions on a starting time of 1800 (6 p.m.) for onset of spawning, with the midpoint of spawning at 2200 (10 p.m.), and finishing at 0200 (2 a.m.). Bolin (1936) observed that cell cleavage in the anchovy embryo occurred at approximately half-hour intervals, thus we assume that the interval between fertilization and first cleavage is about 0.5 h. Since the duration of the Stage II eggs from first cleavage until the onset of epiboly or cellular overgrowth of the yolk is about 7 h and the number of Stage II eggs collected is about seven times the number of Stage I eggs, we assume that the Stage I egg persists about 1 h or 0.5 h to be fertilized and 0.5 h more until the first cleavage.

Table 5 is from a summary of all field data taken in 1980-83, with 1,666 samples positive for some stage of anchovy egg. For each of the first five stages, the mean number of eggs per 0.05 m² is listed for each of 12 2-h periods, and next to each abundance is the cumulative percentage from the onset of the stage to the end of 24 h. For the cumulative percentage of each stage the point at which 5% of the eggs have appeared, 50% and 95% points on the cumulative curve are listed at the bottom of the table. The cumulative effects of temperature have little effect on the early stages. The sets included here are from all temperatures encountered in the surveys, mostly between 13° and 16.5°C in these years with an average temperature of 15.4°C.

In the laboratory experiment, it was not convenient to constrain the gravid anchovies, thus it is not known for certain when the first spawning begins under controlled conditions. Following spawning in the laboratory, the eggs were collected in the outflow in a passive net and were transferred into containers with temperatures controlled at approximately 13.5°, 15.1°, and 16.3°C. This means that the early stages concluded their development at a common temperature of about 15°C and were subsequently placed in controlled temperatures. Thus, for the tables which follow, the temperature is that at which most of Stage III and all of the ensuing stages were passed. The original data were somewhat more finely grouped, but low numbers of specimens in some categories and brevity made some lengthening of observation intervals desirable. To unify these numbers, the raw data were converted to numbers per hundred collected at each time interval, and then the numbers were summed for each stage and the cumulative sum at each reported interval was divided by the total to yield the tabled value of cumulative percent. The 0 in the table means that no specimens of that stage were spotted before that interval; the 1.000 means that no specimens in that stage were found after that interval. All Stage I eggs had developed before the eggs were caught at the outflow and most Stage II and some Stage III eggs were present before the systematic counting and staging began. The experiment was terminated before all larvae had reached the 3.5-mm length.

The actual times of day of the important events were injection of hormones in the afternoon of the preceding day and collection of the eggs in the morning. The first staging ensued at about 1300.

For any small set of anchovy samples, Stage I and Stage XI appear to be too short for any analytical purpose: each appears to be about an hour long. The other stages probably last between 5 and 13 h, and these durations diminish with increasing temperature and development. Thus for other species in lower and higher water temperature, the detection and use of a daily cycle of spawning should include redefined stages which are markedly shorter than one day at the beginning of development. The ensuing stages can be longer as is convenient for data gathering and storage.

Table 5.—Tabulation of field abundance of northern anchovy eggs as a function of time in hours after spawning; 00 refers to the mid-point of spawning.

Age in hours	Stages									
	I		II		III		IV		V	
	eggs/ 0.05m ²	cum. %	eggs/ 0.05 m ²	cum. %	eggs/ 0.05 m ²	cum. %	eggs/ 0.05 m ²	cum. %	eggs/ 0.05 m ²	cum. %
-04-02	0.120	0.014	0.113	0.002						
-02-00	0.919	0.118	1.511	0.026						
00-02	3.265	0.490	6.252	0.125						
02-04	3.303	0.866	11.612	0.308						
04-06	0.464	0.919	10.298	0.471						
06-08	0.462	0.972	8.932	0.613	0.197	0.004				
08-10	0.153	0.989	11.358	0.792	2.029	0.047				
10-12	0.008	0.990	7.426	0.910	4.767	0.149				
12-14	0.039	0.995	3.969	0.973	5.178	0.259	0.109	0.003		
14-16	0.023	0.997	1.023	0.989	7.474	0.419	0.316	0.013		
16-18	0.016	0.999	0.258	0.993	8.945	0.609	1.109	0.048		
18-20	0.007	1.000	0.450	1.000	8.762	0.796	2.378	0.121		
20-22					2.831	0.856	5.056	0.278	0.873	0.026
22-24					1.926	0.897	5.911	0.462	2.482	0.102
24-26					3.408	0.970	7.388	0.691	2.599	0.108
26-28					1.105	0.994	6.842	0.903	3.421	0.284
28-30					0.298	1.000	1.411	0.947	4.570	0.422
30-32							0.318	0.957	5.576	0.576
32-34							1.241	0.995	5.263	0.751
34-36							0.155	1.000	3.318	0.851
36-38									2.078	0.914
38-40									1.181	0.950
40-42									1.109	0.984
42-44									0.543	1.000
Age at which										
5% appeared	-1.5 h		0.5 h		10 h		18 h		22.5 h	
50% appeared	0.0		6.0		16		24		30	
95% appeared	4.5		13.5		25.5		31		40	

Abnormal Embryos

There appears to be a need for stages which can be recognized even with a distorted specimen. A large fraction of the anchovy eggs are decidedly abnormal and stage classification is quite difficult. We have sought to modify sample washing and fixation to diminish this problem, but the specimens are distorted when fresh and alive (Sandknop and Stevens²). Also these abnormal embryos do not seem to be associated with any particular stage or age of egg. Table 9 is a correlation matrix for the disintegrated eggs and the aged ones. The highest correlation is between disintegrated and total eggs, and even this shows no more than 7% of the number of disintegrated eggs can be predicted from the number of eggs in a sample.

Table 6.—Correlation matrix among categories of northern anchovy eggs.

Age of eggs	<8 h	1 d	2 d	3 d	4 d	DIS+ ¹
1 d	0.101					
2 d	0.029	0.396				
3 d	-0.022	0.176	0.277			
4 d	-0.024	0.029	0.002	0.325		
DIS+	0.045	0.150	0.143	0.111	0.073	
Total eggs	0.388	0.786	0.737	0.465	0.130	0.258

¹DIS = disintegrated eggs but with chorion intact.

²E. M. Sandknop, Biological Technician, and E. L. Stevens, Fishery Biologist, Southwest Fisheries Center, National Marine Fisheries Service, NOAA, P.O. Box 271, La Jolla, CA 92038, pers. commun. Dec. 1981.

The time in the field of the proportion of the spawning area covered by spawn (Fig. 2), the mean number of eggs per observation, the standard deviation, the standard error of the mean (Fig. 3), and the dispersion coefficient *k* of the negative binomial (Fig. 4) are consistent with the anchovy laboratory data and that for the Pacific sardine analyzed by Ahlstrom (1943).

SUGGESTIONS FOR FUTURE RESEARCH

Three characteristics of the anchovy make the assignment of ages possible: One is that the anchovy egg is demonstrably spawned during a limited period in each day; another factor is that stages have been assigned so that their duration is about one-third of a day so that ages can be unequivocally assigned if the temperature and time of tow is known; lastly, the total incubation time is about 3 d (see Table 7).

This leads one to the questions: How would one estimate egg production if the eggs were produced at all hours of the day? How would one proceed if the total incubation time were <1 d—more than 10 d?

Research on these questions could proceed along these lines. For the case of the long incubation period, it is not necessary to follow the course of mortality through the entire incubation period. For example, if the incubation period were 14 d and the ages of eggs could readily be determined for only 3 d, the mortality and production rate from that period would suffice for an estimate of the production of spawn; the ensuing stages of eggs would not even have

to be counted or staged.

The rate of production could probably be estimated without knowing the time-of-day of spawning. For example, in Table 8 estimates of rate of production could be determined solely from empirical abundance information and incubation time. For the purpose of this exercise, I have assumed that the 11 stages are incubated in 66 h, or 6 h/stage. Deviations of abundance from the regression estimate of abundance are of two kinds: Sampling variability and duration differences from the even duration assumption. If sampling variability is ignored, then stage lengths longer or shorter than the average stage duration can be estimated.

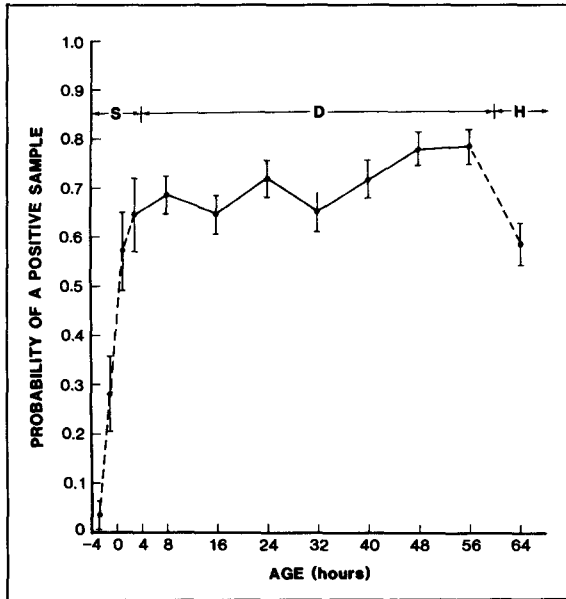
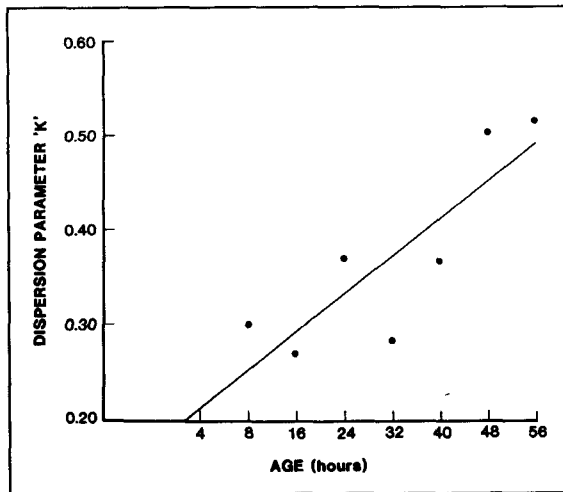


Figure 2.—Time course of proportional occurrence (number of observations with eggs of a given age divided by the number of observations with any age anchovy egg). Dashed lines under "S" indicate observations during the spawning period. The dashed line under "H" represents observations during the hatching period. The process assumed to control the solid line under "D" is dispersal.



ACKNOWLEDGMENTS

We would like to acknowledge the assistance of Carol Kimbrell for organizing and conducting the temperature laboratory experiments, H. Geoffrey Moser and his staff for providing data on staged eggs, Nancy Lo, Susan Picquelle, and Gary Stauffer for determining the age of eggs from field samples, James R. Thraillkill, Rich Charter, and William C. Flerx for planning and conducting the field surveys, designing and constructing the sampling equipment, and Cynthia Meyer and Celeste Santos for entering and checking the egg survey data. We are particularly grateful to Richard Methot who read an early draft of this manuscript.

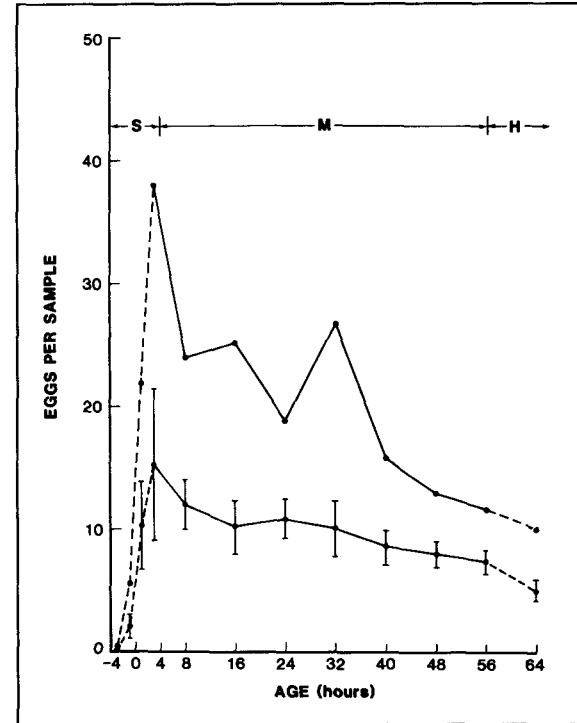


Figure 3.—Time course of the primary statistical parameters, the arithmetic mean (below), the standard deviation of the observations (above), and the standard error of the mean (cross-bars on the arithmetic mean line). Within ages the standard deviation is believed to be a function of the mean, and this function of the mean changes with time owing to dispersal (see Figure 4). Standard error of the mean bars are ± 2 (or approximately the 95% limits). For this illustration the number of observations is 1,666 taken between 1980 and 1983 as part of the egg production method estimate of anchovy spawning biomass. The dashed lines under "S" and "H" represent spawning and hatching as in Figure 2. The principal process controlling the slopes of the standard deviation and mean under "M" is mortality. The convergence of the standard deviation and mean lines is caused by dispersal of eggs.

Figure 4.—Negative binomial distribution is represented by two parameters: the arithmetic mean (see Figure 3) and "k", the "dispersion parameter." The points represent maximum-likelihood fits to sample data in seven 8-h intervals which contain samples unbiased by spawning or hatching. The line represents the linear least-squares fit to these points.

Table 7.—Cumulative incidence of laboratory-spawned and reared northern anchovy eggs and larvae.

Age (h)	Stage of development							Larvae	
	IV	V	VI	VII	VIII	IX	X	2.5 mm	3.0 mm
at 13.5°C									
16	0								
24	0.226	0							
32	0.812	0.204	0						
40	0.995	0.723	0.128	0	0				
48	1.000	0.985	0.759	0.265	0.032	0			
56		1.000	0.992	0.907	0.552	0.032	0	0	
64			1.000	1.000	1.000	0.684	0.174	0.100	0
72						0.947	0.802	0.292	0.004
80						1.000	1.000	0.724	0.014
88								0.880	0.271
96								0.975	0.554
104								1.000	0.763
112									0.843
120									0.888
128									0.977
136									1.000
at 15.1°C									
16	0.028	0							
24	0.591	0.010	0						
32	0.963	0.505	0.075						
40	1.000	0.965	0.459	0	0				
48		1.000	0.974	0.458	0.016	0			
56			1.000	0.981	0.874	0.273	0	0	
64				1.000	0.976	0.929	0.500	0.145	0
72					1.000	1.000	1.000	0.436	0.078
80								1.000	0.230
88									0.527
96									0.810
104									0.868
112									1.000
at 16.3°C									
16	0.025	0							
24	0.766	0.069	0						
32	0.944	0.881	0.231	0					
40	1.000	1.000	0.923	0.570	0.037	0			
48			1.000	0.974	0.880	0.210	0	0	
56				1.000	1.000	0.746	0.387	0.250	0.004
64						1.000	1.000	0.625	0.046
72								0.837	0.367
80								1.000	0.685
88									0.930
96									1.000

Table 8.—Empirically derived stage durations of northern anchovy eggs.

Stage	\bar{X}	2SE	4X	T	RE	D	CUM	F	L	d
I	0.77	0.28	3.08	3	13.78	1.34	0- 1.34	0.67	—	
II	5.34	0.80	21.36	9	12.85	9.98	1.34-11.32	6.33	6.42	-0.09
III	3.86	0.76	15.44	15	11.98	7.73	11.32-19.05	15.19	13.55	1.64
IV	2.79	0.48	11.16	21	11.17	6.00	19.05-25.05	22.05	21.12	0.93
V	2.75	0.62	11.00	27	10.41	6.34	25.05-31.39	28.22	30.70	-2.48
VI	3.06	0.44	12.24	33	9.71	7.56	31.39-38.95	35.17	39.24	-4.07
VII	2.28	0.36	9.12	39	9.05	6.04	38.95-44.99	41.97	47.13	-5.16
VIII	1.65	0.22	6.60	45	8.44	4.69	44.99-49.68	47.34	52.07	-4.73
IX	2.45	0.14	9.80	51	7.87	7.47	49.68-57.15	53.42	56.28	-2.86
X	1.86	0.10	7.44	57	7.34	5.92	57.15-63.07	60.11	62.63	-2.52
XI	0.44	0.074	1.76	63	6.84	1.54	63.07-64.61	63.84	65.65	-1.81

\bar{X} = mean number of eggs by stage ($n = 1,666$) per observation
 SE = standard error of number of eggs by stage.
 4X = daily production by stage if duration is 6 h per stage.
 T = midtime of stage if duration is 6 h per stage.
 RE = regression estimate of abundance (first approximation).
 D = duration of stage in hours if regression error is ignored.
 CUM = cumulated age of stages in hours.
 F = midtime of each stage estimated from field data.
 L = midtime of each stage estimated from lab experiment.
 d = difference in hours.

LITERATURE CITED

- AHLSTROM, E. H.
 1943. Studies on the Pacific pilchard or sardine (*Sardinops caerulea*). 4. Influence of temperature on the rate of development of pilchard eggs in nature. U.S. Fish Wildl. Serv. SSK 23, 26 p.
 1965. A review of the effects of the environment on the Pacific sardine. In Intl. Comm. Northwest Atlantic Fisheries Spec. Publ. 6, p. 53-74.
- ALHEIT, J.
 1985. Spawning frequency of Peruvian anchovies taken with a purse seine. In R. Lasker (editor), An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*, p. 59-61. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.
- BAKUN, A., and R. H. PARRISH.
 1982. Turbulence, transport, and pelagic fish in the California and Peru Current Systems. Calif. Coop. Oceanic Fish. Invest. Rep. 23:99-112.
- BOLIN, R. L.
 1936. Embryonic and early larval stages of the California anchovy, *Engraulis mordax*, Girard. Calif. Fish Game 22:314-321.
- FIEDLER, P. C.
 1978. The precision of simulated transect surveys of northern anchovy, *Engraulis mordax*, school groups. Fish. Bull., U.S. 76:679-685.
 1983. Satellite remote sensing of the habitat of spawning anchovy in the Southern California Bight. Calif. Coop. Oceanic Fish. Invest. Rep. 24:202-209.
- HEWITT, R. P.
 1980. Distributional atlas of fish larvae in the California Current region; northern anchovy, *Engraulis mordax* (Girard), 1966 through 1979. CalCOFI Atlas 28, 101 p.
 1982. Distribution and mortality of northern anchovy larvae in 1978 and 1979. Calif. Coop. Oceanic Fish. Invest. Rep. 23:226-245.
- HEWITT, R. P., and R. D. METHOT, JR.
 1982. Distribution and mortality of northern anchovy larvae in 1978 and 1979. Calif. Coop. Oceanic Fish. Invest. Rep. 23:226-245.
- HICKEY, B. M.
 1979. The California Current System - hypotheses and facts. Progress in Oceanography 8(4):191-279.
- HUNTER, J. R., and B. J. MACEWICZ.
 1980. Sexual maturity, batch fecundity, spawning frequency and temporal pattern of spawning for the northern anchovy *Engraulis mordax* during the 1979 spawning season. Calif. Coop. Oceanic Fish. Invest. Rep. 21:139-149.
- HUNTER, J. R., N. C. H. LO, and R. J. H. LEONG.
 1985. Batch fecundity in multiple spawning fishes. In R. Lasker (editor), An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*, p. 67-77. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.
- KRAMER, D., and E. H. AHLSTROM.
 1968. Distributional atlas of fish larvae in the California Current region: northern anchovy, *Engraulis mordax* (Girard). 1951 through 1965. CalCOFI Atlas 9, 269 p.
- LASKER, R.
 1975. Field criteria for survival of anchovy larvae: the relation between inshore chlorophyll maximum layers and successful first feeding. Fish. Bull., U.S. 73:453-462.
- LASKER, R., J. PELAEZ, and R. M. LAURS.
 1981. The use of satellite infrared imagery for describing ocean processes in relation to spawning of the northern anchovy (*Engraulis mordax*). Remote Sensing of Environment 11:439-453.
- LO, N. C. H.
 1985. A model for temperature-dependent northern anchovy egg development and an automated procedure for the assignment of age to staged eggs. In R. Lasker (editor), An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*, p. 43-50. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.
- LYNN, R. J., K. A. BLISS, and L. E. EBER.
 1982. Vertical and horizontal distributions of seasonal mean temperature, salinity, sigma-t, stability, dynamic height, oxygen and oxygen saturation in the California Current, 1950-1978. CalCOFI Atlas 30, 513 p.
- MACCALL, A. D.
 1983. Population models of habitat selection, with application to the northern anchovy. Ph.D. Thesis, Univ. Calif. San Diego, 170 p.
- MAIS, K. F.
 1974. Pelagic fish surveys in the California Current. Calif. Dep. Fish Game, Fish Bull. 162, 72 p.
- MANLY, B. F. J.
 1974. A note on the Richards, Waloff and Spradbery method for estimating stage specific mortality rates in insect population. Biom. Zeitschr. 17:77-83.
- MILNE, A. A.
 1959. The centric systematic area-sample treated as a random sample. Biometrics 15(2):270-297.
- MOSER, H. G., and E. H. AHLSTROM.
 1985. Staging anchovy eggs. In R. Lasker (editor), An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*, p. 37-41. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.
- PELAEZ, J., and F. GUAN.
 1982. California Current chlorophyll measurements from satellite data. Calif. Coop. Oceanic Fish. Invest. Rep. 23:212-225.
- POWER, J.
 1983. Larval anchovy (*Engraulis mordax*) drift in the California Current. Results of a simulation model. Natl. Mar. Fish. Serv., NOAA, Southw. Fish. Cent., La Jolla, CA, Admin. Rep. LJ-83-23, 36 p.
- SMITH, P. E.
 1978. Biological effects of ocean variability: time and space scales of biological response. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 173:117-127.
- SMITH, P. E., and R. P. HEWITT.
 1985. Anchovy egg dispersal and mortality as inferred from close-interval observations. Calif. Coop. Oceanic Fish. Invest. Rep. 26:97-110.
- SMITH, P. E., and R. LASKER.
 1978. Position of larval fish in an ecosystem. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 173:77-84.
- SOUTHWOOD, T. R. E.
 1978. Ecological Methods: With particular reference to the study of insect populations. Chapman and Hall, Lond., 524 p.



UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE

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April 16, 1987

MEMORANDUM FOR: USERS OF THE EGG PRODUCTION METHOD FOR
ESTIMATING SPAWNING BIOMASS OF PELAGIC FISH.

FROM: REUBEN LASKER *Reuben Lasker*

SUBJECT: ERRATA FOR NOAA TECHNICAL REPORT NMFS 36; "AN EGG
PRODUCTION METHOD FOR ESTIMATING SPAWNING BIOMASS OF PELAGIC
FISH: APPLICATION TO THE NORTHERN ANCHOVY".

A number of printing errors have been discovered by Dr.
Sachiko Tsuji in the published account of the egg production
method. These are important and warrant this memo. Please make
these corrections in your copy.

p. 5, Abstract, 4th line should read:

"be estimable and spawning rate constant over the field
sampling interval."

p. 12, in equation 8, $\hat{\beta}$ should be β .

p. 17, Table 1. on the January line +3.5 should be -3.5.

p. 20, two lines under the formula in the second column,
"sample size" should be "sample scale" and σ_i should read σ_i^2 .
Five lines under the formula "larger observations" should be
"bigger scales."

p. 22, 1st para., No. 3 last line should be simulation, not
stimulation.

p. 23. 1st para., line 7. "Table 9" should read "Table 6."

p. 44. Temperature table in second column on the page.

The temperatures read 13.9
 13.5
 16.2

The correct temperatures are 13.9
 15.2
 16.2.



- p.45. Second column, $Y_{i,t,k}$ should read $y_{i,t}$.
- p.46 1st Para., line 7, change the word "spawning" to "tows, \hat{T} ".
- p.49. Table 5d. Strike out the words "within or" in the second line of the heading.
- p.55. 9th line from the bottom, x_1 should be x_i .
25. p.56. First.para. second column, sixth line, 26 should read
- p.63. Under "Preservation" $Na_2H_2PO_4$ should be Na_2HPO_4 .
- p.93. In table 1, atretic state e, change $>$ to $<$.
- p.97. In the! formula after the second para. change $<$ to $>$.
- p.98. In the formula in the first column change $-Zt$ to $-Zt_h$.