Precision and Bias of Estimates of Larval Mortality

Nancy C. H. Lo, John R. Hunter, and Roger P. Hewitt

ABSTRACT: The results of four ichthyoplankton surveys conducted during January through April 1984 off the coast of California were used as the basis for Monte Carlo simulation of populations of northern anchovy, *Engraulis mordax*, larvae. The simulated populations were sampled and larval mortality rate was calculated, using established analytical procedures. Results may be used to determine the precision of an estimate of larval mortality rate and to determine the number of plankton tows required to detect a difference in mortality rates between two surveys. The estimated mortality rate was found to be biased high when the larval growth rate is overestimated and biased low when the growth rate is underestimated. The bias is asymmetrically distributed and greatest when the assumed growth substantially overestimates the real growth. The results justify interannual comparisons of larval anchovy mortality rates when interannual variation in larval growth is less than twofold. The results also indicate that the sample size required for adequate precision of estimates of mortality rates is modest compared to that required for adequate representation of the spawning season and larval habitat.

The early life stages of several fish have been extensively studied as they are the link between the present adult stock and some future recruitment to the adult stock. Frustrated with the apparent lack of a clear relationship between stock and recruitment, fishery scientists have focused attention on events during the larval stage and their ultimate effect on survival to the juvenile and adult stages. Several hypotheses have been proposed (e.g., Hjort 1913); however, an understanding of the precision and accuracy of estimates of larval mortality rates is necessary to distinguish among them (Gulland 1971). This paper draws upon our experience with the northern anchovy, *Engraulis mordax*, to address this issue.

We focus on three questions: 1) What is the minimum number of plankton tows required to estimate the mortality rate of young larvae (<20 days old) for a given coefficient of variation? 2) What is the minimum number of plankton tows required to detect a difference in the mortality rates of young larvae between two surveys? 3) How does violation of the assumption of a constant growth model affect the estimate of larval mortality?

Several biases associated with sampling northern anchovy larvae have been identified and quantified. Pelagic ichthyoplankton are caught by lowering a fine-mesh net to a depth below the larval habitat and by steadily retrieving it to the surface of the ocean (Smith and Richardson 1977). Variability in the volume of water filtered per unit of depth affects the number of larvae captured; Ahlstrom (1948) formulated the "standard haul factor" to adjust for this bias. Larvae are extruded through the meshes of the sampling gear: retention rates can be expressed as a function of larval length and mesh size (Lenarz 1972; Zweifel and Smith 1981; Lo 1983). Larvae also evade capture as evidenced by differences in the night and day catch rates (Ahlstrom 1954; Smith 1981): retention rates can be expressed as a function of larval length and the diurnal time of capture (Hewitt and Methot 1982). The apparent length of larvae is affected by abrasion from the sampling net and by the preservative solution: live larval length may be expressed as a function of preserved larval length and the duration of the plankton tow (Theilacker 1980).

The application of these corrections yields unbiased estimates of the density of larvae in each of several length categories. Age-specific variations in growth introduce variability in the duration of time that a larva of given length is vulnerable to capture. The density of larvae divided by the duration of growth through each length category yields estimates of the number of larvae of a given age produced per unit sea-surface-area per unit time, which is termed larval production (Hewitt and Methot 1982). Yolk-sac larvae growth has been described as a function of temperature (Zweifel and Lasker

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1976; Lo 1983). Growth of feeding larvae has been described as a function of season (Methot and Hewitt 1980[1]). Interannual variations in growth have not been described, and in the absence of additional information, a larval growth model with constant coefficients is used for all years. The set of coefficients encompassed temperature effects as well as seasonal effects. The rate of decline of larval production with age represents the mortality rate (Hewitt 1981).

In actual practice, a negative binomial-weighted model (Bissel 1972) has been employed to convert length-specific distributions of larval density to unbiased age-specific distributions of larval production, assuming one set of size-specific extrusion and voidance rates (Zweifel and Smith 1981; Hewitt 1982; Hewitt and Methot 1982; Hewitt and Brewer 1983; Picquelle and Hewitt 1983, 1984; Lo 1985). The negative binomial distribution is recommended for describing sample counts of fish eggs and larvae (Smith and Richardson 1977); the distribution is capable of adequately describing patchy spatial distribution patterns. The arithmetic means of these distributions describe the mortality (or production) of larvae with age.

Although the negative binomial-weighted model produces an estimate of the variance of the mean density at a particular age, each age-specific distribution is unique because of the spatial dispersal of the larvae (Hewitt 1981). The variance of the mean density is underestimated as the extrusion and avoidance are assumed to be constant, and the variance about the mortality curve (hence, the variance of the mortality rate) is not easily determined. In the simulation, random variation of avoidance of the net and extrusion through the meshes of the net were included so that the variance of the mortality rate might best be evaluated. The approach used here is to construct a simulated population, sample it with simulated surveys, and estimate the mortality rate of larvae, using the procedures described above. By conducting many surveys, the accuracy and precision of the estimates of mortality rates may be investigated.

Potential biases in estimating larval mortality, introduced by assuming no interannual variation in growth, were our main concern and were investigated by simulation. Growth rates were varied when constructing the populations; mor-

tality rates were subsequently calculated assuming a set of growth rates (i.e., no interannual variation). By comparing the calculated mortality rates to a known rate, the magnitude of biases may be investigated.

METHODS

A Monte Carlo simulation model (Fig. 1) was employed to address the questions pertaining to the biases and precision of the estimate of larval mortality. A population of anchovy larvae was constructed using observed seasonal and geographic distributions. A known mortality rate was imposed on the population and sampling effort was varied over time and space. Known sampling biases were imposed and then adjusted for using the same techniques for calculating larval mortality rate as have been used on real surveys. Several hundred simulated surveys were conducted to assess the accuracy and precision of the estimates of mortality rates. Simulated larval growth was also varied to determine the sensitivity of the estimates of mortality rates to an assumption of constant larval growth. The details of this simulation are outlined in the following paragraphs.

Larval Population

A series of CalCOFI[2] ichthyoplankton cruises conducted in 1984 (Fig. 2) was used as a basis for constructing the population of larvae in the ocean. The total abundance of anchovy larvae at each station was adjusted for extrusion of small larvae through the meshes of the net (Fig. 3) and avoidance of the net by large larvae (Fig. 4). The adjusted catches were then stratified by geographic region (Fig. 2), month, and temperature. The negative binomial distribution was fit to the observations (positive tows only) in each region-month-temperature cell owing to the patchiness of larvae and the fact that the mean larval abundance is less than the standard deviation in general (Table 1). Samples were randomly drawn from these distributions (where the variate was the total number of larvae <9.25 mm per station) to conduct a simulated survey.

[1]Methot, R. D., Jr., and R. P. Hewitt. 1980. A generalized growth curve for young anchovy larvae: derivation and tabular example. SWFC Admin. Rep. LJ-80-17, 8 p.
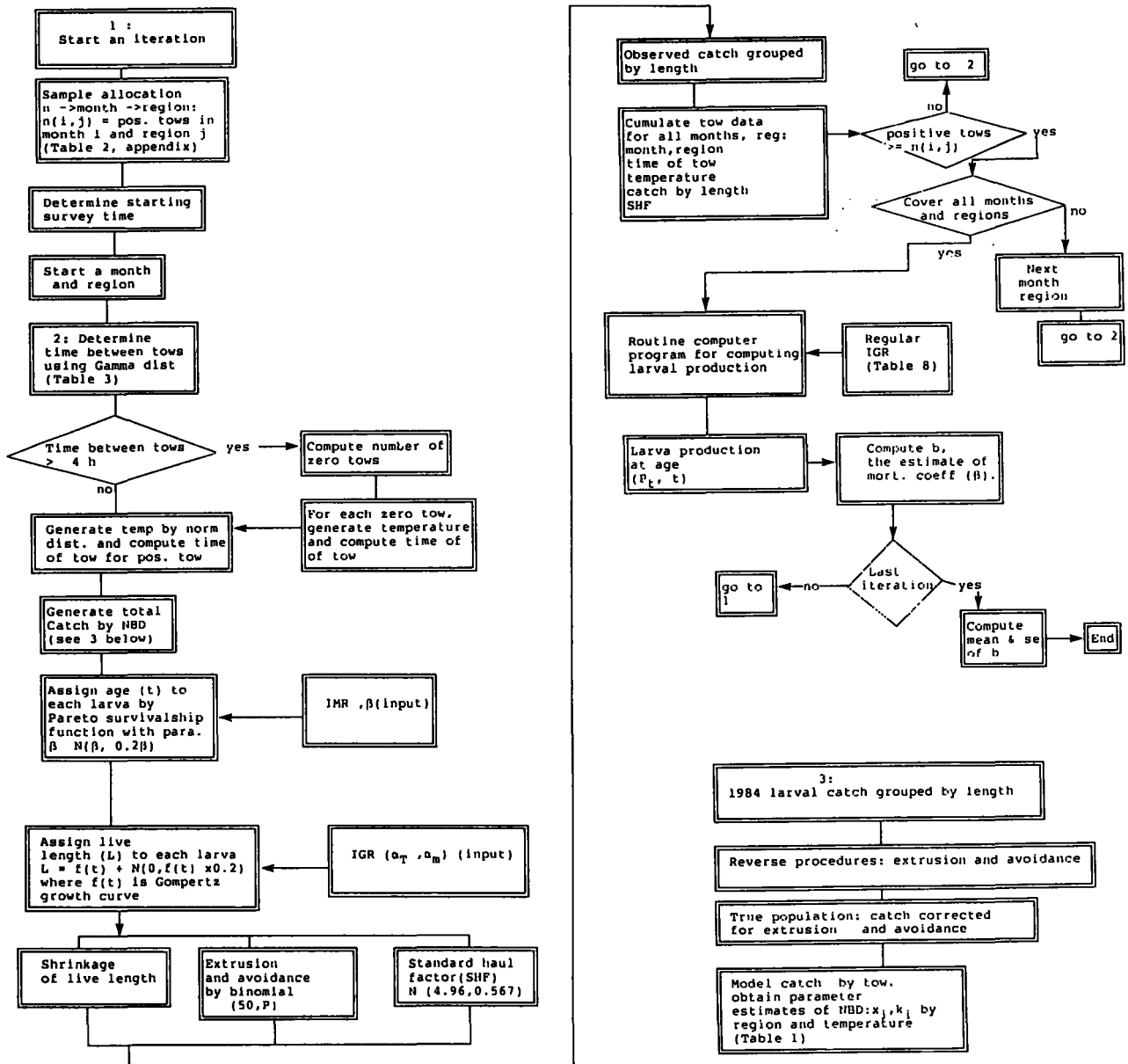
FIGURE 1.—Flow chart of the simulation.

Allocation of Sampling Effort

Simulated population encountered by plankton tows was computed according to their distribution in 1984 by month and region (Table 1). The portion of simulated tows that contained anchovy larvae was similarly determined (Table 2; App.). In this way, the sample size (number of tows) could be varied and yet still retain the spatial and temporal distribution of sampling effort that was used in 1984. The time of the simulated tows was assigned by randomly selecting a value from a Gamma distribution fitted to the actual time between tows in each region (Table 3; App.).

Larval Mortality Rate

Because it was found that anchovy larvae suffer higher mortality during the first-feeding period than during later stages, a Pareto function describes the survival of anchovy larvae younger than 20 days adequately (Hewitt and Brewer 1983; Lo 1985, 1986). In the present study, we used the Pareto function to assign age to the larvae in the population (Table 1; Fig. 5; App.).
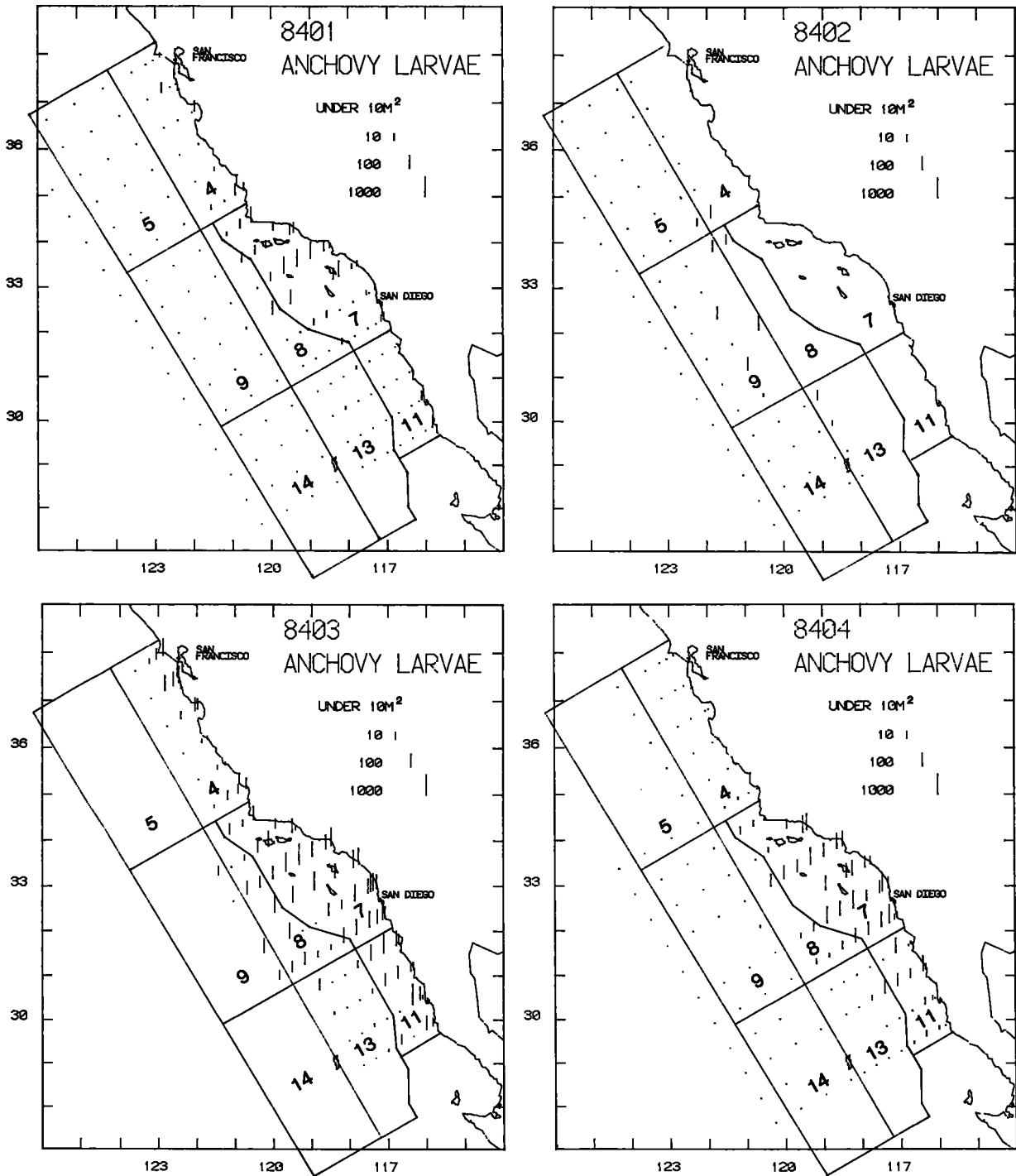
FIGURE 2.—Description of the seasonal and geographic distribution of sampling effort on a series of ichthyoplankton cruises conducted off the coast of California in 1984. The abundance of anchovy larvae at each station is indicated by the height of the "tree." Stations are grouped into geographic regions 4 through 14.

$$R = \frac{1}{1 + \exp\,(4.36 - 1.66\,L_c)}$$
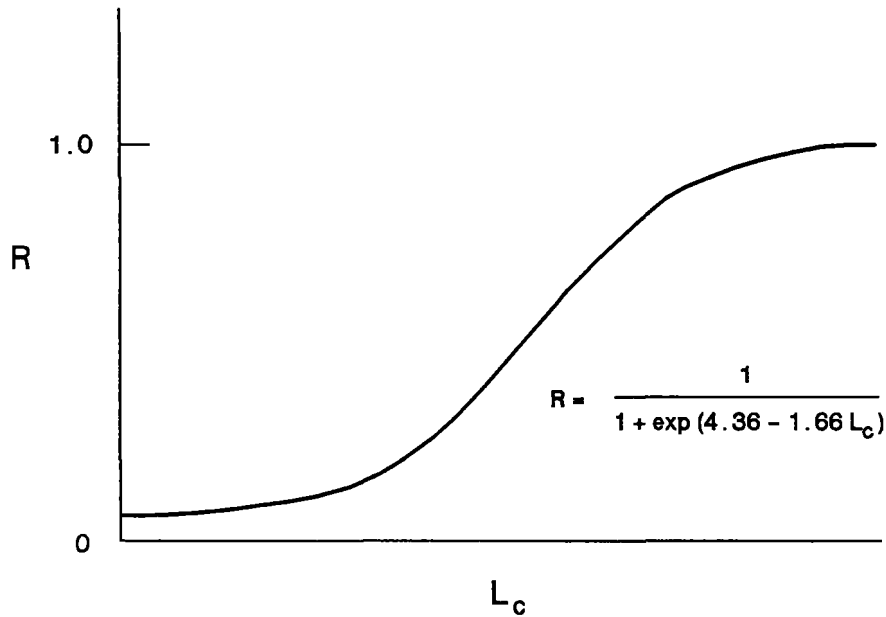
FIGURE 3.—Retention of anchovy larvae not extruded through the meshes of a plankton net constructed of 0.505 mm nylon (Lo 1983). R is the portion of larvae, of preserved length L_c, retained in the net.
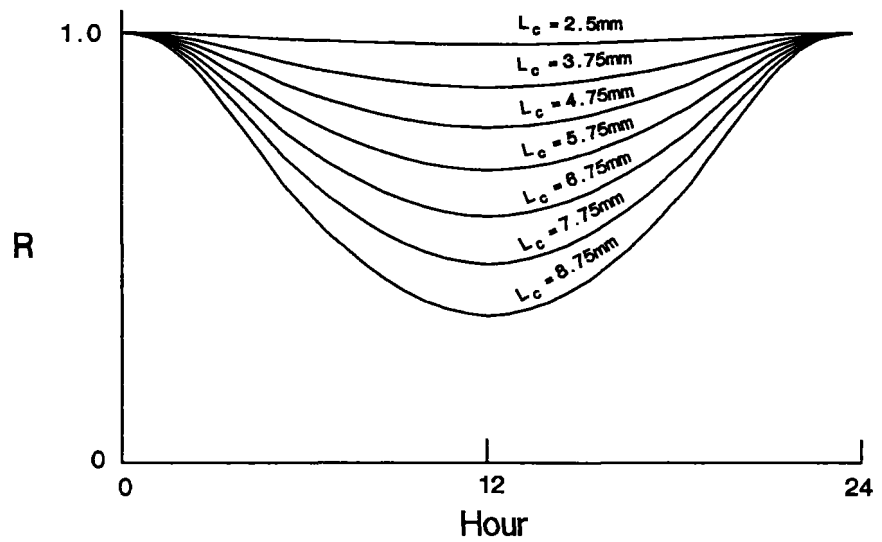
$$R = \left(\frac{1 + DN_L}{2}\right) + \left(\frac{1 - DN_L}{2}\right) \cos\left(\frac{2\pi * Hour}{24}\right)$$

where:

L_c	DN_L
2.5	1.67
3.75	1.47
4.75	1.46
5.75	1.27
6.75	1.21
7.75	1.16
8.75	1.13

FIGURE 4.—Retention of anchovy larvae which have not avoided capture (Hewitt and Methot 1982). R is the portion of larvae, of preserved length L_c, retained in the net. DN_L is the length-specific day/night catch ratio.

TABLE 1.—Simulated population of anchovy larvae based on a series of ichthyoplankton surveys conducted in 1984. Tabulated values are the parameters (m and k) of negative binomial distributions[1] fit to the population stratified by month, region, and temperature.

Month	Region	Temperature °C							
		≤13°		13.1°–14°		14.1°–15°		>15°	
		m	k	m	k	m	k	m	k
1	4	31.25	0.39	63.00	0.34	27.50	0.51	27.50	0.51
	5	[2]0.25	∞	0.25	∞	0.25	∞	0.25	∞
	7	270.50	0.62	270.50	0.62	147.08	0.25	619.10	0.14
	8	2.00	∞	2.00	0.31	44.00	0.22	98.60	0.22
	9	34.00	0.60	34.00	0.60	136.00	0.41	51.00	4.33
	11	56.80	0.55	56.80	0.55	56.80	0.55	56.80	0.55
	13	6.80	0.37	6.80	0.37	6.80	0.37	6.80	0.37
	14	0.25	∞	0.25	∞	0.25	∞	0.25	∞
2	4	558.17	0.64	120.50	0.32	22.50	0.97	22.50	0.97
	5	0.25	∞	0.25	∞	0.25	∞	0.25	∞
	7	270.50	0.62	270.50	0.62	147.08	0.25	619.10	0.14
	8	2.00	∞	2.00	0.31	44.00	0.22	98.60	0.22
	9	34.00	0.60	34.00	0.60	136.00	0.41	51.00	4.33
	11	56.80	0.55	56.80	0.55	56.80	0.55	56.80	0.55
	13	6.80	0.37	6.80	0.37	6.80	0.37	6.80	0.37
	14	0.25	∞	0.25	∞	0.25	∞	0.25	∞
3	4	2.00	0.40	2.00	0.40	2.00	0.40	2.00	0.40
	5	0.25	∞	0.25	∞	0.25	∞	0.25	∞
	7	4.00	5.39	7.33	5.39	522.33	1.07	790.60	1.38
	8	117.50	0.50	117.50	0.50	520.00	0.20	206.30	0.45
	9	0.00	0.00	0.00	0.00	150.50	0.78	150.50	0.78
	11	14.50	2.14	14.50	2.14	147.00	1.22	514.30	0.45
	13	6.80	0.37	6.80	0.37	6.80	0.37	6.80	0.37
	14	0.25	∞	0.25	∞	0.25	∞	0.25	∞
	4	2.00	0.40	2.00	0.40	2.00	0.40	2.00	0.40
	5	0.25	∞	0.25	∞	0.25	∞	0.25	∞
	7	4.00	5.39	7.33	5.39	522.33	1.07	790.60	1.38
	8	117.50	0.50	117.50	0.50	520.00	0.20	206.30	0.45
	9	0.00	0.00	0.00	0.00	150.50	0.78	150.50	0.78
	11	14.50	2.14	14.50	2.14	147.00	1.22	514.30	0.45
	13	6.80	0.37	6.80	0.37	6.80	0.37	6.80	0.37
	14	0.25	∞	0.25	∞	0.25	∞	0.25	∞

[1]Negative binomial distribution where

$$P(X = x) = \frac{(x + k - 1)!}{x!(k - 1)!} * [m/m + k)]^x * [k/m + k)]^k \text{ for } x = 0,1,2,3,\ldots$$

[2]Poisson distribution was used where $P(X = x) = (m^x e^{-m})/x!$ for $x = 0,1,2,3,\ldots$

A two-step Gompertz growth curve (Fig. 6) was used to determine the corresponding larval length. The length at age was generated based on a normal distribution with mean equal to the length computed from the Gompertz growth curve and a standard deviation equal to 0.2 times the length. (The standard deviation is normally proportional to the mean length at age.) The coefficient of variation of 0.2 was arbitrarily chosen because no direct estimate of the standard deviation was available. These simulated larvae, with assigned ages and lengths, composed the catches.

Sampling Biases

The simulated catches were reduced to account for the effects of extrusion and avoidance.

TABLE 2.—Distribution of sampling effort during January through April 1984 by region and month, where $p(i)$ is the *proportion of tows for month i* and $\Sigma p(i) = 1$, $g(j|i)$ is the proportion of tows made in region j during month i and $\Sigma q(j|i) = 1$, and $r(j|i)$ is the proportion of positive tows for region j during month i and $0 \le f(j|i) \le 1$. The number of tows is indicated by N, and the positive tows are indicated by n (i.e., those tows which contained anchovy larvae)

	January		February		March		April		Total
$i =$	1		2		3		4		
$N =$	139		89		67		54		349
$n =$	55		55		47		19		176
$p(i)$	0.40		0.26		0.19		0.15		0.50
Region	q	r	q	r	q	r	q	r	
4	0.14	0.43	0.28	0.29	0	—	0.36	0.14	
5	0.14	0.06	0.20	0.06	0	—	0.15	0	
7	0.25	0.90	0.21	1.00	0.26	0.94	0.26	0.93	
8	0.04	0.67	0.01	1.00	0.14	0.88	0.02	1.00	
9	0.13	0.06	0.16	0.63	0.02	1.00	0.16	0.11	
11	0.14	0.33	0.06	0	0.24	0.88	0.05	0	
13	0.09	0.27	0.02	1.00	0.25	0.41	0	—	
14	0.07	0.08	0.06	0.17	0.09	0	0	—	
	1.00		1.00		1.00		1.00		

TABLE 3.—Two parameters describing Gamma distributions[1] fit to the time between tows minus the constant in each region. Each of these distributions is shifted by the addition of the constant listed. The constant is the minimum time (hours) between two positive tows.

Regional	α	β	Constant
4	0.275	43.71	2
5	0.510	3.92	4
7	0.291	34.93	2
8	0.346	42.19	3
9	0.838	39.47	4
11	0.714	5.03	2
13	0.561	21.08	3
14	0.500	69.00	4

[1]Gamma distribution where

$$g(x) = \frac{1}{\beta(\alpha - 1)!} (x/\beta)^{-(\alpha-1)} \exp(-x/\beta) \text{ for }$$

$$x > 0.$$

$$\frac{1}{P_t} \times \frac{dP_t}{dt} = -\frac{\beta}{t}$$

$$P_t = P_h \left(\frac{t}{t_h}\right)^{-\beta} \text{ for } t \ge t_h$$
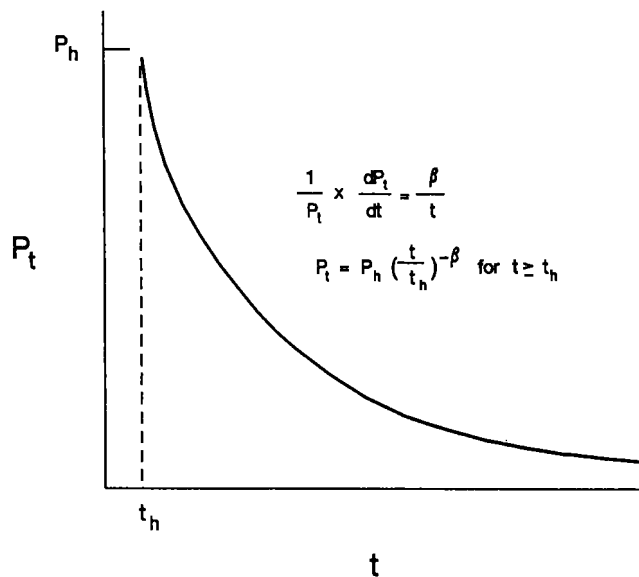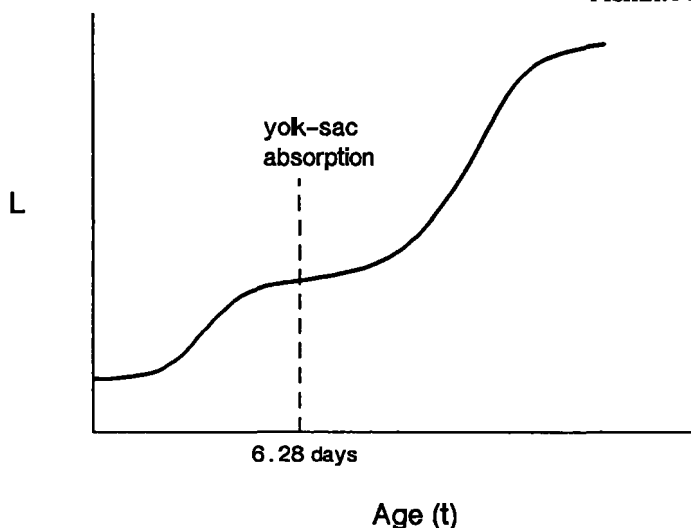
FIGURE 5.—Pareto model of larval production where larval mortality is assumed to decline with increasing age (Lo 1985. and 1986). P_t is the daily production of larvae at age t; β is the mortality coefficient; and t_h is the age at hatch.

$$L = 4.25 \left(\frac{0.32}{4.25}\right)^{e^{-\alpha_T t}} \quad \text{for } t \leq 6.28 \text{ days}$$

$$L = 27 \left(\frac{4.1}{27}\right)^{e^{-\alpha_m t}} \quad \text{for } t > 6.28 \text{ days}$$

Where: $\alpha_T = a_T \exp (b_T \times \text{TEMPERATURE})$

$\qquad = 0.11 \exp (0.12 \times \text{TEMPERATURE})$

$\qquad \alpha_m = (a_m - b_m \times \text{MONTH})^{-1}$

$\qquad = (22.48 - 0.83 \times \text{MONTH})^{-1}$

FIGURE 6.—Temperature-dependent and season-dependent larval growth curves (Methot and Hewitt 1980; Lo 1983). Gompertz models are used to describe each growth phase where α_T is the temperature-dependent growth coefficient and α_m is the season-dependent growth coefficient.

The fraction, p, of larvae extruded through the mesh or avoiding the net was generated by a sample mean of a binomial random variable, y, with parameters N and P. The parameter: N was set to 50 and P was the length-specific extrusion rate or avoidance rate from the same equations used to construct the population from the 1984 surveys. Thus p equaled $y/50$. Although p has a mean of P, it was not necessarily equal to P for each simulation run. The live lengths of larvae were reduced to account for the effects of net abrasion and preservaton effects (Theilacker 1980; Fig. 7). A standard haul factor was selected from the observed normal distribution of this variate (mean = 4.96, SD = 0.567) and used to index the volume of water filtered per unit of depth sampled. These catches then formed the raw material for the mortality estimation procedure.

Estimating Mortality Rate

The larvae in each catch were grouped into 1 mm length categories. A weighted negative binomial distribution was fitted to each length category where the original variate was the number of larvae (of a given length category) per station. Using this procedure, each observation was weighted for the effects of sampling biases (extrusion, avoidance, volume of water filtered, growth and shrinkage). The final variate was the number of larvae (of a given age) produced per day per 0.05 m^2 of sea surface. The rate at which larval production declines with time was defined as the mortality rate. For the Pareto model, the mortality rate was assumed to decline with age and mortality was indexed by the mortality coefficient (β). For the simulations described in this report, β was estimated as the slope of the log-
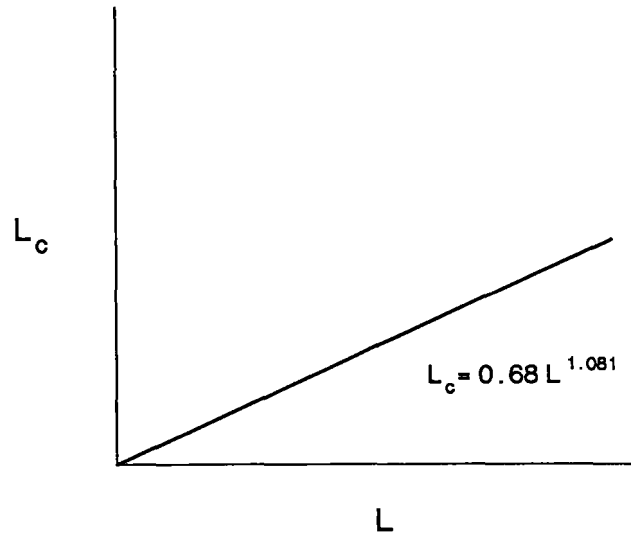
FIGURE 7.—The effect of net abrasion and preservative on the apparent length of anchovy larvae (from Theilacker 1980). L is live larval length; L_c is preserved (captured) larval length, and the length of the plankton tow is 20 minutes.

transformed Pareto function:

$$\ln(P_t) = \ln(P_h) - \beta \ln(t/t_h) .$$

Each simulation that produced an estimate of mortality rate was repeated many times. The collection of estimates of mortality rates was used to assess the accuracy and precision of estimates of mortality rates.

Sample Size for Detecting a Difference of Mortality Rates

The minimum sample size required to detect a difference between two mortalities was computed by two methods.

The *CV* Method

The coefficient of variation (*cv*) of the estimate of the difference between two mortality coefficients ($D = \beta_2 - \beta_1$) was calculated by

$$cv(d) = \frac{[\text{var}(b_1) + \text{var}(b_2)]^{0.5}}{D} \quad \text{for } D \neq 0 \quad (1)$$

where d is the estimate of D, the difference between mortality coefficients β_1 and $\beta_2(D = \beta_2 - \beta_1)$; b_1 and b_2 are the estimates of β_1 and β_2; var(b_1) and var(b_2) varying with sample size are computed in the simulation. The relationship between the sample size (n) and two elements,

$cv(d)$ and D, enables us to determine the minimum sample size for a given $cv(d)$ and D.

The Power Method

The probability of detecting a difference in two mortality rates, given that there is a difference, was calculated as

$$P[d > c(\beta_1,n) \mid D] = P[Z > z(\beta_1,\beta_2,n)] \quad (2)$$

where d follows a normal distribution with a mean of D and a variance of $[\text{SE}(d)]^2$; Z follows a normal distribution with a mean of 0 and a variance of 1:

$$c(\beta_1,n) = 2\,\text{SE}(d) = 2\sqrt{2}\,\text{SE}(b)$$
$$\text{for } \beta_1 = \beta_2(D = 0) \quad (3)$$

$$z(\beta_1,\beta_2,n) = \frac{c(\beta_1,n) - D}{\text{SE}(d)}$$

and $\text{SE}(d) = [\text{var}(b_1) + \text{var}(b_2)]^{0.5}$
$$\text{for } \beta_2 \neq \beta_1(D \neq 0).$$

A normal distribution table was used to obtain the probability values.

Relationship Between Growth and Mortality

The mortality coefficient (β) was fixed. Five

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populations were constructed with data from a single region-month stratum using five combinations of growth coefficients for yolk-sac (α_T, a temperature-specific coefficient) and feeding larvae (α_m, a season-specific coefficient) (see Table 8). Each population was sampled repeatedly and an average mortality coefficient calculated assuming standard growth coefficients. These mortality coefficients were then compared with the fixed mortality coefficient used to construct the populations.

RESULTS

The simulation model was used to estimate the following: 1) the mortality coefficients and their standard errors for various sample sizes when the true mortality coefficient was fixed, 2) the difference between two mortality coefficients and its standard error for various sample sizes, and 3) the mortality coefficients, assuming various growth rates.

Estimates of β with Various Sample Sizes

The mortality coefficient (β) was fixed at 1.5 for the inshore area (regions 4, 7, 8, 11, and 13; Fig. 2) and at 0.05 for the offshore area (regions 5, 9, and 14). The lower coefficient was required to generate simulated catch curves similar to those observed in offshore areas. The low mortality coefficient observed in offshore areas was likely the result of transport of older larvae from inshore to offshore regions (Power 1986). The average mortality coefficient (β), weighted by area of each region, was 1.41.

For each sample size (50, 100, 200, 300, and 400 plankton tows) 100 computer runs were made, and an estimate of the mortality coefficient (b) was calculated. The mean mortality coefficient, its standard error, and the coefficient of variation (cv) are listed in Table 4 for each sample size. The mean mortality coefficient for all sample sizes, except 50, slightly overestimated the true value of β = 1.41. The cv decreased with increasing sample size.

The relationship between cv and the number of positive tows (n) was quantified by assuming that half of the tows contained anchovy larvae (the actual portion of positive tows in 1984 was 0.5) (Table 2).The curve (Fig. 8) may be described by the power function:

$$cv(b) = 0.418 \, n^{-0.47}$$

From Figure 8 and the above expression, cv may be expected to be 0.10, 0.06, or 0.05 for 20, 60, or 100 positive tows. For $n > 100$, cv may be expected to decrease at a slow rate. Thus a survey of 120 tows, yielding 60 positive tows, is sufficient to estimate the mortality coefficient with an expected cv = 0.06. Data from annual surveys conducted between 1980 and 1987, where the portion of positive tows ranged from 0.47 to 0.98, are also shown on Figure 8. The variation of b, as related to sample size during 1980–87, follows the relationship estimated from a single year's data and implies that the relationship can be used as a guide for sample size determination.

TABLE 4.—Mean, standard error (SE), and coefficient of variation (cv) of estimates of the mortality coefficient (b) for various sample sizes (N), with 50% positive for anchovy larvae (n = 0.5 N), from 100 computer runs of each simulated survey.

N	n	mean	SE	cv = SE/mean
50	25	1.39	0.13	0.09
100	50	1.43	0.09	0.06
200	100	1.44	0.06	0.04
300	150	1.44	0.06	0.05
400	200	1.43	0.05	0.03

Estimates of D with Various Sample Sizes

The mortality coefficient (β) was fixed at 1.0, 1.5, 2.0, 2.5, and 3.0 for the inshore area (regions 4, 7, 8, 11, and 13). The inshore area was relatively well sampled and contained relatively high abundances of larvae; the proportion of positive stations in these regions was approximately 0.6 (Tables 1, 2). Estimated mortality coefficients (b) were determined for five simulated populations (corresponding to each of the five mortality coefficients (β)) using sample sizes of 50, 100, and 200 plankton tows with 60% of them positive for anchovy larvae.

The average estimated mortality coefficient and its standard error were determined after 100 computer runs and listed in Table 5. As expected, standard errors decreased with increased sample size. The estimated mortality coefficient was biased slightly low for β < 2 and biased slightly high for β > 2. The biases are negligible although they appeared to increase in magnitude as β departed from 2. The estimates of mortality rates and their standard errors were used to determine minimum sample size by two methods.
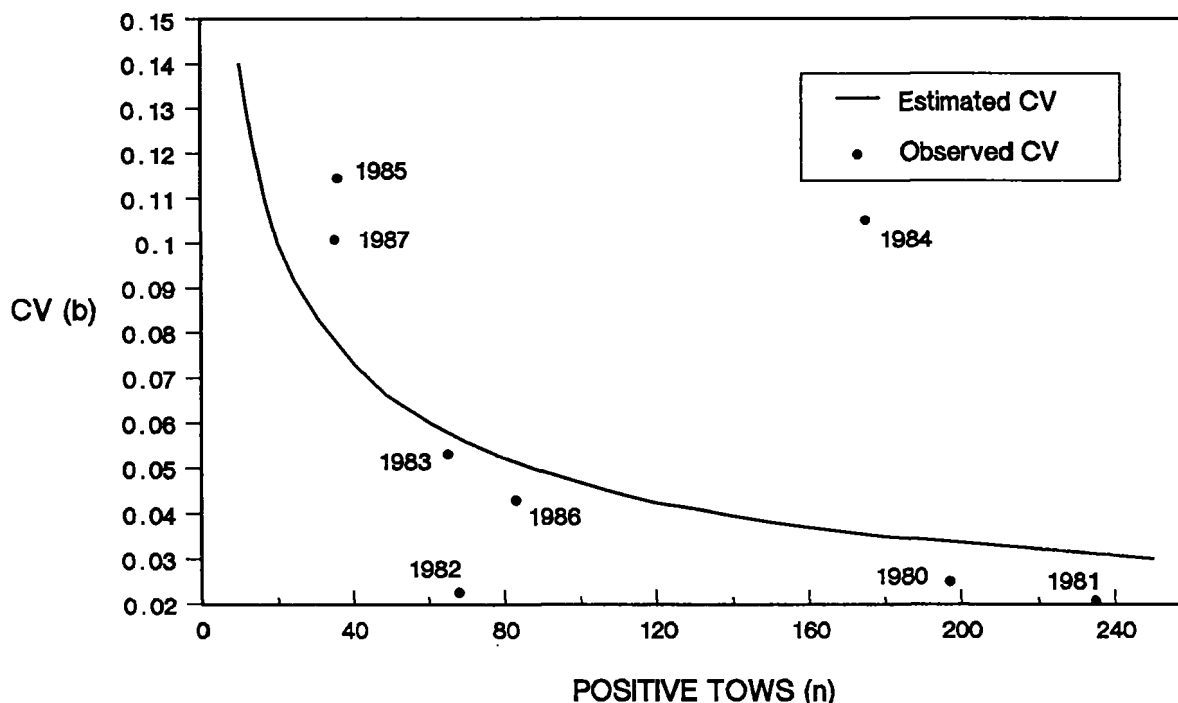
FIGURE 8.—The relationship between the coefficient of variation, cv(b), and the number of positive tows, n, derived from the results of the simulation. 1980–87 survey results are also plotted.

The CV Method

The cv of the estimate of the difference between two mortality coefficients, $cv(d)$ (Equation (1)), was calculated for various mortality differences and sample sizes using the data listed in Table 5. The $cv(d)$ decreases linearly with the difference between mortality coefficients (D), increases linearly with the absolute value of the larger of the two mortality coefficients (β_2), and exponentially declines with increasing sample size (N, n) (Table 6). The required sample size was thus estimated by regressing the number of

positive tows on β_2, D, and $\ln[cv(d)]$:

$$n = -101 + 24.8\,\beta_2 - 150\,D - 128\ln[cv(d)]\,.$$

For example, estimating the difference between two estimated mortality coefficients, when the true mortality coefficients are 3.0 and

TABLE 5.—Mean and standard error (SE) of estimated mortality coefficient based on 100 computer runs. Five populations were simulated, each with a different mortality coefficient (β). Simulated surveys used three sample sizes (N) with 60% of the plankton tows positive for anchovy larvae (n).

β	Sample size N(n)					
	50(30)		100(60)		200(120)	
	mean	SE	mean	SE	mean	SE
1.00	0.90	0.100	0.91	0.075	0.93	0.058
1.50	1.44	0.090	1.44	0.064	1.44	0.060
2.00	1.98	0.120	1.99	0.087	2.01	0.058
2.50	2.56	0.110	2.57	0.097	2.58	0.065
3.00	3.18	0.170	3.18	0.100	3.18	0.075

TABLE 6.—Coefficient of variation of the estimate of the difference between two mortality coefficients, cv(d), calculated for various mortality differences, D, and sample sizes, n. The number of positive tows, n, was 60% of the total number of tows.

$\beta_2 - \beta_1$	Sample size N(n)		
	50(30)	100(60)	200(120)
D = 0.5			
1.5 – 1.0	0.268	0.196	0.166
2.0 – 1.5	0.300	0.210	0.166
2.5 – 2.0	0.320	0.260	0.170
3.0 – 2.5	0.400	0.270	0.190
D = 1.0			
2.0 – 1.0	0.156	0.115	0.082
2.5 – 1.5	0.142	0.116	0.088
3.0 – 2.0	0.208	0.133	0.095
D = 1.5			
2.5 – 1.0	0.100	0.082	0.058
3.0 – 1.5	0.130	0.079	0.064

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2.0 (β_1 = 2.0, β_2 = 3.0, D = 1.0), with a $cv(d)$ = 0.15, will require n = 67 positive tows from each population. With 70 positive tows from each population, approximately 95% of the sample differences can be expected to be between 0.70 and 1.30 (1.0 ± 2 * 0.15).

The Power Method

The standard error of the estimated mortality coefficient, SE(b), was modeled as a function of the number of positive tows, n, and the true mortality coefficient (β) using the data listed in Table 5:

$$SE(b) = 0.356 \, n^{-0.469} \, e^{0.239\beta}.$$

The probabilities of detecting a difference between two mortality coefficients, given that there is a difference (this is referred to as the power of the test), were calculated for various sample sizes and listed in Table 7. The power increases as the difference of mortality coefficients increases, and it is equal to the level of

TABLE 7.—Probability of detecting a difference between two mortality coefficients, given one of the mortality coefficients (β_1), the true difference (D = β_2 − β_1), and the number of positive tows (n). Because of symmetry about D = 0, partial figures are listed.

| n | True difference (D) |
	−2.0	−1.5	−1.0	−0.5	0.5	1.0	1.5	2.0
β_1 = 1.0								
10					0.62	0.96	1.00	1.00
20					0.86	1.00	1.00	1.00
30					0.96	1.00	1.00	1.00
40					1.00	1.00	1.00	1.00
β_1 = 1.5								
10				0.50	0.50	0.97	1.00	
20				0.79	0.75	1.00	1.00	
30				0.93	0.90	1.00	1.00	
40				0.98	0.96	1.00	1.00	
50				1.00	0.99	1.00	1.00	
60				1.00	1.00	1.00	1.00	
β_1 = 2.0								
10			0.96	0.40	0.43	0.92		
20			1.00	0.70	0.70	1.00		
30			1.00	0.86	0.86	1.00		
40			1.00	0.93	0.92	1.00		
50			1.00	0.98	0.96	1.00		
60			1.00	1.00	1.00	1.00		
β_1 = 2.5								
10		1.00	0.91	0.34	0.48			
20		1.00	0.99	0.59	0.58			
30		1.00	1.00	0.76	0.75			
40		1.00	1.00	0.86	0.86			
50		1.00	1.00	0.93	0.91			
60		1.00	1.00	0.96	0.95			
70		1.00	1.00	1.00	1.00			
β_1 = 3.0								
10	1.00	0.99	0.82	0.27				
20	1.00	1.00	0.98	0.50				
30	1.00	1.00	1.00	0.66				
40	1.00	1.00	1.00	0.78				
50	1.00	1.00	1.00	0.86				
60	1.00	1.00	1.00	0.91				
70	1.00	1.00	1.00	0.95				
80	1.00	1.00	1.00	0.97				
90	1.00	1.00	1.00	1.00				

significance (α = 0.05) when the difference is zero. The power is symmetrical about D = 0; thus, only partial figures were given in Table 7.

For example, if the true difference was 0.5 and one of the mortality coefficients was 2.0, with a probability of 0.86, a sample size of 30 positive tows from each of two populations will detect a significant difference in their mortality coefficients. The probability would be only 0.76 if one of the mortality coefficients was 2.5. In general, to achieve the same probability of detecting a given difference between mortality coefficients, a larger sample size is required for a larger β. To detect a significant difference with a probability of 0.96, when the true difference is 0.5 and one of the mortality coefficients is 1.0, 30 positive tows are required from each population. If β = 2.5, however, 60 positive tows are required to detect the same difference with a probability of 0.95. If the difference is greater than 1, at most 20 positive tows from each population would be sufficient.

The two methods serve different purposes. The cv method provides a 95% confidence interval for the difference. The Power Method assigns a probability to the detection of a difference, but provides no information on the magnitude of the difference.

Estimates of β with Various Growth Rates

Mortality is defined as the decline of production with larval age. Thus an overestimate of larval age, predicted from an underestimate of growth rate, will underestimate mortality rate. Similarly, an overestimate of growth rate will result in an overestimate of mortality rate.

The mortality coefficient (β) was fixed at 1.5. Data from February, region 7, temperature 15°C, were used to construct five populations, corresponding to five combinations of growth coefficients for yolk-sac and feeding larvae (Table 8). Each population was surveyed 50 times with a sample size of 50 plankton tows. The estimated mortality coefficient (b) was calculated by assuming standard growth coefficients for February, region 7, temperature 15°C (Table 8). When the population growth coefficients (α_m) were underestimated by the standard coefficients, the estimated mortality coefficient (b) was less than β = 1.5; conversely when growth was overestimated, the mortality coefficient was also overestimated.

Because the yolk-sac stage is short, relative to the feeding stage, we can reasonably assume that the growth coefficient for feeding larvae (α_m) has the largest effect on the estimated mortality coefficient (b). When the estimated mortality coefficient is plotted against α_m (Fig. 9), it is apparent that the bias in estimating mortality rate, caused by errors in the assumed growth rate, is asymmetrical: greater when actual growth is slower than assumed growth and smaller when actual growth is faster than assumed. When the actual growth was half the assumed rate, the mortality coefficient was overestimated by 80%; when the actual growth was double the assumed rate, the mortality coefficient was underestimated by only 16% (Table 8).

The coefficient, α_m, determines the instantaneous growth rate (IGR) at age t as the IGR = $\alpha_m \ln(L_\infty/L_0) \exp[-\alpha_m(t - t_0)]$ where L_∞ is the maximum fish length, and L_0 is the minimum fish length for t > 6.28 days (Fig. 6). Large value of α_m implies that the IGR is large for the small value of age t, and the IGR decreases rapidly as the fish ages. Because both the IGR and the instantaneous mortality rate (IMR = β/t) are two different nonlinear functions of age (t), the relationship between these two coefficients (α_m and β) is also nonlinear and thus the bias is asymmetric.

TABLE 8.—Five sets of coefficients for two-step Gompertz growth curves (Fig. 6) used to simulate five populations. Also listed are the standard coefficients used in the analysis of survey data for region 7 in February with a temperature of 15°C. The estimated mortality coefficient (b) is listed as average of 50 computer runs. The true mortality coefficient (β) was 1.5.

a_T	b_T	α_T	a_m	b_m	α_m	b
0.11	0.06	0.27	44.96	0.83	0.023	2.70
0.11	0.24	4.05	11.24	0.83	0.104	1.26
0.22	0.12	1.33	22.48	0.42	0.046	1.41
0.11	0.09	0.42	33.72	0.83	0.031	1.90
0.22	0.12	1.33	16.86	0.83	0.066	1.31
Standard coefficients:						
0.11	0.12	0.67	22.48	0.83	0.048	

DISCUSSION AND CONCLUSIONS

The simulation model and its methodology have general applicability to larval fish of many species, although these results apply directly to estimates of northern anchovy larval mortality rates derived from CalCOFI surveys. Results may differ because of differences in the param-
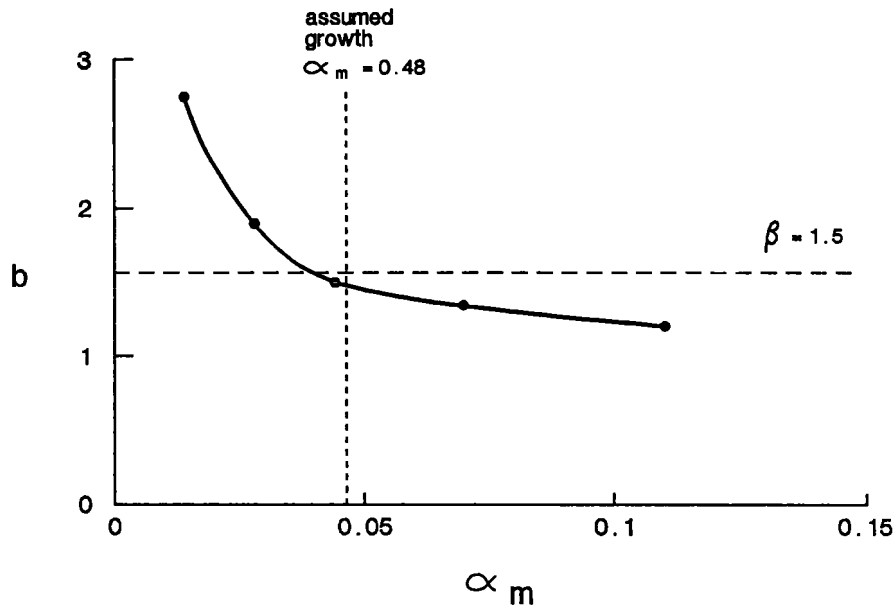
FIGURE 9.—The estimated mortality coefficient (b) is biased by errors in the assumed growth coefficient for feeding larvae, $\alpha_m = 0.48$. The true mortality coefficient (β) was 1.5.

eter values and their variances. Nevertheless, most ichthyoplankton sampling problems are sufficiently similar so that the results derived for anchovy provide a general idea of the sample size required for adequate precision of larval mortality estimates. (When using the regressions derived in this study to estimate sample size, parameter values should be within the range used in the simulation [$1.0 \leq \beta \leq 3.0, 0.5 \leq D \leq 2.0$]. Values outside these ranges could lead to unreliable estimates of sample size.) The results also provide an assessment of the effect of biased growth rates on estimates of larval mortality rate, which has general applicability to many species.

Caveats

Application to Site-Intensive Studies

Small-scale site-intensive studies may be conducted to study underlying mechanisms of larval mortality rate by measuring larval condition, growth, starvation rates, and mortality rate in small segments of the habitat. Such studies have greater problems with bias and precision than the CalCOFI surveys where the entire spawning habitat is sampled. As noted above, an important potential bias is the transport of larvae in or out of the study area. Taggart and Leggett (1987) noted that failure to account for advective losses of larvae from a small bay resulted in a

significant overestimate of mortality.

Another problem arises from the choice of study areas. Many specimens must be collected over a short period to assess growth, starvation, and other condition factors. If sites are selected that contain larval densities that are high, relative to the average density for the entire habitat, and patchy, the effect will be to increase the variance, because the variance is often positively correlated with the density of larvae (Smith and Richardson 1977), and thereby reduce the power to detect differences in mortality rate between sites. The simulations were based on large regions of anchovy habitat and therefore underestimate sample size required to detect mortality rate differences between small areas of high larval abundance.

Application to Other Species

A key difference between larval anchovy and most other species of larval fishes is that anchovy are very abundant. The simulation results indicate that surprisingly few positive tows are needed to detect relatively small differences in mortality rates. In the regions considered in the simulation, 50–60% of the tows were positive, and the number of larvae caught per tow averaged 125, with 88% < 10 mm in length. For a less abundant species, the proportion of positive tows and the average number caught per tow would be much lower, and many more tows

would be required to attain the same level of precision.

Application to CalCOFI Surveys

Three key assumptions underlie the use of the CalCOFI time series of larval mortality estimates for hypothesis testing: 1) a stable age distribution prevails (i.e., abundance of several cohorts of larvae at one moment in time is representative of one cohort as it ages through time), 2) variations in observed mortality rate represent true natural variations and not sampling error, and 3) use of the same larval growth parameters for all years does not bias the estimates of mortality rate.

The first assumption was not addressed in this study. It implies negligible immigration and emigration of larvae and continuous production of spawn. The CalCOFI surveys are designed to encompass the anchovy spawning habitat and thus minimize inaccuracies caused by transport of larvae in and out of the survey area. For a species with a broad temporal spawning curve and with repeated spawning by individuals (9–16% of the females spawn each night; table 7, Fiedler et al. 1986), unbiased estimates of mortality rate may be obtained by pooling plankton tows conducted throughout the spawning season (table 6, Hewitt and Methot 1982). With smaller surveys and shorter time periods, the assumption of a stable age distribution may not be suitable, and estimates of mortality rates may be biased.

With regard to the second assumption, our simulations indicate that the time series of daily mortality rate of anchovy larvae represents predominantly real differences owing to biological variation rather than random variation. Recent CalCOFI ichthyoplankton surveys (Table 9) yielded between 36 and 236 positive tows per spawning season. The simulation model indicates that sample sizes >80 are sufficient to detect a difference of 0.5 or more in the mortality coefficient (β) between years (Table 7). When all possible pairs for the eight surveys (1980–87) are compared, 12 of the 28 comparisons had a difference >0.5 (Table 9). Results of our simulation imply that the precision of past surveys was adequate, and the interannual variation in mortality rate (β ranged from 1.22 in 1980 to 2.14 in 1986) is real.

Because larval mortality rate is age-dependent (IMR = β/t) with high mortality occurring during the onset of feeding and decreasing there-

after, variations in daily mortality rates can be typified by "large differences concentrated in a short period of time" and thus be easily detectable (Gulland 1971). The critical issue in comparing mortality rates does not appear to be one of precision but rather one of obtaining a representative sample.

With regard to the third assumption, the simulation also indicated that the risk of introducing a large bias in estimates of mortality rates by using a single family of standard growth curves is relatively low. A large bias would be expected only when the standard growth curves overestimated the actual growth by a factor of two or more. It is unknown how frequently the standard growth curve generates this large bias, for lack of data on variability of larval growth rates from year to year in the field.

TABLE 9.—Number of tows positive for anchovy larvae (*n*) and mortality coefficients (β) for CalCOFI ichthyoplankton surveys conducted during January through April 1980–87.

Year	n	β
1980	197	1.22
1981	236	1.53
1982	69	1.81
1983	65	2.05
1984	176	1.47
1985	37	2.03
1986	83	2.14
1987	36	1.98

CONCLUSIONS

These simulations validate the use of CalCOFI survey information to test hypotheses regarding larval survival and recruitment (Butler 1987, Peterman et al. 1988). The sample size required for adequate precision of estimates of mortality rates is modest relative to the one required for adequate representation of the spawning season and habitat of a major marine stock such as the northern anchovy. As stated, the critical issue in comparing mortality rates does not appear to be precision of the estimates but rather how well the sample represents the population.

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LITERATURE CITED

Ahlstrom, E.

1948. A record of pilchard eggs and larvae collected during surveys made in 1939 to 1941. U.S. Fish. Wild. Serv. Spec. Sci. Rep. 54, 82 p.

1954. Distribution and abundance of egg larvae populations of the Pacific sardine. U.S. Fish Wildl. Serv., Fish. Bull. 93:83–140.

Bissel, A. F.

1972. A negative binomial model with varying element sizes. Biometrika 59:435–441.

Butler, J.

1987. Comparison of the larval and juvenile growth and larval mortality rates of Pacific sardine and northern anchovy and implications for species interaction. Ph.D. Thesis, Univ. California at San Diego, 242 p.

Fiedler, P. C., R. D. Methot, and R. P. Hewitt.

1986. Effects of California El Nino 1982–1984 on the northern anchovy. J. Mar. Res. 44:317–338.

Gulland, J. A.

1971. Ecological aspects of fishery research. Adv. Ecol. Res. 7:115–176.

Hewitt, R. P.

1981. The value of pattern in the distribution of young fish. Rapp. P.-v. Réun. Cons. int. Explor. Mer 178:229–236.

1982. Spatial pattern and survival of anchovy larvae: implications of adult reproductive strategy. Ph.D. Thesis, Univ. California at San Diego, 187 p.

1988. Historical review of the oceanographic approach to fishery research. CalCOFI Rep. 29:27–41.

Hewitt, R. P., and G. D. Brewer.

1983. Nearshore production of young anchovy. CalCOFI Rep. 24:235–244.

Hewitt, R. P., and R. D. Methot, Jr.

1982. Distribution and mortality of northern anchovy larvae in 1978 and 1979. CalCOFI Rep. 23:226–245.

Hjort, J.

1913. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapp. P.-v. Réun. Cons. int. Explor. Mer 19:1–228.

Lenarz, W. H.

1972. Mesh retention of *Sardinops caerulea* and *Engraulis mordax* by plankton nets. Fish. Bull. U.S. 70:839–848.

Lo, N. C. H.

1983. Re-examination of three parameters associated

with anchovy egg and larval abundance: temperature dependent incubation time, yolk-sac growth rate and egg and larval retention in mesh nets. NOAA Tech. Memo. NMFS-SWFC-31, 32 p.

1985. Egg production of the central stock of northern anchovy 1951–83. Fish. Bull. U.S. 83:137–150.

1986. Modeling life-stage-specific instantaneous mortality rates, an application to northern anchovy, *Engraulis mordax*, eggs and larvae. Fish. Bull. U.S. 84:395–407.

Peterman, R. M., M. J. Bradford, N. C. H. Lo, and R. D. Methot, Jr.

1988. Contribution of early life stages to interannual variability in recruitment of northern anchovy (*Engraulis mordax*). Can. J. Fish. Aquat. Sci. 45(1):8–16.

Picquelle, S. J., and R. P. Hewitt.

1983. The northern anchovy spawning biomass for the 1982–83 California fishing season. CalCOFI Rep. 24:16–28.

1984. The 1983 spawning biomass of the northern anchovy. CalCOFI Rep. 25:16–27.

Power, J. H.

1986. A model of the drift of northern anchovy, *Engraulis mordax*, larvae in the California Current. Fish. Bull. U.S. 4:585–603.

Reid, J. L.

1988. Physical oceanography 1947–1987. CalCOFI Rep. 29:42–65.

Smith, P. E.

1981. Fisheries on coastal pelagic schooling fish. *In* R. Lasker (editor), Marine fish larvae: morphology, ecology, and relation to fisheries, p. 1–30. Univ. Wash. Press., Seattle.

Smith, P. E., and H. G. Moser.

1988. CalCOFI time series: An overview of fishes. CalCOFI Rep. 29:66–78.

Smith, P. E., and S. Richardson.

1977. Standard techniques for pelagic fish egg and larva surveys. F.A.O. Fish. Tech. Pap. 175, 100 p.

Taggart, C. T., and W. C. Leggett.

1987. Short-term mortality in post-emergent larval capelin *Mallotus villosus*. I. Analysis of multiple *in situ* estimates. Mar. Ecol. Prog. Ser. 41, p. 205–217.

Theilacker, G. M.

1980. Changes in body measurements of larval northern anchovy, *Engraulis mordax*, and other fishes due to handling and preservative. Fish. Bull., U.S. 78:685–692

Zweifel, J., and R. Lasker.

1976. Prehatch and posthatch growth of fishes—a general model. Fish. Bull., U.S. 74:609–622.

Zweifel, J. R., and P. E. Smith.

1981. Estimates of the abundance and mortality of larval anchovies (1951–1975): application of a new method. Rapp. P.-v. Réun. Cons. int. Explor. Mer 178:248–259.

APPENDIX

Assignment of Larval Ages Using the Pareto Function

The standing stock (SS) of larvae, between the ages of t_h and 20 days, is the integral of the production curve (Fig. 5) over these ages:

$$SS = \int_{t_h}^{20} P_t \, dt = \int_{t_h}^{20} P_h(t/t_h)^{-\beta} dt$$

$$= [1 - (20/t_h)^{-(\beta-1)}] \frac{t_h P_h}{(\beta - 1)} \qquad \text{for } \beta < > 1,$$

$$= [\ln(20) - \ln(t_h)] t_h P_h \qquad \text{for } \beta = 1.$$

Similarly the number of larvae younger than age t is

$$SS_t = [1 - (t/t_h)^{-(\beta-1)}] \frac{t_h P_h}{(\beta - 1)} \qquad \text{for } \beta < > 1,$$

$$= [\ln(t) - \ln(t_n)] \, t_h P_h \qquad \text{for } \beta = 1.$$

The proportion of larvae that are younger than age t is

$$r(t) = SS_t/SS = \frac{1 - (t/t_h)^{-(\beta-1)}}{1 - (20/t_h)^{-(\beta-1)}} \qquad \text{for } \beta <> 1,$$

$$= \frac{\ln(t/t_n)}{\ln(20/t_h)} \qquad \text{for } \beta = 1,$$

where $0 < r(t) < 1$.

By rearranging terms, t can be expressed as

$$t = t_h[1 - r(t)(1 - (20/t_h)^{-(b-1)}]^{-1/(b-1)} \qquad \text{for } b <> 1,$$

$$= t_h(20/t_h)^{r(t)} \qquad \text{for } b = 1,$$

where b is a normal random variable with mean $= \beta$ and standard error $= 0.2 \beta$ (0.2 is an arbitrarily chosen value for the coefficient of variation (b) and $r(t)$ is a uniform random variable between 0 and 1).

Sample Allocation

The allocation of tows to each region and month was based on the 1984 sampling pattern (Table 2, Fig. 2). For a total of N tows, the number of positive tows allocated to month i and region j was computed as

$$n(i,j) = N * p(i) * q(j|i) * r(j|i)$$

where $p(i)$ is the proportion of tows for month i and $\Sigma p(i) = 1$
$q(j|i)$ is the proportion of tows made in region j during month i and $\Sigma q(j|i) = 1$
$r(j|i)$ is the proportion of positive tows for region j during month i and $0 < = r(j|i) < = 1$.

A uniform random number generator was used to assign each tow to a month and region, to determine whether the tow was positive or not, and thus to produce $n(i,j)$.

Assignment of the Time of Tow

Table 3 lists parameters for Gamma distributions fitted to the actual time between positive tows minus the minimum time between any two positive tows (i.e., plankton tows which caught at least one anchovy larva) in each region because the Gamma distribution takes all values to be greater than zero. Each distribution is shifted to the right by the constant listed (the minimum time between any two positive tows). Actual times greater than 150 hours were assumed to be periods of transit to and from port and were thus excluded when fitting the distributions.

The time of the first tow of a simulated survey was chosen randomly and incremented by time intervals selected from the distributions described in Table 3. If the selected time interval was greater than 4 hours, tows with zero catch were inserted. The number of zero tows inserted was the time interval between two positive stations divided by the average travel time between stations (2 hours).