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A DAILY FECUNDITY REDUCTION METHOD OF BIOMASS ESTIMATION WITH APPLICATION TO DOVER SOLE *MICROSTOMUS PACIFICUS*

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ABSTRACT

A daily fecundity reduction method was applied to *Microstomus pacificus*, commonly known as Dover sole, a pleuronectid flatfish of the upper continental slope (200 to 1,500 m) of the west coast of North America. This method was developed for estimating adult biomass of fishes with determinate annual fecundity and utilizes the daily decline in reproductive potential of the population and the numbers of planktonic eggs. Daily decline in reproductive potential was estimated from the decline of the product of the standing stock of advanced oocytes and the numbers of females with reproductively active ovaries. Daily production of planktonic eggs was estimated from the numbers of eggs in quantitative oblique plankton tows. We describe the survey design, data assembly, statistical estimation procedures, and the possible biases due to the limitation of surveys.

Our objective is to apply a new daily egg production method designed for fishes of determinate annual fecundity (Lo et al., 1992) to Dover sole, *Microstomus pacificus*, a commercial flatfish occurring along the upper continental slope of North America. The potential annual fecundity of Dover sole is determinate (Hunter et al., 1992), consequently, the daily decline in the number of yolked oocytes in the ovary (E) can be considered to be equivalent to the daily rate of spawning. This concept is the heart of the new daily egg production method described by Lo et al. (1992). The new method is called the daily fecundity reduction method (DFRM) to distinguish it from other daily egg production methods in which spawning rates are measured directly using estimates of batch fecundity and spawning frequency. A synopsis of the general concept was presented by Lo et al. (1992). We present the details of procedures for applying the DFRM to Dover sole including survey designs and computations of estimates of biological parameters and their variances.

The major difference between the daily fecundity reduction method and the other daily egg production methods is how daily spawning rates are estimated. Application of the daily egg production method to Dover sole required several modifications since the method was designed originally for northern anchovy (*Engraulis mordax*) and other pelagic multiple spawners. Dover sole spawn at bottom depths of 600 to 1,500 m and their eggs are distributed throughout the water column. Thus, quantitative plankton tows, beginning near the sea bottom at depths as great as 1,500 m and ending at the surface, were required and the developmental rate of eggs had to be adjusted for the temperatures encountered by the eggs during their ascent. Dover sole eggs incubate at much lower temperatures (5 to 13°C) and the egg state lasts longer (20 to 30 d) than northern anchovy (2 to 3 d; Lo, 1985). Thus, transport of Dover sole eggs by currents in and out of the study area was a much more likely source of bias for Dover sole than for anchovy.

In the application of the daily egg production method to northern anchovy and Pacific sardine (*Sardinops sagax*), random mixing of fish of all sizes and reproductive states was assumed. This assumption could not be made for Dover sole because the size, age, and reproductive activity of Dover sole were known to increase with depth (Hunter et al., 1990). Thus, bathymetry was considered in

the design of the sampling plan and computations. Dover sole are neither as abundant nor as fecund as northern anchovy. The northern anchovy stock (ca. 0.5 to 1.0 $\times$ 10^6 mt) produces about 5,000 to 10,000 eggs per gram of female per year, while the Dover sole in our study area (about 17,000 mt according to Butler et al., 1989) produces only 50 to 100 eggs per gram female weight per year, even though they spawn about 10 times. Thus, the 25 cm diameter plankton net used for anchovy egg production surveys, was not suitable because it would not filter a sufficient volume of water and a larger net was required.

Materials and Methods

Model.—The model for the daily fecundity reduction method is:

$$B = (P_0 \cdot A)/[(R/W) \cdot D_t \cdot 10^6] = P_0 \cdot A/K \quad (1)$$

with an approximate coefficient of variation (CV) for the estimate of biomass as:

$$CV(B) \approx \{CV(P_0)^2 + CV(K)^2 - [2 \cdot COV(P_0 \cdot K)/(P_0 \cdot K)]\}^{0.5} \quad (1a)$$

where B is the biomass (mt) for area A, P_0 is the daily production of eggs per 10 m^2 sea surface area estimated from egg abundance adjusted for their development time and mortality during incubation, A is the size in 10 m^2 of the survey area, $K = (R/W) \cdot D_t \cdot 10^6$ in the denominator of (1) is daily population fecundity in units of eggs$\cdot mt^{-1} \cdot day^{-1}$, where R is the female fraction of the population by weight, W is the average female weight (g), D_t is the daily fecundity per female at day of the year t and is expressed as

$$D_t = d(E \cdot G)/dt = E_t \cdot dG/dt + G_t \cdot dE/dt.$$

The constant 10^6 converts grams to metric tons. E is the number of advanced yolked oocytes in the ovary (total fecundity), G is the fraction of females that have ovaries containing advanced yolked oocytes (fraction of females with active ovaries), and t is the elapsed time in days over which changes in G and E are monitored.

A key difference between this model and models for the annual egg production model (AEPM) (Saville, 1964) and daily egg production method (DEPM) (Lasker, 1985) is in the computation of adult reproductive rates. Our model differs from the AEPM model because total fecundity (E) is computed on a daily, rather than an annual, basis and differs from the DEPM model because the number of eggs spawned per day, or daily fecundity, is computed from the decline in total fecundity over the spawning season, rather than from the direct product of spawning frequency (S) and batch fecundity (F).

In the DFRM model (eqn 1), we use two variables (E and G) to measure the decline in total fecundity. As any female taken in a trawl can be included in the denominator of the fraction of females with active ovaries (G), it follows that the biomass (B) is an estimate of the total adult biomass vulnerable to our trawl, which includes all adult fish and an unknown fraction of the juveniles. In the daily egg production method used for anchovy, only the spawning biomass was estimated. While direct formulas were used to compute estimates of parameters and their variances, resampling methods such as the jackknife and bootstrap were also used to estimate the variance of biomass, covariances of adult parameter estimates, and the bias of estimates of parameters (Efron, 1982) because some covariance terms cannot be estimated from the direct method.

Survey Design.—The survey area extended from Point Conception to Monterey Bay, an area of 14,172 km^2 (Fig. 1). This area is a small fraction of the total Dover sole habitat, which extends from the Bering Sea to Baja California, Mexico (Eschmeyer et al., 1983). However, tagging studies indicate a very low level of coastal movement of adults (Quirollo and Kalvass, 1987). The survey area was sampled by trawl during five cruises between 1985 and 1988 (Table 1). A 400-mesh Eastern trawl was used on all cruises to collect Dover sole (Butler et al., 1989). A line transect sampling design was used in 1987 (Jan 8 to Feb 17) to investigate bathymetric patterns, and a random sampling design was used in 1988 (Feb 22 to April 10) as a pilot biomass estimation survey (Fig. 1). Prior to 1987, trawling for Dover sole was a secondary cruise objective, and trawls were taken opportunistically without a formal survey design.

During 1985 and 1986, up to 25 Dover sole from each trawl were randomly selected for sex determination. Additional ovaries were randomly selected so that 25 female Dover sole from each trawl were examined to determine the fraction of females with active ovaries. During 1987 and 1988 we measured, weighed, and classified the ovaries of a random sample consisting of up to 100 Dover

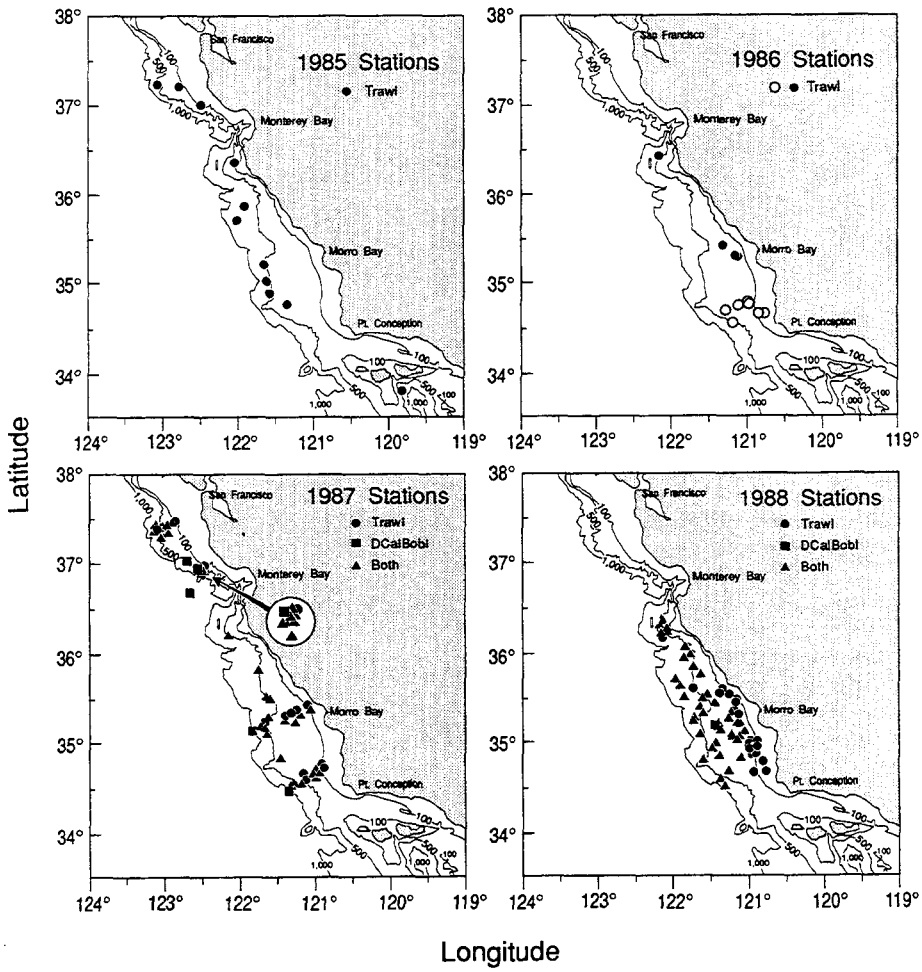

Figure 1. Stations sampled for Dover sole eggs and adults in 1985 to 1988. For 1986, open circles are stations occupied in March–April, and solid circles are stations occupied in May–June.

sole. Sex ratios by weight were estimated, and the total weight of Dover sole in each trawl catch was recorded.

Plankton Tows.—At each station on the 1987 and 1988 cruises, we sampled Dover sole eggs using surface (MANTA) tows, standard oblique bongo (CalBOBL) tows to 210 m depth, and oblique deep bongo (DBOBL) tows, which sampled the entire water column. The DBOBL tow encompassed the vertical range of Dover sole eggs for the purpose of estimating egg production rates. Mature Dover sole spawn near the ocean floor at depths of 600 to 1,500 m, and after fertilization the eggs rise, some reaching the surface. Standard procedure was to take a CalBOBL and a DBOBL tow at every station having a bottom depth >200 m and a CalBOBL tow only at stations where bottom was <200 m deep. A total of 118 CalBOBL tows and 86 DBOBL tows was taken during the 1987 and 1988 cruises and two CalBOBL tows and one DBOBL tow were excluded from data analyses because of incomplete data (Table 1). We sorted the entire contents of most tows for Dover sole eggs. When plankton were unusually dense, ca. 50% of the contents of the CalBOBL and 25% of the DBOBL were sorted.

We used a standard haul factor (SHF) to convert catches of eggs by oblique CalBOBL or DBOBL tows to estimates of egg density (Appendix 1). The lower SHF for DBOBL tows reflects the greater

Table 1. Sources of data used to estimate each of the parameters used in egg production method for Dover sole from central California. Egg production parameters P through E are defined in the text (+, cruise providing data for estimating parameter; −, data not available)

Cruise Year	Month	Sampling type	Number of tows						Depth (fm)	Egg production parameters						
			Plankton										G			
			Bongo		Deep Bongo		Trawl									
			Pos	Total	Pos	Total	Pos	Total		P	W	R	Deck	Lab	Hist	E
85	12	Opportunistic	0	0	0	0	11	17	94–704	0	+	+	−	+	+	+
86	03–04	Opportunistic	0	0	0	0	8	10	52–500	−	−	+	−	+	+	−
86	05–06	Opportunistic	0	0	0	0	3	6	175–537	−	+	+	−	+	+	−
87	01–02	Line Transect	7	54	21	42	49	55	99–705	+	+	+	+	+	+	+
88	02–04	Stratified Random	16	62	22	44	51	63	38–602	+	+	+	+	−	−	+
Total			23	116	43	86	122	151								

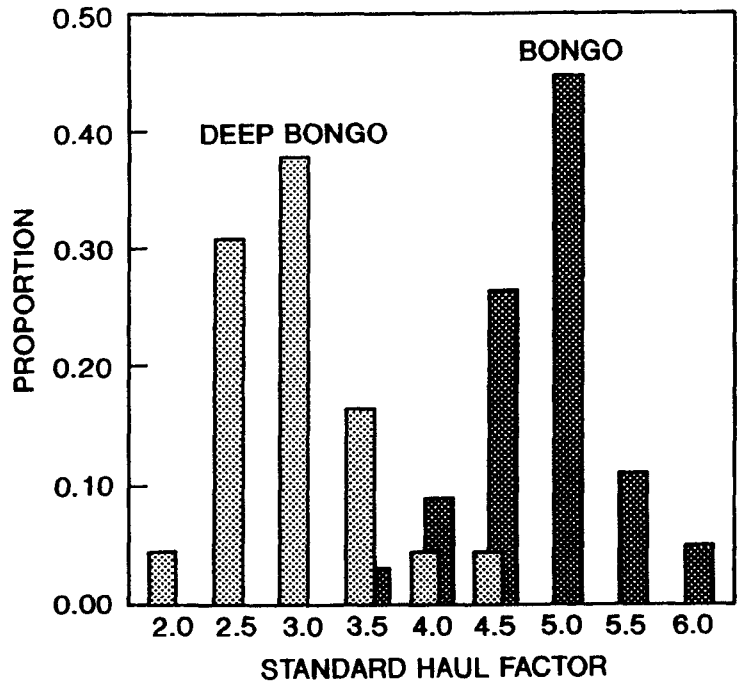

Figure 2. Relationship between standard haul factor (SHF) for the deep bongo and standard bongo net tows.

volume of water filtered per unit depth, not the greater depth (Fig. 2) and the faster speed (1.05 $m \cdot s^{-1}$ rather than 0.85 $m \cdot s^{-1}$ for the standard tow (CalBOBL)). The faster tow allows the ship speed to be controlled more precisely.

The reliability of this sampling procedure depends on the actual vertical distribution of eggs. If eggs were randomly distributed with depth, then the assumption of equal sampling at each depth interval would be unnecessary. If eggs were distributed non-randomly over broad depth zones (50+ m), episodes of over-sampling would probably compensate for episodes of under-sampling, resulting in little or negligible bias. On the other hand, if the egg layer were compact, imprecision could arise from sampling error.

Plankton Sample Processing and Egg Staging.—Each plankton sample was preserved in 5% buffered formalin immediately after each tow. Fish eggs and larvae were later removed from the plankton samples and preserved in 3% buffered formalin. Dover sole eggs were identified in samples by the following: 1) large size (ca. 2.0 to 2.6 mm shell diameter), 2) large perivitelline space, 3) smooth shell, 4) lack of oil globule, 5) homogeneous yolk, and 6) distinctive pigmentation on late stage embryos. Eggs were staged using a modification of the method developed for northern anchovy (Moser and Ahlstrom, 1985). Stages were defined by morphological criteria chosen from the sequence of changes that occur during embryogenesis (Table 2).

The stages of eggs were converted to ages according to a temperature-dependent egg development model (Appendix 1). Data from both CalBOBL and DBOBL were used to model the egg mortality curve. For the stations where both CalBOBL and DBOBL tows were taken, we computed number of eggs $\cdot 10\ m^{-2}$ (y) for each age group as (Fig. 3):

$$y = (y_1 + y_{2u})/2 + y_{2L}$$

where y_1 is the number of eggs per 10 m^2 of CalBOBL, y_{2u} is the number of eggs per 10 m^2 of DBOBL allocated to <200 m level, and y_{2L} is the number eggs per 10 m^2 of DBOBL allocated to >200 m level (Appendix 1). We fitted a Pareto decay function to the number of Dover sole eggs at age because the mortality of younger eggs was higher than that of older eggs. The mortality curve for Dover sole eggs was

Table 2. Criteria for assigning developmental stages to Dover sole eggs

Stage	Criteria
I	fertilization to initial cell division
II	first cell division and blastodisc formation
III	blastomeres minute and blatodisc appears as tissue
IV	germ ring extends ⅓ around yolk
V	germ ring extends ⅔ around yolk
VI	blastopore closure
VII	tail tip rounded and begins to separate from yolk
VIII	tail length = ½ head length
IX	tail length = head length
X	tip of tail reaches ½ yolk diameter
XI	tip of tail reaches ¾ yolk diameter
XII	tip of tail reaches midline of forehead; to hatching

$$P_t = P_0 \cdot (t + 1)^{-\beta} \tag{2}$$

and

$$z_t = \beta/(t + 1)$$

where P_t = the number of eggs per 10 m^2 in the day t age group, P_0 = the number of newly spawned eggs per 10 m^2, β = the mortality coefficient and the daily instantaneous mortality rate at age 0, and z_t = daily instantaneous mortality rate at an age of t days. We used a nonlinear regression procedure of statistical software package BMDPAR (Dixon, 1988) to produce estimates of P_0 and β.

Adult Parameters.—FEMALE WEIGHT (W). Estimates of average fish weight were computed from random samples taken from trawls. The number of fish in a random sample was 25 in 1985 to 1986 and 100 in 1987 to 1988. Total length (mm) of Dover sole in these samples was always measured, but not all fish were weighed. For specimens that were not weighed, weight was computed from a length-weight relationship derived from 1,516 Dover sole taken in the 1987 and 1988 cruises (Appendix 1). Average female weight (W) was computed for each stratum as:

$$W = \sum (q_i \cdot x_i)/\Sigma\, q_i \tag{3}$$

where q_i = number of females in the sample taken from the ith trawl, and x_i = the average female weight in the sample.

FEMALE FRACTION OF POPULATION (R). The female fraction of the population by weight (R) was estimated by the weighted mean of the female fraction in individual trawls by equation 3 where, for the ith trawl, q_i is the total weight of Dover sole sampled and x_i is the female fraction, computed as total female weight in the sample divided by the total sample weight.

TOTAL FECUNDITY (E). Total fecundity (E) was estimated as the product of the ovary weight in grams and the number of advanced oocytes per gram of ovarian tissue. We used the gravimetric method to estimate the number of advanced oocytes per gram of ovarian tissue. Advanced oocytes were counted in each of two weighed tissue samples. Previous statistical analysis indicated that two ovarian tissue samples from each of 70 to 80 females were adequate because advanced yolked oocytes are randomly distributed within the ovary of Dover sole (Hunter et al., 1992).

Advanced yolked oocytes were counted and measured using a digitizer linked by a video camera system to a dissection microscope. Advanced yolked oocytes were distinguished from other yolked oocytes by their high yolk density when viewed on the television screen. Thirty yolked oocytes from each ovary were measured. If the mean diameter of these oocytes was ≥0.86 mm, the fish was used in our fecundity estimates. Ovaries with advanced oocytes averaging <0.86 mm were considered not to be sufficiently mature to accurately identify the advanced stock of yolked oocytes (Hunter et al., 1992). Atretic oocytes were not included in our counts of advanced oocytes. The exclusion of atretic oocytes would not bias the computation of daily fecundity per female (D; eqn 1), as only 1.5% of advanced oocytes were atretic and the rate of atresia did not change with elapsed time.

Total fecundity (E) is the total number of advanced yolked and hydrated oocytes present in the ovary. Fecundity is determinate in Dover sole (Hunter et al., 1992) and decreases during the spawning season as advanced oocytes mature and are spawned. A linear regression was used to model the relationship between fecundity, fish weight, and day of the year (Fig. 3):

$$E = a_0 + a_w \cdot w + a_t \cdot t \tag{4}$$

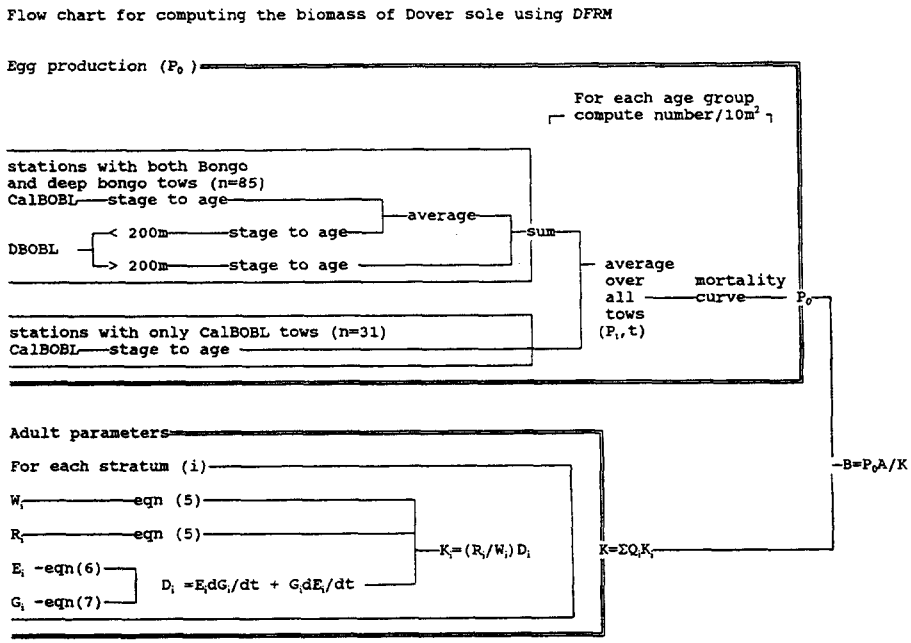

Figure 3. Flow chart for computing the biomass of Dover sole using DRFM. (See Table 3).

where E is the fecundity, w is fish weight (g), and t is the day of the year (t; t = 1 for Jan 1, t = 2 for Jan 2, . . .).

Fraction of Females with Active Ovaries (G). Ovaries from a subsample of up to 25 females from each trawl were examined on shipboard and assigned to one of three classes: *Hydrated,* translucent hydrated oocytes present; *Active,* yolked oocytes present; or *Inactive,* no yolked oocytes present. The fraction of females with active ovaries (G) was the number of active females plus the number of hydrated females, divided by the total number of females in the sample. After each cruise, the ovaries were reexamined and classified in the laboratory to determine the accuracy of the shipboard classification scheme. All ovaries were reexamined, but in 1988 only a subsample was reexamined. The results of these analyses indicated shipboard ovarian classification was reasonably accurate (91%; Hunter et al., 1992). To estimate the fraction of females with active ovaries in 1988, we converted deck-graded data to laboratory-graded (Appendix 1).

The fraction of females with active ovaries (G) would be expected to decline over the spawning season as females spawned. The fraction would also be expected to increase with weight since the attainment of sexual maturity is a function of female size. A Weibull distribution was used to model the relationship between the fraction of active females (G) and day of the year (t) (Hunter et al., 1992). Here, we chose to use a linear equation to obtain an overall decline rate of the fraction of females with active ovaries (G) with respect to female weight and day of the year within the survey period:

$$G = c_0 + c_w \cdot w + c_t \cdot t \tag{5}$$

From a theoretical standpoint, a nonlinear function may be a more suitable model for describing the annual decline of the fraction of females with active ovaries because the rate of decline in the proportion active decreases later in the season (Fig. 4). For the relatively short period under study, however, a linear model gave an adequate estimate of the average decline rate. The coefficient (c_t = dG/dt) for day of the year estimates the average decline rate of the fraction with elapsed time.

Daily Fecundity per Female (D). The fecundity per female is the product of fecundity (E) and female fraction with active ovaries (G). Because both E and G decline during the season and females eventually spawn all of their advanced oocytes, at which time the ovary becomes inactive, the fecundity per female (E·G) declines with time (t). The decline rate of E·G (d(E·G)/dt) measures daily fecundity per female (Fig. 3) and can be expressed as

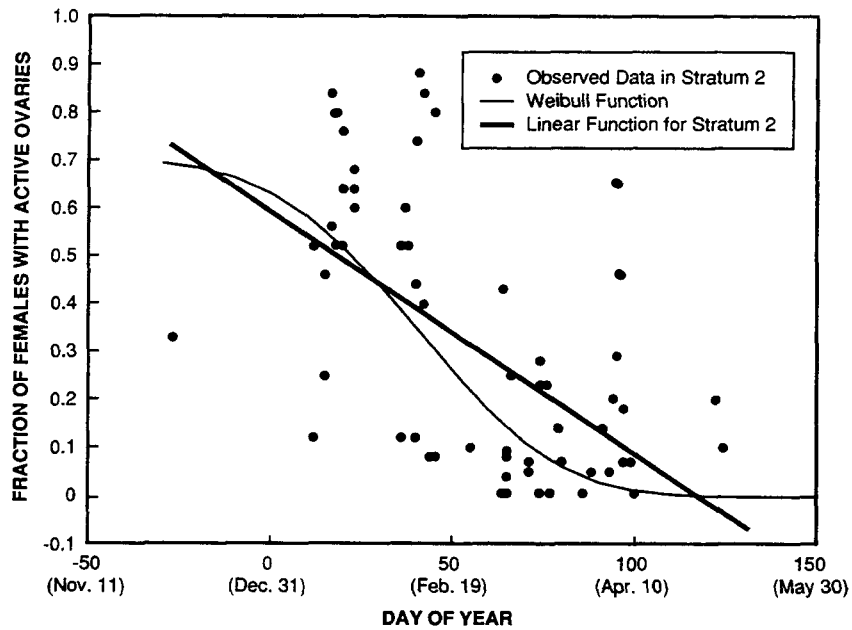

Figure 4. Fraction of females with active ovaries and day of year: Weibull distribution (Hunter et al., 1992) and linear function for stratum 2, 1985 to 1988.

$$D_i = d(E \cdot G)/dt$$

$$= E_i \cdot dG/dt + G_i \cdot dE/dt \quad (6)$$

with approximate variance by the delta method (Appendix 2).

DAILY POPULATION FECUNDITY (K). To estimate the daily population fecundity of Dover sole, we computed the rate for each depth stratum (Fig. 3):

$$K = (R/W) \cdot D_{50} \cdot 10^6 \quad (7)$$

The coefficient of variation of this rate [CV(K)] within each stratum (K) was computed by the delta method:

$$CV(K) = [CV(R)^2 + CV(W)^2 + CV(D_{50})^2 + 2 \cdot COV(D_{50}, R)/(R \cdot D_{50})$$

$$- 2 \cdot COV(R, W)/(R \cdot W) - 2 \cdot COV(D_{50}, W)/(D_{50} \cdot W)]^{0.5} \quad (8)$$

The covariance terms, $COV(D_{50}, R)$ and $COV(D_{50}, W)$, were difficult to compute directly. Thus a bootstrapping method was used to estimate three covariance terms for each depth stratum.

POST-STRATIFICATION OF SURVEY AREA BY DEPTH. We stratified the survey area by bottom depth after the survey because adult parameters changed markedly with bottom depth (Hunter et al., 1990). Three depth strata were used: stratum 1 was area with bottom depth ≤249 fm (456 m), stratum 2 was 250 to 549 fm (457 to 1,004 m), and stratum 3 was ≥550 fm (1,005 m). Each reproductive parameter was computed for each stratum. An analysis of variance (ANOVA) or analysis of covariance (ANCOVA) was performed to test whether the differences in estimates among strata were statistically significant. The covariates were fish weight and day of the year. If estimates for different strata were different, the parameter estimates for each stratum were retained. If they were not different, data were pooled and the parameters were estimated using the pooled data. A complete set of adult parameter estimates was computed for each depth stratum even when the data from different strata were pooled to make the estimate. Each estimate from a stratum was weighted by the product of area covered by the stratum and the mean trawl catch for the stratum to arrive at an overall estimate. The weighted average for each individual parameter (X = R, W, D_{50}, and K) and its variance [V(X)] were computed

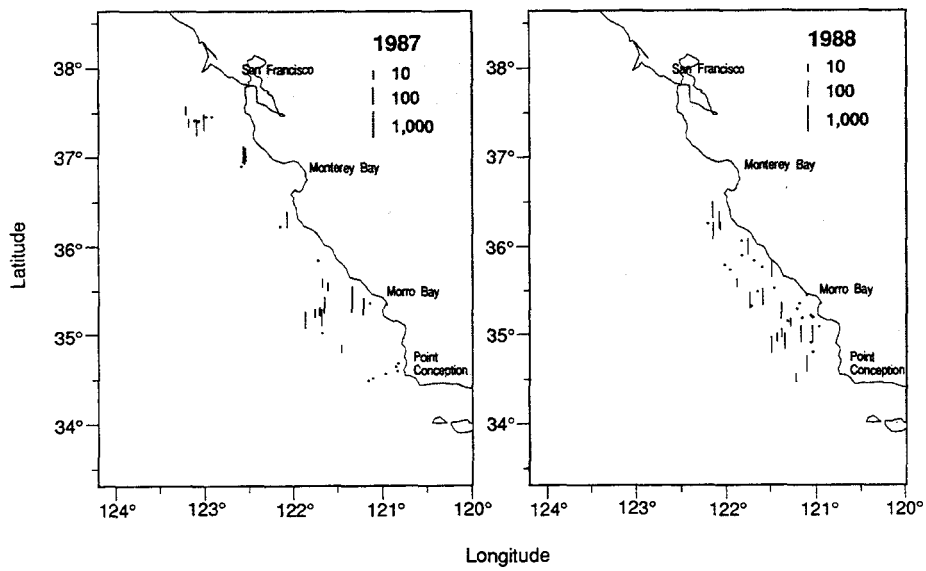

Figure 5. Spatial distribution of Dover sole eggs collected in DBOBL net in 1987 and 1988.

following standard procedures: $X = \Sigma\ Q_iX_i$ and $V(X) = V(\Sigma\ Q_iX_i) = \Sigma\ [Q_i^2V(X_i)]$ where Q_i is the weight, $\Sigma\ Q_i = 1$ and X_i is a parameter estimate in a stratum (Fig. 3).

RESULTS

Planktonic Egg Parameters.—ESTIMATION OF DAILY EGG PRODUCTION (P_0). The eggs taken in each plankton tow (Fig. 5) were first converted to the numbers of eggs per 10 m^2 and then were grouped by their ages into daily age classes. We used the average number of eggs per 10 m^2 per age class over all stations to estimate the production rate, eggs spawned per day (P_0), and the daily egg mortality (z) (Fig. 3). Daily egg production was estimated for the survey area as a whole because eggs were transported between strata making stratification by depth impractical. Data from both CalBOBL and DBOBL were used in computing daily egg production.

The estimate obtained for the number of newly spawned eggs (P_0) was $2.10 \cdot d^{-1} \cdot 10\ m^{-2}$ (SE = 0.24) and the estimate of the mortality coefficient was $\beta = 0.63$ (SE = 0.06) (Fig. 6). The bootstrapped estimate of P_0 (1.94) was slightly lower than the direct computation. The standard error (0.58) of the bootstrapped estimate of P_0 was twice that from the regression. The bootstrapped estimate of the mortality coefficient was 0.65, similar to the direct computation, yet the standard error from the bootstrap method (0.13) was also twice the standard error computed from the direct computation (Table 3, Appendix 2).

Estimation of Daily Population Fecundity (K).—Estimates for parameters used to calculate daily population fecundity of Dover sole ($K = (R/W) \cdot D \cdot 10^6$; $eggs \cdot mt^{-1} \cdot day^{-1}$) are described below.

FEMALE WEIGHT (W). Average female weights for each stratum were used because ANOVA indicated that fish weights were significantly different among strata ($F = 132.14$, $df = 2, 113$). The mean female weight was 250.53 g (SE = 29.81 g,

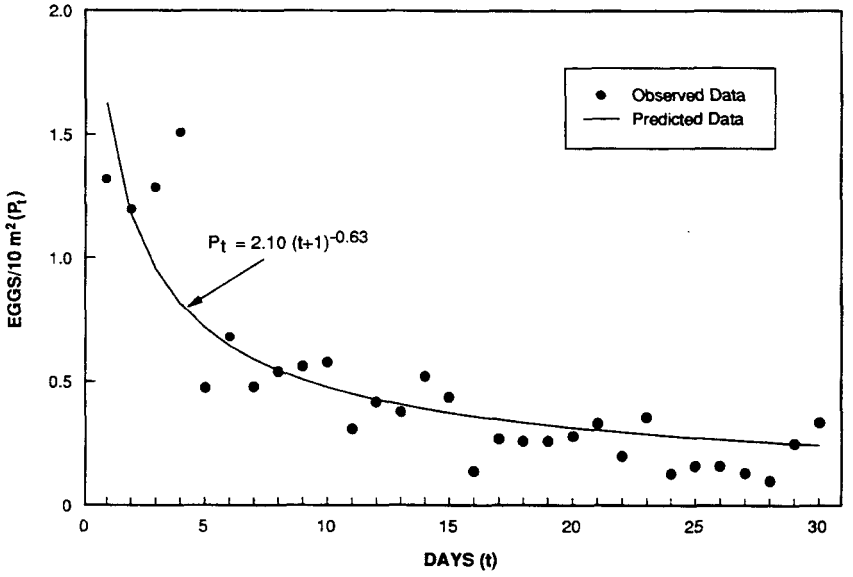

Figure 6. Dover sole egg mortality curve. Each point is the mean abundance per 10 m² by 1-day intervals.

N = 38) for stratum 1, 776.07 g (SE = 31.11 g, N = 62) for stratum 2, and 1,056.7 g (SE = 20.49 g, N = 16) for stratum 3. The weighted average female weight for the entire survey area was 668.51 g (SE = 25.06 g, N = 116), where the weighting factor was the product of stratum area size and mean catch from the swept trawls (Table 3). The standard errors of the estimates were obtained by the jackknife method (Efron, 1982) because the underlying distribution of the estimates was unknown. The bootstrapped estimates were similar to the direct computation, and the variances computed from the bootstrap method were also similar to the jackknife method (Table 3).

FEMALE FRACTION (R). We carried out an ANOVA to determine whether the female fractions were different among strata. The arcsine of the square root of sample ratios was used to normalize the female fraction in each trawl prior to the ANOVA. The results indicated that the female fractions in the three strata were significantly different from one another ($F = 16.33$; df = 2, 113; $P < 0.001$). Thus, we computed R for each stratum. The fraction (R) was 0.67 (SE = 0.034, N = 38) for stratum 1, 0.76 (SE = 0.031, N = 62) for stratum 2, and 0.92 (SE = 0.054, N = 16) for stratum 3 (Table 3). The weighted female fraction for the entire survey area was 0.74 (SE = 0.025, N = 116). The jackknife method was used to compute the standard errors of the estimates. Similar to the estimate of average female weight, the bootstrap method provided point estimates similar to the direct formula and variance close to the jackknife method (Table 3).

TOTAL FECUNDITY (E). An analysis of covariance indicated that the regression coefficients for the day of the year for the three strata were similar ($F = 0.55$; df = 2, 245; $P = 0.58$) but the regression coefficients for fish weight were not the same among strata ($F = 3.40$; df = 2, 245; $P = 0.035$). The difference is due to the high value of regression coefficient in stratum 1 compared with the other two strata (Fig. 7). To accommodate these differences, we used two linear models for

Table 3. Estimates of parameters associated with adult reproduction of Dover sole, egg production (P_0), daily egg mortality coefficient (β), and biomass estimates (B and B_c) in central California by depth stratum, 1985 to 1988* (Sample size, N, is number of trawls)

	Depth stratum (m; fm)							
	0–456 0–249		457–1,004 250–549		1,005–1,280 550–700		Weighted mean†	
Method‡	1	2	1	2	1	2	1	2
W (g)	250.53	253.22	776.07	763.7	1,056.7	1,063.5	668.51	659.5
SE	29.81	17.36	31.11	26.13	20.49	26.3	25.06	22.24
N	38	37	62	60	19	16		
R	0.67	0.70	0.76	0.80	0.92	0.85	0.74	0.78
SE	0.034	0.023	0.031	0.023	0.054	0.07	0.025	0.02
N	38	37	62	60	16	16		
$D_{50} = d(E \cdot G) \cdot dt^{-1}$	100.38	145.93	288.60	282.93	463.52	451.03	250.82	255.84
SE	26.98	33.88	61.63	22.64	92.68	50.83	48.41	18.52
N	37	37	60	16	16			
$K \cdot 10^{-6} = (R/W) \cdot D_{50}$	0.27	0.40	0.28	0.30	0.40	0.36	0.28	0.32
CV	0.30	0.23	0.18	0.07	0.21	0.14	0.15	0.09
A (area, km^2)	4,049		7,204		2,919			
Mean catch·trawl^{-1} (lbs)	67.39		140.14		5.2			
Weighting factor	0.21		0.78		0.01			

Method	1	2
$P_0 \cdot 10\ m^{-2}$	2.10	1.94
SE	0.24	0.57
β	0.63	0.65
SE	0.06	0.13
Stations§	116	
B (mt)	10,629	8,591
CV	0.19	0.31
B_c (mt)	12,567	
CV		0.38

* Abbreviations are as follows: W, the average female weight (g); R, female fraction of the population by weight; E, the total fecundity for average female weight; G, the fraction of female with yolked eggs; D_{50}, the number of eggs spawned per female per day; $(R/W) \cdot D_{50}$, the daily population fecundity per gram; SE, standard error. The biomass estimate (B) was computed based on equation 1. B_c is the bias-corrected biomass estimate.
† Weighted by the product of area and mean catch.
‡ Method 1 is the direct calculation from formulas described in the text. The standard error (SE) was based on regression estimate for P_0, jackknife estimates for R and W, and equation A3 for D_{50}. The CV of K was from equation 8. The SE of B was from equation 1A. Method 2 is the bootstrap method.
§ See Table 1 for number of CalBOBL and DBOBL tows.

fecundity: one for stratum 1 (depth $\leq$ 249 fm), and the other for strata 2 and 3 combined (depth $\geq$ 250 fm) (Table 4).

Fraction of Females with Active Ovaries (G). We used an ANCOVA to determine if it was necessary to use a separate model for each of the three strata, where the covariates were female weight (W) and day of the year (t) and the arcsine of the square root was applied to the fraction (G). This analysis indicated that the active fraction of the same fish weight caught at the same day of the year was statistically different among strata ($F = 6.13$; $df = 2, 108$; $P = 0.003$), although the difference in slopes of two covariates among three strata were undetectable ($F = 2.08$; $df = 4, 104$; $P = 0.08$). As a result, a separate simple linear regression was obtained for each stratum (eqn 5). Our analyses indicated that female weight was an important variable in describing the fraction of females with active ovaries in stratum 2, but not in the other two strata. Thus, the fish weight was included as a variable only in stratum 2 (Table 4).

The overall decline rate of G was 0.0028, 0.0049, and 0.0056 per day for strata 1, 2, and 3, respectively. Except for stratum 1, these rates were similar to the

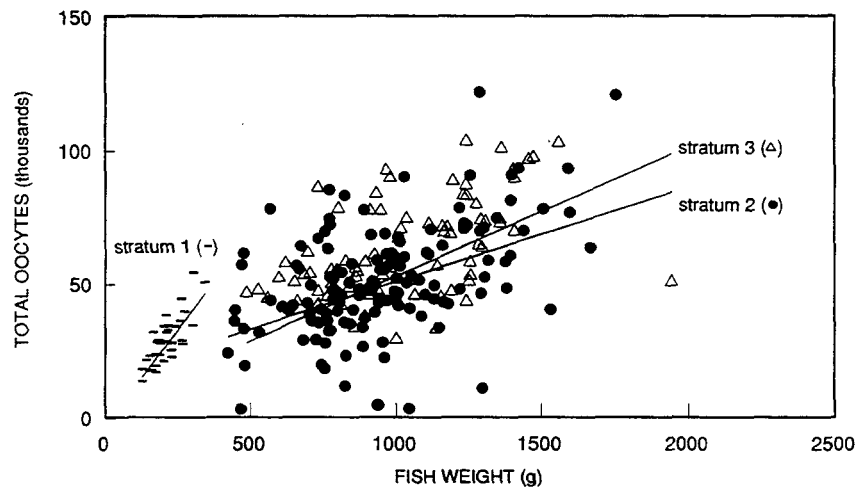

Figure 7. Linear relationship between total fecundity and body weight of Dover sole by depth stratum.

overall rate 0.005 per day computed from the Weibull function (Hunter et al., 1992; Fig. 4). Thus, the simple linear regression is a good approximation for the overall decline rate of G.

Because both E and G decrease as t increases, the mean daily fecundity per female (D) was estimated by D_{50} at t = 50 (day of the year, say Feb 19), which was close to the middle of the study time period. The estimate of average daily fecundity per female for Feb 19, D_{50}, was 100.38 oocytes·female^{-1}·d^{-1} (SE = 26.98, N = 37) for stratum 1, 288.60 (SE = 61.63, N = 60) for stratum 2, and 463.52 (SE = 92.68, N = 16) for stratum 3, where the sample size (N) was the number of trawls (Tables 3 and 5). The average value in each stratum was weighted by the product of the area and average catch per trawl of each stratum. The final estimate was 250.82 eggs spawned per female per day for all strata (Table 3). Except for stratum 1, the bootstrap estimates were similar to the estimates from the direct method and the bootstrapped variances were smaller than the direct computation (Appendix 2; eqn A3; Table 3). In stratum 1, the bootstrapped estimate was 40% higher than that from the direct formula.

DAILY POPULATION FECUNDITY (K). The last three terms in equation 8 were negligible within each stratum. Although our computation of CV(K) (=0.15) in-

Table 4. Linear regression of total fecundity (E) on female fish weight (W) and day of the year (t) and two functional relationships of fraction of females with active ovaries (G) on female fish weight (W) and day of the year (t) by depth stratum. N is number of fish for computing total fecundity (E), and is number of tows for computing G

Stratum	Equation	N	$\sqrt{mse}$	R^2
	Total fecundity (E)			
1	E = 1,334 + 146.53·W − 102.48·t	32	5,677	0.63
2 + 3	E = 23,107 + 40.81·W − 237.5·t	220	16,050	0.37
	Fraction of females with active ovaries (G)			
1	G = 0.22 − 0.0028·t	37	0.152	0.28
2	G = 0.083 + 0.00064·W − 0.0049·t	60	0.190	0.54
3	G = 0.945 − 0.0056·t	16	0.257	0.47

Table 5. Summary of estimates of parameters used in estimating the number of eggs spawned per female per day (D_{50} = |d(E·G)/dt|), 1985 to 1988

Parameter	Depth stratum (m; fm) 0–456 0–249	457–1,004 250–549	1,005–1,280 550–700
E_{50}	32,920.16	42,903.42	54,355.93
SE	2,100.84	1,535.06	1,341.06
dG/dt	−0.0028	−0.0049	−0.0056
SE	0.00075	0.00076	0.0016
G_{50}	0.08	0.33	0.67
SE	0.027	0.031	0.078
dE/dt	−102.48	−237.5	−237.5
SE	86.61	36.71	36.71
COV(E, G)*	133.53	1,130.66	235.26
D_{50}	100.38	288.60	463.52
SE	26.98	61.63	92.68
N (trawls)	37	60	16

* $COV(E_i, G_i) = COV(a_0 + a_w \cdot W + a_t \cdot t, c_0 + c_w \cdot W + c_t \cdot t) = a_t \cdot c_t \cdot V(t)$ for a given fish weight (W) where, t is day of the year, a_i and c_i are regression coefficients of elapsed time (eqns 4 and 5). E_i and G_i are computed for the average fish weight in each stratum. For other abbreviations see Table 3.

cluded all covariance terms, CV(K) computed without covariance terms was 0.18 and thus should be acceptable.

Using equations 7 and 8, we obtained K = 0.27 × 10^6 eggs·mt^{-1}·d^{-1} (CV = 0.30) for stratum 1, 0.28 × 10^6 (CV = 0.18) for stratum 2, and 0.40 × 10^6 (CV = 0.21) for stratum 3. The weighted average was 0.28 × 10^6 (SE = 0.04 × 10^6, CV = 0.15; Table 3). Similar to D_{50}, except for stratum 1, the bootstrapped estimates of K were close to the direct computation. In stratum 1, the bootstrapped estimate (0.4 × 10^6) was higher than the direct computation (0.28 × 10^6; Table 3). The bootstrapped estimate for the whole area (0.32 × 10^6) was higher than the direct computation. The difference (0.04 × 10^6) measures the bias of the estimate from the direct computation. Thus, the estimated K from equation 7 is biased upward. The variation of K was dominated by the variation of D_{50} and the CV of K from the bootstrap method (0.09) was smaller than those computed from equation 8 (0.15; Table 3).

The rate of 0.28 × 10^6 eggs·mt^{-1}·d^{-1}, is much lower than the rate for northern anchovy which is 37 × 10^6 eggs·mt^{-1}·d^{-1} in 1985 (Bindman, 1985). Thus, the daily spawning rate per unit weight of population of Dover sole is slightly less than 1/100th of the spawning rate per unit population of anchovy.

Estimation of Biomass (B).—The estimate of the biomass of Dover sole using daily fecundity reduction method was 10,629 mt (CV = 0.19; Table 3). The coefficient of variation of estimated biomass was computed without $COV(P_0 \cdot K)$ (eqn 1a) because $COV(P_0 \cdot K)$ cannot be estimated from the existing data set. The CV of B from the bootstrap method (0.31) was higher than that computed from equation 1a, primarily because of the high coefficient of variation of P_0 from the bootstrap method (Table 3). The bootstrapped estimate of biomass was 8,691 mt (Table 3). The bias-corrected estimate (B_c) was 12,567 mt (CV = 0.38; Appendix 2). Abundance estimates for Dover sole in the same study area using the swept trawl method in 1987 and 1988 range from 14,000 to 17,000 mt (Butler et al., 1989). Thus the daily fecundity reduction method estimate is close to the lower limit obtained by the swept trawl for the same area.

DISCUSSION

We used an assortment of survey information taken over a number of years to estimate the biomass of Dover sole because data from one single year were inadequate to assess the population, although the bulk of the data were collected from 1987 and 1988. Moreover, the underestimation of biomass resulted from the fact that two requirements for the daily fecundity reduction method were not met fully: 1) the survey does not encompass the offshore limits of Dover sole eggs, and 2) the temperature history of the eggs was approximated for converting developmental stages of eggs to ages. The consequences of the problems, potential improvements, and future directions are discussed below.

Bias Due to Egg Distribution.—Our surveys covered a small fraction of the spawning area of Dover sole (Ahlstrom and Moser, 1975; Savage, 1989). For purposes of this demonstration, it can be assumed that the transport of eggs across the northern and southern boundaries of the survey area caused no bias because no obvious trend existed in their abundance along the coast (Fig. 5a, b). On the other hand, the density of eggs increases as one moves offshore. Surface tows taken with a MANTA net on the same cruises indicate that significant numbers of 1-day-old Dover sole eggs reach the top 16 cm of the sea surface. Assuming that at least some of these eggs originated at 1,000 m, where the spawning biomass is high (Hunter et al., 1990), we estimate that some of these eggs rose 1,000 m in 1 d or about 0.01 $m \cdot s^{-1}$. Such rapid vertical transport of eggs, coupled with long incubation periods of 30 d, would lead to considerable horizontal offshore transport of eggs. This suggestion is supported by the relatively high numbers of eggs found in samples near the outer edge of the Dover sole slope habitat. Thus, our calculation of total egg production may be considerably biased downward because eggs are likely to have drifted offshore from our survey area resulting in underestimation of A in equation 1.

Our estimate of P_0 may also be biased upward because older eggs are more likely to drift into the unsurveyed offshore area than are younger ones. This could cause an overestimate of egg mortality since the number of eggs per age class would be progressively underestimated with increasing age. An overestimate of egg mortality would cause an overestimate of P_0 since it is the extrapolated intercept of the egg mortality curve. If the mortality rate of Dover sole eggs was corrected for offshore transport of older eggs the actual rate would be less than 0.1 per day.

Potential Egg Thermal History Bias.—Surface temperatures were usually highest offshore and to the south in the study area. Thus, a more accurate estimate of spawning biomass of Dover sole will require additional information about water temperatures and the thermal history of eggs. Variability in water temperature, together with transport during the long incubation period, result in uncertainty about thermal history. In this regard, it may be useful to use eggs at ages up to 3 or 4 d in the computations to diminish the effects of transport and temperature change.

The incubation model and the vertical distribution used in this study are first approximations (Appendix 1). Additional data on Dover sole egg development rate over a range of temperatures, together with a quantitative study of the vertical distribution of their eggs in the water column, are essential for developing more precise models. We used the mean temperature over each of two depth ranges (depth <200 m, and depth >200 m) to assign ages to the eggs taken in each of these ranges. This method underestimates the age of the eggs in the upper depth

range since the eggs are spawned at the bottom and rise through the lower range before reaching the upper one. Although, we believe the current method produces only a minor bias in the estimates of biomass, a temperature-dependent, vertical transit model for eggs would be preferable.

Precision, Survey Design, and Vessel Requirements.—A formal analysis of the precision of the daily fecundity reduction method for Dover sole is beyond the scope of this paper. Some information related to the design of sampling program is, however, available. The precision of daily population fecundity estimates (eggs spawned per gram of fish per day) depends on the decline rate of the fraction active (G) and total fecundity (E) during the spawning season, as well as on the number of trawl hauls containing adult Dover sole. Dover sole population fecundity (fraction of females with active ovaries) declines slowly during the spawning season (15% per month) consequently specimens must be collected over a rather long expanse of time to detect a time-dependent change in spawning rates. Our data indicate that 50 positive trawls (trawls in which some fish are caught) over a 100-d interval would provide an estimate of the daily rate of decline of the fraction active (G) with a coefficient of variation of 0.30. On the other hand, if the 50 positive trawls were taken over 30 to 40 d the coefficient for elapsed time is not detectable (CV = 2.50). Even if one doubled the number of trawls it is unlikely that an effect of elapsed time on G could be detected over a 40-d period because the coefficient of variation is so large. We reached similar conclusions for the detection of an effect of elapsed time on total fecundity (E). Thus, the most efficient survey design for Dover sole would be to sample the adults intermittently over 100 or more days. If a species had a much shorter spawning season and spawned fewer times than Dover sole, it would not be necessary to use such an extended survey design.

Estimation of P_0 for Dover sole may require fewer samples than are required for anchovy to achieve the same level of precision even though Dover sole eggs are less abundant than anchovy eggs. This is because the distribution of Dover sole eggs is less contagious than anchovy eggs. The percentage of tows that contains eggs of Dover sole (42%[1]) from both CalBOBL and DBOBL is higher than that for anchovy (36%, an average over 1987 to 1990) and the coefficient of variation for number of Dover sole per unit area (2.02) is lower than that of anchovy (2.50, an average over 1987 to 1990). To obtain a sample size for a given CV of P_0, we analyze data from DBOBL tows only. Our analysis indicates that the CV of P_0 ranges between 0.19 (eqn 2) and 0.37 (bootstrap method) from a total of 85 DBOBL tows (43 were positive) (Lo et al., 1992). We now take the worst case and accept 0.4 as the CV of P_0 from 85 DBOBL tows, then we will need total number of tows equal to $(0.4/CV)^2 \cdot 85$ for a desired CV of P_0. For example, for CV = 0.2, we need 340 DBOBL tows (172 positive stations), which is less than half of the tows required by anchovy (Bindman, 1985). The vertical distribution of eggs may be more variable than assumed in our analysis. If so, sample size may have to be considerably larger than 500.

If P_0 and K are measured simultaneously, a daily egg production estimate of fish biomass requires considerable ship time. Deep, quantitative plankton hauls (DBOBL) require much more time than standard CalBOBL tows (21.5 min for a 200-m standard CalBOBL tow versus about 2.5 h for 1,500 m), and 1-h trawl hauls require considerable time at the greater depths (1.2 h of ship time at 200

[1] Out of 31 stations where only CalBOBL tows were taken, 3 stations were positive. Out of 85 stations where both CalBOBL and DBOBL tows were taken, 46 stations were positive.

m versus 2.5 h at 1,500 m). Under ideal weather conditions, allowing only 1 h running time between stations, about 80 CalBOBL, DBOBL tows, and 1-h trawl hauls could be completed in 20 d, where the bathymetric depth range was 200 to 1,500 m and stations were made at 200-m depth increments. One alternative survey design to increasing the number of net tows for collecting Dover sole eggs would be to use less expensive shallow tows as the basic sampling units with occasional pairing of shallow tows with the DBOBL tows to provide an absolute calibration of shallow tows.

Comparison of Direct Method and Bootstrap Method Estimates.—The biggest discrepancies between the direct computation and bootstrap method were the standard error of estimate of egg production for the whole area and the estimate of D_{50} in stratum 1. The direct method seemed to underestimate D_{50} in stratum 1 and the variance of P_0, and overestimate the variances of D_{50} in strata 2 and 3, and thus in the whole area. The end result was that the biomass from the direct method was biased downward, and its variance was underestimated. The bootstrap method allowed a bias-corrected estimate by providing a measure of bias for the estimate of biomass from the direct method.

CONCLUSION AND FUTURE DIRECTIONS

The daily fecundity reduction method is best suited to species having determinate annual fecundity and a relatively long spawning season, such as the Dover sole. the DFRM is preferred to the DEPM for Dover sole because of the difficulties in estimating spawning rates directly, due to the depth of their habitat and problems of calibrating estimates of the rate of spawning.

The DFRM is preferred to the AEPM owing to the long spawning season of Dover sole, and the relative high cost of the AEPM. The annual method requires ichthyoplankton surveys throughout the entire spawning season, whereas only a portion of the season needs to be surveyed if the DFRM is used. Even if the spawning season were short (1 to 2 months), several advantages may exist in using DFRM over using the annual method. The annual method requires an estimate of the potential annual fecundity and, in making this estimate, one must be certain that no spawning has taken place, that all oocytes considered to be the potential fecundity will actually be spawned, and that atretic losses of oocytes are minor (Hunter et al., 1992). In addition, assumptions may be required concerning the form of the annual egg production curve and the proportion of the year's annual production not covered by ichthyoplankton surveys. Neither the assumptions underlying estimates of annual fecundity nor those related to the annual egg production are usually evaluated. Since the DFRM requires fewer assumptions than the AEPM, it may be the preferable method even when spawning seasons are short.

Compared to trawl or acoustic surveys for estimating biomass, ichthyoplankton methods are usually more expensive but they provide more accurate estimates and require fewer assumptions if all key biological variables are measured in the current year. In trawl surveys, the catchability of fish is usually unknown, only relatively smooth bottoms can be trawled, and some pelagic species may be hopelessly undersampled by trawling. Acoustic surveys also have well known biases and assumptions. In the application of the DEPM to northern anchovy described in Lasker et al. (1985), all key biological variables were measured in the current year but, thus far, our work on DFRM has not achieved this level of accuracy. We lacked the extensive background of knowledge available for anchovy and were forced to make assumptions regarding incubation rates of Dover sole

eggs, and based the estimate on the combined ichthyoplankton and gonad data from different years. Special problems were encountered in the application to Dover sole: quantitative plankton tows had to be taken from 1,500 m depth and adult reproductive activity and size varied with depth. These two problems were overcome and, after some current studies are completed, most of the assumptions affecting the accuracy of the method can also be eliminated. Since all parameters could be measured within a cruise, the DFRM has the potential of being as accurate as the DEPM.

In 1991, surveys were conducted to define the boundary of egg distribution, to obtain a good estimate of area A, and to collect data on the vertical distribution of eggs (Moser et al., in prep.). We are developing a temperature-dependent vertical transit model to refine the procedure for assignment of age to stage. In the future, we recommend simulation studies on estimates of biomass over a range of approximately 25 to 75 days rather than simply using D_{50} as the best estimate of total biomass. Lastly, the final test of the method will be to carry out a DFRM survey in which all parameters are measured during the survey.

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APPENDIX 1

Conversion Factors:

Standard Haul Factor (SHF).—The SHF allows egg count to be expressed as number of eggs per 10 m^2 of sea surface area. The equation for the SHF is:

$$SHF = 10 \cdot D/(R \cdot A \cdot P)$$

where D is the depth of tow in meters, R is the number of revolutions of a flow meter in the mouth of the net, A is the area of the mouth of the net, and P is a flowmeter-specific calibration factor expressed in terms of the length (m) of the column of water needed to effect one revolution of the flow meter (Kramer and Ahlstrom, 1968). The depth of a CalBOBL tow is computed from the trajectory of the net, which is estimated from the cosine of the mean wire angle. The angle of the net is monitored at each 10 m of wire recovered.

In tows deeper than 200 m, wire angles do not provide accurate estimates of depth. To monitor the depth of DBOBL tows we attached a conductivity-temperature-depth (CTD) sensor to the net. The CTD records depth and water temperatures every 20 s. This allowed us to control the ship and winch speeds so that an approximately even volume of water was filtered at each depth. The equation that relates the tangent of the wire angle to the ship speed is:

$$V = V_0 \cdot \exp[r \cdot \tan(a)]$$

where, V is ship speed in $m \cdot s^{-1}$, V_0 is the intercept from a nonlinear least squares fit to the expected equation by the Marquardt algorithm, r is the exponential slope parameter, and tan(a) is the tangent of the wire angle in degrees. The estimates of parameters and their standard errors for the equation are $V_0 = 0.692$ (0.039) and $r = 0.271$ (0.037).

In practice, it was not possible to tow the net in an even trajectory to insure that every depth interval was sampled for the same amount of time on every tow. With a long towing wire, several minutes elapse before a net responds to a change in ship speed. In addition, undersea currents occasionally alter the angle of the towing wire. We believe, however, that no 10-m depth stratum was consistently over- or undersampled so that, on average, the entire water column was equally sampled.

Length-weight Relationship.—A single length-weight equation was applied to both sexes from 1,516 Dover sole taken in 1987 and 1988 because no differences between sexes were evident:

$$\ln(Y) = -14.07 + 3.43 \cdot \ln(X) + e$$

where Y is weight (g), X is total length (mm), and e is the error term with mean 0 and standard deviation $s = 0.1052$.

The model explained 98% of the variation in ln(Y) and there was no evidence of lack of fit. We used $\exp(-14.07 + 3.43 \cdot \ln(X) + e)$ to compute fish weight for a given length. For a nonlinear fit of the length-weight relation, see Hunter et al. (1992).

Conversion from Deck-graded Ovaries to Laboratory-graded.—To estimate the fraction of females with active ovaries in 1988, we computed rates of accuracy for the deck-grading based on a subsample of fish examined later in the laboratory for each of three legs of the 1988 cruise. For each leg, a contingency table was constructed as:

	Laboratory			
Deck	Active	Inactive	Total	Rate of Accuracy
active	m_{aa}	ma_{ai}	$m_{a\cdot}$	$p_a = m_{aa}/m_{a\cdot}$
inactive	m_{ia}	m_{ii}	$m_{i\cdot}$	$p_i = m_{ii}/m_{i\cdot}$

The first subscript refers to the deck grade and the second subscript refers to the laboratory grade; e.g., m_{ia} is number of ovaries which were classified as inactive from deck examination and as active from laboratory examination. To obtain the corrected number of fish with active ovaries (m_c), we used the formula:

$$m_c = m_A \cdot p_a + m_I \cdot (1 - p_i)$$

where m_A and m_I are number of active female and inactive female from deck-grading for each trawl.

Temperature-dependent Egg Development Model.—We used two sets of incubation data to develop an algorithm for the assignment of ages to the 12 Dover sole egg stages: data from 111 Dover sole eggs incubated at 5.9°C (Table A1) and Apstein's (1909) hatching times of North Sea plaice, *Pleuronectes platessa*, at temperatures of 4 to 14°C (Table A2). The latter were used to approximate the relationship between temperature and time required to reach a stage (time-to-stage) for Dover sole because data were available for Dover sole at a single temperature (5.9°C). Incubation data for plaice eggs are a reasonable substitute for those of Dover sole because the diameter of the yolk mass is similar (ca. 2 mm) in the two species, and their eggs incubate in the water column over a similar temperature range, although Dover sole egg development is much slower than plaice at 6°C.

A relationship between temperature and time-to-stage for Dover sole was derived from the relationship between hatching time and temperature for plaice

$$h(T) = 45.06 \cdot \exp(-0.123 \cdot T) \qquad \text{(A1)}$$

where h(T) is hatching time in days and T is temperature in °C (Apstein, 1909). Because the incubation period of Dover sole is longer than plaice at 6°C, we applied only the temperature coefficient of hatching time of plaice (eqn A1) to estimation of development rate of Dover sole eggs.

If one assumes that the proportional change in time-to-stage for Dover sole over a wide range of temperatures is the same as the proportional change in the time to hatching for plaice, then

Table A1. Mean, standard deviation (SD) and coefficient of variation (CV) of time-to-stage (days) for Dover sole eggs from 1987 experiment at 5.9°C; N is number of eggs sampled (Values in parentheses are guessed values)

Stage	N	Mean	SD	CV
I		(0.5)*		
II	38	0.955	0.383	0.40
III	9	3.288	0.575	0.17
IV	6	5.067	0.418	0.08
V	21	6.633	0.551	0.08
VI	18	9.856	1.072	0.10
VII	2	14.40	(1.4)	(0.10)†
VIII	0	—	—	—
IX	4	17.40	(1.7)	(0.10)†
X	7	22.29	1.513	0.070
XI	4	25.50	2.00	0.08
XII	2	28.5	(2.28)	(0.08)†

* 0.5 is the rounded off midpoint of 0 and 0.955.
† The CV of stage VI was used for stages VII and IX. The CV of stage XI was used for stage XII.

$$\begin{aligned} t_s(T) &= t_s(5.9)\cdot h(T)/h(5.9) \\ &= t_s(5.9)\cdot \exp(-0.123\cdot T)/\exp(-0.123\cdot 5.9) \\ &= t_s(5.9)\cdot \exp[-0.123(T-5.9)] \end{aligned} \quad \text{(A2)}$$

where $t_s(T)$ is the time-to-stage for Dover sole in days at temperature T°C. For example, at 10°C, the time to stage 5 is $t_5(10) = 6.633\cdot\exp[-0.123\cdot(10-5.9)]$, or $6.633\cdot 0.604 = 4.0$ d (Table A1).

Allocation of Staged Eggs from DBOBL to Two Depth Segments.—Dover sole eggs experience a wide range of incubation temperatures. Eggs are spawned at the bottom, where temperatures average about 3°C, and then rise in the water column, some reaching the surface where temperatures average 13°C. In order to assign ages to staged eggs, it was necessary to estimate the average temperature experienced by the eggs prior to capture. The water column was divided into two segments: from the bottom to 200 m and from 200 m to the surface. The average mid-depth temperature for the upper segment (<200 m) was 10.3°C (N = 77, SD = 0.49) and for the lower segment (>200 m) was 6.2°C (N = 76, SD = 0.93) (Table A3).

We used mid-depth temperatures at each station in equation A2 to compute the duration of each egg stage although the exact location and thermal history of individual eggs within a water column segment were unknown. The average mid-depth temperature within a stratum was used when no temperature was recorded for a station.

To assign the appropriate number of eggs taken in the DBOBL to each of the two water column segments, we computed a proration coefficient (pc) to be the ratio of number of eggs per 10 m^2 taken in the CalBOBL to that taken in the DBOBL for each stratum. We assumed that at each station both nets swept through the same group of eggs. The ratio was computed for each of three strata, at all the ichthyoplankton stations in which at least one of the two nets contained one or more eggs. The ratio for all positive stations was 1.0 (N = 11) for stratum 1, 0.5 (N = 53) for stratum 2 and 0.34 (N = 21) for stratum 3. Thus, in stratum 1, the catch of DBOBL was the same as that of CalBOBL. In stratum 2, the CalBOBL only catches half of the eggs in the water column, whereas in stratum 3, close to ⅓ of the eggs in the water column was caught by the CalBOBL.

We allocated staged eggs per 10 m^2 taken in each DBOBL tow into upper and lower segments of the water column using the following procedure for each stratum:

Table A2. Hatching time (days) of North Sea plaice at temperatures (T) between 2° to 24°C (Apstein, 1909). Estimated values were computed by using the exponential function $y = 45.06\cdot\exp(-0.123\cdot T)$

Hatching time (d)	Temperature (°C)						
	2	4	6	8	10	12	14
Observed	37	26	20	16	13	11.5	10
Estimated	35.2	27.5	21.5	16.8	13.15	10.28	8.04

Table A3. Mean, standard deviation (SD) of water temperature, and estimated hatching time, and the proration factor for time-to-stage at the surface, mid-depth of water column <200 m, and mid-depth of water column >200 m, off central California, 1987 to 1988 (N is number of tows)

	Sea surface	Mid-depth	
		<200 m	>200 m
Water temperature (°C)			
Mean	13.03	10.27	6.2
SD	0.756	0.493	0.929
N	116	77	76
Hatching time (d)	11.9	16.05	27.30*

* $27.30 = 28.50 \cdot \exp[0.123 \cdot (6.2 - 5.9)]$.

1. $N_u = N \cdot pc$
2. $N_{ui} = N_u \cdot B_i, \quad i = 1, \ldots, 12$
3. $N_{ui} = N_i, \quad$ if $N_{ui} > N_i$
   $N_{ui} = N_{ui}, \quad$ if $N_{ui} < N_i$
4. $N_{Li} = N_i - N_{ui}$

where N is the total number of eggs per 10 m^2 in the DBOBL; pc is the proration coefficient computed for each stratum; N_u is the total number of eggs per 10 m^2 allocated to the upper level, and was computed as a binomial random variable with parameters N and pc; N_i is the number of eggs of the ith stage per 10 m^2 in DBOBL; B_i is the fraction of eggs of the ith stage in the CalBOBL net; and N_{ui} (N_{Li}) is the number of eggs per 10 m^2 of the ith stage allocated to the upper (lower) level.

Step 3 states that if the number of eggs of stage i in DBOBL was fewer than the number of eggs allocated to the upper 200 m, then all the eggs of stage i would be allocated to the upper 200 m. At few stations, either N_{ui} or N_i was zero, then no egg of stage i was allocated to the upper 200 m.

After the eggs caught in DBOBL tows were allocated to each of two segments, the actual age of eggs was then determined according to their developmental stages and the water temperature by a normal distribution. The mean is the average time to reach the stage and the standard deviation is the product of the CV and average time-to-stage where, CV for time-to-stage at 5.9°C was used for all temperatures (Table A1).

Appendix 2

Variance of D_t.—

$$\begin{aligned} V(D_t) &= V(E_t)\cdot(dG/dt)^2 + V(dG/dt)\cdot(E_t)^2 + 2E_t\cdot(dG/dt)\cdot COV(E_t, dG/dt) \\ &\quad + V(dE/dt)\cdot Gt^2 + V(G_t)\cdot(dE/dt)^2 + 2\cdot G_t\cdot(dE/dt)\cdot COV(G_t, dE/dt) \\ &\quad + 2\cdot COV(E_t\cdot dG/dt, G_t\cdot dE/dt) \\ &= V(E_t)\cdot(dG/dt)^2 + V(dG/dt)\cdot(E_t)^2 + V(dE/dt)\cdot G_t2 + V(G_t)\cdot(dE/dt)^2 \\ &\quad + 2\cdot(dG/dt)\cdot(dE/dt) + COV(E_t, G_t) \end{aligned} \tag{A3}$$

In equation (A3), E_t and G_t were computed from equations 4 and 5, respectively, for average female weight and t = 50, the midpoint of the survey. The daily rates, dG/dt and dE/dt, were estimated by separate regressions so that $COV(E_t, dG/dt)$ and $COV(G_t, dE/dt)$ can be assumed equal to zero. The term $COV(E_t\cdot dG/dt, G_t\cdot dE/dt)$ is reduced to $(dG/dt)\cdot(dE/dt)\cdot COV(E_t, G_t)$, and $COV(E_t, G_t)$ was computed by $a_t \cdot c_t \cdot V(t)$ for a given average female weight (eqns 4 and 5), while other covariances were zero when the linear regression coefficients were used for dG/dt and dE/dt.

Parameter Estimates from the Bootstrap Method.—The bootstrap resampling procedure was carried out for plankton and adult samples separately. Bootstrapped point estimate and coefficient of variance were obtained. Covariance was computed for the adult parameter estimates. The bias of biomass was estimated and was used to reduce the bias of biomass computed directly from equation 1.

Daily Egg Production (P_0) and Daily Instantaneous Mortality Coefficient (β).—We bootstrapped the data set of 116 stations, where CalBOBL tows were taken at 31 stations and both CalBOBL and DBOBL tows were taken at 85 stations. Each of the 116 records contains number of eggs per 10 m^2

in 12 stage categories for each net type. One single iteration included a random sample of 116 records (tows) with replacement from the original data set. The egg production (P_0) and mortality coefficient (β) were computed following the procedures described in Results section. From 500 iterations, the point estimate of P_0 was the mean of 500 bootstrapped values for P_0, and the coefficient of variation of P_0 was computed as the standard deviation of 500 bootstrapped values for P_0 divided by the mean of those values.

Adult Parameters.—These include: the fraction of the population being female (R), average female weight (W), daily fecundity per female (D_{50}), and daily population fecundity (K).

Two data files were extracted from 1985 to 1988 adult fish data sets to be bootstrapped for the point estimate and the CV of R, W, D_{50}, and K. Data required to compute R, W, and fraction of females that have active ovaries (S) are contained in one data file, and data required to compute weight-specific fecundity (E) are contained in a separate data file. Both data files contain year and haul number for cross reference. In the first data file, each record contains sample data: sampled total female weight, number of females, sample weight, number of fish in the sample, number of females with active ovaries and number of females with inactive ovaries, depth of the two, day of the year, and year-haul number. In the second data file, each record contains fecundity information of one individual fish: number of yolked eggs, fish weight, and depth of tow, year-haul number, and total number of fish sampled for fecundity for each haul.

The bootstrapping procedures are as follows. For each stratum, a fixed number of tows was selected randomly with replacement for the first data file and the number of tows was the minimum number used for all adult parameters (Table 3): 37 for stratum 1, 60 for stratum 2, and 16 for stratum 3. Any one selected tow was further checked to see if there was any fish sampled for fecundity, i.e., whether there was a record of the same year-haul number in the second data file. If so, from the second data file and within the year-haul number, fish were selected with replacement and the sample size was the original number of fish sampled for fecundity. Otherwise, the resampling process was continued. The total number of fish bootstrapped for each stratum was equal to the actual number of fish sampled in 1985 to 1988: 32 for stratum 1, 141 for stratum 2, and 79 for stratum 3. Within each such iteration, R, W, S, E, D_{50}, and K were computed according to the formula given in the Methods and Materials section. A total of 500 iterations was run and the point estimate and the CV for each parameter estimate was computed. The covariance of any two of R, W, D_{50}, and K wa also computed.

Estimate of Biomass (B).—For each of 500 iterations, the biomass was computed (eqn 1). The point estimate of biomass and its CV were obtained as done for other parameters. The bias of biomass estimate was computed as the difference between the bootstrapped point estimate (B_b) and the biomass estimate (B) directly computed from equation 1. A bias-corrected biomass estimate (B_c) is:

$$B_c = B - (B_b - B)$$

with variance

$$V(B_c) = 4 \cdot V(B) + V(B_b).$$