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STOCK ASSESSMENT OF NEHU, *ENCRASICHOLINA PURPUREA*, USING THE EGG PRODUCTION METHOD

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ABSTRACT

Nehu, *Encrasicholina purpurea*, are short lived, tropical anchovies used as baitfish for the Hawaiian pole-and-line tuna fishery. The spawning biomass of nehu within Pearl Harbor, Hawaii, was estimated weekly using the Daily Egg Production Method (DEPM). Over the 2-year study period, spawning biomass varied between 0.5 and 5.0 metric tons in response to the intensive fishery and a seasonal cyclicity in reproductive effort.

Nehu, *Encrasicholina purpurea*, are among the smallest of anchovies, yet they are commercially valuable as the primary bait used by the Hawaiian pole-and-line fishery for skipjack tuna, *Katsuwonus pelamis* (Uchida, 1977). Similar to other stolephorid baitfishes (Dalzell, 1987), nehu are extremely short lived (<6 months; Struhsaker and Uchiyama, 1976) and spawn almost continuously throughout the year (Tester, 1952; Clarke, 1987, 1989). Unlike the others, nehu occur exclusively within estuarine embayments, migrating daily between diurnal resting areas along turbid shorelines and nocturnal spawning areas in relatively clear channels. The commercial fishery exploits this unusual behavior, and is thereby unique among baitfish fisheries, by using seines to capture nehu in their shallow daytime habitat (Uchida, 1977; Dalzell and Lewis, 1989).

Nehu abundance has fluctuated over time and occasionally has declined to such an extent that vessels have spent nearly as much time fishing for bait as for tuna. The economic hardships resulting from the periodic shortages of nehu precipitated early efforts at stock assessment using either egg and larva data (Tester, 1951, 1952) or commercial catch statistics (Bachman, 1963), but for a variety of reasons, both approaches were not totally successful (Wetherall, 1977; Clarke, 1987). However, a new approach to stock assessment has recently been developed that is particularly suitable for pelagic, schooling species such as nehu.

The new approach, known as the Daily Egg Production Method (DEPM), is especially effective for schooling fishes because it utilizes information from the life-history stage that is the easiest to sample: the egg stage. The underlying premise of the DEPM is that the daily production of eggs by the stock is proportional to its spawning biomass. This concept, which was originally formulated by Saville (1964) and later elaborated by Parker (1980) and Picquelle and Stauffer (1985), can be expressed:

$$B = P/FR \tag{1}$$

where B is the biomass, P is the daily production of eggs by the population, F is the batch fecundity per gram body weight of spawning females, and R is the proportion, by weight, of spawning females in the population. Definition of the population, and therefore the calculation of R, can be in terms of mature fish (spawning stock) or fish larger than some minimum size (commercial stock). In practice, P is estimated by sampling pelagic eggs with a plankton net, and F and R are estimated by sampling adult fish.

In this paper, the DEPM is used to assess the size of the nehu population occurring within Pearl Harbor, Oahu, which supports the second most important baitfish fishery in the Hawaiian Islands. Compared to previous applications on such temperate species as anchoveta, *Engraulis ringens* (Alheit, 1985), and north-

ern anchovy, *E. mordax* (Lasker, 1985), assessments were conducted frequently to capture the rapid population fluctuations produced by nehu's inherently high turnover rate and the occasionally intensive fishery. High-frequency sampling and several design modifications resulted in an approach which differed in some respects form the established approach for DEPM (Lasker, 1985). These differences will be emphasized herein.

Materials and Methods

Pearl Harbor was sampled on 1 day each week from 3 April 1986 to 7 April 1988. Each sampling day was separated into two periods: planktonic egg sampling and adult sampling. Eggs were sampled between 0900 and 1200 with a systematic sampling design (Cochran, 1963) in which a single sample was taken sequentially near the geographic center of each of 39 strata (Fig. 1). The net used measured 5 m long and 1 m in diameter at the mouth and was constructed of 335-μm Nitex. To allow its use from small boats, the net was not towed and instead was simply thrown overboard and allowed to sample as it descended to the bottom (average depth, about 12 m). Compared to a typical towed plankton net, our sampling net had two design features added to increase its effectiveness for this mode of operation. First, to increase the sinking speed, 5 kg of lead weights were attached to the steel ring that held the mouth open. Second, to ensure closure, the retrieval line was attached to a choke collar surrounding the net. Mesh bags of 335-μm Nitex were used as cod ends. When retrieved, these bags were sealed and placed in a 10% buffered formaldehyde solution.

In the laboratory, the plankton samples were examined without subsampling, and all nehu eggs were counted without developmental staging. Egg density at each station was estimated by dividing the egg count by an estimate of the filtered volume. Filtration volume was computed as water depth multiplied by the mouth area of the net.

Adult nehu were sampled in a 1.5-h period preceding sunset when the eggs to be spawned that evening were sufficiently hydrated to allow pre-spawning females to be unambiguously distinguished from non-spawning females (Clarke, 1987). Adults, along with juveniles, were captured by a beach seine with the same mesh size (9 mm) and design characteristics as a commercial nehu seine, except that it was cut to approximately one-third scale (70 m long by 3 m deep) so that it could be set from a small boat. Nehu schools were located in their daytime habitat, with the same searching techniques used by commercial vessels. When found, schools were quickly surrounded with the seine, and a random sample of the catch was taken and immediately preserved in a 10% buffered formaldehyde solution.

In the laboratory, a subsample of nehu was drawn from each field sample: 1) An initial subsample of approximately 50 fish was randomly chosen. 2) In order of size, starting with the apparently largest individual, fish were removed from the subsample and examined microscopically for sex and stage of maturity. 3) If less than 25 mature females were obtained from the initial subsample, additional subsamples were drawn and examined completely in the same manner until at least 25 mature females were obtained. 4) To increase the speed of classifying fish, if six juveniles were drawn in succession from a subsample, all of the remaining fish in that subsample were also classified as juveniles without examination.

The criteria used to classify fish were based upon the following gross morphological features partly adapted from Clarke (1987): 1) largest ova ≥0.7 mm long indicated a mature female with hydrated eggs; 2) largest ova 0.5 to 0.7 mm long indicated a mature female with unhydrated eggs; 3) testis depth (dorsal-ventral) greater than or equal to eye diameter indicated a mature male; 4) largest ova <0.5 mm long or testis depth of less than eye diameter indicated a juvenile.

After the subsample was chosen and classified, the following biological attributes were measured. Standard length (in mm) was measured for all mature fish and for the first 25 randomly chosen juveniles; any remaining juveniles were counted but not measured. All fish measured for length (except females with hydrating eggs) were measured for total body weight (in mg) after being blotted dry; ovary-free body weight was measured for all females. Batch fecundity was estimated for females with hydrating eggs by teasing both ovaries apart and counting under a microscope all hydrating ova. Each count was replicated, and fecundity was estimated as the mean of the two counts. Over the 105 consecutive weeks of sampling, this process was repeated for each of 162 seine samples.

Samples of nehu were also obtained from commercial vessels baitfish fishing in Pearl Harbor during the study period. The methods of collecting these samples varied because the samples were taken by the fishermen themselves, but the general procedure consisted of scooping 100 to 200 fish from the bait well and temporarily preserving them with salt and ice until fishing was completed and they could be frozen. Frozen fish were later fixed in a 10% buffered formaldehyde solution. In the laboratory, up to 100 of the fish were randomly removed from each sample and measured for standard length (in mm). A total of 36 commercial samples was collected.

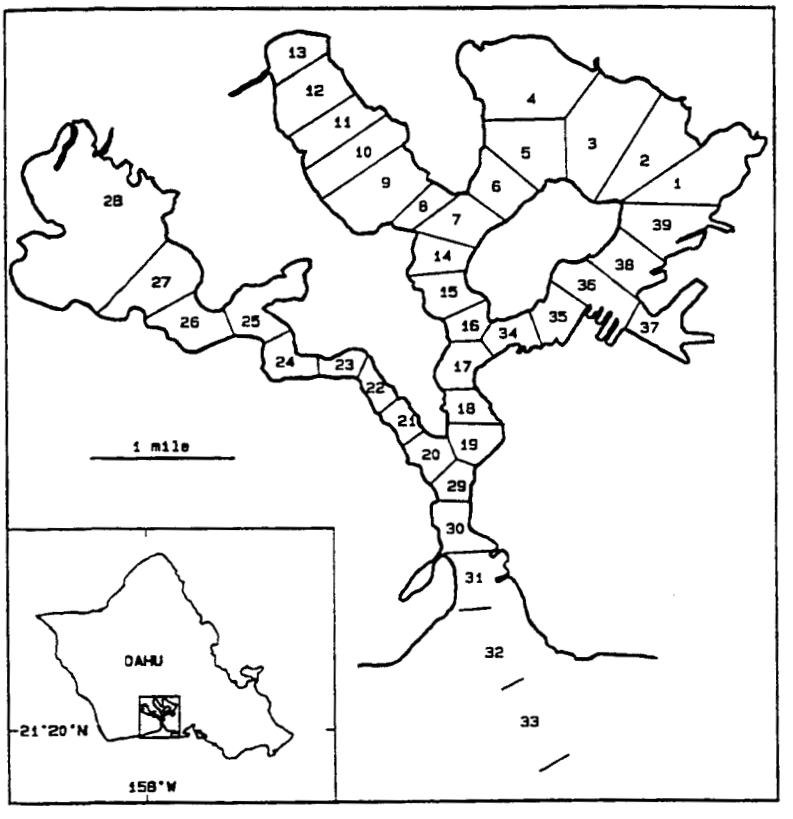

Figure 1. Locations of the 39 sampling sites in Pearl Harbor, Oahu.

Data Analysis and Weekly Biomass Estimation.—Evaluation of Equation 1 requires estimates of P, F, and R. These estimates were obtained as follows. Daily egg production was estimated as

$$P = \sum D_i V_i \tag{2}$$

where D_i is the estimated egg density (eggs per volume filtered) and V_i is the volume of the ith stratum. Strata volumes were estimated as the surface areas measured on a navigation chart times the average sounding depth. The summation is over all 39 strata. Relative fecundity was estimated from each sample as

$$F = \sum E \Big/ \sum W_{fh} \tag{3}$$

where ΣE is the sum of all hydrating ova, and ΣW_{fh} is the sum of the corrected total body weight of mature females with hydrating eggs. Spawning proportion was estimated from each sample as

$$R = \sum W_{fh} \Big/ \left(\sum W_m + \sum W_{fh} + \sum W_{fu} \right) \tag{4}$$

where ΣW_m and ΣW_{fu} are the sums of the total body weights of males and of females with unhydrated eggs. The W_{fh} values were corrected for the temporary weight gain due to egg hydration by regression W_{fu} on ovary-free weight for females with unhydrated ovaries, then predicting W_{fh} from the ovary-free body weights of females with hydrated ovaries.

Since the commercial catch includes juveniles, the commercial population will be larger than the spawning population. To estimate the size of the commercial population, the methods used for research sampling were assumed to be so similar to those used by the fishery that the same size ranges were captured. Therefore when the commercial, rather than the spawning, population was estimated, Equa-

tion 4 was modified slightly to include in the denominator the term ΣW_j or the sum of the total body weights of juveniles.

Variance and bias of the estimated biomass for each week were approximated by using the delta method (Seber, 1973; Parker, 1980). The variance estimator was

$$\text{Var}(B) = B^2(CV(P)^2 + CV(F)^2 + CV(R)^2 - 2\text{Cov}(P, F)/PF - 2\text{Cov}(P, R)/PR + 2\text{Cov}(F, R)/FR) \quad (5)$$

and the bias estimator was

$$\text{bias}(B) = B(CV(F)^2 + CV(R)^2 + \text{Cov}(F, R)/FR) \quad (6)$$

where CV represents the coefficient of variation, and Cov represents the covariance. For both the variance and bias equations, the covariance terms were considered negligible and therefore were omitted.

The variances needed to evaluate Equations 5 and 6 were estimated as follows. The variance of P was estimated as

$$\text{Var}(P) = \sum V_i^2 \text{Var}(D_i) \quad (7)$$

where $\text{Var}(D_i)$ was approximated as the variance in D_i among three adjacent stations, because the plankton net samples were not replicated at each station. The variances of R and F were estimated as

$$\text{Var}(\bar{X}) = \sum (X_i - \bar{X})^2/N(N - 1) \quad (8)$$

where X_i is the ith estimated value of R or F, and $\bar{X}$ is the mean of N values of R or F. Since the number of samples collected each week was always small and sometimes even insufficient to estimate variance, the weekly estimates of the means and variances of the biological parameters were calculated from a pool of all samples within a 5-week moving time window centered on a particular week. Weekly estimates of egg production, which were based on much larger sample sizes, were not smoothed in this manner.

To test whether the nehu length distribution of the research catches differed from that of the commercial catches, the following three steps were taken. First, all commercial length samples and all research length samples taken within each week were pooled. Second, equality of the commercial and research length-frequency distributions for each week was tested by a two-tailed Smirnov test (Conover, 1971). Third, the probabilities associated with the individual weekly tests were combined over all sampling weeks using procedures described in Sokol and Rohlf (1981: 779).

To test whether nehu school according to sex and maturity stage, the number of pre-spawning females in each seine sample was compared to the number of non-spawning females, the number of males was compared to the number of females, and the number of mature fish was compared to the number of juveniles. In each of three cases, a test was made by using a chi-square test of heterogeneity (Sokol and Rohlf, 1981) over all 162 adult samples. To minimize the effect of temporal changes in spawning proportion or sex ratio on the test, expected values were computed from the monthly means rather than the overall mean.

Results and Discussion

Weekly estimates of spawning and commercial biomass (Fig. 2) had similar patterns of variation over time, but commercial biomasses were considerably larger and had wider confidence intervals. The three components of the biomass estimates (i.e., daily egg production (P), proportion spawning (R), and relative fecundity (F) also displayed patterns in their temporal variation (Fig. 3). For P, the pattern is similar to that of biomass and indicates that the biomass estimates are primarily determined by egg production. For R and F, the pattern is largely seasonal although R also displays a shorter period of variation as well as a linear trend. Examined below are aspects of the sampling design that could have led to possible uncorrected bias in these estimates or were strongly influential in determining precision.

Bias in the biomass estimates is a function of bias in P, R, and F in addition to the bias from Equation 6. Egg production could be influenced by three potential sources of bias. First, egg production could be overestimated if the counts of nehu eggs included eggs of other species or included nehu eggs spawned on other nights.

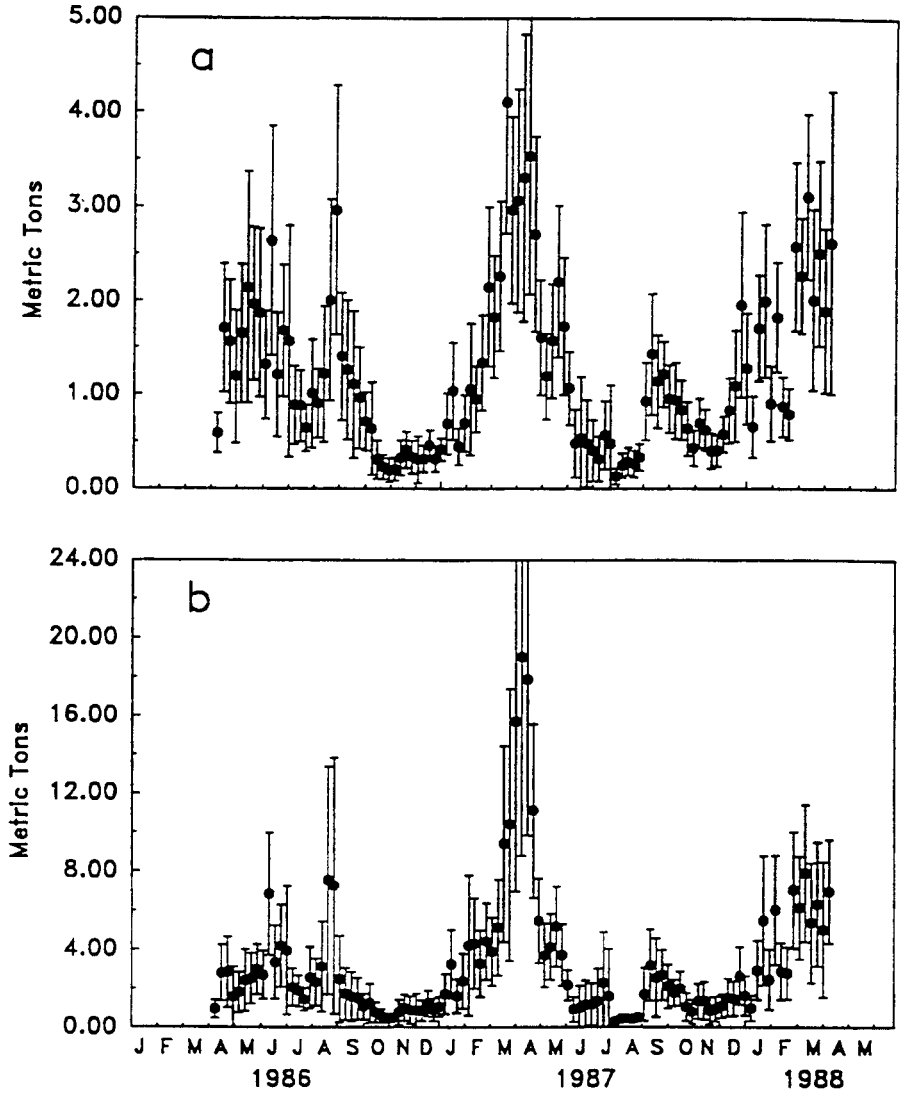

Figure 2. Weekly estimates and 95% confidence intervals of the (a) spawning and (b) commercial biomasses of nehu within Pearl Harbor.

Misidentification, however, is unlikely because no other fishes in Pearl Harbor have eggs similar to those of nehu (Tester, 1951; Clarke, 1987), and inclusion of multiple cohorts in the egg counts is unlikely because nehu eggs usually hatch within 24 h (Clarke, 1987). Second, egg production could be underestimated if egg mortality over the period between spawning and sampling is actually large. Unfortunately, our sampling design was insufficient to allow estimation of egg mortality, and likely values must be estimated indirectly. If the egg mortality rate

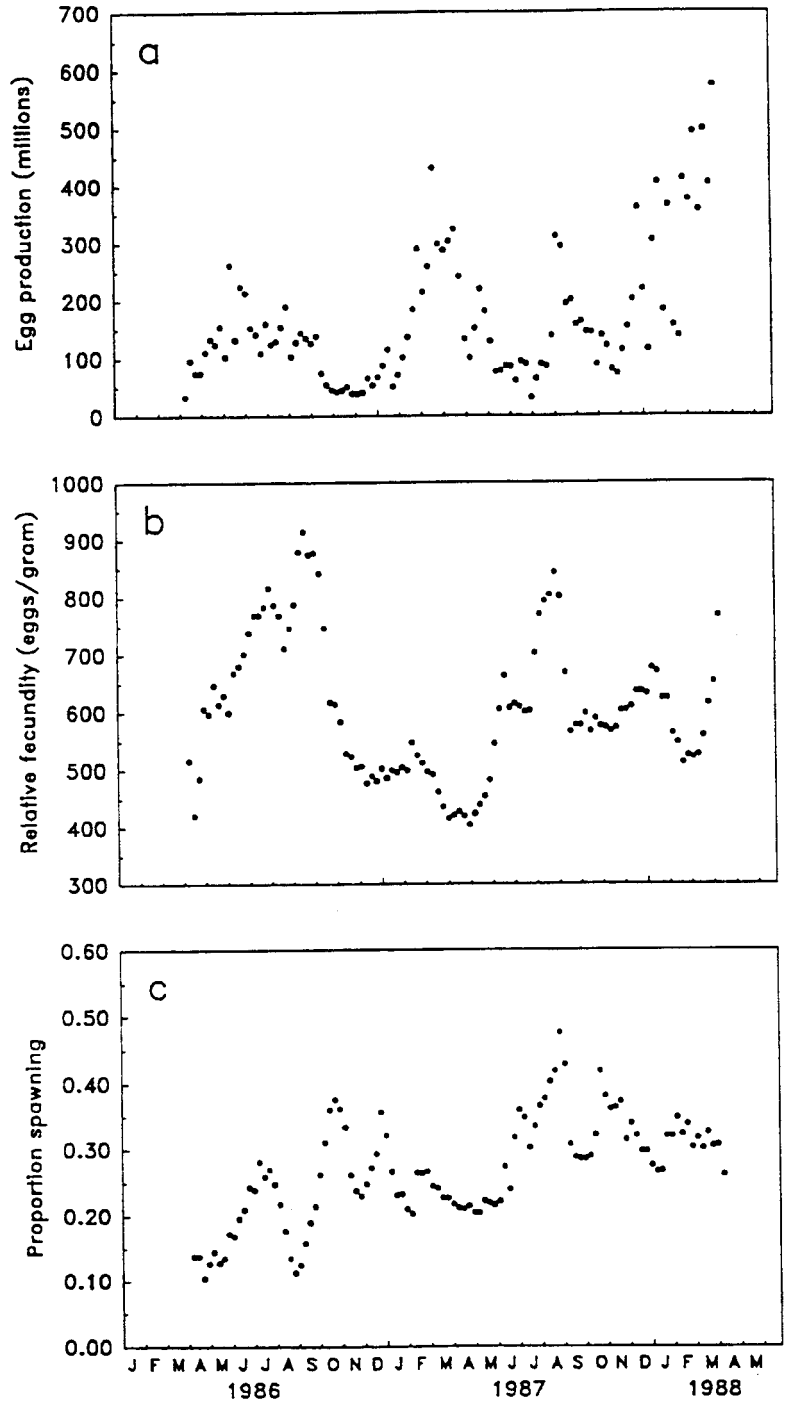

Figure 3. Weekly estimates of (a) daily egg production, (b) relative fecundity, and (c) proportion, by weight, of spawning females in the spawning population.

is the same as it is for northern anchovy (25% per day; Lo, 1986), then approximately 15% of nehu eggs would die during the 16-h period between spawning and sampling (nehu spawn in a 2-h period after sunset; Clarke, 1987). However egg mortality may be considerably less than this because 68% of northern anchovy egg mortality is due to egg cannibalism (based on data from Hunter and Kimbrell, 1980) and nehu rarely cannibalize their eggs (Hiatt, 1951).

Third, egg production estimates could be biased because of a corresponding bias in the estimates of strata volumes. Unlike previous studies on oceanic anchovies (e.g., Picquelle and Stauffer, 1985), egg density was specified in terms of water volume rather than surface area, because nehu eggs are distributed throughout the water column and water depth varies within Pearl Harbor. Strata volumes were measured crudely using a navigation chart and could be considerably biased.

Proportion spawning and relative fecundity are likewise subject to several types of bias. Proportion spawning could be underestimated if pre-spawning females were misidentified. Since pre-spawning females were identified by egg size, sampling had to be restricted to a time of day that the hydrating ova were sufficiently enlarged to be unambiguously distinguishable from unhydrated ova. Previous research (Clarke, 1987) indicated that ova hydrate during the later afternoon, but not until 2 h before sunset do all pre-spawning females have some enlarged (≥ 0.7 mm) ova. Because of the importance of correct timing, we repeated part of Clarke's (1987) study by sampling nehu at approximately half-hour intervals in the late afternoon. On the basis of these samples, we shortened the sampling period to 1.5 h before sunset to ensure correct identification. Although we believe that nearly all pre-spawning females were correctly identified, some of the hydrating ova within these females may not have been recognized because of size variability among hydrating ova in the early part of the period. If so, an occasional underestimate of relative fecundity could have resulted.

Proportion spawning could be biased if pre-spawning and non-spawning females segregate spatially and become differentially available to the sampling gear. For anchoveta (Alheit, 1985) and northern anchovy (Picquelle and Stauffer, 1985), pre-spawning females near the time of spawning segregate from non-spawning females, aggregate with males, and have a higher vulnerability to the sampling gear, perhaps because of a difference either in net avoidance or in depth distribution. Evidence that nehu were similarly segregated during the sampling period was provided by the chi-square tests of heterogeneity in the relative abundance of pre-spawning females to non-spawning females, females to males, and adults to juveniles, which were all highly significant ($P < 0.001$). Although such segregation increases biological heterogeneity among schools and thereby increases the variance in the estimates of proportion spawning, it leads to bias only when the segregated schools are differentially vulnerable. Pre-spawning and non-spawning nehu, however, are apparently equally vulnerable during the sampling period because our overall estimate of the mean proportion spawning based on numbers of mature females, rather than population biomass (0.53), was not significantly different from Clarke's (1987) estimate of 0.50 based on postovulatory follicles and estimated rates of ova development from fish sampled at all times of the day.

Proportion spawning for the commercial population (Fig. 3c) could be biased if the size distribution of fish in the research samples differed from that in the commercial samples. Initially we believed that sampling nehu with the same mesh size as a commercial net ensured catching the same size range of fish. Since mesh size may not be the sole determinant of size selection with seines, we later became concerned that the longer, deeper seines used by the commercial fishery allowed the catch of larger fish. However, the length distributions of the nehu in com-

Table 1. The relative contribution of the error in each of three parameters—P (daily production of eggs by the population), R (proportion, by weight, of spawning females in the population), and F (batch fecundity per gram body weight of spawning females)—to the error in the biomass estimate. Unsmoothed estimates of CV^2 for R and F are averages over the 52 weeks having at least two fish samples. The largest covariance term in Equation 5 (Cov(F, R)/FR) is also shown for the smoothed estimates

	$CV(P)^2$	$CV(R)^2$	$CV(F)^2$	Cov(F, R)/FR
No smoothing				
Spawning biomass	0.032	0.081	0.014	
Commercial biomass	0.032	0.218	0.014	
Smoothing				
Spawning biomass	0.032	0.030	0.007	<0.001
Commercial biomass	0.032	0.066	0.007	0.015

mercial and research samples were not significantly different ($P > 0.05$), and sampling was therefore unbiased.

Variance of the biomass estimates is a function of the variances of P, R, and F. One convenient way of examining how the precision of each parameter contributes to the precision of the biomass estimate is to express precision in terms of CV^2 (Equation 5). When expressed in this way, the average of the weekly estimates of $CV(R)^2$ is considerably larger than $CV(F)^2$ and $CV(P)^2$ (Table 1), especially when the estimate of R includes the weight of juveniles, as it does in the calculation of commercial biomass. There are two reasons why precision in the biomass estimates is dominated by precision in the estimates of spawning proportion. First, the restricted period of fish sampling—bounded between the times of day when hydrating ova became clearly recognizable and when nehu leave the shallow nearshore area to begin spawning—was so short that few samples could be collected (an average of only 1.6 samples collected each week). Second, sampling occurred when pre-spawning and non-spawning fish were segregating in preparation for the evening spawning, and such segregation increases the between-sample variability in proportion spawning.

The covariance between P, F, and R was assumed to be negligible and therefore omitted from the estimated variance of the biomass estimates. As a check on the adequacy of this assumption, the covariance term for F and R (the largest of the three possible terms) was computed for the smoothed estimates of spawning and commercial biomasses (Table 1). For spawning biomass, the covariance term was less than 1% of the sum of the variance terms. For commercial biomass, however, the covariance term was about 14% of the sum of the variance terms and was not negligible. The larger covariance was due to the inclusion of juvenile weights in the computation of P as well as the variation in F with body size. Thus, when a school of small nehu is sampled, R will tend to be low because of the relatively high abundance of juveniles, and F will tend to be low because of the small size of mature females.

To obtain less variable estimates of the parameters and to fill gaps in the time series when insufficient samples were collected (about one-half of the weeks lacked the minimum two samples needed to calculate variance), proportion spawning and relative fecundity were estimated from all samples taken within a running 5-week interval centered on a specified week. Intervals of other widths (3, 7, 9, 11 weeks) were also examined, but 5 weeks was chosen as the best because the sum of the $CV(R)^2$ and $CV(F)^2$ terms was approximately equal to $CV(P)^2$. If proportion spawning and relative fecundity varied slowly with time, then such a

smoothing procedure effectively increases sample size and thereby reduces variance of the estimates. Variation in response to short term events, however, would be lost.

One conspicuous difference between this study and previous applications of the DEPM is the spatial and temporal scales that were employed in the sampling design. For example, applications on anchoveta (Alheit, 1985) and northern anchovy (Lasker, 1985) considered populations distributed over thousands of square kilometers, whereas the nehu population was constrained to an enclosed area of < 10 km^2 that could be sampled in a few hours from a small boat. This difference in spatial scale, and the difference in the rate of biological turnover, dictated the time scales of sampling; thus, nehu were sampled at weekly intervals. Frequent assessment, however, also required rapid processing and analysis of the field samples; this, in turn, required several modifications to the sampling procedures used in previous applications of the DEPM.

The most important modification was the use of hydrated ova rather than postovulatory follicles to identify spawning females. Although this approach was faster and easier because histological preparation was not required, it restricted sampling to a period that occurred when schools were segregating in preparation for spawning and was so brief that few samples could be collected. As a consequence, the variance of the weekly estimates of the reproductive parameters was sometimes not calculable and was always quite large. Temporal smoothing reduced these problems, but also reduced the independence of the weekly estimates of biomass. Regardless of this problem, the results of this study demonstrate that the DEPM can be successfully employed using small boats and hand-operated sampling gear to assess the biomass of small tropical species.

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