**Abstract.**–The potential annual fecundity of Dover sole becomes fixed before the spawning season when the average diameter of the advanced stock of yolked oocytes exceeds 0.86 mm; hence potential annual fecundity is determinate. More central California females had atretic advanced oocytes than Oregon females, but rates of atresia were not sufficiently high to have an important effect on the potential annual fecundity of the population. A 1-kg female matured about 83,000 advanced yolked oocytes at the beginning of the season. Vitellogenesis continued for the advanced yolked oocytes during most of the spawning season while batches were repetitively matured and spawned. About nine batches were spawned over a six-month spawning season (December–May), and spawning ceased when the standing stock of advanced oocytes was exhausted. A 1-kg female released about 10,000 eggs per spawning, except for the first and last batches which were smaller than the rest. Near the end of the season, females may spawn more frequently than earlier in the year, increasing the daily production of eggs by the population even though fewer females are reproductively active. Annual reproductive effort of Dover sole was equivalent to about 14% of body wet weight per year. Fifty percent of the females had become sexually mature when they reached 332 mm total length.

Various methodological issues were also treated in this paper, including validation of key assumptions underlying estimates of annual fecundity; fecundity sample-size requirements; evaluation of criteria and bias in estimating female sexual maturity; and comparisons of classification by histology and gross anatomy.

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# Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision

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Fecundity and sexual maturity estimates are staples of fishery science. Inevitably, they will be estimated for every species of economic consequence because of their importance in the dynamics of the population. A second reason for studying fecundity is that when fecundity estimates are combined with estimates of the abundance of eggs in the sea, they can be used to estimate the biomass of a stock. Our laboratory is currently evaluating such ichthyoplankton methods for estimating the biomass of Dover sole *Microstomus pacificus*, a large demersal resource occurring along the upper continental slope of the west coast of North America. The fecundity of Dover sole from Oregon has been estimated (Yoklavich and Pikitch 1989), but no estimate exists for the segment of the stock living in central California waters, nor have the assumptions underlying fecundity and sexual maturity assessments been studied with the thoroughness necessary for accurate estimates of adult biomass. Thorough analysis of these assumptions is usually lacking in the fecundity literature.

Our objectives were to describe the reproduction of Dover sole off central California and Oregon, and evaluate the assumptions underlying fecundity and sexual maturity estimates. We describe changes in the reproductive state of female Dover sole during the spawning season, estimate annual fecundity, batch fecundity, rates of atresia, annual rates of spawning, and length at 50% mature ($ML_{50}$).

Evaluation of the assumptions underlying annual fecundity estimates requires defining six fecundity terms, and those underlying maturity estimates require defining four terms for reproductive state.

## Fecundity

**Annual fecundity** Total number of eggs spawned by a female per year.

**Total fecundity** Standing stock of advanced yolked oocytes.

**Potential annual fecundity** Total advanced yolked oocytes matured per year, uncorrected for atretic losses. In species with determinate fecundity, potential annual fecundity is considered to be equivalent to the total fecundity prior to the onset of spawning.

**Determinate fecundity** Annual fecundity is determinate when the potential annual fecundity becomes fixed prior to the onset of spawning. In fishes with determinate fecundity, total fecundity decreases with each

spawning because the standing stock of advanced yolked oocytes is not replaced during the spawning season.

**Indeterminate annual fecundity** Annual fecundity is indeterminate when the potential annual fecundity of a female is not fixed prior to the onset of spawning and unyolked oocytes continue to be matured and spawned during the spawning season.

**Batch fecundity** Number of hydrated oocytes released in one spawning; usually determined by counting the number of hydrated oocytes in the ovary.

**Relative fecundity** Fecundity divided by female weight.

### Reproductive states

**Active** Females capable of spawning at the time of capture or in the near future (by the end of the survey or of a season, or other temporal end point). Ovaries of active females contain sufficient number of yolked oocytes for a spawning.

**Inactive** Females not capable of spawning at the time of capture nor in the near future, although some may have been mature in the past.

**Mature** Females that have spawned in the current reproductive season or can be expected to do so.

**Immature** Females that have not spawned in the current reproductive season nor can be expected to do so.

The central methodological issue in fishes with determinate fecundity (Hunter and Macewicz 1985a, Horwood and Greer Walker 1990) is to establish that potential annual fecundity is an unbiased estimate of annual fecundity. For this to be true in Dover sole requires four key assumptions. The first and most important assumption is that fecundity is determinate in Dover sole. This means that potential annual fecundity becomes fixed before spawning begins. Estimation of the standing stock of advanced oocytes (total fecundity) is meaningless if, during the spawning season, oocytes are added to that stock.

The second assumption is that the potential annual fecundity is equivalent to annual fecundity. Strictly speaking, this probably never happens because in any fish population some of the females resorb some of their advanced yolked oocytes rather than spawn them, a process known as atresia. If many females resorbed many of their advanced oocytes, potential annual fecundity would be a serious overestimate of annual fecundity in the population. In addition, not all ovulated oocytes are spawned; a few remain in the ovigerous folds of the ovary after spawning and are later resorbed. Retention of ovulated oocytes is probably seldom a serious bias.

The third assumption is that the females used to estimate potential annual fecundity have not spawned during the current reproductive season. Dover sole females that have spawned some of their stock of advanced oocytes cannot always be distinguished from those that have not begun spawning. Inclusion of partially spawned females in an estimate of potential annual fecundity of the population could be a significant bias.

The fourth assumption is that one is able to identify with certainty the oocytes that constitute the potential annual fecundity. An ovary may not be sufficiently developed to identify all of the oocytes destined to be spawned. On the other hand, if the ovary is highly advanced, spawning may have begun and some advanced oocytes lost. Clearly, an optimal range of ovarian development exists where these risks are minimized.

In addition to evaluating the above four assumptions (determinate fecundity, atresia, spawning, and immaturity) we consider several other methodological issues related to assessment of fecundity and female sexual maturity. These issues are (1) validation of our gross anatomical and histological classification of ovaries into active or inactive and mature or immature states; (2) four precision issues related to total fecundity estimates (number of tissue samples per ovary, number of females, location of ovarian tissue samples, and within-trawl and between-trawl variance); and (3) an evaluation of bias in the assessment of female sexual maturity.

## Methods

### Collections and shipboard measurements

Dover sole were collected along the central California coast (Point Conception to San Francisco Bay) during six research trawl cruises (Table 1). Dover sole were taken off the Oregon coast between Cape Lookout and Heceta Head during two cruises in 1988–89; miscellaneous collections provided by E. Pikitch off the Oregon coast in 1985 and 1986 were also used. Research trawls were one-half hour or one hour long, depending on depth. In central California waters, we used a 400-mesh Eastern trawl (mouth opening ~15 m wide and 1.5 m high; Wathne 1977). In Oregon waters, either an Alaska Fisheries Science Center (AFSC) modified 5-inch mesh, 90/120, high-rise "poly Nor'Eastern" trawl (fishing dimensions ~4.6 m high and 13.5 m wide at wing tips), a 5-inch mesh, 92/83, poly Nor'Eastern trawl, or a 5½-inch mesh, 75/90, high-rise Aberdeen trawl was used. Up to 100 Dover sole from

**Table 1**
Sources of reproductive data on female Dover sole *Microstomus pacificus*. Number of specimens in three levels of ovarian analysis and number of level-3 females with batch fecundity estimates.

| Sampling protocol | | | | Levels of ovarian analysis** | | | | Batch fecundity |
|---|---|---|---|---|---|---|---|---|
| Date (Begin/End) | State | No. positive trawl collections | Selection of females* | 1 | 2 | 3 | Total | |
| | | | | (no. females) | | | | |
| 3 Dec 85<br>12 Dec 85 | CA | 11 | A | — | 39 | 65 | 104 | — |
| 4 Nov 85<br>14 Dec 85 | OR | 4 | Unknown | — | 73 | — | 73 | — |
| 6 Feb 86<br>7 Feb 86 | OR | 3 | Unknown | — | 37 | — | 37 | — |
| 3 May 86 | OR | 2 | Unknown | — | 27 | — | 34 | 7 |
| 5 Mar 86<br>7 Mar 86 | CA | 8 | A | 1 | 135 | 3 | 139 | — |
| 2 May 86<br>4 May 86 | CA | 3 | A | — | 59 | 1 | 60 | — |
| 11 Jan 87<br>24 Jan 87 | CA | 27 | B | 45 | 387 | 103 | 535 | — |
| 5 Feb 87<br>15 Feb 87 | CA | 22 | B | 14 | 391 | 92 | 500 | 3 |
| 23 Feb 88<br>9 Apr 88 | CA | 51 | C | 1716 | 120 | 62 | 1941 | 43 |
| 28 Nov 88<br>14 Dec 88 | OR | 53 | C | 667 | 620 | 152 | 1439 | — |
| 21 Feb 89<br>31 Mar 89 | OR | 21 | C | 104 | 151 | 34 | 292 | 3 |
| All Oregon | | 83 | | 771 | 908 | 186 | 1875 | 10 |
| All California | | 122 | | 1776 | 1131 | 326 | 3279 | 46*** |
| Oregon + California | | 205 | | 2547 | 2039 | 512 | 5154 | 56*** |

*A = Random selection of both females and males until 25 females were collected.
B = Selection stratified by length (*N* 5) in <275 mm class, 10 in 275–424 mm class, and 10 in ≥425 mm.
C = Random selection of ≤100 fish (either females or males).
**Level 1 = gross anatomical; Level 2 = histological with anatomical; Level 3 = total fecundity with anatomical and histological.
***Five females with hydrated oocytes provided by W.W. Wakefield were included in estimate of batch fecundity.

each trawl haul were measured (total length) to the nearest millimeter, sexed, and their gonads classified; some females immediately after capture were also individually weighed to the nearest gram and their ovaries preserved in 10% neutral buffered formalin. Females selected for ovarian preservation were either taken randomly from the trawl catch or selected by length according to a quota for each of three length classes (<275 mm, 275–424 mm, and ≥425 mm) (see Table 1). The preserved ovaries were used to validate our shipboard classification of ovaries, to estimate fecundity, and to provide material for histological descriptions.

## Gross anatomical classification of ovaries

Ovaries that were examined onboard the ship were assigned to one of three classes: no yolked oocytes present; yolked oocytes present; and translucent hydrated oocytes present. Ovaries with hydrated oocytes or other yolked oocytes were considered to be in the active state, while those ovaries in which observers saw no yolked oocytes were considered to be in the inactive state. This simple system based on gross anatomical examination of the ovary is more germane for biomass estimation work than are the more complicated systems which involve many more reproductive stages: for example, the seven-stage scale of Hjort (1910), or the five-stage scale of Hagerman

(1952). Eighty percent of the females that we classified using gross anatomical criteria were also classified as active or inactive using histological criteria. The results were compared to determine the accuracy of identifying active and inactive females by gross anatomical classification.

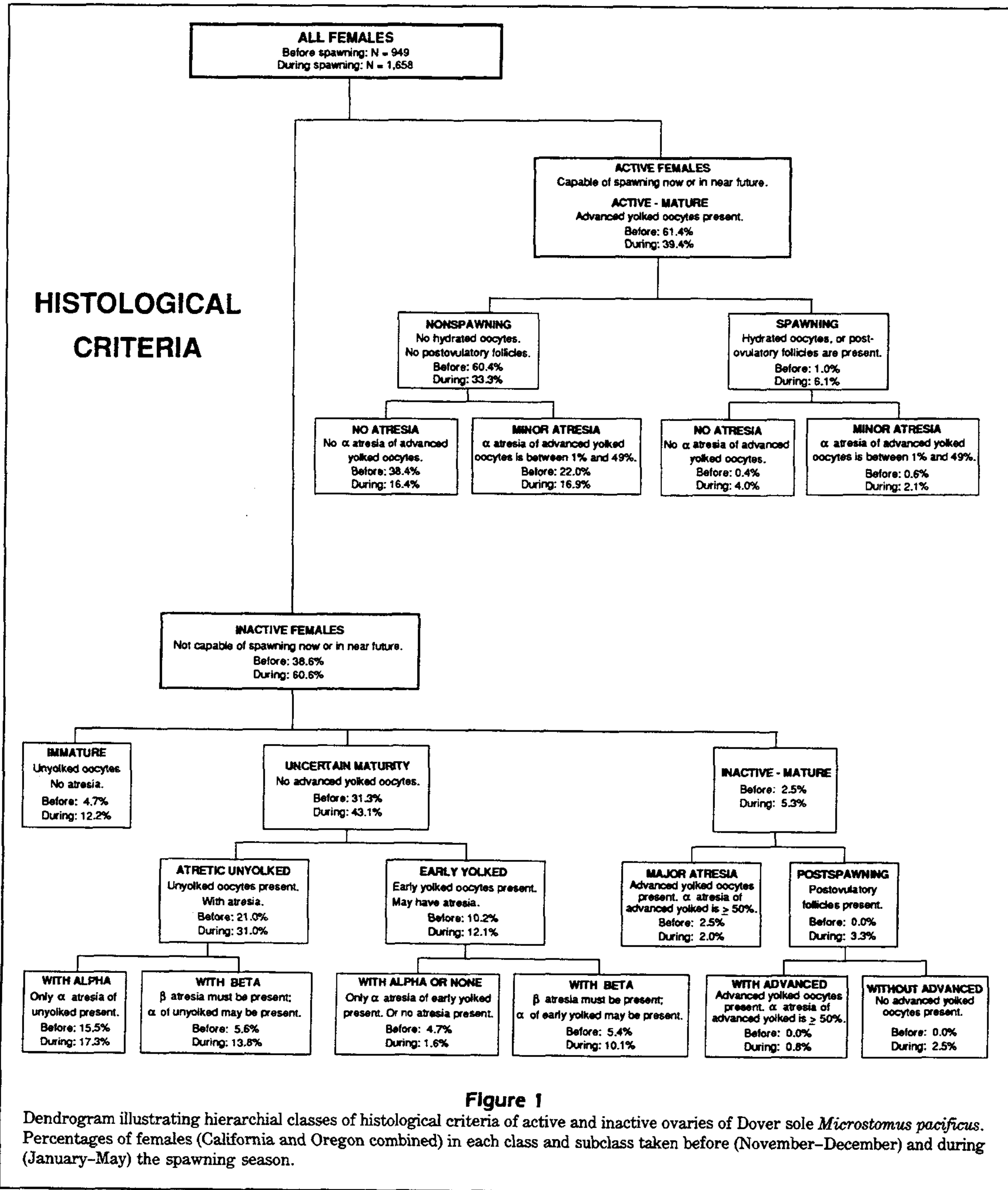



**Figure 1**

Dendrogram illustrating hierarchial classes of histological criteria of active and inactive ovaries of Dover sole *Microstomus pacificus*. Percentages of females (California and Oregon combined) in each class and subclass taken before (November–December) and during (January–May) the spawning season.

### Histological methods

All of the preserved ovaries, regardless of development, had a piece removed for histological analysis. The pieces were dehydrated and then embedded in Paraplast. Subsequently histological sections were cut at 5–6 μm and stained with Harris hematoxylin followed by eosin counterstain (H&E). Each ovary was classified histologically in the manner developed for northern anchovy *Engraulis mordax* by Hunter and Goldberg (1980) and Hunter and Macewicz (1980, 1985ab), with a few modifications appropriate for Dover sole ovarian structure. In the ovary we identified the presence or absence of the following: oocytes that have not begun vitellogenesis; oocytes in the first vitellogenic stages (0.15–0.55 mm diameter); advanced yolked oocytes (0.47–1.4 mm diameter) noting any stages of nucleus migration (precursor to hydration); hydrated oocytes; two stages of postovulatory follicles; and the different stages of atresia. The rate at which postovulatory follicles are resorbed in Dover sole is unknown. Hence no ages were assigned to postovulatory follicles.

### Histological classification

We used histological analysis of the ovaries to assess the accuracy of our gross anatomical classification into active and inactive states, to define the optimal criteria for distinguishing mature from immature females, and to calculate various indices of spawning activity and postspawning states. The dendrogram (Fig. 1) indicates the histological characteristics used to classify ovaries into active and inactive states. The dendrogram also gives the frequency of the classes in each state for the prespawning period (November–December) and for the spawning season (January–May) using combined data from California and Oregon. The data are also given by cruise and region in Table 2.

Females were classed as active when histological analysis indicated that the ovary contained the sufficient number of advanced yolked oocytes for one spawning. Active females were then separated into spawning and nonspawning classes using additional histological criteria. Spawning females were those which showed histological evidence of past spawning

**Table 2**

Numbers of female Dover sole *Microstomus pacificus* in various histological subclasses. Listed by location, before or during the spawning season, and mean cruise date (year and month).

| | Inactive | | | | | | | | | | | | Active | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Immature | Uncertain maturity | | | | | | | Mature | | | | Mature | | | | |
| | | Atretic unyolked | | | Early yolked | | | | Major atresia | Postspawning | | | Nonspawning | | Spawning | | |
| | | Atresia present | | | No atresia | Atresia present | | | | Advanced yolk | | | No atresia | Minor atresia | No atresia | Minor atresia | |
| Cruise | | α only | β only | α and β | | α only | β only | α and β | | With | Without | | | | | | All females |
| **Oregon** | | | | | | | | | | | | | | | | | |
| Before | | | | | | | | | | | | | | | | | |
| 8512 | 1 | 4 | 0 | 9 | 0 | 0 | 2 | 9 | 5 | 0 | 0 | | 20 | 23 | 0 | 0 | 73 |
| 8812 | 32 | 133 | 2 | 37 | 5 | 37 | 4 | 34 | 15 | 0 | 0 | | 316 | 150 | 3 | 4 | 772 |
| During | | | | | | | | | | | | | | | | | |
| 8602 | 0 | 1 | 0 | 9 | 0 | 0 | 2 | 3 | 4 | 0 | 0 | | 6 | 12 | 0 | 0 | 37 |
| 8903 | 23 | 70 | 2 | 16 | 1 | 3 | 7 | 18 | 2 | 1 | 3 | | 24 | 14 | 3 | 1 | 188 |
| 8605 | 1 | 0 | 3 | 4 | 0 | 0 | 5 | 3 | 0 | 4 | 2 | | 0 | 0 | 9 | 3 | 34 |
| Total | 57 | 208 | 7 | 75 | 6 | 40 | 20 | 67 | 26 | 5 | 5 | | 366 | 199 | 15 | 8 | 1104 |
| **California** | | | | | | | | | | | | | | | | | |
| Before | | | | | | | | | | | | | | | | | |
| 8512 | 12 | 10 | 1 | 4 | 0 | 3 | 0 | 3 | 4 | 0 | 0 | | 28 | 36 | 1 | 2 | 104 |
| During | | | | | | | | | | | | | | | | | |
| 8701 | 56 | 65 | 11 | 32 | 0 | 7 | 3 | 37 | 11 | 0 | 0 | | 115 | 138 | 6 | 9 | 490 |
| 8702 | 43 | 99 | 8 | 70 | 2 | 11 | 7 | 36 | 10 | 0 | 1 | | 91 | 86 | 12 | 10 | 486 |
| 8603 | 38 | 24 | 8 | 40 | 0 | 1 | 7 | 4 | 3 | 3 | 7 | | 1 | 2 | 0 | 0 | 138 |
| 8803 | 30 | 18 | 4 | 11 | 0 | 4 | 14 | 17 | 3 | 4 | 15 | | 31 | 28 | 35 | 11 | 225 |
| 8605 | 12 | 9 | 3 | 7 | 2 | 1 | 5 | 0 | 0 | 1 | 13 | | 4 | 0 | 2 | 1 | 60 |
| Total | 191 | 225 | 35 | 164 | 4 | 27 | 36 | 97 | 31 | 8 | 36 | | 270 | 290 | 56 | 33 | 1503 |

(postovulatory follicles present) or imminent spawning (hydrated oocytes or migratory nucleus-stage oocytes present), while the ovaries of nonspawning females showed no evidence of recent or imminent spawning but were capable of spawning in the near future. The fraction of active females classed as spawning was used as a spawning rate index. Spawning performance was also assessed by calculating the mean number of spawning states (postovulatory follicles, hydrated oocytes, migratory nucleus) per female in the spawning class.

Females with ovaries classified as active are considered mature. On the other hand, females with inactive ovaries could be either immature or mature because an ovary may have regressed to an inactive state after the female had attained sexual maturity. We designed our histological classification of inactive ovaries to distinguish as best as possible between mature and immature conditions. Inactive females were grouped into three classes (Fig. 1): immature, uncertain maturity, and inactive-mature. The inactive-mature class included ovaries showing clear histological evidence of past spawning (postspawning subclass) or past maturation of advanced yolked oocytes (major-atresia subclass). Postspawning ovaries contained either postovulatory follicles and no advanced yolked oocytes or postovulatory follicles and mostly atretic advanced yolked oocytes. The fraction of inactive females identified as postspawning was used as an index of the rate at which females passed from the active to the inactive state during the spawning season.

The five major histological classes of active and inactive females were subdivided into atretic subclasses using the first ($\alpha$) and second ($\beta$) stages of resorption as defined by Bretschneider and Duyvene de Wit (1947) and Lambert (1970). One can identify the developmental stage of the oocyte only during the $\alpha$ stage of atresia because the oocyte is completely absorbed by the end of this stage. Subsequent stages ($\beta$, $\gamma$ and $\delta$) involve the resorption of the follicle. Thus, $\alpha$ atresia is of key importance to fecundity studies since the oocyte class can be identified. Subsequent stages may be useful for identifying past spawning activity.

For ovaries containing early yolked or only unyolked oocytes, classification was based solely on presence or absence of the following atresia: $\beta$, $\alpha$ of unyolked oocytes, and $\alpha$ of early yolked oocytes (classes immature and uncertain maturity of the inactive females) (Fig. 1).

Ovaries with advanced yolked oocytes were subdivided into two atretic subgroups using the extent of the $\alpha$ atresia of the advanced yolked oocytes: minor atresia, i.e., females with one oocyte to 49% of their advanced yolked oocytes in $\alpha$; and major atresia, i.e., 50% or more of the advanced yolked oocytes in $\alpha$. We showed in anchovy that the probability of spawning was very low when more than 50% of the advanced oocytes were atretic (Hunter and Macewicz 1985b). Therefore, ovaries with major atresia of advanced yolked oocytes were considered inactive (inactive-mature class) although the ovary contained some advanced yolked oocytes.

### Estimation of total fecundity

We used the gravimetric method to estimate total fecundity of Dover sole. Total fecundity ($Y_F$) was the standing stock of advanced yolked oocytes in the ovary: $Y_F = Z \cdot C$, where Z is the ovary weight in grams, and C is oocyte density (number of advanced yolked oocytes per gram of ovarian tissue). We also measured diameters of 30 of the advanced yolked oocytes in at least one of the 2–5 tissue samples analyzed for each female for which fecundity was estimated. Advanced yolked oocytes were identified, counted, and measured using a digitizer linked by a video camera system to a dissection microscope.

We used the apparent density of yolk in whole oocytes after preservation, when viewed on the television monitor, to discriminate between developmental stages of yolked oocytes. We defined three stages of yolked oocytes: (1) only an initial layer of yolk along the periphery of the oocyte, appearing as a narrow band but not extending over 20% of the distance between the nucleus and the zona pellucida; (2) lightly-packed yolk possibly extending from the periphery to the nucleus with the nuclear area still evident; and (3) yolk dense enough to occlude the nucleus (Fig. 2) which is histologically equivalent to advanced yolked oocytes. Counts of stage-3 oocytes were used to estimate fecundity and measurements to estimate mean diameter of these advanced yolked oocytes.

Alpha atresia of stage-3 yolked oocytes were distinguished from other whole oocytes viewed on the television screen. The yolk within these $\alpha$-atretic stage-3 oocytes appeared mottled and lighter due to yolk liquefaction and subsequent resorption, whereas in normal yolked oocytes it appeared dense, dark, and in compact globules (Fig. 2). In addition, the zona radiata (chorion, or membrane layers surrounding the oocyte) of the atretic oocytes was indistinct and irregular in appearance. It was not possible to accurately identify atretic oocytes in frozen, thawed, or poorly preserved ovaries. Atretic oocytes were not included in counts of advanced yolked oocytes used to estimate fecundity. To estimate rates of atresia, we recorded the number of $\alpha$-atretic yolked oocytes in the random sample of 30 stage-3 oocytes measured. The number of $\alpha$-atretic advanced yolked oocytes divided by 30 was used as an index of the intensity of atresia in all females used for fecundity estimation.

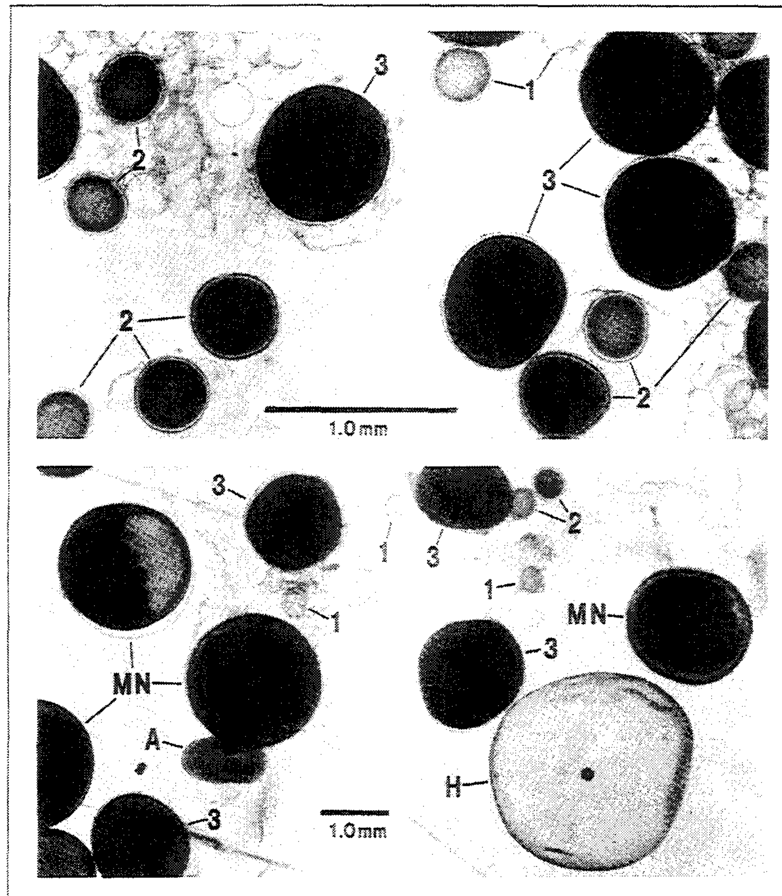



**Figure 2**
Three stages of preserved whole yolked oocytes of Dover sole *Microstomus pacificus* (stages defined in text). Lower panel also shows migratory nucleus (MN) oocytes, a hydrated (H) oocyte, and an $\alpha$-atretic advanced yolked oocyte (A). The small air bubble on the hydrated oocyte is an artifact.

## Batch fecundity

Batch fecundity was considered to be the number of migratory nucleus-stage oocytes or number of hydrated oocytes in the ovary. We used the gravimetric method to estimate numbers of these oocytes. Migratory nucleus-stage and hydrated oocytes stand out as discrete and easily identified oocyte maturity-classes (Fig. 2). Hydrated ovaries that contained new postovulatory follicles were not used to estimate batch fecundity.

We assigned each spawning batch to one of a possible five batch-order designations [1, 2, ($2<B<U-1$), ($U-1$), and U], where B is the batch-order number and U is the total number of spawning batches. The five batch-order designations were defined as follows: first batch (where $B=1$), nonhydrated, advanced yolked oocytes present and postovulatory follicles absent; second batch (where $B=2$), one class of postovulatory follicles and nonhydrated, advanced yolked oocytes present; intermediate batches (where $B>2$ but less than $U-1$), two classes of postovulatory follicles and nonhydrated, advanced yolked oocytes present; the penultimate batch ($U-1$), only two batches were present, one of hydrated and one of migratory nucleus oocytes, with no other advanced yolked oocytes present; and last batch (U), no advanced yolked oocytes present other than a single hydrated batch.

In this classification scheme, we assumed that (1) the presence of a single class of postovulatory follicles indicated one spawning had occurred; (2) the presence of two classes indicated at least two spawnings had occurred; and (3) the absence of postovulatory follicles indicated no spawning had occurred. The assumption of no spawning would not hold if the interval between spawnings was sufficiently long for postovulatory

follicles to be resorbed. We used this batch-order classification system to determine if batch fecundity varied with the order of the spawnings, as it does in some species (Alheit 1986, Hunter et al. 1989).

### Estimation of length at 50% mature

We estimated the total length (mm) of female Dover sole when 50% had become mature using histological criteria. The fraction of females considered to be mature was estimated for 10 mm or for 50 mm length-classes, and the data were fit to a logistic curve (Dixon et al. 1988). We estimated the maturity threshold for females taken off central California and off Oregon, before and during the spawning season. In our analysis, we evaluated the extent to which changes in histological criteria affected the maturity estimate using six sets of histological criteria: (1) advanced yolked

**Table 3**

Conversion equations for Dover sole *Microstomus pacificus* by state, sex, or season.

**Frozen to fresh for length and weight**

| Variable | | | | Linear equation Y = a + bX | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dependent Y | Independent X | State | Sex | a | b | $r^2$ | *F* | *N* | Range of independent variable |
| Fresh length | Frozen length | Cal | All | 9.47 | 1.01 | 0.99 | 25,550 | 251 | 196–512 mm |
| Fresh weight | Frozen weight | Cal | All | 0* | 1.22 | — | 10,229 | 111 | 54–1551 g |
| Fresh weight** | Frozen weight** | Cal | F | 0* | 1.29 | — | 19,575 | 147 | 76–1263 g |

**Length to weight**

| Variable | | | | Exponential equation $W = aL^b$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dependent W | Independent L | State | Sex | a | SE | b | SE | *N* | Range of independent variable |
| Fresh weight (g) | Fresh length (cm) | Cal | F | 0.00198 | 0.00011 | 3.45 | 0.016 | 1245 | 11.8–54.7 cm |
| | | Cal | M | 0.00173 | 0.00018 | 3.49 | 0.029 | 264 | 18.5–47.8 cm |
| | | Cal | Unknown | | | | | 4 | 12.8–23.5 cm |
| | | Cal | F, M | 0.00198 | 0.00009 | 3.45 | 0.013 | 1509 | 11.8–54.7 cm |
| | | Ore | F | 0.00141 | 0.00013 | 3.53 | 0.026 | 991 | 18.8–57.7 cm |
| | | Ore | M | 0.00156 | 0.00015 | 3.51 | 0.027 | 457 | 20.0–52.2 cm |
| | | Ore | All | 0.00159 | 0.00011 | 3.50 | 0.018 | 1448 | 18.8–57.7 cm |
| Fresh weight** (g) | Fresh length (cm) | Cal | F | 0.0038 | 0.00048 | 3.27 | 0.033 | 1198 | 11.8–54.7 cm |
| | | Ore | F | 0.0012 | 0.00026 | 3.58 | 0.056 | 430 | 26.8–56.4 cm |

**Female weight and oocyte volume to ovary weight**

| | Variable | | | | Linear equation $Y = a + bX_1 + cX_2$ | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Season | Dependent Y | Independent $X_1$ | Independent $X_2$ | State | a | b | c | $r^2$ | *F* | *N* | Range of independent variable |
| Prespawning | Ovary weight (g) | Fish weight** (g) | | Ore | 9.07 | 0.013 | | 0.09 | 38.9 | 388 | 122–2017 g |
| | Ovary weight (g) | Fish weight** (g) | Spher. vol.*** ($mm^3$) | Cal | −4.67 | 0.027 | 37.7 | 0.54 | 17.9 | 30 | 202–1124 g<br>0.33–0.70 $mm^3$ |
| | | | | Ore | −34.05 | 0.036 | 95.3 | 0.59 | 45.85 | 64 | 236–1816 g<br>0.33–0.59 $mm^3$ |
| | | | | Cal+ Ore | −26.06 | 0.036 | 76.3 | 0.59 | 68.2 | 94 | 202–1816 g<br>0.33–0.70 $mm^3$ |
| Spawning | Ovary weight (g) | Fish weight** (g) | | Cal | −7.05 | 0.032 | | 0.41 | 826.7 | 1198 | 14–1736 g |
| | | | | Ore | 21.79 | 0.010 | | 0.07 | 4.2 | 42 | 148–1597 g |
| | | | | Cal+ Ore | −5.88 | 0.031 | | 0.39 | 788.0 | 1240 | 14–1736 g |

* Intercept not different from 0, line forced through origin.
** Without ovary.
*** Spherical volume = $4/3\pi r^3$; for mean oocyte diameters $\geq$0.86 mm.

oocytes or postovulatory follicles present; (2) early yolked oocytes with $\beta$ atresia; (3) early yolked oocytes with only $\alpha$ atresia of the early yolked oocytes or no $\alpha$ or $\beta$ atresia; (4) unyolked oocytes with $\beta$; (5) unyolked oocytes with only $\alpha$ of the unyolked oocytes; and (6) unyolked oocytes with no atresia. The sexual maturity for females identified by criterion 1 is certain, but some females may be excluded if only criterion 1 is used. Criteria 2–5, if added to criterion 1, broaden the maturity definition but increase the risk of misclassification. Criterion 6 is considered by definition to be immature. We evaluated these criteria to determine the optimal histological definition of maturity using a regression analysis of the lengths of females identified by each criterion.

### Length, weight, and gonad weight relationships

To enable the reader to convert from one measurement to another, equations are provided to estimate fresh wet weight from frozen wet weight and from length for Dover sole taken in Oregon and central California waters (Table 3). Analysis of covariance indicated that the slope of the regression of the natural logarithms of weight on length did not differ between sexes for either state. The adjusted group mean for males differed from that for females in Oregon ($N$ 1421, $F_{1,1418}$ 64.87, $P<0.005$ for length range 225–522 mm) but not in California. The slope of the regression of the natural logarithms of weight on length did not differ between central California and Oregon females but the adjusted group means were different ($N$ 2215, $F_{1,2212}$ 79.18, $P<0.005$ for length range 188–547 mm). No difference existed between states in the equations for males. We do not attach too much biological importance to these differences; they could be related to differences in the timing of annual reproductive cycle or our sampling of it. Nonetheless, it seemed preferable to use the relationship for a specific sex or region, so all are listed.

An exponential model was fit to these data sets using a statistical program of weighted nonlinear regression (Dixon et al. 1988) where the weighting factor was the inverse of the variance of fish weight because the variance of fish weight increased with fish length. To compute the variance, fish lengths were divided into several segments, chosen so that within each segment the variance of fish weight was homogeneous. We preferred to obtain the estimates of coefficients directly from the nonlinear fitting so that fish weight could be directly estimated from the exponential model (Table 3).

Freezing of Dover sole caused a 9.47 mm shrinkage in total length, independent of fish length (Table 3). A sample of 251 Dover sole was measured just after capture and again, after thawing, four months later. The slope of the regression of fresh total length on frozen total length (after thawing) was not statistically different from 1, but the intercept, 9.47 mm, was significant. Freezing of females, with ovary removed, resulted in about a 22% loss in wet weight ($0.22 = 1 - \frac{1}{1.29}$; see Table 3).

We also provided equations to estimate ovary wet weight (g) from female wet weight (g, without ovary). This conversion is important if one wishes to express fecundity as a function of the total weight of the female, because all fecundity relations in this study are expressed as a function of female weight without an ovary. As ovary weight is a function of the developmental state of the ovary as well as the weight of the female, separate equations are provided for the prespawning period (November–December) when ovaries are less developed and for the spawning season when they are more fully developed. We also provided multiple regression equations to estimate ovary weight from female weight and the spherical volume of the average advanced yolked oocyte (computed from the mean diameter). These equations are used in the discussion to estimate ovary weight when an ovary contains an entire complement of fully matured advanced yolked oocytes.

## Reproductive condition

### Accuracy of gross anatomical classification

We rarely misclassified inactive ovaries using gross anatomical criteria. Of the 1272 females classified as inactive, only 14 (1.1%) were identified as active using histological criteria. This error rate is so low that differences could be attributable to clerical errors alone. A more common error in gross anatomical classification was to misclassify females as having active ovaries. One hundred and fifty-nine females (11.9%) were visually classified as having advanced yolked oocytes and were believed to be capable of spawning, while histological analysis indicated that their ovaries were inactive and future spawning was unlikely. The 159 females misclassified as active fell predominantly into two classes: females with ovaries in the early stages of vitellogenesis (40.8%), and females with advanced yolked oocytes with high levels of atresia (30.1%) (Table 4).

Misclassification of the early stages of vitellogenesis as active is expected because the gross anatomical criterion, "yolked oocytes visible," is not exact; observers are bound to differ on whether to include or exclude females that fall near the visible threshold

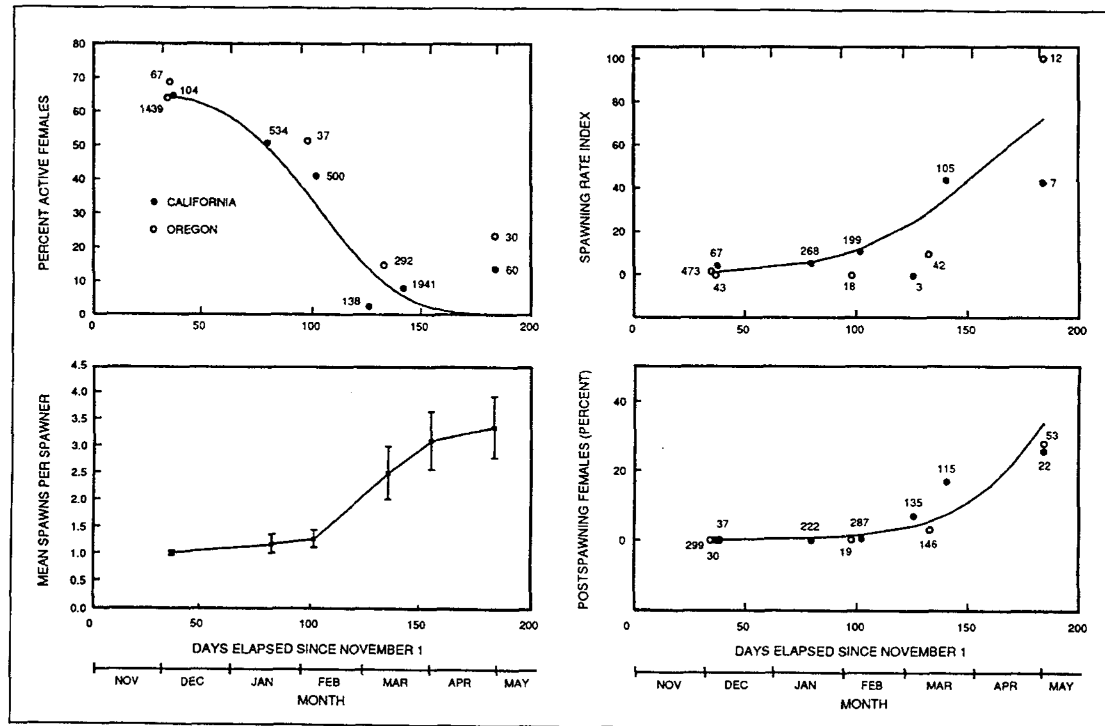
**Table 4**

Histological classification of female Dover sole *Microstomus pacificus* with inactive ovaries that were misclassified using gross anatomical criteria as having active ovaries. Data from central California and Oregon are combined.

| Collection period | Percentage of females misclassified as active per inactive histological class: Immature | Unyolk atretic | Early yolked | Major atresia of adv. yolked | Post-spawning | Total no. misclassed females |
|---|---|---|---|---|---|---|
| Nov–Dec | 0.0 | 2.0 | 48.9 | 48.9 | 0.0 | 49 |
| Jan–May | 4.5 | 23.6 | 37.2 | 21.8 | 12.7 | 110 |
| Nov–May | 3.1 | 17.0 | 40.8 | 30.1 | 8.8 | 159 |

**Figure 3 (below)**

Seasonal change in four indices of reproduction in Dover sole *Microstomus pacificus*. Indices are plotted as a function of elapsed time since 1 November; data are combined from different years; California (solid circles) and Oregon (open circles) data were combined to fit trend lines; numbers are the sample size of females. (upper left) Percentage of Dover sole with active ovaries; trend line is a weighted Weibull model (see text Eq. 1). (upper right) Percentage of females with active ovaries which had one or more spawning states; trend line is logistic model $P = \frac{e^{a+bL}}{1+e^{a+bL}}$, where a = −5.678 and b = 0.036. (lower left) Mean number of spawning states in active ovaries; bars are two standard errors of the mean. (lower right) Percentage of females with inactive ovaries identified as postspawning; trend line is logistic model where a = 8.495 and b = 0.042.




for detection of yolked oocytes in the ovary. An exact criterion, such as oocyte diameter, would be more accurate but would be impractical for production work on the ship. Misclassification of highly atretic ovaries as active is also expected, since α-atretic advanced yolked oocytes are difficult if not impossible to see with the unaided eye. As highly-atretic advanced ovaries were rare in this study, our failure to detect them was a minor systematic error. Under environmental conditions unfavorable to reproduction, however, this could be an error of consequence.

## Changes in ovarian condition during the spawning season

The fraction of females anatomically classed with active ovaries

declined over the spawning season as females expended their stock of advanced yolked oocytes. We fitted a weighted Weibull function to the combined California and Oregon data, yielding the equation

$$P = 0.656\ e^{-\left(\frac{t}{111.5}\right)^{3.76}} \quad \text{(Eq. 1)}$$

where t is days elapsed since 1 November; P, the fraction active, is weighted by $\frac{N}{P(1-P)}$, and pseudo $r^2$ is 0.96. According to the equation, the percentage of females with active ovaries declined from 65% at the onset of the spawning season (about 6 December) to 40% by the end of January; by the end of February only 18% of the females had active ovaries (Fig. 3, upper left).

In California and Oregon, the mean diameter of the stock of advanced yolked oocytes increased steadily from December through April (Fig. 4). Thus reproductively active females continued vitellogenesis throughout most of the spawning season. By March or April the average advanced yolked oocyte is closer to the minimum size at which hydration begins (diameter 1.35 mm). Thus Dover sole may be able to spawn at a higher rate late in the spawning season, because less yolk would have to be added to the advanced oocytes for them to attain the size at hydration.

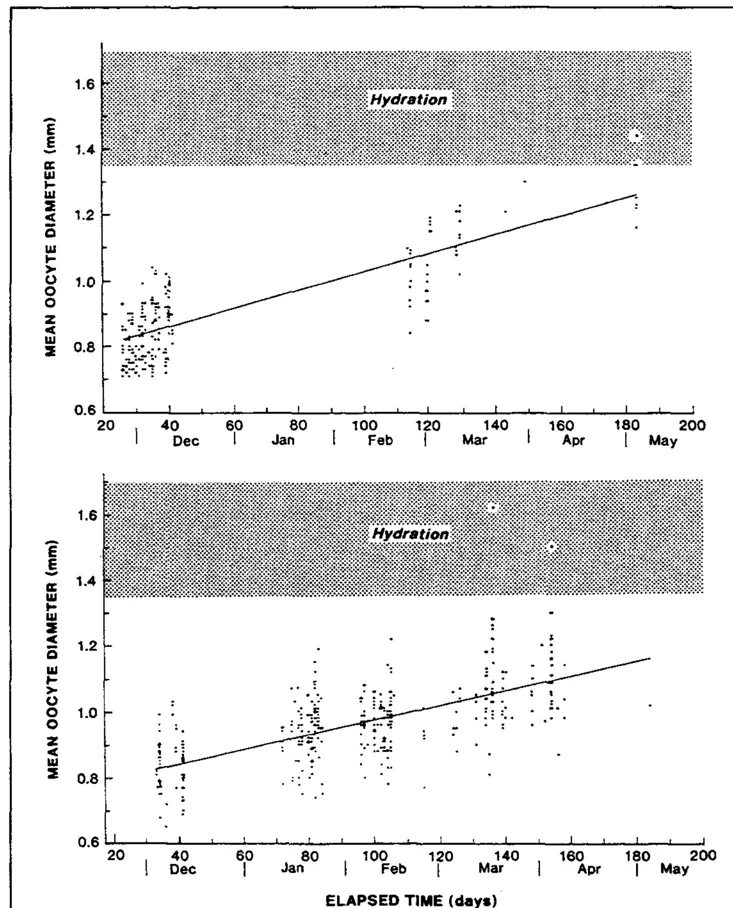



**Figure 4**

Increase in mean oocyte diameter (D) of the advanced yolked oocytes of Dover sole *Microstomus pacificus* from Oregon (top) and California (bottom) as a function of elapsed time (T) since 1 November. Data from different spawning seasons are combined; shaded area indicates size range of oocytes at the onset of hydration; trend lines are Oregon (D = 0.742 + 0.00282T, $r^2$ 0.658, $N$ 195) and California (D = 0.761 + 0.0022T, $r^2$ 0.41, $N$ 365).

Only 10 females taken in November–December were classed as spawning on the basis of their ovarian histology (Oregon and California data combined, $N$ 949). They comprised only 1.0% of all females with preserved ovaries taken during this time and only 1.7% of those classed as active. Clearly, spawning is just beginning in November–December in California and Oregon waters. The spawning rate index increased from 1.3% in November–December to 12% by early February; it accelerated at the end of the season with spawning females comprising about 70% of all active females (percent calculated by the trend line in Fig. 3, upper right). Thus at the end of the season, most of the females with active ovaries had spawned recently. We believe this sharp increase in the index near the end of the season is evidence for a seasonal increase in spawning frequency.

A late seasonal increase also existed in the occurrence of multiple spawning stages within the same ovary. As many as five different past or potential spawning stages could be distinguished histologically in the same ovary: two stages of postovulatory folli-

cles, hydrated oocytes, migratory nucleus oocytes, and other advanced yolked oocytes. These data were expressed as the number of spawning states per spawner (spawns per spawner, Fig. 3, lower left). The average number of spawns per spawner increased from about one in mid-March to about three by early April. These data also indicated that spawning frequency may increase near the end of the spawning season.

The fraction of females with inactive ovaries that were classed as postspawning also increased late in the season (Fig. 3, lower right). This index can be considered a measure of the rate females in the population pass from the active to the inactive state. Although the duration of this stage was unknown, we were certain that it was ephemeral because there were always many fewer females classed as postspawning than the cumulative total of females that had passed from the active to inactive state. This index increased sharply in late-March through April, indicating that the rate females passed into the inactive stage accelerated during the last part of the season.

The sharp increases in the three indices described indicated that the daily production of eggs by the population may be higher in March than February even though fewer fish were spawning. For example, by mid-March (13 March), a half to a third as many females had reproductively active ovaries than in mid-February (10 Feb.). On the other hand, in mid-March as compared with mid-February, about twice as many females with active ovaries were classed as spawning, and the ovaries of the spawners contained evidence of about twice as many past or potential spawnings. Thus the reproductive output of the reproductively active females in the population in mid-March might be four times that of the active fish in mid-February. If this is true, half the number of active females could produce twice as many eggs per day. This is, of course, sheer speculation because the duration of these spawning stages is unknown. Nevertheless, the data presented in this section collectively suggest that the daily production of eggs by the population may increase near the end of the season even though fewer females are spawning.

## Total fecundity

### Location of tissue samples

A key assumption underlying the gravimetric method of fecundity estimation is that oocytes are randomly distributed in the ovary. To determine if advanced yolked oocytes are randomly distributed in the ovary, we compared the densities of advanced yolked oocytes in tissue samples taken from five different locations in the ovary of ten females. The location of a tissue sample within the ovary was defined in terms of three characteristics: longitudinal plane of the ovary (anterior end, middle, and posterior end); cross-sectional plane (interior near the lumen, exterior near the ovarian wall, or interior and exterior combined); and right and left lobes of the ovary. The characteristics of the five ovarian locations along with the mean oocyte density of each location are indicated in Table 5.

Initially we tested the overall effect of location of the tissue sample on oocyte density using two-way ANOVA; the effect of position was insignificant at the 5% level of significance (Table 5, lower). We also tested for possible differences between pairs of location characteristics: posterior end vs. middle; posterior vs. anterior ends of the ovary; right vs. left lobes of the ovary; and interior and exterior sections of the ovary. No significant differences were detectable between any of

**Table 5**
Effect of location of tissue samples within the ovary of Dover sole *Microstomus pacificus* on oocyte density (number of advanced yolked oocytes per unit sample weight) with mean and standard deviation (SD) and, below, two-way analysis of variance on results.

| Position no. | Location in ovary of sample* | | | | Oocyte density | |
|---|---|---|---|---|---|---|
| | | Plane of section | | | | |
| | Lobe | Long. | Cross. | $N$ | Mean | SD |
| 1 | Rt | Post | Int & Ext | 10 | 1803.7 | 468.1 |
| 2 | Rt | Mid | Ext | 10 | 1801.1 | 532.0 |
| 3 | Rt | Mid | Int | 10 | 1754.1 | 488.6 |
| 4 | Rt | Ant | Ext | 10 | 1886.1 | 440.2 |
| 5 | Lt | Mid | Int | 10 | 1839.3 | 537.6 |

Analysis of variance on five locations

| Source | DF | SS | MS | $F$ |
|---|---|---|---|---|
| Fish | 9 | 10,379,034 | 1,153,226 | |
| Position | 4 | 96,638 | 24,160 | 1.373 |
| Error | 36 | 633,493 | 17,597 | |
| Total | 49 | 11,109,165 | | |

*Long. = longitudinal, Rt = right lobe, Lt = left lobe, Post = posterior end, Mid = middle, Ant = anterior end, Int = internal, and Ext = external.

these four comparisons; $F$ values ranged from 0.01 to 0.14, with the degrees of freedom being 1 and 45 for each comparison. Thus the advanced yolked oocytes in Dover sole are randomly distributed within the ovary, and tissue samples can be taken from any location or lobe without bias.

## Optimal number of tissue samples

To develop a procedure for estimating the number of tissue samples needed for estimating total fecundity, we first considered the general fecundity model. The true total fecundity ($Y_F$) is the condition where all the advanced yolked oocytes in the ovary are counted, and the relation between female weight (W) and fecundity is defined as

$$Y_F = f(W) + A \qquad \text{(Eq. 2)}$$

where $f(W) = a+bW$, and A is the error term. The variance of A, $\sigma^2_A$, measures the deviation of the data set $(Y_F, W)$ to the model $f(W)$. As it was impractical to count all advanced yolked oocytes in the ovary, $Y_F$ is estimated from counts of oocytes in weighed tissue samples, expressed as oocytes per gram of tissue or oocyte density. The precision of a fecundity estimate can be increased by increasing the number of tissue samples taken per female. On the other hand, if the amount of labor for fecundity work is fixed, then increasing the number of tissue samples per fish would reduce the number of fish that can be sampled. Thus we needed to know the minimum number of tissue samples necessary to guarantee a goodness-of-fit of the model to the data set.

We determined the optimum number of tissue samples by minimizing the variance of sample variance of A ($\sigma^2(s^2_A)$). This procedure led to using the ratio of the variance of oocyte counts between tissue samples within fish ($\sigma^2_e$) to the variance around the regression line ($\sigma^2_A$), i.e., $\theta = \sigma^2_e/\sigma^2_A$. The smaller the $\theta$, the fewer tissue samples are needed.

Let's denote for the ith fish, $i = 1,..,n$,

- $W_i$ = fish weight,
- $Y_{Fi}$ = total number of advanced yolked oocytes in the ovary,
- $y_{ij}$ = advanced yolked oocyte count in the jth tissue sample, $j = 1,..,m$,
- $z_{ij}$ = weight of the jth tissue sample,
- $Z_i$ = formalin wet weight of ovary,
- $m$ = number of tissue samples from an ovary,
- $M_i$ = maximum number of tissue samples in an ovary,
- $\hat{Y}_{Fij}$ = estimate of total number of advanced yolked oocytes in the ovary from the jth tissue sample $= \left(\frac{y_{ij}}{z_{ij}}\right) Z_i$,
- $\hat{Y}_{Fi}$ = estimated total number of advanced yolked oocytes in the ovary and is used for all analyses in fish fecundity in later sections $= \frac{\sum_{j=1}^{m} \hat{Y}_{Fij}}{m}$, and
- $\hat{Y}_{Fi}$ = estimate of total fecundity from the regression model.

We write $\hat{Y}_{Fij}$ as

$$\hat{Y}_{Fij} = Y_{Fi} + (\hat{Y}_{Fij} - Y_{Fi}) = f(W_i) + A_i + e_{ij} \qquad \text{(Eq. 3)}$$

where $e_{ij} = \hat{Y}_{Fij} - Y_{Fi}$. The estimated total number of advanced yolked oocytes in the ovary is

$$\hat{Y}_{Fi} = \frac{\sum_{j=1}^{m} \hat{Y}_{Fij}}{m} = f(W) + A_i + e_i = f(W) + \xi \qquad \text{(Eq. 4)}$$

and

$$\sigma^2_\xi = \sigma^2_A + \frac{\left(\frac{M-m}{M}\right)\sigma^2_e}{m}. \qquad \text{(Eq. 5)}$$

Thus the variance around the regression line $\sigma^2_\xi$ based upon the data set $(\hat{Y}_{Fi}, W_i)$ is composed of two variance components: one is $\sigma^2_A$ and the other is $\sigma^2_e$. The sample counterparts for $\sigma^2_\xi$ and $\sigma^2_e$ are $s^2_\xi$ and $s^2_e$:

$$s^2_\xi = \frac{[\hat{Y}_F - f(W)]^2}{n-q} \qquad \text{(Eq. 6)}$$

is the mean square error from a regression analysis on $(\hat{Y}_{Fi}, W_i)$ where q is the number of regression coefficients and n is the number of fish, and

$$s^2_e = \frac{\sum_{i=1}^{n}\sum_{j=1}^{m} (\hat{Y}_{Fij} - \hat{Y}_{Fi})^2}{n(m-1)} \qquad \text{(Eq. 7)}$$

is the within-sample variance (Hunter et al. 1985). The estimate ($s^2_A$) of the variance around f(W) when $Y_F$ is known ($\sigma^2_A$) can be estimated by subtraction:

$$s^2_A = s^2_\xi - \frac{\left(\frac{M-m}{M}\right) s^2_e}{m}. \quad \text{(Eq. 8)}$$

According to Hunter et al. (1985), the optimum number of tissue samples can be determined for a given $\theta$ ($= s^2_e/s^2_A$), the cost of processing a tissue sample, and the cost of processing a fish. The ratio, $K = s^2_\xi/s^2_A$, measured the excess variance which is contributed by taking tissue samples rather than counting every advanced oocyte in the ovary.

We used Dover sole collected during January-February 1987 to determine the optimal number of tissue samples. Two tissue samples were taken from the ovaries of 99 Dover sole. The within-sample variance of oocyte density ($s^2_e = 1053 \times 10^4$) was obtained from an ANOVA, and the linear regression of $\hat{Y}_F$ on W ($\hat{Y}_F = 20{,}255 + 40.54W$) gave the MSE ($s^2_\xi = 18{,}469 \times 10^4$) (Table 6; Fig. 5, lower middle). Thus $\theta$ is 0.058 when calculated from equations (7 and 8) where m = 2. Because M was large (range 200–700), $\frac{M-m}{M}$ was assumed to equal 1, and $s^2_A$ was computed as $s^2_\xi - (s^2_e/2) = 17{,}942 \times 10^4$. Hence when two tissue samples are used, the variance within tissue samples is only 5.8% of the variance around the fecundity-fish weight regression line. To quantify the excess variance due to subsampling, we computed $K = s^2_\xi/s^2_A = 1.03$. This means the variance around the regression line which was based on two tissue samples per fish (Eq. 4) is about 1.03 times that of an equation based on counts of all advanced yolked oocytes in the ovary (Eq. 2). Although the within-ovary variance was small, we recommend counting two tissue samples per female because the cost of processing the second sample was minimal.

## Optimal number of females

In addition to the sample allocation based on cost of processing fish and cost of processing tissue samples, the number of females needed for a regression estimate of total fecundity was determined by modifying a procedure suggested by Thigpen (1987) to the 1987 fecundity data for Dover sole. The equation for determining the sample size ($n$) for a linear regression was

$$n - 1 = \frac{\left(\frac{1-r^2}{r^2}\right)}{CV(b)^2} \quad \text{(Eq. 9)}$$

**Table 6**
Within-sample variance ($s^2_e \times 10^{-4}$) from ANOVA and the MSE ($s^2_\xi \times 10^{-4}$) from the regression analysis of fecundity ($Y \times 10^{-2}$) and weight of Dover sole *Microstomus pacificus*. California females taken January–February 1987.

Analysis of variance on total fecundity

| Source | DF | SS | MS |
|---|---|---|---|
| Fish | 98 | 8,559,008 | 87,337 |
| Error | 99 | 104,201 | 1,053 |
| Total | 197 | 8,663,209 | |

Analysis of variance on linear regression

| Source | DF | SS | MS | F |
|---|---|---|---|---|
| Regression | 1 | 2,488,019 | 2,488,019 | 134.72 |
| Residual | 97 | 1,791,468 | 18,469 | |

| Predictor | Coeff. | SD | t |
|---|---|---|---|
| Constant | 202.55 | 32.03 | 6.32 |
| Fish wt. | 40.54 | 3.49 | 11.61 |

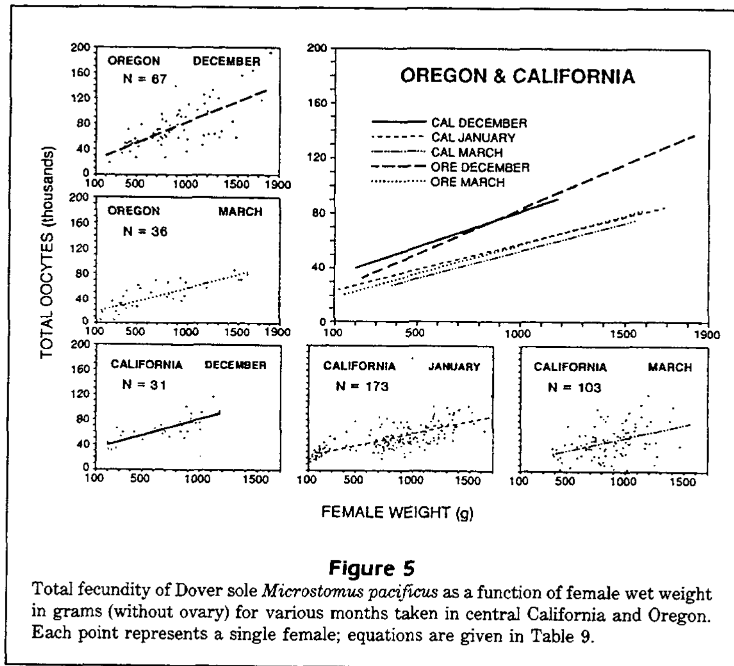



**Figure 5**
Total fecundity of Dover sole *Microstomus pacificus* as a function of female wet weight in grams (without ovary) for various months taken in central California and Oregon. Each point represents a single female; equations are given in Table 9.

where $r^2$ is the coefficient of determination, and $CV$(b) is the coefficient of variation for the regression coefficient (b). The coefficient of determination ($r^2$) for Dover sole total fecundity and fish weight was 0.58. Thus 73 females are required for a $CV(b) = 0.10$. In conclusion, two tissue samples from each of 70–80 Dover sole females were adequate for expressing the relation between weight and total fecundity, if a $CV$(b) of about 0.10 is desired.

## Relation to ovarian development

The optimum time for estimating potential annual fecundity is early in the spawning season when the probability of spawning is low. Estimates taken in this period may be biased because all oocytes may not have been recruited into the advanced stock of yolked oocytes. In this section, we use Oregon Dover sole taken in November–December to examine the recruitment of oocytes into the advanced stock of yolked oocytes.

If substantial numbers of oocytes are maturing from early-yolked to more advanced stages, one would expect an overlap in the size distributions of oocytes in different development stages. When vitellogenesis of the early yolked oocytes does not continue, one would expect that a gap would develop between the less-advanced and the most-advanced oocytes as the yolked oocytes continued their maturation. In Dover sole, the diameter distributions of stage-1, -2, and -3 oocytes broadly overlap when the mean diameter of the advanced yolked oocytes (stage 3) is less than 0.7 mm (Fig. 6). The extent of overlap declines as the stage-3 oocytes grow from 0.7 mm to 0.8 mm. Separation of the advanced stock (stage 3) from the other vitellogenic stages (1 and 2) becomes complete as the advanced stock grows from 0.8 to 0.9 mm. It appears from this qualitative analysis that recruitment of stage-2 oocytes into the advanced stock probably ends when the mean diameter of stage 3 is between 0.8 and 0.9 mm.

We conducted a stepwise multiple regression analysis of total fecundity ($\hat{Y}_F$) on mean diameter of the advanced oocytes (D) and female weight (W) for females taken off Oregon in November–December. The coeffi-

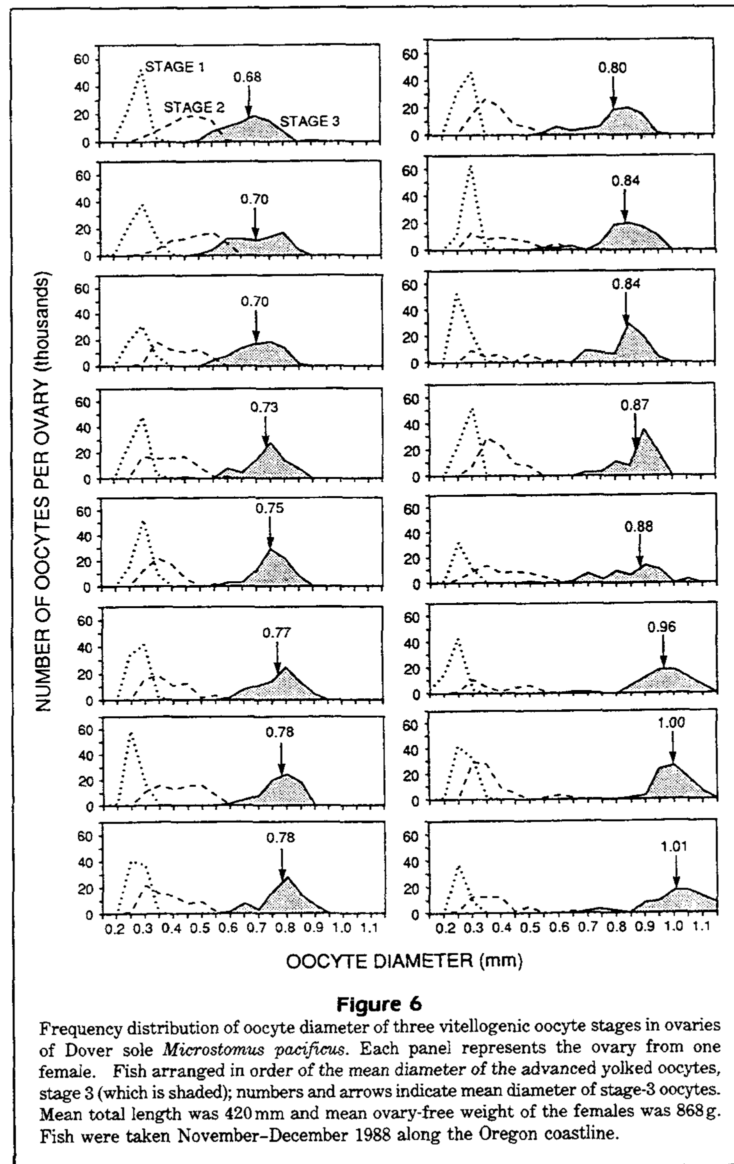



**Figure 6**

Frequency distribution of oocyte diameter of three vitellogenic oocyte stages in ovaries of Dover sole *Microstomus pacificus*. Each panel represents the ovary from one female. Fish arranged in order of the mean diameter of the advanced yolked oocytes, stage 3 (which is shaded); numbers and arrows indicate mean diameter of stage-3 oocytes. Mean total length was 420 mm and mean ovary-free weight of the females was 868 g. Fish were taken November–December 1988 along the Oregon coastline.

**Table 7**

Analysis of the relation between total fecundity ($\hat{Y}_F$) of Dover sole *Microstomus pacificus* and gonad-free body weight (W) and the average diameter of the advanced oocytes (D) using stepwise regression with analysis of variance. Specimens from Oregon in November–December 1988.

| Stepwise regression | | |
|---|---|---|
| Step | 1 | 2 |
| Constant | 22,398 | −88,768 |
| Weight (W) | 45.4 | 47.8 |
| $t$* | 7.56 | 8.80 |
| Diameter (D) | | 129,893 |
| $t$* | | 6.01 |
| S | 25,654 | 23,096 |
| $R^2$ | 27.58 | 41.69 |

| Analysis of variance | | | | | |
|---|---|---|---|---|---|
| Source | DF | SS | MS | $F$ | $P$ |
| Regression | 2 | $5.68\times10^{10}$ | $2.84\times10^{10}$ | 53.27 | <0.000 |
| Error | 149 | $7.95\times10^{10}$ | $5.33\times10^{10}$ | | |
| Total | 151 | $1.36\times10^{10}$ | | | |

| Source | DF | Sequential SS |
|---|---|---|
| Weight | 1 | $3.76\times10^{10}$ |
| Egg diameter | 1 | $1.92\times10^{10}$ |

*For $P = 0.05$, $1.96<t<1.98$., df≥120.

cient for diameter, as well as the one for weight, was positive and significant (Table 7). Thus the potential annual fecundity was not fully recruited as stage-3 oocytes in some of the Oregon Dover sole taken in November–December, since total fecundity increased with the mean diameter of the oocytes used to estimate total fecundity.

To determine the level of ovarian development (oocyte diameter) at which the full complement of oocytes was recruited into the advanced yolked oocyte class (stage 3), we conducted a series of stepwise multiple regression analyses by successively removing the data by 0.01 mm decrements from the lowermost oocyte diameter class starting at 0.71 mm. This analysis indicated that the threshold for a significant effect of oocyte diameter on total fecundity was between mean diameters of 0.85 and 0.86 mm (Table 8). The multiple regression coefficient for oocyte diameter was significant and positive when females with oocyte diameter

**Table 8**

Results of stepwise multiple regression of the total fecundity ($\hat{Y}_F$) of Dover sole *Microstomus pacificus* on gonad-free body weight (W) and mean oocyte diameter (D) for a succession of oocyte diameter-classes using the model $\hat{Y}_F = a + b_1W + b_2D$. Specimens taken along Oregon coast November–December 1988. Line separates oocyte diameter-classes where diameter is a significant variable, from those where it is not.

| Oocyte diameter class (mm) | Constants | | Multiple regression coefficients and their $t$-ratios for: Fish weight | | Oocyte diameter | | |
|---|---|---|---|---|---|---|---|
| | $N$ | a | $b_1$ | $t$ | $b_2$ | $t$* | $r^2$ |
| 0.71–1.04 | 152 | −88,768 | 47.8 | 8.80 | 129,893 | 6.01 | 0.417 |
| 0.72–1.04 | 148 | −90,147 | 48.3 | 8.90 | 130,860 | 5.87 | 0.424 |
| 0.74–1.04 | 133 | −94,375 | 49.6 | 8.34 | 134,295 | 5.14 | 0.415 |
| 0.76–1.04 | 119 | −90,710 | 51.3 | 7.98 | 128,688 | 4.17 | 0.407 |
| 0.78–1.04 | 105 | −83,087 | 53.4 | 7.53 | 118,385 | 3.16 | 0.396 |
| 0.80–1.04 | 91 | −90,344 | 55.9 | 7.29 | 123,891 | 2.60 | 0.400 |
| 0.82–1.04 | 85 | −87,274 | 59.2 | 7.34 | 117,459 | 2.20 | 0.405 |
| 0.83–1.04 | 81 | −111,317 | 56.9 | 7.44 | 145,193 | 2.77 | 0.440 |
| 0.84–1.04 | 77 | −103,009 | 58.7 | 7.55 | 134,678 | 2.42 | 0.460 |
| 0.85–1.04 | 72 | −90,464 | 60.1 | 7.33 | 120,166 | 1.95 | 0.457 |
| 0.86–1.04 | 67 | −38,172 | 65.3 | 7.96 | 60,555 | 0.94 | 0.502 |
| 0.87–1.04 | 64 | −33,982 | 63.5 | 7.30 | 57,891 | 0.87 | 0.482 |
| 0.88–1.04 | 60 | −15,721 | 69.4 | 7.63 | 33,456 | 0.48 | 0.514 |
| 0.90–1.04 | 46 | 45,073 | 82.0 | 7.92 | −40,601 | −0.47 | 0.594 |
| 0.92–1.04 | 34 | 60,339 | 84.0 | 6.92 | −58,185 | −0.49 | 0.616 |

*For $P = 0.05$, $t$ is 1.98 for df = 120, 2.00 for df = 60, and 2.06 for df = 25.

equal to or less than 0.86 mm were included, but was insignificant when only those having a diameter greater than 0.86 mm were considered. We concluded that ovaries in which the advanced stock of yolked oocytes has an average diameter of 0.85 mm or less are not sufficiently developed to be certain that the annual stock is fully recruited. Consequently, to estimate the potential annual fecundity, we used only females in which the average oocyte diameter of the advanced stock exceeded 0.85 mm.

No relationship between oocyte diameter and total fecundity was detected in the females taken off central California during November–December. Oocyte diameter may not have been a significant variable in central California because fewer females were examined and their ovaries were more advanced. In 48% of females from California, advanced yolked oocytes averaged more than 0.85 mm in diameter (*N* 65), whereas only 34% of the fish taken off Oregon had oocytes that did so (*N* 128).

### Seasonal variation in total fecundity

Total fecundity of Dover sole decreased during the spawning season off both central California and Oregon (Fig. 5). Analysis of covariance indicated that equations expressing the relation between female weight and fecundity differed within the spawning season (Table 9). The total fecundity for a 1 kg female declined from about 80,000 advanced oocytes in December to about 50–60,000 during the spawning season (Table 9).

To further describe the decline in total fecundity over the season, we also regressed fecundity on female weight and elapsed time since 1 November. In both central California and Oregon the negative coefficients for elapsed time were significant, indicating that total fecundity declined with elapsed time (Table 9). Analysis of covariance indicated that multiple regression equations for California and Oregon were not different (analysis over a similar weight range of 174–1542 g; $F_{1,388}$ 1.59, *P* 0.208; adjusted mean fecundity for Oregon 57,849, SE 2092; adjusted mean fecundity for California 54,733, SE 1152). When we combined data for the two regions, we found that total fecundity declined on the average about 12% per month. This computation underestimated the actual rate of decline, since it did not take into account females that had spawned all of their advanced yolked oocytes.

### Potential annual fecundity

Potential annual fecundity was considered to be equivalent to the standing stock of advanced yolked oocytes in fully developed, prespawning females. We consider

**Table 9**

Linear regression coefficients, confidence intervals, and estimates for the relationship between female weight (*W*, ovary-free, in g) and total fecundity ($\hat{Y}_F$) of Dover sole *Microstomus pacificus* from California and Oregon. Analysis of covariance for the effect of season on the relation between total fecundity and weight. Multiple regression coefficients are also given for the effect of elapsed time (T; days since 1 Nov.) and female weight on total fecundity.

**Linear regression by month and state**

| | | Linear equation $\hat{Y}_F = a + bW$ | | | | | | | Regression estimate for | Analysis of covariance for effect of month with weight ranges similar | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| State | Mean date of cruise | a | 95% CI | b | 95% CI | $r^2$ | *F* | *N* | 1 kg female | Variables | df | *F* | *P* |
| Oregon | 7 Dec 88 | 17,640 | ±15,460 | 65.5 | ±16.4 | 0.49 | 63.9 | 67 | 83,140 | Weight | 1 | 67.85 | <0.005 |
| | 3 Mar 89 | 14,492 | ±8,530 | 42.9 | ±10.6 | 0.66 | 67.9 | 36 | 57,392 | Month | 1 | 20.06 | <0.005 |
| | | | | | | | | | | Error | 95 | | |
| | | | | | | | | | | Total | 97 | | |
| Central California | 8 Dec 85 | 29,497 | ±6,121 | 51.6 | ±16.1 | 0.58 | 42.9 | 31 | 81,097 | Weight | 1 | 25.20 | <0.005 |
| | 31 Jan 87 | 20,154 | ±6,344 | 38.9 | ±6.8 | 0.42 | 127.0 | 173 | 59,022 | Month | 2 | 18.91 | <0.005 |
| | 23 Mar 88 | 12,072 | ±16,924 | 40.7 | ±18.6 | 0.15 | 18.9 | 103 | 52,772 | Error | 217 | | |
| | | | | | | | | | | Total | 220 | | |

**Multiple regression of total fecundity on weight and days elapsed since 1 November**

| | Multiple regression equation $\hat{Y}_F = a + b_1W + b_2T$ | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| State | a | 95% CI | $b_1$ | 95% CI | $b_2$ | 95% CI | $r^2$ | *F* | *N* | $W_{min}$ (g) | $W_{max}$ (g) | $T_{min}$ (d) | $T_{max}$ (d) |
| Oregon | 35,162 | ±13,378 | 55.2 | ±11.2 | −237 | ±104 | 0.59 | 74.9 | 103 | 147.7 | 1815.9 | 33 | 151 |
| Central California | 41,552 | ±8,142 | 40.3 | ±6.4 | −224 | ±63 | 0.37 | 92.3 | 307 | 120.0 | 1690.3 | 34 | 160 |

**Table 10**

Relationship between total fecundity ($\hat{Y}_F$) and gonad-free body weight (g) or total length (mm) for California and Oregon Dover sole *Microstomus pacificus* females meeting specifications for potential annual fecundity estimation (females taken in November–December with average oocyte diameter >0.85 mm and no evidence of past or imminent spawning). Data are compared with Yoklavich and Pikitch (1989) estimates for Oregon.

| | Fecundity and weight | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Linear equation $\hat{Y}_F = a + bW$ | | | | | | | Estimate for | Gonad-free weight (g) | |
| State | a | 95% CI | b | 95% CI | $r^2$ | $F$ | $N$ | 1 kg female | Mean | Range |
| Oregon | 17,640 | ±15,453 | 65.5 | ±16.4 | 0.488 | 63.86 | 67 | 83,140 | 870.5 | 236.0–1815.9 |
| California | 29,871 | ±12,996 | 50.9 | ±17.1 | 0.544 | 37.06 | 30 | 80,771 | 704.7 | 202.1–1124.2 |
| Oregon + California | 21,124 | ±9,248 | 62.0 | ±10.4 | 0.504 | 98.42 | 97 | 83,124 | 819.2 | 202.1–1815.9 |

| | Fecundity and length | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Exponential equation $\hat{Y}_F = aL^b$ | | | | | | | Estimate for | Total length (mm) | |
| State | a | 95% CI | b | 95% CI | Pseudo $r^2$ | $F$ | $N$ | 453 mm female* | Mean | Range |
| Oregon | $5.667 \cdot 10^{-6}$ | $\pm 1.943 \cdot 10^{-5}$ | 3.806 | ±0.713 | 0.431 | 47.03 | 64 | 72,856 | 436 | 298–551 |
| California | $6.101 \cdot 10^{-4}$ | $\pm 5.042 \cdot 10^{-3}$ | 3.020 | ±1.380 | 0.084 | 2.57 | 30 | 64,094 | 423 | 296–526 |
| Yoklavich & Pikitch** | $1.637 \cdot 10^{-6}$ | $\pm 6.928 \cdot 10^{-6}$ | 4.021 | ±0.684 | 0.818 | 135.13 | 32 | 78,382 | 448 | 358–550 |

* Weight is about 1000 g using equation in Table 3.
** Estimated from Yoklavich and Pikitch (1989) original data.

Dover sole to be developed when the average diameter of the advanced oocytes (stage 3) exceeds 0.85 mm; prespawning females are those taken in November–December which show no histological evidence of recent past or of imminent spawning (no postovulatory follicles nor hydrated oocytes present).

Using only specimens that met these specifications, we regressed total fecundity on female weight (without ovary) for females taken in central California and Oregon. The two regression equations were quite similar; when the data are truncated so that the ranges of female weights were equal, no statistical difference existed between California and Oregon. Combining all data, we obtained the general equation

$$\hat{Y}_F = 21{,}124 + 62.0W \quad \text{(Eq. 10)}$$

where $\hat{Y}_F$ is estimated total fecundity from the regression line, and W is ovary-free female weight in grams. Therefore, the potential annual fecundity for a 1 kg Dover sole is about 83,000 oocytes (Table 10).

Fecundity of Dover sole was estimated recently by Yoklavich

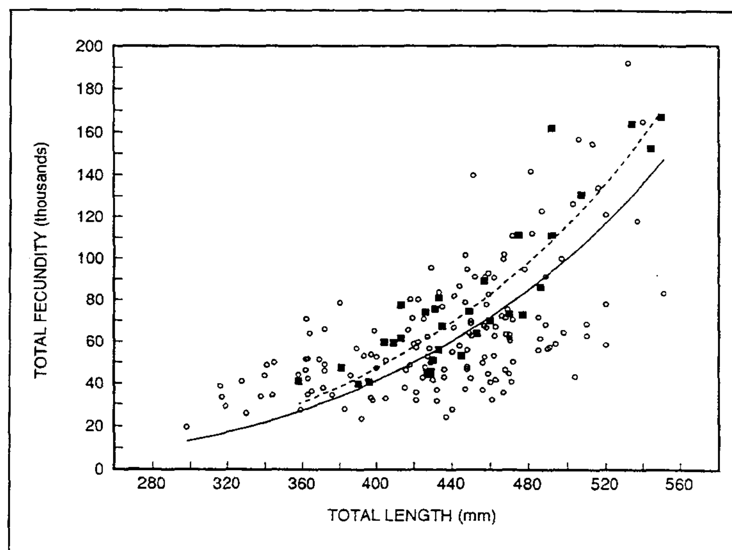



**Figure 7**

Total fecundity as a function of total length of Dover sole along the Oregon coast for our November–December 1988 data (open circles and solid line) and for data given by Yoklavich and Pikitch (1989) (filled squares and dashed line). Equations are given in Table 10.

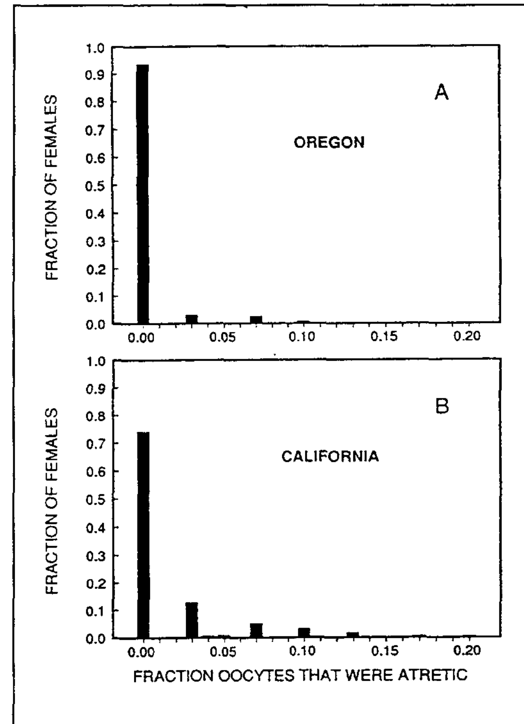



**Figure 8**
Relative frequency distribution of the fraction of the random sample of advanced yolked oocytes measured that were atretic ($\alpha$ stage) from the Dover sole *Microstomus pacificus* females used in estimates of total fecundity shown by state: Oregon females, $N$ 189; California females, $N$ 361.

and Pikitch (1989) for females caught along the Oregon coast. Those authors used an exponential model and expressed annual fecundity as a function of length. The distribution of points in our data broadly overlapped the data of Yoklavich and Pikitch (1989) (Fig. 7). To compare our Oregon data with theirs, we truncated ours so that the length ranges of the two sets coincided and applied an analysis of covariance to log-transformed data. Analysis of covariance indicated that no significant difference existed between the two equations ($F_{1,81}$ 2.03, $P$ 0.158). In Table 10, the exponential equation for fecundity as a function of length is given for our data (not truncated) and for that of Yoklavich and Pikitch (1989). In summary, we found no statistical difference between California and Oregon, nor between our Oregon data and that of Yoklavich and Pikitch (1989).

## Atretic losses

In fishes with determinate fecundity, a key question is whether atretic losses during a season constitute an important fraction of the potential annual fecundity. We identified whole atretic oocytes under a microscope while doing our fecundity work. To measure atretic losses, we counted the number of atretic oocytes ($\alpha$ advanced yolked oocytes) occurring in a random sample of 30 advanced yolked oocytes for each of the females used to estimate total fecundity ($N$ 550).

In the fish used to estimate fecundity, the average fraction of advanced yolked oocytes that were atretic was low with the mean 0.015 (SD 0.032, $N$ 361) in California, and even lower in Oregon females (mean 0.0033, SD 0.014, $N$ 189). Atretic oocytes were observed in only 26% of California females and in only 6% of Oregon females (Fig. 8).

The total fecundity of California females was negatively correlated with the fraction of oocytes in the ovary that were atretic. A stepwise multiple regression of female weight, elapsed time, and fraction atretic on total fecundity (Table 11) indicated that the coefficient for the fraction

**Table 11**
Analysis of the relation between total fecundity ($\hat{Y}_F$) of Dover sole *Microstomus pacificus* ovary-free body weight, elapsed days since 1 November, and fraction of atretic oocytes using stepwise regression. Specimens from California.

| Stepwise regression | | | |
|---|---|---|---|
| Step | 1 | 2 | 3 |
| Constant | 23,335 | 40,296 | 40,976 |
| Weight | 37.7 | 40.8 | 41.4 |
| $t$* | 2.11 | 14.03 | 14.23 |
| Elapsed days | | −205 | −206 |
| $t$ | | −7.85 | −7.94 |
| Fraction atretic | | | −63,278 |
| $t$ | | | −2.17 |
| S | 19,317 | 17,868 | 17,776 |
| $r^2$ | 28.99 | 39.41 | 40.20 |

| Analysis of variance | | | | | |
|---|---|---|---|---|---|
| Source | DF | SS | MS | $F$ | $P$ |
| Regression | 3 | $7.58 \times 10^{10}$ | $2.53 \times 10^{10}$ | 79.99 | <0.001 |
| Error | 357 | $1.13 \times 10^{11}$ | $3.16 \times 10^{8}$ | | |
| Total | 360 | $1.89 \times 10^{11}$ | | | |

| Source | DF | Sequential SS |
|---|---|---|
| Weight | 1 | $6.28 \times 10^{10}$ |
| Elapsed days | 1 | $1.15 \times 10^{10}$ |
| Fraction atretic | 1 | $1.49 \times 10^{9}$ |

* For $P$ = 0.05, $t$ 1.97.

of oocytes that were atretic was significant and negative. According to the equation, when 10% of the advanced oocytes were atretic, total fecundity in a 1 kg female was about 8% lower than when no advanced yolked oocytes were atretic. This analysis indicated that atretic losses of potential annual fecundity occurred, but on a population basis such losses were negligible. No relation between fecundity and atresia existed for Oregon females, probably because atresia was less prevalent in Oregon, with only 6% of females effected compared with 26% in central California.

The ovaries of many more females were examined histologically for atresia (*N* 2607) than were examined using the anatomical method, because we restricted the anatomical work to the fish used to measure total fecundity. Only 2% of all females examined histologically (Table 2) had ovaries in which 50% or more of the advanced yolked oocytes were in $\alpha$ atresia, but minor atresia was more common. Minor atresia occurred in 52% of the nonspawning California females and in 35% of the nonspawning Oregon females (Table 2).

The histological method was more sensitive than the anatomical one. Alpha atresia of advanced yolked oocytes was detected at least twice as frequently using histological techniques. The histological method was more sensitive because we could detect more subtle changes in oocyte structure and because we scanned about 150 oocytes per ovary, compared with 30 oocytes in the anatomical method. Despite the lack of sensitivity, the anatomical method was valuable because the standing stock of atretic oocytes could be easily estimated and directly related to total fecundity.

The histological evidence indicated that females with $\alpha$-atretic advanced yolked oocytes were more common in central California waters than off Oregon. However, season and locality were confounded because most females from Oregon were taken prior to the spawning season while most females from California were taken during the season. To determine if either season or locality affected the relative frequency of atretic females, we combined the minor-atresia and major-atresia classes for California and Oregon and fit the stepwise logistic model

$$P = \frac{e^{\beta_0+\beta_1 X_1+\beta_2 X_2}}{1+e^{\beta_0+\beta_1 X_1+\beta_2 X_2}} \quad \text{(Eq. 11)}$$

to the data (Table 12), where P is the fraction of females with atretic oocytes. The independent variables for location ($X_1$) are $-1$ for California and 1 for Oregon, and for season ($X_2$) are $-1$ for prespawning and 1 for during spawning. The estimates of coefficients for the equation are $\beta_0 = -0.183$ (SE 0.056) and $\beta_1 = -0.288$ (SE 0.056) ($\beta_2$ is not given because effect of season was not significant in an early regression analysis); the estimate of the atresia rate P for California is 0.525 (95% CI 0.479–0.572; Carter et al. 1986) and for Oregon is 0.384 (95% CI 0.338–0.433).

**Table 12**

Histological determination of number of Dover sole *Microstomus pacificus*, with $\alpha$ atresia of advanced yolked oocytes expressed as a percentage of all females with advanced yolked oocytes taken in central California and in Oregon, beginning (November–December) and during (January–May) the spawning season.

| | Spawning season | | | | | | |
|---|---|---|---|---|---|---|---|
| | Beginning | | During | | Beginning + During | | |
| State | % | *N* | % | *N* | % | 95% CI | *N* |
| Central California | 59.2 | 71 | 51.9 | 617 | 52.5 | 47.9–57.2 | 688 |
| Oregon | 36.8 | 536 | 49.4 | 83 | 38.4 | 33.8–43.3 | 619 |

**Table 13**

Mean relative batch fecundity for five batch-order numbers (B), from the first batch to the last batch.

| Batch-order no.[a] (B) | No. of females | Relative batch fecundity (oocytes/female wt(g)) Mean | SD |
|---|---|---|---|
| 1 | 4 | 2.421 | 3.810 |
| 2 | 12 | 11.661 | 4.410 |
| 2<B<U-1 | 19 | 10.489 | 3.184 |
| U-1 | 11 | 12.378 | 8.035 |
| U | 9 | 7.835 | 5.723 |

**Analysis of variance on five batch orders**

| Source | DF | SS | MS | *F* | *P* |
|---|---|---|---|---|---|
| Batch order | 4 | 371.2 | 92.8 | 3.44 | 0.015 |
| Contrast[b] | 1 | 352.6 | 352.6 | 13.08 | 0.001 |
| Error | 50 | 1347.6 | 27.0 | | |
| Total | 54 | 1718.8 | | | |

[a] First batch spawned, B = 1; last batch spawned, B = U; penultimate batch, B = U-1.
[b] Comparison of relative fecundity of the first and last batch to the other batches.

**Table 14**

Comparison of fecundity between the penultimate batch and the last batch within the same Dover sole *Microstomus pacificus* female.

| | Female weight (g) | Batch fecundity* (no. of oocytes) Penultimate | Last |
|---|---|---|---|
| | 270.00 | 4634 | 2121 |
| | 270.00 | 3716 | 323 |
| | 324.50 | 9374 | 1025 |
| | 539.00 | 11445 | 2903 |
| | 703.86 | 3906 | 411 |
| | 713.24 | 12213 | 6455 |
| | 752.11 | 2843 | 124 |
| | 793.54 | 6134 | 5856 |
| | 824.66 | 7022 | 3820 |
| | 1017.70 | 3621 | 29 |
| | 1247.82 | 11047 | 250 |
| Mean | 677.9 | 6905 | 2120 |
| SD | 309.8 | 3525 | 2355 |

*Paired $t$-test: $t$ 4.99, df 10, $P$ 0.0005.

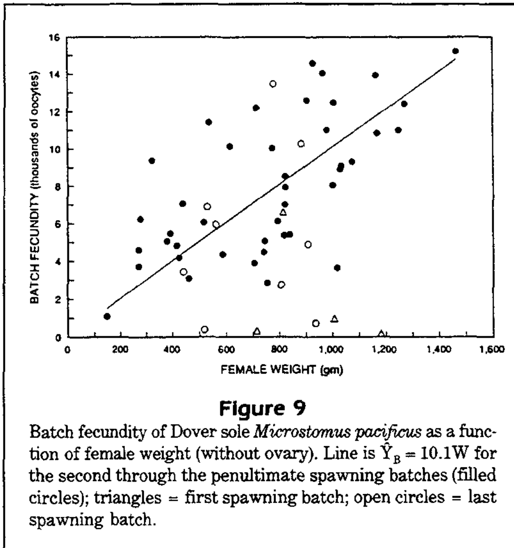



**Figure 9**

Batch fecundity of Dover sole *Microstomus pacificus* as a function of female weight (without ovary). Line is $\hat{Y}_B = 10.1W$ for the second through the penultimate spawning batches (filled circles); triangles = first spawning batch; open circles = last spawning batch.

This computation indicated that the occurrence of females with $\alpha$ atresia of advanced oocytes was significantly affected by locality of the samples but *not* by season. In short, more California females had one or more $\alpha$-atretic advanced oocytes in their ovary than did Oregon females.

## Batch fecundity

The first step in our analysis of batch fecundity ($\hat{Y}_B$) was to determine if the batch size varied with the order of spawning. Analysis of variance indicated that a significant batch-order effect existed (Table 13). The mean relative fecundity of the first (1) and last spawning batch (U) were significantly lower than the other batches (Table 13).

In eleven females, the only advanced oocytes left in the ovary were two "hydrated" spawning batches (Table 14). Each was in a different stage of development: one was fully hydrated (last batch), and the other was in the migratory nucleus stage (penultimate batch). In all of the eleven females, the last batch was always lower than the penultimate batch. The $t$-test for paired differences confirmed the effect of batch order on fecundity indicated by the ANOVA. The $t$-test also had less potential for bias because we used absolute rather than relative batch fecundity. The $t$-test indicated that the fecundity of the last batch differed from the penultimate batch ($t$ 4.99, df 10, $P$ 0.0005).

We concluded that the batch size of a female Dover sole did change over the spawning season, with the last and the first batch being lower than the rest.

We determined the relation between batch fecundity and weight using regression analysis. We did not use the first and last batches since they were lower than the rest. The intercept for the regression of batch fecundity on female weight did not differ from zero ($a$ 2142; $t$ 1.87, df 40, $P$ 0.07). Therefore we forced the regression line through 0, yielding the relationship $\hat{Y}_B = 10.1W$, where female weight ranged from 148 to 1464 g (Fig. 9). This analysis indicated that the relative batch fecundity of Dover sole is about 10 oocytes per gram ovary-free female weight, except for the first and last batch. The relative fecundity for the first and last batches combined was also about 10 oocytes per gram (1 and U in Table 13). Thus the number of potential spawnings (S) per year can be calculated using $S = (\hat{Y}_{FR}/10) + 1$, where $\hat{Y}_{FR}$ is the relative potential annual fecundity ($\hat{Y}_F/W$; $\hat{Y}_F$ from Eq. 10). This means that the average 1 kg female spawns its 83,000 advanced yolked oocytes in about nine batches.

## Sexual maturity

To determine the optimal criteria for sexual maturity in female Dover sole, we established six sets of histological criteria for maturity (Table 15). The first set of criteria selects females with either advanced yolked

**Table 15**

Six sets of histological criteria for female sexual maturity in Dover sole *Microstomus pacificus*, with the mean length of the females in each set. (o) not present; (+) present; (−) not considered.

| | | Advanced yolked oocytes | | Post-ovulatory follicles | Early yolked oocytes | | Unyolked oocytes | | | Mean length (mm) | | No. of females Calif. + Oregon (N 2595) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Criteria set no. | Certainty | with α atresia | no α atresia | | with β atresia | only α atresia or none | with β atresia | only α atresia | no atresia | $\bar{x}$ | ±2 SE | No. in class | Cumulative percent |
| 1 | Certain maturity | + | + | + | − | − | − | − | − | 434 | ±3 | 1343 | 52 |
| 2 | Uncertain | o | o | o | + | − | − | − | − | 414 | ±7 | 218 | 60 |
| 3 | Uncertain | o | o | o | o | + | − | − | − | 397 | ±11 | 77 | 63 |
| 4 | Uncertain | o | o | o | o | o | + | − | − | 379 | ±6 | 279 | 74 |
| 5 | Uncertain | o | o | o | o | o | o | + | − | 350 | ±5 | 432 | 90 |
| 6 | Certain immaturity* | o | o | o | o | o | o | o | + | 297 | ±10 | 246 | 100 |

*Defined as certain immaturity because no histological evidence exists for maturity.

oocytes or postovulatory follicles. The sexual maturity of these females is certain, but some mature females may be excluded if only the first set of criteria are used. Criteria sets 2 to 5, if added to the first set, broaden the maturity definition to include females having ovaries in the earliest stages of vitellogenesis and those showing possible signs of past reproductive activity (β- or α-stage atresia). Each additional criteria set that one might add to the first set increases the risk that immature fish will be classed as mature. Females in set 6 are considered to be immature because they have none of the characteristics mentioned in the other five sets.

Use of β atresia as a possible sign of past reproductive activity seems justified. Females with early yolked oocytes and β atresia (set 2) were larger on the average than those with no β atresia (set 3; $t$ 2.45, $P$ 0.015, df 293); and females with unyolked oocytes and β atresia (set 4) were larger than those with only α atresia of the unyolked (set 5; $t$ 7.69, $P<0.001$, df 709). In addition, the ranking of criteria sets based on our intuitive appraisal of the risk of classification error is largely borne out by the length distributions of the females identified by the criteria set, since mean length decreased with criteria set number (Fig. 10).

To estimate the length at which 50% of the Dover sole are mature ($ML_{50}$) using all six histological criteria sets, we first used a maturity algorithm to estimate the fraction of fish that were mature in a given length-class. This algorithm is a regression method similar to those used to construct age-length keys (Bartoo and Parker 1983, Kimura and Chikuni 1987).

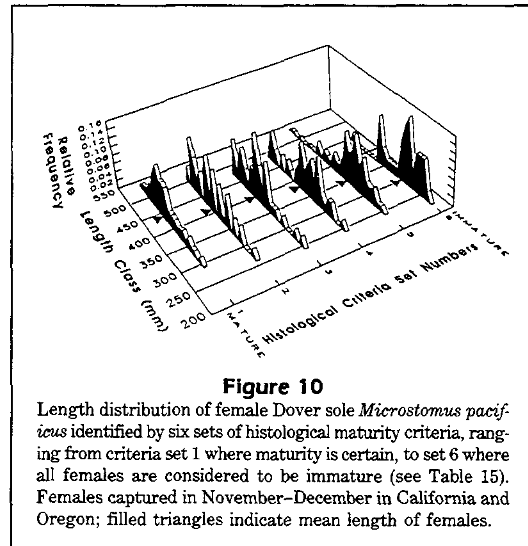



**Figure 10**

Length distribution of female Dover sole *Microstomus pacificus* identified by six sets of histological maturity criteria, ranging from criteria set 1 where maturity is certain, to set 6 where all females are considered to be immature (see Table 15). Females captured in November–December in California and Oregon; filled triangles indicate mean length of females.

This analysis was based on two equations. The first equation was

$$q_{j|i} = q_{m|i}\, q_{j|m} + (1 - q_{m|i})\, q_{j|im} \qquad \text{(Eq. 12)}$$

where $q_{j|i}$ is the fraction of fish of length-class j in the ith criteria set; $q_{j|m} = q_{j|1}$ and $q_{j|im} = q_{j|6}$ because the criteria set 1 consists of all mature (m) fish and the criteria set 6 consists of all immature (im) fish; and $q_{m|i}$ the overall fraction of mature fish in the ith cri-

teria set. The second equation assumed that $q_{m|i}$ changed linearly with criteria set number i:

$$\hat{q}_{m|i} = b_1 + b_2 i. \qquad \text{(Eq. 13)}$$

Combining equations (12) and (13) results in the final equation

$$y_{ij} = b_1 x_{1j} + b_2 x_{2j} \qquad \text{(Eq. 14)}$$

where $y_{ij} = q_{j|i} - q_{j|im}$,
$x_{1j} = q_{j|m} - q_{j|im}$, and
$x_{2j} = i(q_{j|m} - q_{j|im})$.

For each criteria set i, we obtained the estimate of the fraction of mature fish ($q_{m|j|i}$) in each length-class j as

$$\hat{q}_{m|j|i} = \hat{q}_{m,j|i}/\hat{q}_{j|i} = [\hat{q}_{m|i} q_{j|m}]/\hat{q}_{j|i}.$$

We then obtained the estimated number of mature fish of length-class j in the criteria set i ($\hat{N}_{m|j|i}$) as the product of the total number of fish at length-class j ($N_{j|i}$) and the estimated fraction of mature fish at length-class j ($\hat{q}_{m|j|i}$):

$$\hat{N}_{m|j|i} = N_{j|i}\, \hat{q}_{m|j|i}.$$

The summation of $\hat{N}_{m|j|i}$ over all criteria (i) is the total number of mature fish at length-class j ($\hat{N}_{m|j} = \Sigma_i \hat{N}_{m|j|i}$). The total number of fish in length-class j ($N_j = \Sigma_i N_{j|i}$) and the number of mature fish ($\hat{N}_{m|j}$) were used to estimate $ML_{50}$ for all females taken before the onset of spawning (California and Oregon data combined) using BMDPLR (Dixon et al. 1988).

We compared the above estimate with the $ML_{50}$ for each of the five maturity definitions created by including progressively more criteria sets (Table 16). When the definition of sexual maturity is expanded by progressively adding criteria sets 2 to 5 to the definition, the $ML_{50}$ decreased for each additional set of criteria added. Our estimate of $ML_{50}$ from the model was 332 mm and is most similar to maturity definition IV in Table 16. Thus, definition IV is the preferred histological definition of maturity because it is probably the least biased.

Inspection of Table 16 also indicated that the $ML_{50}$ is always greater when measurements are made during the reproductive season than before it begins, regardless of the number of criteria sets used to define sexual maturity. This implied that during the spawning season the ovaries of some postspawning females are reabsorbed to the extent that they become indistinguishable from females defined as immature. Thus maturity should be estimated prior to the onset of spawning, and the definition of maturity should be broader than definition I.

We believe the preferable estimate of $ML_{50}$ is one based on the maturity algorithm because it uses all the histological data, while those based on definitions use only a portion of it. The maturity algorithm should be applied only to data taken before the spawning season, since data collected later in the season will be biased. This method demands detailed histological classification which may be too costly for many purposes. Definition III could be used if tissue were examined microscopically or with a powerful hand lens, and it gives an $ML_{50}$ value close to that provided by the model.

**Table 16**

Estimated length at which 50% of Dover sole females are sexually mature, using six histological definitions of ovarian maturity and a maturity algorithm that uses all data. California and Oregon data are combined; length at 50% mature estimated using logistic model (Dixon et al. 1988).

| | | Before spawning (*N* 854 females) | | | During spawning (*N* 1321 females) | | |
|---|---|---|---|---|---|---|---|
| | | | No. of mature females | | | No. of mature females | |
| Definition no. | Histological criteria sets incl. in maturity definition[a] | Length at 50% mature (mm) | *N* | % of females | Length at 50% mature (mm) | *N* | % of females |
| I | 1 | 373 | 541 | 63 | 419 | 568 | 43 |
| II | 1, 2 | 361 | 582 | 68 | 396 | 692 | 52 |
| III | 1, 2, 3 | 348 | 626 | 73 | 391 | 720 | 54 |
| IV | 1, 2, 3, 4 | 332 | 669 | 78 | 348 | 917 | 69 |
| V | 1, 2, 3, 4, 5 | 258 | 810 | 95 | 255 | 1184 | 90 |
| Maturity algorithm | 1, 2, 3, 4, 5, 6 | 332 | 691[b] | 81[b] | 389 | 742[b] | 56[b] |

[a] From Table 15.
[b] Estimated from maturity algorithm.

**Table 17**

Final maturity thresholds and logistic model parameters* for female Dover sole *Microstomus pacificus*, taken before the spawning season in California, Oregon, and the two states combined.

| Region | Maturity definition | 50% mature | 95% CI | a | SE | b | SE | N |
|---|---|---|---|---|---|---|---|---|
| California | IV | 298 | 215–391 | −14.412 | 4.374 | 0.0483 | 0.0149 | 104 |
| Oregon | IV | 336 | 322–351 | −9.268 | 0.806 | 0.0276 | 0.0022 | 750 |
| California + Oregon | Maturity algorithm | 332 | 305–367 | −14.960 | 1.239 | 0.0450 | 0.0036 | 854 |

$^*P = \frac{e^{a+bL}}{1+e^{a+bL}}$

We combined data from Oregon and California in this analysis because sample sizes (before spawning) were inadequate for application of the model separately. However, in Table 17 we provide estimates and fitting parameters based on definition IV for each region as well as those based on the model using the combined data. The $ML_{50}$ estimated for definition IV was lower in California than in Oregon. However, analysis of covariance of the log transformation of fraction mature, $\mathrm{Ln}\left(\frac{q_m}{1-q_m}+1\right)$, on length and locality indicated that the difference between states was not significant ($P$ 0.625, $F$ 0.26). Thus our $ML_{50}$ estimate for Dover sole along the California and Oregon coasts is 332 mm with 95% CI of 315–349 mm (Carter et al. 1986; Fig. 11). The $ML_{50}$ we estimated from data in Hagerman (1952) for Dover sole from the Eureka California fishery is high (363 mm) compared with our final estimate for Oregon and California coasts (Fig. 11). However, Hagerman collected his specimens during the spawning season, and his estimate is similar to the $ML_{50}$ for females taken during the spawning season (Definition IV, Table 16).

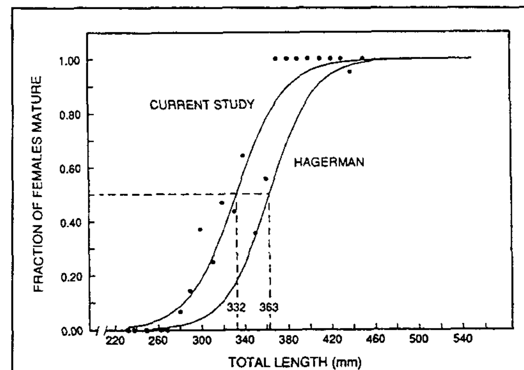



**Figure 11**

Fraction of female Dover sole *Microstomus pacificus* that were sexually mature as a function of total length. Points are for 10 mm length-class intervals; the fraction mature per length-class was assigned using maturity algorithm; data were from California and Oregon in November–December; logistic parameters are in Table 17. Present study is compared with Hagerman (1952), line only (logistic curve parameters, a −17.854, b 0.0450).

## Discussion

### Validation of fecundity assumptions

In the Introduction, we specified four assumptions required for an unbiased estimate of annual fecundity in Dover sole. These assumptions were that (1) fecundity was determinate; (2) potential annual fecundity was equivalent to actual fecundity; (3) females used to estimate annual fecundity had not spawned; and (4) recruitment of oocytes into the advanced stock of yolked oocytes had ceased for the season. The following is a review of the evidence for the four assumptions.

Five lines of evidence support the assumption of determinate fecundity for Dover sole: (1) in mature ovaries (mean diameter of advanced oocytes >0.85 mm), a hiatus existed between the advanced stock of mature oocytes and smaller, less mature oocytes; (2) total fecundity declined over the spawning season; (3) total fecundity was lower in females having postovulatory follicles; (4) the mean diameter of the advanced oocytes increased over the spawning season; and (5) our analysis of the order of spawning batches was consistent with the determinate fecundity assumption.

The second assumption, lack of significant atresia, also was supported by our analysis. Overall, atretic losses of advanced oocytes were negligible during the years of our study. Multiple regression analysis indicated that atresia had a small but significant effect on total fecundity of the California females that had

atretic oocytes. A few females suffered substantial losses in total fecundity, but such fish were rare and they had little effect on population means. Histological and anatomical evidence indicated that females with $\alpha$-atretic advanced yolked oocytes were more common in central California than in Oregon waters. Atresia might be more common in central California Dover sole because bottom sediments are contaminated. Alternatively, females with atretic ovaries may be more common in central California waters because they are living near the southern end of their range where food supply and other habitat conditions may be less than optimal. Both explanations seem equally plausible at present.

The third assumption, that females used to estimate potential annual fecundity have not spawned in the current reproductive year, would be rejected for females taken in January through May. The assumption probably held for the females used to estimate annual fecundity in November–December because only 2.9% of the females from California and only 1% of the females in Oregon showed any histological signs of past or imminent spawning. The few females that showed histological signs of spawning were not used, of course, to estimate annual fecundity. Spawning may have gone undetected in some of the females used to estimate fecundity since postovulatory follicles are eventually resorbed. This does not seem likely for the November–December case because the spawning season had just begun and resorption is probably slow at the low temperatures of Dover sole spawning habitat.

Our fourth assumption, that all the oocytes that constitute the potential annual fecundity were included in our oocyte counts, is supported by two lines of evidence. The first is that no positive correlation existed between the mean diameter of the advanced oocytes and total fecundity. Such positive correlations were eliminated by excluding all ovaries in which the mean diameter of the advanced oocytes was less than 0.86 mm. A positive correlation between diameter and fecundity existed when all ovaries were considered (range in mean diameter of the advanced oocytes, 0.71–1.04 mm). This is evidence that recruitment of oocytes into the advanced class continued until the advanced stock was well separated from early vitellogenic oocytes (stages 1 and 2, Fig. 6). The second source of evidence is the form of the oocyte size-frequency distribution. A prominent gap between stage-2 and stage-3 oocytes existed when the mean diameter of stage-3 oocytes was between 0.84 and 0.96 mm (Fig. 6). The absence of significant numbers of oocytes in the intervening diameter classes (0.55–0.65 mm) indicates maturation of oocytes across this range either had ceased or was proceeding at a very slow pace. We conclude that recruitment of significant numbers of oocytes into the advanced stock probably ceases in Dover sole when the mean diameter of the advanced stock is between 0.86 and 0.96 mm.

Some authors working with other species (Hislop and Hall 1974 on *Melangius merlangus* (L.), Horwood and Greer Walker 1990 on *Solea solea*) consider all yolking oocytes to comprise the potential annual fecundity. In Dover sole this would mean that in addition to stage 3, the most advanced yolked oocytes, stages 1 and 2 would also be used to estimate annual fecundity. Such broad criteria are acceptable if all oocytes that began vitellogenesis ultimately become a part of the mature stock of oocytes that are spawned. This was not the case in Dover sole because oocytes in the early stages of vitellogenesis (stages 1 and 2) occurred in nearly all mature ovaries, including those in which some of the batches had already been spawned. The fate and dynamics of these small partially-yolked oocytes in advanced ovaries is uncertain; their numbers might either decrease due to resorption, increase and become part of next year's production, or remain in stable numbers until later in the year. It would seem impractical to adjust estimates of potential annual fecundity based on all vitellogenic oocytes for the fraction of those oocytes which do not continue vitellogenesis. Therefore, we believe use of the more mature yolked oocytes for estimating the potential annual fecundity is preferable.

An important implication of our discussions of the third and fourth assumptions is that timing the sampling of females is a critical element in estimating potential annual fecundity: Sample too early in the reproductive cycle and the ovaries are not sufficiently mature; sample too late and spawning is prevalent. The optimal time to sample Dover sole ovaries is when the average diameter of the advanced stock is between 0.86 and 1.1 mm (Fig. 12). When the diameter is less than 0.86 mm, the numbers of advanced oocytes are still increasing (indicated by the $t$ value for the diameter coefficient in the fecundity equation, Fig. 12). When the diameter exceeds 1.1 mm, 20% or more of the females show histological signs of past or imminent spawning, and the assumption of no spawning cannot be safely made.

## Spawning rates and reproductive energetics

The spawning season of Dover sole was protracted with postovulatory follicles occurring as early as December and hydrated oocytes as late as May, indicating a season of six months. This is a long season for a fish of determinate fecundity, since typically they are high-latitude species with short, 1–2 month spawning seasons. Batch fecundity was low, averaging about 10 oocytes per gram female weight, except for the first and last batch which average about 5 oocytes per gram. Dover sole spawn about nine times during their

protracted spawning season. Vitellogenesis does not cease when spawning begins, but rather it continues throughout most of the season as the advanced stocks of yolked oocytes are matured and spawned. Spawning frequency appears to increase near the end of the season. This may cause a higher daily production of eggs by the population in late-March or April than in February, even though fewer females have active ovaries in April.

To estimate reproductive effort of Dover sole, we calculated the hypothetical weight of the ovary when the entire advanced stock of oocytes had completed vitellogenesis and hydration had begun. The weight is hypothetical because a Dover sole ovary never contains a full complement of completely yolked oocytes, since vitellogenesis of the smaller advanced yolked oocytes continues after a female begins spawning. To compute the hypothetical weight, we assumed that all oocytes completed vitellogenesis when their average diameter was 1.5 mm. Hydration begins when the advanced yolked oocytes have a mean diameter of 1.3–1.7 mm. We estimated the gonad weight of a 1000 g female with oocytes having a mean diameter of 1.5 mm, using an equation in which gonad weight was expressed as a function of fish weight and volume of the average advanced oocyte (1.5 mm diameter has a volume of 1.77 $mm^3$; California plus Oregon data; Table 3). The ovary was estimated to weigh 144 g, or about 14% of the body weight. In other words, the annual reproductive effort of Dover sole was about 14% per year, and this effort was distributed over about nine spawnings averaging about 1.6% of their body weight per spawning. Gonad weight was considered to be a measure of reproductive effort by Gunderson and Dygert (1988); but they did not adjust the gonad weight for the full complement of yolk, and consequently their estimates are not comparable to these.

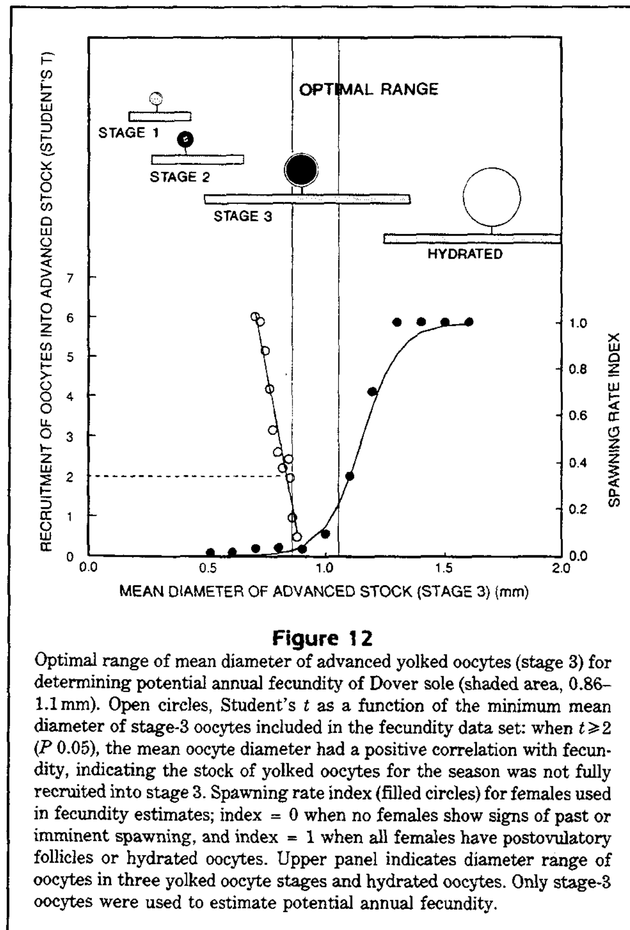



**Figure 12**
Optimal range of mean diameter of advanced yolked oocytes (stage 3) for determining potential annual fecundity of Dover sole (shaded area, 0.86–1.1 mm). Open circles, Student's *t* as a function of the minimum mean diameter of stage-3 oocytes included in the fecundity data set: when $t \geq 2$ ($P$ 0.05), the mean oocyte diameter had a positive correlation with fecundity, indicating the stock of yolked oocytes for the season was not fully recruited into stage 3. Spawning rate index (filled circles) for females used in fecundity estimates; index = 0 when no females show signs of past or imminent spawning, and index = 1 when all females have postovulatory follicles or hydrated oocytes. Upper panel indicates diameter range of oocytes in three yolked oocyte stages and hydrated oocytes. Only stage-3 oocytes were used to estimate potential annual fecundity.

## Assessment of sexual maturity

Our estimates of length at 50% mature ($ML_{50}$) were higher when females were taken during the spawning season than when they were sampled before spawning began, regardless of the histological criteria used. Thus, during the spawning season ovaries of some postspawning females had regressed far enough that they were histologically indistinguishable from immature females. This finding has two important implications: First, it indicates that even the broadest histological criteria, based on analysis of H&E sections, will not identify all postspawning females; second, it means that estimates of length or age at first maturity should always be conducted prior to the onset of spawning, when postspawning females with highly regressed ovaries are rare.

Another limit to our ability to assess sexual maturity is that we do not know how many of the females that begin vitellogenesis actually complete it during the current reproductive season. Dover sole ovaries with oocytes in the early vitellogenic stage occurred throughout the spawning season as well as before it began, indicating some females that begin vitellogenesis may not reach sexual maturity in the current

season. At this time, it is an arbitrary choice to consider as mature all females with vitellogenic ovaries or only those with advanced yolked oocytes. Our analysis showed that this arbitrary decision had a pronounced effect on $ML_{50}$ estimates. Thus the criteria for maturity estimates should be precisely specified. It is particularly important to specify the minimum level of oocyte development necessary for a female to be considered as mature. Our preferred definition of maturity included females in the early stages of vitellogenesis with yolked oocytes as small as 0.18 mm diameter, and also included some females without vitellogenic oocytes (maturity IV, Table 16). Those females without vitellogenic oocytes had $\beta$ atresia in the ovary. We believe that the presence of some $\beta$ atresia is an inevitable consequence of the resorption of an active ovary or ovulation.

No discussion of sexual maturity would be complete without mentioning the gross anatomical systems used to classify ovaries, because they are the chief method used by fishery biologists to measure sexual maturity in marine fishes. Using gross anatomical criteria, we accurately separated active ovaries (advanced yolked oocytes present) from inactive ovaries (no advanced oocytes) with classification errors of 1–12%. Determining sexual maturity is a far more difficult task, however. Identification of mature females using gross anatomical methods has the same problems with post-spawning and early vitellogenesis criteria as histological methods, but the potential for bias is greater. Anatomical criteria are less accurate and may be detectable for shorter periods than histological ones. For these reasons, differences between maturity studies should be interpreted with caution, especially when done by different observers, or with different methods, or when sampling at different times of the year. Many investigators have not been particularly careful to restrict sampling to early in the spawning season. The tendency will be to overestimate the $ML_{50}$ using anatomical methods, especially when samples are taken midseason.

In an earlier paper on Dover sole, Hunter et al. (1990) concluded that size at 50% mature in Dover sole from central California in the 1980s differed from that of Dover sole in northern California in the late 1940s as determined by Hagerman (1952). Although a statistical difference existed between these two data sets, we are inclined to dismiss this difference, since it could be due to differences in criteria and sampling times. Similarly, Yoklavitch and Pikitch (1989) speculated that size at 50% maturity of Oregon Dover sole has changed because their estimate of maturity differed from Harry (1959). We believe that this difference also could easily be due to differences in criteria and timing of sampling. Our analysis of histological criteria for maturity clearly shows that differences in criteria or timing of sampling can produce differences in the $ML_{50}$ as large as any of those seen in the Dover sole literature.

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