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ESTIMATION OF ANNUAL MORTALITY RATES FOR EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*) IN CHESAPEAKE BAY BASED ON BOX COUNTS AND APPLICATION OF THOSE RATES TO PROJECT POPULATION GROWTH OF *C. VIRGINICA* AND *C. ARIAKENSIS*

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ABSTRACT In an effort to restore the ecological role of oysters in Chesapeake Bay and the economic benefits of a commercial fishery, the states of Maryland and Virginia are considering introducing the nonnative Asian oyster (*Crassostrea ariakensis*) into the Bay. As part of an ecological risk assessment (ERA) to evaluate the proposed action and alternatives, demographic modeling is being used to project the change in populations of both the Asian and the native eastern oyster (*C. virginica*) in the Bay across space and time. Annual mortality rates are vital input to the demographic model. We present two approaches for parameterizing mortality rates for *C. virginica* by salinity ranges and disease-intensity categories and discuss how these rates could be applied to project population growth for the Asian oyster. We estimated mortality rates from empirical data collected during annual dredge surveys of oyster beds in Maryland. We compared counts of recent boxes (dead oysters without fouling or sedimentation on the inner valve surfaces, including "gapers" of one or two weeks old with tissue remaining in the shell), old boxes (dead oysters without tissue remnants but with fouling, sedimentation or both on the inner valve surfaces), and live oysters in market-size and small classes. Our mortality estimates based on counts of recent boxes consistently differentiated between years with high disease intensity and those with low disease intensity, between wet and dry years, and between salinity zones. In contrast, traditional estimates of yearly mortality based on total box counts often were out of phase with measured levels of disease intensity and weather (dry or wet). To model populations of *C. ariakensis*, we propose to adjust the mortality rates for *C. virginica* based on research results that provide estimates of differences between the two species' resistance to MSX and dermo and to other mortality factors, such as predation.

KEY WORDS: natural mortality, *Crassostrea virginica*, *Crassostrea ariakensis*, Chesapeake Bay, population model, ecological risk assessment

INTRODUCTION

The eastern oyster (*Crassostrea virginica* [Gmelin 1791]) historically supported a valued fishery and formed an important component of the Chesapeake Bay's ecosystem. In recent years, the abundance of eastern oyster in the Chesapeake Bay has declined to less than 1% of estimated virgin stock because of intense fishing pressure during the 19th and 20th centuries, habitat destruction, degraded water quality, and disease (NRC 2004, Gottlieb & Schweighofer 1996). The states of Maryland and Virginia recognized the need to reverse the decline in oyster stocks to restore the ecological role of oysters in the Bay and the economic benefits of a commercial fishery. To achieve those objectives, managers in Maryland and Virginia have proposed options for increasing the biomass of oyster stocks (NRC 2004), including the deliberate release of diploid Asian oysters (*C. ariakensis* [Fujita 1913]) into Chesapeake Bay on a large scale to establish a self-recruiting population.

A comprehensive study of the potential effects of introducing the nonnative Asian oyster into Chesapeake Bay is being conducted to support a programmatic environmental impact statement (EIS) that will evaluate alternative restoration strategies. The EIS will address the proposed action to introduce diploid *C. ariakensis* and seven restoration alternatives, which include stocking and aquaculture of the Asian oyster and of the native eastern oyster. The comprehensive study, known as an ecological risk assessment (ERA), is being conducted as one element of the overall EIS. The ERA will identify the ecological risks and benefits posed by each of the eight restoration alternatives.

Evaluating the ecological effects of the proposed restoration alternatives requires constructing a demographic model that can project the change in populations of Asian and eastern oyster in the Bay that might result from implementing each alternative. The model must account for the primary population dynamics: growth, stock recruitment, and mortality. The reliability of model projections depends on the validity of the various input parameters, including mortality. Accurate and precise estimates of annual natural mortality rates (M) for Asian and eastern oyster are critical to the performance of the demographic model and the reliability of conclusions to be drawn from the ERA and the EIS.

The accuracy and precision of estimates of mortality are determined by the quantity and quality of the data and the validity of methods used to calculate the estimates. The Maryland Department of Natural Resources (DNR) collects empirical data through annual surveys of oyster beds in Maryland. Samples of oysters are collected by dredging at representatively selected oyster bars during fall. Oysters from each sample are sorted by size and classified into one of two mortality categories: live oyster or "box." The box category refers to dead, articulated shells. DNR classifies boxes further as "recent" (open shells with tissue remaining inside them, known as "gapers," and empty shells without fouling or sedimentation on the inner valve surfaces) or "old" (empty shells with fouling, sedimentation or both on the inner valve surfaces). DNR estimates annual mortality as the proportion of boxes to live oysters (Tarnowski 2003). Natural mortality of oysters in Delaware Bay and Virginia is also estimated from annual collections of live oysters and boxes (Southworth et al. 2005, Ford et al. 2006). Ford et al.

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(2006) found that mortality rates obtained from counts of recent boxes and rates based on total box counts both provided reliable indices of total mortality for the year prior to the survey.

Here we describe a method for estimating annual mortality based solely on counts of recent boxes of small (shell height from 40–76 mm) and market-size (shell height $\geq$ 76 mm) oysters. We compare estimated mortality rates for Chesapeake Bay based on counts of recent boxes with estimates based on total box counts (Jordan et al. 2002, Jordan & Coakley 2004). We estimate oyster mortality rates for specific ranges of salinity and disease intensity to enhance their applicability for spatially explicit modeling of oyster population dynamics, and we discuss how the rates for *C. virginica* could be adapted to model population growth for *C. ariakensis*.

MATERIALS AND METHODS

We analyzed data from Maryland DNR's annual fall survey of oysters for the period from 1980–2005. The survey is conducted mostly during October (early November in some years) using a standard oyster dredge to collect samples of live oysters and boxes from 200–400 representative oyster bars (Tarnowski 2003). A fixed set of 43 "disease bars" has been sampled every year since 1980; length frequency data and tissue samples from each size class (for disease studies) have been collected annually from these bars since 1990. A composite sample of one Maryland bushel ($\sim$0.046 m^3) is collected at each of the disease bars by pooling two, ½-bushel subsamples from replicate tows, and at each bar in seed-production areas by pooling five, 1/5- bushel subsamples from replicate tows. At all other bars, DNR collects a ½-bushel sample from a single tow. DNR has reported counts of recent and old boxes-per-bushel separately for small and market-size oysters since 1991 and for spat (age 0) since 1992. The counts of live oysters and boxes of age 1+ in each sample is classified into two size categories (shell height; market: $\geq$76 mm; small: <75 mm). Spat is identified morphologically by their asymmetric valves. Spat have one valve that is thinner and narrower than the other (the lower valve if they are oriented parallel to the substrate; M. Tarnowski, MD DNR personal communication). All counts are standardized to a volume of one bushel. We estimated mortality rates using only the counts of small and market-size boxes. Box counts for spat are considered to be unreliable because boxes can break apart easily during collection.

We made the following assumptions to estimate mortality from counts of recent boxes: (1) time since death (TSD) is between one and two weeks; (2) the instantaneous (1-wk) mortality rate is constant from June to October; and (3) the cumulative natural mortality from October through May is negligible. Data from an intense mortality study conducted by Maryland DNR in the Choptank River during 2002 provide some support for assumptions 1 and 2. The Choptank study was conducted when freshwater run-off into the Bay was low to estimate the extent to which mortality might increase because of increased *Haplosporidium nelsoni* (MSX disease) infection. MSX is likely to cause additional mortality during years in which salinity is high because of drought. Maryland DNR counted live oysters and boxes from one-bushel samples at each of six oyster bars located in waters that normally are moderately salty every month from June through August, and during the annual fall oyster survey in October 2002. DNR's empirical

data from the study in the Choptank River during 2002 (results summarized later in this paper) support assumptions (1) and (2). Assumption (3) is supported by numerous studies showing that natural mortality from *Perkinsus marinus* (dermo disease) and *H. nelsoni* (MSX) occurs primarily from early summer to October (Andrews 1996, Burreson & Ragone Calvo 1996, Ford & Tripp 1996, Ford & Haskin 1982) and that predation on oysters is greatest during summer months (Carriker 1955, Manzi 1970, Gunter 1979, Garton & Stickle 1980, Pearse & Wharton 1938, Landers & Rhodes 1970). When using total box counts to estimate mortality rates, we followed Jordan et al. (2002) and assumed that the TSD of a box is up to one year. According to that assumption, all boxes observed in the fall survey died within one year prior to the survey, and shells from oysters that died during the year remained articulated until the fall.

To refine the parameters for use in our demographic model, we estimated mortality rates for specific levels of salinity and disease intensity. Dermo infections in Chesapeake Bay are heaviest and cause most mortality at medium and high salinities (NRC 2004). We calculated average annual mortality rates by poststratifying the counts of live oysters and boxes from all oyster bars across years by salinity class and disease tier. The mean salinity from May through September for each bar by year was estimated from the nearest Maryland Chesapeake Bay Monitoring Program station, or interpolated by kriging. We classified the yearly observations of live oysters and boxes from each bar into three salinity (S) classes (low: $S < 11$ ppt; medium: $11 \leq S < 15$ ppt; and high: $S \geq 15$ ppt). The salinity thresholds were defined in consultation with Chesapeake Bay oyster biologists. Maryland DNR uses an index of disease intensity ranging from one to seven based on pathogen concentration in hemolymph or solid tissue (see Gieseker 2001) to classify dermo disease-intensity into three tiers (Tarnowski 2003). Tier 1 (dermo intensity > 2.85) and Tier 2 (2 < dermo intensity $\leq$ 2.85) represent years with high and medium disease intensity, respectively; Tier 3 (dermo intensity $\leq$ 2) represents years with relatively low disease intensity (Fig. 1). Disease tiers 1 and 2 are $\boxed{\text{F1}}$ generally associated with dry (lower 25 percentile of yearly USGS flow estimates from 1937–2003) (USGS 2004) and average (normal range of flow, 25–75 percentiles) years; Tier 3, indicating low disease-intensity, generally is associated with wet years (>75 percentile of yearly flow estimates) (Table 1). $\boxed{\text{T1}}$

We calculated mean annual mortality rates for each size class by salinity zone j and disease tier k based on counts of live oysters and recent boxes from each bar over time ($i = 1, ..., n_{jk}$), using the following equations:

$$S_{jk} = \frac{\sum_i live_{ijk}}{\sum_i (live_{ijk} + newbox_{ijk})} \quad (1.1)$$

$$m_{jk} = -\log(S_{jk}) \quad (1.2)$$

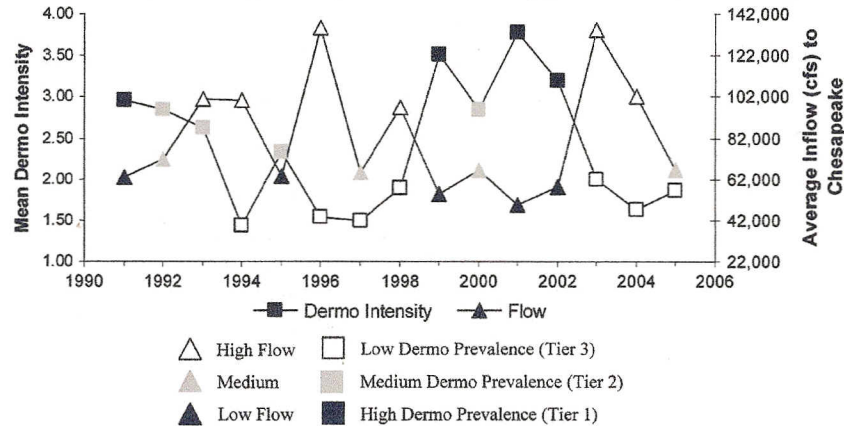$$totm_{jk} = 1 - \exp^{(-m_{jk} \times T)} \quad (1.3)$$

where: s_{jk} = 1- or 2 -week survival rate,

$live_{jk}$ = number of live oysters,

$newbox_{jk}$ = number of recent boxes,

m_{jk} = instantaneous (1- or 2-week) mortality,

Figure 1. Annual mean dermo disease intensity across 43 MD oyster bars and mean discharge (liters/sec) into the Chesapeake Bay from 1991–2005 (USGS 2004). High, medium, and low dermo intensity correspond to disease Tiers 1–3; high, medium and low flow correspond to dry (lower 25th percentile), average (25–75th percentiles), and wet years (upper 75th percentile) based on USGS flow estimates from 1937–2005.

T = expansion factor to total number of weeks (20) when natural mortality occurs ($T = 20$ for 1-wk mortality, and $T = 10$ for 2-wk mortality)

$totm_{jk}$ = mean annual mortality rate

across all bars by salinity zone j and disease tier k during the time series. The numerical representation of instantaneous and annual proportional mortality rates above follows Ricker (1975).

For comparison, we also calculated the annual mortality rates by size class (except spat) from counts of recent and old boxes using the estimator

$$totm_{jk} = \frac{\sum_i allboxes_{ijk}}{\sum_i (live_{ijk} + allboxes_{ijk})}, \qquad (1.4)$$

where $allboxes_{ijk}$ is the total number of recent and old boxes. The ratio estimators (Eqs. 1.1 and 1.4) provide a weighted mean mortality across individual bars, with weights proportional to the number of live and dead oysters at each bar. Mortality estimates for individual bars are highly skewed (Appendix A). We applied the same assumption as Jordan et al. (2002), Jordan and Coakley (2004), and Ford et al. (2006) that shells from dead oysters (boxes) remain articulated for no more than one year, on

average. Our estimates are based on the total box counts from Maryland DNR's dredge surveys of 200–400 bars per year from 1991–2005. Jordan and Coakley (2004) based their yearly estimates on data for the 43 disease bars sampled from 1985–2000. The mean annual mortality rate, or actual mortality rate (Ricker 1975), based on either method is an estimate of the annual expectation of death for an individual oyster.

We calculated the mortality rates from Eqs. (1.3) and (1.4) and the associated variances by bootstrapping (Efron & Tibshirani 1993). We ran 2,000 bootstrap resamples, each consisting of n_{jk} randomly selected observations (with replacement) where n_{jk} is the number of samples collected across all bars over the time series of fall surveys (or yearly) in the respective salinity zone and disease tier. The standard error and the 95% confidence interval (CI) for the annual mean mortality rates were estimated directly from the distribution of the bootstrap estimates. The mean annual mortality rates based on recent box counts were calculated under the assumption that the TSD was 1-wk, and 2-wk. We then pooled the two bootstrap distributions to obtain estimates of mean mortality rates and variances that represent uncertainty in the TSD i.e., allowing TSD to vary between 1 and 2 wk). Our estimates of mortality are expressed as means with associated 95% confidence limits in brackets, unless otherwise noted.

RESULTS

From 1980–1985, a period when disease intensity of dermo and MSX were limited, the estimated average annual mortality rate of market-size oysters across salinity zones based on total box counts was 0.153 (0.147–0.158). The natural mortality increased significantly after 1985, with an average annual rate of 0.293 (0.289–0.298) from 1986–2006. Mortality estimates were generally higher for dry years (e.g., 2002) with elevated disease levels than for wet years (e.g., 1994) with below-average disease levels (Fig. 2 and Fig. 3). The discrepancy between our yearly mortality estimates from total box counts and those reported by Jordan and Coakley (2004, Fig. 2) is due primarily to a bias in their estimates because of an offset of one year (see Discussion).

TABLE 1.

Proportion of years (1991–2005) in each disease tier for three climatic condition classes (dry, average, or wet years) as defined by run-off measured by USGS. Tiers 1–3 represent high, medium, and low disease intensity, respectively.

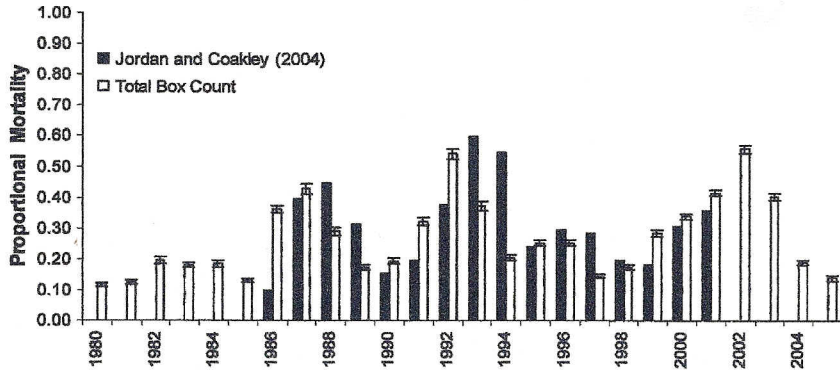| Condition	Disease Tier		
	1	2	3
Dry	0.80	0.20	0.00
Average	0.00	0.75	0.25
Wet	0.00	0.17	0.83

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Figure 2. Yearly natural mortality rates for market-size oysters based on total boxes across all Maryland bars surveyed, versus estimates of mortality reported in Jordan and Coakley (2004). Error bars for our estimates represent 95% confidence intervals.

The annual mortality rate estimated across all years and salinity regimes based on recent boxes (TSD 1–2 wk) averaged 0.37 (0.17–0.56) and did not differ significantly from our estimated average mortality rate based on total boxes, 0.30 (0.29–0.31). The average mortalities by salinity zones based on recent (TSD of 1–2 wk) and total boxes did not differ significantly (Fig. 4), and the 95% confidence intervals for our estimates overlapped with mortality rates provided by Jordan and Coakley (2004). Table 2 shows estimates of the average annual mortality rates by salinity-class and disease-intensity based on recent boxes according to different assumptions for TSD.

Our mortality estimates for market-size oysters by year based on recent boxes (TSD of 1–2 wk) differed significantly from estimates based on total boxes for 12 of 15 y (Fig. 3). Estimates based on the number of recent boxes generally improved the separation of mortality rates for dry and wet years (Fig. 3). Mortality estimates based on recent boxes were significantly lower than those derived using total boxes for 5 of 6 wet years and significantly higher for 4 of 5 dry years. In 2003, a wet year, the mortality rate for market-size oysters based on total boxes, 0.40 (0.38–0.42), was significantly higher than the mortality rate based on recent boxes, 0.17 (0.06–0.28).

Mortality rates based on recent boxes (TSD of 1–2 wk) for market-size and small oysters by salinity zone increased consistently with increasing salinity and differed greatly from mean

mortality rates across salinity zones in dry years such as 1991 and 2002 (Fig. 5). In 2002, for example, which was the second dry year in a row, mortality rates for market-size oysters were 0.88 (0.73–1.00), 0.69 (0.44–0.97), and 0.43 (0.19–0.66) for high, medium, and low salinity classes, respectively, as compared with 0.75 (0.52–0.98) for a mean 2002 annual mortality rate across salinity zones. Using a mean mortality rate regardless of salinity would generally underestimate mortality for oysters in high salinity areas and overestimate mortality for oysters in low salinity areas.

To ensure that results from the predictive demographic model being used in the ERA correctly account for the effects of salinity, the mean annual mortality rate based on recent box counts was estimated individually for each salinity class and disease tier and was compared with mortality rates based on total box counts. In contrast to the mean mortality rates based on total box counts, the mean mortality rates based on recent box counts consistently differentiated between salinity classes and disease tiers (Fig. 6). In high salinity, mean mortality rates based on recent box counts (TSD of 1–2 wk) for market-size oysters were 0.79 (0.57–1.00), 0.51 (0.27–0.75), and 0.23 (0.09–0.37) for disease tiers 1, 2, and 3 respectively; whereas respective mean mortality rates based on total box counts for market-size oysters were 0.45 (0.44–0.45), 0.53 (0.50–0.53), and 0.26 (0.24–0.28).
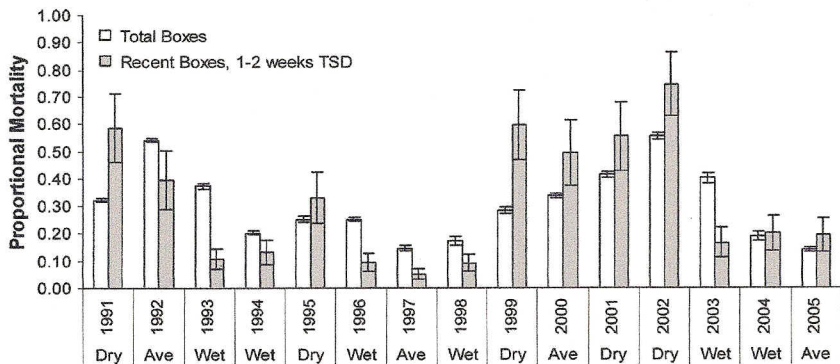

Figure 3. Yearly mortality estimates for market-size oysters (>76 mm) based on recent and total boxes, under the assumptions of average time-since-death (TSD) of 1–2 -weeks and 1-y, respectively. The climatic condition (dry, average, wet) for each year is based on USGS flow estimates. Error bars represent 95% confidence intervals.
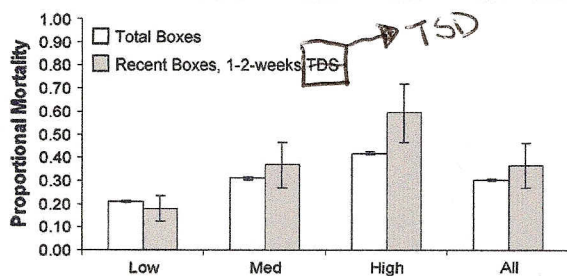
Figure 4. Mean annual natural mortality rates by salinity class for market-size oysters over all years (1991–2005). Error bars represent 95% confidence intervals. We assumed an average TSD of 1 y for total boxes, and 1–2-wk for recent boxes. Mortality estimates per salinity class reported in Jordan and Coakley (2004) are also shown.

Figure 7 shows monthly mortality rates for market-size and small oysters in June, July, August, and October from the study conducted by Maryland DNR in a medium salinity section of the Choptank River during 2002. For comparison, we included the cumulative mortality rate from June to October and an annual mortality rate using our method based on recent box counts (TSD of 1–2 wk) in the Maryland DNR fall oyster survey. Monthly mortality rates for market-sized oysters were 0.31 (0.11–0.51), 0.36 (0.15–0.58), 0.33 (0.14–0.52), and 0.22 (0.08–0.36) for June to August and October, respectively. The cumulative mortality rate from June to October was 0.78 (0.44–0.91); September's mortality rate was imputed using the mean of the August and October estimates. The estimated average annual mortality rate based on the recent box counts from the fall oyster survey for medium salinity and Tier 1 disease intensity was 0.59 (0.34–0.83).

DISCUSSION

Estimates of natural mortality rates for oysters (age 1+) based on counts of recent boxes appear to be more accurate (i.e., closer to the true average mortality) than estimates based on total box counts for Chesapeake Bay. Mortality estimates based on recent boxes increase consistently with increasing disease intensity and salinity, and they are higher during years of drought than during wet years, when reduced salinity commonly decreases disease mortality from MSX and dermo (Matthiessen et al. 1990, Gottlieb & Schweighofer 1996). The mortality rates estimated from total box counts, in contrast, did

TABLE 2.

Estimates of proportional annual natural mortality by size class, salinity zone, and disease intensity (Tier), based on counts of live oysters and recent boxes from 1991–2005 dredge surveys. The relative standard error, RSE = S.E./Mean; LCL, and UCL are lower and upper 95% confidence limits.

| TSD Recent Boxes (Weeks)	Salinity	Tier	Market-Size Oysters				Small Oysters			
			Mean	RSE	LCL	UCL	Mean	RSE	LCL	UCL
1	High	1	0.90	0.01	0.88	0.91	0.81	0.02	0.78	0.84
1	High	2	0.63	0.05	0.57	0.68	0.59	0.03	0.56	0.62
1	High	3	0.30	0.09	0.26	0.34	0.44	0.05	0.40	0.47
1	Med	1	0.71	0.02	0.68	0.74	0.68	0.03	0.65	0.71
1	Med	2	0.54	0.04	0.51	0.57	0.49	0.05	0.45	0.53
1	Med	3	0.17	0.07	0.15	0.19	0.21	0.05	0.20	0.23
1	Low	1	0.44	0.10	0.37	0.52	0.37	0.10	0.31	0.43
1	Low	2	0.28	0.10	0.23	0.32	0.20	0.10	0.17	0.23
1	Low	3	0.13	0.26	0.07	0.19	0.11	0.08	0.09	0.12
2	High	1	0.68	0.02	0.65	0.70	0.56	0.03	0.53	0.59
2	High	2	0.39	0.07	0.35	0.44	0.36	0.04	0.34	0.39
2	High	3	0.16	0.10	0.14	0.19	0.25	0.06	0.22	0.27
2	Med	1	0.46	0.03	0.44	0.49	0.43	0.04	0.41	0.46
2	Med	2	0.32	0.04	0.30	0.34	0.28	0.06	0.26	0.31
2	Med	3	0.09	0.08	0.08	0.10	0.11	0.05	0.10	0.12
2	Low	1	0.25	0.12	0.20	0.30	0.21	0.11	0.17	0.24
2	Low	2	0.15	0.10	0.13	0.18	0.10	0.11	0.09	0.12
2	Low	3	0.06	0.10	0.05	0.08	0.06	0.08	0.05	0.06
1–2	High	1	0.79	0.14	0.57	1.00	0.69	0.18	0.45	0.93
1–2	High	2	0.51	0.24	0.27	0.75	0.47	0.25	0.25	0.70
1–2	High	3	0.23	0.31	0.09	0.37	0.34	0.28	0.16	0.53
1–2	Med	1	0.59	0.21	0.34	0.83	0.56	0.22	0.31	0.80
1–2	Med	2	0.43	0.26	0.21	0.64	0.39	0.27	0.18	0.59
1–2	Med	3	0.13	0.32	0.05	0.21	0.16	0.31	0.06	0.26
1–2	Low	1	0.34	0.29	0.15	0.54	0.29	0.30	0.12	0.46
1–2	Low	2	0.22	0.31	0.08	0.35	0.15	0.33	0.05	0.25
1–2	Low	3	0.10	0.33	0.03	0.16	0.08	0.33	0.03	0.13
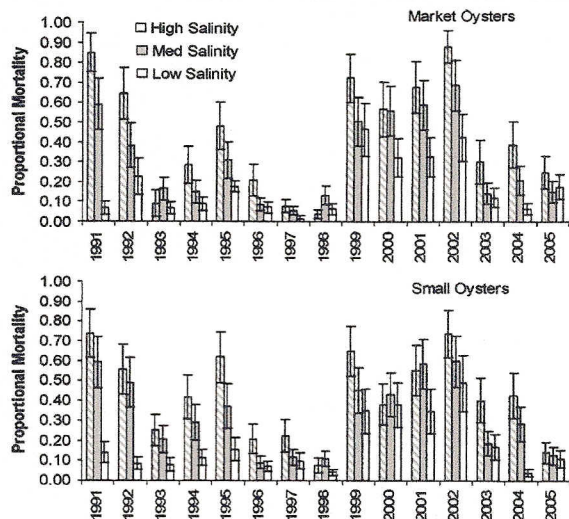
Figure 5. Annual natural mortality rates for small and market-size oysters based on counts of recent boxes (TSD = 1–2 weeks) across all bars in each salinity class. Error bars represent 95% confidence intervals. Market-size oysters are defined as being greater than or equal to 76 mm in shell length. Small oysters are defined as being between 40 mm and 75 mm in shell length.

not always reflect variation in mortality caused by changes in disease levels linked with freshwater runoff into Chesapeake Bay. These mortality estimates were similar for 2003 (wet) and 2001 (dry), for example. The difference between the mortality rates for 2003 based on recent boxes and those based on total boxes might be attributable to a longer time-to-disarticulation than the one year assumed in this study. Estimates based on total boxes may incorporate mortality over more than one year because boxes may take longer than one year to disarticulate (Christmas et al. 1997). Given the high annual mortality rates from 1999–2002 (Fig. 4), which probably resulted from three successive dry years and one year near the 25th percentile of runoff, a significant proportion of the old boxes counted in 2003 could represent oysters that died in 2002 and 2001, thus biasing the estimated mortality rate for 2003. The lower than average estimate of mortality for 2003 based on recent boxes is more credible for a wet year with below-average disease intensity.

One reason for the difference between our estimates of annual mortality based on total box counts and those reported by Jordan and Coakley (2004) is sampling variability. Our estimates are based on counts of live oysters and boxes collected from 200–400 bars per year, whereas Jordan and Coakley (2004) restricted their analysis to data from 43 disease bars. This difference, however, probably is small. Of more concern is a systematic error (bias) in the estimates provided by Jordan and Coakley (2004). They used fall box counts to estimate natural mortality for the following year (i.e., October 1985 counts were used to estimate mortality for 1986); however, most natural deaths in a give year probably occur between May and October, just before the fall survey. For example, by following a cohort of market-size oysters through one year from October 1985 to October 1986, we see

that individuals are subject to mortality because of fishing from October 1985 to April 1986 and to natural mortality from October 1985 to October 1986. Hence, natural mortality during 1985 (i.e., November 1984 through October 1985) would have occurred primarily between May 1985 and October 1985, and therefore should be estimated from October1985 box counts, not from October 1984 counts. The bias caused by the one year offset in Jordan and Coakley's (2004) estimates of M is readily apparent in Figure 3. Estimating natural mortality for the current year based on box counts from the previous year's fall survey could cause a bias in stock assessments of an unknown magnitude and direction. The natural mortality caused by disease is strongly linked to climatic conditions; consequently, the offset could result in very large biases for adjacent wet and dry years, with unknown implications for long-term projections.

Estimates of natural mortality are based on important assumptions, such as a qualitative assumption about the time an oyster shell sits before being subject to sedimentation or fouling (recent-box method) and an assumption about the typical time between death and disarticulation of the shell (total-box method). Our estimates of annual mortality using counts of recent boxes are based on the assumption that most natural deaths within a year occur during a 20-wk period from May to October. We also assumed that instantaneous (1-wk) mortality rates were uniform over the 20-wk time period. This assumption was necessary because live oysters and recent boxes only were counted during the fall each year. The mortality study in the Choptank showed variability in monthly mortality rates based on counts of recent boxes (1–2 wk TSD). Ford and Tripp (1996; Fig. 7) also demonstrated that mortality was not uniform over this period and that samples of recent boxes collected in November for some years may significantly underestimate disease mortality in prior months. An additional source of bias in our estimates of annual mortality from counts of recent boxes is that mortality can occur outside this 20-wk period, for example because of late winter epizootic outbreaks of MSX, and because of winter and spring freshets. Ford and Tripp (1996) showed that increased mortality caused by MSX can occur during the late winter/ early spring; 15% of the small and market-size oysters that survived to the fall survey were killed when MSX outbreaks occurred two or more years in a row. Sources of bias in mortality estimates based on total box counts include violations of the assumption of a one-year disarticulation rate and the assumption that all individuals stay in the same size class for one year. Individuals in a size class (e.g., small oysters) may stay in the same size class between two fall surveys, or grow larger (i.e., achieve market size) within one year. The possibility that oysters will grow to the next larger size class within a year introduces a bias of unknown magnitude in estimates of annual mortality rates. If recent boxes really are only one or two weeks old, then live oysters within the size class of the recent boxes will not have sufficient growth time to reach the next size class before the boxes of their size cohorts are collected and counted. We recognize that our mortality estimates by salinity zones are subject to bias if predation varies geographically, and if the TSD for recent and old boxes varies by salinity and temperature. TSD for recent and old boxes may decrease progressively with increasing salinity (see Christmas et al. 1997, Ford et al. 2006).
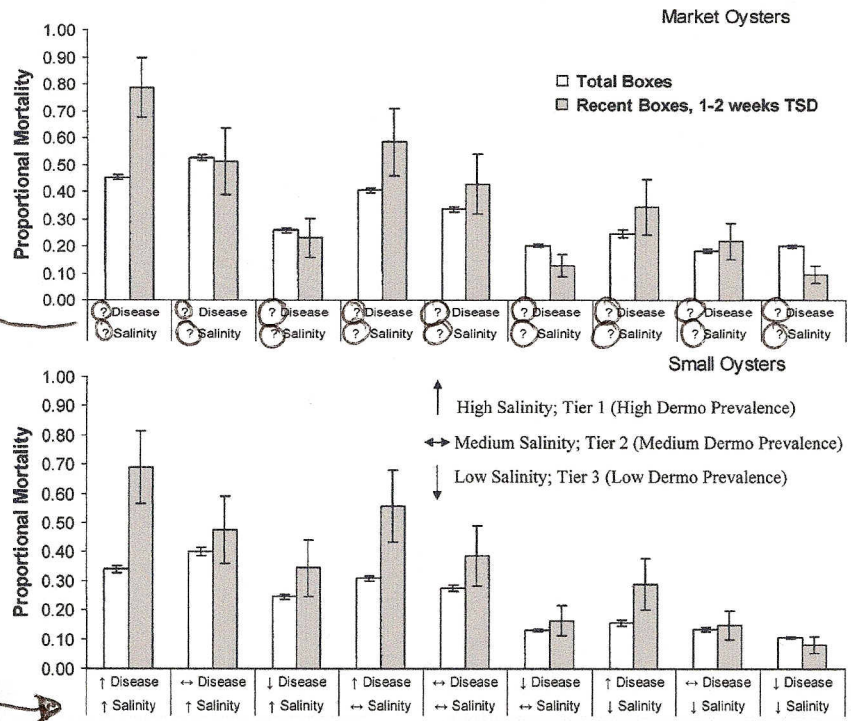
Figure 6. Mean annual natural mortality rates by disease level (tier 1 = high dermo intensity; tier 2 = medium dermo intensity; and tier 3 = low dermo intensity) and salinity class for small and market-size oysters over all years (1991–2005). Error bars represent 95% confidence intervals. We assumed an average TSD of 1 y for total boxes, and 1–2 weeks for recent boxes.

We recommend conducting experiments in Chesapeake Bay to estimate the average TSD of recent and total boxes by salinity zone. Results from experimental studies similar to those conducted by Ford et al. (2006) in Delaware Bay could help to refine the TSD and, hence, the annual mortality estimates for Chesapeake Bay oysters. Mortality rates can then be calibrated through forecasting, starting with the first year in a time series of survey data, for areas where accurate abundance and demographic data are available. Such calibration was conducted using abundance-at-age data from 1994–2004 for 23 oyster bars in the James River, VA, (data provided by R. Mann, VA Institute of Marine Science) to help determine the average TSD of recent boxes. Results indicated that mortality parameters based on the assumption that recent boxes have a TSD of one to two weeks produced population projections that matched the survey observations reasonably.

We used mean estimates of annual mortality by salinity and disease tier and allowed TSD to vary from one to two weeks (Table 2) in the oyster demographic model. After the model randomly selects years between 2004 and 2014 and the climatic condition of each year (dry, average, or wet rainfall year) using block bootstrapping from historic USGS data, the disease tier for each year is assigned randomly; probabilities are based on the historic data (Table 1). Next, the model applies randomly selected mortality rates for the appropriate salinity class and disease tier by drawing randomly from a normal distribution with estimated means and associated variances. The empirical mortality estimates for *C. virginica* presented here can also be

useful for specifying approximate mortality rates for *C. ariakensis* in the Chesapeake Bay for oysters that are one-year old or older. A mortality rate for a disease and salinity category may be selected based on an evaluation of this species' susceptibility to mortality caused by dermo and MSX and to predation. Estimated mortality rates for *C. virginica* for low disease levels (Tier 3; Table 2) could be justified for *C. ariakensis*, because *C. ariakensis* appears to experience low disease-related mortality. The Virginia Seafood Council grew triploid *C. ariakensis* from 2003 to spring 2005, and results from their trials in October 2004 showed no MSX prevalence in the oysters, and low levels of dermo (http://www.vims.edu/vsc/). Mortality rates from these trials supported our assumption that most mortality occurs during the summer months, and mortality rates for *C. ariakensis* were lower than rates for *C. virginica* (S. Allen, personal commmunication). Laboratory studies (Newell et al. in review) comparing the relative susceptibility of juvenile diploids (shell height < 25 mm) of both oyster species to invertebrate predators of eastern oyster juveniles suggest that *C. ariakensis* generally will have weaker shells than *C. virginica*. Both species developed stronger shells in response to cues from predators, but *C. virgincia* showed inducible changes in shell composition that make it comparatively stronger. If this comparative difference in shell strength persists over time after introduction to Chesapeake Bay, juvenile *C. ariakensis* probably would experience greater mortality caused by predation than *C. virginica*, resulting in reduced recruitment to the spawning stock. This differential predation mortality may not
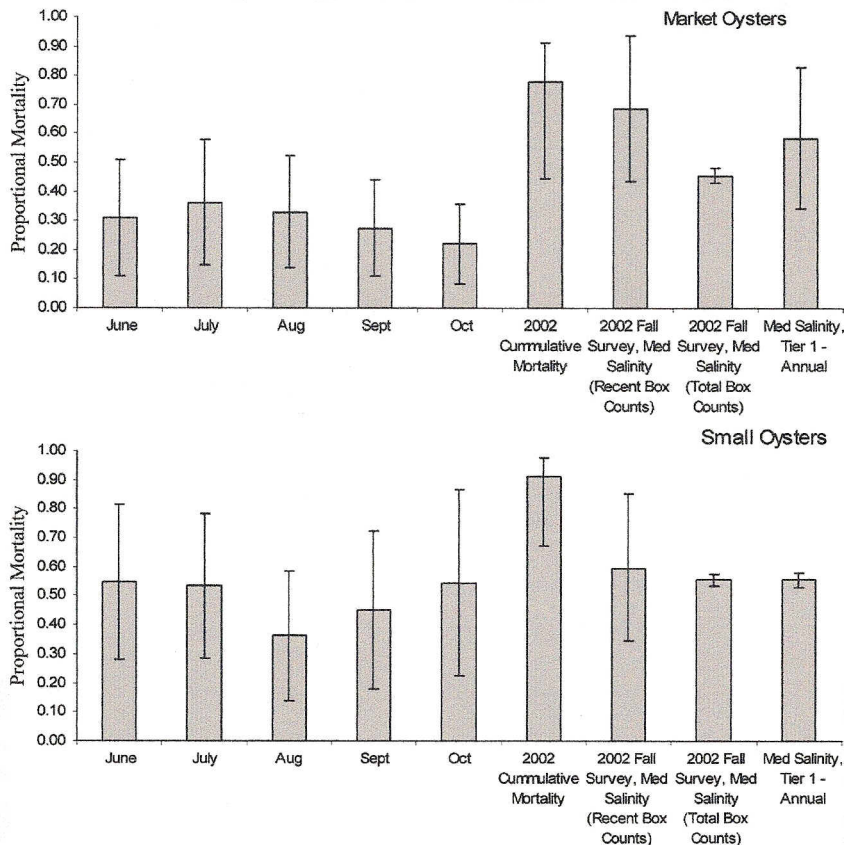
Figure 7. Monthly and cumulative natural mortality at 6 oyster bars in Choptank River (medium salinity) compared with (1) the estimated 2002 mortality rate for medium salinity and (2) the mortality rate across all medium salinity bars in years with high disease intensity. Error bars represent 95% confidence intervals. All estimates are based on counts of recent boxes (TSD = 1–2 weeks).

hold for oysters larger than 40 mm. We note that the study conducted by Newell et al. (in review) covered a fairly short time period. If *C. ariakensis* is introduced to Chesapeake Bay, it may adapt genetically to develop shell strength similar to that of the native oyster. Freeman and Byers (2006) showed that New England mussels can adapt genetically to grow thicker shells in response to invasive predators in fewer than 15 y.

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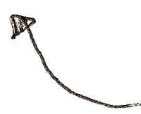
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[Handwritten annotations:]

RE — extra spaces

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AA — reference is now published as:

Newell, R.I.E, V.S. Kennedy, & K.S. Shaw. 2007. Comparative vulnerability to predators and induced defense responses of eastern oysters Crassostrea virginica and non-native Crassostrea ariakensis oysters in Chesapeake Bay. Marine Biology 152: 449-460