Some Implications of Egg Mortality Caused by Symbiotic Nemertean for Data Acquisition and Management Strategies of Red King Crabs, *Paralithodes camtschatica*

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ABSTRACT

Analyses of egg masses from 772 red king crabs (*Paralithodes camtschatica*) collected at 28 Alaskan localities from 1983 to 1985 demonstrated the widespread presence of several species of symbiotic nemertean egg predators. Nemertean intensity was correlated with significant egg mortality. At some localities nearly all crab eggs were consumed. *Carcinonemertes recticulata* was the most abundant of the nemertean egg predators. Its abundance was markedly seasonal.

A visual estimate of the percentage of the maximum volume of eggs that a female red king crab of a given size could carry was recorded as percent clutch size. It provided a rapid, semiquantitative, evaluation of fecundity. The fourth pleopod was collected from ovigerous crabs for a more detailed analysis of the abundance of symbionts in the egg mass. Pleopod fecundity was estimated based on the dry weight of a counted subsample of eggs. Egg mortality was expressed as the percentage of dead embryos based on counts of 1,000 eggs attached to the egg-bearing setae.

Percent clutch was significantly associated with the number of living embryos (r = 0.92). At extreme values (0% or 90%-100%) percent clutch was in close agreement with percent egg mortality. However, percent clutch estimates from 20% to 80% were but weakly associated with percent egg mortality. This variation was apparently due to the relative contributions of two sources of reduced egg counts; either few eggs were oviposited (or retained on pleopods), or heavy embryo mortality occurred (after a full clutch was oviposited). Thus, the correlation between percent clutch and the number of dead embryos was relatively low (r = 0.71), and the number of dead embryos in pleopod egg mass samples with a percent clutch of 2.0% ranged from 0.00%

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38,000 (full clutches ranged from 40,000 - 80,000 living eggs)

The outbreak of symbiotic nemertean egg predators was geographically localized. Epidemic levels were usually reached in enclosed harbors and passages. Larval development of red king crabs appears to considerably longer than that of the nemertean, so that by the time recruitment is complete, there is an infective agent in the water column. Under such conditions effective management might entail the use of larval crab super predators for the infected areas. More studies of larval crab movements, recruitment patterns, and the distribution of the nemertean larvae are required.

Active management of the Alaskan red king crab fishery requires regular surveys yielding information on productivity and recruitment. Inclusion of egg mortality information can be readily accomplished and will enhance interpretation of reduced percent clutch observations. We propose specific and simple sampling procedures to estimate mortality, intensity of nemertean infestations and other factors that affect the survival of the eggs. Such samples would enable retrospective studies of crab fecundity, breeding seasonality, and the relative abundance of clutch symbionts. Most importantly, a routine policy of monitoring egg mortality and causes of future reproductive events.

**INTRODUCTION**

Initial evidence, based on percent clutch observations of Alaska Department of Fish & Game (ADF&G) and U.S. National Marine Fisheries Service (NMFS) surveys, indicated that brood losses of red king crabs in the subsistence fishery were unusually high in some areas in 1983 and 1984 (Kuris et al. 1985a). These indications were confirmed in an extensive quantitative analysis (Kuris et al. 1990) covering 28 locations from 1983 to 1985. These locations generally occurred three to six months into the brooding season (June to September) and were caused by the predatory activity of very high intensities of at least two species of nemertean egg predators, Parathalassodes centnathiscus and P. confinatus. Other nemertean predators, including C. nocilaimis, have been described (Gibson et al. 1989).

Kuris et al. (1990) showed that these predator were widespread over much of the geographic range of red king crabs in Alaska. An outbreak of these symbionts to epidemic levels occurred in certain regions: most notably in Kachemak Bay, certain locations in the Kodiak Archipelago, Southeastern Alaska and near Dutch Harbor. Brood losses were very substantial in these areas. At some places such as Kachemak Bay (Cook Inlet Management Area), Ugashik Bay and Talar Bay (Kodiak Management Area), brood losses were nearly total in the 1983/84 brood season; all ovigerous females lost nearly all their eggs by September. In several samples from different locations nemertean intensities exceeded 100,000 worms per crab. This outbreak subsided in 1985/86 brood season.

Other symbionts were present in the egg masses of red king crabs. A species of turbellarian was abundant, but it did not kill crab embryos. An amphipod (Isocarcinus sp.) was often present and was an egg predator. At three locations it may have been sufficiently abundant to have a significant impact on egg survival. Its overall importance remains to be assessed.

Red king crab stocks are actively managed (Jameson 1986) with annual restrictions on catch. Active management of the red king crab fisheries in Alaska is based on a program of repeated surveys (preferably annual) of red king crab stocks. Sex ratios, reproductive condition, egg output, and population size and structure are assessed (Blau 1983 1986). As the key reproductive statistic in ADF&G and NMFS surveys, biologists routinely record a visual estimate of the size of the clutch, which is related to the size of the female. This is recorded as percent clutch, the relative proportion of a hypothetical full clutch of eggs retained by the brooding female crab.

To improve our ability to acquire information on broad mortality in red king crabs, we present a correlation analysis of the percent clutch statistic with the percentage of egg mortality caused by nemertean egg predators, the total number of eggs present (both living and dead), the number of living eggs, and the number of dead eggs. We also consider some management implications of broad losses caused by nemertean predators, discuss how egg mortality studies complement other components of the continued study of Alaskan red king crab fisheries, and suggest some future lines of research.

**MATERIALS AND METHODS**

Our general sampling procedure was detailed in Kuris et al. (1990). Briefly, pleopod egg samples were obtained from crabs collected by baited crab pots or eastern otter trawls at 28 localities ranging from Adak Island to Southeast Alaska, with most samples being obtained from the Kodiak and Cook Inlet Management Areas during the 1983/84 to 1985/86 brooding seasons.

To provide a sample to quantify egg mortality and the abundance of nemertean worms and other symbionts, the fourth pleopod of each randomly selected ovigerous female crab was excised at its base and preserved in 10% formalin in sea water. The center of the clutch and it carries a large proportion of the eggs. Carapace length, estimate of percent clutch size, depth and location of capture were recorded for each female sampled. Percent clutch is a visual estimate of the percentage of the maximum volume of eggs that a female of such a size could carry (Blau 1986). It provides a semiquantitative record of fecundity. For the statistical analysis of the association between percent clutch and the laboratory egg mortality estimates we used 28 samples collected in the Kodiak Management Area, June to September, 1984, and with high egg mortality (41 to 84 percent) and predatory nemertean worms were shown to be the cause of most of the mortality (Kuris et al. 1990).

Symbionts present in a pleopod sample were extracted by agitation of the pleopod. Symbionts were then identified, counted and preserved in 5% formalin in sea water. If the number of extracted worms exceeded
about 2,000, a subsampling procedure was used to estimate the number of worms per pleopod sample. The worms were poured into a petri dish with a 1 cm² grid. Worms were counted in 6 randomly selected squares. The mean number of worms per cm² was then multiplied by the area of the dish (in cm²) for the estimated total number of worms per pleopod.

To assess egg mortality on individual crabs, all of the egg-bearing setae of each sampled pleopod were carefully removed from the pleopod and a subsample of approximately 1,000 eggs were counted as described in Wickham (1979). Crabs whose clutches were in the process of hatching, or had already hatched, were excluded from this analysis. Egg mortality could not be distinguished from hatched eggs. Intact eggs containing normally developing embryos were counted as live, while eggs containing abnormal embryos, or eggs partially or completely devoid of their contents were counted as dead. Nearly all the dead eggs had slit outer membranes and were empty, indicating that nemerteans had killed the embryos and consumed the contents (Wickham 1978; Roe 1984; Shields and Kuri 1988b). Egg mortality was expressed as the number of dead eggs per total number of counted eggs. Crab fecundity per pleopod was estimated by dividing the total weight of the eggs by the weight of a counted subsample, then multiplying the quotient by the number of eggs in the subsample. The weight of the dead eggs in a pleopod or subsample potentially biased the fecundity per pleopod estimate when mortality was moderate to high (10-100%). To correct for this bias, the mean weight of a dead egg, 0.40 X 10⁻⁴ g (± 0.40 X 10⁻⁴ range = 0.08 - 0.70 X 10⁻⁴ g) and the mean weight of a live egg, 1.90 X 10⁻⁴ g (± 0.19 X 10⁻⁴ range = 1.70 - 2.70 X 10⁻⁴ g) was estimated and the proportional weight of dead eggs (PMD) in a subsample was calculated as follows:

\[
\text{PMD} = \frac{D_e D_{wt}}{(L_e W_{wt}) + (D_e D_{wt})}
\]

where \(D_e\) and \(L_e\) represent the number of dead and live eggs in the subsample, and \(D_{wt}\) and \(W_{wt}\) the weight of a dead and live egg. Then the product of the total weight of the eggs and PMD was divided by the mean weight of a live egg to give the total number of live eggs on the pleopod. Similarly, the product of the total weight of the eggs and PMD was divided by the mean weight of a live egg to give the total number of live eggs on the pleopod. The corrected fecundity estimated the total number of live plus dead eggs.

Margulis et al. (1982) defined "prevalence" as the proportion of infested individuals in the host population, and "mean intensity" as the mean number of symbionts per infested host. Worm density is defined in Wickham (1979) as the estimated number of worms per 1,000 crab eggs and is used here for comparisons with other carinonemertid-host systems. Statistical analyses were computed using the aid of SAS (1982) as per Sokal and Rohlf (1981). Correlation analyses were run on data with and without log-transformation. A value of \(P < 0.05\) was accepted as significant.

RESULTS

Using samples collected in the Kodiak Management Area from June to September 1984, a correlation matrix was developed to examine the predictability of the percent clutch statistic for the number of living eggs, the number of dead eggs, the total number of eggs (live plus dead), and percentage egg mortality (Table 1). Percent clutch was highly correlated with the number of living eggs (\(r = 0.92\)). It was also significantly correlated with the total number of eggs (both living and dead) and was also an excellent predictor of the percentage of egg mortality (\(r = 0.99\)) and the number of dead eggs.

<table>
<thead>
<tr>
<th>Total Number of Eggs</th>
<th>Number of Living Eggs</th>
<th>Number of Dead Eggs</th>
<th>Percentage Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percent Clutch</strong></td>
<td><strong>0.80</strong></td>
<td><strong>0.92</strong></td>
<td><strong>0.71</strong></td>
</tr>
<tr>
<td>Total Number of Eggs</td>
<td>-</td>
<td>0.94</td>
<td>-0.33</td>
</tr>
<tr>
<td>Number of Living Eggs</td>
<td>-</td>
<td>-</td>
<td>-0.64</td>
</tr>
<tr>
<td>Number of Dead Eggs</td>
<td>-</td>
<td>-</td>
<td>-0.80</td>
</tr>
</tbody>
</table>

Further examination, using samples from many Alaskan localities throughout the year \((N = 418)\) provided a correlation of \(r = -0.78\), \((P < 0.01)\), for percent clutch and the percentage of egg mortality. Figure 1 shows the regression of the percentage of egg mortality on percent clutch (mortality = 68.6 - 0.75% percent clutch). Table 2 shows that for percent clutch values 10%, egg mortality generally exceeded 75%. For percent clutch values 75%, egg mortality was usually 10% or less. This suggested that when high percent clutch values \((70-90\%)\) were recorded there was little mortality due to nemerteans. The shipboard observers may have been recognizing differences in the number of eggs oviposited or lost to other mortality causes.

Figure 1 also compared summer samples (May to September) with winter samples (October to March). In general the summer outlier values fell above the regression line indicating that heavy egg mortality from nemerteans predation was sometimes not detected by the shipboard observers, perhaps because the early egg mass retained its full shape even though many eggs were killed. In contrast, the outlier winter values generally fell below the regression line, suggesting that, late in the breeding season, some clutches may be smaller due to mortality factors other than worm predation.
Male red king crabs enter the fishery seven to nine years after they hatch (Powell 1967; Jamieson 1986). It is tempting to try to identify the year in which reduced egg numbers, perhaps caused by nemertean egg predators, may have become a problem for certain Alaskan red king crab fisheries by examining summary data on percent clutch statistics (e.g., Blau 1983). This data set covers 1972-1989 and provides percent clutch estimates for thousands of female red king crabs. However, sampling regions and dates sometimes varied between years. Since Kuris et al. (1990) have shown that egg mortality due to nemertean predation varies between districts of the Kodiak Management Area and that only dates late in the summer survey will detect this source of brood loss, these arequipale data are not suitable for a comparison of different years. However, we suggest that a district-by-district annual summary of the distribution of the percent clutch statistics may help identify where and when the outbreak to epidemic levels of nemertean predation (Blau 1983) have occurred. As Blau (1983) has noted, certain locations, such as Uganik & Vieicked bays, had much lower proportions of females with full clutches (Blau 1983). These locations were consistently and heavily impacted by nemertean egg predation in 1983 to 1985 (Kuris et al. 1990).

Kuris et al. (1990) have shown that brood losses caused by nemertean worms will be substantially underestimated unless samples are taken in the latter half of the brooding season. Worm intensity does not attain high levels until July or August, and egg mortality lags behind worm intensity, often peaking in September for those years in which outbreaks reach epidemic proportions. When outbreaks are less severe peak egg mortality may not be observed until very late in the brooding season, i.e., November to January. Late fall and winter surveys are infrequent generally because weather is inclement.

Reduced egg output, perhaps beginning in the 1970s in some districts, could have contributed to declining catches in the Kodiak Management Area from 1983 to the present. If so, its effects will continue to be a factor until at least 1993. (7 years after our last egg mortality observations in 1986). Unlike other reported episodes of reduced reproductive output (Powell and Lebida 1968; Otto et al. 1983, this volume) the current reduction in egg production appears to be a prolonged event, induced by egg predation.

Recommendations for Improved Assessment of Reproductive Output

Our analysis of the percent clutch statistic shows that it is a valuable predictor of the potential reproductive output of female crabs. As presently recorded, it cannot, however, distinguish between very different causes of egg loss.

The inclusion of an additional sampling procedure in a regular survey or catch monitoring program necessarily competes with the limited resources available to management agencies. To obtain a substantial benefit at minimal cost, it is desirable to focus a new sampling program on probable areas of concern. Additional information on the condition of the egg mass should be sought whenever the mean percent clutch of a location drops below an agreed upon threshold. The percent clutch statistics presented above and in Blau (1983) suggest that detection of less than 70% of the females having full clutches (50% to 100%) should trigger further inquiry. The geographic survey of brood mortality and intensity of nemertean egg predators (Kuris et
al. 1990) suggests that crab stocks in partially enclosed embayments, fjords, and passages are notably at risk. We should not give priority to a regular egg mortality/egg predator sampling program.

To distinguish causes of reproductive failure and quantify nemerteans, we suggest that samples of red king crab be taken in the first two weeks of the brooding season. An early sample, taken in the spring, after oviposition, will enable causes of egg loss, such as brood failure, to be recognized. Mc Mullen and Yoshinaga (1969; Kuris et al. 1990) will also provide a baseline to recognize changes in egg loss. A second set of samples, taken no sooner than August, September, should be sufficient to document the intensity of nemerteans on egg mortality. Nemerteans appear to reach their peak levels at this time, and egg mortality is already substantial (Kuris et al. 1990).

The following is a suggested sampling protocol for monitoring of crab reproduction. Sampling for the presence of nemerteans and the quantification of nemerteans and egg mortality should involve excision and preservation of an egg-bearing pleopod from females selected in a randomized sampling design. Results from samples from Kachemak Bay showed relatively moderate levels of variation for mortality and intensity on nemerteans (Kuris et al. 1990). Thus, counts from relatively few pleopods (n = 25) are likely to provide a simple and adequate sample to estimate brooding success. The simple fact that egg mortality and nemerteans are locations in close proximity revealed that samples from such sites may be grouped for analysis.

Pleopodal samples may be processed as detailed in Kuris et al. (1990). Briefly, egg mortality due to nemerteans that predators may be directly estimated by the proportion of empty egg membranes retained on the egg-bearing setae and subjected to nemerteans, that may or may not be contained within a parchment seam. The adult female nemerteans are also recognized by the alternating arrangement of the pink gut and the white gonads. Length of the nemerteans is variable and depends on the species encountered; but at least some adult females are often fairly long, exceeding 2 mm. Turbellarians are orange, white, transparent, often laterally expanded at mid-body, generally flattened, temporarily adhere to surfaces using a posterior adhesive gland and not enmeshed. Turbellarians are always small, less than 2 mm long. The eggs of these symbionts are also readily recognizable. Nemerteans the are white and oviposited in clusters of hundreds contained within a sac. Turbellarian eggs are deposited on crab eggs. In preserved samples from nemerteans retain their cylindrical shape. The turbellarians are usually expanded posteriorly and are more variable in shape than the nemerteans. Brief transfer of preserved samples permits confirmation of the identity of these two types of eggs.

Management Implications

The additional sampling procedures described above, triggered by low values in the percent clutch statistic, could benefit the active management of red king crab stocks. First, causes of egg mortality may be readily separated, and these causes may have different management implications. Egg mortality in the early stages of reproduction can occur, and if this has happened, restricted fishing would be needed to protect sufficient males and achieve a high proportion of mated females. If high intensities of nemerteans are the principal cause of egg mortality, the fishery may not be the direct source of mortality, and management implications are less certain, requiring further study. Secondly, high intensity nemerteans in the resulting egg mortality and the geographic localization. Certain localities are repeatedly impacted. Since the marked increase in intensity during the brooding period suggests that larval dispersal of Carcinonemertes rectilignis is brief, perhaps egg recruitment is generally restricted to confined bodies of water.

If nemerteans is restricted, then one possible management strategy might be to increase the pressure on these stocks by including ovigerous females in the catch. This might ultimately decrease the abundance of nemerteans (Dobson and May 1987). However, if these stocks are identified as important contributors to larval recruitment, cultural intervention, and defined geographic regions, then increased protection of the breeding females in such stocks may be indicated. We have demonstrated (Kuris 1986; Kuris and Wickham unpublished observations) that nemerteans may be rapidly killed by Dungeness crab larvae. Briefer emersion of the infested host in fresh water or dilute solutions (0.5%) of formalin in sea water. Neither the crab nor its embryos were harmed by this procedure. A stock manipulation to reduce the presence of predatory nemerteans has not been attempted. But, it would seem feasible to dip or otherwise treat the infected females before discharge into the fishery. The several bays around Kachemak Bay and Cook Inlet, where nemerteans infections are intense, might be suitable for such an experiment.
Future Research

If mortality of red king crab embryos continues to be substantial, coordinated studies of reproductive output and brood mortality in studies of larval dispersal and recruitment may be useful. Larval dispersal clearly depends on oceanographic conditions, larval behavior[Ince et al. 1986; Shanks 1982; 1986; 1988; McConnaugha 1989; Clancy and Epifanio 1989]. Some adult populations may contribute disproportionately to the successful recruitment of juvenile crabs, while other adult populations are effectively expatriated by depredation upon larvae produced elsewhere for population persistence (O'Day & Nafpaktitis, 1967). Thus, it would be of interest to assess the reproductive output of the exporting populations and examine whether this productivity predicts subsequent juvenile abundance. Models of local population dynamics should be developed to search for patterns of nemertean abundance, egg mortality, larval abundance and juvenile recruitment.

Studies of nemertean infestations, of the recently recognized bliter crab disease of Chionoecetes spp. caused by a parasitic dinoflagellate (Meyers, et al., this volume; Eaton et al., this volume) and of the rhabdophagous parasite, Priapulus caecus, of king crabs (Sloan, et al., 1984; Meyers and Short, this volume) all indicate that infectious diseases may be very important, geographically localized, mortality causes. We suggest that the geographical mosaic of such infections provides an opportunity to conduct some innovative experiments to try and reduce or eliminate these mortality causes.

The predatory nemerteans are an appropriate first target as they are the main predators of the crabs. The system is quantitatively the most important, and the mortality caused by nemerteans on egg mortality has been most thoroughly recognized. At external symbionts, they are also accessible to removal by appropriate field manipulations. Field manipulations of the red king crab fishery in locations such as Kachemak Bay or the Ugak-Vikeoda-Terror Bay system might include an experimental fishery on female crabs or an attempted reduction of nemertean abundance on ovigerous female crabs caught and released incidentally to the fishery on male crabs. The latter experiment would require further work on methods to kill nemerteans without harming female crabs or their embryos. Additional study is needed to develop the most suitable means of elimination. Possibilities include fresh water, dilute formalin, malexite green and gaultherial, a drug now in widespread use against trematodes and cestodes parasites of humans. Methods of application to be explored include dips, sprays, and slow release pellets that adhere to ovigerous crabs. Rapid shipboard handling procedures need to be developed.

CONCLUSION

Brood mortality of red king crabs has been nearly complete in some areas and years (e.g., Ugak and Kachemak Bay in the 1983/84 brood season) (Kuris et al. 1990). No other life history stage of the red king crab has been shown to suffer such high mortality even when the impact of commercial harvest is included. Elasmobranchs and a symbiotic nemertean egg predators that caused this egg mortality have doubtlessly coexisted with red king crabs for a very long time, their presence and impact has escaped detection until recently (Wickham et al. 1986). We are likely that some past episodes of poor clutch conditions (e.g., Powell and Lebida 1968) have been caused by these symbionts. Crab surveys by ADFG and NMFS have generally been conducted prior to the increase in symbiont intensity and resultant egg mortality in the autumn. Novel approaches, including experimental manipulation of a certain local fisheries now merit careful consideration. It may be possible to substantially reduce the abundance of these mortality agents and perhaps increase the abundance of some red king crab stocks.

Figure Legend

Figure 1. The relationship between the percent clutch statistic and the percentage of eggs killed by predatory symbiotic nemerteans in clutches of the red king crab in Alaska during the 1983/84 brooding season. Eggs masses collected early in the brood season, May to September, (summer) are distinguished from those collected later in the brood season, October to March, (winter). The depicted regression equation is percentage of egg mortality = 68.6 + 0.752 (percent clutch); R = 418 crabs.

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