Pathological Findings in *Crassostrea rhizophorae* from Todos os Santos Bay, Bahia, Brazil

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Types and frequencies of pathologies were investigated in *Crassostrea rhizophorae* before and following heavy oyster mortality in Todos os Santos Bay, Bahia, Brazil. The sporozoan *Nematopsis* sp. was the only parasite present in sufficient numbers to be an important mortality factor, but the intensity of *Nematopsis* infections remained low until after the major die-off. Pathologies such as ceroidosis, reduction or loss of stored glycogen, decreased gametogenesis, edema, and metaplasia of the digestive diverticula were consistent with the hypothesis of an external environmental stress factor that may have caused feeding to cease, the resultant weakness contributing to high mortalities. © 1986 Academic Press, Inc.

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INTRODUCTION

The mangrove environment, a prominent feature of the Brazilian coastline, has a high primary productivity which should be viewed as an utilizable national resource. One of the most representative species of the mangrove areas in Bahia is the oyster *Crassostrea rhizophorae*. At present, all oysters in Bahia come from wild sources and are generally used for local consumption or are marketed as shucked, dried, and salted oysters. Considering the immediate, unsatisfied market and longer-term opportunities for the large scale export of the product, a commercial cultivation technique for the mangrove oyster was developed in an oyster pilot farm implanted in 1974 in the Jacuruna estuary in Todos os Santos Bay (13°00'S,38°30'W), Bahia, Brazil. Under normal conditions (mortality rate of about 15 to 20%/year) this technique can produce, about 20 ton/ha/year of oysters. The system gave a profit of 25% of the input in the first year (Nascimento, 1983).

At the end of 1977 a heavy mortality of various marine organisms, including fish, crustaceans, and mollusks, was reported by residents of the Jacuruna region. By the time investigators arrived in the area the majority of dead benthic and nectonic species had been swept away by tidal currents, and direct investigation of the causes of mortality were no longer possible. In the case of oysters, which also suffered heavy mortality and which were fixed to various substrates, studies which were already underway allowed comparisons to be made of population parameters before and after the major die-off. These oyster mortalities have had economic as well as ecological consequences, detrimentally affecting the living standards of the local fishing communities. This paper reports a search for the causes of mortality in the Bahian mangrove oyster, *C. rhizophorae*.

MATERIAL AND METHODS

Shortly after the major die-off, tissue samples were collected from surviving oysters at 10 sampling stations in the immediate area. Pooled tissue samples and an accompanying sediment sample from each station were sent to the Institute of Water

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Research, Michigan State University, for pesticide and metals analyses.

From September, 1975, to October, 1977, 582 oysters had been collected through periodic random samples of about 24 oysters per month in the Jacuruna estuary and its surroundings. During 1978 and 1979, after the onset of the mortality problem, 404 of the surviving oysters from the same area were sampled at less regular intervals. The oysters were scrubbed to remove sediment and fouling organisms, opened at the hinge by severing the adductor muscle, and the tissues were carefully removed from the shell. Oysters sampled during 1975 to 1977 were used primarily for studies on reproduction. They were cut into two pieces by a transverse section just in front of the adductor muscle, and the anterior part was fixed in Bouins's solution. Oysters collected between 1978 and 1979 were fixed entire in Davidson's fixative. After 24 hr of fixation the tissues were processed for histological studies as described by Shaw and Battle (1957), sectioned at 7 μm, and stained with Harris's hematoxylin and eosin. Stained sections were examined for parasites, pathology, and cellullar responses (Farley, 1968). The slides were examined first at a magnification of $100\times$ for general orientation, to evaluate general condition of the tissues, and to look for large parasites. The entire section was then searched at $400\times$ for smaller microendoparasites. Parasites observed were then studied at $1000\times$. Since we determined the parasite *Nematopsis* sp. to have the highest prevalence and verified that ceroidosis was the most common evident pathological sign, 10 microscope fields in each slide were examined and the number of *Nematopsis* cysts and pigment cells in the micrometer field were counted and recorded. The size of ceroid cell aggregates (pigment cells) was not considered. Slides from 319 Jacuruna Bay oysters collected in the period 1975 to 1977, before the increase in mortality, and from 189 oysters sampled

during the 1978–1979 period, were examined for *Nematopsis* sp. and pigment cells (ceroidosis). The counts were averaged to give the intensity of infection or "stress" each month and to show its change over a period of about 3.5 years.

Fisher's exact probability test (Siegel, 1956) was applied to evaluate the association between the presence of *Nematopsis* and the occurrence of ceroidosis. In addition, the Pearson product-moment correlation coefficients were calculated between the density of *Nematopsis* and the severity of ceroidosis in those oysters which showed both conditions. The statistical dispersion of the *Nematopsis* population was analyzed using the χ^2 dispersion test (Elliot, 1971). When the aggregation of *Nematopsis* was sufficient to be significantly different from the Poisson distribution, a χ^2 test for goodness of fit to a negative binomial distribution was applied (Elliot, 1971).

From October to December of 1979 the rectums of 45 oysters were removed and cultured in thioglycollate medium and examined as described by Ray (1966) for the diagnosis of *Perkinsus marina*, a serious oyster pathogen. Cross-sections of tissues from the visceral mass of these oysters were placed in Davidson's fixative for histologic processing immediately after removal from the left valve. The staining procedures recommended by Farley (1965) were followed to detect spores of *Haplosporidium*.

RESULTS

Results of pesticide analyses revealed no detectable levels of organophosphorus insecticides and only very low levels of chlorinated pesticides in oysters and sediments from the study area. The most contaminated oysters contained a total dry weight pesticide concentration of 100 μg/kg (36 μg pp'DDE/kg + 45 μg pp' DDT/kg + 19 μg TDE/kg), equivalent to a total live weight DDT concentration of about 25 μg/kg (ppb). These pesticide levels are con-

sidered much too low to be the causative agent responsible for the decline in oysters. (Dr. F. M. D'Itri, pers. comm.)

Heavy metal analyses could not be performed on oyster tissues because the entire samples were required for pesticide analyses. In the preparation of sediments for heavy metals analyses, the extraction of trace organics into hexane revealed relatively large quantities of sulfur precipitate upon evaporation of the solvent. Analyses of sediment samples, however, revealed only low to very low concentrations of most metals (Hg, 0.02–0.14 μg/g; Ni, 4–14 μg/g; Cd, 0.6–5.6 μg/g; Cr, < 4–10 μg/g;

Cu, 2–14.2 μg/g; Zn, 4–64 μg/g). Manganese and iron levels were elevated at several sampling stations (Mn, 100–296 μg/g; Fe, 2000–2560 μg/g). It is unlikely, however, that these or any of the other metal ions analyzed could be responsible for the observed decline in the oyster population (Dr. F. M. D'Itri, pers. commun.).

The results of histological examinations summarized in Table 1 refer to the percentage of individual oysters in each year having pathologic responses and/or parasites. Of the 582 oysters examined from 1975 to 1977, before the onset of the mortality problem, only 29 oysters (an average

TABLE 1

FREQUENCY (%) OF PATHOLOGICAL RESPONSES AND PARASITES IN *Crassostrea rhizophorae* FROM THE JACURUNA ESTUARY AND VICINITY (TODOS OS SANTOS BAY, BAHIA, BRAZIL)[a]

Year	1975	1976	1977	1978	1979
No. oysters examined					
histologically	96	276	210	149	255
Pathological responses					
Hemocyte perivascular					
infiltration	3.1	2.5	7.0	24.2	14.9
Hemocyte generalized					
infiltration	0.0	0/0	0.0	6.7	5.7
Aggregation	0.0	0.0	0.0	4.0	2.7
Diapedesis in the					
digestive tract	0.0	0.0	0.0	0.0	3.5
Edema in mantle	0.0	0.0	31.7	95.0	42.7
Generalized edema	0.0	0.0	35.7	2.0	0.0
Ceroidosis	36.5	61.9	69.0	73.8	58.0
Metaplasia in the					
digestive diverticula	32.3	25.0	27.1	75.8	71.8
Necrose in muscle	0.0	0.0	31.9	20.1	23.1
Neoplasia	0.0	0.0	0.0	0.0	0.4
Parasites					
Microsporidia	9.4	11.5	3.2	0.0	0.0
Coccidia like organisms	2.0	0.0	0.0	0.0	0.0
Gregarine like organisms	0.0	0.0	0.9	10.5	0.8
Chlamidia like organisms	1.5	0.0	0.0	0.0	0.0
Nematopsis sp.	40.6	15.6	47.0	60.0	42.7
Amoebula (stage of *Nematopsis*)	0.0	0.7	3.8	10.7	1.9
Ciliate (Sphenophrya like)	0.0	0.0	0.0	2.0	0.0
Ancistrocoma sp.	0.0	0.0	0.0	2.6	0.8
Bucephalus sp.	0.0	0.7	0.0	0.6	0.0
Tylocephalum sp.	0.0	0.0	0.0	0.6	0.8
Unidentified Metazoa	0.0	0.0	0.0	0.6	0.8

[a] Period considered: September 1975 to December 1979. The double line separates pathological responses from occurrence of parasites.

of 5% during the 3 years) presented signs of acute inflammation, characterized by hemocytic perivascular infiltration in the mantle tissue. These signs later increased in frequency, reaching 79 (19.6%) among the 404 oysters studied after the highest mortality had occurred. In addition a generalized hemocyte infiltration was noticed in 25 (6.2%) of these oysters. Only one of the oysters collected between 1975 and 1977 showed this pathology. Other signs of acute inflammation, like aggregation and diapedesis, occurred in 4 and 2.7%, respectively, of the individuals observed in 1978 and 1979. Degenerative processes like ceroidosis, characterized by the formation of pigment cells, increased to a maximum of 73.8% in 1978, and metaplasia in the digestive diverticula walls reached a maximum of 75.8% in the same year.

Other reactive processes like edema (in the mantle and in generalized connective tissues) started to appear in 1977 just before the mortality began. About 32% of the oysters studied in 1977 presented some edema in the mantle. In the years 1978 and 1979, 95.0% and 42.7%, respectively, of the oysters showed edematous mantles.

One oyster collected in 1979 was diagnosed as having a hematopoietic neoplasm. The oyster was in very poor condition, being very edematous and showing metaplasia of the digestive diverticula. Neoplastic cells had invaded all tissues.

Necrosis was noticed in the adductor muscle of 32% of the oysters collected in 1977, before the highest mortality had begun. Muscle necrosis remained relatively common (about 22.5%) in the oysters collected in 1978 and 1979.

The parasites observed are listed in Table 1. Microsporidea, occurring in ova, were noticed only before the heaviest mortality. Other *Sphenophria*-like ciliates, *Ancistrocoma,* and the worm *Tylocephalum,* appeared only during the period 1978–1979. *Bucephalus* sp. were found in three oysters during the total period of studies (1975–1979). *Bucephalus* infections were always

very heavy, resulting in parasitic castration.

Nematopsis, together with its probable amoebula stage (found in oyster gills), was the most prevalent parasite found in oyster tissues examined throughout the study. From 1977 onward the frequency of *Nematopsis* infection became higher than in previous years. Data on the frequency and intensity of infection by *Nematopsis* in monthly oyster samples from the Jacuruna estuary, together with data on occurrence and density of pigment cells, are presented in Figure 1. The intensity of *Nematopsis* infection (number of cysts mm^2) increased greatly from 1977 to 1978. A similar trend in intensity of ceroidosis was observed beginning in 1976. Besides the increase in the number of *Nematopsis* cysts and pigment cells/mm^2, an increase was noticed in the frequency of individuals showing this parasite or ceroidosis.

During various months of 1978 and 1979 the percentage of oysters with *Nematopsis* and/or ceroidosis reached 100% (Fig. 1). Consequently no test of independent assortment was possible. In those cases where the test was possible, Fisher's exact probability test revealed no significant ($p <$ 0.05) departures from independent assortment between the two conditions. Consequently, no direct cause and effect relationship seems likely between the presence of *Nematopsis* and ceroidosis.

The overall correlation between density of *Nematopsis* and the severity of ceroidosis was low but highly significant during the 5 years of the study (Sept., 1975, to Feb., 1979: $n = 186$, $r = +0.2856$, $p <$ 0.0001). Both conditions increased significantly during the period.

Within most monthly samples, however, the density of *Nematopsis* in oysters was not highly, nor significantly correlated with the severity of ceroidosis. Two exceptions were significant:

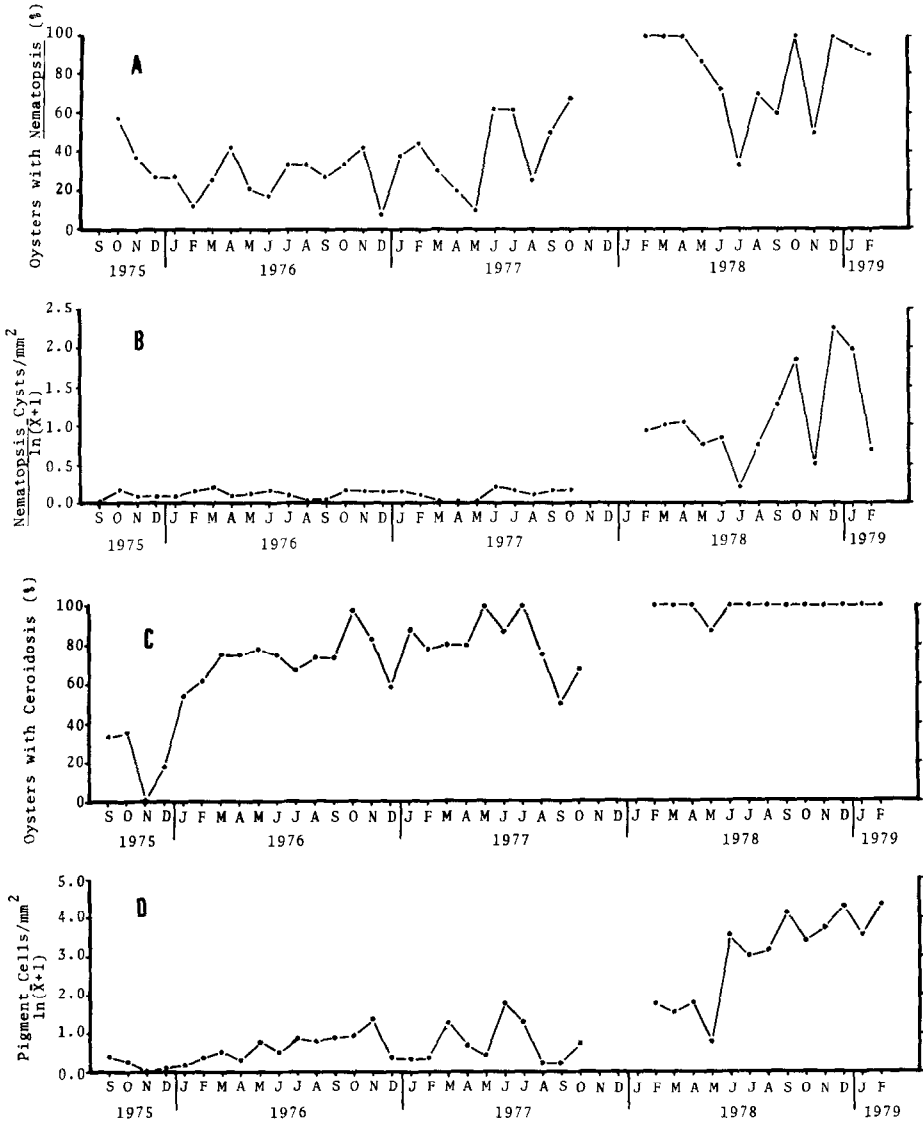October, 1978: $r = +0.6846$
$n = 16$ $p = 0.0034$,

FIG. 1. Trends in *Nematopsis* infections and the prevalence of ceroidosis in *Crassostrea rhizophorae* from Todos os Santos Bay, Bahia, Brazil 1975–1979. (A) Percentage of oysters infected with *Nematopsis* sp. (B) Intensity of *Nematopsis* infections: natural logarithm of number of cysts/mm². (C) Percentage of oysters showing ceroidosis. (D) Intensity of ceroidosis: natural logarithm of number of pigment cells/mm².

February 1979: $r = -0.5676$
$n = 16$ $p = 0.0218$

The results of Ray's (1966) test for the presence of *Perkinsus* (= *Labyintomyxa*, = *Dermocystidium*) sp. were negative. The same was true in the search for *Haplosporidium* spores in oyster tissues using acid-fast staining (Farley, 1965).

DISCUSSION

Surveys of oyster population in the Jacuruna and Jaguaripe estuaries were conducted during the period 1975 to 1979, initially to determine the sexual cycle and main periods of spawning during the year and more recently to permit a search for

causes of oyster mortality, as indicated by the presence of pathological signs and/or parasites. Slides were prepared of the major organs and structures: gonad, stomach, digestive diverticulum, gut, suprabranchial chambers, demibranchs, mantle, and often the kidney. Generally, samples examined from the oysters collected for gametogenesis studies prior to 1977 did not contain all tissues and organs. However, it was possible to judge the general condition and health of these oysters from the tissues available.

The periodic histological examination of oyster tissues from the study areas was of great value, since it permitted comparison of what occurred before, during, and following the heavy oyster mortality in 1978. In general, the oysters collected prior to the end of 1976 were in good condition, since they presented a "normal" histologic picture. In some of these oysters a nonspecific perivascular granulocytic response was observed in the mantle connective tissue. This response became more frequent and more intense in the oysters which survived the heaviest period of mortality. In no case could the response be associated with the presence of parasites. However, it is recognized that mechanisms of defense in the oyster are primarily cellular responses by hemocytes (Farley, 1968). Thus the occurrence of perivascular or generalized hemocytic infiltration might result from some stressing factor. The 36.5% ceroidosis observed before 1976 increased greatly in later years (Table 1), leading to the conclusion that stressing factors have continued high since then.

Abnormalities in the aspect of the digestive diverticula, generally appearing as a thinning of the walls resulting from shrinkage and loss of cells in the epithelial lining, sometimes appeared in oysters collected in the period 1975–1977. More frequent and severe cases, with loss of cilia and then epithelial cells from the large ciliated duct diverticula, occurred in oysters collected after 1977. These degenerative processes, associated with the occurrence

of metaplasia in digestive diverticula, have also been observed in natural *Crassostrea virginica* populations during certain seasons, especially in summer and fall, and in oysters submitted to a sudden increase in temperature and/or salinity under laboratory conditions, without apparently harming the health of the oysters (Quick, 1971).

In the degenerative processes observed in *C. rhizophorae*, leucocytes appeared to be attracted in greater numbers than is usual. Any of these signs could be associated with the presence of parasites, but the conditions described above seem rather to be signs of some other stress, appearing in 1976 and remaining high thereafter.

According to the observed increase of pathological signs, oysters examined from 1977 to 1979 showed a marked decrease in general health. Histologically this was seen as a decrease in gametogenesis, a reduction and eventual loss of stored glycogen, an increase of ceroid (stress) pigment, edema, and metaplasia of the tubules of the digestive diverticula. Some oysters showed necrosis of the adductor muscle and in others some diapedesis was observed associated with bleeding in the intestine. No specific reason for such loss of leucocytes was identified, although it is known that oysters often suffer serum loss through bleeding when under stress (Quick, 1971).

Weakness of oysters has been described as being associated with disease (Farley, 1968). The numerical increases of hyaline hemocytes, which infiltrated affected tissues, and the increase of destructive processes (edema, metaplasia, necrosis) and pigment cell formation stimulated the search for pathogenic organisms in *C. rhizophorae* collected since 1977. Most microparasites that are pathogenic to the oyster cause these kinds of responses, but none of the protozoan parasites observed (Table 1) occurred in significant numbers or frequency to be considered responsible for any major mortality or reduction in the quality of the oyster population. Viral diseases that could be diagnosed with histo-

logic techniques were not observed. The possibility that a virus might be the cause of the mortality in Todos os Santos Bay seems unlikely, since a replicating virus should cause some change in cellular populations. No changes in cell types, cytoplasmic or nuclear changes, nor the presence of cytoplasmic or intranuclear inclusions that could be induced by a virus were observed.

The biggest parasites, like cestodes, appeared as circular to ovoid bodies surrounded by layers of elastic fibers in the connective tissues of the oyster. These structures appear to be metacestode larvae of *Tylocephalum* sp. as described by Cheng (1966). The damage to oysters was only of mechanical nature due to penetration of these cestodes into oyster tissues. Their occurrence was noticed in only 1% of the examined oysters, which suggests that their numbers were too low to be of any consequence. The same pertains to *Bucephalus* sp. which was found in only three of the oysters studied. Sporocyst tubes had completely invaded the gonad and substantial numbers of developing cercariae were present. Even though the gonads were severely ruptured by mechanical presence of the parasite and parasitic castration was observed in all cases, the oysters showed no acute stress from this infection. Phagocyte numbers and other tissues were comparable to those of unparasitized oysters.

The low occurrence of these worms in *C. rhizophorae* from Todos os Santos Bay may be accounted for by the fact that oysters are only an intermediate host. These parasites also need the presence of definitive hosts to complete their life cycle. In addition, *Bucephalus* is a low salinity organism, being found commonly only in waters below 20%o (Quick, 1971), and mean salinities in the study area were approximately 27%o.

Only one oyster was diagnosed as having a probable hematopoetic neoplasm. The neoplastic cells, having a large vesicular nucleous about 8 μm in diameter, had in-

vaded all tissues. The cells found in aggregates showed numerous mitotic figures, which is indicative of a rapidly proliferating cell population. The etiology of this conditions could not be determined by histologic technique. The low prevalence of the neoplasm makes it unlikely to be the cause of the observed oyster mortalities.

Nematopsis sp. was present in the majority of the oysters examined, but at very low to moderate levels. From September, 1978, to the end of 1979, a dramatic increase in the intensity of parasitism by this sporozoan was noticed. The adductor muscle was the most commonly infected organ, but the necrotic response observed in this organ apparently was not caused by *Nematopsis*. Some necrotic areas had no *Nematopsis* and some oysters with necrosis had low *Nematopsis* infection. *Nematopsis* has been reported to contribute to increased mortality, because they close blood vessels and impede normal blood circulation (Prytherch, 1940). However, the oyster mortalities in Todos os Santos Bay began before any significant increase in the prevalence or intensity of *Nematopsis*. The mortalities which began in 1977 may have led to an increase in the intensity of *Nematopsis* parasitism by providing additional infective tissues as food for the mud crab, *Panopeus* sp., which is the definitive host for this parasite (Sprague and Orr, 1955). More infected crabs, in turn, may have magnified the intensity of the disease in the remaining oyster population. It should be pointed out again that *Nematopsis* sp. by itself is not a major oyster pathogen (Owen et al., 1951), and that additional stress is necessary to cause a significant increase in oyster mortalities.

The occurrences of *Nematopsis* parasitism and ceroidosis were independently distributed in the oysters sampled. *Nematopsis* and ceroidosis were associated only by random chance and were not a direct cause and effect.

In October, 1978, the prevalence of both *Nematopsis* and ceroidosis was 100% in

the oysters sampled. In addition, the intensity of *Nematopsis* infections and the severity of ceroidosis were both relatively high. Under these conditions a significant positive correlation existed between the two attributes ($r = +0.6846$, $p = 0.0034$) even though their distributions were independent. This may indicate that they are both responding either directly or indirectly, to the same environmental variable. This general conclusion is also supported by the fact that incidence and intensity of both have shown very similar trends toward higher values during the 4 years of study ($r = +0.2856$, $p < 0.001$; Fig. 1).

In February, 1979, only 4 months later, a significant negative correlation was noted between the same attributes ($r = -0.5676$ $p = 0.0218$). This was accompanied by a sharp drop in the average intensity of *Nematopsis* infections while the incidence of both attributes remained high. This decrease in intensity probably reflects the significant oyster mortality. If *Nematopsis* and stress, as indicated by ceroidosis, act in unison to cause mortality, this could explain the negative correlation. If oysters experiencing high levels of both *Nematopsis* and external stress are more prone to mortality, the survivors would be more likely to have high *Nematopsis* only with low ceroidosis and vice versa. This result would yield a negative correlation between the two.

In this study, low density *Nematopsis* infestations were distributed randomly, providing a good statistical fit to the Poisson distribution. Later, when *Nematopsis* infestations were more severe, distributions often departed significantly from Poisson. Successive waves of random "colonization" may result in a negative binomial distribution (Elliott, 1971), and in many cases parasite populations conform to this model. When dense *Nematopsis* populations were compared with the negative binomial model using the methods described by Southwood (1978), many significant departures from the negative binomial were noted. All departures, however, were in the same direction. The *Nematopsis* distributions were truncated, always being less skewed or more symmetrical than the corresponding negative binomial. That is, the number of severe infestations was fewer than expected. Other lines of evidence from the study, i.e., correlation analysis of intensity of *Nematopsis* infestation with severity of ceroidosis in relation to mortality trends, contribute to the conclusion that mortality was highest among individuals with high *Nematopsis* and ceroidosis levels. This mortality pattern could also produce the truncated negative binomial distributions observed.

In analyzing the spacial distribution (or statistical dispersion) of parasite populations one must bear in mind that the sampling unit, i.e., the host, is not a permanent feature of the habitat. The processes of host movement, natality, and mortality may distort the initial form of a distribution. Kennedy (1974) and others have pointed out that parasite distributions are often truncated. That is, there often exists a shortage of samples with large numbers of parasites or conversely, an excess of samples with few or no parasites. This is often interpreted as evidence of host mortality caused either directly or indirectly by severe infestations (Kennedy terms this a negative feedback control mechanism for parasite populations). On the other hand, an excess of low parasite counts could be induced by the arrival of new host individuals from outside the infected area. In sessile species such as oysters this need not be a consideration if the individuals sampled are restricted to older, more or less uniform and well-established age classes.

With the exception of *Nematopsis* sp., none of the protozoan or worm parasites observed occurred in sufficient numbers to be considered responsible for any major mortality or reduction in quality of the

oyster population. The increased intensity of *Nematopsis* infections began in 1978 after the onset of the heaviest oyster mortalities, suggesting that this parasite was not a significant factor in the major die-off. Symptoms of stress in the remaining oysters, as indicated by high intensities of ceroidosis, continued until at least February of 1979, when sampling was discontinued. The decline in health of the oysters beginning in late 1977 was not accompanied by specific hemocytic responses (cellular defense mechanisms). No cytoplasmic or nuclear changes associated with viral diseases could be detected histologically. The pathological signs observed, such as reduction or loss of stored glycogen, severe decrease in gametogenesis, an increase of ceroidal pigment, etc. might be associated with starvation.

Studies of phytoplankton in the same area, from October, 1977, through July, 1978, revealed no significant changes in the phytoplankton communities (Paredes et al., 1980), but it is possible that the great tidal flux in the area was sufficient to mask local mortality of planktonic organisms. In any event, phytoplankton were sufficiently abundant to preclude the starvation of normally feeding oysters.

Although precipitation during the months of September to December, 1977, was well above average, it did not exceed what would be considered normal during the rainy season (April to June), and no excessive siltation was noted during the period. Salinities in the two estuaries normally exhibit great fluctuations (0.5 to 33%o, depending upon tidal flux), but the mean salinity of 25 to 27%o did not change significantly during the period of major mortality (Paredes et al., 1980; Nascimento et al., 1981), nor did water temperatures (25°–30°C).

Several large oil refineries and chemical factories are located along the shores of Todos os Santos Bay, though all are well removed from the study site. The general mortality of various groups of sessile and nektonic marine organisms reported from the study area suggests the presence of a highly toxic pollutant.

Although pesticide and heavy metals from the study area failed to reveal incriminating levels of these residues, other industrial and/or natural contaminants could not be completely ruled out. One possibility which should be considered is the toxic effect of hydrogen sulfide, which might have been generated by the anaerobic decomposition of organic material deposited in or on sediments in the area. Dissociation of the high sulfur concentrations in the sediments, under slightly alkaline conditions, could also produce significant quantities of H_2S. At a pH of 8, approximately 8% (or about 320 mg/liter) of the total sulfur concentration (H_2S + HS^- + S^-) would be in the form of H_2S. A third possibility is that H_2S may have escaped from a local brine well and its associated submerged pipeline, which crosses Todos os Santos Bay from west to east to a sodium chlorate factory near the city of Salvador, Bahia.

Edema of the mantle, ceroidosis, and metaplasia in the digestive diverticula of oysters continued high for several years after the major die-off, and oyster reproduction and colonization rates were slow in recovering to previous levels. Although no new mass mortalities have been reported, these symptoms in the oyster population suggest that some environmental stress factor may have remained high in the following years. Periodic observations in the area have failed to indicate a specific cause, and in any event the identification of the factor involved in the original mortalities would only be speculative.

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