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Quahog Histopathology Studies 42 pp

Kern (National Marine Fisheries Service)

Narragansett Bay Estuary Program

Current Report

The Narragansett Bay Project

FINAL REPORT

FOR

NARRAGANSETT BAY PROJECT: QUAHOG HISTOPATHOLOGY STUDIES

PREPARED BY

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NATIONAL MARINE FISHERIES SERVICE
NORTHEAST FISHERIES CENTER
OXFORD LABORATORY

PREPARED FOR

U.S. ENVIRONMENTAL PROTECTION AGENCY
REGION 1
WATER MANAGEMENT DIVISION

Mr. Frederick G. Kern, Principal Investigator

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The Narragansett Bay Project is sponsored by
the U.S. Environmental Protection Agency and
the R.I. Department of Environmental Management.

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FOREWORD

The United States Congress created the National Estuary Program in 1984, citing its concern for the "health and ecological integrity" of the nation's estuaries and estuarine resources. Narragansett Bay was selected for inclusion in the National Estuary Program in 1984 and designated an "estuary of national significance" in 1988. The Narragansett Bay Project (NBP) was established in 1985. Under the joint sponsorship of the U.S. Environmental Protection Agency and the Rhode Island Department of Environmental Management, the NBP's mandate is to direct a five-year program of research and planning focussed on managing Narragansett Bay and its resources for future generations. The NBP will develop a comprehensive management plan by December, 1990, which will recommend actions to improve and protect the Bay and its natural resources.

The NBP has established the following seven priority issues for Narragansett Bay:

* management of fisheries
* nutrients and potential for eutrophication
* impacts of toxic contaminants
* health and abundance of living resources
* health risk to consumers of contaminated seafood
* land-based impacts on water quality
* recreational uses

The NBP is taking an ecosystem approach to address these problems and has funded research that will help to improve our understanding of various aspects of these priority problems. The Project is also working to expand and coordinate existing programs among state agencies, governmental institutions, and academic researchers in order to apply research findings to the practical needs of managing the Bay and improving the environmental quality of its watershed.

This report represents the technical results of an investigation performed for the Narragansett Bay Project. The information in this document has been funded wholly or in part by the United States Environmental Protection Agency under interagency agreement #DW13931613 with the National Oceanic and Atmospheric Administration. It has been subject to the Agency's and the Narragansett Bay Project's peer and administrative review and has been accepted for publication by the Management Committee of the Narragansett Bay Project. The results and conclusions contained herein are those of the author(s), and do not necessarily represent the views or recommendations of the NBP. Final recommendations for management actions will be based upon the results of this and other investigations.

INTRODUCTION

Mollusks of several genera and species are important commercial and recreational resources that inhabit all coasts of the United States. Many forms generally are abundant and, at least in their postlarval stages, they are sessile and easily harvestable. More specifically, quahogs (Mercenaria mercenaria), as in the case of several other bivalve mollusks, are efficient filter feeders, tend to be relatively hardy, and can therefore survive in a wide variety of high to medium salinity environments. At the same time they are bioaccumulators capable of concentrating a number of contaminants, including microbial and viral forms, some of importance to human health. Like many other marine animals from coastal environments, quahogs as a group have been studied extensively and information on their biochemistry and genetics are available. Therefore, comparative studies can be readily conducted by those familiar with these disciplines as they apply to this group. For example, physiological changes such as gametogenesis and ciliary action, etc. and pathological alterations such as inflammation, degenerative lesions, and hyperplastic changes to tissues are clearly detectable using proper approaches and methodologies. Furthermore, other changes influenced or associated with environmental alteration or pollution such as parasitic invasion or infection and increased microbial/chemical loads can also be detected.

For this study, quahogs (M. mercenaria) were sampled at the beginning of the spawning season in the spring and again in the fall at the end of their spawning season as they entered the winter dormant period. Clams representing sexually mature individuals were collected in conjunction with age-class population density studies and microbiological, biological, and toxicological studies from the Narragansett Bay Study Committee designated sampling sites (Fig. 1). Information on externally observable lesions along with the results of the microscopic examination will be utilized to assess the biological effects that may occur along the pollution gradient being studied.

METHODS

Quahogs (M. mercenaria) collected on the two Narragansett Bay Project cruises (November 25, 1985 and June 10, 1986) were shipped via next-day express mail service and received in good condition. Samples were immediately processed for histological examination (Howard and Smith, 1983). A total of 963 clams from 12 areas of Narragansett Bay were measured and examined for grossly observable abnormalities, external parasites, and a visual condition index applied.

Each clam was measured along the long axis (from hinge to bill), then opened by carefully severing the adductor muscles, and examined grossly for shell deformities, external lesions, abnormal coloration, and external health conditions. Following these examinations, a 5-mm segment of the animal was excised that contained representative tissues from all major organ systems.

These segments were coded (labeled) and placed into Davidson's fixative. Routine dehydration and paraffin embedding procedures were used. A single section from each tissue was cut at 6 µm, and stained with hematoxylin and eosin for histopathological examination.

The sex of each clam and the stage of gametogenesis was recorded using the National Oceanographic Data Center (NODC) coding system (Computer File 013)[1] for sex and the stage of maturity. This system was used for any pathological lesion or parasite identified and the organ system, distribution, and the severity codes were recorded. The NODC coding system was not developed for the purpose of examining mollusks; it was used when appropriate. Other codes developed at the NMFS Oxford Laboratory were used when necessary (Appendix A).

RESULTS

Of the 12 samples received on the first cruise, only 9 met the contract goal of 30 to 50 clams per sample site. The three "deficient" samples all came from the Mount Hope Bay area. MH-63 (19) had 21 clams, MH-62 (20) had 15, and MH-55 (52) had only 23 clams. All clams were processed and the data recorded. In the second set of samples the Mount Hope Bay area was also deficient of the desired number of 30 clams per site. MH-64 had 21, MH-62 had 28, and MH-55 with only 22. No samples were received from the Ohio Ledge site during this second sampling period (Table 1).

[1]National Oceanographic Data Center, NOAA/NESDIS, E/OC21, Universal South, Room 406, 1825 Connecticut Ave., N.W., Washington, D.C. 20235

GROSS PATHOLOGY

LENGTH: (NODC 0082) The mean length for all clams was 7.02 cm with the largest clams coming from Mount Hope Bay MH-55 (52) at 9.67 cm and the smallest from Greenwich Bay with a mean length of 5.28 cm (Table 1).

PALE DIGESTIVE GLAND: (NODC 362) Two stations from the first cruise had clams with this condition that may indicate physiological stress (Table 2). Providence River station PR-49 had one clam (animal #14) with a pale-appearing digestive gland. This individual also showed signs of abnormal calcium deposits inside the shell, and histologically it showed diffuse and severe ceroidal stress-related pigment throughout the connective tissue. The Greenwich Bay station GB-1 had two clams exhibiting this condition (animals #14 and #27). Neither of these clams showed any other external abnormalities. Histologically clam #14 showed no other signs of stress; it was infected with the procaryote parasite chlamydia (Fig. 3A). Metaplastic changes in the epithelial lining of the digestive diverticula (Fig. 2D) were noted in clam #27 but this condition was not associated with the pale condition of the digestive gland in other clams examined in this study. No clams with pale digestive glands were noted in the clams collected from the second cruise.

SHELL ABNORMALITIES: (OXFORD GC-8) Of primary concern were the rough shell deposits observed on the internal surfaces of the shells (Table 2, Fig. 6). In samples from the first cruise two stations showed differentially high levels of these deposits.

The Providence River station PR-47 had 22 (44%) of the clams with this condition and the Mount View station MV-1 had 19 (38%). Generally shell abnormalities were higher at most of the stations sampled the second time. The highest levels were observed at PR-49 with 22 (52%) and at MH-64 with 14 (58%). The overall prevalence of this condition was 17.65%. No specific histologically observable abnormality or lesion was associated with these shell abnormalities.

CLIONA SP.: (OXFORD EP-10) Infestations of the shell by the boring sponge was seen in only one location during the first cruise. Three clams (6%) from the Mount View station MV-1 were infested (Table 2). These infestations were graded as light to moderate. The sponge was not observed in clams collected during the second cruise. No specific histologically observable abnormalities or lesions were associated with this condition.

ABNORMAL COLORATION: (NODC 352 NOS) Clams from all locations with the exception of Greenwich Bay showed darkened meat coloration. This darkening was not specifically associated with site or clam size. In general the tissues were orange to brown as opposed to the "normal" creamy coloration of the other clams in the sample. Stations PR-47 (54%) and MV-1 (44%) stand out as having the greatest number of abnormally colored clams from the first cruise, and PR-49 (95%), MH-55 (100%), and MH-64 (92%) stand out from the second cruise (Table 2, Fig. 5). No specific histologically observable abnormalities or lesions were associated with this condition.

CONDITION INDEX: The condition index information does not show any significant variation among sites sampled. Clams collected during the second cruise were judged to be in poorer condition than those collected on the first cruise (Table 3, Fig. 7).

NECROSIS: Dead clams, "gapers," were observed in samples collected from five sites during the second cruise. These were as follows: PR-47 (4 dead), GR-2 (2 dead), MV-1 (1 dead), MH-61 (2 dead), MH-55-56 (1 dead), and 8 mud-filled shells were received from PR-49. These animals were very decomposed. The condition was not considered the result of handling problems and may represent ongoing mortalities. Histologically these clams were undergoing nonspecific postmortem degenerative changes accompanied by the invasion of saprophytic bacteria and necrosis (Table 4, 7; Fig. 9). No causative agent (parasite or other pathological condition) could be determined.

HISTOLOGY

SEX RATIO: (NODC 0101) The ratio of male to female varied from site to site; the larger disparities occurred in the smaller samples (Table 6). The overall sexual distribution of clams collected during this study was 47.96 males to 51.00 females.

STAGE OF GAMETOGENESIS: (NODC 0091) Both samples of clams were in various stages of spawning or with spent gonads. Most of the pathology reported was associated with the stress of spawning.

PATHOLOGY: Lesion/etiology (NODC 0382) With the exception of those clams that were observed during the gross examination as gapers, the majority of the clams demonstrated little in the way of significant "health threatening" pathology.

INFLAMMATORY LESIONS: (NODC 200) Lesions were few and localized; none were considered significant to the health of the clams (Tables 4, 6; Figs. 3D, 9).

DEGENERATIVE CHANGES: Ceroidosis, metaplasia, sloughing, edema, and cytoplasmic inclusions were noted in widely varying degrees of intensities in most of the clams examined (Tables 4, 7; Fig. 8).

Ceroidosis (NODC 487) By far the most common lesion noted in the histological examination, averaging 69.13% prevalence for all clams (Fig. 2A). The increased presence of ceroidal pigments has been associated with stress in other animals including mollusks. In this study the stressing condition appears to be spawning.

Metaplasia (NODC 715) Metaplastic changes in the appearance of the tubules and ducts of the digestive gland were noted in 5.08% of the clams examined. This condition has also been used as a nonspecific indicator of stress (Fig. 2D).

Sloughing (NODC 316) Seen as the casting out of dead epithelial cells from the lining of the digestive tubules. This condition was rare, occurring in only 1.08% of the clams examined. The highest prevalence (6%) occurred in clams collected during the first cruise at station PR-30 (Fig. 2C).

Edema (NODC 957) Edema was detected in 1.25% of the clams examined and was not associated with other specific conditions observed. It was more common in clams collected from the Providence River (Fig. 2B).

Cytoplasmic inclusions (NODC 480) Inclusions were noted in the epithelial cells of the digestive tubules in 0.96% of the clams examined from the fall sampling cruise (Fig. 2C).

NEOPLASIA: (NODC 800) Described by Barry and Yevich (1972) and observed in four clams (Tables 4, 7; Figs. 4A, B, 9). One clam from PR-47 (2%), one from OL-1 (2%) collected during the first cruise, one clam from PR-49 (2%), and one clam (3%) from MH-61 were observed in the second collection. These lesions matched their description of gonadal cancer in the quahog. All the "tumors" were contained within the alveoli walls with no evidence of invasion of other tissues.

One clam also from MH-61 collected during the second cruise was diagnosed with a highly invasive neoplastic disorder (Fig. 4C). The cell type involved resembled the sarcoma-like "hematopoietic" neoplastic disease (NODC 860) affecting the soft-shell clam Mya arenaria (Brown et al., 1977; Yevich and Barszcz, 1976) (Fig. 4D).

PARASITOLOGY (NODC 0382)

CHLAMYDIA: (NODC 180) Described by Harshbarger et al. (1977) in quahogs and was present in clams from all sites sampled. No significant pathology was associated with these organisms, and there is no indication that adult clams are adversely affected by these organisms (Tables 5, 7; Figs. 3A, 10).

CILIATES: (NODC 027) Observed in clams from five sample sites. In all cases these organisms were considered as incidental finds and no pathology was associated with the presence of these organisms (Tables 5, 7; Fig. 3C).

SPOROZOA: One clam from PR-30 collected during the first cruise was infected with organisms that resembled stages of Pseudoklossia sp. (NODC 058) (Tables 5, 7). Stages similar to those observed have been reported in other marine bivalve species. There was no significant pathology associated with the presence of this organism in the infected clams.

UNKNOWN #1: (Tables 5, 7, Fig. 3D) Observed in one clam collected from station PR-30 during the first cruise. No positive identification was possible with just the one stage of the organism available for examination. There is a discernible thick cell wall or capsule surrounding the organism. Brown (1977) lists a parasite as "unknown encapsulatum," but gives no further description of the organism that would permit a proper comparison. An inflammatory response was evoked by the host but the impact of the parasite on the host could not be determined from this one specimen.

UNKNOWN #2: (Tables 5, 7, Fig. 3B) Large basophilic amorphous areas were observed in four (0.42%) of the clams examined. No distributional trends could be associated with a specific sampling area. The lesions appeared to be the same as the chlamydia that occurred in the cells of the digestive diverticula, but the exact taxonomic identification is impossible without direct observation using electron microscopy or indirect immunological testing.

DISCUSSION

The parasite and pathology data are presented in two ways: percent prevalence (Tables 2, 3, 6, 7, Figs. 5, 6) and as the average intensity of the lesion in the sample (Tables 4, 5; Figs. 8, 9, 10). The average intensity of a lesion takes into account the severity of each lesion on individual clams in a given sample. Neither method of presenting data can adjust for the differences caused by the large variation in sample size (Table 1).

SAMPLE DATA

Length - No apparent (non-statistical) relationships between size and lesion type were associated with the site data collected. Within a given sample significant lesions (lesions with high enough prevalences to be evaluated) were evenly distributed among the size range of clams collected. Between sample site comparisons were subject to significant error because of the small samples collected from the Mount Hope Bay area.

Gross Pathological Observations

Pale digestive gland - The infrequency of this condition, 0.31% (Table 1), does not permit its use as an indicator of stress in the clam populations studied. Pale coloration of the digestive gland has been associated with nonspecific stress on other molluscan populations (Farley, 1968).

Shell abnormalities - The presence of rough shell deposits, ridges, and chalky material inside the quahog shell were associated by Jeffries (1972a, b) as a response to an irritant. The current study may provide a path to further evaluate that

premise. Although there is considerable variation in the data (Fig. 6), several stations could be utilized to evaluate a cause-and-effect relationship. The Providence River station 47 had consistently high percent prevalences of these conditions, whereas both samples from the Greenwich Bay site were considered free of these abnormalities.

The wide variation in the samples from the Mount Hope Bay area is most likely due to their smaller sample size. Even with the small sample size the same general pattern of abnormalities is repeated in the fall and spring samples. The large differences detected in the two samples from the Providence River site 49 may be that a great deal of variation can occur within a given area, or that the samples were obtained from slightly different areas.

Color abnormalities - Considerable variation occurred between samples taken along the gradient and between the fall and spring samples (Fig. 5). Generally the percentage of abnormal coloration was higher in the samples collected in the spring and may reflect a seasonal pattern associated with filtering rates and/or food or substances being filtered. Jeffries (1972a) reported darker meats in clams from polluted areas when compared with the "white meat" of clams taken from clean water. Again, both samples obtained from Greenwich Bay had few or no clams with abnormal coloration, and may serve as a control area when assessing other data associated with this study.

Necrosis - Dead clams occurred in all areas sampled during the spring sampling period, but none were detected in the clams sampled during the fall sampling period. There is no evidence that samples were treated differently or that dead clams were removed from the fall samples and not the spring collections. It is possible that the dead clams represent an overwinter "natural" mortality that would not have been detected in the fall collection. The condition index data indicate that the clams sampled in the spring were in poorer condition than those collected in the fall. These mortalities seemed widespread and not site-specific, nor were the mortalities associated with any other lesion or parasite. Jeffries (1972b) reported high mortalities associated with clam populations in the Providence River contaminated with hydrocarbons. Two of the dead animals were found at the Greenwich Bay site which had the lowest number of gross abnormalities and also one of the highest condition indexes reported (Tables 2, 3; Figs. 5, 6, 7).

Gametogenesis - Quahogs in the New England area develop gonads in the late fall and overwinter in that condition until spring-summer temperatures induce spawning (Loosanoff, 1937). Gametogenesis and spawning occur concurrently throughout the summer. There was no apparent disruption to this cycle detected at any of the sample sites selected for this study.

PATHOLOGY

Inflammatory lesions - The response by the host clam to a non-self stimulus is a good method of evaluating the relationship between the host clam and parasitic invader; it may not be a good

indicator of the effect on the clam by a toxic substance. All the inflammatory lesions reported in this study were small and localized responses to which no generalized toxic effect could be associated.

Degenerative changes - The most common and intense condition noted in this study was the presence of ceroid (lipofuscin) pigments in the tissues of clams from all areas examined, with the exception of those clams sampled in the fall from the Greenwich Bay site (Table 6). The Greenwich Bay clams were also the lowest in the total intensities of all degenerative changes reported (Table 4; Fig. 8). The association of ceroid with the gonadal tissues seems to indicate that it may be related to the spawning stress, but this is not to say that a cause-and-effect relationship exists. Brown (1977) proposed three hypotheses to explain the occurrence of ceroid/lipofuscin pigments in clams from Quonset Point/Davisville, Rhode Island but was unable to show a cause-and-effect relation to environmenal contamination. The current study, using the Greenwich Bay samples as controls, may be able to show different contaminant levels associated with the intensity levels of degenerative changes reported in this study.

Neoplasia - The low level prevalence of gonadal "neoplasia" in the clams from three of the five areas examined during this study (Tables 4, 7) is consistent with the prevalences reported by Yevich and Barry (1969) and Barry and Yevich (1972). None of the gonadal lesions observed in this study were showing significant pathological effects on other tissues in the affected clams. The

above authors were unable to determine a relationship between the occurrence of this disorder and the quality of the water in which they were collected, nor was there any evidence present that there were any mortalities associated with the presence of these gonadal tumors. Hesselman et al. (1988) found identical gonadal neoplasms in Mercenaria spp. from the Indian River, Florida. They reported a mean annual prevalence of 11.6%, but only one was extensively invasive and indicative of causing the probable death in that clam. Brown (1977) did not describe similar lesions in the gonads of M. mercenaria from Quonset Point/Davisville, Rhode Island, but did report a high level of "hematopoietic" neoplasms in soft clams Mya arenaria from the same location. Farley et al. (1986) have used the term sarcoma to describe the neoplasm in the soft clam, citing the lack of identifying cellular structures to link the neoplastic cells in question with hematopoietic cells of the clam. Since there is no ultrastructural information on the cell type involved in the neoplastic lesion affecting the clams collected during the spring cruise at the Mount Hope Bay site #61, I have used the general term sarcoma to describe this lesion. The highly invasive cell type (Figs. 4C, D) appears anaplastic (loss of cell specialization), has a high rate of proliferation based on the frequent occurrence of mitotic figures (Fig. 4D), and has greatly enlarged hyperchromatic nuclei suggesting polyploidy. This is the first report of this type of cellular disorder in the species M. mercenaria.

PARASITOLOGY

Chlamydia - Brown (1977) reported the presence of "epitheliocystis" in the quahog he examined, but did not describe the lesion or the location of the organism. It is therefore impossible to make a direct comparison of findings with the data reported here, but the same term has been used to describe similar lesions in fish gills caused by chlamydia-like organisms (Paperna and Sabnai, 1980). There were no distinct trends of increased prevalence or specific locations associated with these organisms.

OTHER PARASITES

Ciliates, sporozoa, unknown #1, unknown #2 - These organisms occurred too infrequently to assess the impact on the populations being studied, or to evaluate an environmental cause-and-effect relationship.

CONCLUSIONS

The observation of dead and dying clams collected during the June sampling period, along with the reduced values of the apparent condition index and no significant parasitic diseases, indicates that some stress-induced mortality was affecting the quahog populations in Narragansett Bay. Dead and dying clams were identified in the sample collected from Greenwich Bay which, on the basis of the lower numbers of grossly observable abnormalities, higher condition index, and lower levels of degenerative changes, could have been considered a control area. Whatever caused the mortality was not necessarily

responsible for the other abnormalities recorded in this study. There were no significant alterations in the gametogenic cycles of the clams examined in this study. Whether viable gametes were released and spawning was successful cannot be determined by the use of histological techniques. The hard clam is a hardy and very tolerant species when exposed to many environmental contaminants. Tripp et al. (1984) was able to show only mildly toxic effects on the digestive glands of hard clams experimentally exposed to benzo[a]pyrene, hexachlorobenzene, and pentachlorophenol. The very presence of quahogs in the areas sampled during this study indicates that they are capable of sustaining their present populations under the existing environmental conditions. It is evident, in reviewing the literature, that there is a need for an extensive long-term seasonal study of the clam populations in Narragansett Bay. From the information gained in this study, it should be possible to limit the number of sites and concentrate sampling efforts. The inability to collect complete samples from the Mount Hope Bay area limited the significance of the data collected. Because samples were taken only in the spring and fall, the extensive summer mortalities in Providence River clams, reported by Jeffries (1972b), may have been completely missed by this study. Data obtained from the other project investigations should be correlated with the gross and histological data presented here to see if any or all lesions reported represent sublethal effects associated with environmental contamination.

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Table 1. Summary data on number of clams sampled and their mean lengths by station and area.

SAMP # EPA CODE	SITE	# CLAMS	MEAN LENGTH	HISTO-CODE
		SAMPLE DATA		
1-PR-2	1	50	5.60	NB-1-1-(1-50)
2-PR-1	1	50	6.69	NB-2-7-(1-50)
1-PR-13	2	50	5.36	NB-1-2-(1-50)
2-PR-15	2	50	6.45	NB-2-9-(1-50)
1-PR-30	3	50	5.71	NB-1-3-(1-50)
2-PR-30	3	50	6.84	NB-2-6-(1-50)
1-PR-47	4	50	6.22	NB-1-4-(1-50)
2-PR-47	4	51	7.11	NB-2-1-(1-51)
1-PR-49	5	50	6.00	NB-1-5-(1-50)
2-PR-49	5	42	7.23	NB-2-10-(1-50)
PROVIDENCE RIVER Subtotals		493	6.32	
1-GB-1	6	40	5.28	NB-1-6-(1-40)
2-GB-2	6	37	7.05	NB-2-5-(1-37)
GREENWICH BAY Subtotals		77	6.17	
1-MV-1	7	50	6.66	NB-1-7-(1-50)
2-MV-1	7	42	7.27	NB-2-11-(1-42)
2-MV-2	7	33	7.39	NB-2-8-(1-33)
MOUNT VIEW Subtotals		125	7.11	
OHIO LEDGE Subtotals				
1-OL-1	8	50	5.74	NB-1-8-(1-50)
1-MI-63 (19)	9	21	7.59	NB-1-9-(1-21)
2-MI-64	9	24	9.68	NB-2-3-(1-24)
1-MI-62 (20)	10	15	7.56	NB-1-10-(1-15)
2-MI-62 (20)	10	28	9.13	NB-2-4-(1-28)
1-MI-61 (41)	11	47	6.56	NB-1-11-(1-47)
2-MI-61 (41)	11	38	7.40	NB-2-2-(1-38)
1-MI-55 (52)	12	23	8.34	NB-1-12-(1-23)
2-MI-55 (56)	12	22	9.67	NB-2-12-(1-22)
MOUNT HOPE BAY Subtotals		218	8.24	
NARRAGANSETT BAY TOTALS		963	7.02	
		963	7.02	

Table 2. Summary data of external gross pathologic observations by station and area.

		GROSS PATHOLOGY							
SAMP # EPA CODE	SITE	# PALE DIGEST	% PALE DIGEST	# SHELL ABNORM	% SHELL ABNORM	# CLIONA	% CLIONA	# COLOR ABNORM	% COLOR ABNORM
1-PR-2	1	0	0.00	2	4	0	0	16	32
2-PR-1	1	0	0.00	6	12	0	0	41	82
1-PR-13	2	0	0.00	0	0	0	0	15	30
2-PR-15	2	0	0.00	3	6	0	0	39	78
1-PR-30	3	0	0.00	5	10	0	0	10	20
2-PR-30	3	0	0.00	5	10	0	0	32	64
1-PR-47	4	0	0.00	22	44	0	0	27	54
2-PR-47	4	0	0.00	16	31	0	0	26	51
1-PR-49	5	1	2.00	3	6	0	0	18	36
2-PR-49	5	0	0.00	22	52	0	0	40	95
PROVIDENCE RIVER Subtotals		1.	0.20	84	17.04	0	0.00	264	53.55
1-GB-1	6	2	5.00	0	0	0	0	0	0
2-GB-2	6	0	0.00	0	0	0	0	2	5
GREENWICH BAY Subtotals		2	2.60	0	0.00	0	0.00	2	2.60
1-MV-1	7	0	0.00	19	38	3	6	22	44
2-MV-1	7	0	0.00	8	19	0	0	20	48
2-MV-2	7	0	0.00	4	12	0	0	20	61
MOUNT VIEW Subtotals		0	0.00	31	24.80	3	2.40	62	49.60
OHIO LEDGE Subtotals									
1-OL-1	8	0	0.00	5	10.00	0	0.00	10	20.00
1-MI-63 (19)	9	0	0.00	4	19	0	0	7	33
2-MI-64	9	0	0.00	14	58	0	0	22	92
1-MI-62 (20)	10	0	0.00	2	13	0	0	3	20
2-MI-62 (20)	10	0	0.00	8	29	0	0	25	89
1-MI-61 (41)	11	0	0.00	0	0	0	0	6	13
2-MI-61 (41)	11	0	0.00	8	21	0	0	19	50
1-MI-55 (52)	12	0	0.00	4	17	0	0	5	22
2-MI-55 (56)	12	0	0.00	10	45	0	0	22	100
MOUNT HOPE BAY Subtotals		0	0.00	50	22.94	0	0.00	109	50.00
NARRAGANSETT BAY TOTALS		0	0.31	170	17.65	3	0.31	447	46.42

Table 3. Summary data of apparent condition and the mean condition index by station and area.

SAMP # EPA CODE	SITE	CONDITION INDEX	# FAT	% FAT	# MED	% MED	# WATERY	% WATERY
1-PR-2	1	2.08	4	8	46	92	0	0
2-PR-1	1	1.42	0	0	21	42	29	58
1-PR-13	2	2.10	5	10	45	90	0	0
2-PR-15	2	1.34	0	0	17	34	33	66
1-PR-30	3	2.20	10	20	40	80	0	0
2-PR-30	3	1.66	3	6	27	54	20	40
1-PR-47	4	2.00	0	0	50	100	0	0
2-PR-47	4	1.58	0	0	30	59	21	41
1-PR-49	5	2.20	11	22	38	76	1	2
2-PR-49	5	1.31	0	0	13	31	29	69
PROVIDENCE RIVER Subtotals		MEAN 1.79	33	6.69	327	66.33	133	26.98
1-GB-1	6	2.60	24	60	16	40	0	0
2-GB-2	6	1.70	5	14	16	43	16	43
GREENWICH BAY Subtotals		MEAN 2.15	29	37.66	32	41.56	16	20.78
1-MV-1	7	2.26	14	28	35	70	1	2
2-MV-1	7	1.55	5	12	13	31	24	57
2-MV-2	7	1.48	2	6	12	36	19	58
MOUNT VIEW Subtotals		MEAN 1.76	21	16.80	60	48.00	44	35.20
OHIO LEDGE Subtotals		MEAN						
1-OL-1	8	1.98	0	0	49	98	1	2
1-MI-63 (19)	9	2.29	7	33	13	62	1	5
2-MI-64	9	1.21	0	0	5	21	19	79
1-MI-62 (20)	10	2.20	3	20	12	80	0	0
2-MI-62 (20)	10	1.29	0	0	8	29	20	71
1-MI-61 (41)	11	2.11	5	11	42	89	0	0
2-MI-61 (41)	11	1.68	0	0	26	68	12	32
1-MI-55 (52)	12	2.26	6	26	17	74	0	0
2-MI-55 (56)	12	1.14	0	0	3	14	19	86
MOUNT HOPE BAY Subtotals		MEAN 1.77	21	9.63	126	57.80	71	32.57
NARRAGANSETT BAY TOTALS		MEAN 1.82	104	10.80	594	61.68	265	27.52

Table 4. Summary data of the mean intensities of microscopically observable parasites by station and area.

		MICROSCOPIC PATHOLOGY					
SAMP # EPA CODE	SITE	INT INFLAM	INT DEGEN	INT NECROSIS	INT REPAIR	INT PROLIF	INT NEOPLAS
1-PR-2	1	0.30	1.96	0.00	0.00	0.00	0.00
2-PR-1	1	0.00	3.24	0.00	0.00	0.00	0.00
1-PR-13	2	0.00	1.88	0.00	0.00	0.00	0.00
2-PR-15	2	0.00	2.06	0.00	0.00	0.00	0.00
1-PR-30	3	0.06	1.22	0.00	0.00	0.00	0.00
2-PR-30	3	0.00	3.04	0.00	0.00	0.00	0.00
1-PR-47	4	0.06	4.64	0.00	0.00	0.00	0.10
2-PR-47	4	0.06	4.39	0.02	0.00	0.00	0.00
1-PR-49	5	0.16	5.46	0.00	0.00	0.00	0.00
2-PR-49	5	0.00	3.25	0.00	0.00	0.00	0.16
PROVIDENCE RIVER Subtotals		MEAN 0.06	MEAN 3.11	MEAN 0.08	MEAN 0.00	MEAN 0.00	MEAN 0.03
1-GB-1	6	0.00	0.97	0.00	0.00	0.00	0.00
2-GB-2	6	0.00	0.81	0.37	0.00	0.00	0.00
GREENWICH BAY Subtotals		MEAN 0.00	MEAN 0.89	MEAN 0.19	MEAN 0.00	MEAN 0.00	MEAN 0.00
1-MV-1	7	0.10	3.58	0.00	0.00	0.00	0.00
2-MV-1	7	0.00	2.33	0.14	0.00	0.00	0.00
2-MV-2	7	0.21	2.09	0.00	0.00	0.00	0.00
MOUNT VIEW Subtotals		MEAN 0.10	MEAN 2.67	MEAN 0.05	MEAN 0.00	MEAN 0.00	MEAN 0.00
OHIO LEDGE Subtotals		MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
1-OL-1	8	0.00	3.00	0.00	0.00	0.00	0.10
1-MH-63 (19)	9	0.00	6.90	0.00	0.00	0.00	0.00
2-MH-64	9	0.12	9.16	0.00	0.00	0.00	0.00
1-MH-62 (20)	10	0.00	4.40	0.00	0.00	0.00	0.00
2-MH-62 (20)	10	0.00	6.46	0.00	0.00	0.00	0.00
1-MH-61 (41)	11	0.00	3.36	0.00	0.00	0.00	0.00
2-MH-61 (41)	11	0.00	2.92	0.34	0.00	0.00	0.26
1-MH-55 (52)	12	0.00	6.60	0.00	0.00	0.00	0.00
2-MH-55 (56)	12	0.00	4.55	0.00	0.00	0.00	0.00
MOUNT HOPE BAY Subtotals		MEAN 0.02	MEAN 5.54	MEAN 0.04	MEAN 0.00	MEAN 0.00	MEAN 0.03
NARRAGANSETT BAY TOTALS		MEAN 0.04	MEAN 3.68	MEAN 0.07	MEAN 0.00	MEAN 0.00	MEAN 0.03

Table 5. Summary data of the mean intensities of microscopically observable parasites by station and area.

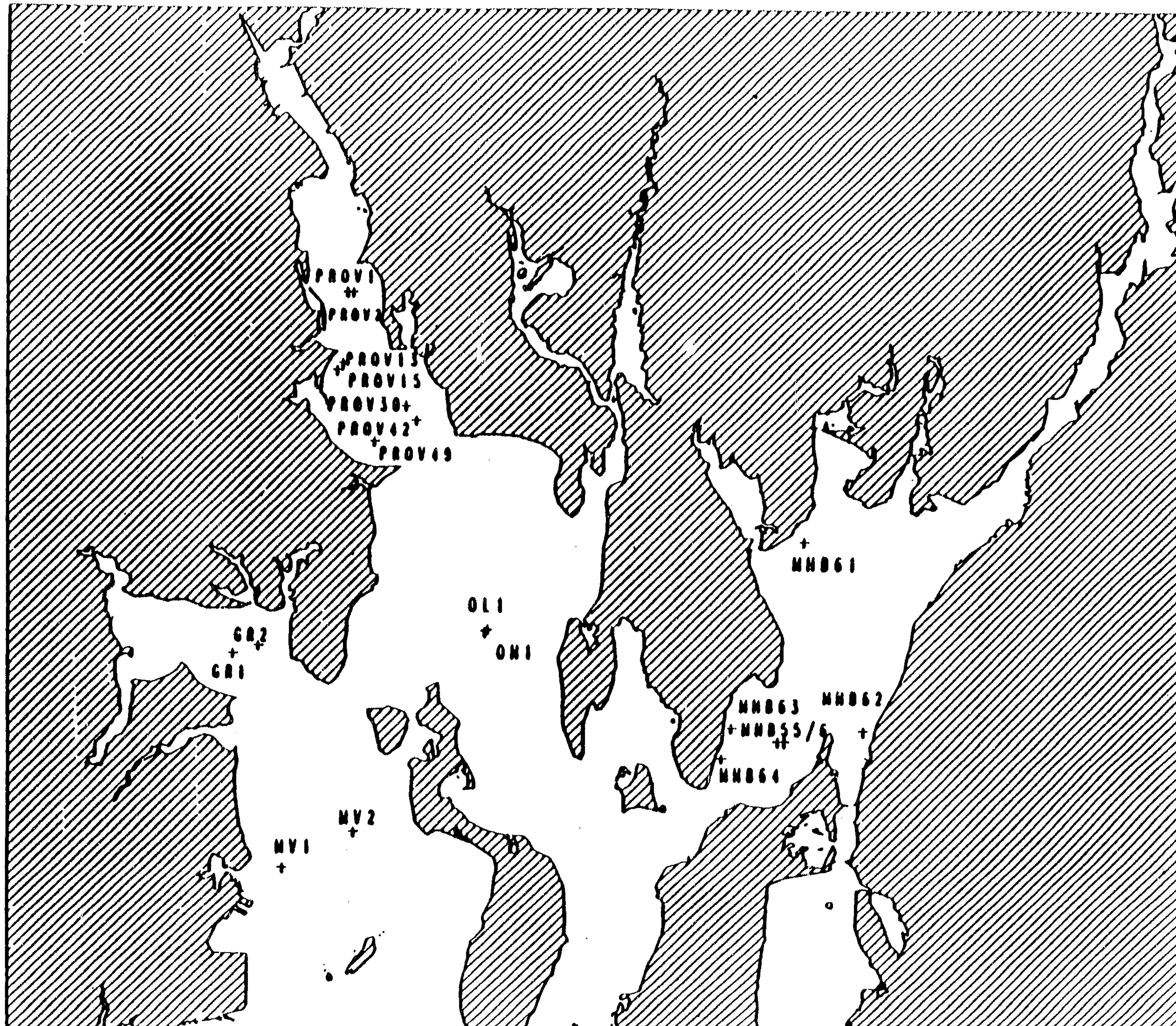
SAMP # EPA CODE	SITE	PARASITES INT VIRUSES	INT CHLAMYD	INT BACT	INT PROTOZ	INT SPOROZ	INT CEST	INT TREMAT	UNK #1	UNK #2
1-PR-2	1	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-PR-1	1	0.00	0.56	0.00	0.02	0.00	0.00	0.00	0.00	0.00
1-PR-13	2	0.00	0.52	0.00	0.12	0.00	0.00	0.00	0.00	0.00
2-PR-15	2	0.00	0.60	0.08	0.00	0.00	0.00	0.00	0.00	0.00
1-PR-30	3	0.00	0.56	0.00	0.00	0.02	0.00	0.00	0.08	0.00
2-PR-30	3	0.00	0.62	0.00	0.04	0.00	0.00	0.00	0.00	0.00
1-PR-47	4	0.00	0.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-PR-47	4	0.00	0.64	0.52	0.00	0.00	0.00	0.00	0.00	0.08
1-PR-49	5	0.00	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-PR-49	5	0.00	0.50	0.00	0.04	0.00	0.00	0.00	0.00	0.02
PROVIDENCE RIVER Subtotals		MEAN 0.00	MEAN 0.66	MEAN 0.06	MEAN 0.02	MEAN 0.002	MEAN 0.00	MEAN 0.00	MEAN 0.01	MEAN 0.01
1-GB-1	6	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-GB-2	6	0.00	0.73	0.38	0.05	0.00	0.00	0.00	0.00	0.00
GREENWICH BAY Subtotals		MEAN 0.00	MEAN 0.60	MEAN 0.19	MEAN 0.03	MEAN 0.00	MEAN 0.00	MEAN 0.00	MEAN 0.00	MEAN 0.00
1-MV-1	7	0.00	0.88	0.00	0.02	0.00	0.00	0.00	0.00	0.00
2-MV-1	7	0.00	0.19	0.07	0.00	0.00	0.00	0.00	0.00	0.00
2-MV-2	7	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MOUNT VIEW Subtotals		MEAN 0.00	MEAN 0.57	MEAN 0.02	MEAN 0.01	MEAN 0.00	MEAN 0.00	MEAN 0.00	MEAN 0.00	MEAN 0.00
OHIO LEDGE Subtotals		MEAN	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
1-OL-1	8	0.00	0.72	0.00	0.00	0.00	0.00	0.00	0.00	0.04
1-MI-63 (19)	9	0.00	0.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-MI-64	9	0.00	1.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1-MI-62 (20)	10	0.00	0.40	0.60	0.00	0.00	0.00	0.00	0.00	0.00
2-MI-62 (20)	10	0.00	1.21	0.00	0.00	0.00	0.00	0.00	0.00	0.04
1-MI-61 (41)	11	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-MI-61 (41)	11	0.00	1.00	0.32	0.00	0.00	0.00	0.00	0.00	0.00
1-MI-55 (52)	12	0.00	0.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-MI-55 (56)	12	0.00	0.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MOUNT HOPE BAY Subtotals		MEAN 0.00	MEAN 0.84	MEAN 0.04	MEAN 0.00	MEAN 0.00	MEAN 0.00	MEAN 0.00	MEAN 0.00	MEAN 0.01
NARRAGANSETT BAY TOTALS		MEAN 0.00	MEAN 0.71	MEAN 0.06	MEAN 0.01	MEAN 0.001	MEAN 0.00	MEAN 0.00	MEAN 0.00	MEAN 0.01

Table 6. Percent prevalence of males and females, and prevalence of pathological lesions by station sampled.

NARRAGANSETT BAY PATHOLOGY DATA

PERCENT PREVALENCE

SITE	MALE	FEMALE	CEROID PIGMENT	METAPLASIA DIVERTICULA	EDEMA	SLOUGHING	INCLUSIONS	INFLAMMATION
1-PR-2	66	34	46	10	4	2	0	10
2-PR-1	62	38	86	0	0	0	0	0
1-PR-13	60	40	38	4	2	2	0	0
2-PR-15	50	50	66	2	0	0	0	0
1-PR-30	54	46	28	0	0	6	0	2
2-PR-30	50	50	74	12	0	0	0	0
1-PR-47	48	46	80	4	10	0	0	2
2-PR-47	58	32	78	10	0	0	4	2
1-PR-49	38	62	96	2	4	0	0	4
2-PR-49	40	60	95	7	0	0	0	0
1-GB-1	50	50	0	15	0	2	0	0
2-GB-2	62	38	8	14	0	0	3	0
1-NW-1	48	52	56	4	0	0	0	2
2-NW-1	45	55	45	7	0	0	0	0
2-NW-2	52	48	64	0	0	0	0	3
1-[illegible]-1	42	58	74	0	0	4	0	0
1-MI-54	44	56	100	17	0	0	0	0
2-MI-55	35	65	95	5	0	0	5	0
1-MI-61	45	55	74	0	0	2	0	0
2-MI-61	47	53	63	0	0	0	3	0
1-MI-62	47	53	93	0	0	0	0	0
2-MI-62	34	53	100	4	0	4	0	0
1-MI-63	24	76	100	5	10	0	0	0
2-MI-64	50	50	100	0	0	4	8	4
	47.96	51.00	69.13	5.08	1.25	1.08	0.96	1.21



... [illegible] designated sampling sites.

Table 7. Percent prevalence of pathological lesions and parasites observed by station sampled.

NARRAGANSETT BAY PATHOLOGY DATA

PERCENT PREVALENCE

SITE	NECROSIS	NEOPLASIA	SARCOMA	CHLAMYDIA	CILIATES	BACTERIA	SPOROZOA	UNK #1	UNK #2	BOXES
1-PR-2	0	0	0	36	0	0	0	0	0	0
2-PR-1	0	0	0	30	0	0	0	0	0	0
1-PR-13	0	0	0	20	0	0	0	0	0	0
2-PR-15	2	0	0	32	0	2	0	0	0	0
1-PR-30	0	0	0	16	0	0	2	2	0	0
2-PR-30	0	0	0	22	4	0	0	0	0	0
1-PR-47	0	2	0	26	0	0	0	0	0	0
2-PR-47	0	0	0	18	0	0	0	0	2	0
1-PR-49	0	0	0	34	0	0	0	0	0	0
2-PR-49	0	2	0	23	2	0	0	0	2	0
1-QB-1	0	0	0	18	0	0	0	0	0	0
2-QB-2	5	0	0	24	5	5	0	0	0	0
1-WW-1	0	0	0	30	2	0	0	0	0	0
2-WW-1	2	0	0	10	0	2	0	0	0	0
1-WW-2	0	0	0	33	0	0	0	0	0	0
1-QL-1	0	2	0	24	0	0	0	0	2	0
1-MI-54	0	0	0	43	0	0	0	0	0	0
2-MI-55	0	0	0	35	0	0	0	0	0	9
1-MI-61	0	0	0	30	0	0	0	0	0	0
2-MI-61	0	3	3	34	0	5	0	0	0	0
1-MI-62	0	0	0	33	0	0	0	0	0	0
2-MI-62	0	0	0	39	0	0	0	0	4	0
1-MI-63	0	0	0	33	0	0	0	0	0	0
1-MI-64	0	0	0	41	0	0	0	0	0	0
	0.92	0.38	0.13	28.50	0.88	0.92	0.08	0.08	0.42	0.38

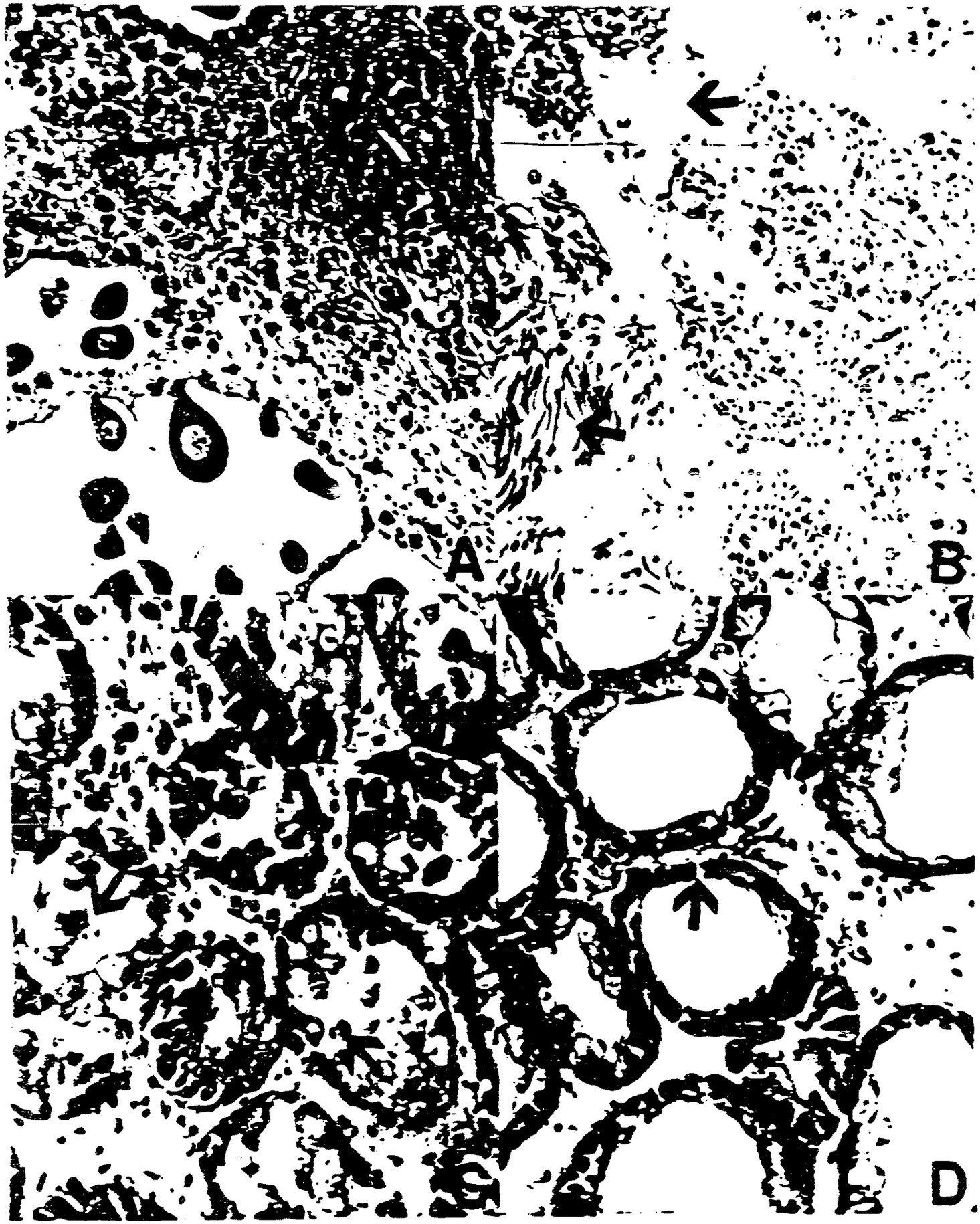


Fig 2

FIGURE 2

Figure 2A. Aggregations of ceroid containing cells (arrow) in connective tissue adjacent to ovary. 400X.

Figure 2B. Arrows indicate open sinus spaces and separated muscle fibers indicative of an edematous condition affecting the clam. 400X.

Figure 2C. Tubules of the digestive diverticulum with epithelial cells (arrows) sloughing into their lumens. 400X.

Figure 2D. Arrow indicating the metaplastic changes in the epithelial lining of the tubules of the digestive diverticulum (tall cuboidal to squamous) giving the digestive gland a swiss cheese appearance. 400X.

Fig 13

FIGURE 3

Figure 3A. Arrows indicate large basophilic areas in the epithelial lining of the digestive gland tubules caused by the presence of chlamydia-like organisms. Uninfected tubules appear normal. 400X.

Figure 3B. Large basophilic lesion (unknown #2, arrow) adjacent to the epithelial lining of the clam's muscular foot. 400X.

Figure 3C. Ciliated organisms (arrows) in the area of gills. 1000X.

Figure 3D. Arrow indicates thick wall organism (unknown #1) in connective tissue adjacent to the digestive gland. Note the inflammatory response surrounding the organisms. 400X.

Fig 4

FIGURE 4

Figure 4A. Gonadal neoplasm in the alveoli of the testis (arrows). 400X.

Figure 4B. Gonadal neoplasm in the alveoli of the ovary (arrows). 400X.

Figure 4C. Invasive sarcoma-like neoplasm in the connective tissue of the clam. 200X.

Figure 4D. Arrow indicates a mitotic figure of neoplastic cell. Note nuclear enlargement and hyperchromatic appearance of these cells. 1000X.

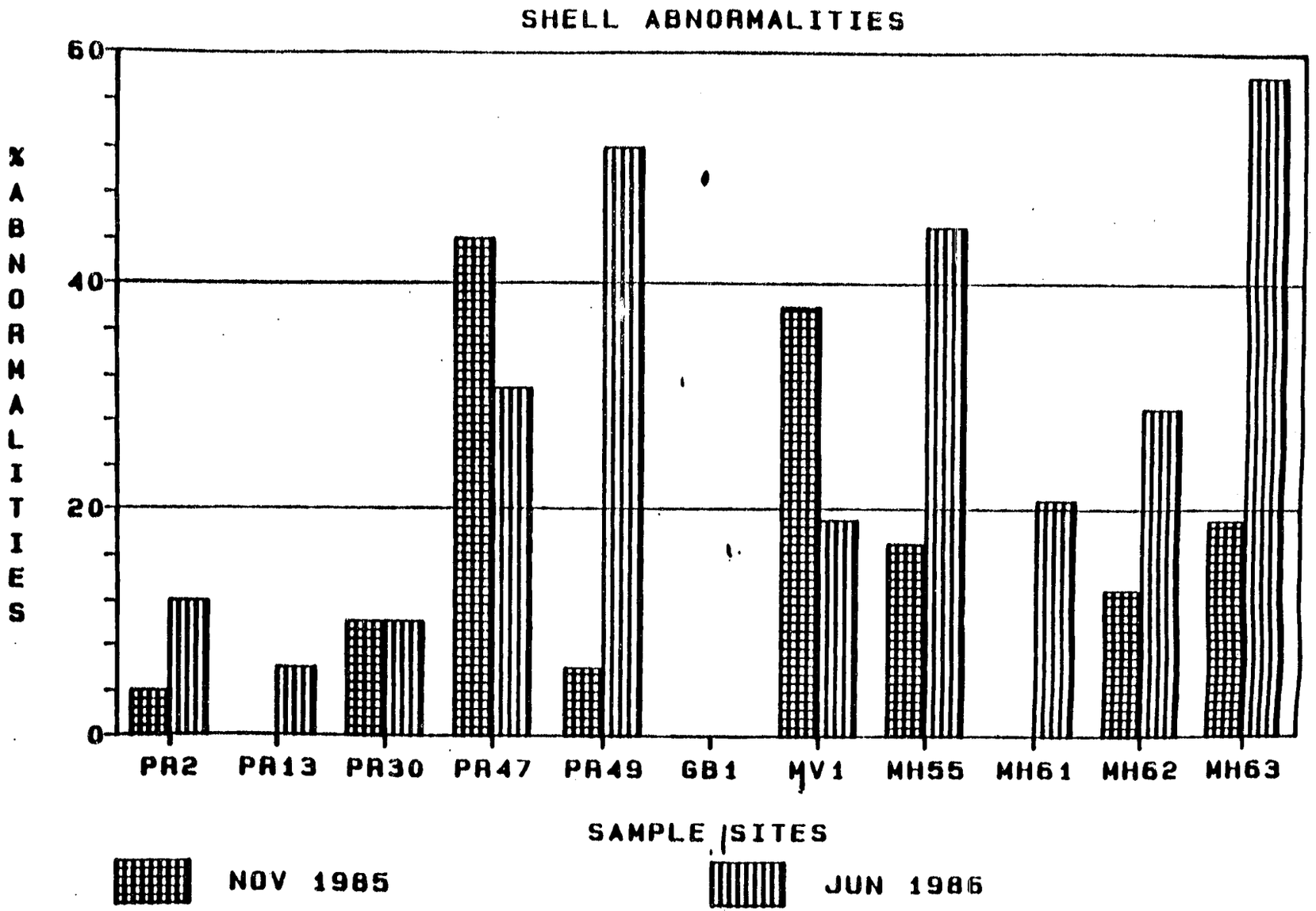


Fig. 6. Percent prevalence of shell abnormalities.

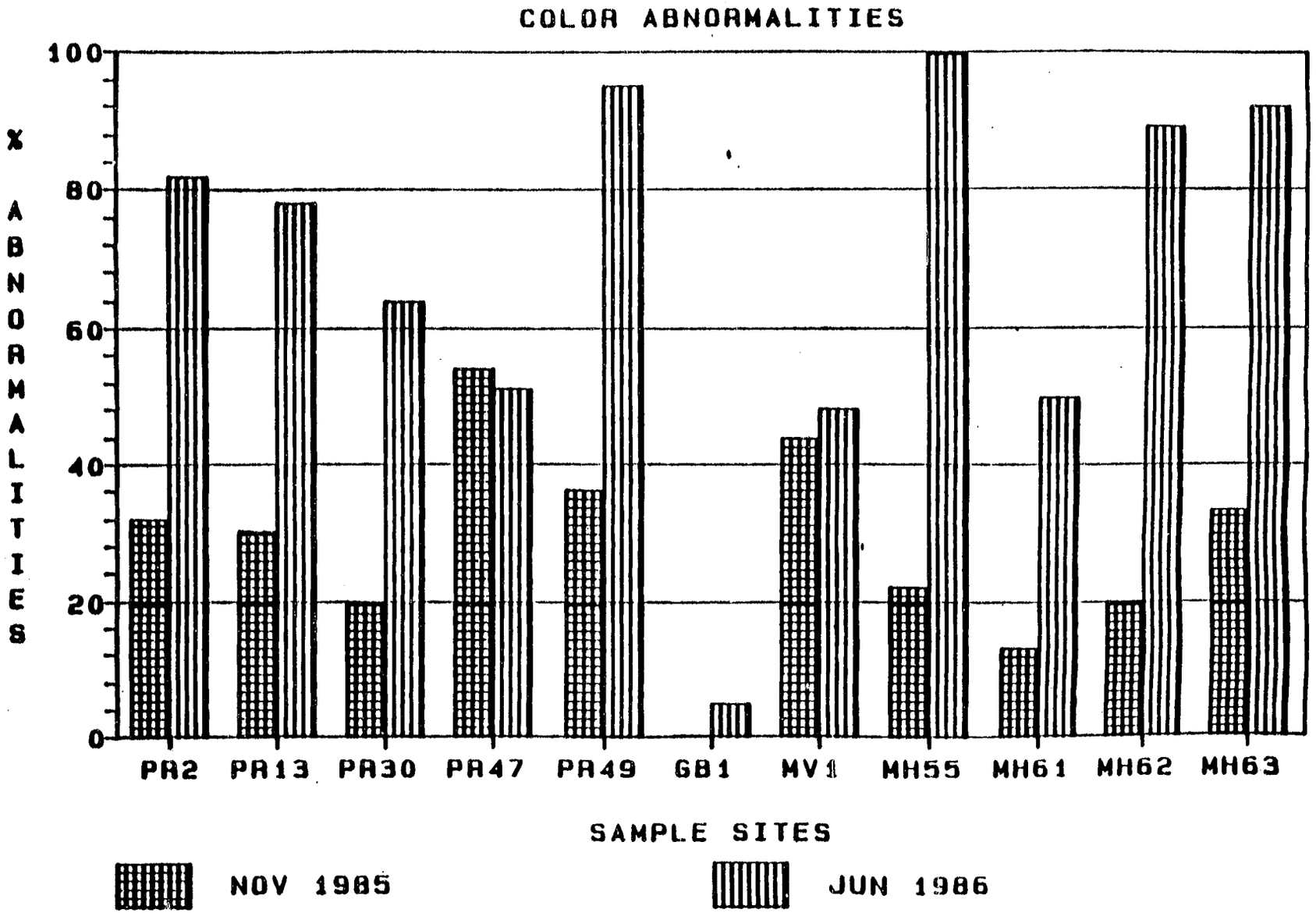


Fig. 5. Percent prevalence of color abnormalities.

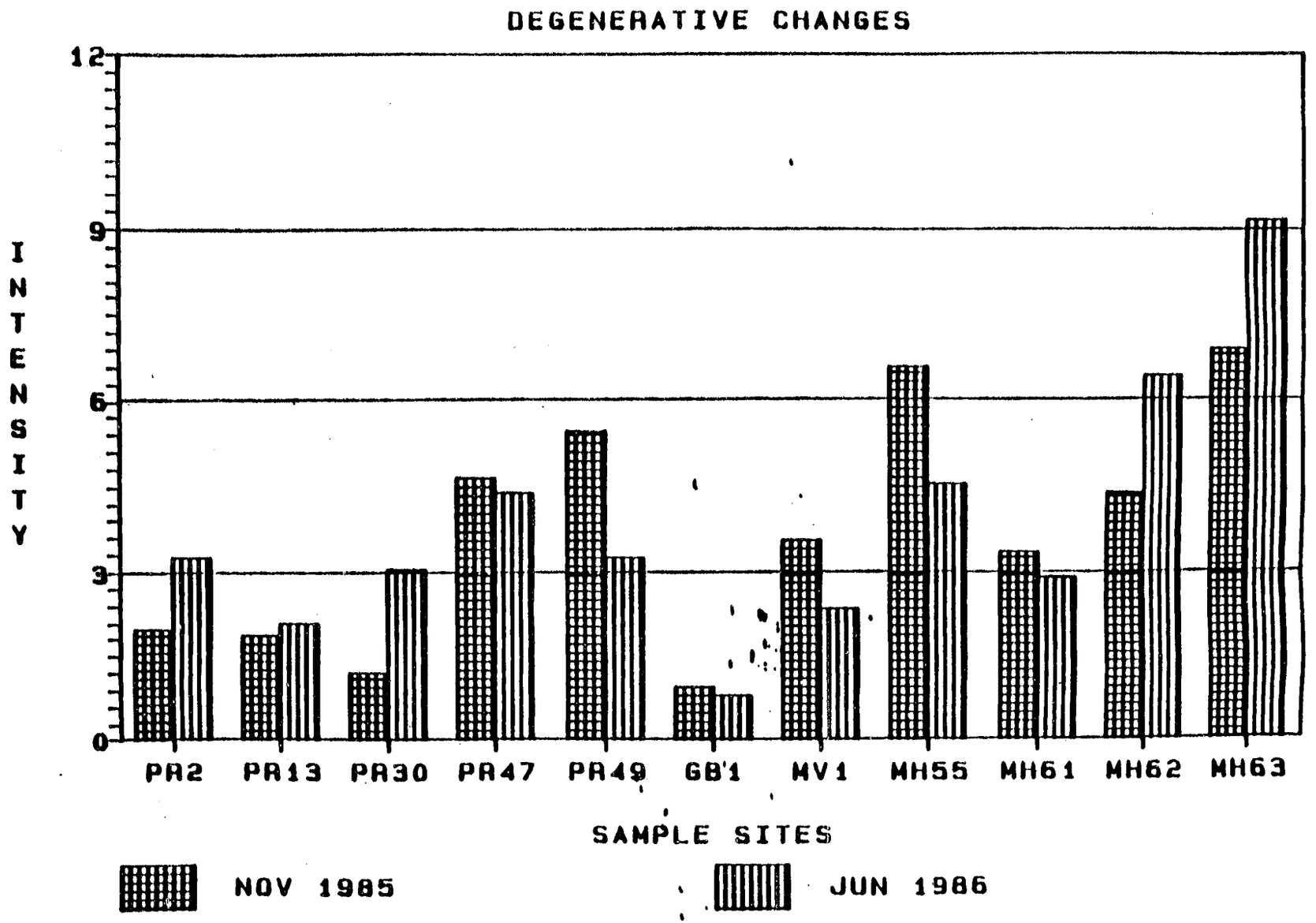


Fig. 8. Mean intensity of total degenerative changes (ceroidosis, metaplasia, sloughing, edema, and cytoplasmic inclusions) per clam per sample.

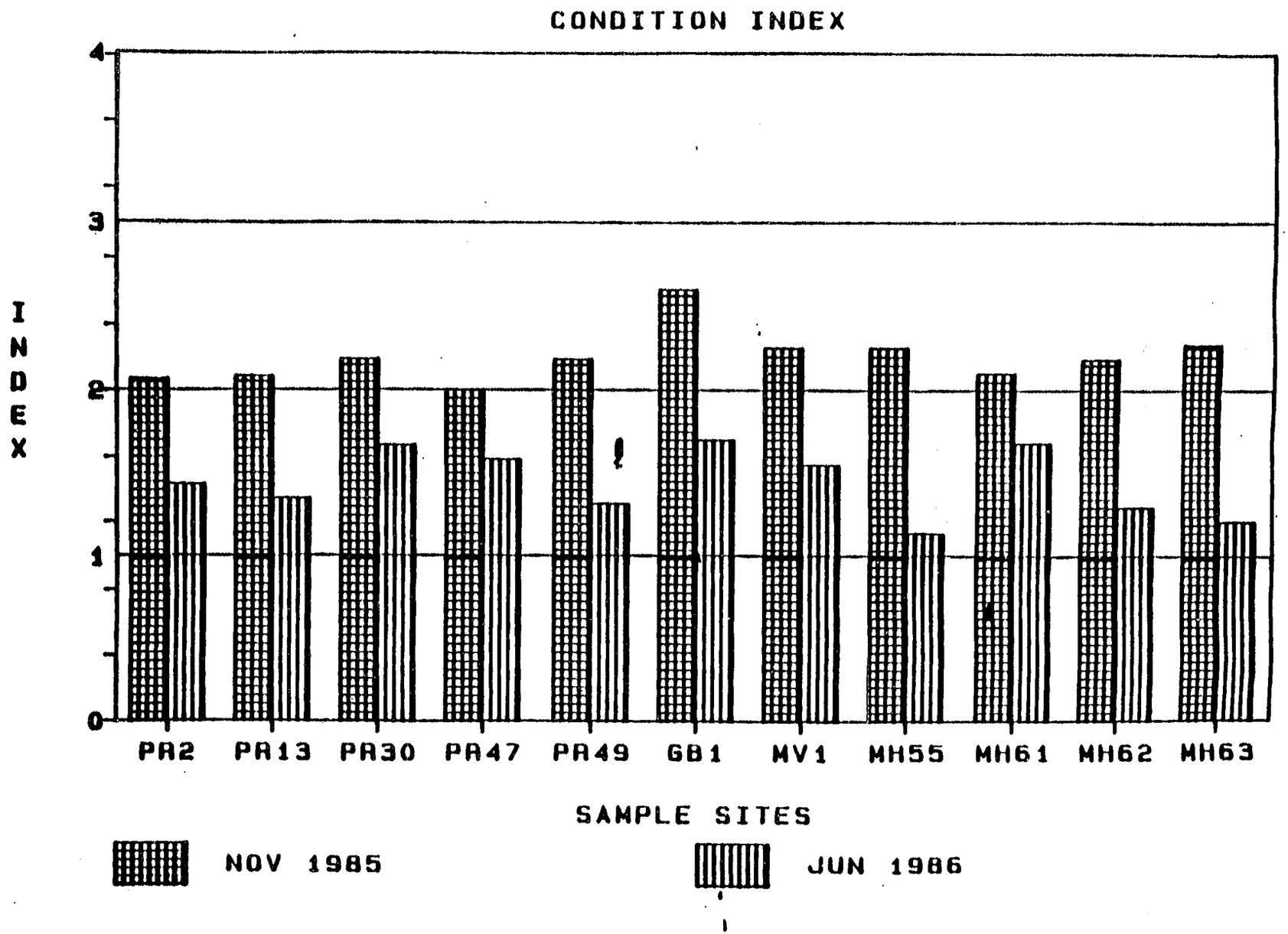


Fig. 7. Mean visually applied condition index (3 = fat, 2 = medium, 1 = watery).

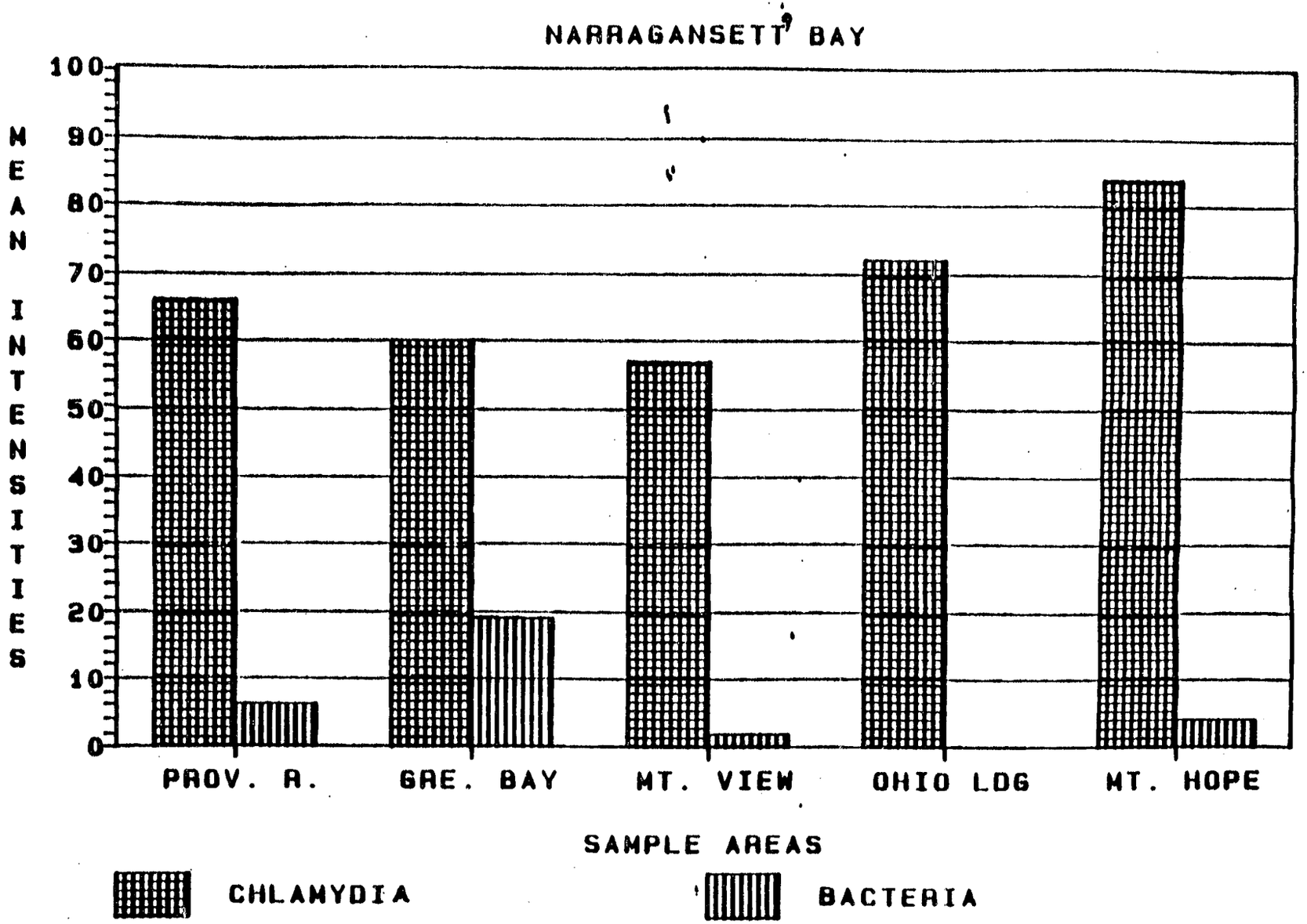


Fig. 10. Mean intensity of chlamydial and bacterial lesions per clam per sample.

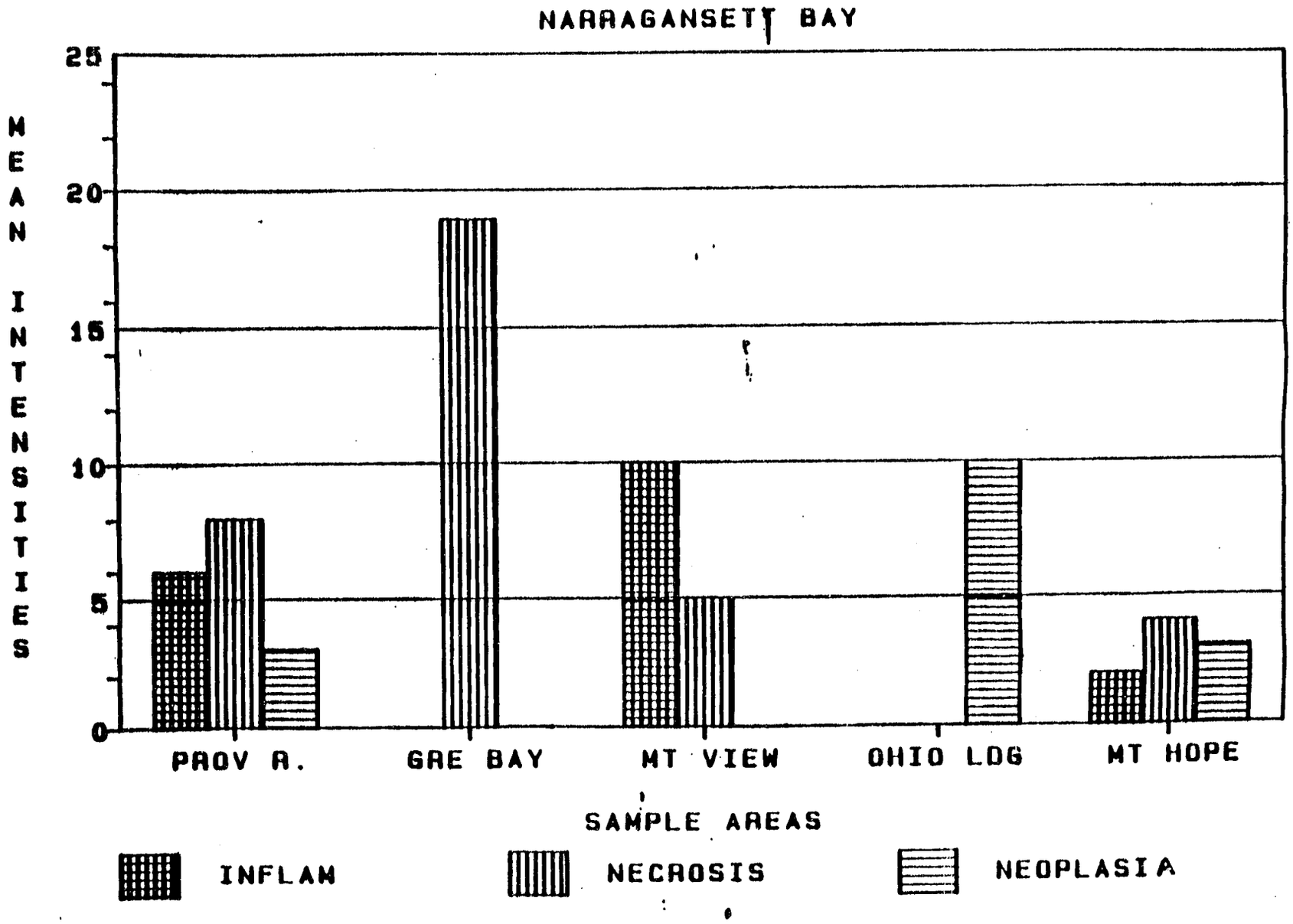


Fig. 9. Mean intensity of inflammatory, necrotic, and neoplastic lesions per clam per sample.

APPENDIX A

NODC AND OXFORD CODES

LENGTH (NODC 0082)

SEX CODE (NODC 0101)

SEX MATURITY (NODC 0091)

GROSS PATHOLOGY (NODC)

- COLOR ABNORMALITIES (NODC 352 NOS)
- PALE DIGESTIVE GLAND (NODC 362)

ORGAN CODES (NODC 0380)

- DIGESTIVE GLAND (OXFORD 014)
- GILL (NODC 033)
- TESTIS (NODC 035)
- OVARY (NODC 036)

TISSUE CODE (NODC 0381)

- CONNECTIVE TISSUE (NODC 030)

LESION/ETIOLOGY (NODC 0382)

- NECROSIS (NODC 102)
- INFLAMMATION (NODC 200)
- SLOUGHING (NODC 316)
- CYTOLOGIC INCLUSION (NODC 480)
- CEROID (NODC 487)
- METAPLASIA (NODC 715)
- NEOPLASIA (NODC 800)
- CILIATES (NODC 027)
- COCCIDIA (NODC 058)
- TREMATODE (NODC 106)
- CESTODE (NODC 106)
- BACTERIA (NODC 175)
- CHLAMYDIA (NODC 180)
- VIRAL (NODC 183)

SARCOMA (NODC 860)

EDEMA (NODC 957)

LESION DISTRIBUTION (NODC 0383)

LESION SEVERITY (NODC 0384)