Mussel Watch Program

Histological Studies Of Mussels

Disposal Area Monitoring System Damos

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Experimental field populations of mussels (either Mytilus edulis L. or Modiolus modiolus L.) were deployed and maintained on or near open water dredge spoil dumpsites for up to thirteen months. The reproductive tissues of the experimental animals were compared with those of reference mussels by means of a quantitative assay based on measurements of numbers and sizes of ova. The reproductive development of the experimental populations generally was not impaired, and data analyses showed that dates of sample collection were more

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important influences on reproductive condition than either station types or locations. We also present evidence that the gonadal condition of *Modiolus modiolus* varies seasonally; however, the changes are not as pronounced as those evident in the tissues of *Mytilus edulis*.

SCIENCE APPLICATIONS, INC.

DAMOS
MUSSEL WATCH PROGRAM

HISTOLOGICAL STUDIES OF MUSSELS FROM DREDGED MATERIAL DISPOSAL SITES

CONTRIBUTION #20

April 25, 1984

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1.0 INTRODUCTION

The biological impacts of open water dredged material disposal have been assessed by studying structural changes in benthic communities (e.g. Saila et al., 1972; Oliver et al., 1977; Rhoads et al., 1976) and by examining indicator organisms for chemical or physiological changes (e.g. Ancerlini et al., 1975; DeGoursey and Vernberg, 1975; Engler, 1979; Arimoto and Feng, 1982). We have developed a quantitative histological assay of mussel reproductive tissues to study the condition of experimental field populations deployed at the DAMOS (Disposal Area Monitoring System) disposal sites along the New England Coast from New Haven, CT. to Rockland, ME. The assay of reproductive condition was based on measurements of numbers and sizes of ova, and the data enabled us to study the variance within and between populations.

Laboratory studies by Bayne et al. (1978) showed that when mussels (*Mytilus edulis*) were subjected to temperature and food ration stresses, they produced smaller eggs (by weight) than unstressed controls. In the stressed animals, ripe gametes occupied a smaller proportion of mantle tissue than in the controls, and when the stressed animals were induced to spawn, they released fewer eggs than controls. Clearly, animals living on or near dredged material disposal sites may be affected by the physical and chemical changes induced by the disposal operations. Consequently, we conducted investigations to determine whether reproductive tissues of mussels deployed on or near several disposal sites differed from those of reference (control) animals.

Mussels and other bivalve molluscs have been used as sentinel organisms in monitoring programs such as the Mussel Watch (Goldberg et al., 1978), and, for another part of the DAMOS program, the trace metal and polychlorinated biphenyl (PCB) concentrations of certain populations have been monitored (Arimoto and Feng, 1982). The research presented here complements the chemical monitoring studies because reproductive cycles of indicator organisms can affect the body burdens of chemical contaminants (Phillips, 1977 and 1978).

2.0 METHODS

2.1 Field Procedures and Site Locations

Two species of mussels were used to establish the experimental field populations: the blue mussel, *Mytilus edulis*, for the stations south of Cape Cod, MA. and the horse mussel, *Modiolus modiolus*, for the northern stations (Figure 2.1-1). The experimental populations of *Mytilus* were established by attaching 25 to 30 polythylene mesh bags (2 cm mesh), containing 50 mussels each, to 1 meter high polyvinyl chloride frames. The frames were then lowered to the seafloor, and they were anchored to the bottom by concrete footings (Figure 2.1-2). The monitoring arrays were equipped with renewable subsurface sonic beacons (Model PMC-50, Johnson Laboratories, Southold, NY) and also with subsurface buoys that released if the surface buoys were pulled free.

Located in eastern Long Island Sound, the New London disposal site recently has been the most heavily used dredge material disposal area in New England waters. The site was first

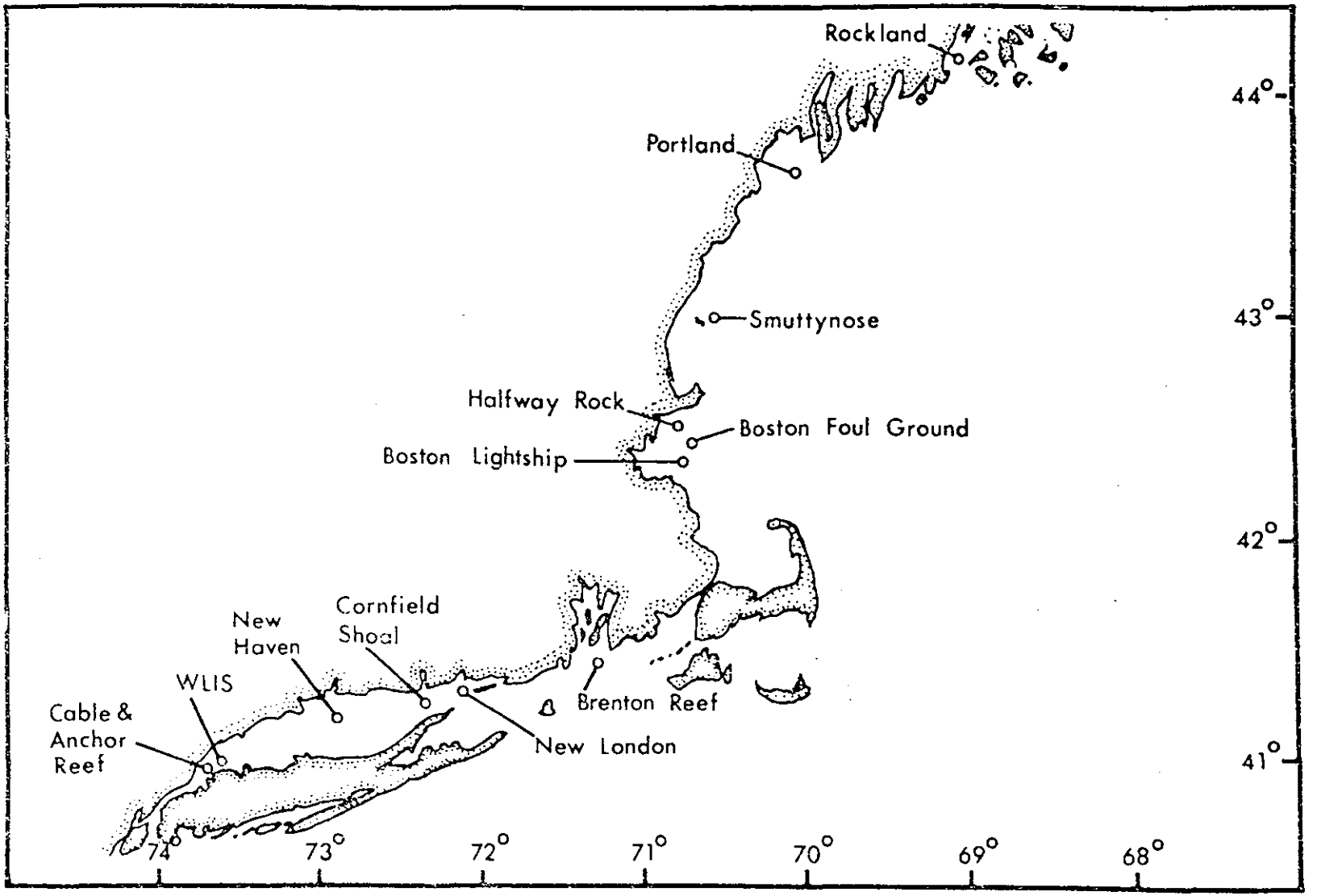

Figure 2.1-1. Disposal Area Monitoring System (DAMOS) Stations in New England Coastal Waters.

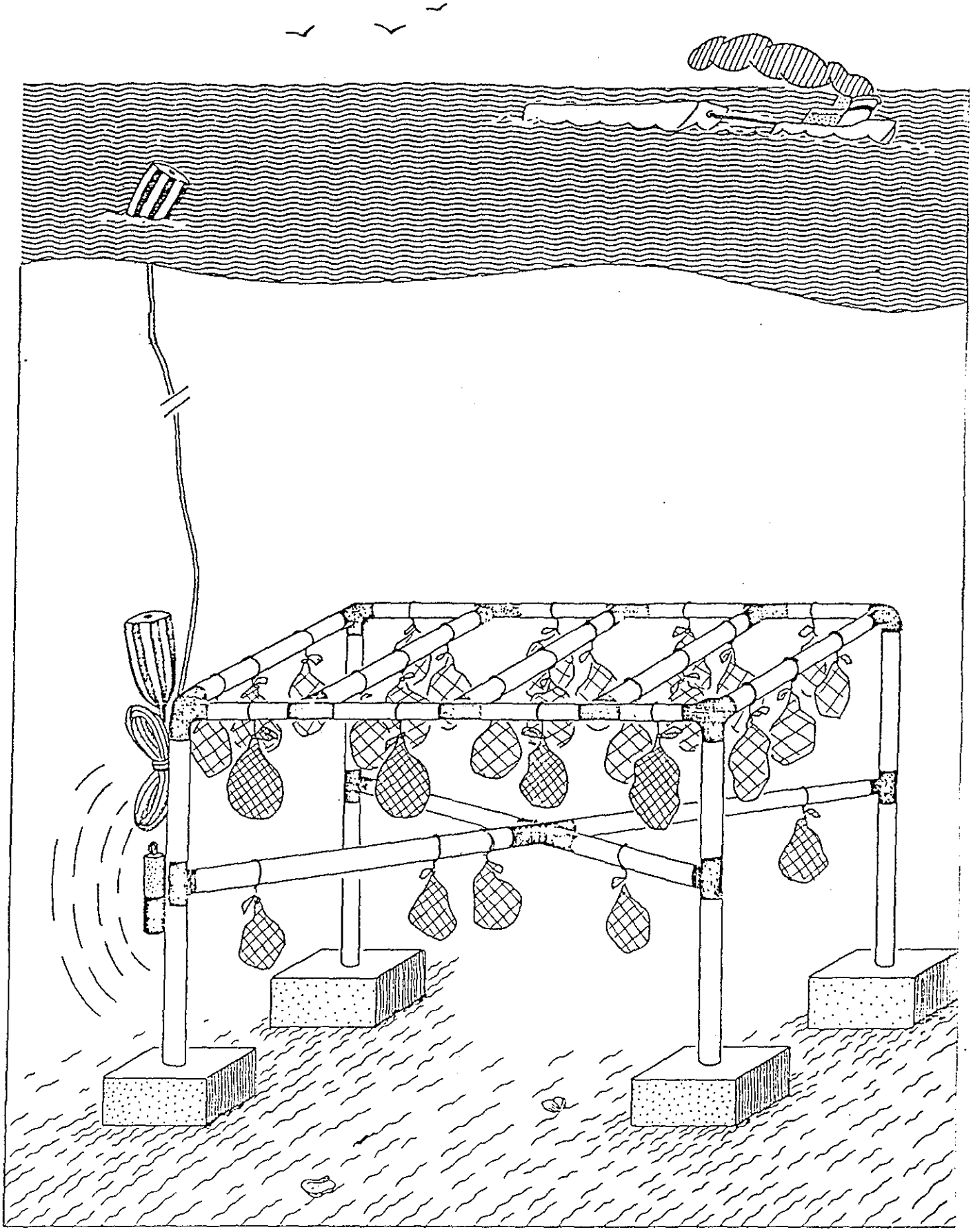

Figure 2.1-2. PVC platform used in maintaining shellfish at various monitoring stations.

used for the disposal of dredged material in 1943, and during two disposal operations, which occurred between August 1974 and June 1979, more than two million cubic meters of dredged material were disposed. Blue mussels from a subtidal reference population at Latimer's Light were transplanted to New London and to three other sites in Long Island Sound: an historic disposal site at Cable and Anchor Reef, an active disposal area south of New Haven harbor, and a prospective site in the western basin of the Sound. We also established and monitored a population of Mytilus at the inactive Brenton Reef disposal site at the mouth of Narragansett Bay. A brief description of these and the other DAMOS sites is presented in Table 2.1-1, and details of usage and site characteristics have been compiled (Naval Underwater Systems Center and Army Corps of Engineers, 1979 and New England River Basins Commission, 1981).

The study site near Portland, ME. was still under consideration for use as a regional disposal site during our monitoring program, and disposal did not begin there until 1980. We stocked the Portland station with mussels from a natural bed at Bulwark Shoals, and we designated Bulwark Shoals as a reference population. The Rockland, ME. site was last used in 1974, and a mussel bed at Drunkard's Ledge served as the stock and reference population for Rockland. The Portland and Rockland stations were stocked with horse mussels, and for them, fifteen mesh bags containing fifteen Modiolus each, were attached to the monitoring platforms. These two stations were too deep to be serviced by divers; consequently, acoustic releasers (Model 431, Innerspace Technology, Inc., Waldwick, NJ) were attached to the

Table 2.1-1

Disposal Area Monitoring System (DAMOS) Stations

Mytilus edulis stations

Station	Depth (m)	Last Usage[a]	Reference Station(s)	Depth
New London	20	active	Latimers Light	10
			Fishers Island	10
			Pine Island	5
New Haven	20	active	New Haven Reference Array	20
Cable and Anchor Reef	20	1973	Western Long Island Sound	30
			Greens Ledge	
Brenton Reef	30	1971	Outer Bridge	5

Modiolus modiolus stations

Station	Depth	Last Usage	Reference Station	Depth
Rockland	75	1974[b]	Drunkards Ledge	10
Portland	60	----[c]	Bulwark Shoals	10

[a] usage as of June 1979

[b] subsequent disposal occurred in 1981

[c] disposal at Portland began in 1981

platforms, and they were later used to retrieve the mussels. The sampling of these and several other stations was co-ordinated with the Naval Underwater Systems Center, Newport, RI.

2.2 Laboratory Procedures and Data Analyses

Immediately after collection, the shells of the mussels were cracked, and the soft tissues were fixed in either Bouins solution (on the first two sampling dates) or phosphate buffered formalin. The fixed tissues were prepared for observation by the histology laboratory of the Department of Pathobiology, University of Connecticut. The tissues were embedded in paraffin, and to standardize the preparations, cross sections of each animal were cut just anterior to the foot. Sections were stained with hematoxylin and eosin.

The mussel tissues were examined with a Millipore II MC particle measurement computer system interfaced to a Bausch and Lomb microscope and a Setchell Carlson video monitor. Specimens were examined at a magnification of 400X, and two types of measurements were made for each preparation. First, the number of eggs in each of twenty fields was counted (each field was 57909 μm^2), and second, the area in μm^2 of one egg in each of the twenty fields was measured.

For the Modiolus samples, separate two-way analyses of variance (two-way ANOVAs) were conducted to determine whether the numbers or areas of ova in mussels from the disposal site and reference populations differed significantly. For these analyses, mussels from the Rockland disposal area were compared with animals from Drunkard's Ledge, and mussels from Portland

were compared with samples from Bulwark Shoals. Samples from the two disposal site stations were collected in three different months; thus, the month of sample collection was the second classification variable for the two-way ANOVAs. Further information on the temporal variability in numbers and sizes of ova in Modiolus was obtained by analyzing data for horse mussels from the reference stations at Bulwark Shoals and Drunkard's Ledge. These reference stations were each sampled five times between May 1978 and June 1979.

During the early stages of oogenesis, the ova of Mytilus could not be accurately measured with the image analyzer. The reproductive cycle of Mytilus has been well documented (Seed, 1976), however, and we were primarily concerned with comparisons between experimental and reference populations. Therefore, the data for each sampling cruise were grouped by station, and one-way classification analyses of variance (one-way ANOVAs) were used to test the significance of the differences in station means. For the ANOVAs that produced significant variance ratios (F-values), least significant difference (LSD) tests (Snedecor and Cochran, 1967) were used to compare the mean numbers and areas of ova in mussels from the experimental and reference populations.

The data for both species of mussels also were examined using a program for cluster analysis (BMDP2M--Biomedical Computer Programs P-series Program 2M, Engelman, 1979) which was executed on the IBM S-370 computer of the University of Connecticut. For those analyses, the samples were labeled by the station, and the month and year in which they were collected. Samples were

grouped according to Euclidean distances computed from standardized values for egg numbers and areas.

The objective of the cluster analyses was to determine whether the mussel samples formed natural groupings based on collection sites, or dates, or a combination of sites and dates. Cluster analysis was employed as an interpretive or heuristic aid; it was used to generate hypothesis about the structure of the data, but it was not used to test the significance of the differences among the samples.

3.0 RESULTS

3.1 Modiolus

Qualitative examinations of Modiolus samples revealed no marked variability in reproductive tissues. Small concretions of undetermined composition were present in the kidney tissue of some individuaals, but we did not quantify them or attempt to determine either their causes or their effects. Of 207 Modiolus specimens examined, 82 were females with ova that could be measured with the image analyzer. The mean numbers of ova per image analyzer field and the mean areas of the ova are summarized in Table 3.1-1.

The gonadal tissues of female Modiolus from the Rockland disposal site and Drunkard's Ledge reference populations were not measurably different; neither the mean numbers of ova nor the mean sizes of the ova differed significantly (two-way ANOVAs, Table 3.1-2). For the three pairs of concurrent samples from these sites, the numbers of ova ranged from 8.60 (s.d. = 4.36) to 13.25 (s.d. = 1.52), and the sizes of ova (in μm^2,

Table 3.1-1

Numbers and areas of ova in *Modiolus modiolus* from experimental and reference populations

Station	Month and Year of Sample	n[a]	Exposure (months)	Mean ± Standard Deviation: Number of ova	Mean ± Standard Deviation: Area of ovum (μm^2)
Rockland	August 1978	3	3	8.60 ± 4.36	4581 ± 222
	November 1978	3	6	10.62 ± 1.83	4143 ± 540
	June 1979	4	13	13.25 ± 1.52	4559 ± 396
Drunkards Ledge	May 1978	6	na[b]	10.29 ± 0.85	3910 ± 422
	August 1978	4	na	9.80 ± 1.34	4212 ± 386
	November 1978	6	na	10.56 ± 1.82	4606 ± 576
	February 1979	3	na	15.32 ± 2.05	3190 ± 445
	June 1979	2	na	11.73 ± 1.80	3871 ± 499
Portland	August 1978	2	3	10.25 ± 2.19	4692 ± 858
	November 1978	2	6	8.98 ± 1.17	3989 ± 549
	February 1979	4	9	11.84 ± 0.98	3573 ± 364
Bulwark Shoals	May 1978	4	na	12.89 ± 1.49	3505 ± 315
	August 1978	5	na	7.81 ± 0.56	3924 ± 486
	November 1978	5	na	10.86 ± 0.98	4281 ± 421
	February 1979	5	na	13.38 ± 1.00	3720 ± 266
	June 1979	2	na	18.45 ± 9.62	2718 ± 753

[a] n denotes the number of individuals examined. Twenty fields were counted and twenty ova were measured for each individual.

[b] na: not applicable

Table 3.1-2

Summary of two-way analyses of variance for *Modiolus modiolus* samples

Rockland Disposal and Drunkards Ledge

Source of Variation	ANOVA (F-value, p) Number of Eggs	Egg Area
Main effects		
Study Site *A*	0.01, ns	0.91, ns
Sampling Date *B*	2.76, ns	0.27, ns
Interaction		
A x *B*	0.47, ns	2.85, ns

Portland Disposal and Bulwark Shoals

Source of Variation	ANOVA (F-value, p) Number of Eggs	Egg Area
Main Effects		
Study Site *A*	0.49, ns	0.29, ns
Sampling Date *B*	24.10, $p < 0.0001$	4.38, $p < 0.05$
Interaction		
A x *B*	8.21, $p < 0.01$	2.49, ns

Table 3.1-2 (continued)

Drunkards Ledge and Bulwark Shoals		
	ANOVA (F-value, p)	
Source of Variation	Number of Eggs	Egg Area
Main Effects		
Study Site A	2.46, ns	8.30, $p < 0.001$
Sampling Date B	9.29, $p < 0.0001$	4.66, $p < 0.05$
Interaction		
A x B	3.88, $p < 0.05$	2,51, ns

Table 3.1-1) ranged from 3871 (s.d. = 499) to 4006 (s.d. = 576).

In contrast, analyses of the three concurrent samples from the Portland dumpsite and Bulwark Shoals reference site showed that the reproductive condition of the animals changed with time. At Portland, the largest ova (in μm^2) occurred in the August sample (mean = 4692, s.d. = 856) and the smallest ova were in the February sample (mean = 3573, s.d. = 304); at Bulwark, the largest ova occurred in November (mean = 4281, s.d. = 421) and again, the ova were smallest in February (mean = 3720, s.d. = 266). At Portland and Bulwark the numbers of ova also varied temporally (range = 7.81, s.d. = 0.56 to 13.36, s.d. = 1.00, $p < 0.001$), but a significant disordinal site by date interaction ($p < 0.05$) indicated that the timing of development for the two populations was asynchronous.

Analyses of five sets of concurrent samples collected from the Bulwark Shoals and Drunkard's Ledge reference populations provided further evidence that the gonadal tissues of female Modiolus underwent developmental cycles. Areas of ova (in μm^2) ranged from 2718 (s.d. = 753) to 4606 (s.d. = 576), and these changes were strongly related to the month of collection ($p < 0.001$, Table 3.1-2); the difference in station means also was significant ($p < 0.05$). Numbers of ova ranged from 7.81 (s.d. = 0.56) to 18.45 (s.d. = 9.62), but for these data, the difference between the two stations was not significant. For the analyses of numbers of ova, a significant interaction between site and sampling date showed that the reproductive cycles of the two reference populations were not synchronized.

Cluster analysis (Figure 3.1-1) indicated that the

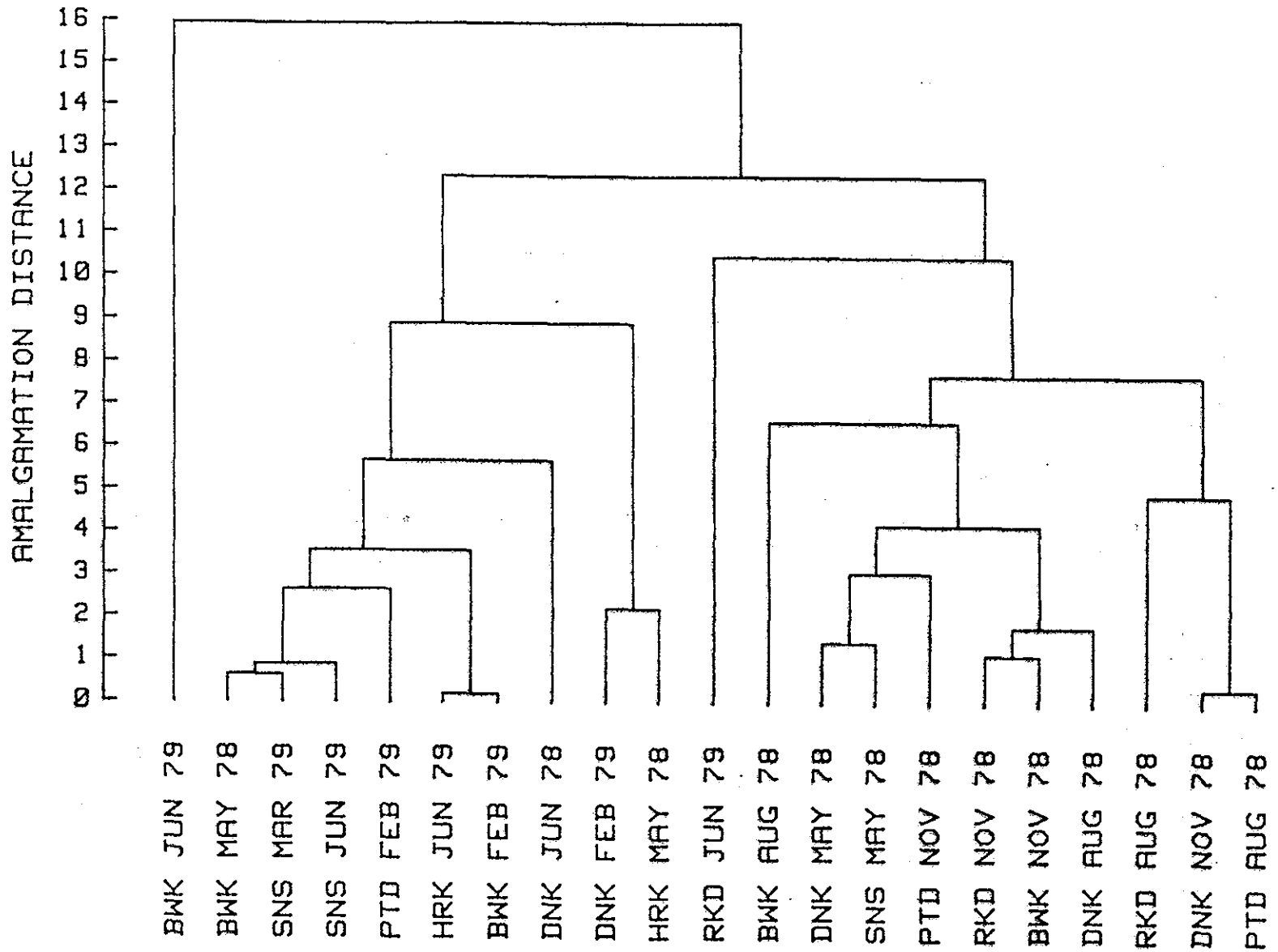

Figure 3.1-1. Cluster Analysis of Modiolus modiolus Reproductive Conditions from DAMOS Stations in Northern New England Coastal Waters.

reproductive condition of horse mussels from all stations, including Smuttynose and Halfway Rock (Figure 2.1-1), changed with time; the Modiolus samples formed two major clusters based on measurements of numbers and sizes of ova. One cluster (Group I) was primarily composed of samples from August 1978 and November 1978, and the other cluster (Group II) contained samples from February 1979 and June 1979. The samples collected in May 1978 were evenly split between the two clusters. The ova of Group I mussels were larger (mean = 4220 μm^2, s.d. = 312) than those of Group II animals (mean = 3521 μm^2, s.d. = 466), but Group I animals contained fewer eggs per field (mean = 40.19, s.d. = 16.41) than Group II mussels (mean = 49.84, s.d. = 15.37).

3.2 Mytilus

Tissue abnormalities were observed in the Mytilus samples collected from the New Haven disposal site on 29 July 1978, but in general, the reproductive tissues of mussels from the experimental populations were indistinguishable from those of reference animals. The gills of blue mussels from the July 1978 New Haven sample showed extensive epithelial necrosis and cell sloughing. In some specimens, only the chitinous supporting structures of the gills remained because the epithelium had completely detached. Sperm follicles of the experimental mussels were abnormal: the spermatids and spermatazoa were unevenly packed, and breaks in the periphery of the follicles were evident. Ova of mussels from this disposal site sample were small and granular, and their appearance suggested that gametogenesis had not proceeded normally or that the eggs had

degenerated. Deterioration of the periphery of the egg follicles also was evident in these animals, and Leydig tissue, which stores glycogen and normally surrounds gonadal and other tissues, evidently had degenerated.

The gonadal tissues of female Mytilus from the July 1978 New Haven disposal site sample also differed quantitatively from those of reference animals (Table 3.2-1). The New Haven reference station, Western Long Island Sound site, and Cable and Anchor Reef also were sampled in July 1978, and the sizes of ova in the samples from these populations differed significantly (one-way ANOVA, Table 3.2-1). Moreover, the ova in mussels from the New Haven experimental site were smaller than the ova in New Haven reference site mussels (LSD test, $p < 0.05$).

The New London disposal site and two reference populations were sampled in mid-November 1979. The numbers of ova in the mussels from these stations were not significantly different, but the sizes of the ova were significantly different at $p < 0.05$. The gonads of mussels from the disposal site could not be distinguished from those of reference animals from Latimer's Light, but mussels from the Fishers Island population had larger eggs than animals from the other two populations.

The Mytilus samples were separated into two groups by cluster analysis (Figure 3.2-1), and as was true for horse mussels, the dates of collection evidently were more important influences on reproductive condition than were station types or locations. The samples comprising group A were collected in July, August and September: these mussels had a relatively large number of ova in each image analyzer field (mean = 20.88, s.d. =

Table 3.2-1

Numbers and areas of ova in Mytilus edulis from experimental reference populations

July 1978

Station	n[a]	Exposure (months)	Mean ± Standard Deviation Ova per Field	Area of Ovum (μm^2)
New Haven dumpsite	5	3	14.84 ± 5.01	1182 ± 197
New Haven Reference Site	4	3	15.44 ± 2.46	1522 ± 137
Cable & Anchor Reef	2	3	17.98 ± 7.04	1866 ± 149
Western Long Island Sound	3	3	19.08 ± 3.93	1744 ± 252
F(3,10)[b], p			0.71, ns	8.80, <0.01

November 1979

Station	n[a]	Exposure (months)	Mean ± Standard Deviation Ova per Field	Area of Ovum (μm^2)
Latimers Light	4	1	11.30 ± 6.94	2055 ± 194
Fishers Island	3	1	12.95 + 1.57	2426 ± 50
New London	5	1	11.68 + 5.92	1997 ± 238
F(2,9), p			0.08, ns	4.86, <0.05

Table 3.2-1 (continued)

Other Stations Sampled

Station	Month and Year of Sample	n	Exposure (months)	Ova per Field	Area of Ovum (μm^2)
Latimers Light (natural bed)	October 1978	3	na	11.21 ± 0.76	2413 ± 155
	December 1978	3	na	8.40 ± 6.64	2589 ± 263
	December 1978	3	na	9.82 ± 5.47	2663 ± 405
	September 1978	7	na	23.16 ± 5.35	2159 ± 253
Pine Island	August 1979	11	1	28.95 ± 3.07	2011 ± 108
Greens Ledge	May 1979	3	na	15.10 ± 7.64	2125 ± 467
Brenton Reef	December 1978	3	7	13.12 ± 1.66	2417 ± 308

[a] n denotes the number of individuals examined. Twenty fields were counted and twenty ova were measured for each individual.

[b] F-values computed by analyses of variance to test the null hypothesis that station means were not significantly different. Degrees of freedom in parentheses.

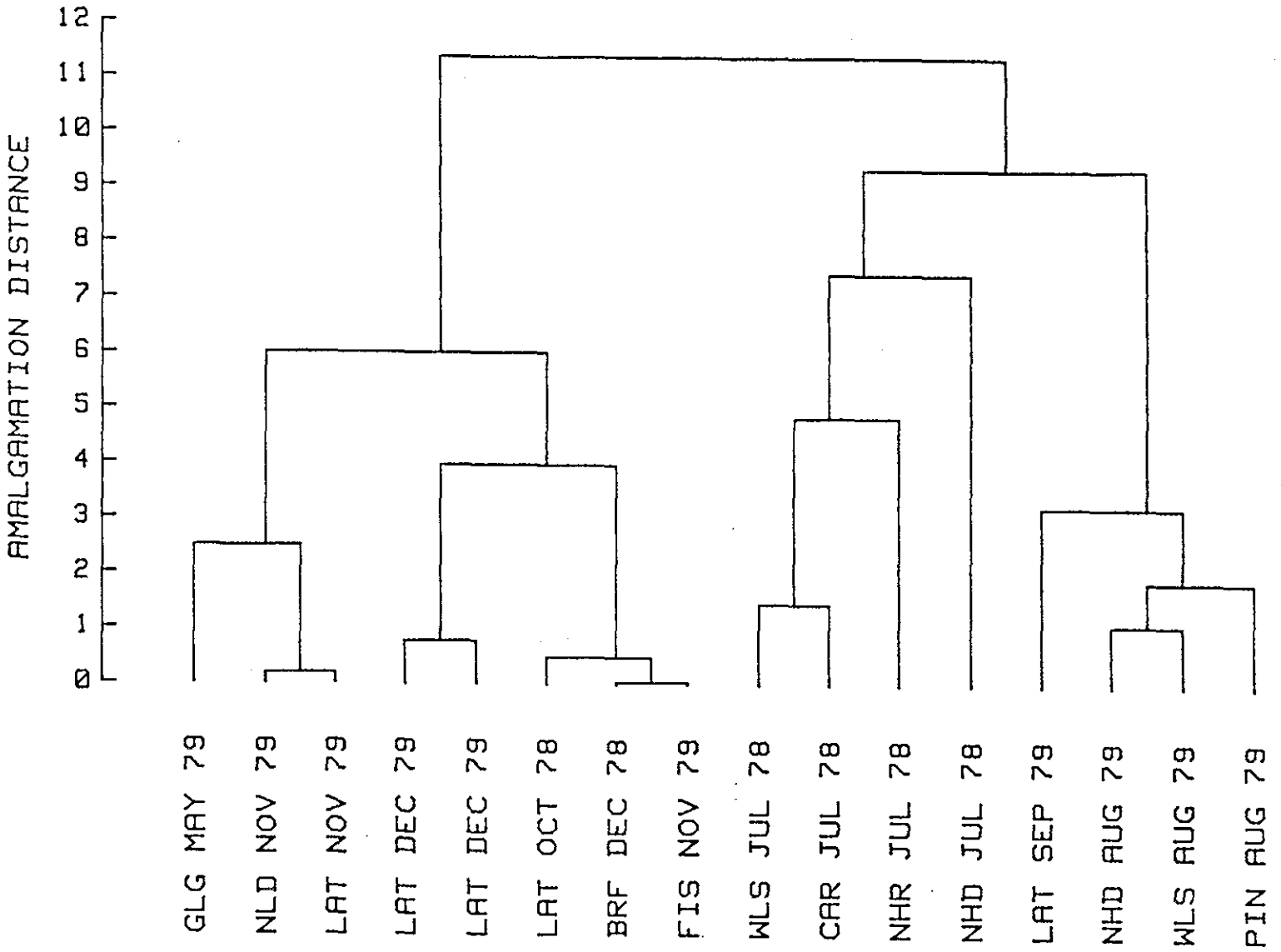

Figure 3.2-1. Cluster Analysis of Mytilus edulis Reproductive Conditions from DAMOS Stations in Southern New England Coastal Waters.

5.09), and the ova were smaller than those of group B mussels (mean = 1812 μm^2, s.d. = 315). Most of the cluster of samples designated as group B were collected in October, November or December, but one sample was from May. The ova in these mussels were large (mean = 2333 μm^2, s.d. = 252), but the number of ova in each field was small (mean = 11.70, s.d. = 2.07). The cluster map also showed that the New Haven disposal site mussels, in which tissue abnormalities were evident, comprised the last sample to be added to a cluster.

4.0 DISCUSSION

In coastal New England waters, the numbers and sizes of ova in Modiolus vary seasonally; however, the reproductive cycle is not pronounced, and routine histological examinations probably would not reveal the temporal differences in condition. Quantitation of egg numbers and sizes in the hard clam, Mercenaria mercenaria, by Keck et al., (1975) showed that when clams became sexually ripe, the sizes of ova increased and the numbers of ova per given unit of tissue reached a maximum. They also showed that when spawning occurred, the gonads contained relatively few, but large, eggs. The ova in Modiolus from subtidal populations at Bulwark Shoals and Drunkard's Ledge were largest in November, and the numbers of ova were small at that time. Consequently, our data indicate that there is a peak of spawning activity for Modiolus in autumn. Analyses of the data also show that the reproductive development of geographically separated horse mussel populations are not synchronized. The

asynchronous development of the populations may reflect differences in ambient temperatures and associated parameters, but we have not collected any data to test this possibility.

The spawning period of Modiolus is generally thought to be poorly defined (de Schweinitz and Lutz, 1976), and we found that the changes in gonadal condition of Modiolus were, indeed, not as distinct as in mussels of the genus Mytilus. Nevertheless, there is additional evidence, suggesting that the reproductive condition of Modiolus varies seasonally and that peaks in spawning occur in autumn and winter (Seed and Brown, 1977).

Our studies indicated that the reproductive development of horse mussel populations deployed on or near dredge material disposal sites was not impaired. At Rockland, captive populations of horse mussels were deployed to depths greater than sixty meters without measurably affecting the reproductive development of the animals. Evidently, the dredged material that was disposed at Rockland several years before our monitoring program began had little or no effect on the sexual development of the mussels. At Portland, the numbers of ova in the mussels varied with time, and a significant site by date interaction indicated that local environmental factors may have affected the reproductive condition of the animals. The site was not used for dredge material disposal until after our sampling was completed, however, and the differences between stations cannot be attributed to disposal operations.

Blue mussel populations from dredge material disposal sites generally exhibited normal reproductive development, but

the tissues of one set of samples from the New Haven disposal site were abnormal. Unfortunately, the causes of the tissue abormalities could not be investigated because the samples were processed and examined several months after their collection. In addition, samples from another experimental population subsequently established at the New Haven disposal site appeared normal, and presumably, the stresses causing the abnormalities were in some manner alleviated. At the New London disposal site, conditions were suitable for the growth of mussels: Mytilus has colonized a portion of disposal dredged material (Stewart, 1979), and juvenile mussels have been found attached to the shells of the captive monitoring animals (personal observation).

Our method for assaying reproductive condition anabled us to quantify changes in the tissues of mussel populations, but there are limitations to the procedure. First, we could only use the technique for studies on female mussels, and during the early stages of gametogenesis, the eggs of Mytilus could not be measured. Second, many of the mature eggs could not be isolated and measured with the instrumentation we used. Most important, the viability of the eggs could not be determined.

A chief advantage of our analytical scheme is that it enables us to study the continuous nature of reproductive development in certain animals. Prior histological studies on bivalve molluscs have lead to the recognition of four main stages of gonadal condition: developing, ripe, spawning and spent (Chipperfield, 1953 and Seed, 1976). Our method for assessing reproductive condition by counting and precisely measuring ova enables us to study how development progresses and to compare

populations subjected to various treatments or conditions. This approach could be used to learn how mussels, and perhaps other animals, respond to disturbances and to study the processes that govern their reproductive development.

5.0 SUMMARY

Experimental field populations of mussels (either *Mytilus edulis* or *Modiolus modiolus*) were deployed and maintained on or near open water dredge spoil dumpsites for up to thirteen months. The reproductive tissues of the experimental animals were compared with those of reference mussels by means of a quantitative assay based on measurements of numbers and sizes of ova. The reproductive development of the experimental populations generally was not impaired, and data analyses showed that the dates of sample collection were more important influences on reproductive condition than either station types or locations. We also present evidence that the gonadal condition of *Modiolous* varies seasonally; however, the changes are not as pronounced as those evident in the tissues of *Mytilus*.

6.0 REFERENCES

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