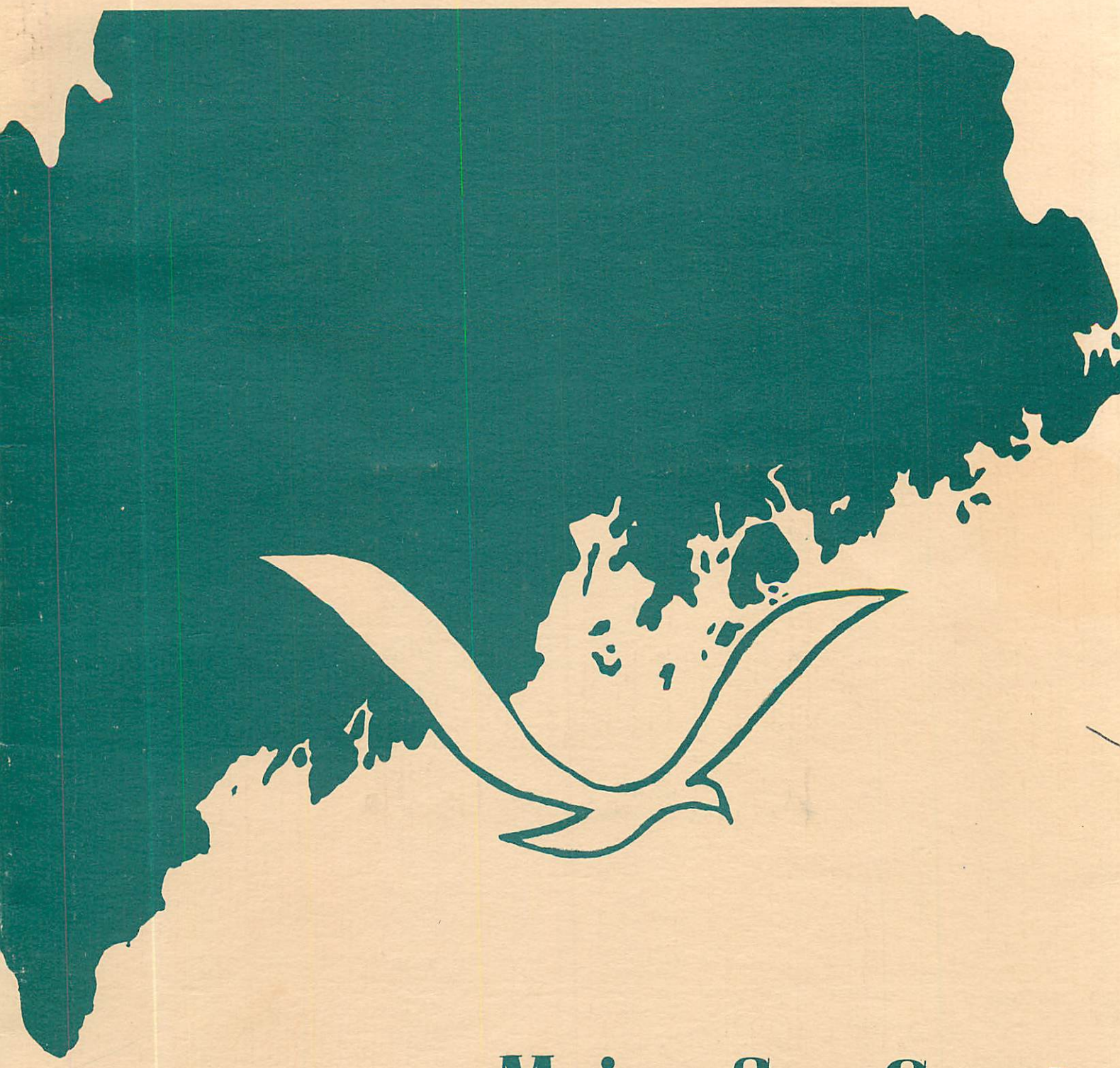
A University of Maine
Sea Grant Publication



Maine Sea Grant

a report

January 1 - December 31, 1975

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Ira C. Darling Center Reference No. 76-29

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Director's Message

This report covers Sea Grant activities at the University of Maine at Orono for the calendar year 1975. Coherent project support was received for marine research, education, and advisory services. Sea Grant activities occurred at the main campus of the University at Orono, at the University's Ira C. Darling Center in Walpole, and at a number of field locations about the state.

Productive use of the living marine resources contiguous to the coast of Maine continued to provide a focus for the research program. Research in direct response to this focus included both that on aquaculture and on indigenous species. Progress continued toward "Commercial Implementation of Aquaculture in Maine" including the support activity of "Shellfish Hatchery Seed Stock Production and Development of Hatchery Techniques for Maine". Research on "Cytogenetics of Selected Marine Species" and on "In Vitro Cell Culture of the Oyster" complemented the shellfish aquaculture effort. "Preservation of Rainbow Trout and Atlantic Salmon Gametes" provided a broadening of the aquaculture effort beyond the earlier program in shellfish.

Two research efforts looked at the impact of the discharge of a large nuclear power plant into Maine's coastal environment and aid in the assessment of both positive and negative aspects of such facilities. "Measurement and Modeling of Accumulation of Power Reactor Associated Radionuclides in Sediments and Aquacultured Oysters in Montsweag Bay, Maine" has provided valuable data for evaluating potential productive uses of the heated effluent. Influence of the discharge on two economically valuable species was studied in a project entitled "Effects of Thermal Addition on Sandworms and Bloodworms".

Complementary to the research program three Marine Advisory Services projects including both field services and publications efforts were successfully undertaken. These projects together with a project for the development of a graduate course in aquaculture increased the usefulness of the Coherent Project Sea Grant Program to the people of Maine.

The University of Maine Sea Grant Program has continued in its cooperative role with the University of New Hampshire Coherent Project Sea Grant Program. A Cooperative project for "Development of a Sustained Blue Mussel Industry in the Gulf of Maine" while producing good research results has also acted as a catalyst for cooperation not only between the marine programs of the Universities of Maine and New Hampshire but also with the Department of Marine Resources and Abandoned Farms, Inc., a commercial aquaculture firm in Maine.

In 1975 progress toward more productive use of Maine's living marine resources was accomplished and a contribution made not only to the overall objectives of the National Sea Grant Program but also to the State of Maine. Through development and expansion of the Sea Grant Program at the University we hope that the new knowledge gained and delivered through the program will benefit many.

Frederick E. Hutchinson

Director

RK Seaberg

Assistant Director

Maine Sea Grant worked with existing fishing industries and sport fisheries in a problem solving capacity and continued its established interest in creating new fisheries through aquaculture and the technology to support these new industries.

Commercial Implementation of Aquaculture in Maine
Shellfish Development of Hatchery Techniques for Maine

Shellfish Hatchery Seed Stock Production and
Development of Hatchery Techniques for Maine

We have been working four years now on our oyster culture projects and have made very significant progress in creating a new industry for Maine. It is time now to assess what has been accomplished, to allow us to set some new directions and emphasis for the future.

Very briefly, in the early 1970's, we felt that Maine was blessed with a super abundance of diverse, clean, estuarine waters that might support grow-out operations for cold-water varieties of American and European oysters. Indeed, our early environmental evaluation projects indicated that market half-shell European oysters and American oysters could be produced in 2-3 growing seasons, depending on specific environments. Long, cool summer-fall periods, contrasted to hot summer periods to the south, favored superior oyster growth. Thus, with the availability of cultchless seed from California, the logical place to start here was with commercial oyster grow-out.

A considerable portion of our early research-extension strategy was based on the exceptional performance of oysters in the Maine environment. First, we felt that we should involve private citizens to help us with environmental evaluation and, more important, to find combinations of ability and motivation coupled with a good growth area in which to actually begin cooperative experimental and pilot commercial grow-out operations. We felt that the best catalyst for large scale success would be to first get one or two coastal citizens to turn a profit at commercial grow-out. Second, since Maine lacks a consistent natural seed source, we began working on adapting world hatchery techniques for the sub-boreal environment to allow us to make proper hatchery recommendations should the grow-out operations become a commercial reality. Finally, we have used graduate student research as a mainstay of these projects. We have asked students to do projects that would solve the practical problems of hatchery and grow-out, but retain enough flexibility to make the research intellectually rewarding and worthy of University-quality research.

The results of our projects have been very significant. In four years we have involved about 80 private citizens in experimental oyster grow-out. Of the 80, three commercial companies have evolved with several more contemplating commercial activity in 1976. The prestige of Maine's European half-shell oyster has been established in the New York City restaurant trade with the Maine-grown "Balon" oyster commanding the highest unit price (85¢ each) of any half-shell oyster from North America. Further, Mr. Mark Richmond of the Maine Coast Oyster Corporation has been designated as Maine's JayCee "Farmer of the Year" for 1975 and will soon go for the national title in 1976. Presently, the

Maine grow-out operations have given rise to a five million seed demand, which has been supplied by California hatcheries; however, it would be very desirable to eventually have a Maine-based source to avoid possible disease introduction. Our Sea Grant program is providing back-up commercial seed now to our grow-out people should the West Coast sources fail. Two entrepreneurs will begin pilot commercial seed production in 1976 with our help.

Our involvement with the developing industry in Maine has allowed our staff and students to develop a wide variety of relevant research topics.

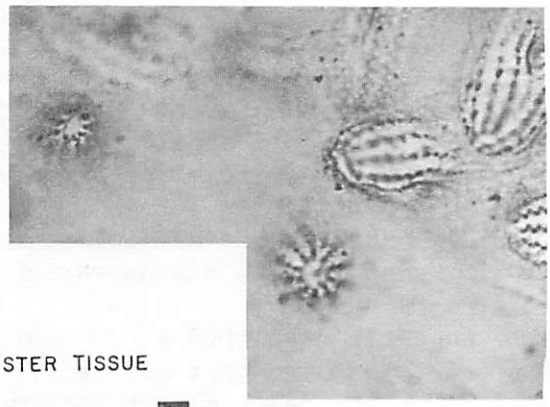
We have recently developed a new cultchless setting method using polished marble. It was found that mature oyster larvae were stimulated to set, apparently by the calcium carbonate of molluscan shell. Marble, which is metamorphosed calcium carbonate, also is highly stimulatory to setting oysters. Setting larvae will completely blacken marble in short order. Metamorphosed larvae can then readily be removed with a blade a day after setting. Very recently we have found a way to improve the efficiency of the process by orienting the setting larvae. It was found that larvae will set umbo uppermost if presented with a vertical cultch surface. Oriented spat are removed much more efficiently because less shell damage results from scraping from the umbo side first.

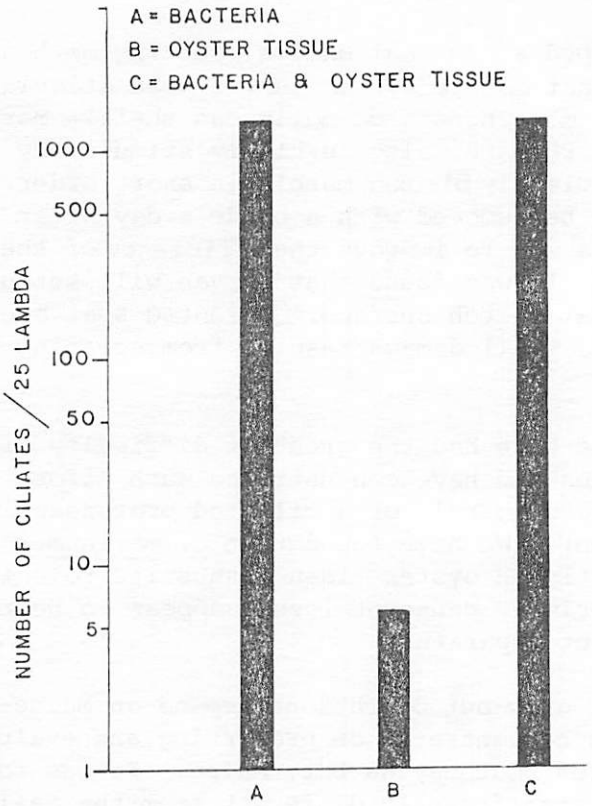
With hatchery systems we have had the greatest difficulty with the very early cultchless nursery stages and have concentrated much effort in this area. We have investigated the feeding role of a ciliated protozoan invader, Uronema marinum (see description). We have found that U. marinum is a bacterial feeder and does not feed directly on oyster tissue, thus its role in our hatchery is as a secondary invader. Primary cause of losses appear to be related to toxic materials in our grow-out apparatus.

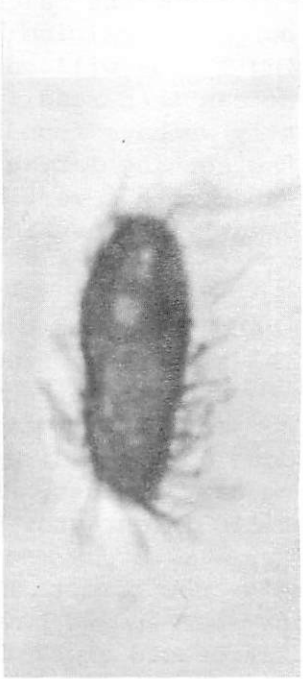
Maine's new commercial grow-out operations depend on Maine-adapted European oyster seed and we have concentrated on preserving and evaluating the 25-year old introduced stock from Boothbay Harbor, Maine. It was found that the Boothbay population is significantly different from the California hatchery stocks with respect to the genetic control of glycogen metabolism. The Boothbay population may lack genetic diversity, but appears to be selected for better overwinter survival. We are considering this information in our brood stock strategy and attempting to outcross the Maine groups with California stocks of European oysters.

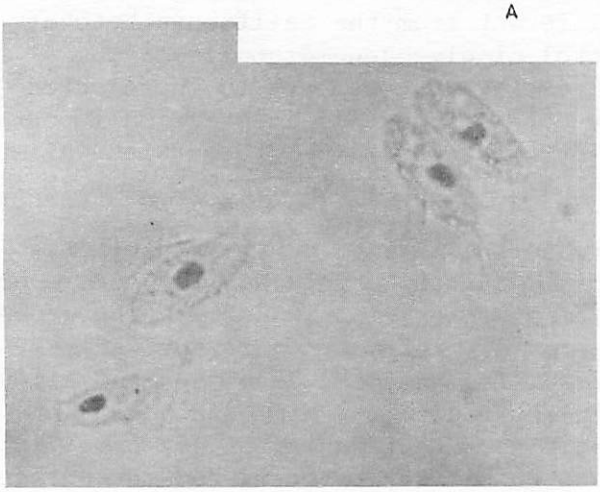
In the future we intend to shift our emphasis somewhat. Our early efforts have been geared to stimulating commercial activity. This has now been accomplished and we now have a very fragile first generation industry. The emphasis in the next several years should be to provide infrastructure for the new industry. Inevitably, problems will arise: for example, diagnosis and alleviation of possible disease problems, efficient fouling removal, and a host of other biological problems. Several people now are thinking of, or have begun, pilot operations of shellfish hatcheries to satisfy the new seed demand here. We are working closely with them and are refining our methodology to allow the best recommendations to industry.

Herbert Hidu, Principal Investigator

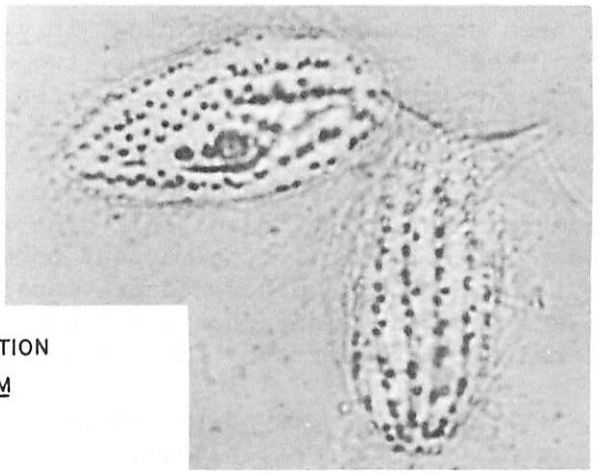


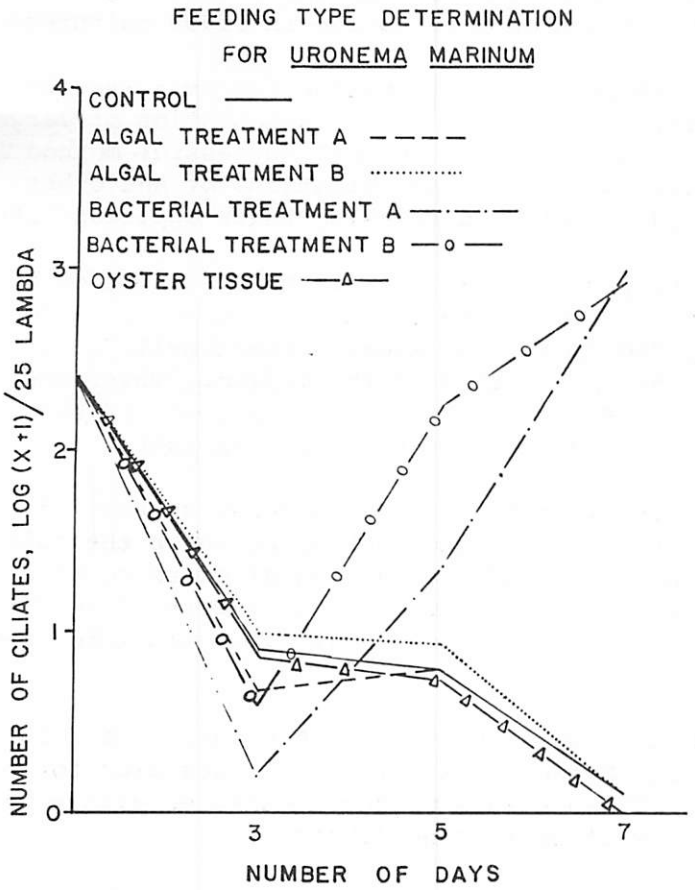


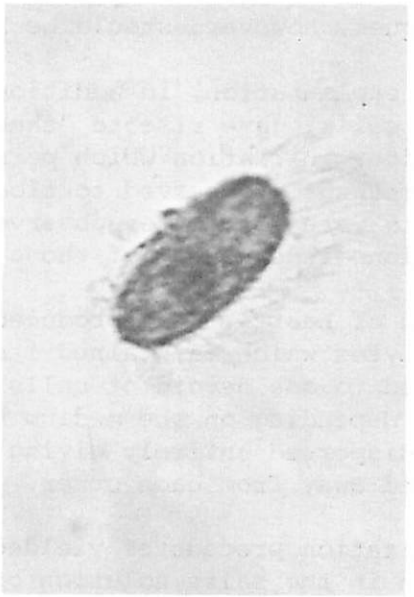




A protozoan ciliate, Uronema marinum, frequently infesting early cultch-less, juvenile oysters was stained and identified. We determined it to be a bacteriophage, thereby defining its trophic niche and its role in oyster hatchery production.







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In Vitro Cell Culture of the Oyster (*Crassostrea virginica* and *Ostrea edulis*)

We have been successful in culturing, in vitro, cells from oyster (Crassostrea virginica) heart tissue. In general, two cell types predominate. One type is small, amber colored, granulated and often highly vacuolated. The other cell type appears larger with few granules and no vacuolation. The smaller cells were most often present in a rounded form but appeared to give rise to several other forms depending on the growth medium. Many of these cell forms exhibited extensive movement. The second cell type was always round and movement was not observed. These two cell types appear to correspond with the leucocytic and lymphocytic amoebocytes described previously (3,4). The smaller leucocytes were always more prevalent than the larger lymphocytes and rounded leucocytes outnumbered extended forms by five or ten or more to one in older cultures.

Several methods were employed to prepare the heart tissue for culture; these included mincing into small pieces, trysinization, and explantation of larger tissue chunks. Tissue explantation was clearly the most successful method in terms of the numbers of viable cells obtained. Modifications of the other techniques, however, should be studied before dismissing their applicability.

Tissue explantation, in addition to providing a generalized distribution of single cells, gave rise to "sheets" of interconnected amoebocytes two to four days after initiation which persisted up to four weeks. These cell "sheets" were frequently observed to float on the surface of the medium. These sheets of cells were most often observed in Medium 6 (explanation below) and exhibited pulsations independent of those which occurred in the heart explant itself.

Mincing of heart tissue produced, in all media tested, a dense culture of amoebocytes which maintained itself for about one week after which the culture declined to one devoid of cells in the extended condition after three to six weeks, depending on the medium used. Small clumps of tissue were often noted which dispersed entirely giving rise to patches of "healthy" cells which then migrated away from each other.

Trysinization procedures yielded the poorest cultures. A solution of .25% trypsin in the salts solution of Li, Stewart and Drinnan (2) was used for periods of one to thirty minutes. These always produced cultures with very few extended cells and large amounts of extraneous debris.

Six different media formulations were used, each containing medium 199(2X) dissolved in salts solution (2X) (2) and made up to volume by the addition of 15% fetal calf serum, antibiotics, and various supplements as described below.

Medium 1 - Basal Medium

Medium 2 - Basal Medium and Lactalbumin Hydrolysate

Medium 3 - Medium 2 + Oyster Hemolymph

Medium 4 - Medium 3 + Bovine Embryo Extract

Medium 5 - Medium 3 + Whole Oyster Extract

Medium 6 - Medium 3 + Oyster Heart Extract

The various media were evaluated for their ability to support cell maintenance and migration from heart tissue explants. The best media were six, one and two, in decreasing order. Medium six, as noted above, gave rise to extensive cell monolayers. Several leucocyte forms were observed, the earliest (1-2 days) being a polygonal shaped cell with short, narrow and highly branched extensions issuing from the apieces of the cell contour. These cells were observed in all media but seemed least vacuolated and most extended in medium 1. No visible movement was noted but patches of these cells dispersed with time. The second cell type appeared later and was typically four to six times longer than the original rounded cell. This cell subtype has one or two long, thin, linear extensions ending in a hyaline, barely visible fans of cytoplasm. These were noted only in medium 6 and movement was quite noticeable.

Media 1 and 2 were similar to cells produced. In addition to the polygonal type, a highly motile, rounded type typified by many short motile projections (bristle cells) were noted. These often appeared in entangled clumps of five and six. In addition to amoebocytes, ciliated cells appeared on the surface of the explant after one week in Medium 1 and two weeks in Medium 2, and have persisted for fifty-two days to date.

Medium 3 tended to give rise to clumps of tissue which dispersed into polygonal amoebocytes after several days.

Medium 4 yielded fewer polygonal cells than the other media and those that did appear persisted for a maximum of four weeks as compared to five to seven weeks in the better media.

Medium 5 failed to show any cells at all for the first two to three weeks after explantation. After this period, however, polygonal cells were observed similar to those in medium 6. Apparently, something which inhibits cell growth is present in the whole oyster extract added to Medium 5.

Of the several vessels tested, plastic (30 ml) flasks gave the most consistent results. Leighton tubes with coverslips were not as effective. In all cases, plastic surfaces proved best, if not essential, for culturing oyster cells.

Some attempts were made at culturing other tissues such as gonads, mantle, pericardium, and embryonic juvenile tissue. All such attempts yielded few cells but were hindered by bacterial and/or protozoa contamination. The culture of cells of these tissues should be investigated further.

Extensive contamination with yeast and protozoa were encountered throughout these experiments and appears to be the major impediment to the routine culture of oyster cells. We have, however, brought this problem largely under control through the development of improved methods of handling tissues used for explantation.

Cultures established at 12-15C yielded fewer cells and less variety of cells than cultures maintained at 18-20C. The higher incubation temperature appears optimum.

Preliminary studies indicated few, if any, cells undergoing mitosis in any culture.

Bruce L. Nicholson, Principal Investigator

Cytogenetics of Selected Marine Species

Emphasis on this Sea Grant project has been on isozyme analysis, particularly of Mytilus edulis. Initial attempts to identify populations of M. edulis using starch-gel electrophoresis indicated that intrapopulation genetic variation was significant and should be studied if interpopulation differences were to be understood. Consequently, studies of the population biology of M. edulis were undertaken in conjunction with the electrophoretic work to see if variations within population structures could be related to genetic variations found by electrophoresis in these populations.

Sampling at several locations along the Maine coast demonstrated that the size distribution of M. edulis varied from site to site and within the tidal zone at each sampling site. It appeared that this size variation could be due to age differences, selection by larval cohorts for particular substrates or tidal zone levels, or local post-settling movement of mussels from one portion of the tidal zone to another. Indeed, in different sampling locations, each of these four mechanisms appeared to play a major role in producing this variation.

The questions considered are, one, whether or not the allelic frequencies of the enzymes leucine aminopeptidase, phosphoglucose isomerase, malate dehydrogenase, and acid phosphatase vary with the age of the mussels within a single population; and two, if such variation occurs, can it be related to environmental selection forces.

Both LAP and PGI were found to give best results using Selander's phosphate buffer at pH 6.7 (Selander et al., 1969). Three LAP loci could be resolved using this system, two of which migrated anodally and one cathodally. The two anodally migrating loci were usable but the migration rate of the cathodally migrating enzymes was too slow to adequately separate alleles and this locus was not used in the study. Each of the two anodally migrating loci contained several alleles, two of which were predominant, yielding zymogram patterns with single-banded homozygotes and two-banded heterozygotes. The zymogram patterns obtained from the LAP study appear consistent with a two-locus, multiple allele model, and do not appear to fit a single locus model. It should be noted that when using buffers other than the phosphate buffer, only one LAP locus could be resolved. This locus appeared to coincide with the faster of the two anodally migrating loci found with the phosphate buffer system.

This interpretation of the LAP data to fit a two locus genetic model (three loci, if the cathodally migrating locus is included) may differ from other models proposed for the LAP system in M. edulis. For individual specimens at least one band was always present at each anodally migrating locus with heterozygotes and homozygotes occurring in all possible combinations in different specimens. The results gave combinations of two, three and four bands occurring on the zymograms with never less than two bands for each specimen. In addition, staining density at these two loci was consistent with a two locus interpretation, with single banded homozygotes exhibiting a stain density greater than either of the two bands of the corresponding heterozygote at each locus. The stain density of the two bands of each heterozygote were equal, and where three bands occurred, that band interpreted as homozygous for its locus was usually found to be more densely stained than either of the two bands representing the heterozygous condition at the other locus.

The cathodally migrating locus was always present and densely stained. Variation in migration rates could be found among different specimens, but the migration distances were not adequate to determine any further information from this locus.

PGI proved to be a single locus, multiple-allele system exhibiting single-banded homozygotes and three-banded heterozygote zymogram patterns. A total of eight alleles were found at the PGI locus, but three were predominant.

MDH and AP were best resolved using a Poulik buffer system modified from Schaal and Anderson (1974). Both of these enzymes exhibited a single locus and were monomorphic in all animals tested.

The results of the LAP study are shown in Table 1, with observed phenotypic frequencies for the two LAP loci together with expected frequencies (in parentheses) on the basis of a Hardy-Weinberg equilibrium model. Also shown in the table are the allelic frequencies corresponding to the observed phenotypes. As presented in the table, Locus A represents the faster of the two anodally migrating loci, and allele A represents the most rapidly migrating alleles. Alleles which fit neither the A nor B migration pattern were included with the allele they corresponded to most closely. The low frequencies (less than five percent total) of these alleles justified this approach.

Both LAP loci exhibit large deficiencies of heterozygotes in the age groups containing young-of-the-year animals, while the older groups which have successfully overwintered show increasing percentages of heterozygotes. Tests for Hardy-Weinberg equilibrium show that for both loci, the young-of-the-year age classes were not in equilibrium ($p < .005$) while the two older age classes were in equilibrium. Contingency Chi-Square tests show that a significant difference ($P < .01$) exists among the three age groups at each LAP locus. It is obvious from the gene frequencies given in Table 1 that this difference lies between the first group and the two older age groups for the fast LAP locus, and between age group three and the two younger age classes for the slower of the two LAP loci.

Table 2 gives the results obtained from the PGI study, again showing observed phenotypic frequencies and those expected (in parentheses) on the basis of a Hardy-Weinberg equilibrium model. Since the number of alleles occurring in addition to the three predominant ones was large and constituted collectively a significant portion of the total, a separate allele class (X) was created using the pooled counts of these alleles. The alleles are presented alphabetically in the table in decreasing order of migration rate, the fastest being allele A.

Mortality factors which were observed in this study were relatively few in number and largely non-selective in nature, and it is our belief that these factors exert a randomizing and mixing effect on mussel populations with the result that older age classes will be closer to random distribution and hence closer to Hardy-Weinberg equilibrium and contain higher percentages of heterozygotes than young-of-the-year age classes.

The gross deficiency of heterozygotes shown in the PGI date (Table 2) suggests that other factors in addition to individual post-settlement movements and randomizing, non-selective mortality must play a role in producing such a de-

ficiency. The most likely explanations are that heterozygotes do not survive as larvae or that they fail to survive subsequent metamorphosis. Although our laboratory has been successful in making individual genetic crosses with M. edulis and obtaining growth rate data from the progeny, it has not yet been possible to get genetic data from individual mussel larvae, but an hypothesis can be made for homozygote selection on the basis of spatial compression of ecological variables within the tidal zone. In this zone, environmental changes occur over a vertical distance measured in meters comparable to changes occurring in terrestrial systems measured over distances of hundreds of meters. To colonize and survive over the entire range of environmental variables found in the tidal zone undoubtedly requires a combination of physiological adaptation and genetic variability. That genetic changes occur within the width of the tidal zone in mussels has been shown by both Koehn et al. (1973) in Modiolus demissus and by Gravem (1975) with Mytilus californianus.

It is possible that competitive demands placed upon juvenile mussels settling in the intertidal zone are magnified by the narrowness of the zones within which particular genotypes can survive. The effect of such magnified competition could be the survival of only those mussels most fit to compete in each narrow area: homozygotes for particular alleles. That heterozygotes are found is evidence of physiological adaptation and the variable nature of selection forces Environmental factors which exert selective pressures are not constant, and the intensity of their selection will vary over time. This could allow the accumulation of heterozygous individuals which could survive in the population over brief periods but which might be eliminated when selective pressure is intensified. In addition, as mussels increase in size they become less susceptible to environmental damage: they are more difficult to prey upon, by invertebrate predators at least, and their increased mass makes them less apt to be killed by extremes of weather. Thus, once grown to adult size, even if selective pressures intensify, large heterozygous animals might be able to survive as well as smaller homozygous ones, with the result that heterozygotes will exist in larger numbers in older age groups, as this study and others (Koehn, 1972; Graven, 1975)have indeed found.

Based on this analysis, we conclude that aquaculturists should continue to establish breeding schemes to maximize heterozygosity, as has become standard with nearly all agriculture species; but they should also pay particular attention to the environment in which the species will exist. With sufficient narrowness of the environmental zone selected homozygosity might lead to improved survival. Thus, caution should be used in applying breeding schemes based on heterosis and should not be unequivocally accepted.

Table 1. Leucine aminopeptidase alleles and phenotypic frequencies observed (expected) in three age groups from a single population of Mytilus edulis.

Age group	N	Phenotypic frequencies observed (expected)			Gene frequencies	
		AA	AB	BB	A	B
Locus A						
1	95	51(45.2)	29(40.6)	15(9.1)	.67	.31
2	71	20(17.7)	31(35.5)	20(17.7)	.50	.50
3	100	22(25)	56(50)	22(25)	.50	.50
Locus B						
1	73	62(56.3)	4(16.5)	7(1.2)	.87	.13
2	79	62(61.4)	14(17.9)	3(1.3)	.87	.13
3	104	95(93.9)	8(9.8)	1(.26)	.95	.05

Table 2. Phosphoglucose isomerase alleles and phenotypic frequencies observed (expected) in three age groups from a single population of Mytilus edulis.

Age Group	N	Phenotypic frequencies observed (expected)							Gene Frequencies		
		AA	AB	AC	BB	BC	CC	X	A	B	C
1	55	13(4.0)	0(10.4)	2(5.6)	16(6.2)	1(7.3)	8(2.0)	15(3.0)	.27	.35	.19
2	82	5(1.2)	0(2.6)	5(6.9)	7(1.4)	2(7.5)	23(10)	35(52)	.12	.13	.35
3	87	16(6.6)	0(8.5)	3(15.6)	12(2.8)	3(10.2)	22(9.5)	31(34)	.27	.18	.33

Franklin L. Roberts, Principal Investigator

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Development of a Sustained Edible Blue Mussel Industry in the Gulf of Maine

In 1975 the University of Maine was happy to join with the University of New Hampshire, the Maine Department of Marine Resources and Abandoned Farm, Inc., a private aquaculture enterprise, to study various aspects of the development of a new industry for the Gulf of Maine. This project, administered through the University of New Hampshire Sea Grant Program, examined several phases of this growing fishery.

Under the direction of Reginald Bouchard of the Maine Department of Marine Resources, consumer education projects were carried out in Maine and New Hampshire. A breaded mussel for restaurant use was developed by MDMR food technologists. Increased demand for the blue mussel in the New England area has resulted. Abandoned Farm conducted test marketing with two restaurants and did test shipping as well as retailing mussels locally.

As market development continues and mussels are sent farther afield,extending the shelf life of M. edulis becomes necessary. Dr. Bohdan Slabyj researched this aspect of shellfish handling. His studies resulted in the publication "Handling and Storage of Blue Mussels in the Shell".

Research and commercial culture of mussels were allied through the co-operative efforts of the University of Maine research team and Abandoned Farm. New co-operators were brought into the experimental raft program. Assessment was begun of growth, mortality and recruitment of M. edulis in 6 Maine and 2 New Hampshire environments including the heated effluent waters of nuclear and fossil fuel power plants. Twelve rafts were placed along a gradient from an upper estuarine environment to the open ocean in order to determine the most favorable conditions for mussel growth. With Abandoned Farm the research unit made monthly measurements of meat yield, pearl incidence and condition of 50 - 70 mm cultured mussels; monitored mussel growth and mortality at various depths; and initiated hatchery operations where various culture techniques were tried.

See also: The University of New Hampshire Sea Grant Program 1975: A Report on the University of New Hamphsire Sea Grant Program January 1, 1975 to January 1, 1976.

Co-Principal Investigators:

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publications:

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Myers, Edward A. and Paul D. Ring. Maine Mussels.

Dietary Intake and Nutritional Parameters in the Lobster

The development of a suitable low cost feed for lobsters (Homarus sp) is becoming more necessary with the increased holdings of lobsters. Lobster fishermen are discussing the formation of lobster co-ops which will hold lobsters over from times of low demand, with low net worth, to times of peak demand, February through September, and high value, in order to stabilize the market prices. The lobster is being used regularly in scientific research in areas such as neurophysiology and medical assay. Studies indicate that the lobster fishing industry has achieved its maximum sustainable yield yet demand is increasing. Therefore, the market cost is rising, making the farming of lobsters more economically feasible with each price increase. In all of the above cases, lobsters will be held in reserve tanks for some period of time and during this holdover they must be fed.

Although lobsters have been maintained on feeds such as brine shrimp and lobster meat and other natural foods these feeds are far too expensive. A low cost feed source is required. Research to this end is in progress primarily by trial and error methodology. Rather than base a test diet on feeds established for other species, we are studying the natural dietary intake of the lobster through the analysis of the stomach contents of lobsters taken directly from their natural environment. This study could then be used as a more accurate basis for the development of artificial diets for lobster holding, shipping or for aquaculture.

The lobster, like any domestic animal, requires certain amounts of energy--protein, minerals and some vitamins. These are the nutrients we are analyzing in the lobster stomach. Protein is being further analyzed as to its amino acid content. A precise amino acid balance is necessary for efficient protein utilization and protein is usually the most expensive part of a feed.

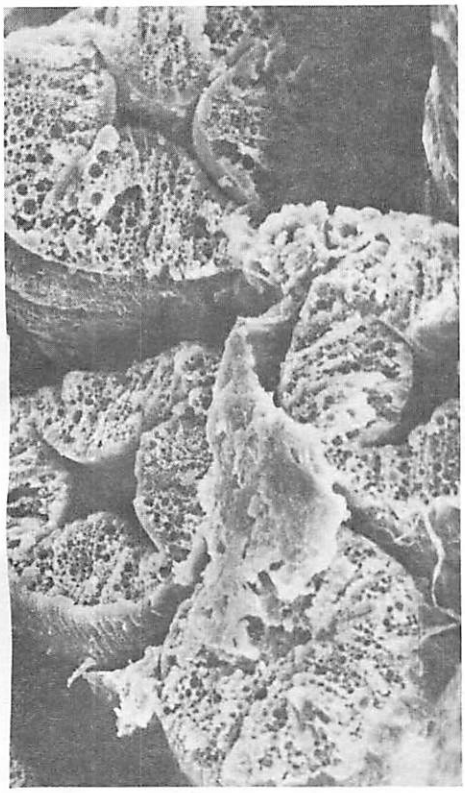
Preliminary stomach samples taken in the spring indicate that the lobsters were consuming a diet very high in minerals, relatively low in protein with varying levels of fat when compared to diets of domestic animals.

The primary nutrient absorptive structure of the lobster is the midgut gland. The physiological condition of this gland may be influenced by improper diets. One method that may be used to evaluate the physiological status of this gland is examination of the absorptive surface by scanning electron microscopy (SEM). In a study funded in part by the Maine Agricultural Experiment Station,

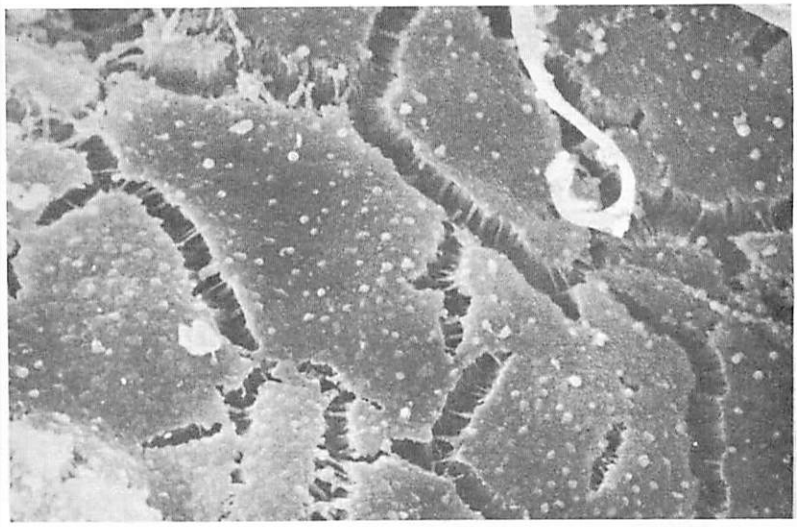
we have examined midgut glands from several lobsters. Tubules, finger like projections into a sinus, were observed (see photo #1). Feed passes through the hollow lumen (Photo #2) and absorption takes place at the fine filamentous microvilli that line the tubule lumen (Photo #3).

Robert Bayer, Principal Investigator

(Bayer, photo 1)



(Bayer, photo 2)



(Bayer, photo 3)

Preservation of Rainbow Trout and Atlantic Salmon Gametes

Long-range uses for salmonid gamete and embryo preservation are many. One immediate and pressingly urgent value of this research is the immediate impetus successful gamete preservation techniques would provide for the genetic engineering of the Atlantic salmon. When genes, rigorously selected by nature, in fish with particular migratory behaviour in specific rivers can be stored, distributed and manipulated by fish geneticists, the restoration of the Atlantic salmon to North America will proceed more efficiently than is now possible.

Salmonid Spermatozoa

Incubation. The fertility of fresh, unfrozen spermatozoa, after incubation in a diluent solution, reveals what effects various parameters such as osmolality, pH and ionic content of the extender may have on the viability of spermatozoa. The data in Table I show that rainbow trout milt, incubated in a modified Poulik's solution at either at 1/1 or 1/4 dilution, is as fertile as fresh, unincubated milt. In fact, by comparison to a sample of fresh milt from another group of rainbow trout that had a low fertility, an incubated sample had greater than 100% fertility (40% compared to 30%). Note also in Table 1 that Hank's diluent reduced the fertility of nonfrozen spermatozoa, but incubation of milt in modified Poulik's subsequent to incubation in Hank's restored much of the lost fertility so long as the final dilution of milt was 1/8 and not 1/16.

Freezing and thawing. Rainbow trout eggs, fertilized with spermatozoa, frozen to -80°C in Hank's diluet, had 7% hatching success (per 1100 eggs in duplicate) compared to 30% for eggs fertilized with untreated, control milt. As one would expect from the incubation data cited above, in another experiment with rainbow trout gametes, the post-thaw incubation of milt, frozen in Hank's diluent, did increase the fertility of the milt. Atlantic salmon spermatozoa were motile after being frozen to -196°C and thawed, but their fertility was lost. Apparently critical properties of Poulik's diluent had been altered.

Vacuum drying. After an initially high fertility obtained with sperm that were vacuum dried, this procedure has had minimal success. Nonetheless, dried, reconstituted sperm stored for as long as one year at -11°C have produced normal fry. Vacuum drying can work, but details of the procedure need improvement.

Salmonid Eggs and Nascent Embryos

Freezing and thawing. During this study salmonid eggs and embryos have been successfully frozen to temperatures as low as -50°C. Eyed rainbow trout eggs survived -40°C temperatures and eyed Atlantic salmon eggs survived -55°C. The eggs which were clearly frozen solid, subsequently hatched into normal fry. Two samples of salmon eggs, frozen to -20°C immediately after fertilization in a balanced salt solution, had 57% and 90% of the eggs eye and hatch into normal fry.

	Dilution	# of eggs	Percentage eying
Hanks	1/2	722	37
	1/2	965	58
	1/4	1309	24
	1/4	1342	25
Pouliks	1/2	1183	98
	1/2	1278	97
	1/4	1354	97
	1/4	1196	98
1 (Hanks 1/2) into 3 Pouliks			
	1/8	943	64
	1/8	988	68
1 (Hanks 1/4) into 3 Pouliks			
	1/16	935	11
	1/16	924	17

Table 1. Fertility of Rainbow trout milt expressed as percentage of total number of eggs present that eyed. (Nearly 100% of eyed eggs hatched in all groups). All milt was incubated for 30' at 0°C. Double dilutions incubated 15 min. in each solution for a total of 30 min.

Susan Zell, Principal Investigator

Sea Grant's concern is not only for Maine's economy as it works to create new, non-polluting aquaculture industries but also for Maine's environment. In 1975 Sea Grant-funded research worked to save Maine's beaches, educate the public and, in part, monitor environmental impact of a nuclear power plant.

Effects of Thermal Addition on Sandworms and Bloodworms

In 1975 Sea Grant provided partial funding for the continuation of environmental surveillance studies in the area around the Maine Yankee atomic power plant. The field studies were designed to determine whether warm water discharge from the plant has altered nearby populations of sandworms and bloodworms. The harvesting of these bait worms constitutes the principal commercial fishery (in excess of $1 million annually) near the plant. Although other species were monitored in the intensive studies, Sea Grant's involvement was with bloodworms and sandworms and we will limit our discussion to the methods used to monitor the quantitative distribution, growth and reproduction of these species and the temperature and salinities of intertidal benthic habitats.

Bi-weekly water temperatures and salinities were recorded at or near slack high tide at 22 field stations to determine temperature and salinity conditions over tidal flats where worms were being sampled. At each station, measurements were taken at 1 m intervals from surface to bottom using a Beckman RS5B salinometer-thermometer which was checked periodically against a laboratory thermometer and a Beckman RS7 laboratory salinometer calibrated with standard sea water.

A modification of the method used by Archambault and Johnson (1972) was used to monitor tidal flat temperatures. Thermistor probes were attached to a wooden dowel at 1) 6 cm above tidal flat surface, 2) 2 cm below tidal flat surface where sediments show the broadest daily temperature fluctuations and where the juvenile bait worms reside, and 3) 24 cm below tidal flat surface where daily temperature fluctuations are minimal. The dowel, inserted in the flat, linked the probes to a battery operated telethermometer and a strip-chart recorder housed in an enclosure atop a piling. Strip-charts were made of pressure-sensitive paper and were printed with a time overlay to facilitate recording and reading. Charts provided seven days of recording between changes.

A new roll of chart paper was placed in the recorder every two weeks. The used roll was taken to the laboratory to process the data from analog to digital form. Digital data were then plotted by Computing and Data Processing Services at the University of Maine in Orono with the use of a Cal-comp high speed plotter.

Bloodworms, Glycera dibranchiata, and two species of sandworms, Nereis virens, and Nereis diversicolor, were sampled quantitatively during the spring, summer and fall from tidal flats at 12 stations. At each station worms were taken from 25 m^2 plots at 3 intertidal levels along a line transect. A bait digger's fork was used to dig each plot to a depth of at least 15 cm. Worms were gathered by hand and placed in labeled containers. Bloodworms and sandworms were kept in separate containers. The worms were then taken to the laboratory in a thermally insulated box where they were refrigerated at 4-5^{o}C and processed within 24 hours of collection.

To process, worms were placed in a relaxant (0.15% propylene-phenoxytol - sea water solution) then identified to species, counted, and the body length measured to the nearest 0.5 cm. The various methods used to make observations about reproduction of sandworms and bloodworms were: 1) looking for spawning worms, 2) sampling sediments for young of the year, 3) collecting plankton in search of embryos and larvae, 4) determining seasonal changes in the development of gametes of N. diversicolor and 5) determining the growth and development of N. virens juveniles living among rafted oysters.

In laboratory studies the worms were subjected to physical conditions similar to those near the plant. Worms were studied under both constant and fluctuating temperature regimes. Investigations included acclimation (from ambient to 17 and 27°C), modifications of metabolic rates, behaviour (at ambient temperatures), reproduction and development of the sandworm N. virens (worms were raised to 2 inches) and determination of critical thermal maxima on various life history stages. A ventilation machine was developed for the measurement of worm activity.

On July 1, 1975 only those aspects covered by NRC license specifications were continued. It is hoped that the data collected will assist state and federal agencies in the planning, siting, licensing, and regulation of future power plants.

See also: Maine Yankee Atomic Power Company Environmental Surveillance Report, Semi-Annual Report No. 6. January 1, 1975 through June 30, 1975.

Michael Mazurkiewicz, Principal Investigator

Measurement and Modeling of Accumulation of Power Reactor Associated Radionuclides in Sediments and Aquacultured Oysters in Montsweag Bay, Maine

Fuels used in the release of energy are becoming valuable as their availability decreases. The utilization of the waste heat from electrical generating stations in the production of protein (through aquaculture) should be examined as a method of increasing the net efficiencies of the economic and energy cycles involved in the generation of electricity.

During 1975 we have continued a project designed to evaluate the use of thermal effluent of the Maine Yankee Atomic Power Plant in the culture of the American oyster Crassostrea virginica.

Results to date point to the possible use of effluents from atomic power plants for the efficient production of protein. Oysters located under the influence of the thermal plume continued to show accelerated growth when compared with controls. The sites most directly influenced by the heated effluent produced oyster growth significantly greater than sites more distant from the discharge point.

A factor limiting the use of the thermal effluent is the proliferation of the marine worm Polydora at the warmer water sites. These worms adversely affect the commercial value of the oysters.

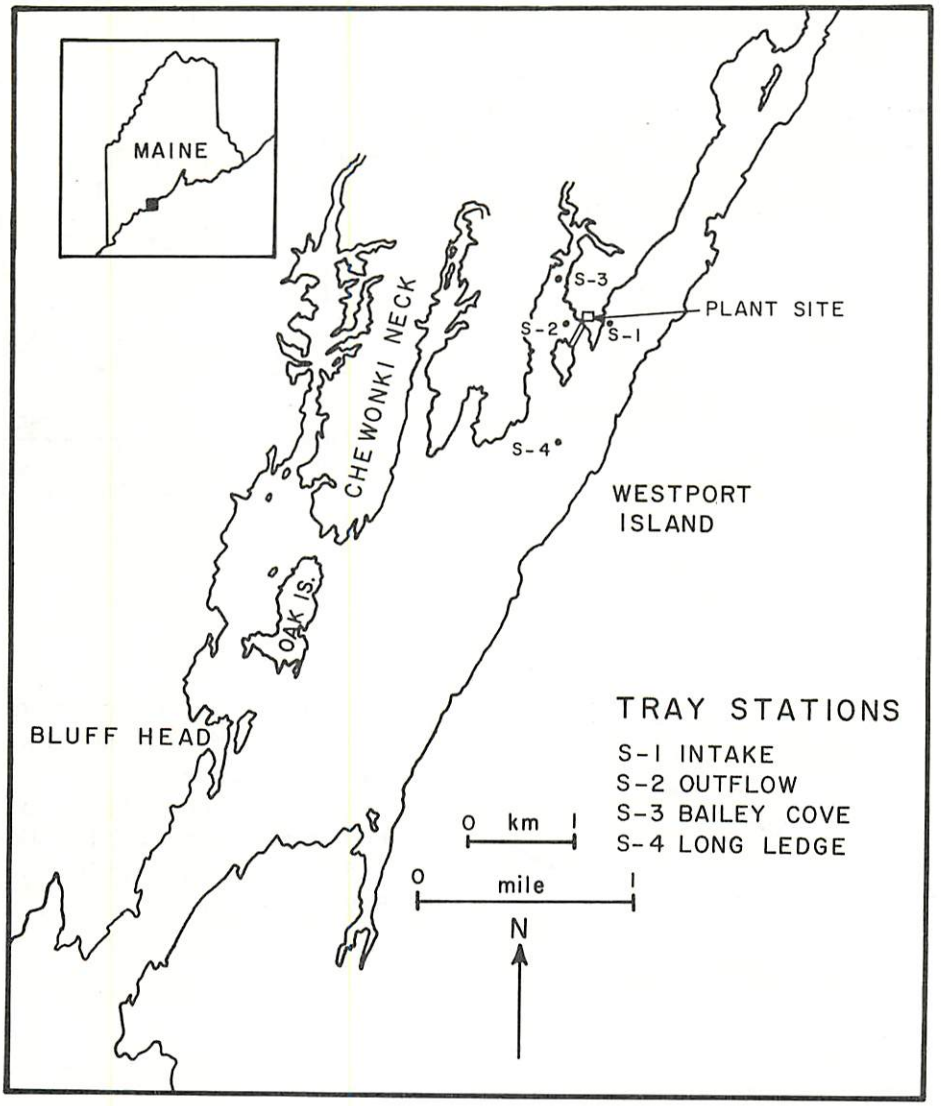
Another limiting factor is the uptake of gamma-ray emitting radionuclides by the oysters. We have modeled the effects of variations in reactor releases of gamma-ray emitting radionuclides in the effluent on the uptake and retention of these nuclides by oysters at the various sites.

The comparison of measurements and theory allows a projection of the radionuclide content of oysters in time, and with distance from the discharge point. The results of this mathematical model suggests a release schedule, based on oyster physiology, that could minimize up-take of the radionuclides by the oysters cultured in the heated effluent.

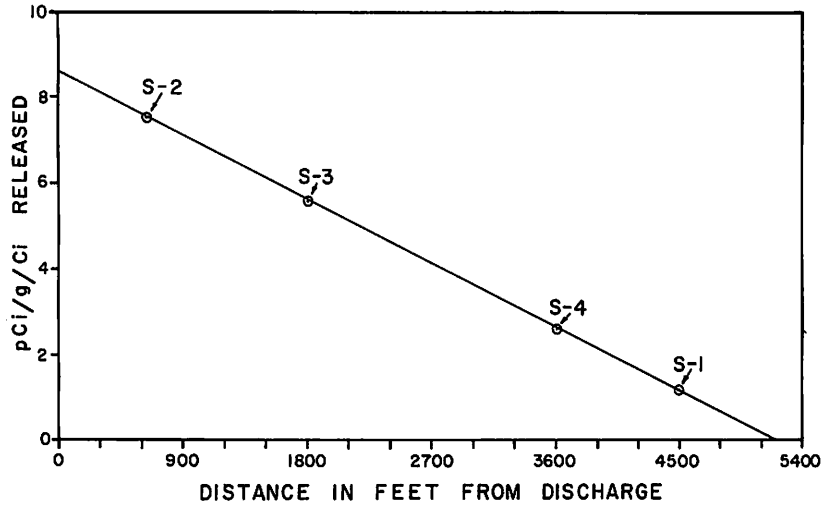
C. T. Hess and C. W. Smith
Principal Investigators

publication:

Hess, C. T., C. W. Smith and A. H. Price, II. Model for the accumulation of radionuclides in oysters and sediments. Nature, *258, No. 5532 (Nov. 20, 1975), 225-226.*



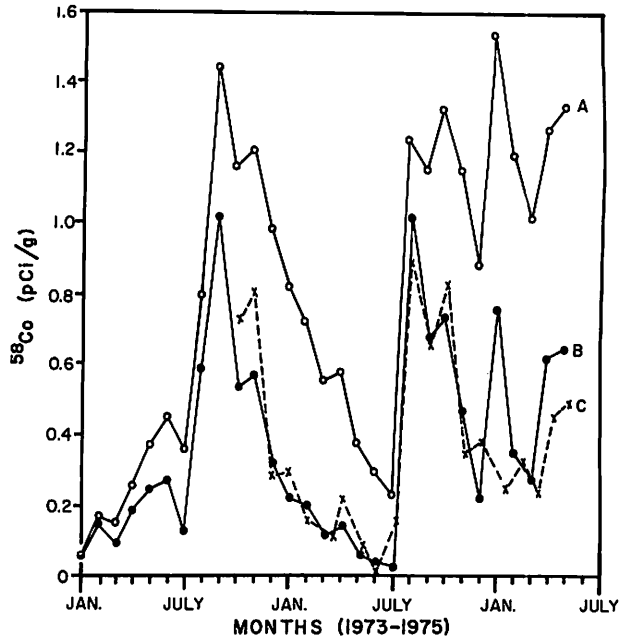
Map of Montsweag Bay showing location of oyster tray stations.

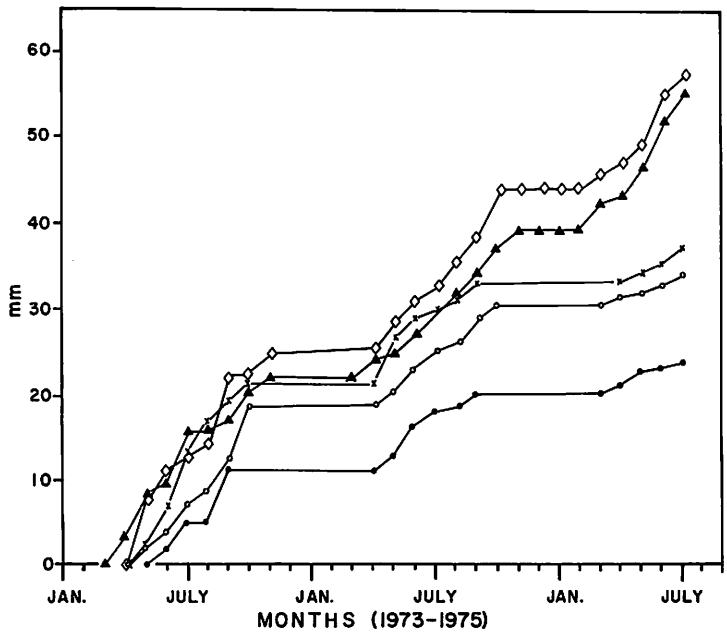


Ratio of retention, U, versus distance from the discharge point measured along the most direct water route.

Pulsed relaxatory theory using:

A. Only physical decay constant (o-o-o).
B. Theory using physical decay constant plus biological decay constant (●-●-●).
C. Experimental results (x--x--x).





Comparison of cumulative oyster shell growth at the control site (●-●-●), intake of the plant (x-x-x), outflow of the plant (Δ-Δ-Δ), above the outflow in Bailey Cove (◊-◊-◊), and below the outflow in Montsweag Bay (o-o-o).

Maine's Beach Systems — Options for Use and Management

Beach systems perform several functions. In the ecosystem, they serve to protect the vital and highly productive salt marsh areas behind them. For man, they serve as a unique open space and transition zone between land and sea. Over the decades, Maine's beaches have been the focus of an intensive, economically important tourist industry. Each year, however, demands are made on this resource for increased recreational use and private development. Often these demands compromise both a beach's function in nature and its service to man.

Thoughtful use and informed land use planning are essential to protect this valuable coastal resource. Unfortunately, too little is known about the state's beach systems to provide a factual basis for making knowledgeable management decisions. A comprehensive beach assessment program was initiated during the last four months of 1975. The project was primarily designed to collect and disseminate data on the physiographic properties of Maine's beaches and associated marsh areas. Providing objective information to local groups and state agencies is the aim of the beach assessment program; these groups must then use this information to draw up sound beach use and management policies in the months and years ahead. Work has progressed in three broad but related areas.

First, all known reports, studies, and research efforts made on Maine's beach ecosystems were compiled. These provide an important backlog of data with which our own findings can be compared. In addition, state agencies and personnel with an interest in beach processes were told of the objectives of the project. The intent was to promote cooperation in achieving common goals and to avoid duplication of effort. Subsequently, a close working relationship was established with the Maine Bureau of Geology and the geology department on the Portland campus of the University of Maine.

Second, an intensive program of field work was initiated. Vertical profile data were recorded for nine beaches south of Ogunquit. These data provide a graphic picture of prominent beach features as well as the slope and absolute height of the beach face. Taken over a series of years, the profiles can indicate the nature and extent of changes a beach system is undergoing.

Sand samples were taken at marked intervals along beach profiles and later analysed for their grain size distributions. Such information is useful in determining sand movement and sorting processes within a beach system. It also characterizes the type of sand that should be used in any beach replenishment program.

A ground level photographic record was kept to identify each profile range and to show existing beach features. Aerial photographs were taken to help delineate geological provinces and characteristic vegetational zones.

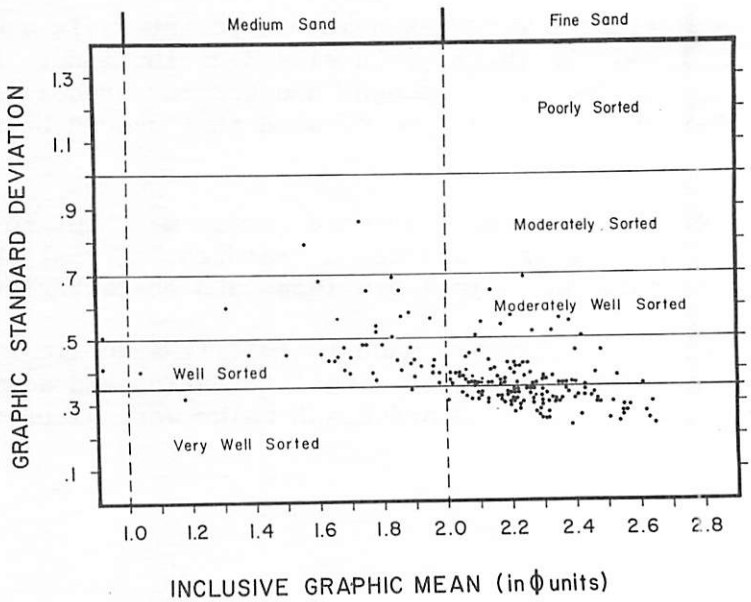
Third, efforts were made to fulfill a public service role. Help was given to three local groups seeking information and advice on specific beach problems. In addition, talks and beach walks were given to members of the Natural

Members of the Board of Environmental Protection get first hand introduction to the dynamics of Maine's beaches.

Resources Council of Maine, the York County Audubon Society, Sierra Club, and Phippsburg Conservation Commission concerning beach features, dynamics, and politics.

At present, plans call for the continuation of the project with emphasis on the accumulation of baseline data. Close attention will be given to the effect of man-made structures on beach morphology and dynamics. With the construction of the dike at Ogunquit Beach, a unique opportunity exists to follow the consequences resulting from the major alteration of a natural frontal dune ridge which exhibited aeolian features, unique to East Coast beach systems. In addition, recently acquired wind data will be used to assess the role of prevailing winds on the transport and cycling of sand within the Popham and Seawall Beach systems.

L. Kenneth Fink, Principal Investigator



Scattergram illustrating the correlation between sorting and the mean grain size of beach sand in Maine.

Major reconstruction of the Ogunquit frontal dune ridge presents unique opportunity to monitor long term effects on the beach system.

April, 1974: Only the first section of dike (arrow) had been constructed.

September, 1975: The completed dike is shown.

Marine Advisory Services — Public Education

The major effort for our Marine Advisory Service during 1975 was to conduct Fishing Business Management workshops along the Maine coast. The workshops were designed to acquaint fishermen with the maintenance of appropriate financial records and effective business practices. Twenty-eight workshops were held in barns, town halls, schools, etc. from Kennebunkport to East Machiasport. Over 300 participants utilized materials produced specifically for this project including: The Fishermen's Account Book; leaflets on fishing business management, Capital Construction Fund, etc.; and videotapes on fishing business management, NMFS financial assistance, vessel documentation, Production Credit Association financing, using the fishermen's account book, etc. Individuals from many federal and state agencies as well as from private industry (bankers, accountants, bookkeepers, etc.) helped at workshops and in the preparation of written and video materials.

The secondary effort for this year was in coastal resource education. Working with local, state and regional personnel from The Nature Conservancy, the first Preserve Managers' Workshop was held. Twenty-eight preserve managers attended the workshop. Training sessions were held on how to inventory, plan the use of and manage a preserve. This two-day workshop was the first such event ever to take place in the United States.

Adult workshops on resource inventories were given at the Rachel Carson Salt Pond Nature Conservancy Preserve in Bristol, Maine. Topics ranged from mapping and compass work on aerial photographs and on the preserve to supra and subtidal geology of the preserve. In all, nine topics were addressed and over eighty people were involved. Youth activities are being planned for next summer as a continuation of this project.

Three work study students were involved in resource inventorying this year. Two students spent the summer surveying and producing maps of two Conservancy Preserves. A third student lived on the Damariscove Island Preserve to keep a log on the use of the island and surrounding waters by summer transients. All three students assisted in workshop instruction and made recommendations on preserve management at the Salt Pond and Damariscove Island Preserves.

Another major effort was in the area of coastal beach management education. Meetings were held with park commissions, state officials, public interest groups, school and community youth groups. Work sessions were held at Pemaquid Beach, Maine to: build fences to guide pedestrian activities, replant denuded dunes with American beach grass, fertilize dunes with seaweeds, and rebuild destroyed dune areas with sand and seaweed. Volunteer efforts by Miss Bonnie Davidson in the construction of educational signs at Pemaquid Beach are gratefully acknowledged.

NEMAS (New England Marine Advisory Service) became operational during 1975 with the hiring of a full-time director. Our combined efforts led to the 5th Annual Marine Recreation Conference held in Portland, Maine. Sixty participants attended workshops with the basic theme "Shoreline Management and Boating: Can the Industry Survive?"

Other educational experiences were arranged for fourteen groups to discuss: marine occupations, environmental studies, the University of Maine's marine program, model boat building, aquaculture, intertidal ecology, and other marine related topics of special interest to the participants.

Administrative changes included: identifying the need for, gaining support for, and finally hiring another Marine Specialist to serve the "Down East" counties, hiring an editorial assistant to take charge of the Maine Sea Grant Publication program, and realigning administrative support through the Assistant Sea Grant Director rather than through the chairman of the Department of Oceanography.

The Maine Sea Grant Publications office is an adjunct of the Sea Grant Marine Advisory Service and tries to respond to public needs as defined by that program. One such need to be met in 1975 was answering the demand for non-technical information on Maine's principal commercial species. These low-budget leaflets have found wide distribution through lobster pounds and seafood dealers. Also, groundwork was laid for a new leaflet series dealing with topical marine problems. This series will be used in secondary schools. A proposal was written and information gathered for a series of publications to be co-sponsored by The Nature Conservancy. These will deal primarily with resource inventories and coastal resource management.

An effort was made to broaden the range of topics in both the Sea Grant information leaflet and bulletin series and to make these more responsive to some of the informational needs of businesses and the general public as well as the scientific and scholastic communities. Publications were produced on the topics of aquaculture, fishing business management, marine occupations, history and a cookbook on underutilized species.

In 1975 Maine Sea Grant Publications office continued to disseminate technical information gathered through Sea Grant funded research. It also increased circulation of its popular educational materials by initiating coastal library and secondary guidance counselor programs and by participating in such events as the Rockland and Boothbay seafood festivals.

Paul D. Ring, Principal Investigator

Pemaquid Beach

UMO Sea Grant Budget

January 1, 1975 - December 31, 1975

	NOAA Grant Funds	University Matching Funds
RESEARCH		
Aquaculture Research	$154,065	$ 48,048
Marine Environmental Research	23,732	60,285
MARINE EDUCATION & TRAINING	800	2,448
MARINE ADVISORY SERVICES	70,905	25,261
PROGRAM MANAGEMENT & DEVELOPMENT	77,816	38,764
	$327,318	$174,806

MAINE SEA GRANT PUBLICATIONS

Information Leaflets

MSG-IL-1-73	Ring, Paul D. Marine Research and Development Projects Being Conducted at the University of Maine	Free
MSG-IL-2-73	Ring, Paul D.(ed.) Marine Related Films Available from the University of Maine Film Rental Library	Free
MSG-IL-3-74	Reynolds, Clark G. Nautical Historians Consider Organizing	Free
MSG-IL-4-74	Ring, Paul D. Problems in Marine Science I. Ocean Currents: Water Movements in the Atlantic Ocean	Free
MSG-IL-5-74	Richmond, Mark S. Oyster Culture in Maine--Specifications and Approximate Costs of Rearing Equipment	Free
MSG-IL-6-74	Bender, Norman K. The Capital Construction Fund*	Free
MSG-IL-7-74	Reynolds, Clark G. Law of the Sea/Nautical History	Free
MSG-IL-8-74	Smith, Frederick J. Fishing Business Management	Free
MSG-IL-9-75	McAlice, B. J. Preliminary Check List of Planktonic Microalgae from the Gulf of Maine	Free
MSG-IL-10-75	Roberts, Kenneth J. Understanding the Emergency Energy Shortage Loan Program*	Free
MSG-IL-11-75	Ehrbar, Charleen. The New Maine Oyster Industry	Free

Bulletins

MSG-B-1-73	Ring, Paul D. (ed.) Maine's Fresh and Salt Water Pleasure Boat Service Industry Boating Business Workshop, March 26, 1973	$1.00
MSG-B-2-74	Hidu, Herbert and Mark S. Richmond. Commercial Oyster Aquaculture in Maine	$2.00
MSG-B-3-74	Ring, Paul D. and Barbra McCandless (ed.) Maine's Fresh and Salt Water Pleasure Boat Service Industry Boating Business Workshop II, October 29, 1973	$2.00
MSG-B-4-74	Lutz, Richard A. Raft Cultivation of Mussels in Maine Waters--Its Practicability, Feasibility and Possible Advantages	$1.00
MSG-B-5-74	Hamlin, Cyrus and John R. Ordway. The Commercial Fisheries of Maine	$3.50
MSG-B-6-75	Reynolds, Clark G. and William J. McAndrew. The 1973 Seminar in Maritime and Regional Studies	$4.00
MSG-B-7-75	Darling, Dana R. and Gregory C. Griffin. Maine's Marine Industries--Vocational Education Planning Guide	$2.00
MSG-B-8-75	Coggins, Phyllis (ed. and illus.) The Uncommon Cookbook	$1.50
MSG-B-9-76	Coggins, Phyllis, Paul D. Ring and Jane D. Simonds (eds.) Marine Occupations Conference	$1.00

Technical Reports

MSG-TR-1-72	Riley, John G., Richard J. Rowe, and Herbert Hidu. Oysters: Reattachment as Method of Rearing Cultchless Hatchery Oysters. Comm. Fish. Rev. 34 (5-6):41-43.	$ .50
MSG-TR-2-72	Riley, John G. A Mechanized System for Oyster Production. Amer. Soc. of Agr. Eng. Paper NA 72-106.	$ .50
MSG-TR-3-72	Riley, John G. Mechanical Handling Techniques for Shellfish Production. Amer. Soc. of Agr. Eng. Paper 72-574.	$ .50
MSG-TR-4-73	Blamberg, Donald L. and David C. O'Meara. Dehydrated Scallop Viscera, A Potential Component of Poultry Rations. Poultry Sci. 52(3):1203-1205	Free
MSG-TR-5-73	Blamberg, Donald L., David C. O'Meara, Richard W. Gerry, Paul D. Harris and Thomas A. Bryan. Preliminary Observations concerning the Nutritive Value of Dehydrated Scallop Viscera as a Component of Poultry Rations. University of Maine Life Sci. and Agr. Expt. Sta. Misc. Rept. 129	Free
MSG-TR-6-74	Dunham, Wallace C. and Munden M. Bray. An Evaluation of the Potential for Maine Raised Oysters. University of Maine Life Sci. and Agr. Expt. Sta. Bulletin 709	Free
MSG-TR-7-75	Slabyj, Bohdan and Carolyn Hinkle. Handling and Storage of Blue Mussels in Shell. University of Maine Life Sci. and Agr. Expt. Sta. Vol. 23, No. 4, January 1976	Free
MSG-TR-8-75	Hess, C. T., C. W. Smith and A. H. Price, II. Model for the Accumulation of Radionuclides in Oysters and Sediments. Nature, 20 November 1975, Vol. 258, No. 5532:225-226	Free
MSG-TR-9-76	de Schweinitz, Elizabeth and Richard A. Lutz. Larval Development of the Northern Horse Mussel, Modiolus modiolus (L.), Including a Comparison with the Larvae of Mytilus edulis L. Bio. Bull., June 1976	Free
MSG-TR-10-76	Packie, Robert, Mark S. Richmond and Herbert Hidu. The Suitability of Maine Waters for Culturing American and European Oysters, Crassostrea virginica Gmelin and Ostrea edulis L.	Free
MSG-TR-11-76	Lutz, R. A. Annual Growth Patterns in the Inner Shell Layer of Mytilus edulis L. J. mar. biol. Ass. U.K. (1976) 56, 723-731	Free

Miscellaneous Publications

MSG-M-5-73	University of Maine Coherent Project Sea Grant Program Annual Report for the Year Ended April 30, 1973	Free
MSG-M-6-74	A Proposal to Review the University of Maine's Coherent Project Sea Grant Program. Vol. I and II. September 1974	Free
MSG-M-75	Magill, Robert N., Jr. A Short History of Damariscove Island. August 1975	Free

* Out of print

Single sheet information leaflets are available on the following species: The American Lobster, Homarus americanus; The Rock and Jonah Crabs; The Northern Shrimp, Pandalus borealis; The Sea Scallop, Placopecten magellanicus; The Atlantic Herring, Clupea harengus L.

The publications listed were produced by the Sea Grant Program at the University of Maine, Orono, Maine 04473.

These publications are available from: Maine Sea Grant Publications, Ira C. Darling Center, Walpole, Maine 04573.