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Summer 1987

## Explorations, Vol. 3, No. 3

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# EXPLORATIONS

A JOURNAL OF RESEARCH  
AT THE UNIVERSITY OF MAINE

Summer 1987

*Cover: Marcia Spencer, University of Maine art student.*

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# Characterization of Normal and Carcinogen Induced Neoplastic Cells of Teleost Origin

by Tim Lyden

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Neoplasia, the abnormal uncontrolled growth of cells within an animal or plant, has been known since antiquity. Early medical texts from China to Greece describe *tumorous growth* in both humans and the animals around them. By the advent of the modern era of experimental science, cancerous growths in humans were widely recognized and began to be documented. Slowly neoplasia, more commonly called cancer, was shown to have environmental and occupational connections. The first well-documented example of this connection in the modern era came from Dr. Percivall Pott, a London surgeon who reported in 1775 that he had noted an inordinate number of cases of cancer of the scrotum among chimney sweeps. After careful analysis he concluded that constant contact with, and resultant irritation from, coal soot was the major causative factor in the onset of these neoplastic lesions. This was the first such documented report implicating an occupational exposure to a specific environmental agent as being a causative factor in the induction of neoplasia. It was not until the 1920's, however, that a polycyclic hydrocarbon was isolated and identified as the precise carcinogenic chemical component of soot and coal tars. (1)

Continued research throughout this and the last century has expanded the list of known potentially carcinogenic chemical agents to include a wide range of compounds including but not limited to alkylating agents, nitroso compounds, azo dyes, as well as many additional polycyclic hydrocarbons. It has also been found that a variety of physical agents is able to induce neoplastic changes in cells. Such changes from controlled normal growth to uncontrolled neoplastic growth are referred to as neoplastic transformations.

Advances in understanding cellular transformation were made possible with the advent of functional understanding as to the means and mechanisms of cellular genetics. This

new insight was triggered during the early 1950's by the elucidation of deoxyribonucleic acid (DNA) as the genetic material by James Watson, Francis Crick, and others. These findings led to the perception that chemical and physical carcinogenic agents possess a common property in relation to the mechanism of transformation; all interact either directly or indirectly with the DNA of the target cell, thereby altering genetic expression and so the cell's normal function. (2)

Today, it is understood that the cellular genome, coded for by chemical messages within the DNA, provides the cell with a master plan or program containing the information needed to produce all of a cell's potential protein products. Many of these proteins then function in the production or assembly of yet other products, both protein and nonprotein, the sum of which make up the whole cellular structure. Therefore directly or indirectly the genome codes for all possible cellular proteins and protein products which in turn determine all potential patterns of growth and differentiation.

Differentiation is the process of development through which a cell passes from relatively nonspecific functional states to very highly specialized functional states. Each individual differentiation state represents the unique expression of a portion of the cell's genomic content. It is important to understand that this expression can be unique in several ways, including expression of genes particular to that differentiation stage, repression of genes particular to other differentiation stages, as well as quantitative differences in expression of ubiquitous structural and other genes. Hence, the structure and function of a cell at any given stage of differentiation represents the precisely controlled expression of a unique gene set. It follows then that any change in a gene set or in the expression of a gene set leads to new and different cellular differentiation states. Such mechanisms form the basis of controlled regulation of normal cellular growth and differentiation as well as the general mechanism underlying carcinogenesis. Regardless

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*Tim Lyden is working toward his PhD in Biological Sciences.*



of the transforming agent, an oncogenic (tumor-producing) modification in the gene set or expression of the gene set yields a new and radically different cell behavior in which a cell leaves the normal bounds of growth control, dedifferentiates and becomes neoplastic in character.

The study of oncogenic modification of cellular gene sets through mutational processes has been predominant in research efforts since before the 1950's. In the past two decades however, through insights gained in a variety of areas including the new field of Molecular Biology, (which studies the chemical and structural aspects of the genome), the role of modification of gene expression without the need for mutational events, which produce structural changes in the genes themselves, has gained in importance. This new perspective, with distinction made between mutational and non-mutational modification of gene set expression will, in the opinion of the author, figure strongly in the next stage of understanding to occur in the study of cellular neoplasia. From this new insight will, I believe, flow functional understanding as to the basic mechanisms of control of cellular differentiation and quite likely the ability to intervene in those mechanisms in such a manner as to arrest or reverse the neoplastic process as a whole.

Toward this end, I am engaged in a Ph.D. degree program in Biological Sciences at the University of Maine with a research project which has as a central objective the elucidation of certain aspects of eukaryotic cellular biology that bear directly on mechanisms of control of gene set expression at the whole cell level. This project is designed to answer several basic questions concerning cellular behavior and will contribute directly to the question of neoplastic transformation control and differentiation state modification. A number of cultured fish cell lines and whole animals have been selected as the working systems for these studies. At this stage of planning, the project will seek in these systems to examine and contrast a diverse set of cellular parameters related to normal and transformed cell states. Included in this group of parameters are the cellular and extracellular fibronectin levels, various lipid and fatty acid levels associated with cellular membranes, membrane-associated and extracellular glycosphingolipids, cell growth properties and morphological modifications associated with transformation.

It is expected that such studies in basic cellular biology conducted on these systems will improve the current understanding of neoplastic transformation in teleost species (bony fishes) which, unlike mammalian species, have not been extensively or systemically studied in this regard. This last point is of particular importance in light of the position relative to man that fish hold in the global foodchain and ecosystem. This position, combined with an environmental intimacy much closer than mammals normally have, makes fish an interesting potential monitor for environmental changes, since we are able to detect those

changes at very low levels. Some species of fish also display extreme degrees of sensitivity to the effect of chemical carcinogens, a property which forms the basis of one aspect of this research project. For example, in the case of Rainbow Trout as little as 15 minutes exposure to Aflatoxin B1 during embryogenesis produces hepatic cancers in 40 percent of the resultant adult fish, yet identical treatment of other species produces little or no evidence of neoplastic disease in later life. (3) One particularly promising aspect of study in this area involves histopathological examination and documentation of the genesis of neoplastic hepatic lesions in whole animals during development. A recent review of the literature pointed up the need for a clarifying study of the precise stages that occur in preneoplastic states of teleost hepatic lesions. The literature on teleost neoplasia is also replete with statements of need for further study in almost all areas of basic cancer biology. In addition, such studies can be useful in the elucidation of neoplastic transformation as a whole in other species which undergo these cellular changes.

In all areas of this project a fundamental goal will be to compare and contrast gene set expression as a whole through the modification of differentiation states in the several cell types under study. A preliminary investigation of cellular fatty acid composition in relation to cell growth density and therefore, indirectly, differentiation states which I conducted as an independent study research project during my senior year here at U.M., provided considerable impetus to my current research project. In that study RTG-2 cells (Rainbow Trout Gonad, a continuous cell line also included in my current thesis research) were grown in culture to several levels of cell growth density. After 72 hours these cells were harvested by standard methods and analyzed for cell size, cell count, total cell protein concentration, and total cellular fatty acid composition using gas chromatography. Growth density related changes were clearly indicated by the observed results. As a general pattern, fatty acids involved with membrane rigidity were replaced by those involved with membrane flexibility when the cell culture density decreased. By examining these cells at several levels of growth density, this study measured changes principally in the plasma-membrane of the cell, which resulted from the expression of distinct, albeit similar, gene sets. Since the sedentary behavior and morphology of normal cultured cells crowded by neighboring cells at high growth density is distinctly different from the motile and dividing behavior of cells which are widely separated from other cells in the same flask, a correlation between these cells and those of distinct differentiation states can be asserted. Such a correlation then provides an example of extracellular environmental changes (*i.e.*, cell density) producing a modification of differentiation state behavior in the RTG-2 cells of this earlier study. These changes in differentiation behavior were, however, transitory and

limited in scope. In order to explore such modifications of gene set expression and the larger ones attendant with neoplastic transformations in my thesis research, chemical carcinogenesis will be employed to provide sustained differentiation modifications for study. Although this project is still in the very early stages, preliminary data are already coming in which suggest that successful transformation of the cultured cell lines included in this study will be possible. In fact, one such transformation may have resulted from a cellular toxicity study now being completed. The data so far collected on this cell line are suggestive of a successful transformation with aberrant cellular behavior including an apparent lack of contact inhibition and low levels of substratum adhesion being observed. At this writing however, conclusive results have yet to be established.

In addition to the above mentioned toxicity studies and possible transformation of cell cultures, a preliminary experiment was begun in February aimed at the induction of neoplastic hepatic lesions in Atlantic Salmon embryos exposed to Aflatoxin B1. These lesions will provide the material for detailed histopathological studies as well as source material for the establishment of primary and continuous cell culture lines which are crucial for later stages of the project. This experiment is also a pilot effort from which similar work using Rainbow Trout embryos will follow.

Short-term experimental goals over the next year will involve the establishment of at least three subpopulations of transformed continuous cultured cell lines derived from the RTG-2, CHSE (Chinook Salmon Embryo), and EPC (Epithelial Papilloma-Carp) lines normally cultured in our laboratory. In addition to these cells, establishment of both primary and continuous cell culture lines from Rainbow

Trout and Atlantic Salmon will be undertaken. For the purposes of this project it is anticipated that both transformed neoplastic as well as nontransformed normal hepatocyte cultures will be established.

Concomitant with these efforts in cell culture will occur a series of biochemical and physiological experiments concerned with building a large data base on the normal and later neoplastic characteristics of the various cell lines. This work will involve both quantitative and qualitative analytic procedures including, among others, gas chromatography, gel electrophoresis, column chromatography of several types, electronic cell counting and size distribution analysis, photomicroscopy, computerized image analysis, histochemical and immunochemical analysis and eventually scanning electron microscopy. Of course, as with any scientific research, new avenues of investigation will undoubtedly present themselves as the project proceeds; wherever applicable and practical these will also be pursued.

This research is being supported by the Migratory Fish Research Institute and a U.M. Graduate Student Board Grant, with additional support pending from Sigma Xi Grants in Aid of Research, U.S.D.A., Hatch Funds from the Maine Agricultural Experimental Station, N.I.H. Biomedical Research Science Grants, The Center for Marine Studies and The College of Life Sciences and Agriculture.

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# Attitudes and Opinions of Maine Dairy Farmers

by John Muth and James Leiby

The dairy industry is, in terms of total sales, the largest agricultural enterprise in the state. In spite of its importance, little is known concerning the details of commercial dairy production in Maine. To overcome this lack of knowledge, the Maine Agricultural Experiment Station provided funding for a five year study of Maine dairy farms. As part of this study, detailed questionnaires are periodically sent to farmers who receive payments from the Maine Milk Pool. The discussion that follows is based on the voluntary comments made in response to a part of the questionnaire.

Questionnaires were mailed to 861 Maine dairy farmers in April 1986. Of these, 260 were returned as of August 1986. The questionnaire included questions relating to farm operation, management style, feed production, milk production, etc.

In addition to the standard survey questions, there were questions that solicited the opinions of the respondents, and space was provided to discuss any other issues that the respondent thought might be of help or of interest, such as the problems facing dairy farmers today or the future of dairy farming. Of the 260 questionnaires returned, 119 included written comments expressing attitudes and opinions. In addition to the space for written comments, there were eight questions that asked the respondents to express their opinions about certain aspects of the dairy industry in Maine. For these questions, between 206 and 238 of the respondents answered the questions. This paper presents some of the more interesting results of these sections of the questionnaire.

Of particular interest in this study is the health of the dairy industry in the state; thus, the focus of most of the questions concerning the opinions of the respondents is related to this issue. As background, the reader should be aware that the recession that ended for most domestic industries in the early 1980s has persisted in agriculture. Because of the administered pricing structure present in the dairy industry, surpluses of dairy products grew to proportions that were difficult to maintain in a period of fiscal

restraint. As a result, dairy farmers have experienced rapidly decreasing prices, and a series of assessments on the payments that they receive.<sup>1</sup>

## The Future of Dairy Farming in Maine

The respondents appeared to have mixed feelings about the future of the industry, that is, they seemed to feel confident about their ability to continue dairy farming, but felt that the industry in general would fail to grow. Responding to the statement *I feel that I will remain in dairy farming until I am able to retire*, more than 68 percent of those responding to this statement agreed, including almost 12 percent who strongly agreed. Less than 11 percent of respondents disagreed with this statement. On the other hand, responding to the statement *I feel that the dairy industry in Maine will grow in the next five years*, more than 47 percent of respondents disagreed; almost 15 percent strongly disagreed. Table I presents the details of the responses to these statements. It should be noted that, on average, the respondents to the survey plan to increase the size of their dairy herds by about 20 percent over the next five years.

Of the 119 written comments, thirty, or about 25 percent, said that they felt that the future of dairy farming was not good. Among the comments made:

- *I feel there is no future in New England dairy farming especially for young people.*
- *I feel that dairy farming in Maine will end up like poultry production and the shoe industry.*
- *It is a depressing business to be in . . .*
- *Our farm is a six-generation farm (since 1820). Probably (we) will not be able to continue much longer at this location.*
- *Future???*

## Current Problems Associated with Dairy Farming in Maine

The problems felt by these people are from many sources. In dairying one of the major difficulties has been sharply declining prices. In Maine, the money price for raw milk increased by about 40 percent from 1976 to 1981, and has remained about constant since then. Because of inflation, the real value of the milk sold by farmers in Maine had declined by about 18 percent during the five years prior to the survey. In addition to the reduction of the real price

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The authors are Work Study Assistant and Assistant Professor in the Department of Agricultural and Resource Economics at the University of Maine.

received, government, in response to growing milk surpluses, levied fees to be deducted from the prices received by farmers. As a result, the revenue received by dairy farmers for 100 pounds of milk fell by more than 20 percent in about five years.

Many of the respondents commented about the association of the government with their problems. Of all the written responses, the most common single unsolicited opinion was that the source of the problem was government intervention. Almost 40 percent of all respondents who wrote comments (45 of 119) expressed the thought that the current problems were caused by government interference in the dairy industry. Concern over the role of government in the industry was aimed at both the State and Federal levels. About the Federal government, it was said:

*I feel as long as the federal government controls the farm prices and does not get out of agriculture, the future of agriculture is very bleak. Let the product, be it milk, grain, or beef, seek its own price without the C.C.C.. (Commodities Credit Corporation).*

and,

*I think if the government would get out of it all eventually things would be ok.*

Some comments about the State included the following:

*The State of Maine is doing everything to destroy dairy farming. Until they back out and let free enterprise reign the future is very doubtful.*

*The Maine Dept. of Agriculture is still talking about the inefficient farmers and dairy plants and they probably still will until we only have one of each under the present (Brennan) administration.*

It is clear, however, that feelings in the industry are somewhat mixed toward government intervention. Although, the most common unsolicited opinion was that the current problems were caused by government intervention, many felt that the government should do something to alleviate the situation. Slightly more than 30 percent of the written comments (37 of 119) suggested governmental action to help solve the problems.

The most common suggestion for government intervention was the imposition of a quota system for dairy farms. This was mentioned in about 22 percent of the written responses (26 of 119). A quota system is one in which a government establishes a set of rules enabling either the government itself, or producers of the commodity to regulate production of a commodity. By regulating production, the price may be controlled without the usual surpluses or shortages generally caused by simply regulating prices. Under such a system, if prices fall, the amount of the quota is usually reduced, and when prices rise, the quota is increased, so that prices are more stable.

In the United States, this system has been applied to several commodities, notably tobacco. The most common

practice is to establish some quantity of product that each producer has the right to market (most often, surpluses are sold at a much lower price). The *right to market* is usually transferable to other producers, and may have significant economic value. If producers wish to expand production, they must purchase, or lease that right from other producers who wish to reduce production.

Such a system is probably not useful at the state level, since if the price of milk becomes much higher than in surrounding states, milk will be brought into the state from areas not subject to the quota. The tobacco quota system functioned quite well until several foreign countries began marketing tobacco to the United States. Since the foreign production was difficult to control, it became more and more difficult to regulate prices by this means (for details of the recent problems of the tobacco program see Sumner and Alston).

An important recent attempt by the Federal government to limit milk production took place at about the time of the survey, and was frequently mentioned in the survey



(more than 23 percent of the written responses). This program was known as the *Whole Herd Buyout*. Under this program, producers would submit bids of the price for which they would be willing to surrender their entire herd to the government, and promise that their farm would not be used for milk production for a period of a least five years. In Maine, 86 farms had bids selected in the buyout. Most of the opinions about the buyout were that it would be ineffective, at least without imposing a quota.

Statements ranged from simply *The buyout is bad, to I have some question as to the long-term effects of the whole herd buyout . . . a lot depends on how much the herds left increase their cow numbers.*

and,

*The buyout sounds good, but with the farmer paying for part of it, he is forced to get more cows to make up the difference in the check.*

The survey also included the opinion question, *I feel that the whole herd buyout program is a good way to help solve some of the problems of the dairy industry.* More than 52 percent of the respondents who answered this question disagreed with the statement; more than 32 percent strongly disagreed, while less than 25 percent agreed. Details of the responses to this question are presented in Table II.

Many of the above concerns stem from the current lack of profitability of dairy farms. Responding to the statement *My dairy farm produces a fair return on my investment*, more than 50 percent of the respondents to this question disagreed, more than 20 percent strongly disagreed. In contrast, less than 3 percent strongly agreed; however, about 40 percent did agree with the statement. Details are presented in Table II.

It seems, however, that the respondents were more optimistic about the future returns to their investment, at least for those farms that plan to remain in dairying. Responding to the statement *I feel that the profits on this farm will increase in the future*, almost 49 percent agreed, while only about 13 percent disagreed. For the statement, *I feel that the profitability of dairy farming in general will increase in the future*, about 35 percent agreed, about 18 percent disagreed, while more than 45 percent were uncertain. See Table II for details.

At least with respect to their own farms, the opinions of Maine dairy farmers seemed to be that the current problems will be solved in the future and, given the many statements against government interference in the marketplace, it seems that the faith in the future is faith in their own ability and will to survive difficult times and improve their own farm's situation.

Several described the efforts they were undertaking to weather the current storm. Among the more memorable statements:

- . . . to do this (farming) . . .
- Wife teaches two nights per week.
- Daughter runs farm stand.
- Daughter-in-law farms and tests for DHIA . . .
- I run a farm supply business.
- Wife and daughter run a computerized knitting machine.
- I sell computerized feeders and mulch systems.

A total of ten respondents mentioned the need to hold second outside jobs to continue to operate the farm.

Fourteen respondents mentioned their current debt loads as significant problems. Typically, farmers are quite conservative in their willingness to enter into debt, but with the capital requirements of operating modern farms, they require external financing. In the late 1970's and early 1980's interest rates reached record high levels in recent United States history. These high interest rates were primarily due to the unprecedented inflation of the period. High nominal interest rates are only a problem if the revenues of borrowers fail to continue to increase. Since the early 1980's the rate of inflation has decreased greatly, and with this decrease, market interest rates have decreased as well. For those who are able to refinance their debt, the disinflation has caused few problems; farmers, however, are frequently involved in long-term debt that is difficult to refinance. Since the disinflation and government levies have reduced the rate at which milk prices have increased, the real cost of long-term debt of dairy farmers has increased dramatically.

At the time that some of our dairy farmers went into debt, product and input prices were rising rapidly, so that it seemed reasonable to borrow at the prevailing high interest rates. Unfortunately, shortly after this occurred, these prices stopped increasing, and in some cases declined. The value of the assets of farmers decreased, but the value of the debts incurred to acquire these assets increased. Because of the decline of farmer's equity, it was difficult to refinance at lower interest rates. As a result, many farmers have been forced to operate at a loss until the debt situation can be cleared up.

Although this situation is probably somewhat less severe than in some other parts of the country, (more than 32 percent of respondents stated that their farm was currently debt-free) it is, nevertheless, particularly hard on those who are in this situation. Of the dairy farmers who responded



**Table I**

Relative Frequencies of Responses to  
 "Opinion" Questions on the  
 1986 Survey of Maine Dairy Farms

	STRONGLY AGREE	AGREE	UNCERTAIN	DISAGREE	STRONGLY DISAGREE	NUMBER RESPONDING
I feel that I will remain in dairy farming until I am able to retire.	11.7%	56.5%	21.1%	4.9%	5.8%	223
I feel that the dairy industry in Maine will grow in the next five years.	4.5%	14.4%	33.8%	32.4%	14.9%	222

**Table II**

Relative Frequencies of Responses to  
 "Opinion" Questions on the  
 1986 Survey of Maine Dairy Farms

	STRONGLY AGREE	AGREE	UNCERTAIN	DISAGREE	STRONGLY DISAGREE	NUMBER RESPONDING
I feel that the whole herd buyout program is a good way to help solve some of the problems of the dairy industry.	4.8%	19.7%	23.2%	19.7%	32.5%	228
My dairy farm produces a fair return on my investment.	2.3%	37.1%	10.0%	30.3%	20.4%	221
I feel that the profits on this farm will increase in the future.	13.2%	35.5%	38.2%	7.3%	5.9%	220
I feel that the profitability of dairy farming in general will increase in the future.	6.3%	29.6%	45.7%	10.3%	8.1%	223

to the questionnaire, about 15 percent owe more than half of the value of their farms, while about 4 percent owe as much as or more than the total value of their farms.

#### Summary and Conclusions

Based on the opinions expressed in the 1986 Survey of Maine Dairy Farms, the outlook for the Maine dairy industry as a whole is less than optimistic. Most respondents felt that the industry as a whole would not grow in the future. On the other hand, several farmers' outlooks toward their own futures in the industry were considerably more optimistic.

There seems to be a strong current of opinion that the dairy industry problems stem largely from government interference in the industry, but many feel that the solution to these problems lies with the government as well. Relatively few consider the most recent government efforts to help solve the problems of the industry, *The Whole Herd Buyout*, to have been particularly effective in solving the problems. Many of the respondents felt that perhaps some form of a quota system for dairy farms held promise for eliminating some of the problems.

Many of the respondents felt that their farms were not providing adequate returns to them, but most felt that the situation for their farms would improve in the future. They were less sure about the future for other dairy farms. Some concern was expressed about the debt levels of farms, but it seems that most of the farms that participated in the survey were financially sound, despite some current problems.

This review represents only a preliminary exploration into the Maine dairy industry. Several more detailed reports of the analyses of the data of the 1986 Survey of Maine Dairy Farms are currently in process.

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## Note

1. The details of these are far too numerous and complicated to document here, but see, for example, the *Economic Report to the President* (Council of Economics Advisors) or Babb, Emerson M. (see References).

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# Background: THE QUEST FOR THE EIGHTEEN MONTH OYSTER

The American oyster (*Crassostrea virginica*) has a wide habitat range extending from the Canadian Maritimes to the Gulf Coast. This species is generally the one referred to by Easterners when they speak of "the oyster." Other edible species are *Dendroostrea frons*, the coon oysters common in Florida and the crested oyster, *Ostrea equestris*, found in salty waters from Maryland south. However, these are minor species compared to *C. virginica*. The famous European oyster (*Ostrea edulis*) has been cultured in eastern U.S. waters, but due to high mortality in certain situations, growers have lost interest in it.

The American oyster was never abundant in Maine preferring warmer and less salty water than most of our coastal areas provide. But it did thrive locally in the warmer estuarine conditions. Oysters were a favored food of Maine Indians and large shell middens can still be seen on the Damariscotta River where Indians gathered for oyster harvesting. The animals were shucked and eaten on the spot or were dried for future use. However, like so many of our natural resources the oyster has been over-exploited and no commercial fishing has occurred in Maine since 1968. American oysters consumed in the northeast are pro-

duced by commercial growers in New England or are shipped north from Chesapeake and Delaware Bay areas.

The American diet has been undergoing a change for the past few years. The traditional red meats (beef, pork and lamb) are declining in consumer appeal while consumption of chicken and sea food are on the rise. This change has led to a renewed interest in the *farming* of fin-fish and shellfish. For several years the Department of Animal and Veterinary Sciences has been active in lobster research through the expertise of Dr. Bob Bayer and his graduate students. In 1984 the department acquired Dr. Herb Hidu, a shellfish expert, who transferred from the Department of Zoology at UMaine. In 1985 when the Maine Agricultural Experiment Station became interested in *shellfish genetics* Dr. Hidu and Dr. Robert Hawes cooperated to organize a selection program for the genetic improvement of growth rate in the American oyster. The commercial mariculturist is still farming a *bunch of wild animals* compared to the improved lines of farm animals currently in use by land agriculturists. We hope to change that situation with respect to at least one species. Our aim is to have an oyster at market size at 18 months instead of the two to three years that it now requires.

## The Student . . .

*Kevin Scully holds a B.S. degree in Biology from Cornell University and came to the University of Maine in August 1985 to start a Masters Program in shellfish genetics. He has always been interested in the sea and in boating, and he is a licensed diver. Scully spends the months from September to May taking course work on the Maine campus, but spends his summers at the University's Ira C. Darling Center for Marine Studies in Walpole, Maine. At the Darling Center Kevin has taken charge of the spawning and the early care of the young oysters. His work has been invaluable in insuring that the breeding program is off to a successful start. Following the completion of his Master Degree Kevin hopes to work in some phase of Maine aquaculture.*

## The Advisor . . .

*Dr. Robert Hawes was trained as a flat-land farmer in the field of poultry breeding. He holds a B.S. degree from UMaine in poultry husbandry, an M.S. from U. Mass. and a Ph.D. from Penn. State in the areas of poultry breeding and genetics. Prior to coming to the University of Maine in 1978, Dr. Hawes was for nine years a teacher and researcher in the Dept. of Animal Science at Macdonald College (McGill University) and for seven years a commercial research geneticist for a large midwest poultry breeding company. His conversion to shellfish from poultry has allowed him to again be involved in an animal breeding program at a university setting. He finds oysters much less flighty than chickens, but then again chickens don't stick to his fingers as baby oysters do.*

# The Quest for the Eighteen Month Oyster

by Kevin Scully

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Nearly every article concerning genetic selection experiments in shellfish aquaculture begins by asking the same question—*Why have there been so few attempts to improve shellfish stocks genetically, especially when one considers the tremendous genetic gains achieved in land agriculture?* (In fact, land agriculture could not survive without improved breeds and strains of animals and plants.) Most replies to the question focus on a lack of laboratory space or funding or that the time span necessary for significant gains is prohibitive. For these reasons and others, no selected lines of shellfish are available for general use by the commercial grower. This has the potential to change since recent findings suggest that shellfish appear little different from farm animals in their response to selection and other genetic manipulations.

Improvements should be forthcoming (in at least one species) as the Department of Animal and Veterinary Sciences at the University of Maine has recently undertaken a long-term selective breeding program for the genetic improvement of growth rate in the American oyster, *Crassostrea virginica*. The project's first year centered on procuring several geographically widespread stocks of adult oysters, spawning these animals and gathering baseline data on the performance of both pure lines and crosses under varying environmental conditions. Preliminary results suggest that considerable improvement in key production traits is possible through a selection program, findings that will benefit Maine's aquaculturists and state economy directly and provide important genetic information for similar projects elsewhere.

Today Maine's aquaculture industry is expanding with total annual production in which cultured mussels, salmon and oysters predominate, with a worth of between \$5 and 10 million. While significant commercial activity began in 1972 with the passage of the Maine Aquaculture Act, the formation of the Maine Aquaculture Association in 1977 has helped to unify the industry. Many factors make Maine an ideal location for aquacultural enterprises. Maine can boast of an abundance of clean, unpolluted water over much of its 4000 mile coast. Warmer estuaries, protected bays, and open coast provide a wide variety of environments for the culture of various species. Maine also has a lower incidence of several major shellfish diseases that plague production farther south. The state is also near major population centers of the Northeast; given their large populations,

concentrations of ethnic groups, comparative wealth, and a willingness to try new foods, these metropolitan areas should offer receptive markets for high quality cultured products.

Several factors were instrumental in deciding to work with the American oyster rather than one of Maine's top three species of shellfish: soft shelled clams, blue mussels and sea scallops. Although town clam flat management programs have supplemented wild clam populations with hatchery seed, a low unit price and fairly abundant sources of natural seed would tend to make the hatchery production of both clams and mussels uneconomical. The sea scallop poses another problem: aquaculturists cannot successfully complete the scallop life cycle in captivity, at least not on a commercial scale. Another possibility was the European oyster, introduced into the United States in the 1940's and cultured extensively throughout the 1970's. Two factors, a wild population discovered in Casco Bay and high mortality in cultured animals prior to reaching a marketable size, have decreased efforts to culture this species.

The American oyster became a logical choice for genetic improvement. First commercial hatcheries already produce American oyster seed, primarily due to a lack of natural seed in the Northeast. Secondly, a high market price, appealing to aquaculturists, should lead to a greater demand for fast growing, hatchery produced stock. Finally many traditional oyster grounds are facing plummeting production levels, the result of both natural and manmade problems. The Chesapeake Bay fishery, long the heart of the United States' oyster industry, has declined sharply because of pollution, overfishing and the oyster disease organism, *Minchinia nelsoni* (MSX). Parts of the Gulf Coast have suffered through devastating back-to-back hurricanes in 1985 and the oyster drill, a predatory snail, has further decreased production in this area. These factors should lead to a greater demand for fast growing hatchery produced American oyster seed stock.

The greatest question when beginning a selective breeding program is in deciding which traits to select for. The commercial breeder must choose traits of high economic importance, both to himself and the consumer. We will be examining important production traits including growth rate, desirable shell shape and performance in Maine's cooler estuarine waters.



Correlations between the traits being selected for must also be determined. The breeder must be careful not to select for negatively correlated traits, where an improvement in one trait would simultaneously degrade a second valuable trait. Hypothetically, selecting for growth rate could cause a change in meat quality or cause shells to become more fragile. Calculating correlations early in a program could save considerable time and frustration later on.

The traits that we are examining are called quantitative traits; they are controlled by many genes, and their control is quite complex. These traits, shell length for example, are generally normally distributed, with most animals near the average length and increasingly few oysters measuring toward either extreme. For breeding purposes, the genetic effects that contribute to a trait must be separated from the environmental factors. Under optimal environmental conditions even a genetically inferior animal may appear above average, while the oyster with the best genetic makeup may seem average when grown under poor conditions. The manifest characteristics of an organism that result from both its genetic makeup and its environment are known as its phenotype.

The fraction of total phenotypic variation in a trait that is controlled by genetic differences is called heritability ( $h^2$ ). Traits with heritabilities greater than 0.25 can be changed effectively by selection. Generally traits linked to growth rate, such as weight or length at a specific age, have a high  $h^2$  ( $h^2 > 0.25$ ), whereas reproductive traits such as egg size and fecundity have low heritability values and are harder to select for.

Knowing heritability allows you to calculate response to selection, a value which produces an estimate of the potential gain which can be achieved each generation toward reaching your goal. Response to selection ( $R$ ) equals the heritability ( $h^2$ ) multiplied by the selection differential ( $S$ ). The selection differential is the superiority of the selected broodstock over the population average. Oysters are notorious for their high phenotypic variation and high fecundity, up to 10 million eggs per female per year. Having thousands of highly variable individuals available will allow the breeder to choose only the best animals for selection as broodstock. Coupling traits having a high heritability with the ability to incorporate a high selection differential should allow rapid gains in the desired traits.

The relatively few selection programs conducted on shellfish hint at the potential gains that could be realized. Workers at Dalhousie University in Nova Scotia have selected for increased growth rate in the European oyster. After a single generation the selected line averaged 23 percent larger than the controls. A similar study using American oysters at the National Marine Fisheries Service (NMFS) Laboratory in Milford, Connecticut, has shown that in 5 of 7 selected lines there was a positive response

to selection, with progeny from large parents ranging from 53-238 percent larger for shell surface area than those of small parents. In a study at Rutgers University aimed at increasing resistance to a particular disease organism *Minichinia nelsoni* (MSX), the survival of selected stocks has steadily increased through four generations of selection to be almost nine times that of susceptible stocks. These studies suggest that a steady response to selection can be expected for shellfish and seem to indicate that substantial gains would be possible through a well-conceived selective breeding program.

In the spring of 1986 founding broodstock was secured from a wide geographic area. Our only native Maine stock, collected from the upper Sheepscot River, came from an area once densely populated by American oysters. Although other sources of American oysters still exist in Maine, such as the Piscataqua and New Meadows Rivers, health restrictions prohibit their removal. For example, to prevent the introduction of diseases the stocks would have to be quarantined, a costly and space-consuming operation for any hatchery. With the cooperation of William Mook, owner of Mook Sea Farm in Walpole, Maine, we have obtained



**Maintenance of holding trays and oyster sampling on the Damariscotta River.**

an unselected commercial Long Island stock, designated the *Flowers stock*. Our third line, called the *Delaware stock*, also supplied by Mook, consists of individuals from the line developed at Rutgers for resistance to MSX. The final line consists of third generation fast-growth selected oysters provided courtesy of the NMFS Laboratory in Milford, Connecticut, previously mentioned.

The first oysters were spawned in early spring, 1986, at Mook's hatchery. The process began about a month earlier when broodstock were conditioned to reproductive readiness. Oysters which normally would spawn in late summer in the wild had to be coerced into thinking summer was upon them. This was accomplished through elevating the water temperature and increasing food availability by supplying cultured algae. Under these conditions the oyster's gonads rapidly developed and were soon ready to spawn.

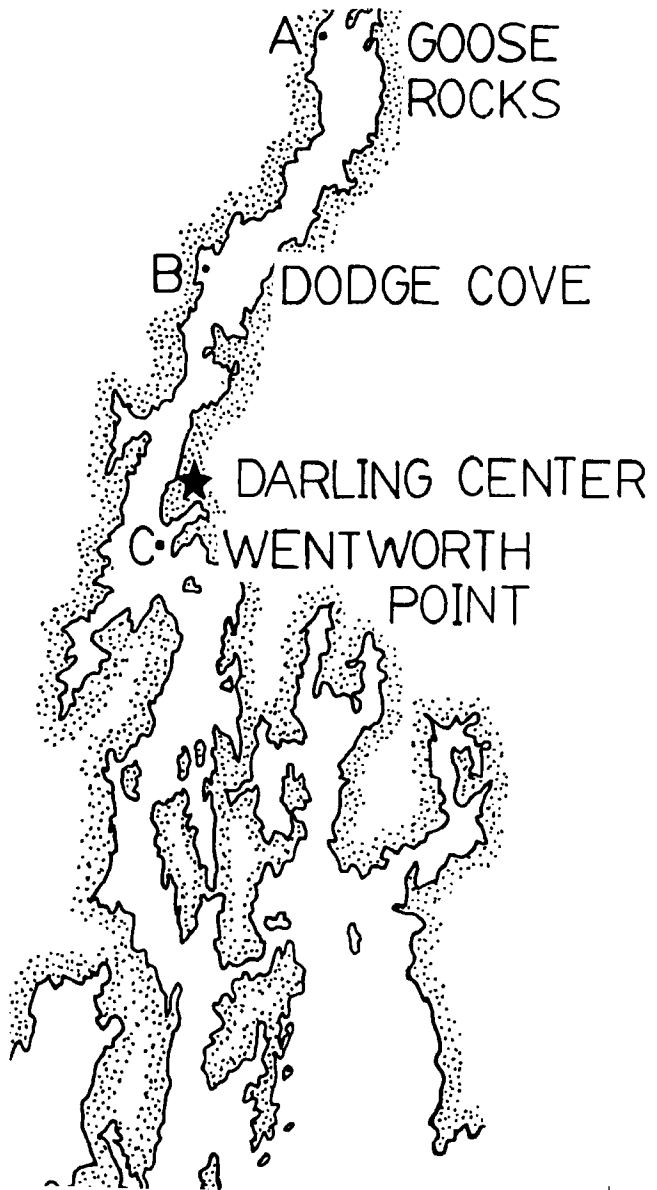
For spawning each stock was placed in its own shallow pan of seawater heated to about 28°C. In some cases this may be the only impetus necessary. With the spawning of the first oyster others in the tub will often become synchronized for simultaneous spawning. Simultaneous spawning means that eggs and sperm will be at the same stage of development ensuring the fertilization process. Other tricks such as adding food or deactivated sperm to the water may also help to initiate the release of eggs and sperm. Above all, patience is mandatory, since it may be hours before anything happens in the spawning tray.

The sex of an oyster cannot be determined from external features and may even change from year to year. Once spawning starts, males and females are separated to prevent premature fertilization of the eggs. This allows us to then fertilize each batch of eggs with the appropriate sperm to obtain either pure lines or crosses. Since oysters are extraordinarily fecund, with each female capable of producing millions of eggs per year, few animals need actually spawn in a commercial situation. For our experiment it was necessary to have as many oysters as possible contribute gametes in order to maintain a high genetic diversity and to prevent inbreeding in future generations.

Within 24 hours the fertilized egg becomes a weakly mobile planktonic larva, already possessing the two shells or valves characteristic of bivalve shellfish such as oysters, clams and scallops. Depending on both temperature and food supply this stage may last from 2-3 weeks. Throughout this period the larvae have a ring of hairlike cilia which they use for locomotion and feeding. Our larvae were grown in aerated 400 liter containers at a density of 5 per milliliter and were fed two species of cultured algae, *Isochrysis galbana* and *Chaetoceros gracilis*.

After 2-3 weeks the larvae were ready to metamorphose from the free living stage to the attached juvenile or spat in a process called *setting*. Until recently the larvae were generally set on whole oyster shells or other suitable sub-

strate called cultch. If many oysters were to set on a single shell they would become crowded and misshapen as they grew, resulting in less valuable animals. With today's technology, larvae are set on chips of ground-up shell, approximately the same size as the larvae themselves: about 3/10 of one millimeter. Due to their minute size the shell chips become insignificant as the oyster grows. As a result the animals are unattached and their growth is unrestricted, allowing them to develop their optimal shape. After setting, the spat were grown until they were large enough to be retained on window-screen sized mesh. At this point they were placed in 3' x 4' floating wooden trays and were moved from the relative safety of the hatchery to our experimental sites on the Damariscotta River in south central Maine.



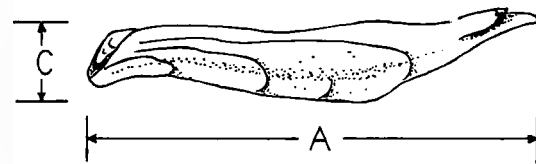
Location of experimental sites on the Damariscotta River estuary, Maine.

The Damariscotta River is a 16 kilometer *arm of the sea* estuary that is the center of Maine's blossoming oyster aquaculture industry. The river has been well-characterized for both physical and biological parameters in past studies by researchers at the University of Maine's marine laboratory, the Ira C. Darling Center, located directly on the river. Peak summer temperatures range from 23-25° C at the headwaters to 15-16° C at the mouth. The general characteristics of our three sites are as follows: The upper site at Goose Rocks displays the warmest summer temperatures and has the most variable salinity due to fresh water inputs from upriver. Conversely, the lower site at Wentworth Point is the coldest and overall it has lower temperature and salinity fluctuations being closer to the open coast. The middle site at Dodge Cove experiences conditions intermediate to the two extremes. The five miles that separate the upper and lower sites represent an ideal natural laboratory to study the growth rate of diverse populations of the American oyster under varying environmental conditions. Existing aquacultural enterprises run by Carter Newell (Pemaquid Oyster Company) and Richard Clime (Dodge Cove Marine Farm) at the upper and middle sites, respectively, have helped out tremendously by allowing us to deploy our gear at their sites giving us some protection against loss of gear.

During the first summer oysters were spawned on three different occasions. Mating I consisted of two pure lines, pure Flowers (FxF) and pure Delaware (DxD) and their two reciprocal crosses (male F x female D and female F x male D) and were randomly placed in one of four sections of a floating tray at an initial density of 2000 animals per section. These were set out in June with three replicate trays at each site. Mating II, two replicates of pure Milford stock (MxM) and a second pure Flowers stock (FxF), were placed at the upper site only, beginning in July. The final spawning of the summer (Mating III), various combinations of Flowers and Sheepscot Stock (FxF, FxS, SxF, and SxS), were too small to be placed out in the river.

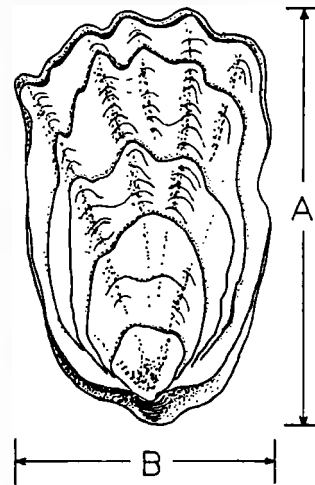
The sites were visited on a weekly basis to record temperature and salinity and for tray maintenance. The small mesh screen was subject to clogging by algae and tunicates, in spite of enclosing an herbivorous snail, the common periwinkle, *Littorina littorea*, within the trays to graze them clean. Fouling decreases water circulation in the tray, limiting food and oxygen reaching the oysters and thus decreases growth. The trays were sampled monthly from July to November for a total of five sample periods. Random samples of 30 animals per section per tray were placed in numbered plastic bags and frozen for future analysis. The oysters from each monthly sample have since been measured for four parameters including shell length, the longest distance from hinge to bill; shell width, the longest distance perpendicular to shell length; shell inflation, the thickness of the animal and total wet weight.

SIDE VIEW



A = SHELL LENGTH  
 B = SHELL WIDTH  
 C = SHELL INFLATION

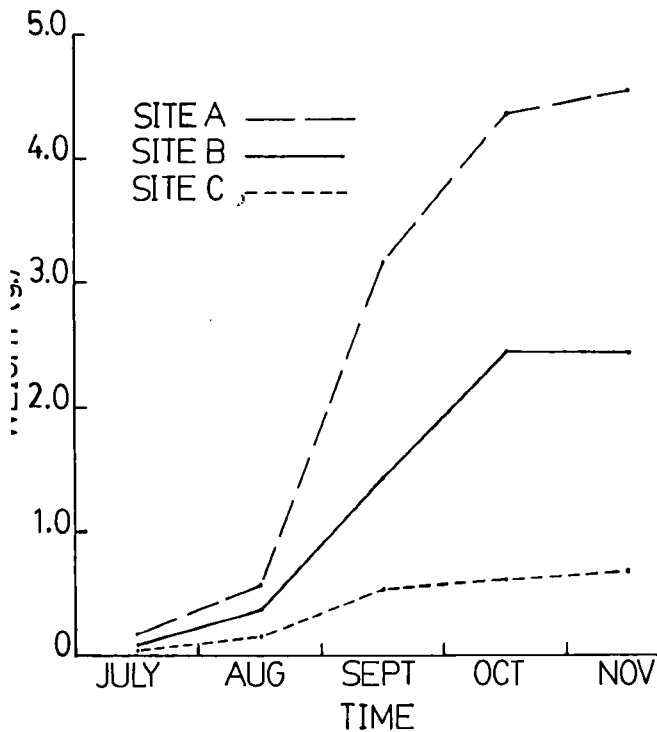
TOP VIEW



Dimensions of shell parameter measurements.

As early as August we began to notice dramatic differences in growth rate between sites and even between lines. From September onward there were statistically significant differences among all three sites. American oysters grow best in 20-25° C water, a criterion normally found further south, but met by our upper site. Since temperature and food availability are the two most important variables governing oyster growth, faster growth in the warm upriver sites could be expected, especially since the food supply is relatively constant between the sites. A decline in growth at all three sites throughout the fall probably coincided with a rapid decline in water temperature and possibly food availability.

Examining the Flowers and Delaware pure lines and crosses, the pure Delaware (DxD) line was significantly smaller than the other three by October. Members of the pure Flowers group (FxF) were never significantly different from the two crosses. Since the Delaware stock is native to much warmer Delaware Bay it may be less physiologically adapted to these colder waters. We hypothesized that the Delaware stock may exhibit an increased growth rate once a temperature threshold is reached, but no evidence of this was found.



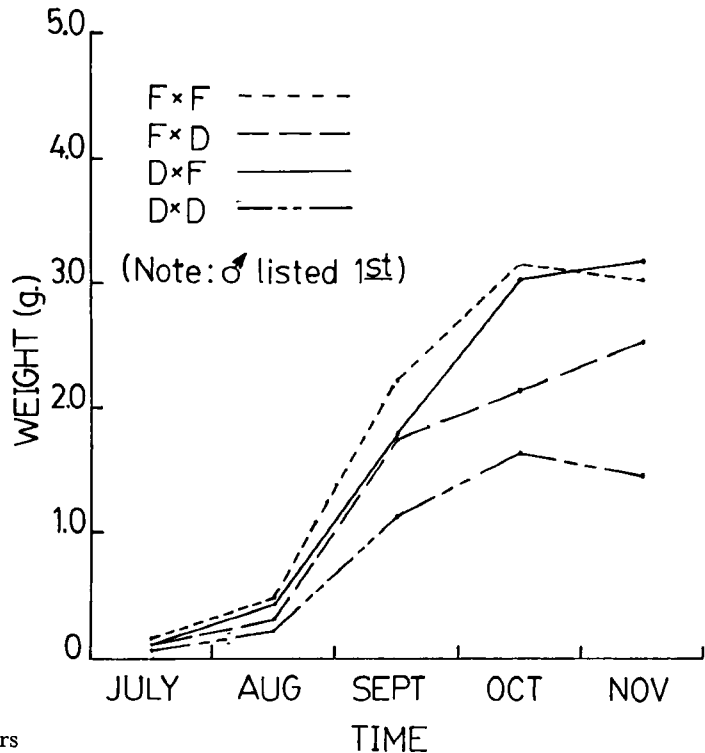
Total weight as a function of time between experimental sites.

Of the two crosses in Mating I, the one in which Flowers females were used as mothers grew somewhat better than the reciprocal cross, though not significantly so. This edge may have been due to some reproductive trait such as egg size which may lead to more rapid early development. Studies by other researchers have suggested a positive correlation between larval and spat growth rates, but most saw this advantage diminish with age.

When the mean lengths of the pure lines were combined and compared with the combined means for the crosses, the mean for the crosses was higher than the pure line mean. Being from geographically and thus genetically isolated stocks, the crosses are actual hybrids and seem to indicate some degree of heterosis or hybrid vigor. Knowing that positive heterosis exists for these cross-combinations may prove valuable in the future. We plan to breed selectively the pure lines for three generations. Once we have improved the animals through selection, forming hybrids between lines may allow us to boost performance even higher.

Finally, in Mating II, comparing pure Milford and pure Flowers, the Milford stock outperformed the Flowers stock, and since the Milford animals came from the third generation of a group selected for increased growth rate, this was to be expected. On the other hand the shell shape for the Milford stock is less than optimal, being long and narrow compared with the preferred rounder Flowers line oyster.

The fact that the various lines performed differently and exhibited high variability even within groups, seems to indicate that plenty of scope exists for improvement of certain traits through a selective breeding program.



Total weight as a function of time between pure lines and crosses of Mating I.

The remaining oysters are now overwintering in the aquaculture building at the Darling Center. Random samples of 250 oysters from each section of each tray were secured in mesh bags and placed in flowing ambient sea water. In spring the lines will be returned to their respective positions in the river for another season of growth. We will then choose our first selected parents in fall, 1987, and these will serve as brood stock for our first selected generation.

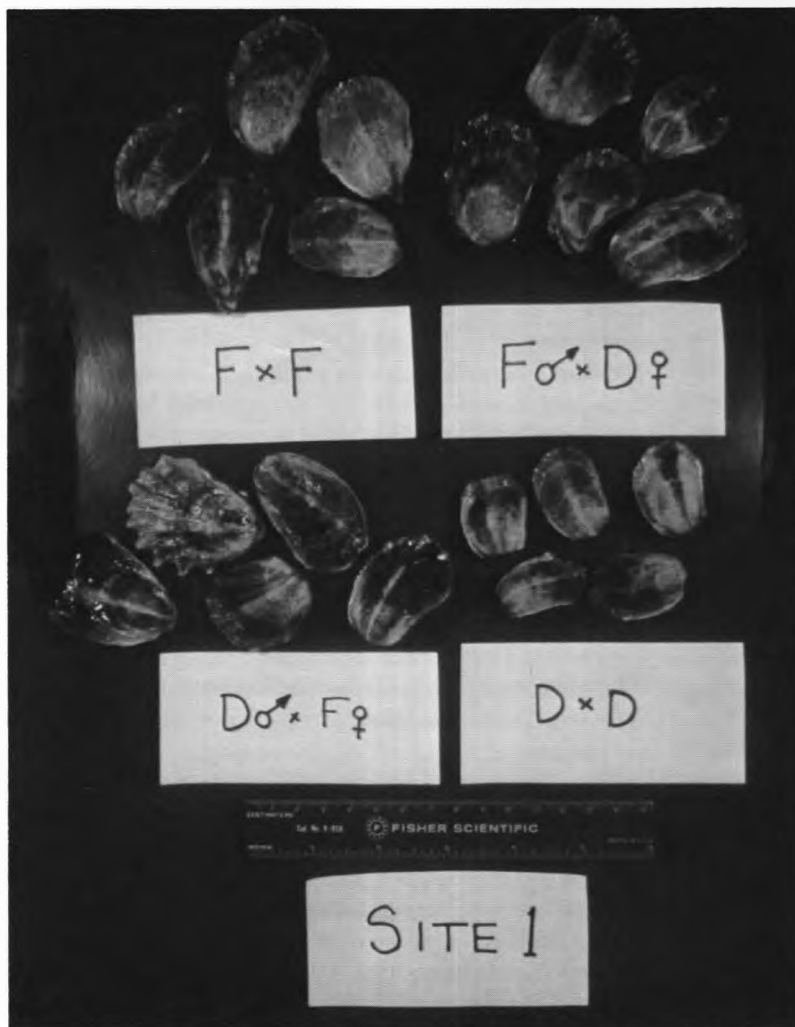
The ultimate goal of any selection program is to produce a population many standard deviations from the original mean. For Maine's growing aquaculture industry this goal is easy to justify. For example, increasing growth rate has many advantages. Faster growth at a young age will decrease the time necessary to reach market size. As with land agriculture, once outside the hatchery, cultured marine organisms are at the mercy of predators, disease, storms and other environmental processes. The ability to market animals in less time may increase the odds of having a successful aquaculture venture.

Selection for shell shape will produce the most desirable combination of shell characteristics. Uniformly sized,

rounded, deeply-cupped oysters will command top dollar in the valuable half shell trade. Deep cupped, high volume oysters should contain large meats, and consumers have grown accustomed to a rounder oyster.

Finally and of great importance especially for Maine is increasing growth rate specifically at colder water temperatures, a commodity which shows no shortage in Maine. Today, slow growth at the lower experimental site would prohibit commercial operations from using this portion of the river or similar areas elsewhere in Maine. Time to market would be at least four years and possibly longer. Development of cool water (15-18° C) varieties would greatly expand the area open to the culture of this valuable species.

Currently aquacultural genetics is at the point where land agriculture genetics and breeding were in the 1800's. Shellfish appear to react similarly to chickens and cows relative to the heritability of certain production traits. Although more preliminary information and experiments are needed, aquaculturists have a long history of livestock breeding to draw from, and early gains should be rather large. Any selection experiment needs to be integrated with continued research on nutrition, health and technological developments. Given its sparsely populated 4000-mile coastline, pristine water quality, and advances in shellfish genetics, Maine may become a leader in the production of high quality cultured shellfish like the American oyster.



Differential growth between lines of American oysters at one experimental site.

# MEASUREMENT OF SURFACE TENSION OF KRAFT BLACK LIQUOR

by *Jaya Krishnagoplan, Ph.D.*

A fundamental property of liquid surfaces is that they tend to contract to the smallest possible area. This tendency is shown in the spherical form of small drops of liquid. The fact that a liquid surface contracts spontaneously shows that there is free energy associated with it and that work must be done to extend the surface. This free energy associated with a liquid surface is of fundamental importance; a vast number of problems relating to the equilibrium of surfaces can be solved without knowing more than the magnitude of the free energy. In the solution of such problems, a mathematical device is almost invariably employed to simplify calculations; it is to be substituted for the free energy of a hypothetical tension, acting in all directions parallel to the surface and equal to the surface free energy. This is what is generally known as surface tension. Boundary tensions between air/liquid interfaces and liquid/liquid interfaces are generally termed as surface tensions and interfacial tensions respectively.

**PROJECT OBJECTIVES:** The overall objectives of the droplet formation project, funded by the Department of Energy (Contract no. DEACO2-83CE40626) and the American Paper Institute, is to conduct a comprehensive program of research to develop technical information with which nozzles can be better designed for Kraft recovery boilers and operated to deliver a specified rate of flow of droplets of black liquor of specified size, velocity and direction, and within narrow ranges of statistical variation. These parameters should be capable of regulation in the presence of disturbances in the physical properties of black liquor feed. They should be capable of control to accommodate changes in furnace conditions and production requirements.

Formation of liquid droplets can be considered the combination of two processes: energy input to the liquid to transport and fragment it; and the action of surface tension to resolve the individual fragments into drops. Surface tension is important to any drop formation operation such as the spraying of black liquor into the recovery boiler or spraying of black liquor in the cyclone evaporator. In the recovery boiler, the rate of drying and pyrolysis and combustion processes will depend upon the drop size of the sprayed black liquor. The cyclone evaporator scrubber's particulate removal efficiency and rate of evaporation will depend on the size of drops formed. As with other physical

properties, knowledge of surface tension would be useful in the characterization of black liquor and may give some insight into the surface active agents present in the black liquor.

Very little surface tension data are available for black liquor, especially at high solids concentrations. Table 1 lists the researchers, methods used, concentrations and temperatures of samples tested along with their approximate results. Methods available for measuring surface tension were evaluated critically for their suitability to high solids black liquor. The following factors were considered:

1. High solids black liquor is very viscous. At each solids concentration, there would be a minimum temperature, below which surface tension measurements cannot be made, using any of the available methods, as the sample would be a semi-solid.

2. At high temperatures, evaporation of water and other volatiles takes place, thus changing the concentration of the black liquor. The system environment has to be controlled to suppress this volatilization.

3. Black liquor, due to its high alkalinity, will react with glass, therefore critical measuring components will have to be constructed of materials that are resistant to a high pH environment.

4. Black liquor degrades when maintained at high temperatures for prolonged periods of time.

Keeping these factors in mind, the maximum bubble pressure method has been chosen for the measurement of surface tension of high solids black liquor at elevated temperatures.

**MAXIMUM BUBBLE PRESSURE METHOD:** Pressure is applied on a capillary which is immersed in the test liquid and as the pressure is increased a bubble is formed at the bottom of the capillary. For small capillaries (inside radius 0.1 cm), the maximum pressure occurs when the bubble is very nearly hemi-spherical at which point the radius of the bubble can be assumed to be the same as that of the capillary, and therefore the maximum pressure across the bubble interface can be written as a function of surface tension as follows:

$$P_{\max} = 2 \sigma / r$$

where  $r$  is the radius of the capillary. This relationship is derived from Young and Laplace's fundamental equation

of capillarity. Since the tip of the capillary is at some arbitrary distance  $l$  below the surface of the liquid, the pressure difference between the inside of the capillary and the liquid surface will be

$$P_{\max} = 2 \sigma / r + \rho g l$$

where  $l$  is the distance between the liquid surface and the tip of the capillary.

Significant departures from this limiting equation have been observed for larger capillaries, as the bubble is more ellipsoidal and not spherical. More accurate measurements can be made by deriving the maximum bubble pressure/surface tension relationship from the Schrodinger equation.

$$\sigma = 1/2 P_{\max} r - 1/3 \rho g r^2 - 1/2 \rho g r l - (\rho^2 g^2 r^3) / 12(P_{\max} - \rho g l) \quad (1)$$

If equation (1) is written for two orifices and then the equations subtracted, the result may be rearranged as follows:

$$\begin{aligned} \sigma = \{1/((1/r_1)-(1/r_2))\} \{ & (P_{\max 1} - P_{\max 2}) + \\ & \rho g(l_1 - l_2) + 1/3 \rho g(r_1 - r_2) + \\ & [(\rho g r_2/3 + \sigma/r_2)^2 + 3/2(\rho g r_2/3)^2]^{0.5} - \\ & [(\rho g r_1/3 + \sigma/r_1)^2 + 3/2(\rho g r_1/3)^2]^{0.5} \} \end{aligned} \quad (2)$$

The advantage of using two capillaries of different radii, can be seen from the second term in equation (2). It is very difficult to measure the immersion length accurately during each measurement, but on the other hand, it is much easier to either have the two immersion lengths the same (which will make the second term zero) or to measure the difference in the immersion lengths very accurately. Schork and Ray (1983) have used an approximation of equation (2) for on-line measurement of surface tension during emulsion polymerization.

Density values are necessary for the determination of surface tension using any technique. This same instrument can be used to measure the density by using two capillaries of the same radii but with different immersion depths. The differential maximum pressure between these two capillaries will be proportional to the liquid density. Equation (2) can be rearranged with  $r_1 = r_2$  to give the following:

$$\rho = (P_{\max 1} - P_{\max 2}) / g(l_1 - l_2) \quad (3)$$

**EXPERIMENTAL APPARATUS:** The maximum bubble pressure surface tensiometer/densitometer has been designed with the following features to accommodate the requirements of high solids black liquor.

Figure 1 is the schematic of the experimental apparatus. The bubble cell consists of a stainless steel container with a tight-fitting lid in which three stainless steel capillaries are fixed. The two large capillaries have a diameter of 0.476 cm, and the third narrow capillary has a diameter of 0.199

cm. One large diameter capillary and the small diameter capillary are fixed to give similar immersion lengths. The other large diameter capillary is fixed such that its immersion length is about 0.8 cm higher than the other two capillaries. All three lengths are measured accurately from a common reference point. The tips of the capillaries are cut uniformly and square; the outside is ground and tapered to keep the ratio of outside to inside diameter the same for all three capillaries and the wall thickness to the minimum. The cell is immersed in heat transfer oil (Syltherm 800) contained in a Haake F3 circulation/heater. A vent tube is fixed in the lid so that the void space inside the cell will be at atmospheric pressure.

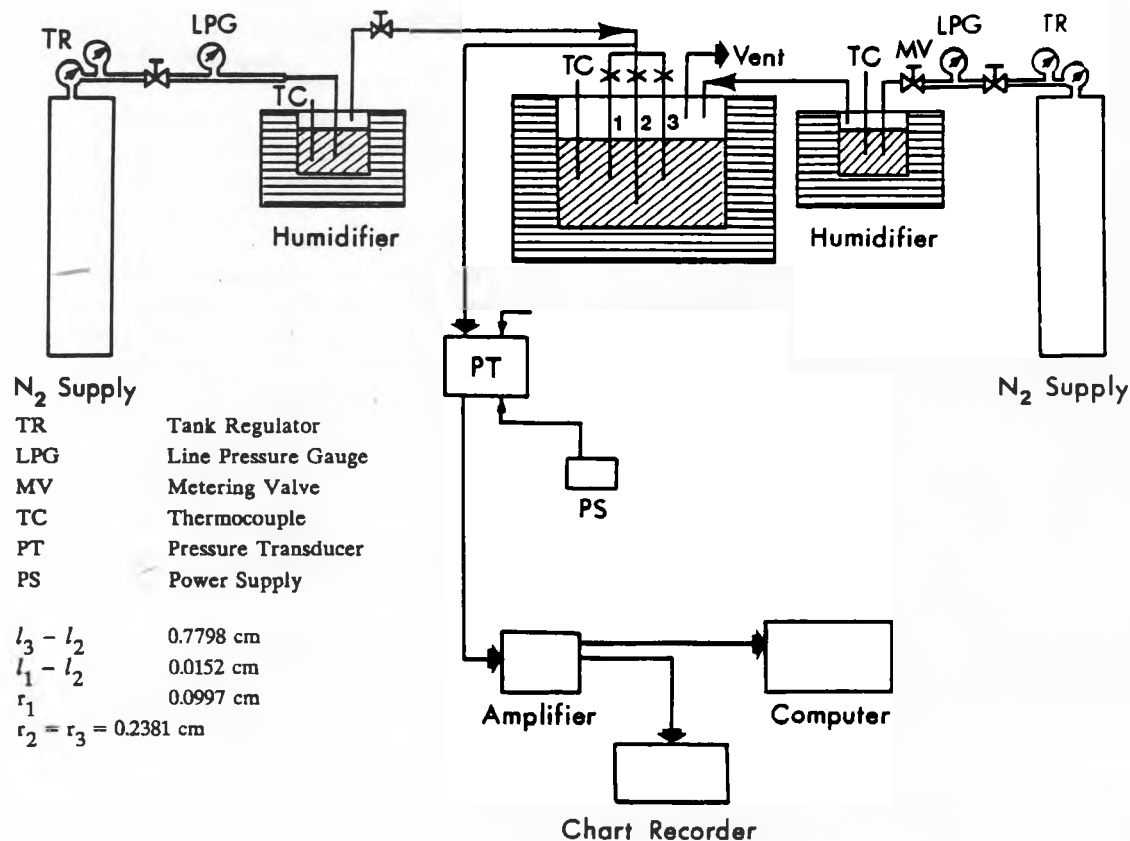
Some investigators have felt that accurate results can be obtained only if the gas bubbling through the sample liquid is saturated with the vapor of the liquid before reaching the capillary. Since oxygen will react with the black liquor components and oxidize some of them, we chose nitrogen for bubbling through the test samples. This gas is bubbled through some black liquor held at the same temperature as that of the test sample. Since most test samples are at high solid concentrations, we chose not to use the same liquor in this saturation chamber. Instead we chose a dilute liquor (16 percent solids) mixed with additional NaOH to increase its boiling point. This enabled us to have a workable liquid for presaturation that had low viscosity but at the same time a high boiling point elevation. For example, a 30 percent NaOH solution, has a boiling point of 130 to 135° C at atmospheric pressure.

Using appropriate connections and valves, bubbles are blown into one capillary at a time. The pressure transient is monitored using a low range pressure transducer (0 to 0.2 psid or 0 to 13800 dyne/cm<sup>2</sup>), which is recorded with a chart recorder and simultaneously collected into a microcomputer via an A/D converter.

Since gas is continuously blown into the sample, the bubble cell has to be vented. To reduce evaporation of the volatiles while the sample is maintained at a high temperature, a steady stream of saturated vapor is fed to the head space of the bubble cell to help maintain an equilibrium environment. This vapor is generated by bubbling nitrogen gas from another tank into a chamber containing the same liquor as that used in the first humidification chamber.

**TEST SAMPLES:** Two black liquor samples were obtained from a Wisconsin paper mill, at 16 percent and 50 percent solids content. The liquors were evaporated at our laboratory to various solids levels, using an Artisan wiped film evaporator. Both the 16 percent and 50 percent (designated as samples #1 and #2 respectively) samples were collected from the same process stream and at the same time. Apart from having gone through part of the mill evaporation cycle, the 50 percent liquor has had its soap removed at the mill at about 30 percent level.

Figure 1



Schematic of the Complete Maximum Bubble Pressure Surface Tensiometer/Densitometer

**RESULTS AND DISCUSSION:** The observed maximum bubble pressure increases as the frequency of bubbling is increased, which is obtained by increasing the gas flow rate. The flow rate is adjusted by means of a metering valve placed at the outlet from the humidification chamber. In the absence of viscous and inertial forces, the differential maximum pressure between the small and large capillaries is proportional to the surface tension, and this difference increases as the flow rate (and thereby the bubble frequency) is increased. Many researchers have attributed this increase to the fact that a newer surface is generated as the bubble frequency is increased. On the other hand, at low bubble frequency, the surface is generated slowly, and therefore has an opportunity to age, *i.e.*, the components with lower surface energy have a chance to migrate to the surface, thereby lowering the surface tension which manifests itself in lower differential maximum pressure. Initial investigations into the magnitude of the change in observed surface tension between high and low bubble frequencies indicated that there are factors other than diffusion of surface-active components that contribute

to the change. Engineering estimates were made to calculate the pressure contributions due to inertial and viscous forces. At very low bubble frequencies, these corrections are negligible, and therefore the surface tension calculated from the limiting differential pressures as gas flow rates approach zero, would be a good approximation of the static or equilibrium value. Density and surface tension are therefore calculated using the extrapolated maximum pressures corresponding to individual capillaries in the limit of zero gas flow rate.

Eight different concentrations each of black liquor samples 1 and 2 were tested. In each case, the test temperatures ranged from a minimum temperature (depending on the solids content) to a maximum near the elevated boiling point for that sample. As expected, surface tension decreases with increasing temperatures at all concentrations. Figure 2 is a representative plot of surface tension versus temperature, for sample 1. As expected, surface tension decreases with increasing temperatures. Figure 3 is a plot of surface tension versus solids content, at several constant temperatures. Values for these plots are obtained

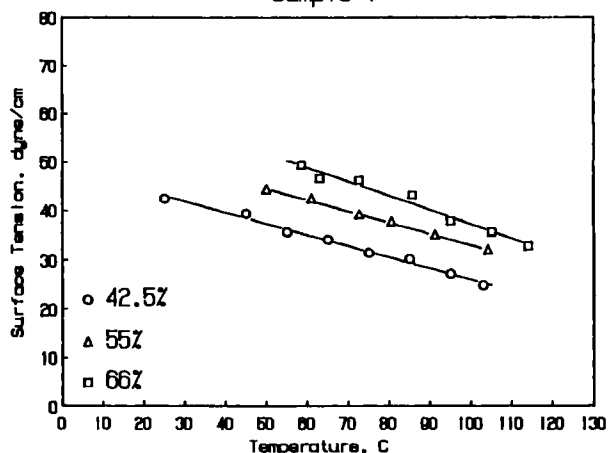


from interpolating  $\sigma$  (vs) T data. Data for water are from literature and are chosen as the basis at zero solids content. With this basis, it can be seen that surface tension decreases with increasing concentration at lower solids concentrations and starts to increase again with further increase in concentration, showing a minimum in  $\sigma$  (vs) solids content relationship. Similar minima were observed by Soderhjelm (1983). She found that a pine liquor exhibited a minimum between 30 and 40 percent solids content and birch liquor, around 20 percent solids content.

This trend is observed in both of the samples. This means that the surface tension values of samples 1 and 2, which are collected from two different locations in the mill are surprisingly similar. Sample 2 not only has undergone some processing (evaporation for a duration of 30 to 60 minutes with a total residence time of 4 hours from the weak liquor tank to the collection point), but has had its soap removed. Tall oil analysis showed that sample 2 contained about 2 percent less tall oil than sample 1 (samples 1 and 2 contained 3.79 and 1.59 percent tall oil based on dry solids content, respectively). It may be inferred from the results, that this difference in tall oil content has not affected the *near equilibrium* values of surface tension significantly for the two liquids tested.

Figure 2

### Surface Tension vs Temperature Sample 1

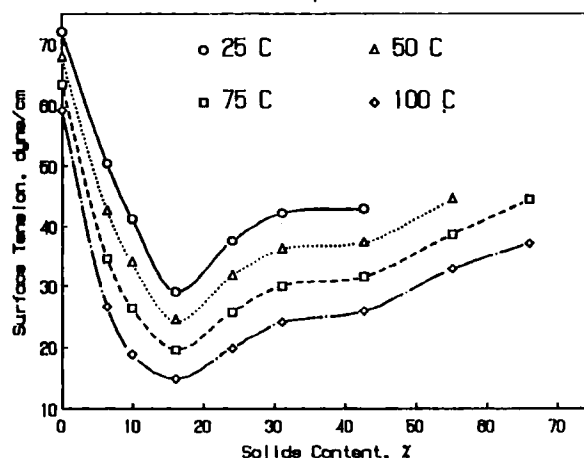


Surface Tension versus Temperature for Sample #1 at 42.5%, 55% and 66% Solids Contents

In the case of multicomponent solutions, lowering of surface tension with an increase in temperatures is affected by two means: (1) surface tension of all pure liquids is lowered by an increase in temperature; (2) an increase in temperature increases the diffusion of lower surface energy components to the surface. In black liquor, the effect of temperature can thus be explained easily.

Figure 3

### Surface Tension vs Solids Content Sample 1



Surface Tension versus Percent Black Liquor Solids Content at Four Different Temperatures for Sample #1

The effect of solids content on surface tension, on the other hand, is not straightforward. Black liquor is a multicomponent aqueous solution containing organics (*e.g.*, organic acids, lignin compounds and extractives) and inorganic salts. Water has a high surface tension value of about 72 dyne/cm at room temperature. At low solids concentrations, the organics, including the surfactants which have fairly low surface tension values, dominate the solution surface properties. Therefore, it is understandable that low solids black liquor has significantly lower surface tension compared to that of water. As the concentration of black liquor increases, there is less water, and this results in a further decrease in surface tension, which is substantiated by the results at the lower percent dry solids.

The reversal in the trend as evidenced by the minimum is interesting. Several things happen as black liquor is concentrated:

1. The fraction of water, a component with high surface tension value, decreases.
2. The fraction of inorganic salts, components with higher surface tension values than water, increases.
3. The fraction of organics, components with much lower surface tension values than water, increases.
4. Evaporation increases the solids content of black liquor and brings about significant structural changes in the components. Molecular structural changes, which result in viscosity increase, generally are accompanied by a decrease in diffusivity. In black liquor a significant increase in viscosity is observed as the solids content increases. Decrease in the diffusivities of the surface active components may very well be encountered as a result of higher concentrations.

5. It has been observed that the mutual attraction of constituent molecules plays an important role in the surface tension values of solutions.

6. Like the inorganics, the surfactants undergo change as black liquor is concentrated. Increase in surfactant concentration could promote micelle formation and critical micelle concentrations. In a surfactant mixture, as the concentration of surfactant is increased, the surface tension decreases and reaches a constant value at the critical micelle concentration. Beyond this stage, it is possible that the effect of inorganics could become more pronounced with increased total solids concentration.

7. Surface tension is known to increase with increasing molecular weight for a given homologous series. Black liquor contains several lignin and cellulose degradation products with a distribution of molecular weights. The smaller molecules possessing lower surface energy might tend to seek the surface under favorable conditions. Higher viscosities and lower diffusivities associated with higher concentrations, could decrease the influence of lower molecular weight fractions.

**SUMMARY AND CONCLUSIONS:** One of the objectives of this study was to select a suitable technique for the measurement of surface tension of high solids black liquor at elevated temperatures. The maximum bubble pressure method was selected after reviewing several available techniques, keeping in mind the physical and chemical nature of high solids black liquor. From an experimental point of view, the maximum bubble pressure method is a convenient method for the determination of near equilibrium values of surface tension of high solids black liquors up to their corresponding elevated boiling points. Saturated conditions both in the bubbling gas and above the liquid surface eliminate excessive evaporation, drying, *skin formation* on the liquid surface and swelling.

Because of the dynamic nature of the bubble pressure method, the total pressure measured during bubble formation is not just the pressures due to surface tension and hydrostatic forces. Pressure contributions due to inertial and viscous forces have to be subtracted before surface tension as a function of surface ages can be measured. Engineering estimates to separate out the inertial and viscous pressures have been inadequate. Therefore, in this study, effect of surface age on surface tension has not been studied. The measured pressures were extrapolated to values corresponding to zero flow rates and the surface tensions were calculated using these extrapolated pressures. Consequently, the results reported in this study are near equilibrium values.

If the non-surface tension pressures can be adequately estimated, this method may be suitable for a wide range of surface ages, in which case this method would be useful in the estimation of surfactant concentrations and for the study of diffusional characteristics and adsorption kinetics

of the surfactants.

The results of this study show that surface tension of black liquor decreases with increasing temperatures at all solids levels. This is an expected behavior of all liquids. The surface tension-temperature relationship is found to be linear in all cases.

A graph of surface tension versus black liquor solids content curve passes through a minimum. Black liquor is a multicomponent solution containing several organic compounds (lignin and cellulose degradation products and extractives) and inorganic compounds (several sulfur based sodium salts and salts of few other cations) in approximately a ratio of 3:1. The minimum was observed in both the samples tested in this study which occurred around the 15-20 percent solids level. Evaporation of black liquor decreases the concentration of water, which has a high surface tension value of about 72 dyne/cm at room temperature. Therefore, the initial decrease in surface tension is to be expected. The occurrence of a minimum is probably due to the effect of inorganic compounds (which have significantly higher surface tensions than water) dominating over the effects of surfactants. Reduction in the solubilities of the inorganics, promotion of micelles of the surfactants and reduction in the diffusion of surfactants to the surface all could contribute to this phenomenon.

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TABLE 1  
SUMMARY OF PREVIOUS STUDIES ON BLACK LIQUOR SURFACE TENSION

Researcher	Black Liquor Source	Black Liquor Solids %	Temperature °C	Surface Tension dyne/cm	Comments & methods used
Maksimov et al (1966)	From several Russian mills	0.5-46 20	40-80 80	— 32 - 38	Maximum bubble air pressure. Surface tension decreased rapidly with temperature
Finnish Research Institute (1968)	Calcium based spruce sulfite liquors	10 to 60	20-90	38 - 50	$\sigma$ vs. surface age. $\sigma$ increased with increasing solids
Mehrotra et al (1977)	From Indian mills /Bamboo	10-30 30	30-95 30-95	30 - 50 20 - 55	Capillary rise method
Foliodova et al (1979)	From Russian mills Hardwood & Softwood	15-50	40-80	48 - 30	Method not stated.
Beckwith et al (1981)	From southern mills/softwood	11-45	25	34 - 32	duNouy ring. Found to decrease with increasing solid content. Could not test higher solids at room temperature due to high viscosity
Soderhjelm, L. & Koiveniemi, U. (1982)	Hardwood & Softwood from several Finnish mills	22, 57, 61	20-90	—	duNouy ring. $\sigma$ reduced as drop formation time increased. Effect of soap skimmings was studied.
Liva Soderhjelm (1983)	Hardwood & Softwood from several Finnish mills	10-60 55	25-90 90	Pine A 29.8 Birch B 27.0 Pine C 23.0	Falling drop method Surface tension depends on temperature and composition and not on species. Adding inorganics increases $\sigma$ (work is being continued in this area).
Jim Wearing, PPRIC	—	20	Room	N/A	Pendant drop method

## From the former student . . .

I was born in Nagapatam, India on November 20, 1944, in a Hindu family. I graduated from Vaidheeswara High School in Mettur Dam, India at the top of my graduating class. In my generation not many women studied to get a professional degree, except in medicine. Women doctors have been very common in India for many many years, and medicine has been considered a very natural profession for women, since women would go to only lady doctors for childbirth and other gynaecological treatment. I considered going to medical college, but getting admission in one was very difficult and I thought it would be an undue burden on my father to support me for several years and also spend money for my marriage (it is traditional to spend a good amount of money for a daughter's marriage in India). So, I decided to get a B.S. degree in Home Science, since I thought that it would be the only degree that I could use in my future as a housewife. I did not foresee myself in any career and I was not very interested in teaching school, which would have been the only thing I could have done with my degree. My family background did not encourage me in becoming a nurse or a secretary. So, I received a B.S. degree in Home Science, in an all women's college, came first in my class and second in the whole university (which included several colleges). This was in May 1964. I got married in June 1965 and had two sons in 1966 and 1970.

Things started changing in 1971. My husband Gopal, who is a chemical engineer, was working in a pulp and paper mill in India, came to the University of Maine, to get a master's degree and left me behind with the boys at my father's place. Thinking that he would be returning in 1½ to 2 years, I joined him in 1972, to see the country, this time leaving my sons with my parents. Gopal decided to continue with his Ph.D. and we brought our children to Maine. Our visa status prohibited me from taking up employment, and I was very bored and wanted to go to school. Having had enough chemistry and physics in my home science curriculum, and because of my husband's influence, I got a Fifth Year Certificate in Pulp and Paper Technology, in 1973. Then I decided to get a master's degree in chemical engineering. I enrolled as a special student, and fulfilled my undergraduate chemical engineering requirements and received my master's degree in 1976. Gopal and myself had our thesis defenses (his Ph.D. and my master's) on the same day, in fact. Gopal taught as an Assistant Professor for 1½ years and we returned to India in 1978.

After a year of settling down in India, I took up a job as a Research Officer in a pulp and paper research institute, affiliated with Gopal's company and worked for 2 years. I realized that if I wanted to be fully satisfied with a career,

which I was destined to have by now, I had to get ahead, and a doctoral degree was very important for me, wherever we decided to live. Especially, in India, a woman engineer most often ended up in a research facility or a design or consulting office, a desk type job. Most companies did not hire women in plant operations. If we decided to return to USA and live, my age was such that a research or an academic position would have been more suitable than a plant job. So I left my family behind in India and came back to University of Maine in 1981 to get my Ph.D. degree in chemical engineering.

After a year of course work and looking around, I started working in a funded project which I planned to use for my Ph.D. dissertation. I started working as an Instructor in chemical engineering, devoting my time both in teaching and research. This was meant to help me both financially and professionally, as I wanted to teach after my graduation. Meanwhile, my husband had decided to come to USA and settle down, and had been applying to universities and industries for a job. He was offered a teaching position at Auburn University in Alabama and had to start in Spring 1984 quarter. It was impossible for him to wind up his job and make this deadline, so I took a leave of absence from University of Maine and taught at Auburn University in Gopal's place. Auburn was trying to start a program in pulp and paper and I had the honor of teaching the first course in pulp and paper engineering as an elective. My husband and two sons came to U.S. and I returned to Maine in July 1984 with my sons and learnt that my advisor was leaving UM for another University. I had four options (1) to go with him and continue my research, (2) to transfer to Auburn, (3) to quit my Ph.D. program and lastly (4) to stay at UM, change the project and start my research all over again. I chose the last option as I owed it to myself to finish my program and owed to UM to get my degree here. I had my eldest son enrolled as a freshman in electrical engineering and admitted my younger son in Old Town High School. I worked on "Measurement of Kraft Black Liquor Surface Tension" for two years under the guidance of Dr. Ivar H. Stockel and finally graduated in December 1986.

So, I have been a non-traditional student in more than one way. I come from a society where traditionally there have not been many women engineers. I belong to a generation and a middle class society, where women did not go for higher education and careers. Women do not leave their husband and children to pursue higher studies. It is common for me to do so. I interrupted my studies several times. I was the first female master's and Ph.D. student in chemical engineering in UM. I am not sure, if the whole college of engineering at UM (including chemistry) had a female

Ph.D. before me. I have not checked the statistics.

It was not easy to pick up the studies after long interruptions and continue. It was not easy to be separated. What was easy was living in Maine and being part of UM and part of our chemical engineering department. It is a wonderful state, and a wonderful university. The department is filled with people who are very supportive of everyone, men and women. Never once in my long stay in Maine and my association with the University have I come across discrimination personally, because of my race,

religion or sex. I have to personally mention my gratitude towards Dr. Stockel for his support, guidance and advice. I am proud to have been part of UM and will always cherish the fact.

My son Yadhunandh is a junior in electrical engineering and is very happy to be part of the EE program here. At this time I have left Maine with my other son and have taken up a position of Assistant Professor at Auburn University Chemical Engineering department.

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## From the Advisor . . .

Jaya was a graduate student in Chemical Engineering (PhD candidate) for five years from September 1981 through December 1986 when she completed all of the requirements for the PhD in Chemical Engineering. Throughout this period she was engaged in teaching or teaching assistance in some form. She has taught the first semester of a two-semester sequence in Chemical Engineering Unit Operations four times and the second semester, twice. She has taught a Papermaking Laboratory course and assisted in a Pulping Laboratory course.

Her teaching received good evaluations by the students; the tenured faculty members who taught other sections of these laboratory courses (including myself) and other faculty for whom she was a teaching assistant have been consistently enthusiastic about the high quality of and obviously strong motivation which drives her teaching.

Dr. Krishnagopalan did her research on an experimental study of the surface tension of kraft black liquor, an important process stream in the manufacture of kraft papermaking pulp. I was her advisor and chairman of her committee. She worked very hard, identified her experimental approach and designed and

supervised the construction of her apparatus. She carried that design through several iterations as she generated data which pointed up weaknesses in the original, an experience which all experimentalists enjoy and which I believe is one of the several advantages to the student of an experimental dissertation subject. She concluded the work successfully and reported it at the Fall (Boston) meeting of AIChE.

It is Jaya's attitude which makes her outstanding in everything she does. She is highly motivated to achieve an outstanding performance and to assist others in the same pursuit. However, she is patient and kind in all of her dealings. The result is that she becomes the naturally elected leader of any group in which she participates. During her time as a graduate student in Chemical Engineering, she was the agreed-upon leader of the graduate student body in the department. Jaya accomplished these good things while she lived away from her children for four years and her husband for six years, all for the sake of a PhD in Chemical Engineering. Jaya realized her goal last December when she completed all of the requirements for her PhD. She returned here in May to receive her degree.

# Aquatic Fungal Decomposers in Two Adjacent Maine Lakes of Different Acidity

by Peter Wagner

In 1986, a \$15,000 grant was awarded by the Land and Water Resources Center to Dr. Richard L. Homola, Professor of Botany, and Joyce Longcore, a graduate research assistant in Botany, to study aquatic fungi and their role as decomposers of the dead plant and animal matter in two adjacent ponds (Mud and Salmon Ponds) located in Cherryfield, Maine. Mud Pond's water is more acid (pH 4.68) and lacks fish, while Salmon Pond's water has near neutral (pH 6.78) and contains brook trout.

Microorganisms such as saprotrophic bacteria and fungi break down animal excreta and dead plant and animal matter (detritus). Also, saprophages as aquatic insects help by feeding on the detritus. Saprophages and decomposers rid the aquatic system of garbage accumulation. (Saprophage: eater of decaying organic matter.)

Homola and Longcore believe that aquatic fungi are the primary organisms in the decomposition of organic matter and even make the detritus more palatable for the aquatic saprophages in lakes, ponds and streams and that the numbers and kinds of fungi could vary with changes in acidity.

To carry out this study various organic debris was collected from the two ponds. Leaves, twigs, pollen from seasonal pollen blooms which settled on the water surface, cast off skins (exuviae) of mayflies and other aquatic insects and even dead frogs and fish have been examined in the laboratory under the microscope for the aquatic fungi. This was done several times during the ice-free months of the year. At the same time net bags containing baits of cellulose, chitin, and keratin were placed in each pond, left for a week or so, removed and again examined for aquatic fungi. Cellulose is a polysaccharide found in cell walls of plants and the most abundant organic compound found in plant debris. Chitin is a carbohydrate found in the exoskeletons of insects and crustaceans. Keratin is a protein found in hair and feathers. Fungi have the proper enzymes to break these large polymeric compounds down into simple sugars that can serve as food.

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Peter Wagner relates his experiences with research as an undergraduate student at the University of Maine.

During the first semester of my sophomore year here at the University of Maine, I was approached by Dr. Richard Homola about working with him and his graduate student, Joyce Longcore, on this study. At that time I had very little idea as to what particular area of botany I was interested in studying further. To improve my training and background in botany, I took a variety of courses such as Mycology, Pathology, Phycology, Plant Anatomy, Ecology and many more. I accepted Dr. Homola's suggestion, and decided to find out just what the scientists were going to do. In doing so, I could get a good picture of what scientific research was all about, as well as fulfill a botany major requirement of doing a sophomore tutorial. I was able to kill two birds—or two fungi—with one stone.

The whole subject of aquatic fungi was all very new to me. I had never thought of the possibility of the presence in nature of water-inhabiting fungi. Up to this point, when I thought of fungi I thought mainly of the most common and most visible Basidiomycetes which include the very commercialized mushrooms of the Agaricales. To my surprise, after spending a short while with Joyce Longcore, I became aware of an entirely new area of fungi which blew away my initial, very narrow view, of what comprised the fungus kingdom.

My first exposure to aquatic fungi was with what are called Chytrids from the order Chytridromycetales, which are considered to be lower fungi. What I was looking at were microscopic, usually single rounded cells, which had affixed themselves onto pollen grains. Looking further I learned that it is possible to categorize these minute fungi, for they may vary greatly structurally. They range from those very simple ones to those that are highly ornamented. I found that these Chytrids develop into sporangia, containers of spores, and in time will release zoospores (motile asexual reproductive cells). The way in which these zoospores are released is also useful in identifying species. Release mechanisms range from mere cracking of the sporangia to the formation of an opening with a lid, to the overall disintegration of the entire wall of the sporangium.

As I mentioned before, the Chytrids were found on pollen grains and they can also grow on other material such as seeds, chitin, and forms of cellulose. These fungi could be captured by baiting the collected water debris sample with

various forms of the materials mentioned before. Pine pollen seemed to work well, hemp seeds were used, dead dried shrimp exoskeleton could be used for chitin, and cellophane cigarette package wrappers could be used as a form of cellulose. Even blonde baby hair which is a source of keratin was found to be an effective bait. With the removing of adhering debris, the Chytrids were easily observable on the clear cellophane. During the summer months many of these materials could be placed in mesh bags which were submerged below the pond surface to allow the free movement of zoospores into the bait bags. This sampling method is used by Joyce Longcore to identify characteristic species of the two lakes. Also, if the bags were checked periodically from the spring through the fall months, the seasonality of these aquatic fungi could be determined, and it was found that there is quite a variation of fungi species due to changes in the environment.

After the fungi are collected it is necessary to grow them in culture either on agar plates, liquid nutrient media or in sterile water. In order to obtain a pure culture it is necessary to clean up the fungi, to try to rid the particular fungus of any bacteria, other fungal spores, or various other extraneous materials. The job of cleaning these spores is a very tedious one. There are many methods of doing so, but the one I proceeded with involved placing a sample on a sterile agar plate, finding a particular sporangium, and finally rolling it over the agar to wipe away any possible infectants. This may not sound too difficult until you remember that this is all done under a microscope and the rolling of a sporangium is accomplished with needles. Also, this procedure must be repeated a number of times to ensure that at least one of the cultures will indeed grow and be pure in its growth. More often than not, either the sporangia will not release zoospores to produce a large sample of fungi, or the fungal culture will become contaminated with bacteria.

I wondered at one point whether there are marine aquatic fungi. Joyce Longcore supplied me with several articles about marine fungi found in the Antarctic. This piqued my interest in marine biology. I proceeded to a couple of places along the Maine coast, specifically Brooklin and Sandy Point, and obtained various samples from the different intertidal zones, as well as salt water samples. After bringing those samples back to the lab, I baited them and made sea water agar on which to grow the potential fungi. After examining the pollen I used as bait, I discovered that indeed there were marine fungi off the coast of Maine and that they could be grown in culture. The problem with this was that the cultures were overrun with bacteria. Because of this it was impossible for me to create a pure culture of these marine fungi, so identification was impossible.

So ended my first year of working with Dr. Homola and Joyce Longcore.

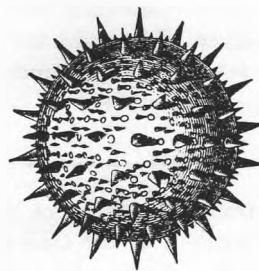
Two years later, in my senior year, I still participate in

some of Dr. Homola's and Joyce Longcore's work. I received a work merit scholarship, so I now get paid for the work I do. Not unlike most work-study positions in scientific labs, a great deal of the work I do consists of washing thousands of slides and hundreds of petri dishes, continually autoclaving utensils, mixing up batches, batches and batches of nutrient agar, and doing tedious library information searches. The menial jobs must be done and being low man on a research team, one doesn't always get the most lucrative and most interesting jobs.

Besides the fairly ordinary, menial work I do, I have also done drawings for a particular article that Joyce Longcore and Dr. Homola were publishing about a new species of Oomycete. My drawings were done by using what is known as a camera lucida. This is an attachment on a microscope that magnifies the object and appears to project the image onto paper, so one can trace the outline of the image. This technique is very much like the very old camera obscuras. After the outline which corresponds to the actual proportions of the object is drawn, details are added free-hand.

My latest area of interest in lab work is photography. I am now relearning darkroom techniques which I hope are useful skills. I feel that there is little artistic skill involved in producing an adequate image of these aquatic fungi. This is somewhat discouraging because in art lies one of my greater interests. The drawings require artistic skill and are more satisfying to make than the photographs, but both techniques are certainly legitimate ways of conveying an idea.

I believe working for Joyce Longcore and Dr. Homola has been a valuable experience. It has given me a great exposure to what science and research in a professional world are about. I have been made aware of the successes, such as gaining a research grant, and the disappointments, such as being *scooped* on the publication of an article. Through this experience I have come to the conclusion that a career in research is probably not where I am headed, at least not now. Even though I have come to this conclusion, I do not feel that my work in the lab was a waste of time. I provided help to the project and I certainly gained many insights. Finally, I would encourage any undergrad who has an interest in a particular field to try to obtain practical experience while still in school. I believe it to be very useful in determining future goals.



# STUDIES ON A NEW MOUSE MUTATION: In Search of the Primary Defect

by Luanne L. Peters

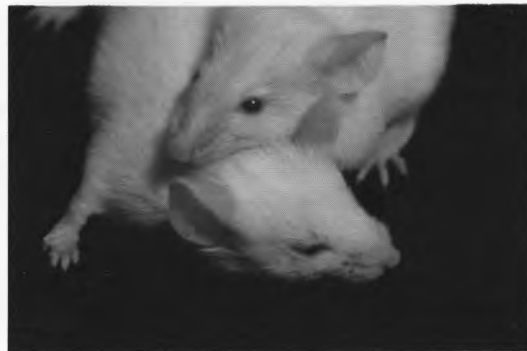
A new mutation recently occurred in the BALB/c-By mouse strain at the Jackson Laboratory. The genetic defect is overtly expressed in an intermittent fashion in the affected (homozygous recessive,  $-/-$ ) animals as a bleeding problem accompanied by severe anemia. Bleeding occurs into the gastrointestinal tract, extremities, and brain. The animals are severely affected at birth, but many recover fully. After a remission period of approximately two to three weeks, they suffer a second crisis episode and die as a result of massive hemorrhaging. Less frequently, the mutants suffer several crisis episodes with intervening periods of remission prior to death. Mutant animals in crisis ( $-/-$ -Cr) are easily distinguished from normal ( $+/+$ ) mice by their extreme pallor and bruised feet, tails, and ears. Mutants in remission ( $-/-$ -Rem) cannot be distinguished from  $+/+$  mice. They show no evidence of bruising and have a healthy pink coloration to the skin. Indeed, peripheral blood smears taken from  $-/-$ -Rem mice are indistinguishable from normal smears and show no signs of the severe anemia seen during crisis episodes (Figure 1).

Propagation of this mutation presents a challenge. Heterozygous ( $+/-$ ) carriers cannot, as yet, be identified. They show no symptoms of the defect. The affected, readily recognizable homozygous recessive ( $-/-$ ) animals rarely survive until puberty. Those that do are too fragile to survive pregnancy and delivery unless they stay in prolonged remission, which is rarely the case. To circumvent these

*Luanne L. Peters completed her undergraduate education at the University of Maine majoring in zoology. She was a promising student who demonstrated extraordinary interest in the biomedically related sciences and research. Luanne went to Indiana University, completed a master's program in physiology and while there, did the practicum year required to become a certified medical technologist. Her experiences in the diagnostic laboratory at the Indiana University Medical Center convinced Luanne that biomedical research was her ultimate goal and she returned to the University of Maine in 1984 to pursue a doctorate. Luanne's academic background in physiology and the technical expertise gained while working as a medical technologist have prepared her very well for the dissertation problem she has undertaken. Luanne is also a competent and enthusiastic teacher and she is looking forward to a career in an academic institution which will provide opportunities for her to teach and pursue her research. Her immediate plans are to complete her Ph.D. and then to obtain a postdoctoral position in a laboratory where she will gain expertise in molecular biological techniques.*

problems, ovaries from homozygous recessive animals are transplanted into ovariectomized adult recipients that are genetically identical to the mutants but do not carry the gene defect. These recipients are then mated to  $+/+$  males. Their offspring are heterozygous for the mutant gene. When these heterozygotes are mated to each other, 25 percent of their progeny are homozygous recessive and express the mutation (Figure 2).

With a supply of mutant mice in hand, Dr. Jane Barker and her associates at the Jackson Laboratory sought to determine the cause of the bleeding. Partial thromboplastin times (PTT) and prothrombin times (PT) in the mutants did not differ from  $+/+$  controls. These results rule out a disease such as hemophilia (in which there is a deficiency of clotting factors in the blood) as the cause of the bleeding episodes. However, bleeding times were elevated in the mutants, indicating the presence of a qualitative or quantitative platelet defect. When platelet counts were performed, some mutant animals had normal counts but subsequently died anyway. Others had decreased platelet counts (thrombocytopenia). Taken together, these observations suggest the presence of a qualitative platelet disorder in which the resultant bleeding eventually leads to anemia and thrombocytopenia due to blood loss. Finally, the disease was found to be transferable through the hematopoietic stem cells; bone marrow from mutant mice caused identical symptoms when transplanted into lethally irradiated  $+/+$  recipients.



Note pale ears.



It was at this point that we began collaborating with Dr. Barker to identify precisely the primary physiological defect produced by the gene mutation. We began by establishing some normal sex and age related hematological parameters in BALB/c-By mice. When we compared normal white blood cell (leukocyte) counts and platelet counts to those of mutants classified as either in crisis or in remission by overt criteria, we found a consistent and marked decrease for both parameters in  $-/-$ Cr mice (Figures 3 & 4). Furthermore,  $-/-$ Rem mutants showed a complete recovery of both white blood cell and platelet numbers. This data made it impossible once again to rule out a quantitative platelet disorder, as had been presumed from earlier data in which  $-/-$ Rem animals and  $-/-$ Cr animals were considered as one experimental group. Furthermore, it became apparent that  $-/-$ Rem and  $-/-$ Cr animals had to be considered separately in order to avoid misleading and conflicting results. The  $-/-$ Rem animals are remarkable in the extent of their recovery: to date only one parameter we have studied has differed from  $+/+$  controls (see below). Again, however, we were not in a position to determine whether the low platelet counts in  $-/-$ Cr mice were the primary cause or simply the result of the bleeding.

In human beings, qualitative platelet disorders are attributed to platelet membrane abnormalities (Thrombasthenia and Von Willebrand's Disease, for example) and to deficiencies in platelet internal granule contents (Storage Pool Disease, Gray Platelet Syndrome). Platelet ultrastructural changes have been correlated with some of these disease states. Thus, we began screening for qualitative platelet defects by preparing normal,  $-/-$ Rem, and  $-/-$ Cr platelets for electron microscopical study. Anticoagulated (citrate) whole blood was centrifuged at 800 RPM for ten minutes to obtain platelet rich plasma. The platelet rich plasma is removed and centrifuged at 2000 RPM. In this way, a pellet highly enriched for platelets is obtained. Red blood cells (erythrocytes) and some white blood cells are also present. Following fixation in 3 percent glutaraldehyde and 1 percent osmium tetroxide in phosphate buffer the platelet pellets are embedded in plastic (Spurr's), sectioned, stained with uranyl acetate and lead citrate, and viewed on the electron microscope at magnifications up to 70,000 X. Platelets from  $-/-$ Rem animals appeared normal ultrastructurally and did not differ from  $+/+$  platelets in any respect. However, despite several attempts, no platelets were ever seen in the pellets prepared from  $-/-$ Cr animals. Only erythrocytes and occasional leukocytes were seen.

A closer examination of peripheral blood smears revealed why no platelets were recovered from  $-/-$ Cr animals. Platelets can be fairly accurately estimated from peripheral blood smears. In a field of approximately 100 erythrocytes, the number of platelets is counted and multiplied by 20,000 to give an estimate of the actual count. For the  $+/+$  and  $-/-$ Rem mice, smear estimates closely correlated with the

actual counts, but the estimates were always far below the actual counts obtained for mice in the  $-/-$ Cr group. In fact, the estimates were always close to zero- no platelets present. The actual counts shown in Figure 3 for  $-/-$ Cr mice are falsely elevated. The discrepancy is due to the failure of erythrocytes in the  $-/-$ Cr animals to lyse completely in the platelet counting medium. Microcytic erythrocytes, fragments, and intact erythrocytes with membrane spicules (acanthocytes and burr cells), all of which are present in the  $-/-$ Cr group, appear as small, dot-like structures under the light microscope and are erroneously counted as platelets.

Fortunately, sections of spleen were also prepared for viewing under the electron microscope. In mice, the spleen, as well as the bone marrow, is a normal site of hematopoiesis. In  $-/-$ Cr animals, the spleen is markedly enlarged (Figure 5). We felt this enlargement simply reflected an increased demand for RBCs. However, under the electron microscope no increase in the number of erythrocyte precursors in the  $-/-$ Cr group was found. Instead,  $-/-$ Cr spleens appeared completely infiltrated by mononuclear cells of unknown origin. Furthermore, structurally normal platelets appeared within the splenic parenchyma. The presence of platelets within the spleens of animals during crisis episodes, in which the peripheral platelet count is near zero, suggests splenic hypersequestration (hypersplenism). In human beings hypersplenism is associated with lymphomas, hairy cell leukemia, and a variety of other malignant disorders. The hypersplenism, in turn, contributes to the pancytopenia (a condition in which all the formed elements of the blood are decreased) associated with these conditions. Our investigation led to the conclusion that the mutation is not a primary bleeding disorder or an inherited anemia, but a neoplastic proliferation accompanied by secondary hemorrhagic tendencies. The finding that the mutation is transferable through the hematopoietic stem cells is consistent with this conclusion.

The marked changes in the structure of the spleen in  $-/-$ Cr animals is best appreciated upon examination by light microscopy (Figure 6). The proliferation of the mononuclear elements is so pervasive that the normal white and red pulp areas cannot be discerned, and the lymphatic nodules are nearly, if not completely, obliterated. In  $-/-$ Rem and  $+/+$  spleen sections, erythrocytes and their precursors are readily demonstrable in the red pulp areas by benzidine staining, which produces brown deposits in the presence of hemoglobin. In spleens of  $-/-$ Cr mice, benzidine staining is negative (Figure 7). To add further evidence that the mononuclear proliferating elements are of leukocytic origin, frozen spleen sections were stained histochemically to demonstrate the leukocyte enzyme, acid phosphatase. Strong activity was found throughout the cellular elements of  $-/-$ Cr spleens, and in the lymphatic areas of  $+/+$  and  $-/-$ Rem spleens. Lymphocytes and

histiocytes are positive for acid phosphatase activity, suggesting the presence of a malignant lymphoma in the mutant animals.

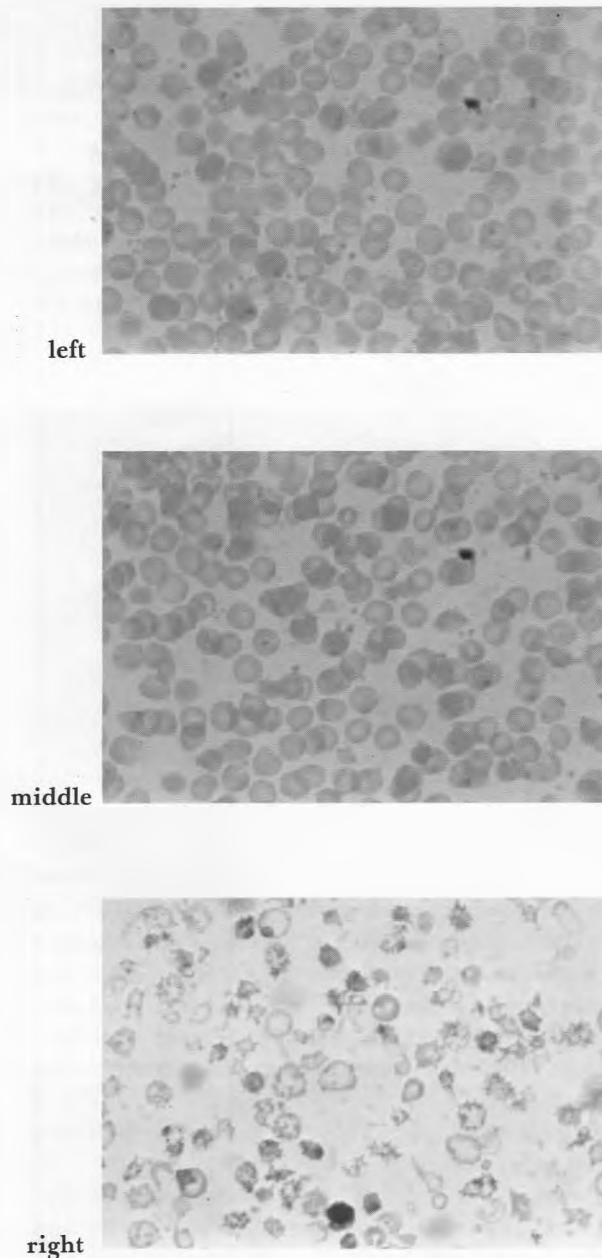
In human hairy cell leukemia, proliferating *hairy* cells (derived from B lymphocytes) produce a diffuse pattern in the spleen which is very similar to that seen in  $-/-$ Cr mutants. Hairy cells are acid phosphatase positive in the presence of tartrate, an inhibitor of acid phosphatase activity in other lymphocyte subtypes. Acid phosphatase staining of  $-/-$ Cr frozen spleen sections in the presence of tartrate is negative, thus eliminating hairy cell leukemia as a possibility.

Histochemical staining for the demonstration of alkaline phosphatase and alpha-naphthyl acetate-esterase (ANAE) activity reveals high activity for both enzymes in the peripheral areas of the lymphatic nodules in  $+/+$  and  $-/-$ Rem animals. Positive activity is seen only in widely scattered cell clusters in animals in crisis (Figure 8), suggesting the malignant proliferation is expanding the red pulp areas of the spleen and that the proliferating cells are negative for these two enzymes.

Mouse T-Lymphocytes are strongly ANAE positive, whereas only approximately 5 percent of B-lymphocytes show significant activity. On this basis, we can speculate that a B-cell or Null cell lymphoma is present in the mutants. A histiocytic or mixed histiocytic-lymphocytic neoplasm seems unlikely, as histiocytes are also ANAE positive. Definitive classification of the neoplasm awaits immunocytochemical staining for specific marker antigens. Indirect immunoperoxidase staining for surface immunoglobulin (an identifying marker for B cells), Thy-1 antigen (an identifying marker for T-cells) and Mac-3 antigen (a marker for histiocytes) is planned in the near future. The demonstration of immunoreactivity for one of these antigens is critical for a definitive classification of the malignant cell type. The absence of immunoreactivity for all the above antigens would suggest a lymphoma of the Null cell subclassification.

While determining the nature of the splenic mononuclear elements, we did not forget about the secondary manifestations (anemia and thrombocytopenia) of the genetic defect. Hypersplenism alone did not seem to account for the severity of the thrombocytopenia. Further electron microscopical studies did not indicate above normal numbers of platelets within the  $-/-$ Cr spleens. Cellulose acetate serum protein electrophoresis at pH 8.6 (barbital buffer) indicates an elevated gamma globulin fraction in the  $-/-$ Cr animals. The  $-/-$ Rem animals have levels intermediate between the crisis animals and the  $+/+$  control animals. This is the first parameter studied in which an abnormality has been found in the  $-/-$ Rem mice.

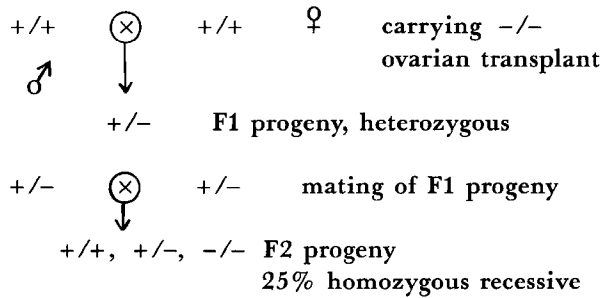
The specificity of the elevated antibody fraction is presently being investigated. In a preliminary study, 0.1 cc. of heparinized plasma was obtained from a mouse in each



**Figure 1.** Wright's stained peripheral blood smears from  $+/+$  (left),  $-/-$ Rem (middle), and  $-/-$ Cr (right) mice. Note the hypochromic, deformed erythrocytes, the presence of many reticulocytes (darker staining, young erythrocytes) and the absence of platelets in the  $-/-$ Cr animals. (oil immersion, 1000X).

group and injected into an adult  $+/+$  recipient by way of the tail vein. Platelet counts were performed at times 0, 30 minutes and 90 minutes following injection. In recipients of plasma from  $-/-$ Cr and  $-/-$ Rem animals, there is a large drop in the number of circulating platelets; no change in platelet counts occurred in the mouse receiving  $+/+$  plasma (Figure 9).

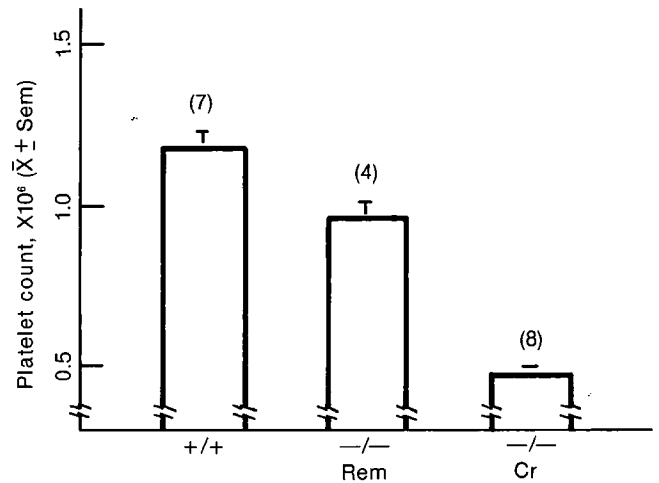
The above observations suggest a secondary autoimmune disorder of platelets in the mutant mice. Autoimmune complications of platelets and red blood cells occur in human malignancies. We intend, therefore, to investigate the possibility that a secondary autoimmune hemolytic anemia may also accompany the mutation. Direct antiglobulin (Coomb's) testing and plasma free hemoglobin determinations are planned as preliminary screening tests for erythrocyte hemolysis.



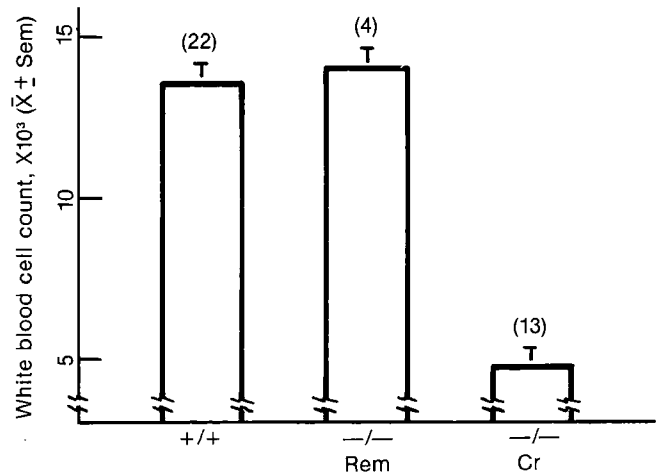
**Figure 2. Propagation of the mutation using ovarian transplant recipients.**

Inherited mouse anemias are frequently associated with red blood cell membrane defects. Specifically, deficiencies of the membrane structural proteins called spectrins and anchorins have been documented. Polyacrylamide gel electrophoresis of red blood cell ghosts by the method of Fairbanks reveals no deficiencies of these structural proteins in the mutant mice. The gross structural deformations of the red blood cells seen during crisis episodes, therefore, may result from antibody mediated hemolysis.

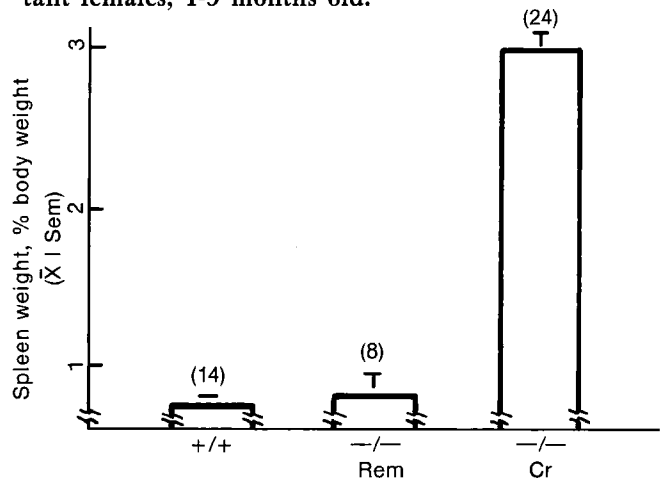
It appears we have a possible model for the study of lymphoma and secondary autoimmune diseases of platelets and possibly red blood cells. In addition, this mutation may provide answers to questions regarding the mechanisms of malignant transformation at the molecular level. The phenomenal recovery of the mutants during remission periods suggests that an on-off switching mechanism is associated with the gene mutation and may provide clues to the control of regulatory genes and the expression of oncogenes. Such long-range studies await definitive characterization of the physiological expression of the mutation. The studies described here are a start in this direction.



**Figure 3. Platelet counts in normal and mutant mice. As platelet counts vary with age and sex, the data shown are for females one month old. Similar results are obtained in males and in older mice.**



**Figure 4. White blood cell counts in normal and mutant females, 1-3 months old.**



**Figure 5. Spleen weights, (as percent of body weight) in mutant and normal mice.**

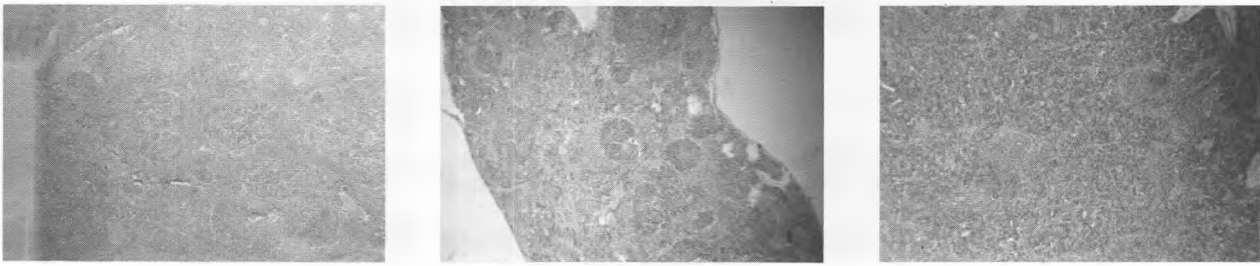


Figure 6. Hematoxylin and eosin stained paraffin sections of spleen (40X magnification). Left to right: +/+,

-/-Rem, -/-Cr. Note the absence of clearly defined lymphatic nodules in the -/-Cr mice.

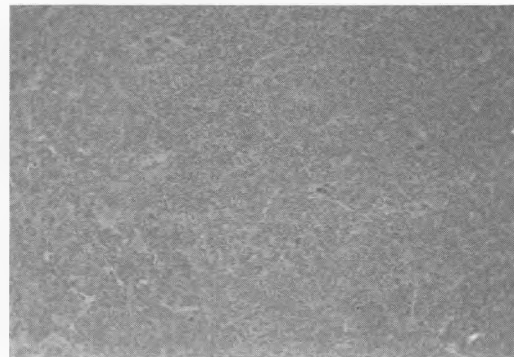
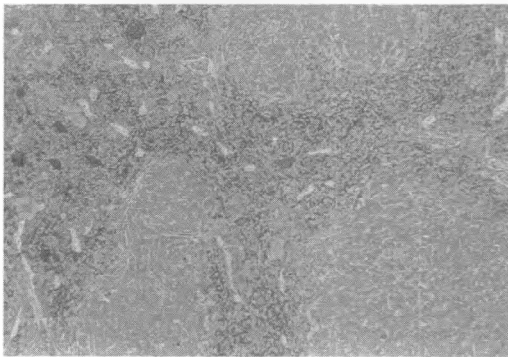


Figure 7. Benzidine stains of plastic embedded spleen sections in +/+ (left) and -/-Cr (right) mice (100X magnification). The counterstain is 1% toluidine blue. Note the complete absence of hemoglobin in the -/-Cr

mice, while extensive staining in the red pulp areas is apparent in +/+ animals. Remission animals (not shown) stain identically to the +/+ mice.

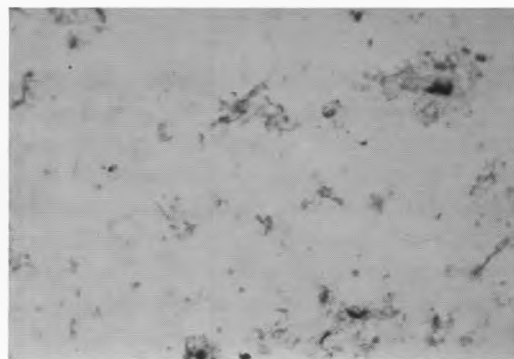
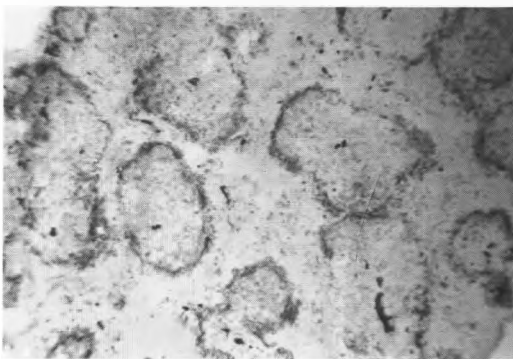


Figure 8. Alkaline phosphatase stains in +/+ (left) and -/-Cr (right) mice. Note the strong positive reaction (purple) in the +/+ mice in the nodular areas and the

widely scattered groups of positive cells in -/-Cr mice. This pattern of expanded red pulp areas in the -/-Cr mice is also evident in ANAE stained sections.

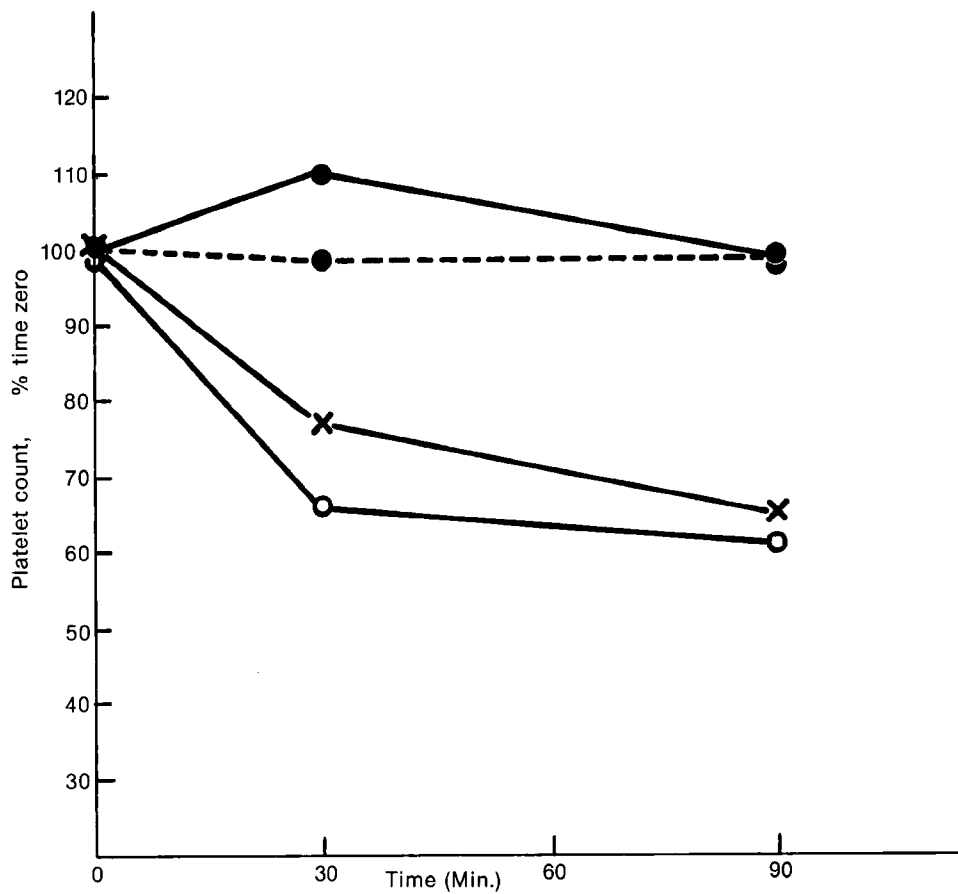


Figure 9. Platelet counts in +/+ recipients at 0, 30, and 90 minutes following injections with 0.1 cc saline (●- - -●) or plasma from +/+ (●—●), -/- REM (○—○), and -/-Cr (x—x) donors.

# OPPORTUNITIES FOR STUDENTS:

## Maine Agricultural Experiment Station Research Programs

by Mark W. Anderson

The Maine Agricultural Experiment Station has a pivotal responsibility in the University of Maine System for research in agriculture, forest resources, and rural economic development. The Experiment Station is in its one hundred and second year of operation on the Orono campus and at field sites and experimental farms across the state. Students in a variety of academic programs participate in more than one hundred projects that constitute the Experiment Station's research program.

Table 1. presents a brief numerical summary of student involvement in station research this academic year. More than one hundred graduate students, at both the Masters and Ph.D. level, work closely with scientists from the thirteen academic departments at the University of Maine that participate in Experiment Station research. Additionally, another one hundred undergraduate students are actively involved with research projects.

Table 1. STUDENTS INVOLVED WITH MAES RESEARCH PROGRAMS 1986-87 ACADEMIC YEAR

	MALE	FEMALE
GRADUATE STUDENTS		
Ph.D.	18	13
M.S.	49	32
UNDERGRADUATE STUDENTS	<u>69</u>	<u>72</u>
	136	117
TOTAL		253 students

These students are involved with research projects in the following disciplines, in which many are pursuing their degrees:

- Agricultural Engineering
- Agricultural and Resource Economics

- Animal and Veterinary Sciences
- Biochemistry
- Botany and Plant Pathology
- Entomology
- Food Science
- Forest Biology
- Forest Management and Wood Utilization
- Human Development
- Microbiology
- Plant and Soil Sciences
- Wildlife

The stories behind these numbers are as diverse as the research projects the students are working on, some of which are reported elsewhere in this issue. The graduate students are intimately involved in the research projects from inception through final documentation. Most are addressing specific objectives in larger Experiment Station projects as the foundations for their own doctoral dissertations or masters theses. Many receive financial support from graduate research assistantships supported by the Experiment Station or by grants and contracts awarded to MAES scientists by outside funding sources. These graduate students are the young investigators who will be making important contributions to the science of agriculture and forest resources for years to come. They are making important contributions to Maine's natural resource-based industries while they are here.

The undergraduate students involved in Experiment Station research often have a different role to play. Many have jobs through the College Work-Study Program, and as such, their work is part of their financial aid package. These students are frequently involved with technical support aspects of a research program such as cleaning glass, running analyses, data collection, and data entry. Many work-study students will spend the four years of their undergraduates careers in one MAES lab, undertaking increasingly more responsible activities each year. More than a dozen of the undergraduates working on MAES research projects this academic year were merit scholars, honors students, or pursuing a special course or independent study

*Mark Anderson is Assistant Director of the Maine Agricultural Experiment Station.*



Cindy Wiggin, Ph.D. candidate in Animal and Veterinary Sciences, works with Dr. Harold Gibbs taking a blood sample from a bull calf. Wiggin's dissertation research involves the links between the immune system and dairy cattle parasites. Gibbs is an internationally renowned parasitologist on the MAES faculty.



Undergraduate students don't have to be majoring in a subject area to make an impact on research. Here Laura Downey, a freshman History major, is carefully transplanting blueberry bushes which will be used in the tissue culture studies of Professor John Smagula.

with an Experiment Station scientist. These students function more like graduate students in their respective labs. Although their research projects may be of a smaller scope than that of a typical graduate student, independent studies projects provide undergraduates with a comprehensive research experience. Students frequently find this experience helpful in deciding whether they want to pursue graduate studies in a particular field of science.

Since much of the research conducted under the auspices of the Experiment Station relates directly to Maine's agriculture, forest resources, and marine industries, students have considerable opportunity for field research. From Ph.D. candidates to work-study students, many of the students are involved with data collection at one of the

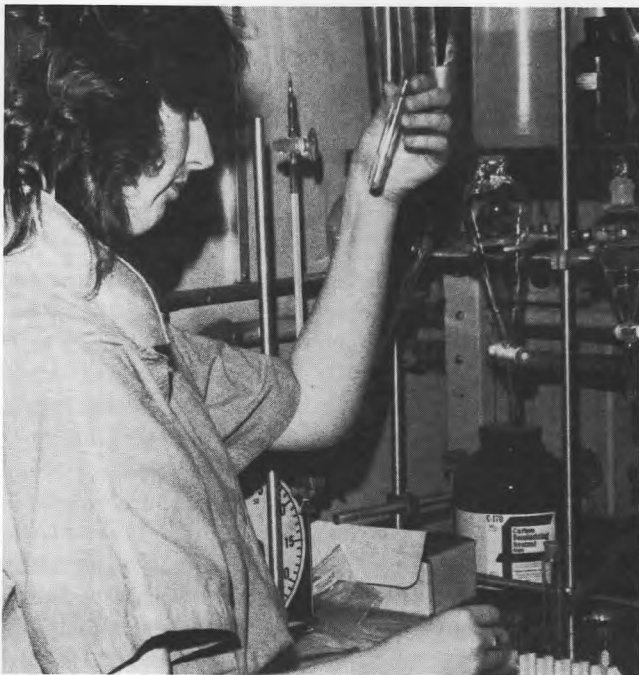
Experiment Station's four experimental farms, at the University Forest, or on site with one of the many private cooperators whose facilities are offered to assist in the conduct of research. This hands-on experience enriches the education of these individuals while it provides crucial input for the research project itself.

In the final analysis everyone benefits from student involvement in the research programs of the Experiment Station. Students receive invaluable training and exposure to the ways of science. Faculty members get an *extra pair of hands*, which is often critical to the success of a particular research project. Finally, the people of Maine benefit from the results of Experiment Station research, all of which is aimed at improving the quality of life for Maine citizens.



**Dawn Beaton looks over the shoulder of Kerry Black as they review the sequence of DNA in the lab of Assistant Professor Keith Hutchinson. Both undergraduate**

**students are actively involved in lab procedures related to Hutchinson's study of conifer tree genetic changes which occur as trees mature.**



**Sharon Savage, an exceptional high school student from Howland, assists in the work of Food Scientist Rod Bushway as he evaluates a new test kit intended to spot pesticide residue in fresh produce. Sharon intends to study medicine after high school.**



# Experimental Embryogenesis in Red Pine

by Judy C. Gates

An embryo is the rudimentary plant contained in a seed. A fully developed pine embryo consists of a whorl of about eight cotyledons, or seed leaves, surrounding an epicotyl or shoot apex, a short hypocotyl or shoot axis, and a primary root or radical. The embryo is imbedded in the tissue of the female gametophyte, which is surrounded by the seed coat (Figure 1). The term embryo encompasses the initial developmental phases following the zygote, or fertilized egg, in all classes of plants. Studying the inception and formation of the embryo, known as embryogenesis, leads to a greater understanding of plant development and maturation.

Maturation is a continuous process; it begins in the relatively simple one-celled stage of development and continues throughout the increasingly complex adult stages. Maturation occurs in distinct patterns in response to physiological, morphological and genetic factors. These patterns have their inception in the process of embryogenesis. Characterizing some of the developmental patterns occurring during embryogenesis in red pine (*Pinus resinosa* Ait.) is the focus of my doctoral research at the University of Maine. Identifying some of the physical and environmental influences on embryo development is the major emphasis of this research project.

Conifers are of significant economic and taxonomic importance. The economic importance of conifers in the northeast lies in their use for timber and paper products. As a major taxonomic group, conifers are unique from angiosperms in many morphological characters, including their reproduction. Within the conifers, many pines have distinct reproductive cycles, often spanning up to three years. In red pine, the cones require two complete growing seasons to mature. Fertilization of the egg cell occurs

in the early spring, approximately one year after pollination, marking the beginning of embryogenesis. Pollination, fertilization and subsequent embryo development have been described for many species of *Pinus*, including red pine, but the development triggers for embryogenesis remain a mystery.

The seed used in my research was collected from the U.S. Forest Service seed orchard located in Greenbush, Maine. Collections begin in April when fertilization occurs. The seeds are collected and stored in a biological fixative that kills the material while preserving cellular integrity. The preserved material is dehydrated with an alcohol series and then infiltrated with paraffin. The sample is then sectioned on a microtome and mounted on microscope slides. Using biological stains that adhere to specific cellular substances we can look at the cytochemistry of the seed. The sectioned material is also used to determine the timing of embryo development (Figure 2).

By June the single-celled egg, or zygote, is fully formed. Embryogenesis begins in early July. The initial stage of embryogenesis is marked by the appearance of tiers in the zygote. Two of these tiers elongate to form the suspensor cells, which push the embryonic tier into the female gametophyte. The cells in the center of the female gametophyte break down to form the corrosion cavity in which the embryo will mature. The cells of the embryo continue to divide and elongate until the embryo becomes torpedo-shaped. Following the torpedo stage, cotyledons begin to form at the apex of the growing embryo. The cotyledons are the embryonic needles; they continue to grow until the embryo fills the corrosion cavity. At this stage the embryo is mature.

During embryogenesis, the female gametophyte is going through observable changes. Before fertilization, the seed enlarges and the female gametophyte initiates as a watery, translucent gel. As the embryo matures, the female gametophyte accumulates starch, fats and proteins. By late July the gametophyte surrounding the mature embryo is firm, white and opaque. How the timing of the development of the female gametophyte influences embryogenesis is poorly understood.

Because the interaction between the environment created by the surrounding gametophyte and the genetic constitution of the embryo appears to determine normal embryogenesis, describing the environment of the developing

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**NAME:** Judy C. Gates

**DEGREE SEEKING:** Ph.D. in Forestry, Forest Biology Department

**FORM OF SUPPORT:** McIntyre-Stennis research assistantship

**BACKGROUND EDUCATION:** B.S. in Agriculture, West Virginia University  
M.S. in Plant & Soil Sciences, University of Maine

embryo becomes crucial to understanding the process of embryogenesis in conifers. If the zygote contains all of the genetic information necessary to generate a seedline then embryogenesis must occur in response to an environmental cue, such as water, oxygen, carbon dioxide, or light. These varying environmental influences might provide a trigger for normal embryogenesis. While there have been some descriptive studies of developing pine embryos, the microenvironment of the embryo within the gametophytic tissue has not been characterized. One part of my research focuses on identifying some of the unique physical characteristics of the embryo's microenvironment within the female gametophyte.

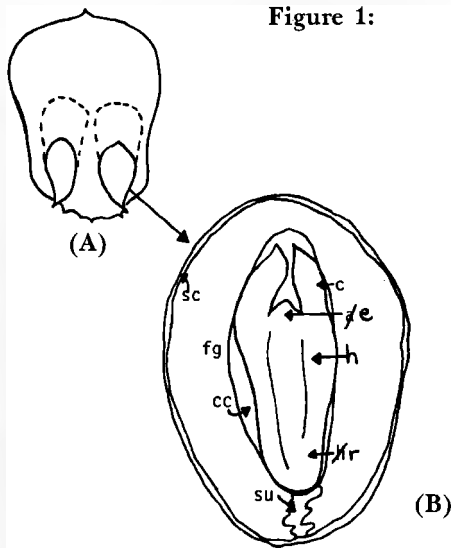
development if the impermeable seed coat is removed as in tissue culture experiments? These are two of the complex questions I hope to address in the course of this research project. Observing embryo development under conditions of enhanced or reduced oxygen might further our understanding of environmental cues for embryogenesis.

To begin to assess the impact of changes in water content during embryo development, we began weekly measurements of the changing water content of whole seeds, or ovules, and excised female gametophytes. These observations indicate that the water content of both the gametophytic tissue and the whole ovule decline during embryogenesis (Figure 3). The drying of the seed coat may contribute to the decreasing water content of whole ovules. The decreasing water content of the female gametophyte is probably a result of the cellular water being replaced by accumulating solids. This theory is supported by the increase in gametophyte dry weight as soluble carbohydrates, fats, and proteins accumulate. The gradual change in the composition of the gametophyte may buffer the embryo from drastic shifts in its microenvironment.

The changing water and nutrient content within the ovule might be affecting the osmotic potential within the seed. Osmotic potentials determine the movement of solutes across cell membranes, or in this instance, between the gametophyte and the developing embryo. Movement of solutes to and from the embryo could be related to the timing of embryogenesis. Nutrients required as building blocks during different stages of embryogenesis may move in and out of the embryo according to the ability of a specific nutrient to cross membranes under certain osmotic conditions. Osmotic potentials during embryogenesis have not been measured for conifers. Estimating or measuring the osmotic changes during embryo development could be an important component in characterizing the physical microenvironment of the red pine embryo. A changing osmotic environment within the female gametophyte during embryogenesis has important implications for manipulating embryo development in tissue culture. If we can vary the *in vitro*, or artificial, osmotic environment with the changes that occur *in vivo*, or in the plant, then we should be able to induce embryos to mature from zygotes in the laboratory.

In tissue culture, sucrose or a similar sugar is used to vary the osmolarity of the tissue culture medium and as a source of carbon, which is ultimately converted to energy for plant development. Mature embryos of some herbaceous plant species will continue to develop *in vitro* even in the absence of sucrose, while precotyledonary embryos require progressively greater concentrations of sucrose in the medium. Conifer embryos younger than the torpedo stage, when cotyledons begin to appear, have never matured *in vitro*. Young embryos may require a different *in vitro* environment than is provided by known methods. The

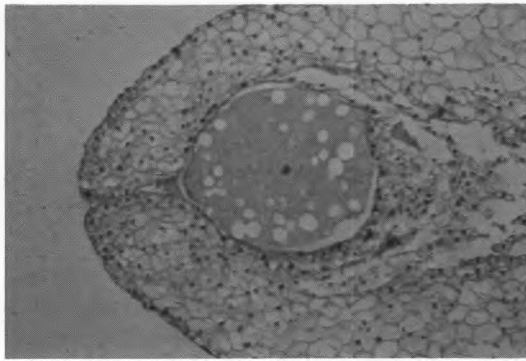
Figure 1:



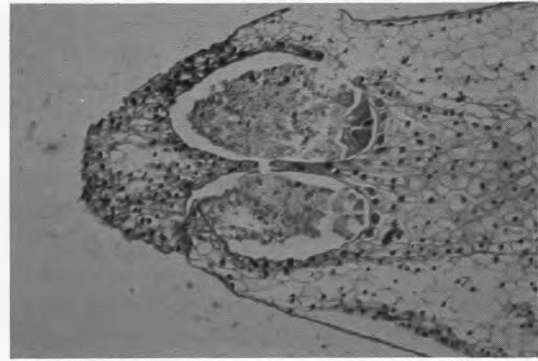
A - An ovuliferous cone scale (red pine)

B - A mature red pine seed: sc = seed coat, fg = female gametophyte, cc = corrosion cavity, su = suspensor cells, c = cotyledons, e = epicotyl, r = radical, h = hypocotyl.

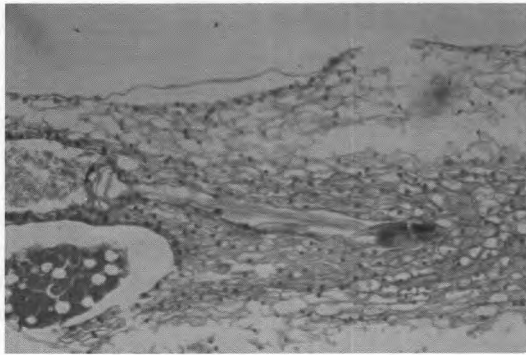
The amounts of water and oxygen could be constantly changing as the gametophyte and seed coat develop. During embryogenesis, the seed coat hardens and becomes less permeable to water and gases, including oxygen. The hardening of the seed coat might result in a decreasing amount of oxygen moving into the female gametophyte as the embryo matures. This has important consequences for the developmental metabolism of the embryo. Oxygen is involved in the metabolic processes of glycolysis and oxidative phosphorylation within the seed; these cycles produce ATP using the starches and fats stored in the female gametophyte. The ATP is ultimately used for energy needed for growth and development. As the seed matures, its metabolism begins to slow in preparation for dormancy, a resting phase. What are the effects of the changing oxygen relations on embryogenesis? What happens to embryo



a = zygote in female gametophyte



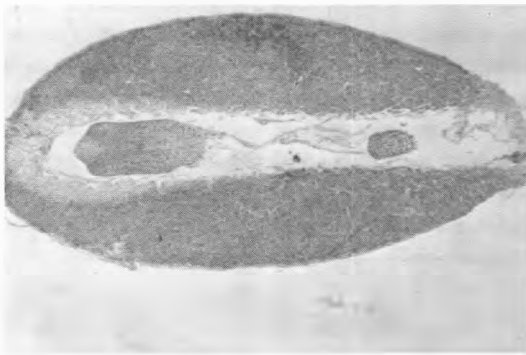
b = formation of tiers in zygote



c = suspensor cells elongate and move 4-celled embryo into female gametophyte  
Phases a through c usually occur during the first week of July.



d = many globular to torpedo stage embryos (polyembryony)



e = initiation of cotyledons



f = mature embryo, second week of August

Figure 2: Embryogenesis in Red Pine (*Pinus resinosa* Ait.)

possibility that a relationship may exist between normal red pine embryogenesis and *in vitro* sucrose concentrations formed the basis for one of the tissue culture studies completed for my project.

A basic tissue culture medium contains macronutrients and micronutrients thought to be required for growth plus essential vitamins, sucrose, agar for gelling, and sometimes plant growth regulators. A tissue culture medium developed by Murashige and Skoog in 1962 has been very successful in many areas of plant tissue culture, including studies with woody plants. To look for a relationship between *in vitro* embryogenesis and sucrose concentration we varied the sucrose concentration from 0-12 percent in a basic Murashige and Skoog tissue culture medium, hoping to find a level of sucrose suitable for young embryo development. Gametophytes containing embryos at different developmental stages were cultured on the medium and maintained at constant light and temperature under sterile conditions for six weeks. We hoped that the female gametophyte would protect and nourish the embryo by retaining some of its proposed buffering capacity.

None of the embryos in this study appeared to mature in culture. It was common to find no evidence of a zygote or embryo in gametophytes cultured on media with greater than 6 percent sucrose. Many of the gametophytes turned brown and soft. The cells within the gametophytic tissue appeared very disorganized, and starch and proteins did not accumulate normally at greater sucrose concentrations. Although embryos did not mature at lower sucrose concentrations, the female gametophytes retained cellular integrity. The gametophytes cultured on media with 3 to 6 percent sucrose also grew to almost normal sizes by the end of the study. This growth can probably be accounted for by water uptake from the media because the gametophytes did not accumulate the starches, proteins, and other cellular substances. We concluded that when culturing embryos in the gametophytic tissue, 3 to 5 percent sucrose provided the most favorable environment for gametophyte integrity. We also questioned whether there was a medium more suitable than the Murashige and Skoog medium for inducing normal red pine embryo development.

The next major tissue culture study, in July 1986, compared three different tissue culture media thought to induce *in vitro* development: Murashige and Skoog (1962) medium, developed primarily for tobacco culture, Litvay's (1981) medium, developed for conifer culture, and Von Arnold and Ericsson's (1981) medium, also a conifer medium. These three media differed primarily in the amounts of some macro- and micronutrients. All contained 3 percent sucrose, identical vitamin concentrations, and no growth regulators. On the assumption that the browning seen on the gametophytes in the first study was a result of toxin accumulation (*i.e.*, tannins or phenolic compounds) we tried the three media with and without adding activated char-

coal (AC). Activated charcoal absorbs some of the toxic substances that can occur in culture. Gametophytes were cultured at all stages of embryogenesis, from zygote to mature embryo.

The gametophytes cultured on the media with activated charcoal retained cellular integrity and did not turn brown. The youngest embryos did not degenerate, but development was incomplete. Embryos remained at the developmental stage that they were in at the time of culture. Some of the more mature embryos, in which cotyledons were already beginning to differentiate, continued to develop in culture. The primary shoot axis elongated and in a few cases the embryonic cotyledons turned green (Figure 4). A few of

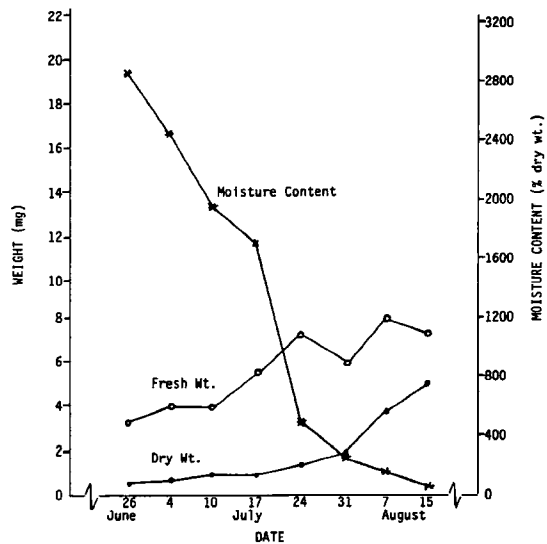


Figure 3a Changes in fresh weight, dry weight, and percentage of moisture of developing female gametophytes of red pine during embryogenesis (1986).

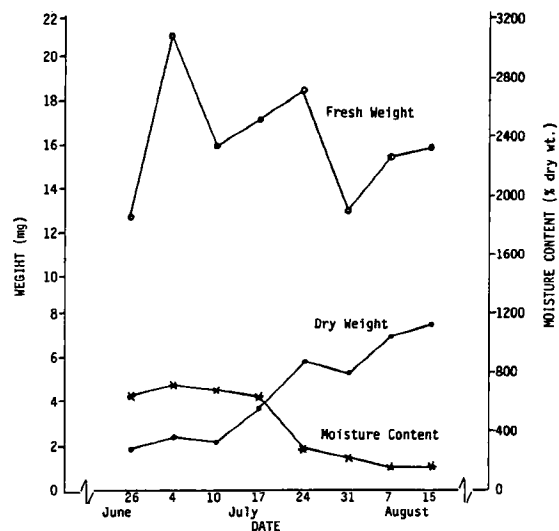


Figure 3b Changes in fresh weight, dry weight and percentage of moisture of developing red pine seeds during embryogenesis (1986).

the embryos developed callus tissue. Callus is a mass of unorganized parenchyma cells that can form on cultured tissue. The growth of callus tissue on a medium without growth regulators is not common. A specific combination of auxins and cytokinins, two types of growth regulators, is usually required to initiate callus growth. The development noted in this study occurred primarily on the Von Arnold and Ericsson (1981) medium with AC. Based on the results of this study, we are now using this medium for our tissue culture studies on red pine embryogenesis.

A similar medium, labeled HM2/1, is currently being used for studies on somatic embryogenesis in conifers being conducted at the Institute of Paper Chemistry, Appleton, Wisc. *Somatic embryogenesis is the process by which haploid or diploid somatic cells develop into differentiated plants through characteristic embryological stages without fusion of gametes.* A somatic cell is a cell that forms the basis of a plant tissue. Somatic embryogenesis can occur naturally in many species or be induced through tissue cultures as a method of regeneration. Regeneration can be indirect, through callus or protoplasts, or direct from cells of an organized structure, such as stem segments of zygotic embryos.

Direct somatic embryogenesis from a zygotic, or seed-derived, embryo occurs from cells that are already determined for embryonic development before culturing; they require favorable conditions to induce cell division and embryogenesis. Direct somatic embryogenesis has not been reported in conifer species. Indirect somatic embryogenesis requires redetermination of differentiated cells and callus growth followed by the development of embryogenically-determined cells. In somatic embryogenesis via callus, a zygotic embryo is placed on a defined medium and gives rise to a compact clump of *embryogenic* cells (Figure 5). This callus clump has the potential to produce many somatic embryos, which *germinate* like zygotic embryos, developing green cotyledons, a hypocotyl, and a primary root.

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**This research project is giving me the opportunity to continue learning about plant systems. The challenge of solving new problems is always present in developmental plant physiology and it's a good motivator for learning. My primary interest is in basic rather than applied science or how trees grow rather than how to grow trees. Dr. Greenwood has given me the opportunity to apply this interest to experimental embryology in conifers.**

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Indirect somatic embryogenesis from callus is currently being pursued as a possible method of mass regeneration for some conifer species, including Douglas-fir, loblolly pine, and Norway spruce. Embryogenic callus has the potential to produce many somatic embryos identical to the originally cultured embryo. Callus can be maintained and

subcultured for an extended time without reducing its reproductive capacity. We have successfully induced somatic embryogenesis of Norway spruce in our lab using callus provided by the Institute of Paper Chemistry. This callus was originally induced from torpedo-staged zygotic embryos using the following protocol:

- 1) The embryo is cultured on a basic HM2/1 medium containing 2, 4-D, an auxin, and benzyladenine(BA), a cytokinin. This medium induces callus growth.
- 2) The callus is transferred to a medium containing activated charcoal, but no growth regulators. This medium induces the formation of somatic embryos from the callus.
- 3) Callus is transferred to a second embryogenic medium without activated charcoal after one week. This medium contains another auxin, indole acetic acid, and abscisic acid. On this medium the embryoids develop and germinate.
- 4) The germinating embryoids are transferred to a quarter-strength HM2/1 medium without growth regulators to develop into plantlets.

This protocol for somatic embryogenesis has not been applied to red pine. Beginning this spring, we will try to induce embryogenic callus on red pine embryos following this procedure.

The success of somatic embryogenesis raises many questions. While it appears to provide a method of rapid clonal propagation, it has not been shown that the genome of the parent remains unaltered in somatic embryos. There is the potential for genetic rearrangement in the rapidly proliferating callus cells, resulting in offspring that differ from the parent. The conifer plantlets that have been generated through somatic embryogenesis are not old enough to predict their resemblance to the parent in genetic or morphologic features. Why somatic embryogenesis occurs in response to specific media components, such as growth regulators, is a question for development plant biologists. A formerly undifferentiated parenchyma cell in embryogenic callus will become determined and form a complete embryo *in vitro*, but a predetermined zygote will not continue along its developmental pathway in the same environment. If a zygote could be isolated before it divides, genetic engineering could be used to modify its genome. Genes could be inserted or regulated to produce a plantlet with disease resistance or herbicide resistance. The implementation of genetic engineering in conifers depends on greatly expanding the sparse knowledge that exists on the structure and function of plant genes. Information on conifer gene arrangement will bring genetic engineering of conifer zygotes closer to reality.

Genetic engineering and mass propagation through *in vitro* embryogenesis might also overcome some of the difficulties encountered in traditional clonal propagation of

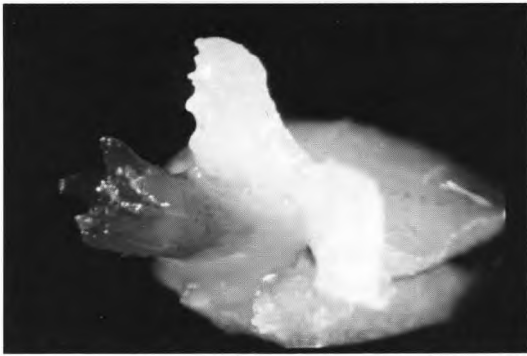


Figure 4: Mature red pine embryo emerging from a female gametophyte in vitro.

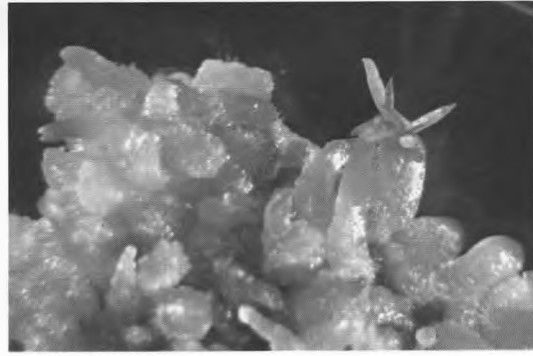


Figure 5: Embryogenesis callus of Norway spruce undergoing somatic embryogenesis.

conifers. In clonal propagation, cuttings are taken from mature trees that show desirable growth characteristics. Unfortunately these cuttings usually continue to grow slowly and often horizontally, still resembling branches. Seedlings grow upright, but because only the maternal parent is usually known, growth can vary among seedlings. If we were able to propagate conifers through direct *in vitro* embryogenesis, the embryos could be genetically identical, but retain the faster, upright growth of seedlings.

Experimental embryogenesis has applications in both applied and basic science. Applied to forest tree propagation, it becomes a tool for rapid multiplication of selected genomes. In basic scientific research, it provides an opportunity to observe development and maturation from a fundamental level. But before these goals can be realized, research must focus on identifying developmental controls over normal *in vivo* embryogenesis and the potential for using these cues to induce successful *in vitro* embryogenesis.



# THE V-NOTCHED LOBSTER IN MAINE

by Cheryl Waltz

Lobsters are the highest value fishery in Maine. Since 1950, an average of 20 million pounds of lobster, with an approximate value of \$50 million, has been landed annually. There are nearly 8000 licensed lobstermen in Maine, fishing an estimated 2 million traps (Krouse, 1987).

For decades, lobster biologists have predicted a crash in the lobster fishery due to overfishing. Currently there are four conservation measures enforced in Maine, designed to prevent stock depletion.

Since 1874 Maine has had a minimum size restriction for lobsters, below which they are illegal to land. The current minimum legal size is 3-3/16 inches (81mm) carapace length (CL). Carapace length is measured from the eye socket to the end of the carapace. There is also a maximum legal size of 5 inches (127mm) CL. These size restrictions are designed to protect both juvenile lobsters until they are capable of reproducing and larger lobsters that are capable of carrying a great number of eggs.

Another effort to protect juvenile lobsters is the escape-law. All lobster traps fished in Maine must be fitted with a vent large enough to allow sublegal lobsters to escape. This cuts down on the handling, and therefore injury, of juveniles, and avoids the high predator mortality on lobsters that are thrown back.

Since 1903 Maine has had a V-notching program aimed at protecting the known reproducing females in the population. When a Maine lobsterman catches an egged lobster in his trap, (egged lobsters cannot be landed anywhere in the U.S.), he will voluntarily notch the right uropod (tail flipper) and return the lobster to the sea. Once a lobster has been notched, it can no longer be legally landed in Maine.

One of the concerns voiced by lobster biologists is that the minimum size restriction is ineffective as it now stands. At minimum legal size (which is the same in all New England states) less than ten percent of female lobsters are sexually mature. For this reason managers in most states

have advocated raising minimum legal size; most Maine lobstermen are opposed to this. The maximum size restriction, while supported by most inshore lobstermen, is generally considered by biologists to have minimal impact on the fishery, due to the small number of lobsters that reach 5 inches carapace length.

Maine is currently the only state with a V-notch law, a program strongly supported by Maine lobstermen. For years they have unsuccessfully attempted to encourage other states to adopt this law, for once a V-notched lobster migrates beyond the Maine border, it is no longer protected and can be landed in any lobstering state. Until recently, however, there has been no scientific data to support the fishermen's conviction that the V-notch program increases the number of eggs produced by the lobster population.

In 1986 the Maine Legislature passed a bill to increase minimum legal size, provided that other states agree to honor the V-notch. The New England Regional Fisheries Management Council is now considering a plan to increase minimum legal size and introduce the V-notch law in all lobstering states. The existence of scientific evidence to support the V-notch program would greatly increase the chance that this plan will be agreed upon by the Council.

The Maine Lobstermen's Association and the University of Maine have been working together to investigate the impact of V-notched lobsters on the lobster fishery. Since 1982 a survey has been conducted to determine the abundance of V-notched lobsters in the population. Lobstermen reported numbers of V-notched and egged lobsters in their trap catches during two days each October. The proportion of V-notched lobsters to landed lobsters in the catch was used to calculate the number of V-notched lobsters in the population. This was then used to estimate the number of eggs contributed to the fishery by V-notched lobsters.

Two assumptions were made in this survey: that no lobsters were recaptured, and that the size distribution of V-notched lobsters was similar to that of egged lobsters. The first assumption is important because any recaptures would cause an overestimate of the size of the V-notched population; the second assumption is important because size af-

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Married, 26, from Damariscotta, ME

Thesis will be lobster population model to evaluate  
management techniques

Other work includes study in burrowing behavior in dif-  
ferent sediments

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*Cheryl's research on the significance of V-notch lobsters is part of a larger effort to determine the factors that contribute to lobster egg production and future abundance. The overall goal of the research is to maximize the stable yield of lobsters. Participants in these studies include Sea-Grant, the newly formed Maine Lobster Institute, the Maine Agricultural Experiment Station and the Maine Lobstermen's Association.*

**Table 1:** V-Notched and Egged Lobster Sizes Over the Fishing Season

<i>V-Notched Lobsters</i>	<i>June</i>	<i>August</i>	<i>October</i>	<i>Overall</i>
Mean CL*	95.80	94.42	95.26	95.10
with no eggs	94.23	92.78	93.48	93.40
with eggs	104.02	101.61	103.70	103.11
-% total V-notch	16.1	18.6	17.4	17.5
% recaptured				11.66
 <i>Egged Lobsters</i>				
Mean CL	104.57	101.69	105.64	104.38
non-V-notched	106.18	101.95	109.51	107.41
V-notched	104.02	101.61	103.70	103.11
-% total egged	74.5	93.4	66.6	70.5
% recaptured				9.24

\* all measurements are in millimeters

fects the number of eggs that a lobster can carry. As the size of a female increases, the number of eggs she will carry increases logarithmically. This is in part due to the increase in width of the abdomen, where a female lobster cements her eggs.

Last summer the Maine Lobstermen's Association and the University of Maine conducted a study to test these two assumptions and to examine the size distribution of V-notched and egged lobsters along the Maine coast.

Fifteen lobstermen in four areas along the Maine coast participated in the study. Area 1, the Damariscotta area, was the southernmost area in the survey. Most fishing was carried out at the mouth of the Damariscotta River, a very productive warm water estuary. Area 2, the South Thomaston area, was primarily fished within the harbor. In area 3, the Southwest Harbor area, most fishing occurred nearby offshore islands, as in the northernmost area, area 4, near Jonesport.

Each lobsterman recorded the carapace length, in millimeters, of lobsters in three categories in their catch: V-notched lobsters without eggs; V-notched lobsters with eggs; and non-V-notched lobsters with eggs. These were the same categories used in the October surveys. Each lobster that was measured was marked with a blue band on the claw or knuckle and returned to the sea. This mark indicated that the lobster had already been measured, and any lobsters caught with a mark were recorded as recaptures in their categories. Lobstermen also recorded the number of pounds landed, the number of traps fished, and the location that was fished.

Data were recorded over three periods of ten consecutive fishing days in early June, in late July and early August, and in late September and early October. These times roughly coincide with premoult and postmoult periods, and the period of greatest landings.

On thirteen days during the study, researchers accompanied fishermen and collected the data requested, in an effort to confirm the accuracy of the data recorded by the fishermen.

Upon analysis, it was discovered that the most important contributor to variation in size was category; *i.e.*, V-notched lobsters without eggs, V-notched lobsters with eggs, and non-V-notched lobsters with eggs. Sizes of V-notched lobsters with eggs were not different from non-V-notched lobsters with eggs, but were different from V-notched lobsters without eggs. The average size of V-notched lobsters was significantly smaller than the average size of egged lobsters, as shown in Table 1.

Area also had a significant effect on the size distribution of V-notched lobsters. In the southern areas 1 and 2, V-notched and egged lobsters were significantly smaller than in northern areas 3 and 4 (Table 2). The sizes of lobsters caught in area 3 were significantly different than those caught in area 4.

Season had an effect on lobster size as well. During the postmoult period, late July and early August, the sizes of measured lobsters were significantly smaller than in either the premoult period or the period of highest landings.

There was no significant difference between sizes measured by lobstermen and by researchers.



**Table 2 : Summary of Egged and V-Notched Lobster Sizes Along the Maine Coast**

<i>Category</i>	<i>Area</i>			
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>
V-notched	94.30	94.36	97.77	97.59
with eggs	101.85	96.56	107.42	109.78
with no eggs	93.07	93.52	94.84	94.04
Egged	101.53	96.87	107.33	112.78
with V-notch	101.85	96.56	107.42	109.78
without V-notch	99.69	97.49	107.08	115.52

All sizes presented are mean CL in millimeters

**Table 3: Proportion of Trap Catch that is V-Notched or Egged**

	<i>October 1982-1984*</i>	<i>1986</i>
V-notch with no eggs <sup>1</sup>	23.8	16.0
V-notch with eggs <sup>1</sup>	5.2	3.4
Egged with no V-notch <sup>1</sup>	2.4	1.4
Landed females <sup>1</sup>	69.3	79.2
Total trapped females <sup>2</sup>	58.7	55.8
Total V-notched lobsters <sup>1</sup>	29.0	19.4
Total egged lobsters <sup>1</sup>	8.1	4.8
Egged V-notched lobsters <sup>3</sup>	68.0	70.5

\*Mean % - from Daniels *et al*, 1985

1. as % of total trapped females
2. as % of total trapped lobsters
3. as % of trapped egged lobsters

Of all V-notched lobsters captured in the study, 17.5 percent carried eggs. However, 70.5 percent of all egged lobsters were V-notched.

Over the summer, 11.66 percent of all marked V-notched lobsters were recaptured, while 9.24 percent of marked egged lobsters were recaptured. Non-V-notched lobsters with eggs were recaptured less frequently than were V-notched lobsters with eggs.

During the survey, participating lobstermen landed 60,590 pounds of lobsters in 74,400 traps, averaging 0.9

pounds per trap. In area 3 (Southwest Harbor) lobstermen averaged only 0.56 pounds per trap, while in other areas lobstermen averaged nearly one pound per trap.

Traditionally, data collected by fishermen have been viewed with skepticism by the scientific world. Our analysis showed that the data recorded by fishermen were no different than that recorded by researchers. These results help add validity to the experiments conducted by fishermen, and will enhance cooperation between fishermen and scientists. Fishermen are a valuable resource to scientists and can contribute data which would otherwise be unavailable to researchers.

The first objective of this study was to test the two assumptions made in the October V-notch surveys. Recapture rates in this study were determined only for the entire season, but do indicate that recaptures during the October survey were a possibility.

Table 3 represents comparison of the results of this study with the results of the October MLA/UM V-notch surveys. Consistently, the percent of total trapped females that are V-notched or egged is lower in the present study than in the V-notch surveys. This may be partly due to the presence of recaptures in the data of the V-notch surveys. However, it is unlikely that recaptures alone are responsible for this difference. Another possible explanation might be the difference in study techniques. The V-notch survey is conducted only over a two-day period, while the results of this study represent a seasonal average. Whatever the reason, the higher proportion of V-notched and egged lobsters in the October surveys would cause an overestimation of the total V-notched population. Notice, however, that the percent of egged lobsters that are V-notched is similar in both studies, indicating that the ratio of V-notched to egged lobsters is consistent.

Analysis showed that the size distribution of V-notched lobsters is definitely different from the size distribution of egged lobsters. This refutes the second assumption made in the October V-notch surveys.

While the average size of V-notched lobsters, 95.10 mm CL, is significantly smaller than that of egged lobsters, it is still larger than the average size of landed lobsters, 89.1 mm CL (Thomas *et al*, 1983). This shows that V-notched lobsters can contribute more eggs per lobster than can the legal lobsters before they are landed. In addition, the V-notched lobsters, due to Maine's protection, will be able to reproduce many times, while most legal lobsters will be able to reproduce only once or twice before being landed.

From Table 3 we know that 70.5 percent of egged lobsters are V-notched. Obviously the V-notched lobsters are contributing a significantly greater proportion of the eggs produced than are non-V-notched lobsters. Because the number of eggs a lobster will carry increases logarithmically with size, the results of this study indicate that the V-notched lobster contribution to egg production is much more significant than previously predicated on the basis of numbers of V-notched lobsters alone.

The second objective of this study was to examine the size distribution of V-notched and egged lobsters along the Maine coast. As discussed earlier, the lobsters caught in areas 1 and 2 were significantly smaller than those caught in areas 3 and 4. One explanation for this is that fishing pressure is higher along the southern Maine coast. Because of this, more lobsters would be caught at minimum legal size, and fewer would grow to any great size. This might also account for the difference between areas 3 and 4; few lobstermen work as far north as Jonesport and Lubec.

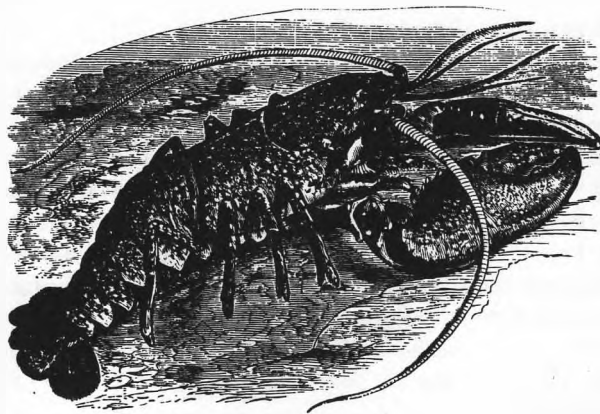
Another explanation for this area difference might be difference in habitat. In the southern areas, fishing mainly occurred near shore, where water would be warmer. The

northern areas were mainly fished near offshore islands, where the water temperature would be lower and the bottom substrate could be different.

The combination of higher fishing pressure and lower water temperature in the Southwest Harbor area (area 3) likely produced the lower average of pounds landed per trap fished.

The results of this study indicate that V-notched lobsters contribute significantly to total egg production in Maine. While the numbers and size of V-notched lobsters are lower than estimated by the Maine Lobstermen's Association/University of Maine V-notch surveys, the high percentage of egged lobsters that are V-notched and the increased size of V-notched lobsters over landed lobsters, show the V-notched lobsters' egg contribution to be considerable. This information will be valuable input to the decision of the New England Regional Fisheries Management Council.

In recent years, the trend in management of exploited species has been to construct a model of the energy flow within the population and to use a computer to simulate the population cycling in order to evaluate the status of the exploited population under present and proposed management techniques. Several models have been created for the Maine lobster population, but due to the lack of data, the V-notched lobsters have been conspicuously missing from these models. The results of this study have shown that V-notched lobsters make up a significant proportion of the total population and greatly contribute to the total number of eggs produced by the Maine lobster population. The ultimate goal of this project is to construct a model of the lobster population, based on the results of this study, which will include V-notched lobsters and will allow a more accurate evaluation of present and future management techniques.



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# Undernutrition in a Pediatric Population

by Paula Quatromoni

A major focus of nutrition education today is concerned with increasing the public's awareness of the clearly established links between the foods we eat and our subsequent health status. For this reason, numerous health promotion campaigns have been launched to help individuals realize that too much of a good thing can be detrimental to one's well-being. With much of the educational efforts being turned towards avoiding nutritional excesses in order to reduce the morbidity associated with obesity, diabetes, hypertension, and coronary artery disease, it is easy to overlook the fact that some Americans are experiencing a different kind of malnutrition - *undernutrition*.

The picture of undernutrition in the United States may be difficult for many Americans to visualize since this concept of lack of available, wholesome food is oftentimes associated with starving and diseased children struggling for survival in underdeveloped third-world countries. Although undernutrition in the United States is not typically manifested by such severe clinical evidence as is depicted in countries such as Ethiopia, it nevertheless does exist. Usually, specific nutrient deficiencies among our populations are not visually observable. Thus, they may remain undetected for a period of time, allowing a sub-clinical deficiency to progress and the overall clinical picture to worsen.

Socioeconomic status is one of the most significant environmental factors known to have an impact on the nutritional status of populations. The socioeconomic level of an individual or family may influence the type and amount of food available, the health status of individual family members, the quality of medical care available, the adequacy of housing facilities, and the availability of numerous additional resources that enable individuals to purchase, plan, prepare, and provide well-balanced, nutritious meals on a regular basis. It is generally the case that low-income persons have a less varied dietary pattern as compared to upper-income persons. Lack of variety in the diet may be associated with an increased risk of nutritional inadequacy since a well-balanced nutritious diet is one which includes many different foods from each of the various food groups. With an increasing rate of poverty, unemployment, and homelessness in parts of the United States, it is appropriate to wonder how nutritional status and overall health are being affected.

One group of particular concern to nutritionists and public health professionals is the pediatric population liv-

ing below the defined poverty level. High infant and childhood mortality rates for a developed nation are causing health providers to examine more closely the wide range of risk factors associated with chronic diseases and nutritional inadequacies among this population. Health risks such as malnutrition may inhibit the child's growth performance and restrict the child's ability to reach his/her growth potential. Growth velocity is one functional aspect of development which may be retarded as a result of chronic undernutrition secondary to a low socioeconomic status. Equally important, however, is the reduced intellectual development which often times accompanies the malnourished state. Various studies have documented evidence of growth retardation and impaired intellectual capacity among children from low-income families.

What can be done to help individuals from economically depressed families provide enough high quality foods to promote good health, proper growth, and mental development among infants and children? In the United States, public health programs incorporate an emphasis on improving the health status of pregnant women and their children through nutritional supplementation and education. The largest and most specifically targeted public health nutrition program in the country today is the Special Supplemental Foods Program for Women, Infants, and Children (WIC). Since its enactment in 1972, the WIC Program, which is administered by the U.S. Department of Agriculture, Food and Nutrition Service, has provided cash grants to state health departments and approved local health agencies for the specific purpose of providing nutrient-dense food supplements and nutrition education to low-income, high-risk pregnant and lactating women, infants, and children up to five years of age. The program is designed to serve as an extension of good health care during critical times of growth and development. Its goal is to optimize the health, growth, and development of an individual from conception through childhood. By providing appropriate foods or coupons which enable families to purchase nutritious foods, the WIC Program strives to improve the health status of persons identified as being *nutritionally or medically at risk*. Examples of specific risk conditions used as a basis for determining WIC eligibility include anemia, underweight, overweight, low birth weight, growth stunting, failure-to-thrive, and inadequate dietary intake patterns.

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# Paula Quatromoni

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- Member, Phi Kappa Phi Honor Society
- Member, Society of the Sigma Xi
- Graduate Research Assistantship appointment in the School of  
Human Development under Dr. Richard A. Cook

## PRESENTATIONS (related to this research project only):

- May, 1986 Assessment of Nutrient Intake Levels Among A Pediatric WIC Population in Maine. Presented at the Twelfth Annual Maine Biological and Medical Sciences Symposium in Portland, Maine
- June, 1986 Assessment of Nutrient Intake Levels Among A Pediatric WIC Population in Androscoggin County. Presented at the summer meeting of Maine WIC Nutritionists in Augusta, Maine
- July 1986 Food Consumption Patterns of a Pediatric WIC Population Identified As Being Nutritionally At Risk. Presented at the Nineteenth Annual Meeting of the Society for Nutrition Education in Washington, D.C.

## PUBLICATIONS: (related to this research project only):

- Quatromoni, P.A., Cook, R.A., Parvanta, I., and Halteman, W.A.: Growth deficit among low-income preschool children in Maine. *Nutrition Reports International*, March, 1987 (in press).

## INTERVIEW:

- Interviewed by WNBC Nightly News (New York) for a news segment on malnutrition and poverty in Maine.

## On a personal note

- \*Currently enrolled in a dietetic internship program at Beth Israel Hospital in Boston, Massachusetts
- \*Serving as a consultant for the follow-up research project on growth retardation among Maine WIC children
- \*Plans to pursue a doctoral program in public health nutrition to continue to build a strong background for education and research endeavors

Since poor growth may be the first clinical sign of malnutrition in the child, state nutrition surveillance systems have been essential to enable ongoing data collection (including heights and weights of children) to assess the prevalence of growth deficit and underweight among pediatric populations. The Maine WIC Program has participated in a National Pediatric Nutrition Surveillance System conducted by the Centers for Disease Control in Atlanta, Georgia, since 1979. Public health nutritionists in Maine were alerted to the high prevalence of short stature (height-for-age below the fifth percentile, according to growth standards prepared by the National Center for Health Statistics) among Maine WIC children by the findings of surveillance data collected in the early 1980's. On the average, the incidence of growth retardation was 200 percent higher among Maine WIC children (10.8 percent incidence) than the expected rate in a national reference population (5.0 percent incidence). The presence of short stature in Maine ranged from a high of 22.9 percent of WIC children in Androscoggin County to a low of 6.3 percent in Aroostook County (Table 1). Although the surveillance system was able to document the existence of growth problems among low-income children in Maine, the underlying causes of growth retardation remained a mystery. Since the significant occurrence of short stature may be an indicator of long-term illness or chronic undernutrition within this population, intervention was warranted.

Public health nutritionists employed by the Maine Department of Human Services, Division of Maternal and Child Health were anxious to develop and initiate a statewide research protocol to investigate the high prevalence of growth retardation among Maine's WIC population. However, inadequacy of funds and lack of available research personnel prevented such widespread investigation. For this reason, the state public health nutritionist sought the assistance of nutrition researchers at the University of Maine to make a collaborative investigative effort to evaluate the identified public health problem. This important link between state and university researchers enabled the timely implementation of a pilot study to examine the potential role of malnutrition in the retardation of growth documented among economically disadvantaged preschool children in Maine. The scope of this initial pilot project was limited to Androscoggin County, the Maine county identified with the highest rate of short stature (22.9 percent), due to financial constraints. Funds were provided by the Maine Department of Human Services, Division of Maternal and Child Health, and the Maine Agricultural Experiment Station.

Parents of eighty growth-stunted Androscoggin County WIC children identified by a January, 1985, nutrition surveillance report were contacted in June, 1985, and asked to participate in the study. A cash incentive of \$15. was offered to encourage participation and successful completion

of the requirements of the study. Parents were assured that their decision to enroll in the project would not affect their WIC status. Thirty percent of the families who were eligible agreed to participate (n=24). A control population was established from a pool of more than 100 randomly selected normally developing WIC children (n=19).

Assessments of each child's general health status, growth, family history, and usual food intake pattern were incorporated into the experimental design. Two interview sessions were conducted at the home of each family participating in the study. The first interview consisted of measuring the child's height and weight. Information was gathered to examine the medical background of both the family and the child. Parents were required to keep a detailed record of all foods consumed by the child over a three-day study period in order to characterize the usual food intake pattern. The procedure for recording food intake was thoroughly explained to the parent. The second interview was scheduled following the completion of the diet records. At this time, a questionnaire was administered to collect additional data concerning the child's usual dietary habits and food preferences.

As the researcher responsible for data collection in the community setting, I was able to gain insight into the lifestyle and experiences of young mothers and their children living below the poverty level. One of the many challenges associated with this type of data collection was to establish an empathetic, sincere, and trusting relationship with the mother and child. It was hoped that this type of relationship would facilitate the interviews and encourage honesty and accuracy in reporting necessary information. For the most part, families were very receptive to my visits and did not seem to feel invaded by my presence in their homes. One mother felt so comfortable with my call that she invited me into their third-story apartment via her kitchen window leading from a fire escape! This was not an easy entrance to make carrying a physician's scale and measuring equipment. However, if this was how she had friends and neighbors enter her home, it must have been a sign of acceptance and lack of affectation.

The majority of parents were very interested in learning the results of their child's assessment following completion of the project. Most parents did not have difficulties accurately recording their child's food intake, although illiteracy was a problem for one family, resulting in incomplete food records. In this case, the parents were asked to recall what the child had eaten over the past three days and make a verbal report. Working closely with economically deprived families in the community setting enabled me to conceptualize the many factors that affect food choices and the ultimate adequacy of nutrition resources available to such individuals. For instance, one family recently reported running out of Food Stamps and having a few boxes of macaroni and cheese left to get them through the

week. One can begin to imagine the effects that this lack of food availability can have on the growth and development of the three young children in that household. Simple nutrition education and basic information about food purchasing, preparation, and meal planning would greatly benefit such families and help them to provide more nutritious foods despite their financial limitations. One other example that reinforces the need for basic nutrition education is the number of women who reported that their children were consuming *juice*, when in fact they were referring to Kool-aid or other fruit-like drinks containing a good deal of sugar and little nutrition.

Analysis of food consumption data revealed some general but distinct trends among both the growth stunted and the normally developing control children. Both population groups were found to be consuming adequate amounts of total calories. In most instances, the food selections supplied generous amounts of protein, vitamins, and minerals. However, extremely low intake levels of vitamin D, marginal to low levels of zinc, and somewhat low levels of iron were documented among both growth stunted and control children. Vitamin D, zinc, and iron are all critical to normal growth patterns. Thus, sustained, depressed intake levels of these key nutrients would be undesirable for young growing children. For this reason, it is important to focus nutrition education efforts on ways to increase childhood consumption of these nutrients. For example, vitamin D-fortified milk and dairy products should be encouraged, in addition to lean meats, fish, and poultry which are excellent sources of available dietary iron and zinc.

It was interesting to note that the most significant contributor of iron in the diets of WIC children surveyed was the grains food group. Thus, not only were the iron intakes of many of the children inadequate, but the majority of the total iron consumed was supplied by non-heme iron sources such as iron-fortified cereals which are made available by the WIC program. Because non-heme iron is less biologically available to the child than heme iron from animal products, this observation is important from a physiological perspective. Parents need to be informed of the importance of providing either a source of heme iron or a source of vitamin C (orange juice for example) with the non-heme iron food source in order to enhance absorption and bioavailability of iron from grains and cereals.

At the opposite end of the spectrum, both groups of Androscoggin County WIC children consumed higher than desirable amounts of sodium, total fat, and refined carbohydrate (sugar). It would appear prudent to investigate further the overconsumption of such nutrients which have been identified as potential health risks for older Americans. Food consumption patterns learned in early childhood are very difficult to change later in life when health problems such as obesity, hypertension, coronary artery disease, and diabetes may emerge. Undesirable dietary patterns such

as these should be the focus of nutrition intervention and parent education to reduce the risks of developing nutrition-related health problems later in life. The major supplier of sodium in the diets of WIC children was the grains group. The dairy group was responsible for providing the largest amounts of total fat and saturated fat in the diets of children surveyed, partially as a result of whole milk and cheese products made available through WIC.

While large percentages of children in both study groups had intakes of vitamin D, zinc, and iron that fell within the range considered to be inadequate (less than 60 percent of the Recommended Dietary Allowance), other

nutrients including vitamin A, vitamin C, calcium, folacin, vitamin B<sub>6</sub>, and magnesium were consumed by many WIC children at levels thought to be inappropriate for growing children. The fact that these children were from a disadvantaged socioeconomic background and had already been classified by WIC as being nutritionally at risk compounds the seriousness of these dietary insufficiencies. Overall, undernutrition appeared to be a problem for many of these children, regardless of their growth status and despite WIC intervention.

Parents were asked to rate the quality of the food supply available to their children during the past year. Availability of food was considered adequate in terms of quantity by 66-74 percent of the parents interviewed. Not enough food was reported by 16-34 percent of the parents, with the majority of reports coming from families of growth stunted children. It appeared that inadequate nutrient intakes of some children, as assessed by the diet intake data, were reflected in parental reports of an inability to provide adequate amounts of food or the right kinds of food to feed their children properly. In fact, growth retarded children whose parents reported poor food availability had significantly lower intakes of protein, iron, folacin, phosphorus, and various B-vitamins as compared to stunted children whose parents reported an adequate food supply (p 0.05).

Data collected from the health history questionnaire indicated that parents generally rated the health of their children as excellent or good. Very few reports of disabilities, handicaps, or chronic medical problems were documented among this population. A low prevalence of prematurity was found among control and short children (11 percent and 17 percent, respectively). Since the total population was voluntary, and not random, prematurity cannot be assumed to be of minimal importance among all WIC or all low socioeconomic children in terms of growth effects.

Birth weights and birth lengths of control and short children were not found to be significantly different. Thus, short children were not growth stunted due to a significantly depressed birth length as compared to normally developing control children. This finding may implicate the role of postnatal environmental factors, including socioeconomic level and nutritional status, in determining the pattern of growth among infants and children. However, the quality of the intrauterine environment must not be overlooked as an important component of WIC's prenatal education. The incidences of maternal cigarette smoking and pregnancy complications were significantly greater among mothers of growth stunted children, although birth weights and birth lengths were not significantly affected. Since growth deficit was found to be expressed post-natally, poor nutritional status could be a contributing factor to the stunting process occurring after birth.

Due to the nature of this experimental design, it was dif-

Table I. Maine WIC Program Nutrition Surveillance Data 1982 Annual Summary

Rank Order of Counties by Prevalence of Short Stature

Rank	County	Ht/Age below 5th percentile (Percentage)
1	Androscoggin	22.9
2	Waldo	16.1
3	Franklin	15.8
4	Sagadahoc	15.4
5	Oxford	13.3
6	Lincoln	13.1
7	Hancock	12.9
8	Cumberland	12.7
9	York	12.5
10	Kennebec	11.8
11	Somerset	10.6
12	Knox	8.6
13	Penobscot	8.1
14	Washington	7.0
15	Piscataquis	6.7
16	Aroostook	6.3
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	Maine State Rate	10.8
	Centers for Disease Control (1980)*	8.5
	U.S. Reference Population	5.0

\*White population only

difficult to assess directly the impact of poor nutrition on growth during the preschool years since all of the children surveyed were receiving the benefits of WIC intervention. As a result of WIC participation, low-income families were provided nutrient-dense foods to enhance the quantity and quality of the needy child's food intake to improve overall nutritional status. Therefore, although this research confirmed the existence of undesirable and inappropriate present food intake patterns, assessment of children already enrolled in WIC yielded little information which may have been used to differentiate between high-risk and control children on the basis of a history of dietary inadequacy. Poor nutrition and lack of food availability appeared to be generalized problems for many low-income children from both population groups examined. In addition, malnutrition is strongly suspected as a major environmental factor contributing to the inhibition of growth observed among

## **From the Advisor . . .**

My association with Paula Quatromoni has been a truly rewarding experience. Our mutual interest in human nutrition was first realized through her selection of me as an advisor for her undergraduate honors program. Through readings and research we established a close working relationship and Paula developed a strong interest in community nutrition research. It was soon apparent to me that Paula was an exceptional student academically, but she also expressed maturity and professionalism rarely found in an undergraduate which allowed her the opportunity to develop research skills early on. We found that we could both be equally demanding of each other on a daily basis to achieve ambitious goals.

Paula has great organizational skills and strong leadership capabilities. She is self-motivated and works rapidly, but won't compromise time at the expense of quality. These attributes allowed her to excel academically, be active in leadership roles in honorary societies, be eagerly sought after by the University for committee work, and to learn how to plan and carry out research and to report research results. Her efforts went beyond any other undergraduate student having worked for me.

Because of her enthusiasm, dependability and dedication, Paula was included in state-level discussions for an upcoming project of growth deficit among preschool children in Maine. She was also given responsibility for overseeing a Northeastern Regional research project involving portion estimations in the study of food consumption patterns. These experiences were extremely influential in stimulating her desire to continue training at the graduate level.

Paula prepared for graduate study before entering Graduate School through advanced coursework and research planning. She began her graduate career with her research project already planned out (and approved by her

this group.

A new research project is currently being initiated which will involve studying diet histories of WIC-eligible children without present or previous WIC involvement. An assessment of high-risk children prior to WIC intervention is necessary to elucidate further the interrelationship between nutritional status and the development of nutritional risk from birth. Future research efforts will continue to rely on the combined expertise of the University-based research team and the public health nutritionists employed by the state agency. Maintaining and strengthening research ties between the University of Maine and the Maine Department of Human Services is a vital component of the ongoing research protocol. The ultimate goal is to perpetuate effective intervention strategies in hopes of reducing the prevalence of nutritional risk among low-income children in Maine.

graduate committee) and with the experience of reporting her undergraduate research at state and national levels and through the submitting of her work for publication. Ironically, her research grew out of the state-level discussions she had been allowed to partake in as an undergraduate, and it was the beginning of a linkup with the state for community nutrition research, a goal long sought after by myself. Her advisor at the state level was a former graduate student of mine, M. Ibrahim Parvanta, currently Director of the Public Health Nutrition Unit, with whom I had maintained close ties concerning research needs in the state following his receiving his Master of Science degree in 1981.

Paula performed admirably in her Masters program with all the qualities expressed as an undergraduate to complete her degree at an accelerated pace. She gained experiences in teaching and research that were so rewarding that her future goals seem destined to include seeking a doctorate for future education and research endeavors.

Although Paula usually outproduced students around her, she was highly regarded by those students who, by association, became more motivated to do their own work. She was equally comfortable in a one-on-one situation or in a group situation with colleagues and became recognized by researchers within and without the state as a respected professional. Even after obtaining her degree and having left the state, she continues to keep close contact with me and to offer her expertise for follow-up research as an outgrowth of her graduate work.

My praise for Paula as a colleague and friend is the highest. She embodies the best of what one could experience in an educational institution. I have been most fortunate to have worked with her and the experiences shared will be long remembered and transcend to others who come after her. I'm thankful to EXPLORATIONS for allowing her this opportunity to express her story for others to read and appreciate.

# ARCHAEOLOGY OF THE CENTRAL MAINE COAST

by Douglas Kellogg

*As usual it was a foggy morning downeast. We ferried ourselves out to the research vessel for the trip from our tent camp on Great Spruce Island to the prehistoric archaeological site we were exploring on Roque Island near Jonesport. I went below to catch some extra sleep. I should have known it was a mistake - every other time I had been below to take a nap, something had happened. This time there was a high-pitched squeal and the smell of burning rubber. It was a fan belt - our bilge pump had seized up. Some fussing around underneath the deck revealed that the pump not only kept the hold from filling with water, but also supplied water to the exhaust to cool it as it passed under the cockpit deck. As we were an hours' cruise from anywhere even without engine problems, there was only one thing to do. We took the pump out to try to figure out what was wrong with it. Earlier in the summer we had lost an impeller in one of our water pumps. I figured that it was probably the same problem this time. On dismantling the pump though, it turned out that the grease fitting had simply gone dry causing the pump to grind to a halt. We added some grease, installed the pump, and were on our way.*

*The purpose of this story is that the rest of the day, as I walked the shoreline searching for undiscovered prehistoric sites, it occurred to me that I go about archaeology in the same sort of problem-solving fashion as we had used to diagnose the problem with the pump.*

Archaeology, like any other scientific endeavor, is the search for answers to specific questions. In my case, the questions are concerned with the ways that pre-European Native peoples interacted with their environments. *Why did they choose to live at this particular spot and not another one?* is something I ask myself when I find a site. To answer such a question, one goes through a problem-solving process. As with the broken pump, a series of hypotheses is tested. The results of the tests usually lead to new questions, or perhaps to a solution. Unlike the pump, however, the behavior of prehistoric peoples is never simple or mechanistic. There is rarely a definite answer. There are, however, wrong answers, and the scientific method provides a means for eliminating unlikely answers, or erroneous explanations.

I earned a M.S. degree through the Institute for Quaternary Studies, University of Maine in 1982. My thesis asked the question, *Why did prehistoric peoples choose to live where they did?* My data set consisted of 190 archaeological sites along

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*Doug Kellogg was raised in Louisiana and Arkansas. He received a B.A. degree in Physics and Anthropology from the University of Arkansas in 1976. Doug has worked on archaeological projects in Texas, Arkansas, and Colorado, as well as in Maine over the past ten years. In addition to continued archaeological and paleoecological research, Doug's other interests include wild mushrooms.*

the shores of the Damariscotta and Sheepscot Rivers. My first concern was *Is this sample of archaeological sites representative of where people chose to live?* It is widely recognized that erosion is affecting the sites. Could it be that the sites in my sample were only those left after erosion had done its damage? To answer this question I undertook a study of the ways in which coastal erosion proceeds in my study area on the Maine coast. The findings (Figure 1) were that archaeological sites tend to be found at rather rapidly eroding locations. Therefore, I could make the assumption that less rapidly eroding locations had not been the scene of intensive prehistoric occupation. The reason for this relationship between shore erosion and site locations became clear in the second half of my study.

The next major step was to test to see if certain types of locations were actually chosen for occupation over others. From a literature review I found that many ideas had been expressed bearing on site choices. For example, it had been reasoned that access to a fresh water source would be a critical factor in the selection of a campsite. Another variable was thought to be a south-facing aspect. I phrased these ideas as formal hypotheses to test. The problem was, how could I test these hypotheses to show that they were or were not supported by the data? To do this I divided the study area into numbered coastal segments approximately equal in length to the shore occupied by the average prehistoric campsite. See Bibliography. From these I chose a statistically random sample. Thus, I had two samples of the coastal environments in my study area that I could compare. The first sample was made up of the 190 archaeological sites. I assumed that this data set contained cultural biases for particular environmental factors that were important to the Indians. The other sample consisted of 190 randomly distributed locations. This sample contained no biases for particular environmental features. The two samples were compared by statistical tests (Figure 2). As a result I was able to show that the archaeological sites are found at relatively rare locations where several environmental factors coincide. For example, many sites are found at beach-type shores, facing south or east, with clam flats nearby. Fresh water, interestingly, does not appear to have been a critical factor in determining the locations that the Indians chose to occupy. Apparently, fresh water was sufficiently abundant so that it could be readily obtained from almost any camping spot that was chosen.

In addition to testing the hypotheses it became clear that the archaeological sites are more susceptible to erosion because the Indians chose to occupy beach type shores



rather than bedrock shores. The archaeological deposits are, therefore, rapidly eroding because they rest on unconsolidated sediments exposed to wave and tidal action.

The results of my Master's degree research led to a research appointment at the University of Maine studying the problem of coastal erosion in another area of the coast. One weakness of my Master's degree research was that it assumed that the present coastal environment was similar enough to past environments so that I could proceed to make the comparison between 1000 to 3000 year old archaeological sites and modern environments. The erosion coast is due to relative sea level rise; thus, environments have surely changed over the past several thousand years. I decided, therefore, to enter the Individualized Ph.D. program at the University of Maine in order to undertake studies of paleoenvironmental reconstructions. The goal of my Ph.D. research is to reconstruct the coastal environments of Muscongus Bay, Maine, at critical times in the past to assist in the interpretation of prehistoric cultural adaptations to the coast. To do this I have obtained more than 60 kilometers of high resolution seismic profiles from the bay and nine cores from the sediments of the bay. The seismic waves penetrate the sediments underwater and allow cross sections to be drawn (Figure 3). The cores are used to verify the seismic data and to obtain samples for study and radiocarbon dating. Maps of sediment thicknesses can be drawn and interpreted to show the topography of the bay when sea level was lower.

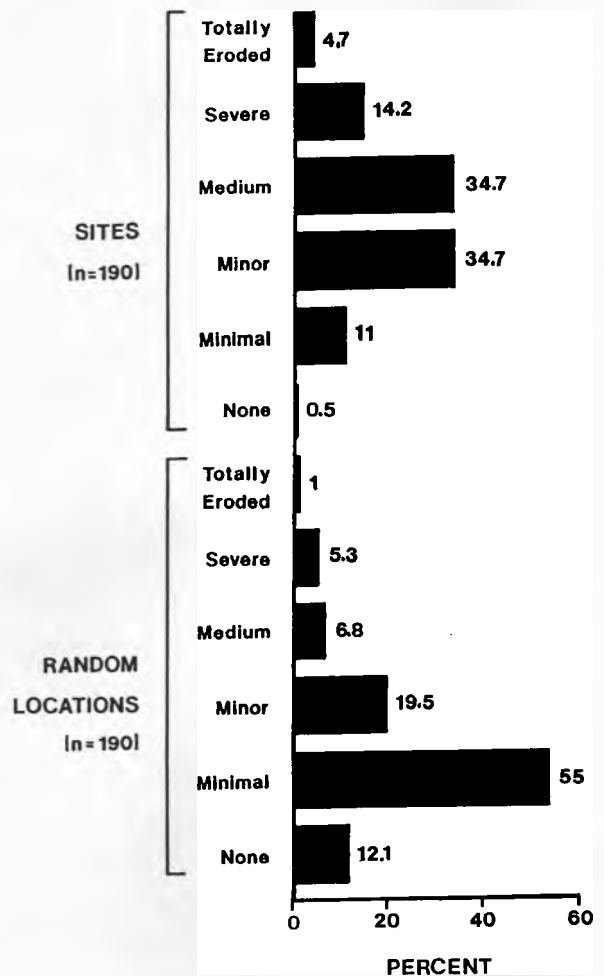
The vegetation of the area is being reconstructed through analysis of the pollen content of the sediments in a lake on nearby Pemaquid Neck. The varying amounts of pollen from different trees and other flowering plants can be quantified by microscopic counts (Figure 4). Through correlation of the geology and the vegetation reconstruction, a relevant framework for interpreting the prehistoric occupations will be established (Figure 5).

Just as repairing that bilge pump on our research vessel involved a problem-solving sequence, so have my investigations of prehistoric archaeology followed a similar sequence. To fix the pump it was first dismantled to see how it worked. My Master's degree thesis took the archaeological settlement pattern apart piece by piece. The result was that I was able to understand a little better how the prehistoric system may have worked. The archaeological record, consisting of bits and scraps of durable material evidence, can never give us a complete picture of past cultural systems. However, careful examination and consideration of particular problems yield insightful results. The Ph.D. research follows up my Master's degree research, by trying to resolve some of the problems that arose during that initial study. This time I am dismembering the geological and ecological context of the past, hoping to formulate a better working model of the past human ecosystems on the coast.

A better analogy for archaeology might be engine design

rather than engine repair. The difference is that I have neither a physical machine to repair, nor a clear idea of what kind of machine I am trying to design. The task of the archaeologist is to apply knowledge of human behavior to the interpretation of bits and pieces left from a past system. Starting with analogs from present systems or theoretical models, explanations are tested until a satisfactory working model is obtained. Further refinement is constant and falsification is always possible with new information. My goal is to come to as clear an understanding of human interactions and adaptations to past environments as is possible.

Figure 1. Erosion of Boothbay Shores



BOOTHBAY 15' QUADRANGLE

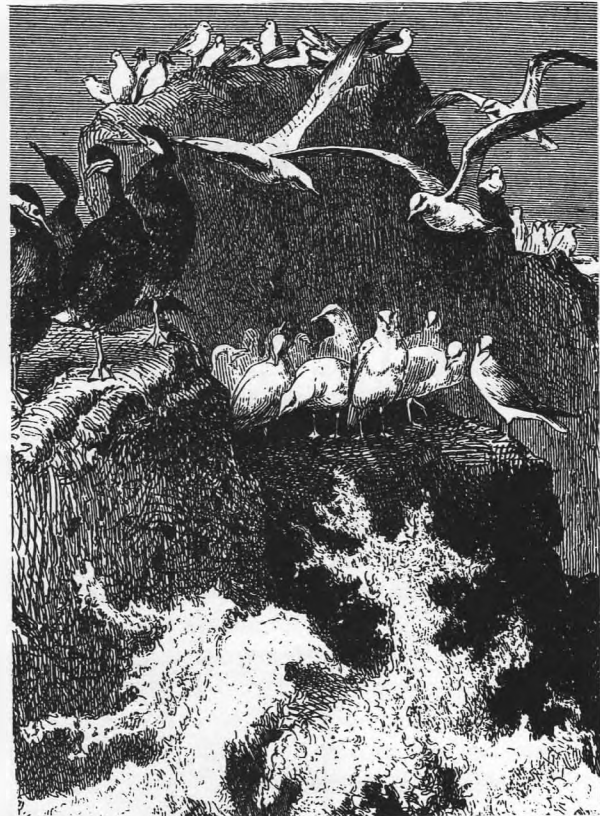
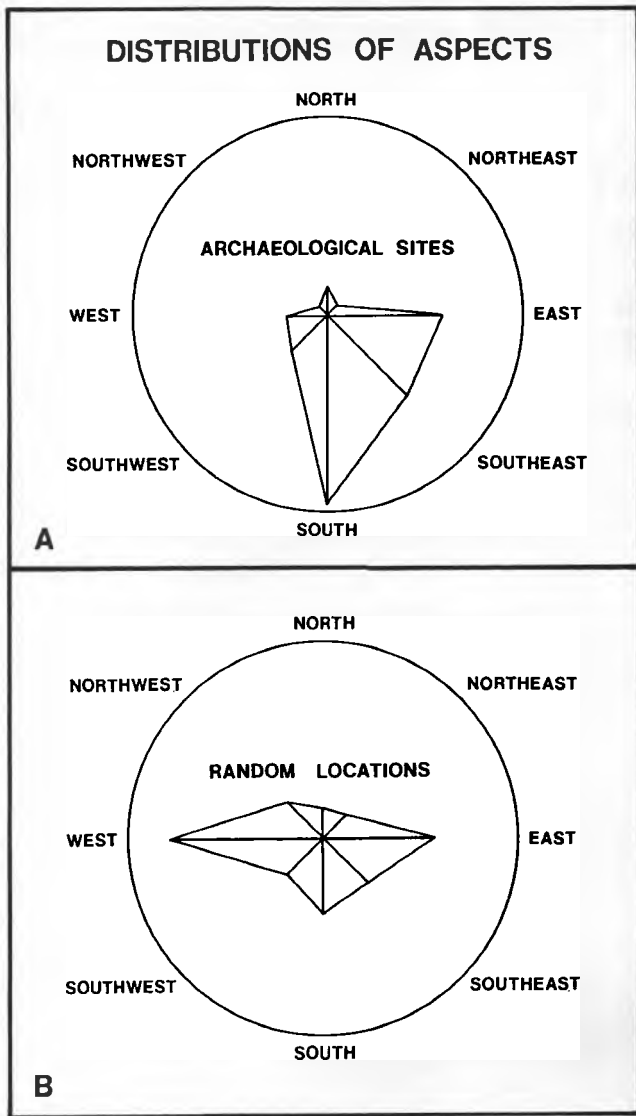


Figure 2.

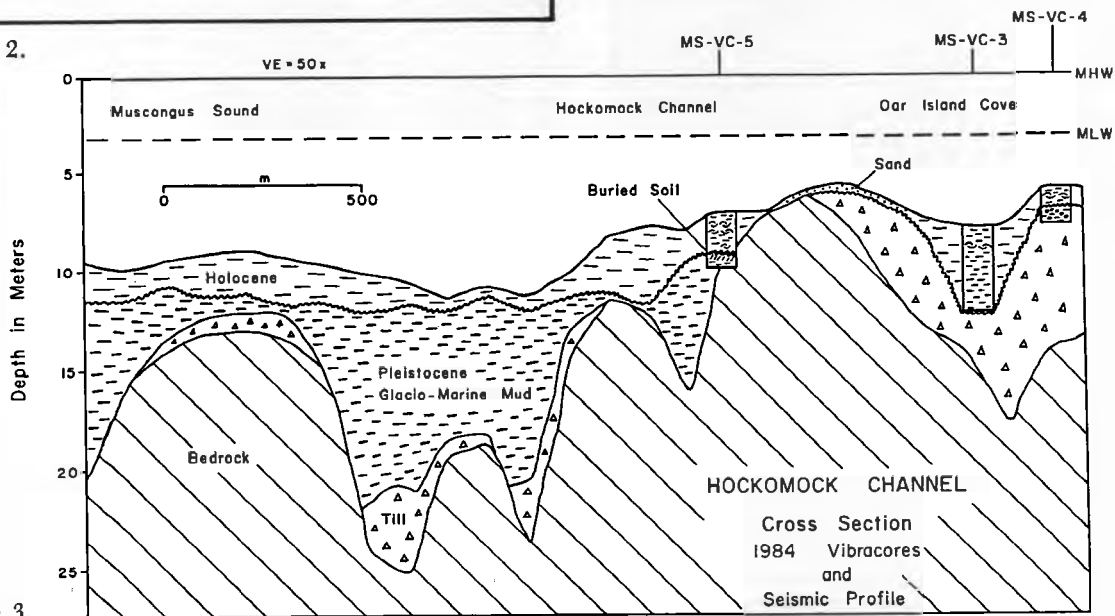


Figure 3.

# ROSS POND, PEMAQUID NECK, MAINE

DOUG KELLOGG, FEB. 1987

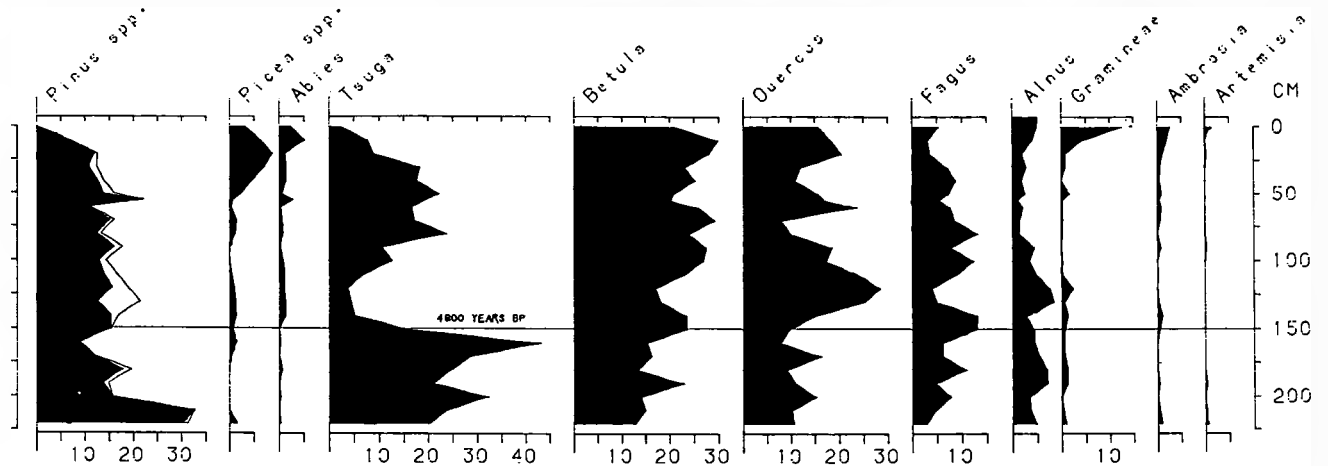


Figure 4

PERCENTAGES

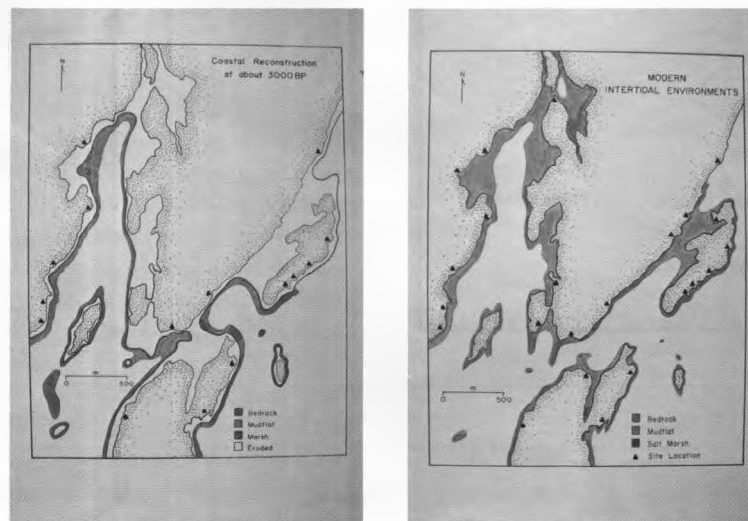


Figure 5

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# Marketing Strategies for Computer Consultants in Small Business

by Kimberly Dagher

The recently increased use of microcomputers by small businesses has produced a potential need for computer automation consulting services. Previous research reveals the level of computer expertise among small business owners and managers is limited. The problem has been compounded by an unwillingness on the part of many vendors to provide the amount of extra support needed.

This study attempts to test the premise that a need exists among small businesses for independent microcomputer consulting. The data collected are compared to other national studies. A series of marketing strategies is generated, designed to aid the marketing of computer consulting services to small business owners and managers.

## THE ROLE OF SMALL BUSINESS COMPUTER CONSULTANTS

Personal computers have become the largest part of the \$60 billion a year computer market, and small business is

the fastest growing segment. According to some estimates only 10-16 percent of the nation's 3.2 million small businesses currently have computers (1). There is considerable evidence that most small business personnel lack the knowledge necessary to make intelligent computer automation decisions (2) (3). As a result, owners and managers are reluctant to invest in equipment they do not understand. Another barrier is time, or more aptly put, lack of it. Often small businesses have neither qualified help to take the new gear on board nor managers with time to do it themselves. One recent study (Peterson, 1984) found that small businesses whose primary business line was not in retailing indicated that *using computers* was their area of greatest need for management assistance.

While some computer dealers are emphasizing their consulting services and user-training centers to cash in on the growing small business market, an equal number are trying to avoid them. Some dealers complain that marketing computer systems to small businesses is expensive. They need substantial persuasion before making a purchase, and extensive attention after the sale to get the system working properly. (1) Selling and service are so expensive that anyone trying to serve small businesses must charge customers two to three times the market price of a basic computer to begin to realize a profit. Frequently, dealers do not provide the unbiased support, individual attention and patience needed to assist small businesses in analyzing all of the alternatives relevant to their needs. The marketplace is vast and confusing to the uninitiated small business owner or manager.

It seems obvious that a market exists for small business computer consultants. Unfortunately, small businesses are typically suspicious of paying for a consultant's time. When asked to list the management assistance sources used (Peterson 1984), only 12 percent of small business owners and managers indicated that they had used private consultants. Instead, they tend to use and consider most important those sources that are convenient and require minimal effort to employ. Sources used most frequently include accountants or CPA's; lawyers or bankers; books, magazines, or trade journals; suppliers; and other business people.

## LITERATURE REVIEW

Existing literature concerning small businesses and their use of microcomputers has concentrated either on selec-

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*My undergraduate studies were completed at the University of Wisconsin-Madison, where I received a Bachelors of Business Administration (BBA) in 1981. My majors were Personnel Management and Industrial Relations, Administration, and International Business.*

*In December, 1986, I graduated from the University of Maine with a Masters of Business Administration (MBA). During my graduate studies I was a Graduate Assistant for Dr. Virginia Gibson, Professor of Information Systems.*

*Prior to my graduate studies I was the Director of Research at the University of Wisconsin Foundation (UWF) for three years. UWF is the fund-raising branch of the University of Wisconsin.*

*Immediately after my undergraduate studies, I was an AIESEC (International Business and Economic Student Association) student intern at Ansvar Insurance Company in Stockholm, Sweden.*

*My interests in conducting the microcomputer research project were twofold. First, I feel that it is very important for all businesspersons to be knowledgeable in the area of conducting scientific research. When the opportunity arose for me to design my own project for a Small Business Course, I wanted to take advantage of the guidance my professor could provide in undertaking a research project.*

*The second reason was because of my interest in and experience with small business microcomputer consulting. Should I decide to start my own business in microcomputer consulting, this research will provide the core of my marketing feasibility study.*

tion and implementation, frequencies and reasons for failure, attitudes of owners and managers toward computers, or the impact of microcomputers on the work behaviors of owners and managers.

Farhoomand and Hrycyk (1985) conducted a survey the aim of which was to reflect accurately the experiences of small business computer users.

Research by Cheney (1983), on the other hand, attempted to determine the frequencies of and reasons for success or failure of introducing micro- and mini-computers into small businesses.

By 1985, little information was available regarding the impact of microcomputers on the work behavior of owners and managers of small businesses. Malone (1985) surveyed owners and managers of ten small building material retailers currently using computers. Malone's study highlights some of the differences between large and small businesses in using computer technology. Small businesses used applications classified as operational and managerial rather than those of strategic orientation used by larger companies. In general, respondents thought the computer enabled them to make decisions more quickly and improved the quality of decision making within their organizations.

Computer attitudes and applications of small business owners and managers were again researched by Nickell and Seado (1986). They found that the majority of small businesses surveyed were using computers, and again that applications differed from those used by large companies. Small business owners and managers in this study were using computers for basic functions such as word processing and mailing lists. Eighty percent of the owners and managers personally use the computers, thus indicating they must be able to do a variety of tasks. Most had a positive attitude toward computers, which was effected by past experience with them.

Arthur D. Little, Inc., in market research commissioned by the Digital Equipment Corporation, found that small businesses want customized computer solutions to the specific needs of their industries. These results were reinforced by a recent article in *Fortune* (1985) describing the computer industry's hot new marketing strategy of vertical integration. Computer systems tailored to the unique needs of many industries which have concentrations of small businesses have already been made. For example IBM and Burroughs serve the banking and insurance industries. Tailored software has also been developed for auto dealerships, hospitals, and professional services such as lawyers, accountants and doctors. The Little, Inc., report concluded that dealing with small businesses requires much individual attention and support - treatment that has traditionally been reserved for very large customers because of the costs involved.

## THE SURVEY

*Subjects* A random sample of 500 was selected from the

population of small businesses (1) in the Bangor, Maine, area. The sample was chosen from the *Greater Bangor City Directory* and retains the same proportions of each business category (e.g., real estate vs. auto dealerships vs. restaurants) found in the population.

*Instrument* Each business in the sample was mailed two separate one-page questionnaires and asked to answer the appropriate one. One of the questionnaires was for current users of microcomputers, including those in the process of installing, and the other for non-users. The surveys were color coded for clarity.

The users were asked questions regarding brand(s) and model(s) of computers used; the number of computers in use; years of use; applications; individual most influential in the purchase decision; level of satisfaction overall and with specific services provided by vendors; problems with hardware, software, implementation, and dealer support; cost to introduce the system and breakdowns of hardware vs. software vs. conversion.

Respondents not currently using computers were asked to indicate what has prevented them from doing so; how record-keeping is currently done; future plans for computer purchases; and the types of record-keeping systems used.

Both groups were asked to respond to demographic questions, including the number of employees; 1985 annual sales; industry classification; familiarity with computers; and approximate levels of business activities in terms of number of credit customers, vendors with credit, inventory items, accounts, letters sent per month, and the number of people on mailing lists. Finally, space was provided for comments.

*Methodology* The questionnaires were sent with a cover letter addressed to Owner/Manager, in late October, 1986. Respondents were asked to indicate whether or not they would like to receive a summary of the results. This request was seen as a major motive to ensure an acceptable response rate. Funding was provided by the Small Business Institute at the University of Maine. A follow-up was not mailed.

*Responses* Of the 500 questionnaires mailed, 95 (19 percent) were completed and returned. An additional 15 were returned as undeliverable because the businesses were no longer at the indicated locations and had no forwarding addresses. Only one of the completed surveys could not be used, because of failure to complete the correct form. Of those responding, 51 percent were non-computer users, and 49 percent were either current users or in the process of installing a system. The results were analyzed using frequencies, means, and correlations from SAS.

## ANALYSIS OF RESULTS

### *Profile of the Respondents*

Tables 1 and 2 compare size characteristics of the small business users and nonusers of microcomputers. Questions were asked relative to the number of employees and an-

nual sales in an attempt to determine what relationship size of business has on the introduction of computers.

Using both sales and number of employees as a proxy for size reveals that some level of activity is critical before decisions were made by small businesses to introduce computers. The level of sales appear more important than employee size. Slightly more than one half (53 percent) of the users reported annual sales in excess of \$500,000 whereas 56 percent indicated they had sales less than \$250,000. Eighty percent of nonusers have less than 10 employees, and 97 percent less than 20.

Table 3 shows that major industry classifications for nonusers include Trade/Retail (34 percent), Professional/Technical Services (16 percent), Health/Education (16 percent), and Construction (11 percent), while users were concentrated in Professional/Technical Services (28 percent), Trade/Retail (19 percent), Finance/Insurance (12 percent), and Construction (12 percent). A 1986 COMTEC survey of microcomputer penetration for all businesses, based on industry groupings, shows similar results. The highest usage of microcomputers was found among banking/financial/insurance firms, with 48 percent penetration. Professional/technical service firms had a 41 percent penetration rate.

Almost half the nonusers indicated no previous exposure to computers, while 13 percent said they were very familiar with them. On the other hand, 90 percent of users were at least somewhat familiar with computers. These results are presented in Table 4.

In lieu of the larger size computer users versus nonusers, one would expect that their business activities would also be on a larger scale. This was indeed the case, as can be seen in Table 5 from the comparison of the mean amounts under each category.

#### NONUSERS

##### *Reasons for not computerizing*

Table 6 indicates that the most frequently stated reason for having not computerized is that the owner or manager is unsure of the benefits of computerization. Other commonly cited responses: cannot afford the cost involved (35 percent), do not have time (35 percent), and business too small (33 percent).

##### *Success Predictors*

The majority of nonusers (65 percent) indicated they have personnel who are capable and interested in operating computers. The presence of personnel who are qualified and interested in operating a computer is a good predictor for how successful the business will be when implementing a system. Resistance to computerization will be lower and transition to an automated system smoother. The importance of minimizing conversion difficulties is crucial because these problems usually persist after the start-up phase (Farhoomand and Hrycyk, 1985).

Another success predictor included in the questionnaire was the presence of systematized and documented pro-

cedures for record-keeping. The results are presented in Table 7. Twenty-eight percent of the businesses indicated that they do not have any systematized procedures. Accounting systems were among the most frequently formalized with Personnel records next.

Malone (1985) found that smaller firms which have adopted more formal manual record-keeping systems have a less traumatic conversion when switching to computer-based systems. Ein-Dor and Segev (1978) also argue that lack of formalization can lead to time lags in decision making which further imperil success.

##### *Future microcomputer purchases*

Nonuser small businesses were asked if, and when, they planned on purchasing a microcomputer. Table 8 reveals that 56 percent expect to purchase a microcomputer. Nine percent will do so in less than one year. Within two years 38 percent plan on making a purchase. Several significant differences distinguish those who will not computerize from those who will. Respondents with no plans to purchase a microcomputer were more likely to feel their businesses too small for computerization (52 percent versus 14 percent for those who plan on purchasing) and that they have a lack of computer expertise by current employees (38 percent versus 18 percent). However, businesses with purchase plans were more frequently small compared to those which will not computerize. This underscores that smallness in size is not a valid factor for businesses that have not computerized. A final point of interest is that industry classifications do not have a bearing on whether a small business will purchase a computer in the future.

#### USERS

##### *Brands Used and Years of Use*

This study once again shows the popularity of IBM and IBM compatibles. Table 9 presents the frequency of microcomputer use by brand. None of the ten miscellaneous brands were used by more than 3 percent of respondents. Major differences from national trends are a higher percentage of Kaypros and a lower percentage of Apples in use.

More than half the users purchased their systems within the last two years. Table 10 shows that 18 percent purchased their system within one year and 43 percent within 1-2 years. These results indicate users are still relatively inexperienced and also reflect the recent rapid growth in purchases of microcomputers by small businesses.

Applications software in use is concentrated in accounting, word processing, and mailing lists. These findings, shown in Table 11, reinforce the notion that small businesses use computers for more basic functions than large businesses. (Nickell/Seado 1986, Malone 1985, Chenny 1983.) If anything, the frequency of use between advanced and basic applications among small businesses is becoming more pronounced.

### *Influence on Purchase Decision*

Not surprisingly, the individual most influential in making the decision to computerize is the owner or manager. Also showing considerable influence in Table 12 are salespersons (11 percent) and accountants (7 percent).

The influence of accountants in the decision to computerize is noteworthy. Small businesses utilize accountants and CPA's as a primary source of information (4).

### *Satisfaction*

Table 13 indicates that the general level of satisfaction was very high, with 53 percent indicating that they are satisfied and 40 percent strongly satisfied. This high degree of satisfaction may be explained in part by the theory of cognitive dissonance. Since the respondent and purchaser of the system are usually one and the same, post-purchase doubts are either ignored or overcome by some other means.

In spite of the high degree of satisfaction, 80 percent of users indicated they had experienced at least one major problem with their system. The frequency of specific problems is presented in Table 14. Training is most frequently cited, followed by software limitations and lack of dealer support.

Insight as to the cause of these problems can be gained by reviewing Table 15, respondents evaluation of vendor services. Training for example, is either not provided by the vendor or when provided evaluated as being poor 62 percent of the time. Two other vendor services are not provided or evaluated as poor when provided, more than half of the time: Pre-Purchase Needs Analysis and System Design Consultation. Hardware maintenance is the vendor service most frequently provided (78 percent of the time) and the one most often evaluated as excellent (36 percent of the time).

### *Attitude toward Consultants*

The acceptance rate of 38 percent for private computer consultants, shown in Table 16 is higher than that for management consultants in general (Peterson, 1984 and Franklin/Goodwin, 1983).

Small businesses often view the hiring of an outside management consultant as an admission of incompetence. However, seeking outside assistance for computerization may be increasingly viewed as a necessity. The degree of familiarity with computers by small business is low, as shown in Table 4. When combined with the complexity of the microcomputer marketplace, computerization is too confusing to implement alone.

### *Costs*

Table 17 indicates that 56 percent of the computer systems in use cost less than \$10,000. The total amounts spent on the computer systems in this study are on average considerably less than reported in other research (Farhoomand and Hrycyk 1985, Cheney 1984). A likely explanation is that the cost of computer systems has been decreas-

ing by 10-20 percent annually since the last surveys were conducted. It may also be explained by the newness of the systems in the study (heavy software expenses have not been incurred) or the indirect nature of conversion and training costs.

On the average, respondents spent 63 percent of total system costs on initial hardware, 16 percent on initial software, 9 percent on additional hardware, 7 percent on additional software and 2 percent on conversion. Percentages spent on different components of the system seem to be unrealistic, although not unusual. Malone (1985) states that smaller firms tend to overinvest in hardware and underinvest in software. According to other research, small businesses can expect to spend up to 80 percent of the total price on software and 100-300 percent of start-up costs on conversion and training.

Fifty-nine percent spent approximately what they had originally budgeted; 26 percent spend more, and 10 percent spent less than anticipated.

## MARKETING IMPLICATIONS

### *Services*

This study provides considerable evidence that microcomputer vendors are not providing small businesses with the degree and quality of services they need. Respondents have indicated varying degrees of need by a non-vendor source for the following computer consulting services: Pre-purchase Needs Analysis, Design Consultation, Training, Hardware Maintenance, Software Programming and Post-Purchase Technical Assistance. A discussion of each service follows.

A pre-purchase computer system needs analysis was provided to only 67 percent of the respondents in this study. When provided, 20 percent evaluated it as being poor. Thus, more than half of the current micro users did not receive the quality or degree of assistance needed at this important stage of the computerization process. Computerizing is not the solution for all small businesses. However, when vendors provide this service they have a strong incentive to sell - their own products. Hidden costs frequently are not mentioned. Unfamiliarity with computers, uncertainty as to their benefits, a lack of time in which to analyze the problem, and the complexity of the micro marketplace all point to an independent computer consultant as the solution. In conducting this analysis, the consultant should examine the economic, technical, and organizational feasibility of introducing a computer to the business.

The degree and quality of design consultation services provided by computer vendors is essentially the same as for needs analyses. The first step in this phase is to develop an understanding of the businesses' current operations and their future needs. Input by the small business owner and manager is essential. Unless the owner or manager is a computer expert, which is not likely according to this study,

outside assistance will be needed to put the best computer system together. With more than 35 brands and models of microcomputers on the market and thousands of software packages the benefits of hiring an independent computer consultant are eminently clear. An error at this stage will be costly. The consultant should be knowledgeable about the software packages designed and specifically for the needs of the industries of their clients. Consultants can be particularly effective in providing clients with accurate cost estimates of a proposed system.

The need for training by an independent computer consultant was discussed earlier. While many universities and vendors provide group training sessions, the need seems to be more for tailored training packages. The small business owner or manager and staff have indicated a desire for one-on-one training to meet their unique needs.

Hardware is one area of support that is being satisfactorily met by computer vendors. Similarly, software programming does not seem to be an area of great need. Ninety percent of the small businesses in this study used packaged software.

Post-purchase technical assistance, while provided frequently by vendors, is another area of dissatisfaction by small businesses. Farhoomand's research (1981) indicated even deeper dissatisfaction with technical assistance. In fact, businesses in his study felt that it was their most significant problem. Although the nature of this service may pose some difficulties for the independent to offer, in special circumstances it may be worthwhile. In fact, handling limited technical problems free of charge for existing clients may be a good marketing device.

#### *Target Market and Market Potential*

The small business computer consultant has a large potential market, which includes all of the small businesses in his/her area. The market for businesses which have not computerized, 84-90 percent nationwide, is the largest. A 1985 Dataquest survey reveals that 90 percent of them are potential clients. The market shows great potential growth within the next two years. Thirty-eight percent of the non-computerized businesses in this study plan on purchasing a computer within that time. Current users are concentrated in the areas of Professional/Technical Services, Trade/Retail, Construction and Finance/Insurance. Before taking on clients, consultants should determine the business' potential for successfully converting to a computerized system. Two success predictors utilized in this study are qualified/interested personnel and the existence of formalized record-keeping systems.

#### *Competition*

The primary competition for the computer consultant will come from computer retailers, computer programmers, and systems consultants. The consultant should become familiar with the services provided gratis versus an additional charge by local vendors. Then a determination as

to the appropriate marketing approach can be made.

#### *Marketing Approach*

Small businesses have typically been reluctant to hire independent consultants. The results of this survey indicate that computer consultants may provide an exception to this rule.

Advantage should be taken of small businesses' reliance on their accountants. Forming a business relationship with one or more local accountants could lead to subcontracting their computer-related jobs. Educational institutions often hire independents to facilitate software training seminars or courses. Offering free seminars through local Chambers of Commerce or business associations will also provide an opportunity to establish a reputation and meet local small business owners and managers. The importance of traditional methods such as direct mailings of descriptive brochures along with telephone solicitations should not be overlooked. Fees must be kept in line with what the small business can afford. Finally, the consultant must continually monitor the small businesses' computer support needs and the rapidly changing microcomputer marketplace.

#### *CONCLUSION*

This study tested the premise that a need for small business computer consultants does exist. The results indicate that small businesses do need management assistance when converting to a computerized system. Furthermore, a review of the literature reveals many computer vendors are not interested in the small business market because the costs of selling and servicing are so high. Thus, private computer consultants can provide the expertise and unbiased support needed.

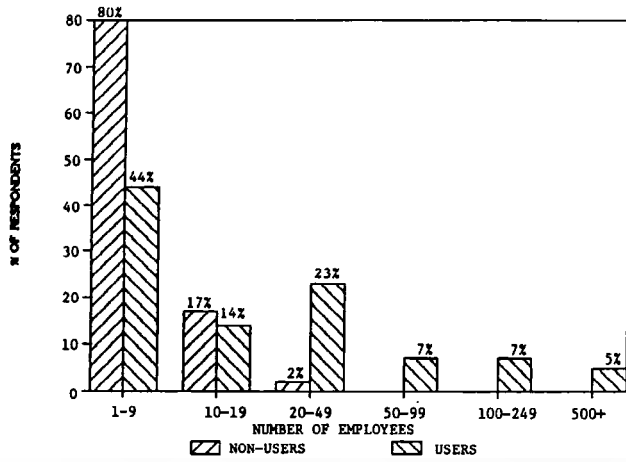
## **From the Advisor . . .**

When asked, *What would you like to be doing 10 years from now?* an increasing number of MBA students report they would prefer to be in business for themselves. Although our graduate program does emphasize the development of managers, the Special Projects course I directed during the 1986 Fall Semester was designed specifically for those students who expressed an interest in becoming entrepreneurs or directly involved with small businesses.

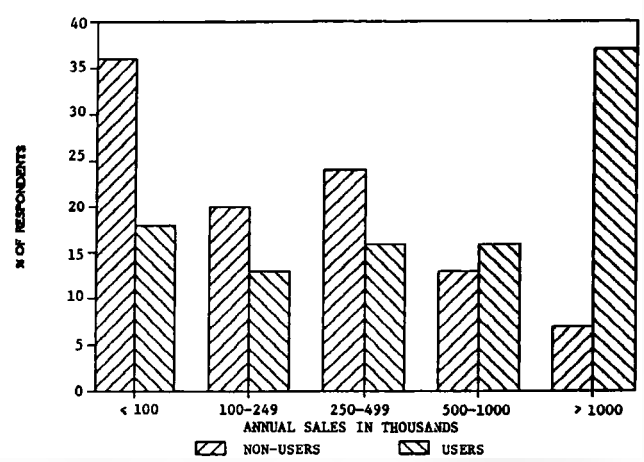
Kim Dagher's interest in computer applications to business problems fit in perfectly with the course objectives. The basic question was, *is there a market in the Bangor area for computer consultants for small business?* Her paper describes the market evaluated, the methodology used, and the marketing strategies to be used to capture that market. The research project permitted her to apply concepts incorporated in the methodologies of market research in addition to testing out a real life issue: *is there a potential for her to consult to small business?*



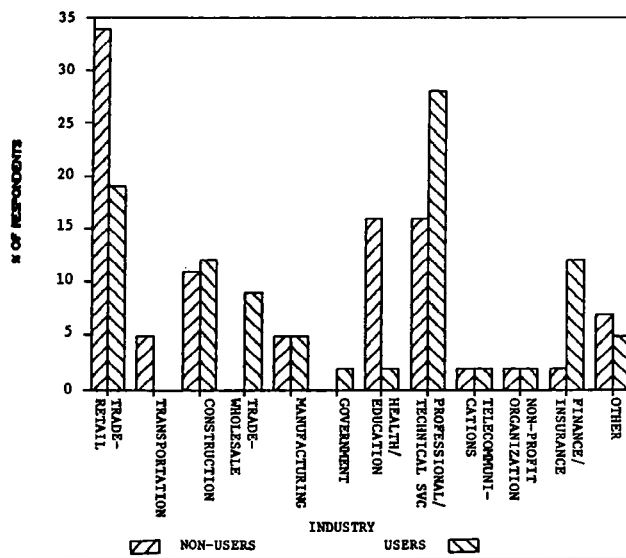
**Table 1 NUMBER OF EMPLOYEES**



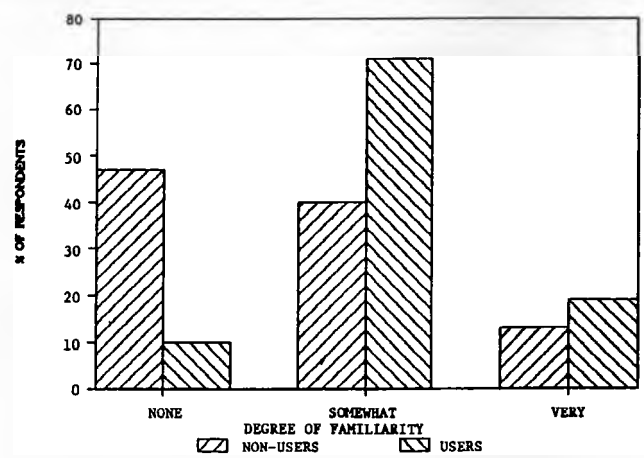
**Table 2 ANNUAL SALES IN 1985**



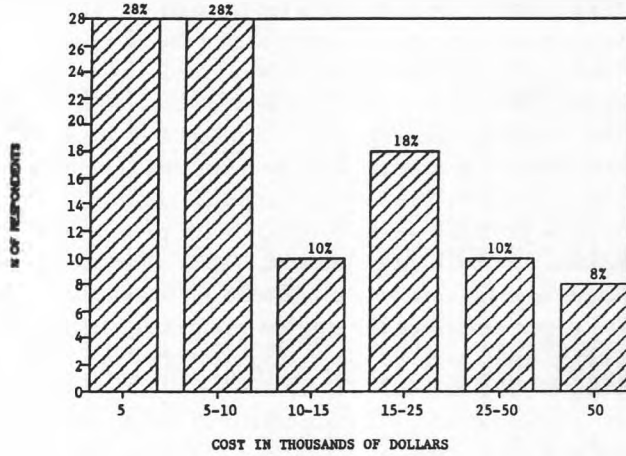
**Table 3 INDUSTRY CLASSIFICATION**



**Table 4 FAMILIARITY WITH COMPUTERS**



**Table 5 TOTAL COST OF MICROCOMPUTER SYSTEM**



**Table 6 FACTORS WHICH PREVENTED COMPUTERIZATION**

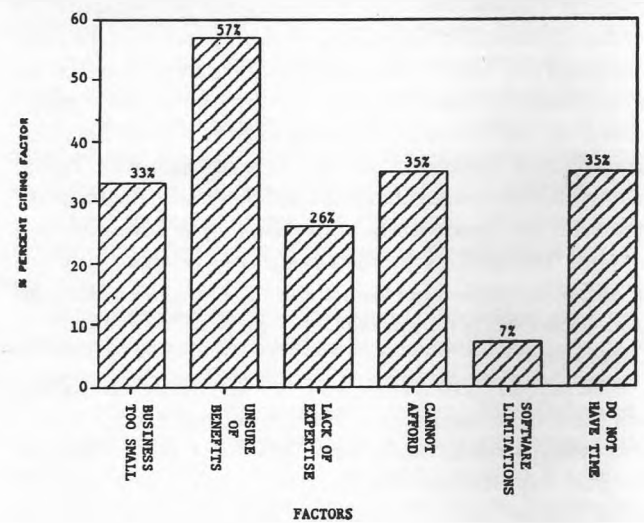


Table 7 % HAVING SYSTEMATIZED PROCEDURES FOR:

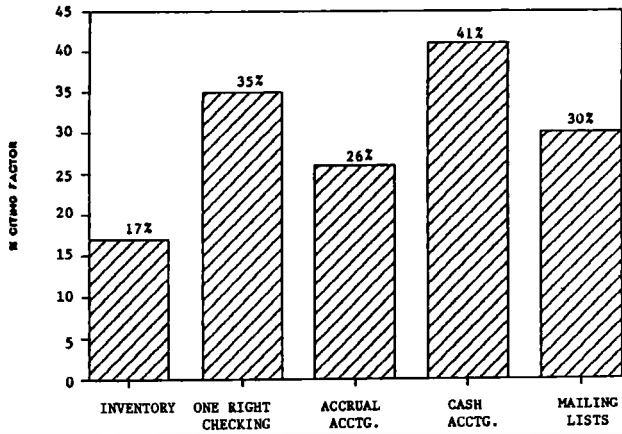


Table 8 WHEN PLAN ON PURCHASING COMPUTER?

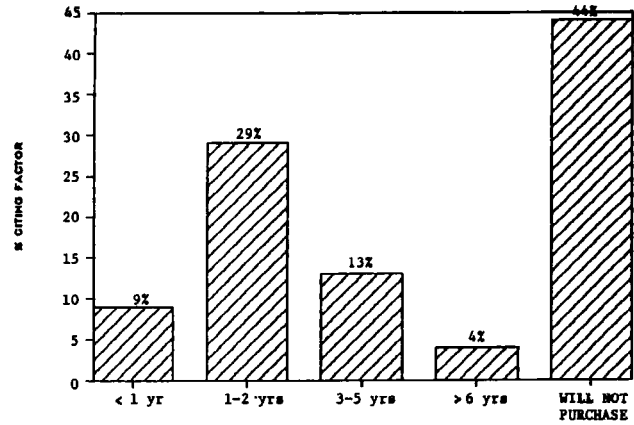


Table 9 BRAND OF MICROCOMPUTER USED?

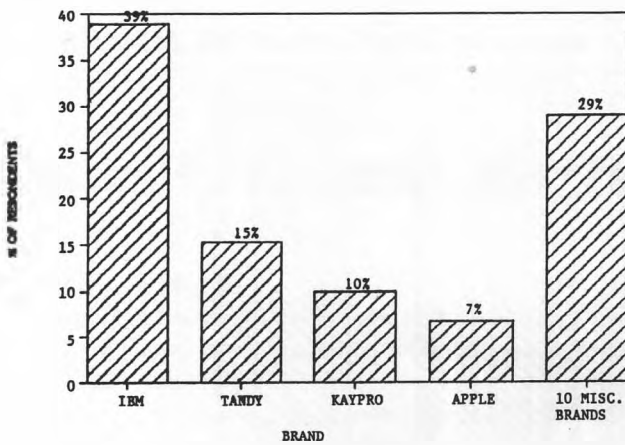


Table 10 YEARS OF COMPUTER USE

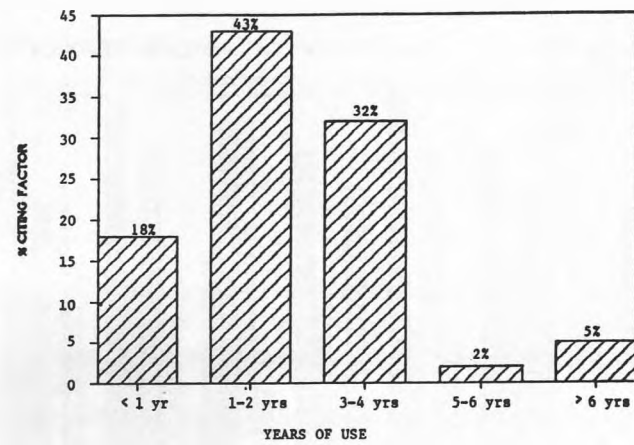


Table 11 MICROCOMPUTER APPLICATIONS USED

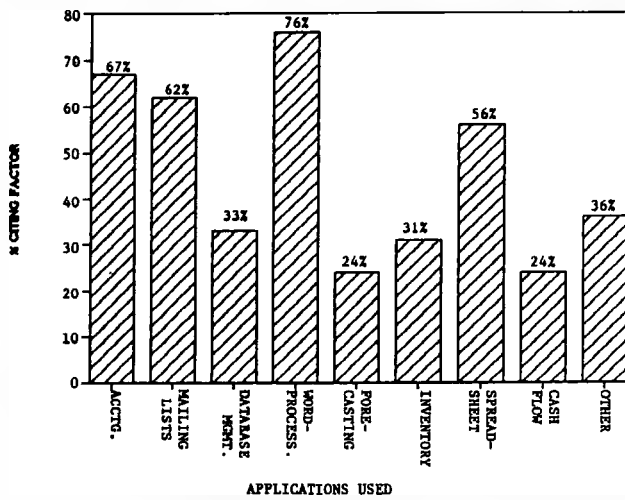


Table 12 MOST INFLUENTIAL IN PURCHASE DECISION

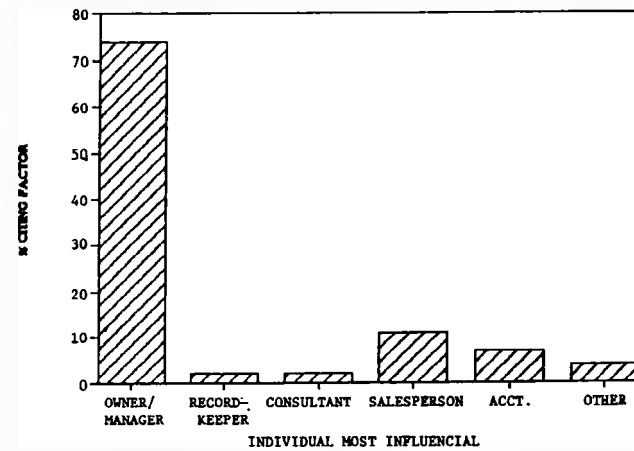


Table 13 LEVEL OF SATISFACTION WITH COMPUTER

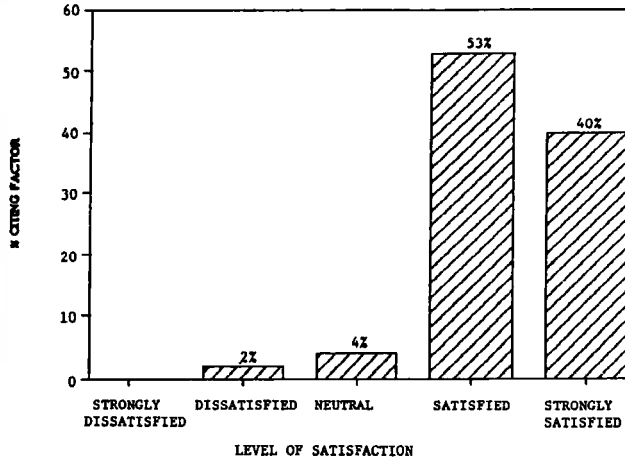


Table 14

PROBLEMS WITH COMPUTER SYSTEM

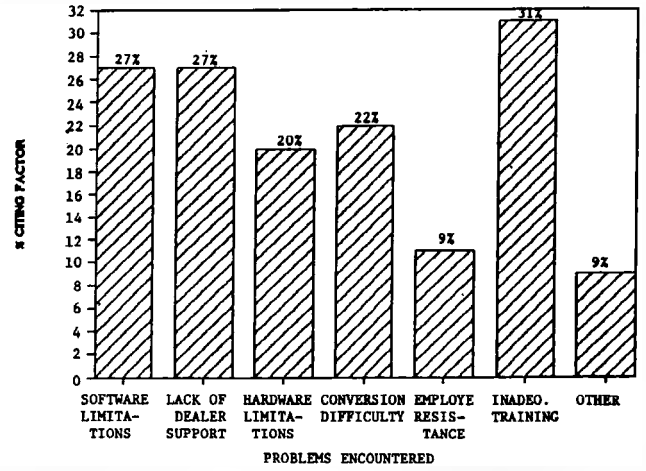


Table 15

EVALUATION OF VENDOR SERVICES

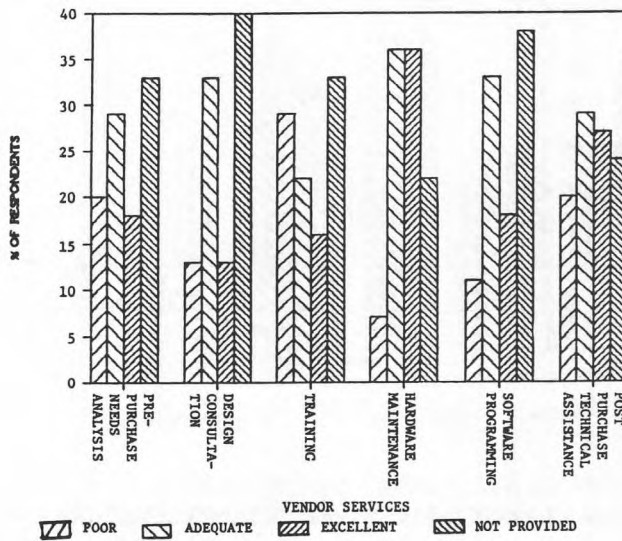
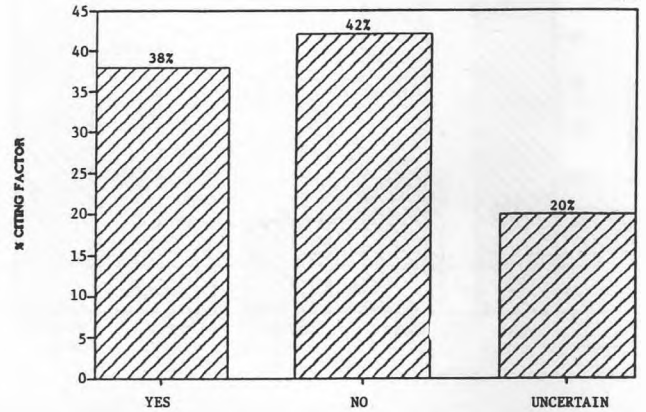


Table 16

WOULD YOU HIRE A COMPUTER CONSULTANT?



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- (4) Peterson, Robert A. "Small Business Management Assistance: Needs and Sources" *American Journal of Small Business* (Fall, 1984): 35-45

## FOOTNOTE

- (1) Small businesses as defined by SBA Guidelines.

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# Our Cover Artist

## MARCIA SPENCER

The production of artwork has been an active part of my life since I was quite young and I have pursued it both educationally and independently for many years. Attending the University of Maine gives me the opportunity to work more closely with other artists and to learn from their accomplishments. My intention is to provide the environment for my work to grow and improve and to increase the possibilities for a career. I hope to continue to teach and ultimately to work into a Master of Fine Arts program.

I initially studied at the Philadelphia College of Art from 1967 to 1969, majoring in Illustration. In 1979 and 1980 I furthered my education career by taking courses in printmaking at the University of North Carolina at Greensboro. Returning to school once again in 1985 at the University of Maine I hope to finish my undergraduate degree as an art major. Having lived for the past ten years in Washington County, Maine, I have found job opportunities in the arts to be few and far between. Most recently I have spent the last three years teaching art to elementary school age children in the Gifted and Talented Program of School Administration District 77. Other teaching experiences have included an adult summer art program in Lubec, Maine, a children's art program in Machiasport, Maine, a children's art workshop in Machias, Maine, and art activity director at Washington Academy in East Machias. I have

also found occasional work as a free-lance artist as well as spending a period of time learning something about Graphics in the work-study program at the University of Maine.

As well as being involved in the arts through job situations, I have had the chance to show my work in various galleries and exhibits in Maine, North Carolina, and New Brunswick, Canada. Some of these include having had work shown at the Frost/Gully Gallery in Portland, a one-person show at the Leighton Gallery, Castine, a group show at Sunbury Shores Art & Nature Center in St. Andrews, New Brunswick, and two 2-person shows at the Bear & Crow Gallery in Lubec. Some of my pieces are in various private collections, and I have participated in and received awards in numerous student exhibits and art exhibitions, including having one piece in Orono's 1986-87 Traveling Art Exhibition.

My interest in teaching art revolves around a strong conviction that the creative process is an absolute necessity in the educational experience. This does not necessarily mean the production of artwork so much as thinking creatively and independently. Often in my classes the situation occurs where the student must take responsibility for finding solutions and must rely on his or her own motivation to do so. In return I have been personally enriched by being actively involved in this process.

# From the Advisor . . .

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by James Linehan

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The practice, or habit, of teaching in the visual arts is as tricky to comment upon as it is to engage in. At times I am sure the process seems deceptively simple to students (“Here, draw this!”) or intentionally vague (“What are you trying to say? How do you *feel* about this?”) or downright arbitrary (“Too much blue!”). After eleven years of teaching young artists, I am still mystified and electrified by the challenge of the business. It is an unapologetically subjective process, which when done well is as challenging to the teacher as it is to the students. Over the years, some of my students have been extremely gifted, but many have been perversely underequipped for the intellectual rigor (yes - it’s true!) and the technical challenges involved in making sound art. Perhaps the most difficult tool of all for students, and professionals, to master, and the most necessary to possess, is the capacity for self-criticism. Without this, it is impossible to make art.

Marcia Spencer, the artist featured on the cover of this issue of EXPLORATIONS, has worked with me for several semesters in Advanced Independent Study courses. I think she is a very good painter. She possesses a lot of technical facility as a painter, and more importantly she does possess the aforementioned capacity for self-criticism. Over the past year, we have discussed a number of issues in relation to the development of these landscape-based paintings. I have been pleased to see them evolve thus far, and I fully expect that the ideas she is working with here relating to image, color, and surface will continue to develop into some very fine paintings.



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