and Algal Herbivores
Ground Water as an Algal Culture Medium in North Clare

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All algal cultures require a growth medium. In small cultures autoclaved water is used, while in aquaculture filtered or ultra violet radiated water is the commonest medium. The expense of treating very large quantities of natural seawater is one of the barriers to large scale algal cultures.

In some aquaculture installations, an alternative source of suitable water is a subterranean supply. In France fossilised water in tertiary deposits has been shown to have salinity, pH and nutrient characteristics which are near ideal for algal cultivation. This example suggested trials which might be rewarding at our company site.

At Redbank Shellfish an alternative supply of water has been developed. The company hatchery is built over Carboniferous limestone. This rock formation is very porous, and many nearby houses draw their freshwater from wells. A test well drilled within ten metres of the shore yielded a water of 20 ppt salinity. Nutrient analyses showed an average of 90 μg at/litre nitrate nitrogen, the figures for silica and phosphate were 18 and 0.15 μg at/litre. While bacteria were detected, no algae or other eukaryotic cells occurred. Heavy metal concentrations, zinc, lead, copper, cadmium and iron, were comparable to seawater. pH is lower than seawater, 6.8 compared to 7.9 to 8.3.

During the year salinity levels fluctuated between 12 and 25 ppt, although temperature was almost constant at 10 to 12 degrees Centigrade. Nitrate was highly significantly correlated with low salinity. Low salinity only occurred during very heavy rainfall, a salinity of 15 to 20 ppt was more usual. Flow rates of 20,000 litres per hour have been maintained indefinitely, and at present, phytoplankton is cultured using this water in 25m x 25m x 2m plastic lined ponds.

This water has several characteristics suitable for algal culture. Nitrate concentrations, and to a lesser extent, silicate concentrations, are far higher than winter seawater concentrations. The almost constant temperature reduces problems of over heating or chilling. The lower pH implies a high CO₂ content, while the absence of any indigenous algal population removes any serious danger of contamination. The low salinity is suitable for many coastal or estuarine forms. To date, this water has only been used for phytoplankton culture but macroalgal cultivation is a possibility which deserves consideration.

The origin of the water is unclear. It appears to be a mixture of freshwater runoff and trapped sea water. The fluctuating salinity suggests an active rather than fossilised reservoir. The correlation between low salinity and high nitrate shows that this nutrient has a terrestrial origin. The age of the sea water is uncertain, several features suggest some aging occurs. The low pH suggests a breakdown of organic material, this is supported by low levels of organic nitrate and phosphate relative to sea water. The absence of eukaryotic life also suggests a long subterranean residence time.

Whether this water supply is confined to the North Clare coast or whether other supplies could be found remains to be seen. Redbank Shellfish would like to thank Bord Iascaigh Mhara for financial support to this project.
The Proposed Northern Ireland Centre for Marine Resources and Mariculture

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The proposals forwarded to the International Fund for Ireland following the 1989 Ir-Am-Aqua meeting in Galway included a joint venture between University College Galway and the Queen's University of Belfast to establish an outreach, research and development service interfacing the universities with the Irish aquaculture industry. The 'northern' component of this included the establishment of a mariculture unit at Portaferry as part of the Queen's University School of Biology & Biochemistry's facilities there. Although the overall proposal was not accepted in its original format, encouragement was given to continue seeking funding for a modified Portaferry proposal. Following further discussions with interested parties including the Department of Economic Development (N.I.) a modified proposal is being assessed.

The overall objective is to establish a centre to 'facilitate and stimulate environmentally acceptable development of existing and new mariculture enterprises in Northern Ireland' and to 'encourage the further development of expertise and marine resources in an all-Ireland and wider international context'. To this end new and updated facilities and appropriate staffing are being sought. The initial five-year programme proposal has five main headings, namely:

1. Coastal Marine Resources, to include a Northern Ireland marine resource inventory, assessment of ocean quahog (Arctica) potential and artificial reef practicality.

2. Bivalve mariculture, particularly local Mytilus and Pecten.

3. Prosobranch mariculture, especially whelk (Buccinum) and winkle (Littorina) culture and fouling control by Calliostoma.

4. Seaweed cultivation in relation to nutrient stripping.

5. Services including environmental impact assessment, quality control monitoring, and technology transfer and training.

The outcome of this proposal should be known in early 1993, and if accepted, should provide a considerable boost to Irish mariculture and marine resource use.
The Effectiveness of *Calliostoma zizyphinum* (Gastropoda) in Controlling Mariculture Mesh Fouling

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Preliminary experiments demonstrating the effectiveness of *Calliostoma* in decreasing the number, frequency and abundance of organisms growing on plastic mesh bags used for bag culture of the scallop *Pecten maximus* were reported to the first IrAmAqua conference in Galway, 1989. Since then further work has been undertaken in relation to selectiveness of this control, appropriate stocking density, and artificial rearing and maintenance of *Calliostoma*.

A test site with moderately fast current flow and a low-tide water depth of approximately 7m overlying sand was selected, in an embayment off the Strangford Lough Narrows, Northern Ireland. Eighteen 9mm mesh bags of 50 x 90 cm size were set at 1m depth in February, 1992. Densities of from 0-20 *Calliostoma* were placed in the bags which were left *in situ* until mid-September, 1992. The bags were then examined, the number of *Calliostoma* still alive determined and the large macro-fouling organisms assessed on an abundance scale. Three transects were taken on each bag, recording on each mesh section each species present and its abundance on a 0-3 scale.

Results showed the % of meshes remaining unfouled was directly related to the number of *Calliostoma* per bag (p<0.01) with up to 40% reduction of fouling the higher densities tested. The hydroid *Tubularia* was particularly affected, its frequency and density being markedly reduced with very little being present at the higher *Calliostoma* densities. The occurrence of *Laminaria* and other algae was not strongly affected, therefore other means of controlling these such as increasing depth or introducing herbivores are being sought.

If the potential mariculture use of *Calliostoma* is to be realized it may well prove important to protect natural stocks of the species since they play a key role in their environment.

Individual *Calliostoma* have been kept in laboratory conditions for up to one year on a diet of herring. The species is clearly not a herbivore as stated in various texts. Spawning has been induced by temperature increase and the directly developing young raised to 8 weeks. Attempts are being made to increase viability, decrease ciliate infection rate and rear young through to maturity.

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*Laminaria longicurulis*, a North Atlantic kelp with rapid growth rates and large biomass, is of both economic and ecological importance in subtidal coastal ecosystems, and is a key component of a variety of food webs. This species is known to suffer sporophyte deterioration at a temperature of 23°C, and summer temperature is known to constrain its southern limit of distribution. Our research shows that reproductive output in kelp is partially regulated by temperature as well. The role of temperature in determining the allocation of blade surface to meiospore production was quantified for populations of *Laminaria longicurulis* at three study sites along a temperature gradient in the Northwestern Atlantic. Using a stratified random sampling technique, adult sporophytes were collected at sites in Long Island Sound (41° 17' N), Portsmouth, New Hampshire (43° 03' N) and Halifax, Nova Scotia (44° 38' N) in the spring of 1992. Photoperiod and insolation were roughly equivalent for all three sites. The ratio of sorus area (reproductive tissue) to blade (vegetative tissue), was determined by morphometric analysis and used as a measure of reproductive effort. Nonparametric statistical analysis revealed significant variation between sites with least reproductive effort occurring near the southern limit of the geographical distribution range (Figure 1). With increasing temperature and decreasing latitude, fewer resources were allocated to reproduction. A regression line fit to the data for the three latitudes shows a positive correlation between reproductive output and latitude, with an x-intercept occurring at 37.8° N, roughly equivalent to the known observed limit of the species (Figure 2). A second regression line fit to the data (Figure 3) shows that, theoretically, reproduction does not occur at spring temperatures above 12°C, corresponding roughly to a summer temperature of 23°C. A global temperature warming of 1 to 2°C could eliminate the species from the Long Island Sound estuary by causing its geographical range to retreat northward.

*Figure 1. Box-and-whisker plots showing comparison of allocation of blade surface to reproduction in Laminaria longicurulis for spring 1992 samples at three latitudes in the Northwestern Atlantic Ocean. Circles represent medians; horizontal lines are means.*
Figure 2. Regression of latitudes vs. ratio of sorus area to blade area for samples from three locations along a temperature gradient in the western Atlantic Ocean.

Figure 3. Regression of temperatures vs. reproductive output for L. longicuris samples from three locations along a temperature gradient in the northwestern Atlantic Ocean.
Biotechnology in Seaweed Research and Aquaculture

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Recent developments in biotechnology have enabled the production of square tomatoes, bioluminescent plants, and giant mice and fish. They have also made possible the selection of disease resistant strains of domesticated organisms, and the propagation of virus-free fruit trees and ornamental plants. These useful techniques for manipulation of genetic traits of plants and animals are slowly being adapted for the domestication and improvement of aquatic primary producers, the seaweeds.

Studies and pilot scale testing of domesticated seaweeds are being attempted around the globe. In China, Korea and Japan, where seaweed farms have been in existence for generations, improved strains are being selected and propagated with traditional spore seeding and macropropagation (Fang et al., 1978; Fei, 1987; Funano, 1980; UNDP/FAO, 1989). Some researchers, however, have been looking into technologies for micropropagation and selection of new mutated varieties with single cell and protoplast technology. In the Institute of Oceanology in Qingdao, China, efforts are in progress to select for improved genetic strains of edible marine macrophytes such as Porphyra, Laminaria, and Undaria. Several research groups from Africa, Australia, Canada, France, Israel, New Zealand, Sweden and the USA are selecting strains of commercially recognized green, brown and red seaweed. Some of this work is supported by private funds and is unpublished. In many of these projects emphases are placed on: 1) massive testing of methods for microtissue vegetative-propagation; 2) selection for disease resistant strains; 3) selection for fast growing varieties; 4) selection and enhancement of seaweeds rich in gelling agents, or pharmaceutically active substances (Levy and Frindler, 1990). A recent summary of activities in applied phycology at European institutes of higher education and research centers appeared in Applied Phycology Forum (1992), a newsletter for algal biotechnology.

To achieve the desirable biotechnological manipulations, our group and others have been developing techniques for reliable manipulation of tissues in culture, induction and culture of calluses, single cell and protoplast isolation and culture, and their re-differentiation (Polne-Fuller and Gibor, 1987, 1990; Coury et al., 1991).

Although the potential is great, and interest has been expressed, government funding for these efforts is hard to come by. The lack of funding discourages many researchers, and the extent and speed of the research is greatly limited.

Enzymes from seaweed grazers and pathogens have been tested for gentle degradation of the complex polysaccharide cell walls of the targeted seaweeds, while retaining viability, vigor, and totipotency in the isolated cells and protoplasts. Abalone species such as Haliotis rufescene and H. cracherodii, the sea hares Aplysia californica and A. vaccaria, the sea urchins Strongylocentrotus franciscanus and S. purpuratus, the turban snail Astrea ungulata and the kelp snail Norissta norrisit, all contain useful enzymes for seaweed cell wall degradation. These enzyme systems produced viable and totipotent protoplasts from tissues as well as calluses.

Methods for efficient extraction of intact nucleic acids from several commercial seaweeds are now available (Hong et al., 1992; Mayes et al., 1992). These tools are steps towards genetic manipulation of isolated cells and protoplasts. Also techniques for the development of antibodies for specific polysaccharids, or other desirable compounds, have been developed as markers for selecting the best sporelings in a crop (Vreeland et al., 1992).

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