CANADIAN AQUATIC BIOTECHNOLOGY NETWORK

AQUATECH '92

RÉSEAU CANADIEN DE BIOTECHNOLOGIE AQUATIQUE

ABSTRACTS AND SUMMARIES
RÉSUMÉS ET SOMMAIRES

PROGRESS AND PROFITS FROM RESEARCH

LA RECHERCHE: SES PROGÈS ET SES AVANTAGES

HALIFAX, NOVA SCOTIA/NOUVELLE-ÉCOSSE

MARCH 1-3, 1992/LE 1-3 MARS, 1992

AQUATECH '92

PROGRESS and PROFITS from RESEARCH

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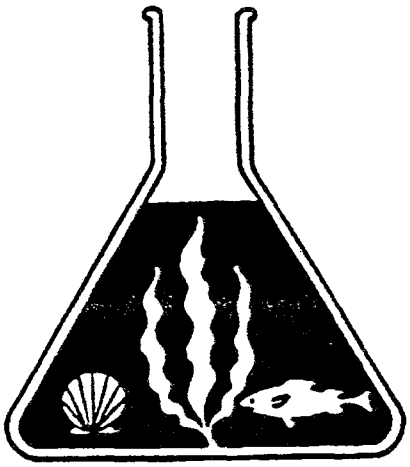
La recherche: ses progrès et ses avantages



Halifax, Nouvelle-Écosse
Du 1er au 3 mars 1992

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AQUATECH '92

HALIFAX

MARCH 1-3/1er AU 3 MARS
1992

THE AQUATECH NETWORK

AQUATECH is a network of Canadian companies, research institutes and government organizations dedicated to promoting and commercializing biotechnology related to aquatic organisms. Since its founding in 1987, AQUATECH has hosted an annual conference to foster interactions and collaborations among Canada's university, business and government sectors. The network's ultimate goal is to provide an environment that nurtures the development and growth of Canadian aquatic biotechnology enterprises and thereby aiding Canadian industry in meeting global competition.

LE RÉSEAU AQUATECH

AQUATECH est le nom d'un réseau d'entreprises, de centres de recherche et d'organismes gouvernementaux canadiens qui se consacrent à la promotion et à la commercialisation de la biotechnologie reliée aux organismes aquatiques. Depuis sa création en 1987, AQUATECH organise une conférence annuelle visant à favoriser les rapports et la collaboration entre les universités, les entreprises et le gouvernement au Canada. Son principal objectif est d'offrir un milieu propice à la création et au développement d'entreprises canadiennes de biotechnologie aquatique pour ainsi aider l'industrie canadienne à se tailler une place sur le marché mondial.

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PROGRAM

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Speakers:
N. Gray, Co-ordinator for BIOFOR
R. Brousseau, Co-ordinator for BIOCIDE
P. Darrah, Co-chair, Canadian Construction Research Board

Support Programs for Aquatic Biotechnology R & D. Chair: M. McInerney-Northcott, Canadian Aquaculture Producers' Council, Halifax, NS.

Funding Representatives:
D. Healey - National Research Council
C. Murray, D. Blair, I. Langlands - Industry, Science & Technology Canada
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A. McCulloch, NRC Institute for Marine Biosciences, Halifax, NS
E. Donaldson, West Vancouver Laboratory, Dept. of Fisheries & Oceans, Vancouver, BC
N. Sherwood, University of Victoria, Victoria, BC

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G. Friars, Atlantic Salmon Federation, St. Andrews, NB
R. Devlin, West Vancouver Laboratory, Dept. of Fisheries & Oceans, Vancouver, BC

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Conférenciers:
N. Gray, Coordonnateur pour BIOFOR
R. Brousseau, Coordonnateur pour BIOCIDE
P. Darrah, Coprésident, Commission canadienne de recherche sur la construction

Programmes d'appui pour la recherche et le développement en biotechnologie aquatique.
Présidente: M. McInerney-Northcott, Halifax (N.-É.), Conseil Canadien des aquiculteurs

Les représentants étaient:
D. Healey: Conseil national de recherches du Canada
C. Murray: Industrie, Sciences et Technologie
I.M. Price: Ministère des Pêches et Océans
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M.A. Ragan, CNRC, Institut des biosciences marines, Halifax (N.-É.)
A.W. McCulloch, CNRC, Institut des biosciences marines, Halifax (N.-É.)
E. Donaldson, Laboratoire de Vancouver ouest, MPE, Vancouver (C.-B.)
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Participants:
G. Fletcher, OSC, Memorial University of Newfoundland, St. John's (T.-N.)
G. Friars, Atlantic Salmon Federation, St. Andrews, (N-B.)
R. Devlin, Laboratoire de Vancouver ouest, MPE, Vancouver (C.-B.)

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Conférencier: J. Risley, Président, Clearwater Fine Foods Inc.

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ABSTRACTS/RÉSUMÉS

Merging Conventional Mating and Selection Practices with Transgenic Technology. G.W. FRIARS, J.K. BAILEY & F.M. O'FLYNN, Salmon Genetics Research Program, Atlantic Salmon Federation, St. Andrews, NB, Canada, EOG 2XO.

Genetic gains, resulting from classical breeding practices, are cited for animals and plants. The gains described for Atlantic salmon involved both mass selection, for a single trait, and a linear-selection index for multiple traits. The research discussed, in connection with the rat-growth-hormone gene in mouse populations, indicated that this transgene tended to disappear over generations, even where selection was applied to growth rate. Hence, the inclusion of transgenes in classical breeding programs may include monitoring for their presence as a component of multiple objective selection.

Natural Sciences & Engineering Research Council of Canada. C. ARMOUR & J. WALDEN, Strategic Grants and Networks, NSERC, 200 Kent Street, Ottawa, ON, Canada, K1A 1H5.

The Natural Sciences & Engineering Research Council of Canada (NSERC), the country's largest research granting agency, provides a balanced funding program that supports a full spectrum of research in Canadian universities. The Council is committed to supporting high quality research in the natural sciences and engineering including in strategic areas of national and economic importance, promoting industrial R & D through its partnership programs, and training the next generation of Canadian researchers.

The Strategic Grants Program of NSERC targets selected fields of research for support, and includes biotechnology and aquaculture. This program is aimed at accelerating high quality research of socio-economic benefit to Canada, training highly qualified personnel, and encouraging the transfer of knowledge to the user sector. Funding is provided for research projects of specific duration, and although eligible applicants must hold an academic appointment at a Canadian university, in the case of collaborative research initiatives, co-investigators may be from outside the university sector. Multidisciplinary research and cooperation with industry and/or government is encouraged.

Through its research partnership programs, the Council, together with industry and sometimes with other federal agencies and departments, funds research activities in the university that will yield technical advances and commercial benefits, and ultimately boost the Canadian economy. The flexibility of these programs is attractive to both small and large companies. It allows industry to develop a project it could not do in-house, either partially or completely, and smaller companies with little or no previous R & D experience benefit from collaboration with universities. The partnership programs encompass a flexible array of grants for university-industry cooperative R & D activities, technology diffusion activities, and industrial research chairs.

For more detailed information on the Strategic Grants Program, you may call (613) 996-2717 or on the Research Partnership Program (613) 996-1898.

Microbiotests in Aquatic Ecotoxicology: Characteristics, Utility, and Prospects. C. BLAISE, Centre Saint-Laurent, Longueuil, QC, Canada, J4K 1A1.

Small-scale biological tests (microbiotests) have steadily increased in development and application over the last 30 years in the field of aquatic ecotoxicology. Multitrophic level assessment requirements, attractive features of microbiotests, and the constant search for

simplicity and cost efficiency of testing are reasons explaining the expanding use of microbiotests. In this article, the major characteristics that confer popularity on microbiotests are presented and 25 currently applied aquatic toxicity microbiotests are listed. Conducted with bacteria, protozoans, microalgae, small invertebrates, and fish cell lines, these microbiotests represent a realistic cross section of those that are now becoming an essential part of ecotoxicological assessment. Microbiotests can be profitably employed for ranking and screening chemicals, for novel applications enabling rapid detection of ecotoxic effects in complex liquid samples, and for increasing the cost efficiency and diagnostic potential of hazard assessment schemes. Microbiotesting research, development, and applications will continue to surge in the 1990's, driven, among other factors, by the imperative need for cost effectiveness in environmental programs. Research in the fields of ecotoxicology, biotechnology, and immunochemistry should provide interesting breakthroughs to further enhance the specificity and diagnostic value of microbiotests.

BIOCIDE: A Dickensian Network. R. BROUSSEAU, Biotechnology Research Institute, 6100 Royalmount Avenue, Montreal, QC, Canada, H4P 2R2.

BIOCIDE has existed since 1984 and regroups Canadian researchers involved with *Bacillus thuringiensis*, a naturally occurring insecticidal bacterium widely used in Canadian forestry. BIOCIDES's fundamental characteristic lies in its minimalist approach; no newsletter, no administrative staff, no paperwork, not even an agreement on intellectual property. Despite BIOCIDE's low profile, and non-existent funding, the synergy between the participating institutions has led to remarkable scientific achievements, and places Canada well at the forefront of *B. thuringiensis* research. It is, therefore, tempting to consider BIOCIDE both as the worst network (no funding and no legal existence) and as the best network (no lawyers, and no administration).

Parasite Control: Problems and Possible Biotechnological Solutions. M.D.B. BURT, University of New Brunswick, Fredericton, NB, Canada, E3B 6E1.

A number of parasites threaten the salmonid aquaculture industry. Those that infect freshwater stages are relatively easy to control and will not be discussed; those that infect the marine stages are much more difficult to control and are considered below. In this latter group are certain roundworms, or nematodes, as well as sealice.

The nematodes in question are the sealworm (*Pseudoterranova decipiens*) and the whaleworm (*Anisakis simplex*). Both of these species are found in fish at the larval stage and both species have been reported to be increasing in wild fish. At present, there are but few reports from cultured salmonids which could be infected by eating crustaceans which inadvertently enter the sea cage or by eating fresh food made from infected fish such as herring. Control strategies could involve: (1) biological control, using a different but related worm; (2) development of vaccines for both species; and (3) killing worms in food before it is fed to salmon.

Two sea lice that are potential problems: the larger *Lepeophtheirus salmonis*; and the smaller *Caligus elongatus*. Although the larger species does more damage and is mainly responsible for the problems in Scotland and Norway, it is less of a problem in eastern Canada due to low numbers. The smaller species (*C. elongatus*) currently poses a real threat with potentials for serious losses this summer. A number of control strategies are being examined with

emphasis on biological control (based on their behaviour) rather than chemical control.

Industrial Perspective: Dealing with Shellfish Toxins. P. DARNELL, President, Indian Point Marine Farms, Indian Point, Mahone Bay, NS, Canada, BOJ 2EO.

Marine toxins in shellfish still present a challenge for aquaculture producers and processors. This past year alone, the marketing of cultured scallops was both delayed and prohibited by the presence of PSP toxins. The marketing of cultured mussels was severely compromised by the presence of DSP producing organisms and the detection of DSP toxins. The presence of domoic acid stopped the sale of some cultured oysters and mussels.

As a grower, my primary concern is to ensure that a safe product reaches consumers. The question is "Are present testing regimes adequate and how may they be improved?". In order to produce better testing for industry, several points must be addressed:

- Who has responsibility for testing - government or industry, or a combination of both?
- There is a need for quicker, and less expensive testing methods.
- As industry grows and international markets open up, the demands for frequency of testing and type of testing will increase.
- Industry is realising the need for phytotoxin testing as a management tool, not just as a regulatory procedure.
- The Quality Management Programs with which industry is working do not deal with phytotoxin testing. This is an obvious omission which would provide a level playing field for all sectors of the industry in all regions.

In Canada, the tools to manage phytotoxins are in place, or the expertise is here to develop them. What is lacking is a framework in which governments as regulators, private industry, researchers and growers are coordinated to ensure product quality and safety.

FINS (Forensically Informative Nucleotide Sequencing): Its Application to Aquaculture and Seafood Processing. W.S. DAVIDSON, Bio-ID Corporation, St. John's, NF, Canada, AlB 3X9.

We have developed a procedure called FINS that has wide applicability to aquaculture, quality control of fish processing, and the fishing industry as a whole. FINS can be used to measure the amount of genetic variation within and between different populations, and it provides an analytical tool for identifying the species origin of a biological sample. In this talk, I shall give an overview of the procedure and then give examples of how it has been applied to questions such as stock identification, the comparison of mixed fisheries, and the analysis of processed seafood. Further information concerning FINS and its applications may be found the references provided below.

1. "Identification of Thunnus tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome b genes". Bartlett, S.E. and Davidson, W.S. (1991). Can. J. Fish and Aquatic Sci. 48: 309-317.

2. "Polymerase chain reaction/direct sequence analysis of the cytochrome b gene in Salmo salar": McVeigh, H.P., Bartlett S.E. and Davidson, W.S. (1991) Aquaculture 95: 225-233.

3. "FINS (Forensically Informative Nucleotide Sequencing): a procedure for identifying the animal origin of biological specimens": Bartlett, S.E. and Davidson, W.S. (1992) BioTechniques 12:(March issue).

The Development of Nova Chem. D.H. DAVIES, President, Nova Chem Ltd., 1 Research Drive, Dartmouth, NS, Canada, B2Y 4M9.

Nova Chem Ltd. was incorporated by three professors in 1978 to commercialize knowledge obtained by academic research at Saint Mary's University. Since incorporation, it has operated with its own financing obtained through grants, contracts, and funds provided by the principals; has developed processes to produce biomedical grades of chitin and chitosan; and has invented a water-soluble derivative of chitosan, N,O-carboxymethylchitosan (NOCC), on which it holds composition of matter patents in nine countries. It has a small plant to produce these products in Dartmouth and is trying to interest larger companies to use its products in cosmetics, biomedical devices, and agriculture. This paper describes the continuing up hill struggle!

Biotechnology for Aquaculture. Production of Transgenic Salmon with Improved Freeze-resistance and Enhanced Growth. G.L. FLETCHER, C.L. HEW & P.L. DAVIES, Memorial University of Newfoundland, St. John's, NF, Canada, A1C 5S7; Department of Biochemistry, Queen's University, Kingston, ON, Canada, ; Department of Clinical Biochemistry, University of Toronto, Toronto, ON, Canada, M5G 1L5.

It is evident that the potential economic benefits of transgenic technology to aquaculture are paramount. The isolation and construction of genes responsible for desirable traits, and their transfer to broodstocks could provide a quantum leap over traditional selection and breeding methods. In addition, new traits not present in a genome can be transferred to it from unrelated species, enabling the production of new, economically valuable phenotypes. Two ways in which transgenic technology can benefit commercial fish culture are increased growth rates and increased freezing resistance. We have isolated antifreeze genes, transferred them to the genome of Atlantic salmon, and demonstrated that stable germ-line transformed fish containing low levels of blood antifreeze can be produced. We have also developed an antifreeze protein-growth hormone gene construct and demonstrated that salmon transgenic for this condition grew an average four times faster than non-transgenic controls. Research supported by NSERC, MRC and DFO, Canada.

Challenges for the Implementation of Transgenic Fish. R.H. DEVLIN, Department of Fisheries and Oceans, 4160 Marine Drive, West Vancouver, BC, Canada, V7V 1N6.

Research into the production of transgenic fish for commercial aquaculture has intensified over the past few years. Several challenges need to be met, including technical improvements to the methodology, shortening the time required to produce true-breeding lines (using gynogenesis or androgenesis), expansion to new biological areas, developing and meeting regulatory requirements, and educating and informing the public during the entire process.

While most transgenic fish have been made by microinjection of foreign DNA into fertilized eggs, alternative methods are being explored such as electroporation, liposome-mediated transfer, biolistic gene guns, and transfection of toti-potent stem cells coupled with cell or nuclear transplantation. The low efficiency with which stable lines are produced requires that large numbers of individuals be cultured, and screening is usually done by labour intensive biochemical methods, consequently, improvements are needed to either increase the efficiency of transgenic production or simplify the screening methods used to identify them (i.e. development of visible markers). New gene vector systems are

also required that can provide a range of options for controlled expression of introduced genetic information, with promoter systems that can provide different levels of protein production, tissue specificity, and inducibility.

Transgenic fish must be evaluated with respect to their safety to humans as well as to their potential impact on the environment. Human safety issues are primarily those associated with food quality, including such issues as the source of the donor DNA, the type of protein being synthesized, and the nutritional value of the flesh. These considerations should be regulated as for any other new food product under Health and Welfare guidelines. The environmental concerns are primarily associated with the escape of transgenic fish from culture facilities, and reproductively interacting with their natural counterparts or establishing new feral populations. This concern has been well recognized, and a significant effort is being applied to the development of effective physical and biological (sterilization) containment measures.

NRC Institute for Marine Biosciences. R.A. FOXALL, Director General, NRC Institute for Marine Biosciences, Halifax, NS, Canada, B3H 3Z1.

NRC's Institute for Marine Biosciences, located in Halifax, has a mandate to perform R & D in the marine biosciences and related disciplines relevant to the utilization of marine resources and protection of the marine environment. Collaboration with industry, universities, and other government departments is strongly encouraged. The research program focuses on marine biology, biological chemistry and analytical chemistry. Much of the work in marine biology involves studies of marine plants, both seaweeds and microalgae, and includes aquaculture, cell-culture, cell biology and molecular biology. The Biological Chemistry Section performs research on bioactive compounds (agrochemicals, pharmaceuticals and shellfish toxins extracted from a wide range of marine organisms, including microorganisms. Finally, the Analytical Chemistry Group studies the development and application of various methods of chromatography and mass spectrometry (and combinations thereof) for the analysis of many types of organic compounds, including shellfish toxins, PACs, PCBs, peptides and proteins. This group also manages NRC's Marine Analytical Chemistry Standards Program, which provides analytical methods, standards and reference materials for determining pollutants in the marine environment and for analysing for shellfish toxins. The presentation will give examples of current research projects with an emphasis on recent work in marine biotechnology.

Merging Conventional Mating and Selection Practices with Transgenic Technology. G.W. FRIARS, J.K. BAILEY & F.M. O'FLYNN, Salmon Genetics Research Program, Atlantic Salmon Federation, St. Andrews, NB, Canada EOG 2XO.

Genetic gains, resulting from classical breeding practices, are cited for animals and plants. The gains described for Atlantic salmon involved both mass selection, for a single trait, and a linear-selection index for multiple traits. The research discussed, in connection with the rat-growth-hormone gene in mouse populations, indicated that this transgene tended to disappear over generations, even where selection was applied to growth rate. Hence, the inclusion of transgenes in classical breeding programs may include monitoring for their presence as a component of multiple objective selection.

Recovery of Valuable Organic Constituents by Membrane Separation Technology. J.D. HAZLETT, T. MATSUURA, T.A. TWEDDLE, S. CROTEAU, C.M. TAM, A. FOUDA & O. KUTOWY, National Research Council of Canada, Institute for Environmental Chemistry, Ottawa, ON, Canada, K1A 0R9.

Recovery of organic species from process waters has implications in energy conservation, the environment and has economic potential. The use of membranes for these separations further enhances the efficacy of the separations. Membranes, present a low energy, rapid and non-degrading means of separation.

This paper discusses some of the history of membrane application at NRC as well as some operational considerations of membranes in practical separation examples. Ultrafiltration (UF), Reverse Osmosis (RO) and Gas permeation will be specifically mentioned. A new high temperature RO application for the recovery of valuable compounds from cook waters will be introduced in more detail.

The Potential of Natural Microorganisms for Oil Spill Bioremediation. K. LEE, Department of Fisheries and Oceans, Maurice Lamontagne Institute, P.O. Box 1000, Mont-Joli, QC, Canada, G5H 3Z4.

Recent events have clearly shown that despite our advances in technology, oil spills can and will occur in the future. In the long-term, contaminant hydrocarbons in the environment are primarily removed by processes mediated by microorganisms. Thus, bioremediation procedures to enhance the rate of biodegradation of pollutants have been suggested as one of the new tools for the cleaning of shorelines fouled with oil following a spill. to develop and evaluate the effectiveness and operational limits of bioremediation technologies in the environment, the Department of Fisheries and Oceans is currently conducting field experiments with spill of condensate (Scotian Shelf) and waxy crude oils (Terra Nova, Hibernia) within the intertidal zone of low-energy coastal environments in Nova Scotia. Result of recent field trials in a sand beach environment have shown that low concentrations of a waxy-crude oil (0.3% Terra Nova, by volume) could be degraded in a matter of days by the indigenous biota, while higher concentrations (5%) were much more persistent (components ads light as n-C_{11} remained after six months). These results suggest that cleanup of code oil spill at low concentrations in sand beaches may be left to nature, since natural biodegradative processes occur rapidly. At higher oil concentrations, however, the natural biodegradative rates for oil could be accelerated by the application of fertilizers, without adverse effects. Nutrient enrichment was also found to be effective in the treatment of low concentrations of waxy code oils stranded in salt marshes, provided that the oil did not penetrate beneath the aerobic surface layer.

The Potential of *Lux* Genes in Building Light-emitting Sensors for the Detection of Aquatic Pollutants. E.A. MEIGHEN, McGill University, Montreal, QC, Canada, H3G 1Y6.

Bacterial *lux* genes from luminescent bacteria have now been expressed in eukaryotes as well as a wide variety of prokaryotes as extremely sensitive reporters of a wide range of metabolic functions including gene expression. *Lux*A and *lux*B code for the α and β subunits of luciferase while *lux*C, D and E code for the fatty acid reductase catalyzing the synthesis of the aldehyde substrate for the luminescent reaction, $FMNH_2 + O_2$+aldehyde → light. Luminescence in different prokaryotes can be generated by transfer of the *lux*A-E

genes or addition of aldehyde to bacteria containing only the *lux*A and B genes. In eukaryotes, a monocistronic fused luciferase (*lux*A-B) is used so expression is under a single promoter; however, in most cases, the cells must be lysed to measure expression as sufficient $FMNH_2$ does not appear to be present in eukaryotic cells. The presence of the lux reporter system provides a convenient and highly sensitive method to measure the growth, distribution and viability of different cells as well as the response of different promoters to a variety of nutritional and environmental conditions including specific pollutants.

Research Progress for Fish Disease Detection, Diagnosis and Control. G. OLIVIER & J. DALY, Department of Fisheries and Oceans, Fish Health Laboratory, Halifax, NS, Canada, B3J 2S7.

Application of biotechnology in the field of fish disease is still considered experimental. In time, biotechnology will provide interesting tools that can be applied a aquaculture, particularly to resolve problems caused by fish diseases. In the last two years, publications have described DNA probes for the detection of some bacterial pathogens. Thai application will undoubtedly increase and bacterial pathogens including *Aeromonas salmonicida* and *Renibacterium salmoninarum*, and viral diseases like infectious pancreatic necrosis (IPN) or infectious hematopoietic necrosis (IHN). Recent results indicate that biotechnology could be instrumental in the development of new fish vaccines. this presentation describes recent biotechnological advances in fish disease diagnostic, how these new techniques will help our understanding of the epidemiology of certain fish diseases, and possibly help in detecting fish in a viral or bacterial carrier state. The impact of biotechnology in the detection and diagnosis of fish disease will be discussed including its limitations and some of the problems that may be caused when fish disease management decisions arise.

The Commercial Potential of Enzymes and Proteins from Aquatic Sources. T.R. PATEL, Memorial University of Newfoundland, Departments of Biochemistry and Biology, St. John's, NF, Canada, A1B 3X9.

Recently, fish farming and the fishing industry have attracted many investigators seeking cheap sources of enzymes and proteins of commercial value. Organic waste generated by the fishing industry is an excellent source of products including enzymes and proteins readily usable in industrial processes. Dumping the fish waste into the sea or lakes only causes environmental problems and may be considered wasteful. With the rapid growth in the Canadian fishing industry, accompanied by increased world markets for such products, efforts should be made to obtain optimum benefits by effective use of this waste. The discussion will focus on enzymes and proteins of fish origin.

Commercializing Aquatic Biotechnology. W. PATERSON, President, Aqua Health Ltd., Loretto, ON, Canada, LO6 1LO.

In the past few years, biotechnology, and in our instance, aquatic biotechnology, have become popular scientific catch words and interest areas. The number of actual applications of this biotechnology into aquaculture in a commercial sense are still limited, however.

The following flow chart outlines, from the example of Aqua Health, some of the

constituents necessary to commercialize biotechnology. Care should be taken in choosing the focus or direction since this will dictate the product, and the progress will be dictated by the knowledge and competence that you have going in, as well as the estimated market values in that area. Having chosen the biotechnology topic the company can be formed. This requires several aspects simplified into venture capital, finances, and a location.

AQUATIC BIOTECHNOLOGY	
Focus -	Fish Vaccines
Company Formation -	Venture Capital Research Facilities and Support Location
Product Development -	Choosing Products Development Regulatory Hoops
Production -	Approved and Adequate Facilities
Marketing -	Distribution Service
Expansion & Improvement by Joining Forces -	Partnerships, Joint Ventures, etc.

Then the hard work of product development comes in. Future products must be carefully chosen, dictated by the size of market and the technical feasibility in their development. You must construct accurate development time frames for all steps between the initiation of experimentation to the licensing and the implementation or commercialization of the product. Attention must be paid to production in certain areas of biotechnology. Some areas are regulated to a varying extent. For veterinary vaccines, very tight regulatory control dictates that production plants be modern and of a specific design so that international regulatory agencies can approve them.

Having developed products to the license stage, one sometimes thinks that the job is now over; however it is just beginning. Without successful marketing, the value of developed products will never be realized. You must decide what market to penetrate, how diverse geographically, whether you wish to provide the distribution yourselves or go through a distributor, and you must decide on what type of service level you are going to provide.

To move ahead after initial successes, one must consider expansion and improvement by joining forces. This could utilize partnerships, joint ventures, formation of new companies in foreign countries, etc.; the aim always being to have more products available in more market places. There are commercial opportunities in biotechnology.

Research Perspective: Quantitative Measurement of Toxins and Residues. M.A. QUILLIAM, NRC Institute for Marine Biosciences, Halifax, NS, Canada, B3H 3Z1.

Canadian aquaculture products such as shellfish and salmon are generally regarded as safe and healthy. This is in part due to clean environments and careful controls at most aquaculture sites, and in part due to monitoring programs for contaminants. With the

increasing use of antibiotics in aquaculture operations, there is a growing demand for the development of analytical methods for the identification, monitoring and confirmation of toxic contaminants. This presentation will focus on recent developments in the analytical chemistry of phycotoxins and antibiotics in seafood products. Various analytical approaches and techniques (e.g., bioassay, immunoassay and instrumental methods) will be discussed and compared.

Business Perspectives: Views on Aquatic Biotechnology Martek Corporation. R. RADMER, President, Martek Corporation, Columbia, MD, USA, 210452.

Martek Corporation is a biotechnology company whose mission is to identify, modify and culture microalgae to produce pharmaceutical, diagnostic, nutritional and biochemical products. A fundamental challenge in carrying out this mission is the rational application of the company's core microalgal technology to a widely diverse suite of products and markets.

The company currently has four major product areas (labelled Biochemicals, Nutraceuticals, Breath Test Diagnostics and New Pharmaceuticals), each with its own technical and market characteristics. The technical development of these products is being funded primarily through grants and contracts from the U.S. Government. In addition to providing the necessary monetary resources, this mechanism provides an excellent scientific audit, since these funds are only disbursed after vigorous peer review. The success and promise of these technical programs has provided the company with access to private venture capital funding. This money is being used to market the products and develop the company into a more mature enterprise.

Status and Potential for Better Utilization of Fisheries Waste in Canada. F. SHAHIDI, Memorial University of Newfoundland, St. John's, NF, Canada, A1B 3X9.

Fisheries wastes account for 70-85% of the total weight of catch and these have generally been dumped inland or hauled into the ocean. However, environmental restrictions have brought about a need for "green disposal or value-added utilization of these traditional wastes. Discards included by-catch of unwanted species, carcass from some fish after roe extraction, and offal from processing of fish, shellfish and other underutilized species, including seal. While preparation of meal and silage as well as composting provide a means for low-grade utilization of fisheries discards, preparation of value-added components from wastes has been progressing slowly. Processing discards of fisheries from different species include proteins, lipids, minerals, chitin/chitosan and their derivatives, flavourants/flavour enhancers, enzymes and enzyme support materials, pigments, and biologically active compounds. Isolation of value-added ingredients and their potential utilization in food, agriculture, pharmaceuticals, health care commodities, waste management, water purification, aquaculture, etc. will be discussed.

Surimi Processing of Atlantic Canadian Fish. K. SPENCER, Canadian Institute of Fisheries Technology, Halifax, NS, Canada, B3J 2X4.

Traditionally harvested fisheries resources are limited and yet the demand for high quality seafood products continues to grow. Surimi processing technology has been used to improve the sensory attributes and functional properties of many underexploited species.

Commercial development has primarily applied to lean fish, e.g., Pacific pollock, cod and hoki; however, research has shown that products with good gel-forming ability may be manufactured from pelagic fish. Pilot plant scale processing trials conducted at the Technical University of Nova Scotia investigated factors influencing the quality of surimi made from silver hake (*Merluccius bilinearis*), herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*). Silver hake surimi exhibited the functional characteristics required to prepare kamaboko-style surimi-based products. Herring processed within approximately 14 h after harvest resulted in surimi with excellent resiliency, good cohesiveness and moderate gel-strength. Mackerel processed at one and three days postmortem age gave moderately functional products. The research delineated methods for manufacturing surimi from lower-valued species of Atlantic fish.

Industrial Perspective: Dealing with Drug and Pesticide Residues in Cultured Fish. D. TILLAPAUGH, BC Salmon Farmers' Association, #506-1200 West Pender Street, Vancouver, BC, Canada, V6E 2S9.

Coincident with the development of salmon farming in Canada was the need to provide assurances to consumers that our product was safe to eat, wholesome and high quality. Industry took the lead in convincing government that formal regulation and inspection programs were required to provide acceptable customer assurance. From an industrial perspective, consumer safety is paramount; at the same time, communication of our safety programs has a direct bearing on product demand and, therefore, industry success. Environmental awareness, food wholesomeness and safety issues will demand increased attention by the fish farming industry in the 1990s.

The recent Consumers Report's article "Is Our Fish Fit to Eat" (February, 1992), will be examined to focus on industry responses to media attention and requirements for promoting farmed salmon in the demanding marketplace of the 1990s.

Canadian Centre for Fisheries Innovation. L.P. VISENTIN, Dean of Science, Memorial University of Newfoundland, St. John's, NF, Canada, A1B 3X7.

Established in 1989, the Canadian Centre for Fisheries Innovation is a non-profit, federally-incorporated body whose aim is to address the problems and opportunities of the Atlantic fishing industry through science and technology. The Centre is unique in that it speaks directly to the Atlantic fishing industry and with industry input sets out to translate problems into solutions. Furthermore, the Centre links the human resources and expertise of both Memorial University of Newfoundland and the Marine Institute with that of the fishing industry in order to collectively solve problems and pursue opportunities identified by the Atlantic fishing industry.

The efforts of the Centre are concentrated in four main areas: aquaculture, harvesting, processing and equipment development. The pursuit of excellence in these areas is addressed through demonstration projects, and research and development projects which pool the expertise of industry, educational institutions and government. The Centre persists in technology transfer and information dissemination initiatives so that the fishing industry may avail of scientific discoveries and state-of-the-are equipment.

Bioactives from Aquatic Organisms. J.L.C. WRIGHT, NRC Institute for Marine Biosciences, Halifax, NS, Canada, B3H 3Z1.

For years, the search for important biologically active compounds from nature, "chemical prospecting", has fuelled the pharmaceutical industry. Like any prospecting venture, the work can be long and painstaking, but if successful, the rewards can be spectacular.

Such successes have been recorded for natural products isolated from terrestrial organisms, and this success maintained the pharmaceutical industry and latterly the agrochemical industry through several decades. However, in the seventies, as successful discoveries became less frequent, there was a wide wing towards the laboratory synthesis of novel chemicals with unusual chemistry that might display biological activity.

The synthetic approach met with limited success and in the late seventies and early eighties, interest in the chemistry of marine natural products began to grow. This interest was stimulated by the frequent discovery of unusual compounds containing novel chemistry, usually with not terrestrial equivalent.

At the same time, industry was developing new and novel assays with which to screen biological activity. These new assays, many of them enzyme based, allowed chemists to probe deep into extracts of biological tissue in the search for new bioactives. Chemical prospecting became mechanised. Coupling these new, extremely sensitive bioassay methods with the development of highly sophisticated spectroscopic instrumentation for structure elucidation studies, has permitted the discovery of many unusual and fascinating chemical compounds from the marine world. The strategy for developing such a bioactives program and some interesting successes will be described.

POSTER ABSTRACTS/RÉSUMÉS DES AFFICHES

Toward the Production of Antibodies for the Marine Toxin, Domoic Acid. A. ACEL, G. LAMOUREUX, Institut Armand-Frappier, Laval, QC, Canada, H7N 4Z3; Université de Montréal, Montréal, QC, Canada, H3C 3J7.

Domoic acid (DA) is the toxin responsible for 150 cases of intoxication and four deaths following the consumption of contaminated mussels. Efficient technology to detect toxins is important; consequently, we studied the possibility of an immunological detection assay for DA in shellfish species. DA was coupled to several carrier molecules to serve as an immunogen for antibody (Ab) production in rabbits and Balb/c mice. The rate of Ab production against DA was measured in serum samples using a modified ELISA and polystyrene supports for the antigen. Only rabbits immunized with the complex of DA-FNPS-Poly-L-lysine produced Abs in sufficient quantity in rabbits and no production was observed in mice. In standardized conditions, the titre of the pooled positive sera was 1/800 compared to that of the control sera. The Abs were specific to DA since they did not react with other molecules used as negative controls. Unfortunately, these Abs do not absorb DA in solution, but only when DA is fixed on a solid support, suggesting that they have a low affinity for DA. In conclusion, it was difficult to produce anti-DA Abs since no mice and only 20% of the rabbits yielded Abs with only one having adequate titre; moreover, the anti-DA Abs are of poor quality and lack affinity for DA.

Towards an 18S ribosomal RNA gene phylogeny of the red algae (Rhodophyta). C.J. BIRD, C.A. MURPHY, M.A. RAGAN, National Research Council of Canada, Institute for Marine Biosciences, Halifax, NS, Canada, B3H 3Z1; E.L. RICE, Department of Fisheries & Oceans, Halifax, NS, Canada, B3J 2S7; R.R. GUTELL, Dept. of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO, USA, 80309.

Nuclear-encoded small-subunit ribosomal RNA genes (18S rDNAs) have been PCR-amplified, cloned, and completely sequenced from 37 red algae so far, many of which represent type genera of red algae orders. Among these are representatives of commercially important genera, *Ahnfeltia*, *Chondrus*, *Gelidium*, *Gracilaria*, *Gracilariopsis*, *Mastocarpus*, *Palmaria*, and *Phophyra*. Sequences were aligned according to secondary structure. Phylogenetic trees were derived by parsimony, distance and maximum-likelihood procedures, and bootstrap estimates of confidence intervals made where appropriate. Red algae are monophyletic vis-a-vis other eukaryotes. 18S rDNA sequence divergence among red algae is comparable to that among animals, and greater than among green plants. Red algae did not diverge especially early within the eukaryotes, and are not specifically related to fungi. Variation of 18s rDNA sequence with taxonomic level has been explored in depth within order Gracilariales (19 sequences).

Marine Analytical Chemistry Standards Program Certified Reference Materials. M.D. LEBLANC, Institute for Marine Biosciences, National Research Council of Canada, Ottawa, ON, Canada, K1A 0R6.

In 1976, the National Research Council established the Marine Analytical Chemistry Standards Program (MACSP) in response to needs of the scientific community for certified standards and reference materials required to monitor marine environmental pollutants. The objectives of MACSP are to develop the analytical methods and materials required for high-quality chemical analysis in support of marine environmental protection, marine resource management, and marine science. At the same time, NRC established the Canadian

Committee on Marine Analytical Chemistry (CMAC) as a forum to provide MACSP with ongoing advice and program direction. Composed of marine and analytical chemists representing government, university and commercial laboratories, CMAC aids in identification of problem areas in environmental analysis. MACSP is managed by NRC's Institute for Marine Biosciences (IMB) in Halifax and is operated jointly with NRC's Institute for Environmental Chemistry (IEC) in Ottawa. IEC's role is to develop methods and reference materials for the determination of trace elements and other hazardous constituents in marine sediments, biological tissues and seawater. At IMB, analytical methods, reference materials and standards are developed for organic constituents such as shellfish toxins, polychlorinated biphenyl compounds (PCBs) and polycyclic aromatic hydrocarbons (PAHs).

These reference materials and standards, as well as the analytical methods we develop, from a solid base for analysis to draw upon in monitoring the environment. Thus far, response to the MACSP has been favourable. Over the years, the National Research Council's Marine Analytical Chemistry Standards Program has become internationally known. MACSP methods as well as the program's certified reference materials and standards are now used by analysts worldwide to monitor the marine environment. We continue to develop new standards, reference materials, and methodologies based on users' recommendations for new materials coupled with advice provided by the Committee on Marine Analytical Chemistry.

The Influence of Prolonged-release Recombinant Porcine Somatotropin Therapy on Growth and Sexual Maturation in Coho Salmon. E. MCLEAN & E.M. DONALDSON, West Vancouver Laboratory, Department of Fisheries & Oceans, 4160 Marine Drive, West Vancouver, BC, Canada, V7V 1N6; I. MAYER, Department of Zoology, University of Stockholm, Stockholm, Sweden; E. TESKEREDZIC, Ruder Boskovic Institute for Marine Research, Bijenicka 54, 41000 Zagreb, Croatia; C. PITT & L.M. SOUZA, Amgen Inc, 1900 Oak Terrace Lane, Thousand Oaks, CA, USA, 91320.

It has long been recognized that injection of exogenous somatotropins (st) stimulates appetite, accelerates growth, and improves feed conversion efficiencies in teleosts. Moreover, salmonids exogenous st assists in mediating those physiological responses that prepare juveniles for saltwater transfer. Technically, however the major restriction to the use of st in the commercial production of salmonids is the lack of practical methods of administration. While st injection induces decisive growth acceleration in fish, the necessity for frequent handling limits the use of this procedure. A reduction in the need of for manipulation, however, is afforded by prolonged-release devices, but no information exists concerning the long-term effects of sustained-release st therapy on fish. Accordingly, five groups of coho salmon *Oncorhynchus kistuch* were implanted with a placebo or one of 4 types of recombinant porcine st-containing slow-release pellet. Each type of rpst-based pellet was coated with polyvinyl alcohol, the thickness of which controlled the liberation kinetics of rpst. Following implantation, the growth response of each group was monitored over 2 years. All fish in receipt of rpst therapy expressed significant growth acceleration relative to placebo implanted animals, for 100 days after treatment. This growth advantage was subsequently maintained for in excess of 70 weeks. At the termination of the trial gonadosomatic, visceral and hepatosomatic indices were calculated, together with an examination of relative gut lengths, for all animals. The results of this study will be discussed with reference to the potential employment of sustained-release rpst therapy by the salmon culture industry.

Chemotaxis in Zoospores of two Fish-egg Pathogenic Strains of *Saprolegnia diclina* (Oomycotina: Saprolegniaceae) Toward Salmonid-egg Chorion Extracts, and Selected Amino Acids and Sugars. T.G. RAND, Department of Biology, Saint Mary's University, Halifax, NS, Canada, B3H 3C3.

Recent work in my laboratory has indicated that settlement and subsequent development of zoospores of *Saprolegnia diclina* on brook charr, *Salvelinus fontinalis*, egg surfaces are important contributing factors to the establishment of egg mycosis. This study determines whether zoospores of two strains of this fungal species demonstrate positive chemoresponses toward concentration gradients of brook charr egg chorionic membrane extracts, selected amino acids and simple sugars, under experimental conditions. Zoospores of both strains exhibited significant positive chemotaxis toward the chorionic membrane extracts, the amino acids arginine and alanine, but not toward the other amino acids nor any of the sugars used in the assays. The results indicated that chemotaxis may have an important role in attracting zoospores of *S. diclina* toward living salmonid eggs. They also suggest that chemotaxis might be used in the development of a strategy to control or eliminate saprolegniaceous infestations among salmonid eggs raised in hatcheries.

Interaction of [D-ARG^6, PRO^9-NEt]-sGnRH Ethylamide and Domperidone on the Induction of Gonadotrophin Secretion and Spawning in the Thai Carp (*Puntius gonionotus*, Bleeker). N. SUKUMASAVIN & E.M. DONALDSON, West Vancouver Laboratory, Department of Fisheries and Oceans, West Vancouver, BC, Canada, V7V 1N6; W. LEELAPATRA, Pathumthani Freshwater Fisheries Station, Department of Fisheries, Thailand.

The effects of intraperitoneal injection of various combinations of [D-Arg^6,Pro^9-NEt]-sGnRH (sGnRHA, 0-10O µg/kg) and a dopamine antagonist, domperidone (Dom, 0-10 mg/kg) on the induction of gonadotropin (GtH-II) secretion and spawning in mature female Thai carp were investigated. Administration of sGnRHA or Dom alone at 10 µg/kg or 10 Mg/kg respectively, significantly increased plasma GtH levels at 3 and 6 hr after injection, but failed to induce spawning. Combinations of sGnRHA and Dom stimulated GtH secretion in a dose-related manner, and combinations at dosages equal to or greater than 5 µg/kg and 5mg/kg respectively were very effective in stimulating plasma GtH secretion and inducing spawning in this species.

In the peak spawning season, we were able to induce ovulation in 30% of the fish treated with domperidone alone. We conclude that dopamine plays an important role in the regulation of GtH secretion in this species.

Evaluation of the Fluidaire Biofilter for Commercial Production of Arctic Char. W. VAN TOEVER, J.N. BOYER, Waterline, RR2, North Wiltshire, PE, Canada, C0A 1Y0.

Replicate 2 m^3 tanks equipped with 0.125 m^3 biofilters were stocked with 60 kg (30 kg/m^3) of Arctic char of 162 g average weight. Inlet water flows were set at 1.5l/min, flows through the biofilter were ca. 60 l/min (hydraulic loading of 0.69 m^3/m^2/day), and airlift aeration flows were ca. 90 l/min. Fish were fed to satiation daily. After 3.5 months, the fish had grown to an average weight of 352 g with total biomass of 130.4kg (65 kg/m^3). The predicted size of these char using our hatchery growth rate (unpublished data) was 337 g. Therefore, the char in biofilter systems grew as well if not better than those on flow-through. By the end of the trial, more than 1 kg of feed at 46% protein content was being

consumed daily, resulting in the production of ca. 18 g NH_4-N/day. The average specific activity of the biofilters was 260 g-N/m^3/day, for a total biofilter capacity of 32 g-N/day. Unlike trickling filters, the specific activity of the biofilter increased (9-260 g-N/m^3/day) with hydraulic loading (0.05-0.69 m^3/m^3/day). We believe that higher water flows through the biofilter cause the media to expand and fluidize resulting in an increase in the active specific surface area of the bed.

Isolation and Fusion of Protoplasts from *Porphyra haitanensis* and *Porphyra yezoensis*. Y. ZHOU, East China Sea Fisheries Institute, Shanghai 200090, People's Republic of China; S. WANG, Shanghai Fisheries University, Shanghai 200090, People's Republic of China.

Porphyra haitanensis and *Porphyra yezoensis* are two staple seaweed species cultivated on nets in China. Using an enzyme-based method, large numbers of viable protoplasts were isolated from both of these species. Protoplasts of the two species were fused using a polyethylene glycol (P6) treatment. Differences in the colour and other physical characteristics of the protoplasts were used as markers to detect fused cells. Many heterokaryocytes and homokaryocytes derived from *P. haitanensis* and *P. yezoensis* appeared in the preparation following washing in high Ca^{++} medium and addition of protoplast culture medium. The frequency of completely fused cells was 8-10%. Fused cells generally survived for 2-3 days. One heterokaryocyte divided after 40 days, and generated filaments after two months in culture. Although the survival and growth of fused cells needs considerable improvement, we are optimistic that physiologically-based colour and starch differences used in this study will prove to be valuable tools in the future selection and documentation of fusion cells.

Final Report to the AQUATECH Steering Committee on the "Building R&D Networks" Workshop

Held Sunday, March 1, 1992, 3:00-5:30 pm.

General:

- three invited speakers presented 15-20 minute talks on the networks they co-ordinate. Afterwards, an AQUATECH Steering Committee member, Dr. John Spence, was asked to present his proposal for an aquaculture R&D network
- these presentations were followed by over 1 1/2 hours of discussion on networks in general and on AQUATECH in particular
- the workshop forum did provide a good opportunity to explore the successes of other networks and suggest means of strengthening AQUATECH

Highlights of Presentations:

Dr. Neil Gray, Team Leader of ICI Bioproducts and Chair of BIOFOR Network:

- BIOFOR's 6-member Executive of the Steering Committee is very active and meets frequently. In general, the sponsoring organizations of Steering Committee members pay for expenses associated with their Cttee activities
- BIOFOR has theme-oriented conferences which attract over 200 attendees as well as smaller ongoing discussion groups and networks. These conferences, groups and meetings are piggybacked onto existing forums where possible. Dr. Gray suggests that AQUATECH and BIOFOR might meet jointly - especially to explore marine environmental issues
- research projects have been funded by network participants to tackle problems of interest to industry members
- there is strong industrial support for the network and for its annual meetings, including direct financial input.

Dr. Roland Brousseau, Head of the Gene and DNA Synthesis Group at NRC's Biotechnology Research Institute, and Co-ordinator of the BIOCIDE Network:

- BIOCIDE is an active network of about 25 researchers
- the network does not include commercial 'users' of the technology although network participants are able to pursue these linkages on their own
- research is very focussed on *Bacillus thuringiensis* (Bt) with short to medium range research goals
- BIOCIDE has resulted in notable research achievements
- members' participation is mostly sponsored by their parent organizations
- BIOCIDE has no newsletter, administration or other infrastructure.

Mr. Patrick Darrah, Executive Director of the St. John Construction Association and Member of the Canadian Construction Research Board (CCRB):

- the CCRB and other construction R&D networks have grown very quickly and are now successful in linking scientists to the relatively non-technical users of their technology
- construction networks focus on the most important problems of the sector
- the construction industry sponsors university research chairs

- rapid communications have proven to be key to this success. Electronic mail, including electronic bulletin boards, was seen as a particularly effective means of spreading information and getting answers to technical questions quickly
- networks' activities must be strongly promoted to potential users and funders

John Spence, Director of the BC Aquaculture R&D Council (BCARDC):

- outlined BCARDC's work to: (i) form networks to assist in problem solving on behalf of the aquaculture industry; and (ii) develop research consortia to produce tools and services for this sector
- suggested that AQUATECH might assist these networks

Points Arising from the Discussion:

- that AQUATECH was underused and not well-known but that it could indeed provide the forum for discussing the major aquatic biotech R&D needs of its industry client communities. To achieve this, workshop participants suggested that the AQUATECH Steering Committee should:

  - become more active in shaping AQUATECH to meet industry's needs
  - re-think AQUATECH's mission statement to determine what the network wants to achieve (i.e. does AQUATECH want to take an an advocacy role for industry, provide a forum for co-ordinating research activities or for discussing R&D issues, etc.)
  - work with industry to identify their major problems/opportunities and form and promote AQUATECH discussion groups or nodes around these issues. Some suggested that these nodes could be regional to enhance communications. These AQUATECH nodes would then communicate independently between annual AQUATECH meetings
  - organize meetings/symposia/workshops more frequently than annually to discuss specific issues of interest to AQUATECH members
  - organize annual meetings around themes (e.g. shellfish toxins, regulatory issues)
  - organize joint meetings/workshops with related networks or other relevant conferences (in particular, determine which meetings AQUATECH's industry communities attend and piggyback on these)
  - piggyback on existing newsletters or e-mail networks (which are read/used by client communities)
  - strive to ensure that each AQUATECH conference becomes a "must-attend" meeting for its business opportunities content
  - publicize AQUATECH and expand membership to include more of its client communities and promote the benefits of belonging
  - examine opportunities to link with other networks, e.g. the BC Biotech Alliance and the Canadian Institute of Biotechnology (CIB). The suggestion was made that AQUATECH consider sending an industry representative to sit on CIB's Board of Directors.

JCY March 2/92

Notes for Building R&D Networks Workshop

AQUATECH's Mandate, Role, and Membership (from DFO - see also description of AQUATECH attached):

- AQUATECH was formed in 1987 as one of several biotechnology networks under the National Biotechnology Strategy

- AQUATECH is a network of Canadian companies, research institutes and government organizations dedicated to promoting and commercializing biotechnology related to aquatic organisms. Since its founding, AQUATECH has hosted an annual conference to foster interactions and collaborations among Canada's university, business and government sectors. The network's ultimate goal is to provide an environment that nurtures the development and growth of Canadian biotechnology enterprises thereby aiding Canadian industry in meeting global competition

- membership is open to those who are interested in biotechnology R&D focussed on aquatic species related to aquaculture, fisheries and other commercial endeavours for producing food products, aquatic bioactives, and other byproducts, as well as ancillary technologies

- in the short term, AQUATECH's role is to establish a communications network to promote interactions among members and foster national and international collaboration in aquatic biotechnology

- proposed activities include:
  - annual meetings
  - workshops focussed on commercial and R&D opportunities
  - encouraging the development and maintenance of bibliographies, mailing lists and inventories
  - identifying research priorities and policy issues to national policy and funding agencies

AQUATECH Resources:

- AQUATECH receives an annual grant of $25K from Industry, Science and Technology Canada (ISTC) which is administered by DFO - Aquaculture and Resource Development Branch. This money is used mainly to cover the costs of AQUATECH's annual meeting including speaker/chair and Steering Committee expenses. Annual meetings are organized by volunteer committees mostly headed/championed by a Steering Committee member

- AQUATECH '92 applied for and received funding from the Canadian Institute of Biotechnology to cover increased costs of: speaker/chair and some Steering Cttee. travel and general conference expenses. This source of $ may be available for future AQUATECH activities

- there is little likelihood that government funding ($'s and/or personnel) for AQUATECH will be increased

- the Director of DFO's Aquaculture and Resource Development Branch also serves as the Secretary of AQUATECH's 12-member Steering Committee. This Cttee meets about once a year usually at the time of AQUATECH's annual meeting

AQUATECH's Activities and Effectiveness:

- to-date, AQUATECH activities have been centered primarily around an annual meeting with about 65-100 people in attendance from university, government and academia. Little formal interaction takes place outside this forum. Recently, however, AQUATECH provided the "network" for members to discuss an industry problem (sea lice).

- it seems to be generally held that AQUATECH is a relatively unknown and underused organization. Conference agendas have been variable and usually reflect the interests of the organizing committee

- AQUATECH has received little private sector funding

Various Networks Surveyed:

- various networks and associations surveyed or reviewed as background to the AQUATECH R&D Networks workshop include: aquaculture and fisheries organizations, NSERC Centers of Excellence, software networks, various engineering networks, construction association networks, biotechnology networks (e.g. BIONET, BIOCIDE, BIOFOR), the Canadian Advanced Technology Association, NRC-Industrial Research Assistance Program field officers and national elements co-ordinators, Canadian Institute of Advanced Research, and Industry Science and Technology Fisheries Sector Campaign and other technology networks.

Common Features of Successful R&D Networks:

- clear common **need** and **benefit** to participants, i.e. these networks offer something to participants which allow them to do their jobs better

- "buy-in" from participants, active participation and $ contributions - $ is available for travel, meetings, graduate students, etc.

- dedicated personnel (for such activities as maintaining databases, producing newsletters/publications, organizing get-togethers, circulating relevant material, etc.)

- real use of membership lists to share information, e-mail especially growing in popularity as communications tool

- regional or 'interest' nodes or chapters

Some Features of Failing R&D Networks:

- *usually* lack of features listed above!

- competitive field - participants unwilling to share information

AQUATECH as Seen by Those Surveyed:

- difficulty in defining the aquatic biotech audience, i.e. especially hard to address the relatively nuts and bolts technical problems of aquaculture operators and the commercial opportunities of advanced biotechnological applications such as transgenics at the same meeting
- no dedicated personnel to propel network and capitalize on opportunities
- conferences not scientific enough, need more leading edge and international science discussed and less commercial/market information
- conferences too scientific, need more nuts and bolts industry-relevant discussion and more commercial/market information
- some believed, however, that AQUATECH was one of the few places where aquatic biotechnology is discussed in Canada and where a good mix of technical / commercial topics was discussed
- little communication outside of annual meeting
- need for more private sector input and leadership - too dependant on public funds

JCY Feb. 28/92

FULL PAPERS/TEXTES COMPLETS

INDUSTRIAL PERSPECTIVE: DEALING WITH SHELLFISH TOXINS

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Marine toxins in shellfish presents a serious challenge for aquaculture producers and processors. In 1991, the marketing of cultured scallops from Newfoundland was both delayed and prohibited by the presence of PSP toxins. On our own farm in Nova Scotia, we had to interrupt the marketing of mussels for two months because of PSP toxins. The presence of domoic acid or Amnesiac Shellfish poisoning stopped the sale of cultivated oysters and mussels in parts of PEI. As a shellfish producer in Nova Scotia my primary concern is to be certain that when my product goes to market, it is safe to eat. So the question I would like to ask today is whether present testing regimes and procedures are adequate to ensure that all shellfish going to market is safe and if the answer is not an unequivocal yes, how may they be improved?

Presently, Health and Welfare Canada sets the standards for food safety but with regard to seafood, Fisheries and Oceans Inspection Branch determines what resources are dedicated to testing and what procedures to follow. DFO is divided into regions (Scotia-Fundy, Gulf, Newfoundland, Quebec, Central & Arctic and Pacific), and there are different standards and regimes from region to region.

DFO does not have unlimited resources to dedicate to shellfish testing and the fact is that we have a rapidly growing shellfish industry while Government departments, including DFO's Inspection Branch, are facing cutbacks. Some might also agree that phytotoxin blooms are increasing worldwide, creating an increasing need for vigilance by shellfish producers. In this context DFO has not been able to test individual batches of mussels before marketing but rather has adopted a sector approach, whereby they monitor larger areas on a periodic basis and the safety of products from individual firms is inferred from the process. (In actual practice, sampling aquaculture operations is the easiest way to carry out this sector approach and many of us do get our product tested on a periodic basis.)

As our industry grows, and resources are stretched, new approaches to shellfish testing must be developed. This is happening on an ad hoc basis. For example, in Nova Scotia, DFO Inspection Branch initiated an introductory phytoplankton monitoring program which proved successful in forecasting toxic blooms, but DFO had no money to continue and expand the program. Because the growers thought the program was critical, the Nova Scotia Aquaculture Association obtained money (again for one year) from the Nova Scotia Department of Fisheries for a technician to carry out the program in the DFO lab in Halifax. Industry and two levels of government successfully collaborated in a program deemed important by the industry. On PEI, growers can have PSP and ASP tests carried out by the Atlantic Veterinary College at a subsidized rate - third party testing. In Nova Scotia, the offshore scallop industry which wanted to market roe-on scallop from George's Bank took advantage of a private company, Fenwick Laboratory, who were accredited by DFO to do the testing of their products. This was done, as I understand it, because DFO would not take on the responsibility for testing this new product.

Who has the responsibility for shellfish testing? Government, industry or a combination of both? DFO states in its Aquaculture Strategy that it will "continue to concentrate on marine toxins research and on the control of molluscan shellfish harvesting, shipping and

processing" and "will continue the present focus of research and the monitoring of toxins in molluscan shellfish (such as paralytic shellfish poisoning and domoic acid)". Yet there remain many shellfish growers across Canada who are not satisfied with the level of testing done and who want more. The industry has identified the lack of consistency in testing offered by DFO from region to region as a problem. While one region may be very satisifed with the service offered by DFO, another region may not be and would like to supplement it with third party testing, but cannot afford it when their competitors do not have similar expenses.

Atlantic Canada's mussel industry has started marketing in Europe in countries in which the government bears the testing cost. Many in the industry feel that if the producer bears the cost of testing, they will be uncompetitive in this new marketplace. Shellfish entering Europe is routinely tested for Diarrhetic Shellfish Poisoning, yet DSP testing is not routine in Canada. In fact, DSP is a good case for illustrating some of the problems phytotoxins pose for the aquaculture industry.

DSP was positively identified at two sites on Nova Scotia's Atlantic Coast, approximately 100 miles apart (Mahone Bay and Ship Harbour). At one site it has occurred for two consecutive years. The causative agent is thought to be Dinophysis, which seems to be ubiquitous throughout Atlantic Canada. However, Dinophysis is not always toxic. At one site, DSP was present in mussels when Dinophysis levels were as low as 600 cells/litre but was not present when levels were as high as 32,000 cells/litre. At this moment, scientists do not understand what makes Dinophysis toxic only sometimes. To exacerbate the Canadian producers' problem, DSP is not an officially recognized problem, despite the fact that it has been identified here for nearly two years. Health and Welfare Canada has not given official status to DSP nor set regulatory limits. Until it does, DFO's Inspection Branch cannot routinely test for the toxin, although it has done and will continue to do so in the two sites where it has occurred before. DFO's mouse bioassay for DSP is expensive, time-consuming and very imprecise. Chemical testing methods have been developed but are very expensive and difficult. Clearly, it is imperative that Health and Welfare Canada must do its job more quickly and set regulatory limits and DFO must start to routinely monitor for DSP in Atlantic Canada.

Conversely, ASP or domoic acid seems to be handled quite well in the areas of PEI where it routinely occurs. Phytoplankton monitoring of the causative agent, Nitzchia, indicates when testing must be intensified. The testing method is a chemical one and it is straightforward and precise. Closures are carried out without negative publicity and most importantly, without consumer illness. The pattern of occurrence is becoming recognizable in eastern PEI, and I feel that a comprehensive phytoplankton monitoring program on all shellfish farms will provide early warning of potential problems.

I cannot stress too strongly the importance of phytoplankton monitoring programs. Carried out by either the farmers, or contractors, this monitoring gives farmers and regulators the notice that intensifed product testing must occur. While not foolproof, this is cheap insurance for producers and consumers, and I feel that ongoing phytoplankton monitoring should be a condition that must be met by producers in order to be in the shellfish business.

Paralytic Shellfish Poisoning (PSP) is certainly the most frightening of the shellfish toxins because of its extreme toxicity and the complicated nature of the toxins. Ten years ago when I started growing mussels, we thought that PSP only occurred in the Bay of Fundy, Gulf of Maine and some remote parts of the upper Gulf of St. Lawrence. In other words, somebody else's problem. Then PSP occurred in Shelburne Harbour - but we thought that it

was an anomaly, probably due to stocking of scallops from George's Bank. But last year there was PSP in Ostea Lake on the eastern shore, and in Newfoundland. Gone is the sense that "it won't happen here". In fact, that sense of security was shaken by the occurrence of domoic acid in PEI and gone for good when our mussels had DSP. I think it only prudent to presume that anything can happen, at any time. That requires constant monitoring and testing which is neither required of shellfish farmers, nor offered by DFO Inspection Branch. And, there is not much room in the profit pictures of most operations for expensive third party testing.

PSP testing is time consuming and expensive. The mouse bioassay is used, so testing must be centralized and there can be several days between shellfish being taken from the water and the receipt of results. And the mouse bioassay can only pick up PSP toxins at 40 mouse units, where the regulatory limit is 80 mouse units, and under bloom conditions, this gap can be bridged very quickly. Once again, I think that phytoplankton monitoring can serve as an early warning of a problem.

My primary goal is to ensure a product that is safe to eat for my customers and as a to make some money. If I discover a problem with my product only after harvesting some large quantity which then has to be dumped, the already thin profit margin disappears. Therefore, industry is becoming much more aware of the need for phytotoxin testing as a managment tool. We need to test products at the farm so harvesting decisions can be made intelligently but quickly. At present this capability just does not exist. Indeed, the Canadian shellfish industry finds itself in a Catch-22 situation. Rapidly increasing supplies, both domestic and imported, put downward pressure on price while consumers areshowing a greater awareness of food safety issues and are demanding higher standards.

One last problem is that in some provinces the so-called "fisherman packer" is exempt from provincial inspection regulations and if he/she sells their products within the province they are not subject to federal regulations either. So it is quite possible that shellfish can get to the market place having by-passed all inspection measures. Clearly this loop-hole must be closed.

I originally asked, "Are present testing regimes adequate?" I feel that they are not. Yet at a meeting in Ottawain 1991, senior people from DFO's Inspection Branch indicated that they felt that their inspection mandate was being adequately fulfilled. This indicates a gap between what government feels is required and what industry feels it needs. How is this gap to be narrowed? This most obvious requirement is for more accurate, quicker and less expensive testing methods, particularly for PSP and DSP. A very ambitious program dealing with those needs is about to start. Fenwick Laboratories of Halifax supported by the National Research Council and industry will attempt to develop better methodology for shellfish toxin testing. Yet even this is controversial. Many in the industry think that this research should be carried out by Fisheries and Oceans, that they should fulfill the obligations they took on in their Aquaculture Strategy. Some in the industry feel that new methods developed and owned by companies like Fenwick will force a shift from DFO being the tester to the third party. This will happen, not as a result of a change in policy, but because DFO will be left behind on technological development. We should address this question of responsibility now.

DFO Inspection has recently embarked with industry on a new inspection concept - that is Quality Management Programs or QMPs. All federally registered processing plants must have a QMP in place and there is a very different philosophical basis to this. Under QMP, the plant polices itself and the new DFO role is one of monitoring and auditing the QMP.

Yet for shellfish producers, the whole question of product testing has not been addressed. Yet QMPs may be the perfect vehicle for addressing the industry's problems.

I feel that a national conference of producers, regulators and scientists should be convened to develop a Quality Management Program for each sector of the shellfish industry. Hypothetically, for the mussel industry, the QMP may be something like this: (1) Weekly or bi-weekly phytoplankton monitoring, (2) As cell counts increase of potentially dangerous phytoplankton, product testing would be stepped up. Perhaps weekly testing for PSP should be carried out regardless, as it is so potentially dangerous. The actual mechanics of the QMP would be developed by the conference, and the conference would also address the question of who would do the testing, and who would pay for the testing. As better technologies are developed, these would be incorporated into the QMPs. The important thing would be that all shellfish on the market would be treated equally - the level playing field would be established. And the "Canada Inspected" logo which QMP plants are allowed to use would have real meaning for the consumer.

In Canada, all the tools to manage the problem of phytotoxins are in place, or the expertise to develop them is here. What is lacking, is a clearly defined framework in which regulators, producers and researchers act in a coordinated manner to make best use of our own world-leading knowledge to produce the safest possible products for our customers. I really feel that this is the shellfish aquaculture industry's number one challenge. We are a new industry and we need innovative approaches and cooperative solutions in order to provide safety assurances for our products.

MERGING CONVENTIONAL MATING AND SELECTION PRACTICES WITH TRANSGENIC TECHNOLOGY

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Introduction

The application of breeding systems that control both mating and selection practices have produced significant genetic gains in animals and plants. Documented examples of realized genetic gains, through the use of controlled breeding programs are cited in Schnell (1988) in plants, Olivier (1988) in several farm animal species, McMillan (1990) in layer-stock chickens and Friars (1990) in Atlantic salmon.

The successful transfer of metallothionen-growth-hormone-fusion genes from rats to mice by Palmiter, Brinster, Hammer, Trumbauer, Rosenfeld, Birnberg and Evans (1982), and Sabour, Ramsey and Nagai (1991) has stirred the interests of animal scientists. Similarly, several exciting results with transgenics in fish have been cited by Fletcher (1991), particularly with respect to growth-hormone and antifreeze-protein genes.

The very credible advances in scientific knowledge and technology pertaining to transgenics, in the last ten years, have come to pass without much alliance with proven breeding practice. Included in this paper are: 1) examples of realized responses to selection in Atlantic salmon, 2) a reference to selection in mice where difficulties have been encountered in maintaining the frequency of the rat growth-hormone gene in both selected and non-selected populations of mice and, 3) proposed approaches to merging transgenic effects with technology involving mating systems and selection.

Realized Responses to Selection

Mass selection is a simple method of choosing breeders, where the best individuals, in terms of some trait such as weight, are selected and mated together to produce offspring. A mass-selection experiment for fork length in Atlantic salmon, that had matured after one sea winter (grilse), was conducted in the Salmon Genetics Research Program (SGRP) between 1985 and 1988. The founding population was pedigreed, allowing the avoidance of brother-sister matings. A control line was established by the sampling of a son and a daughter from each of approximately 50 full-sib (same mother and father) families. These matings produced progeny that could be used as a genetic "bench mark" for comparisons to progeny from selected parents. A similar number of selected parents, based on fork length, were mated, again avoiding brother-sister matings, to produce select progeny. The selection differential or reach (difference between the mean of the selected parents and the total population which is represented by controls between generations, Fig.1) was 6.70 cm.

The realized response, in the select compared to the control progeny after 70 weeks in sea water was 1.84 cm. The ratio (realized response/selection differential= 1.84 cm/6.70 cm) yielded a realized heritability of .27 (Anonymous, 1989), a result that is exciting from the standpoint of a breeder wishing to improve growth rate.

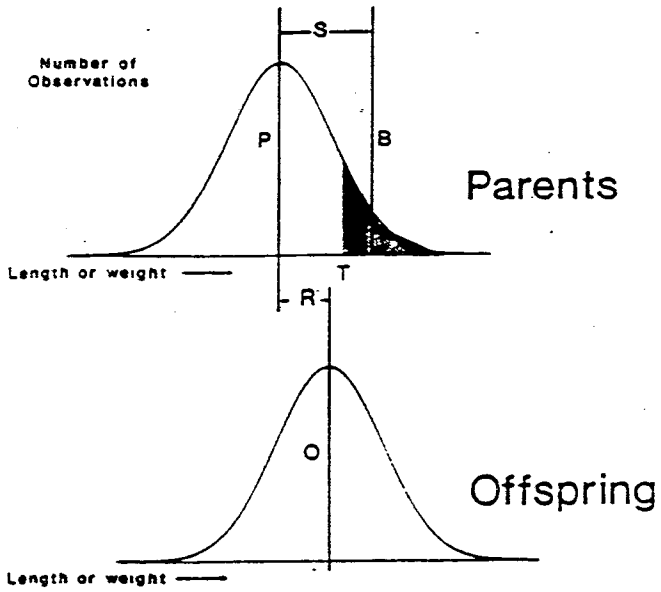

Fig. 1. Frequency distributions of a continuously varying trait, such as weight or length, where mass selection has shown a positive response. The upper panel shows the parental distribution and the lower panel depicts the progeny generation. The symbols are defined as follows: T is the point of truncation (the minimum acceptable value for selection); P, B and O are mean values of the total parental population, selected parents and offspring respectively; S is the selection differential or reach (B-P); and R is the response to selection (genetic gain).

A second selection experiment was conducted in the SGRP between 1988 and 1992. Estimated and assumed values of genetic and phenotypic variances and covariances and economic weights for each trait were used to form a linear selection index (eg. Van Vleck, 1981). The traits involved in the index were parr length, percent smolt, harvest length (after about 16 months in sea water) and mature length (after about 29 months in sea water). The fish in the second experiment were derived from the Saint John River (SJR), where the early run denotes fish returned to Mactaquac before August 1st and the late run after August 31st. The greatest selection emphasis was placed on harvest length. The selection differential on the index was 1.27 and .95 standard deviations in the early and late groups respectively. The SJR stock is desired in the Bay of Fundy aquacultural industry because its low frequency of grilse allows a high incidence of sizes that fit predominant markets (8-9 pounds after 16-18 months in sea cages). The responses to selection are apparent in both fresh and sea water traits (Table 1).

The genetic gains from selection for the traits in the index ranged from .24 standard deviations for fry length in the late run to .60 for percent S1 smolt in the early run (Table 1). The gains in the number of eggs per female and egg size represent correlated responses at the phenotypic level, since these traits were measured on the parents. The gain of .33 to .37 standard deviations for length and weight at market time (after 18 months in sea water) is of consequence. The mean difference between the control and select lines is approximately 340 grams or three quarters of a pound. Such a gain combined with progress in other traits is of substantial economic significance, amounting to several million dollars for the Atlantic salmon marketed each year from the Bay of Fundy cage-culture industry.

Table 1. Mean index values of traits (*) and correlated responses in fresh and sea water (SW) in Saint John River fish. Gains in standard deviations (SD).

Trait	Early Run			Late Run		
	Control	Select	Gain (SD)	Control	Select	Gain (SD)
No. of eggs per female	14,594.00	15,258.00	0.15	14,343	15,513	0.45
Egg size (mm^3)	102.68	107.30	0.42	106.83	108.78	0.18
Fry length (cm)*	6.34	6.49	0.22	6.46	6.80	0.50
Fry weight (g)	2.78	2.98	0.19	3.08	3.62	0.51
Parr length (cm)	10.98	11.60	0.55	11.52	11.42	-0.12
Presumptive Smolt length (cm)	16.72	16.94	0.19	16.80	16.99	0.12
Early survival (%)	47.50	57.70	0.26	65.60	48.40	-0.44
Late survival (%)	23.30	46.00	0.10	40.50	59.50	0.08
S1 Smolt (%)*	34.91	48.67	0.60	46.50	50.90	0.25
Length-4 mo. SW (cm)	37.39	37.79	0.20	37.98	38.26	0.12
Weight- 4 mo. SW (kg)	0.62	0.64	0.16	0.66	0.65	-0.02
Length- 18 mo. SW (cm)*	69.21	71.28	0.33	70.14	72.61	0.37
Weight- 18 mo. SW (kg)	4.15	4.47	0.35	4.35	4.75	0.35

Part of these data were presented at the 4th International Symposium on Genetics in Aquaculture, Wuhan, China, Apr. 29-May 3, 1991 and are in review for a special edition of Aquaculture, dealing with the Symposium.

Rat Growth Hormone Gene in Populations of Mice

The publication by Palmiter et al. (1982), dealing with very large increases in the size of mice carrying a growth hormone gene from rats, caused considerable excitement among animal scientist. Research by Sabour et al. (1991) involved this gene in populations of mice of different origin from that used by Palmiter et al. A control and a line selected for 42-day weight were derived from synthetic gene pools made up of inbred lines 15 generations earlier. These two lines were crossed and backcrossed to mice carrying the rat-growth-hormone gene (rGH) to form two base gene pools. These gene pools were randomly mated for two generations to establish approaches to genetic equilibria. From these bases, respective controls and lines selected for 42-day weight were developed over six generations. The frequency of the rGH gene decreased from 0.075±0.042 to 0.050±0.034 in both the control and select lines from the weight-selected gene pool, and from 0.300±0.072 to 0.025±0.024 and 0.125±0.052 in the respective control and select lines derived from the control gene pool. The decrease in the latter gene pool is striking, particularly where the gene tended to disappear from the population even in the presence of selection for growth.

Involving transgenics in practical breeding programs would appear to require special monitoring for the gene in selected animals. Such a procedure would be particularly important where work by Nagai, Sabour and B. Benkel (Pers. Comm.) indicates that mice with the rGH gene, even though heavier than contemporaries that did not carry this gene, had lower fitness and productivity.

Combining Information On Single-Genes With Data on Quantitative Traits

Multiple objective selection is usually required in a conventional breeding program, where simultaneous improvement in several traits is required. The results in Table 1 show responses to such selection, through the application of a linear selection index. The

addition of information on a major effect, such as that pertaining to the growth-hormone gene, could be accommodated with index selection. In fact, theoretical models involving major gene effects along with multiple traits and information on relatives have been studied (Kennedy, 1990).

A simpler but less efficient form of selection would entail independent culling levels for the major gene and the quantitative traits. For instance, all breeders might be required to possess a transgene before being considered with respect to the other traits. However, the effective numbers of parents will need to be given attention in order to avoid serious inbreeding. Hence, a relaxation of culling levels may be required.

In light of the experience of Sabour, Ramsey and Nagai (1991) with the rGH gene, monitoring for the presence of major genes will probably be required in order to keep transgenes in a population. Decisions relative to mating and selection programs will need to go "hand-in-hand" in efficient integration of transgenics into practical populations.

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STATUS AND POTENTIAL FOR BETTER UTILIZATION OF FISHERIES WASTE IN CANADA

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Processing discards from fisheries account for 70-85% of the total weight of catch and have generally been dumped in-land or hauled into the ocean. However, environmental restrictions have brought about a need for "green" disposal or value-added utilization of processing wastes. Discards include by-catch of unutilized species, carcass from some fish after roe extraction and offal from processing of fish and shellfish as well as carcass from underutilized species, including seal. While preparation of meal and silage, and composting provide a means for low-grade utilization of fisheries discards, preparation of value-added components from wastes has been progressing steadily. Processing discards of different fish species include proteins, lipids, minerals, chitin/chitosan and their derivatives, flavourants/flavour enhancers, enzymes and enzyme support materials, pigments, and biologically-active components. Isolation of value-added ingredients and their potential use in food, agriculture, pharmaceuticals, health care commodities, waste management, water purification, aquaculture, etc. is of prime importance both from an environmental point of view for waste disposal and for economic return to fishermen and processors.

Fisheries Waste Classification

Fisheries wastes may include (1) processing by-products of existing catches and these include ground fish and shellfish, (2) unutilized species and these are species and/or stocks which are not fished by Canadian or foreign fleets, and (3) underutilized species and these are species which have directed fisheries but may be considered as "surplus". Unutilized species include Arctic cod, sand lance and dog fish. Capelin, Atlantic mackerel and herring are considered as underutilized. Foreign utilized species include roundnose grenadier and silver hake.

Utilization of Fisheries Wastes

Fisheries wastes may be used in the following manner: (a) dumping (in land or in the ocean), (b) composting/fertilizer, (c) preparation of meal and oil, (d) ensilation, (e) value-added use. Value-added use of fisheries waste is possible, however, a thorough knowledge of compositional characteristics of waste and potential utilization of components thereof is required. Three main examples of seafood products/by-products will be considered in this paper; these are (1) fish, (2) shellfish, and (3) seal.

Fish Processing Discards

Fish processing discards may account for up to 75% of the weight of the catch after filleting. Thus, development of fish by-products and processes to economically support the fishing industry is necessary. While composting fish wastes for the bedding industry, production of hydrolysates by acidification as a fertilizer, and meal preparation has been practised, clearly there is a need for better use of the waste. Production of high protein products in the weaning of piglets and recently for aquaculture feed has been practised.

Offal from cod fishery may be used in their entirety as feed component for aquaculture. Filleted cod (with heads and guts) may be ground, perhaps with the addition of some acid to

prevent microbial growth, and be included in feed formulation for aquaculture species. A typical representation of the % composition of the offal so produced is: moisture, 77.6; crude protein, 14.3; lipid, 4.3; ash, 4; caloric value, 413 kJ/100 g; and PER value (calculated), 1.88-2.36 [1]. Although the feed value of offal proteins is less than that of the fillet, nonetheless its total energy is comparable or superior to that of the fillet due to the inclusion of the fatty livers in the final product.

Some fish are "gutted and headed" prior to filleting. In such cases, cod cheeks and tongues may be extracted and collected for food use. In addition, livers and stomachs may be separated. Livers may be used for preparation of cod liver oil or may potentially be used for preparation of pastes and pates. Stomachs are well valued in some cultures as a delicacy. In particular, preparation of "changi" or "shiokara" in the Japanese and Korean restaurants, respectively, is of interest. This partly hydrolysed product has an appealing texture and possesses an "umami" taste. Preparation of bouillon from fully hydrolysed fish stomach is also practised.

Preparation of fish mince from frames intended for production of fish cakes or fish flour is possible. Furthermore, the deboned muscles may be used for production of surimi. This topic is addressed in contributions from other scientists.

Other value-added components of fish offal include skin and collagenous materials. Fish skin may be used for production of unique leather products. Furthermore, preparation of the so-called "pearl essence" from shiny materials present in the skin of many pelagic fish species for incorporation into cosmetics and other applications is practised. Gelatin of different grades may also be produced from collagenous materials in the bones and skins of fish. However, fish gelatins have low gelling ability.

Enzymes present in fish gastrointestinal tract may be used as a clotting agent for milk. However, recovery of enzymes from fish may not lend itself to general commercial applications due to unfavourable economy of the process. However, fish enzymes may be used in specialized applications.

Finally, fish oil may be produced from liver or from fish body in processing of fatty fish in fish meal preparation. Fish oil so produced may be used in different application. Fish liver oils are of three different grades. First grade fish liver oil is prepared from fresh livers by heating to 82-85°C. The leached out oil is rich in vitamin A and has a light straw-coloured appearance. This oil has use in the pharmaceutical industries. Further processing of the residue yields an orange-red coloured oil due to its partial oxidation. Third type oil is obtained from partially decomposed livers and has a dark colour. This oil competes with that obtained from fish body and has a high content of free fatty acids. Its applications are in the industries for production of leathers, paints, lubricants, etc.

Shellfish Processing Discards

Shellfish processing discards contain mainly proteins, minerals and chitin. Minor ingredients of shellfish wastes include flavourants, lipids and pigments. Typical % composition of discards from crab and shrimp are: moisture, 72.1; crude protein, 44.12 (on dry weight basis, dwb); lipid, 8.4 (dwb); ash, 29.0 (dwb); chitin, 17.0 (dwb); pigments, 147.7 (μg/g dwb); flavourants, 1.6 (% of proteins); and PER value, 2.79-2.88 for shrimp; corresponding % values for crab discards in the same order are: 42.5, 19.1, 0.9, 30.7, 18.7, 139.9, 1.4, and 2.30-2.42 [2, 3]. Chitin is the most abundant natural polymer after cellulose and is made up of N-acetyl-D-glucosamine units. It is found abundantly in the exoskeleton

of crustaceans.

Chitosan is the deacetylated form of chitin. It may be produced from chitin by its treatment with caustic solutions. Chitosan and its derivatives have many applications in foods, pharmaceuticals, agriculture, water purification, waste treatment, adsorption of radioactive compounds, cosmetics, health-care, wound healing, juice and wine clarification, clothing, flocculant, etc. At present, the use of chitosan is primarily for recovery of proteins from waste waters, in potable waters and for recovery of radioactive materials. However, their food applications is practised only in Japan [3-5]. Our own work on some chitosan derivatives has indicated their effectiveness in retarding development of rancidity in muscle foods. Chitosan derivatives may also be used as a coating material for extending the shelf-life of fruits and to delay ripening.

Chitosans may be used as support for slow-release drugs. Their hypocholestrolemic action has also been reported in the literature. They are also found to have excellent moisturizing and nutrient-holding effects and have widespread application in health-care commodities and cosmetics. Some chitinous compounds may have anti-inflammatory properties.

The content of chitin in shell wastes, as mentioned earlier, on a dry weight basis, was about 17% for shrimp and varied between 18.7 and 32.2% for crab. Backs and claws were rich in minerals and had a lower chitin content. Soft-shelled crabs had a larger content of chitin.

The proteins in shrimp and crab discards accounted for up to 1/3 of the weight of raw materials, on a dry weight basis (dwb). The proteins of shrimp shell were well-balanced in their amino acids composition, however, crab proteins were of lesser quality due to the presence of smaller amounts of lysine, leucine and isoleucine. Of the total nitrogen-containing materials present in the discards, water-extractable substances accounted for 1.40±0.20 and 1.58±0.11% (see above) of the total crude proteins from crab and shrimp offal, respectively. The water-extractable proteins of shrimp and crab are flavour active and upon recovery may be used in the preparation of pastes intended for incorporation into surimi for production of kamaboko products. Flavourants may also be recovered from the cooking water.

Minerals present in shell wastes from crab and shrimp were mainly calcium salts. Sodium, potassium, magnesium, phosphorus and strontium were present in smaller amounts. While calcium salts have applications in the pulp and paper industries, their recovery from the waste may not be economical.

Lipids in the shellfish discards are of minor importance. However, monounsaturated fatty acids are the major components. The content of EPA + DHA to the total lipids in the wastes was 23.7% for shrimp and 18.3% for crab.

Pigments in shellfish discards account for only 119.6 to 147.7 mg/kg of the raw materials. Carotenoid pigments found in shellfish discards are astaxanthin and its mono and diesters, cathaxanthin, leutcin and zeaxanthin. These pigments are also present in the flesh and skin of salmonoid fish such as salmon, trout and Arctic charr. Pigments from shell wastes may be extracted into oils intended for incorporation into aquaculture feed for salmonids. Inclusion of isolated astaxanthin and its derivatives in the diet of Arctic char and their subsequent uptake by flesh and skin of fish has already been reported from our laboratory. A nine week feeding on pigmented diets (at 75 ppm pigment level) was found necessary for obtaining an adequate visual colour impression in the flesh of Arctic char. However, feeding for up to 18 weeks on pigmented diets resulted in more intense colouration of the

flesh which is considered as desirable.

Seal Carcass

Seals (*Phoca groenlandica*) are potentially a major source of raw material for many reasons. Pelts may be used for production of fur, leather and other food/pharmaceutical products. Blubber lipids may be used for preparation of seal oil which could subsequently be employed for production of omega-3 capsules. We have successfully prepared omega-3 enriched seal oils containing some 45-47% long-chain omega-3 fatty acids. It is particularly rich in docosapentaenoic acid (DPA) [6]. We plan to examine the cholestrolemic activity of this oil after fractionation. However, the carcasses after skinning and removal of blubber are generally dumped, except for flippers which have been traditionally used as food.

Our research has shown that seal meat is a good source of high-quality muscle food and is fairly lean with a high content of vitamin B-12 and hemoproteins [7-12]. Its contents of cholesterol and nucleic acids are reasonably low. In short, seal meat is rich in the content of beneficial components and it is deficient in the content of potentially harmful substances. Unfortunately, the dark colour and intense flavour of seal meat has deterred its widespread utilization in human foods. While preparation of seal surimi with a light colour has been successful, we have not been able to remove its intense flavour. Work on the identification of seal flavour components is planned. Meanwhile, it has been demonstrated that nitrite curing of seal meat does not produce any N-nitrosamine in the products. Thus, a new line of processed meats could be introduced. We have also prepared decolourized protein products from seal meat which once fractionated afford components with attractive neutraceutical/ pharmaceutical properties.

Internal organs of seals and/or their components may have potential applications in different areas. Gall bladders are generally a rich source of cholic, deoxycholic, and dehydrocholic acids as well as cortisone, all of which possess medicinal and pharmaceutical applications. While cholic acid and its related compounds are important suppressants of synthesis of cholesterol stones, cortisones have anti-inflammatory effects. Prostaglandins may also be present in the seminal vesicles located in the bladder of male seals. Some cultures may consider the cholesterol stones and also male organs of seals to possess a mystical aphrodisiac value.

Rennet-like enzymes present in seal gastrointestinal tracts have proven effective in preparation of cheddar cheese. We are presently attempting to use these enzymes, after immobilization, in a continuous process for milk clotting. Seal bones from mechanical deboning of meat are potentially suitable for the immobilization process.

In the preparation of seal surimi, a large amount of haemoproteins may be separated. Furthermore, we have quantitatively isolated the haeme pigments of seal meat in preparation of decolourized seal protein products. The haeme pigments so obtained have food, chemical and pharmaceutical applications. In addition, the resultant protein preparations have been found to possess attractive functional and medicinal properties. A fraction of polypeptides so prepared is considered to possess muscle softening effects and the ability to enhance the blood flow as shown by the Japanese. Thus, seal carcass may be used in its entirety in production of value-added products.

Conclusions

Compositional characteristics of fisheries discards indicate presence of adequate quality and quantity of value-added components which could be isolated. Methods of separation of useful components of fisheries discards are available. Application of many of the isolated components have been explored; however, regulatory approval and development of more economical isolation/application procedures require further investigation.

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THE DEVELOPMENT OF NOVA CHEM

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When I joined the faculty at Saint Mary's University, Ernie Hayes was there and was working on chitin and chitosan. I collaborated with him and we had research grants from the Fisheries Research Board from 1974 to 1977 for "Chemical Products from Crustacean Wastes". The last grant was for work on the ion exchange properties of chitosan. During this period, Clive Elson also started to collaborate with us on chitin research and we became a triumvirate which was most unfortunately reduced by Ernie's untimely death in 1991.

In the summer of 1978, I obtained a number of "real" waste water samples from plants in Dartmouth. One of these was from a galvanizing plant, and we were able to reduce the zinc content from 800 ppm to below 1 ppm using a flotation process involving chitosan. The plant was very interested in our work, since they had negotiated a DREE grant which was contingent on the effluent being cleaned up. Within two weeks we had demonstrated the process on a 20 gallon scale and had incorporated Nova Chem Limited to provide chemicals and consulting services. There were three equal partners: Don Davies, Clive Elson, and Ernie Hayes. We were naive and did not tie the galvanizing plant down with a contract and, after about two years, they decided they knew what the chemicals were and that they didn't need us any more. Nevertheless, we made a few thousand dollars for research and gained experience.

In 1982 we were successful, in collaboration with Geortec of Newfoundland, in obtaining a contract with the Department of Health and Welfare under which Nova Chem tested all of the homes containing urea formaldehyde foam insulation (UFFI) in the three Maritime provinces (Geortec tested in Newfoundland). That bid made us sweat, since we had to make up-front financial commitments and take out loans. We hired seven testers in June of 1982 and the program lasted until July of 1984. We were very successful, largely because the principals drew very little remuneration. All of the profits were used to finance company research and development. Because our financial projections were accurate, this contract provided a track record which assisted us in our dealings with the bank.

In 1982, we also hired our first full time research technician. She and a second technician hired in 1983 are still with us and their combined experience in chitin chemistry is invaluable. Both have B.Sc.'s, one from Acadia and one from Saint Mary's.
Research was performed at both Saint Mary's University and Acadia University, since Ernie Hayes moved to Acadia in the early 80's. We used the regular faculty space at both universities and a rented laboratory at Acadia. In all cases, we purchased our own chemicals and equipment and made sure that we were seen to do so. When we used the University telephones or photocopiers, we paid for the services. The University administrations approved our activities, were supportive, and were kept advised. At both Universities the collective agreements stated that, under these conditions, the University had no claim to any inventions. In activities of this type, it is essential that everything be above board, that proper business practices be followed, that the banker and the university be kept informed, and that good relations are maintained with granting agencies and investors. A business can only succeed if it builds on firm business foundations, and good technology is not sufficient.

Our company research established procedures for producing biomedical grades of chitin and chitosan (the procedures are proprietary). Our chitosan is being used in wound dressings which are being tested for the Department of National Defence. According to the company making the dressings, our product is the only one that works!

Chitin is found in crustacean shells, insect cuticles, and fungi. In the case of crustaceans, it is a waste product of the fishery which used to be used as a fertilizer but which now has to be dumped at sea in most cases. It is therefore generally free for the taking, since this eliminates a cost. However, the waste will eventually command a price as products from it are commercialized. In our case, since we are producing biomedical and food grade products, the "waste" has to be treated as a food/biomedical product. We are dependent on a supply of a high quality fresh crustacean shells and we produce potentially valuable products from what is now a pollutant.

Chitin is insoluble in all common solvents and chitosan, which is deacetylated chitin, is soluble in dilute acids. We developed a water soluble derivative, N,O-carboxymethylchitosan (NOCC) on which we have composition of matter patents in Australia, Canada, France, Germany, Italy, Japan, the Netherlands, the United Kingdom, and the United States. We have shown that, when it is used to coat fruit, it establishes a controlled atmosphere within the fruit which allows the fruit to be stored in normal cold storage and retain the freshness that it would have retained in controlled atmosphere storage. There is also no room seal to break and you can remove the fruit one at a time without losing the controlled atmosphere. Agriculture Canada supported field trials across Canada to prove its effectiveness, and we own the trade mark Nutri-Save™ for it in Canada and the United States. With the assistance of CIDA, we will carry out trials in Chile this year on fruit whose peel is never eaten (avocado, kiwi, melon, & chirimoya) since it is much too expensive at this time for us to obtain approval for a food additive. However, we had trials carried out by Hazleton Laboratories America under good laboratory practices in which the gastrointestinal tract was examined and which indicated that for 14 days there was no adverse effect with NOCC as 5% of the diet in rats. NOCC has also been injected into the ocular cavities of rabbits with no adverse effects. We produce a biomedical grade of NOCC which has viscoelastic properties similar to those of hyaluronic acid and which might replace hyaluronic acid in some applications since NOCC is cheaper to produce.

Applications for NOCC include: a preservative coating for foods and seeds; a slow release system for drugs, pheromones, hormones, etc.; a moisturizer/protein binder in personal care products; an amphoteric resin to separate cations and to recover and separate amino acids by class; and as a viscoelastic substance in various surgical and medical applications.

There are things that can be done as a company that cannot be done as a faculty member. We were able to access the National Research Council's IRAPM and IRAPH programs on numerous occasions for our research. The former provides research grants for specific company projects and the latter funds summer students. We also received support from the NSERC Industrial Postdoctoral Fellowship Program and the NSERC Industrial Undergraduate Research Awards Program for summer students. This is particularly interesting for faculty members in small universities since NSERC operating grants are not a prerequisite. These programs also assisted students in our departments by providing summer employment. However they are not research grants in the normal academic sense and do not pay the full costs of the project. They only assist with salaries.

As previously stated, Agriculture Canada provided a contract to conduct field trials of Nutri-Save™ and also did their own research which resulted in published reports which have

been of great value to the company's credibility. We are presently negotiating a contract with Agriculture Canada to develop a slow release system for pheromones based on a NOCC gel. We also had four contracts with the Department of National Defence to develop a sonar transducer fluid to eliminate acoustic cavitation, and a contract with the Canada Mortgage and Housing Corporation to develop air purifiers. These contracts assisted the company and allowed staff members in the government departments concerned to develop additional projects since, in some cases, the funds were not departmental.

Nova Chem needs large international companies to develop and market its products. However, these companies will not evaluate material produced in the laboratory. Nova Chem therefore needed a small plant and, in September of 1990, it took possession of 2,850 square feet in the Technology Innovation Centre at 1 Research Drive Dartmouth. This is an excellent facility operated by the Province to get small technology companies established, and it provides all of the office services required at a very reasonable cost. We were able to obtain support from ACOA for 50% of the equipment and leasehold improvement costs (they liked the project since the normal level of support at that time was 30 to 35%). They also provided an interest buy down for the lesser of half of the interest or 6% of the outstanding principal over three years. In addition, we received assistance from the Technology Advancement Program (TAP) which is a joint Federal Provincial Program to assist with research non-capital costs. They also have programs which we have used that will assist with travel expenses for conferences and with travel and other expenses for trade shows. ACOA also have programs for trade shows and for marketing which we have not yet used. The Province of Nova Scotia has hired a marketing firm to assist N.S. companies sell in the U.S. market and we intend to use the program this year. These programs are essential if technology companies are to develop in Canada since there are virtually no private venture capital funds available under reasonable conditions in Canada.

Another source of funds is the Federal Scientific Research Tax Credit. Thirty-five percent of research expenditures qualify as a refundable tax credit, i.e. if there are no taxes payable, you get a cheque. Unfortunately, the Province has a 10% non-refundable scientific research tax credit and, unless you formally renounce it, the federal government considers that you have received it and reduces your refund accordingly. The tax credits also apply to research equipment, but only forty percent of that credit is refundable. A company claiming these credits should probably hire a major accounting firm since there are many grey areas and since, at least on their first claim, there will be both a financial and a scientific audit by Revenue Canada.

Nova Chem is now on the launch pad and we hope that it will go. However, we need additional investors. The company has generated over one and a half million dollars in revenue and the three principals have donated their time and have injected capital over a 13 year period. It could not have come this far if we had not had university appointments and the moral support of our universities. At Saint Mary's University, for example, there are three companies in which faculty members in the Chemistry Department are involved all of whom have served as Chairmen of the Department, Dean of Science, or Academic Vice-President.

Universities exist to provide an education for students and to produce research. Since they are publicly funded, their activities should stimulate, or at least sustain, the economy by providing educated workers, new knowledge, and spin-off companies such as Nova Chem. Saint Mary's University and Acadia University have done so, and we thank them. We also thank the various government agencies and individual scientists who have helped.

THE COMMERCIAL POTENTIAL OF ENZYMES AND PROTEINS FROM AQUATIC SOURCES

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1. INTRODUCTION

In recent times more and more emphasis has been placed on methodologies applied to better handling of wastes from fishing industry and to new and innovative technologies to better the utilization of waste, by producing end products which add to the general economy. There is a great potential to add value to the fishery resources and create new biotechnological products and markets. Hence a close examination of fisheries waste must be made in order to survive in todays competitive markets. Fish waste is a gold mine if appropriate cost-effective technologies are applied to recover a variety of by-products such as fish proteins, enzymes, peptones, nucleotides, nucleosides, flavouring agents and natural food colorants. Below is a brief discussion of enzymes and proteins of fish origin.

Applications of enzymes in industry is a very rapidly moving technology and there is a proportionately increased demand for the industrial enzymes. Today, of about 25000 different enzymes known to man about 21000 have officially been recognized by the International Union of Biochemistry (1,2). Based upon their substrate specificities and functions they are classified into six major groups. Proteases which are the focus of this discussion belong to one of the six classes called 'hydrolases' (2) Proteases can be derived from plants, animals (guts) and microorganisms (bacteria, fungi and yeast). Proteases are equivalent to 'peptide hydrolases' since they catalyse the cleavage of proteins (proteinases)and peptides (peptidases). There are about 490 different types of proteases described in the literature. Hydrolases wake up to about 78% of the total industrial enzymes (3). They are economically the most important industrial enzymes with a market valve between 1-5 - 2.0 million U.S. dollars (Table 1). Proteases are the most extensively used enzymes in the food industry where they function to improve the solubility, stability and quality of foods. For the same reason it is not surprising that they represent 44% of total sale of enzymes (Table 2).

2. Fish Waste as Source of Enzymes

In Newfoundland and labrador alone the annual value of fish products is about 550 million dollars. Hence, a large potential source of raw material exists from fisheries industry. The most important waste is the fish frames and the fish guts. Approximately 40% of head-on gutted fish is the frame i.e. the portion left after the fillets have been removed. In 1986, almost 382,000 metric tons of groundfish were landed in Newfoundland. Using standard conversion factors this translates into about 150,000 tons of fish frames available as a source of raw materials. The large quantity of ground fish landed means that proportional volume of raw material is readily accessible without further investment in harvesting fish. This resource is ready for putting into an appropriate use. The only cost involved is the processing and transportation.

To explore the possibility of recovering enzymes of industrial importance the group at Memorial University of Newfoundland initiated a project during early 1980's in the food science programme of the Biochemistry Department. The initial work focused on characterizing the proteases from Greenland cod (Gadus ogac) (4) and harp seal guts (Pagophilus groenlandicus) (5). Greenland cod is a subarctic species that thrives year round in the North Atlantic waters. Harp seals are harvested annually by Greenland, Iceland and some European countries. Canada was included in this list at the time when the project was undertaken.

The digestive enzymes from poikilothermic fish have unique properties which make them attractive as sources of proteases for applications in certain food processing operations. The proteases from cold adapted fish carry two important properties that give them some advantages in specific applications. Their high molecular activity at low temperature and their thermal instability make them unique over other proteases from conventional sources.

Tables 3-6 illustrate some of the salient features of gastric proteases from cold-adapted fish. These tables demonstrate that poikilothermic fish from cold waters have proteases with altered biochemical properties. It is apparent from these tables that low temperature environment results in enzymes that can effectively utilize food at the habitat temperature of the fish. This is exemplified by temperature dependent substrate binding affinity (Km values), low energy of activation (Ea), higher turnover number, and improved physiological efficiency-defined as the ratio of its substrate turnover number to its substrate binding affinity (Vmax/Km).

It has also been shown that Protease A from gastric mucosa of young and adult harp seals has properties very similar to that of chymosin (calf-rennet) (5). The seal protease A behaves much like the chalf enzyme in its milk clotting properties, cheese yield, fat retention and specificity but differs from bovine and chicken pepsins. A closer examination of seal protease suggests that its molecular size, amino acid composition, isoelectric point (pI), and stability in 6M urea resembles that of chymosin but differs from pepsins. However, the seal enzyme behaved differently from chymosin in its stability property in different buffers.

An adult harp seal gut mucosa has four zymogens of acidic proteases A,B,C and C'. Occurrence of multiple zymogens is not unique to harp seal since stomachs of other animals species have been shown to contain multiple zymogens. All four isoenzymes of harp seal gastric proteases exhibited milk clotting and haemoglobin hydrolysing activities. Stability and milk clotting activities near neutral pH are considered distinguishing characteristics of chymosin. Seal gastric protease A was much more stable at pH 7.0 than porcine pepsin. Seal protease A and C clotted milk up to pH 7.0 whereas under similar conditions porcine pepsin failed to clot milk above pH 6.5. Bovine pepsin clotted milk up to pH 6.9.

Considering the industrial application of these enzymes the crude seal gut protease and protease A were found to resemble calf rennet in their milk clotting properties. In an actual cheese - making trials the yield of cheese and fat retention were similar in cheese made with calf rennet and seal gut protease A. The sensory preference scores of the cheese made with crude seal gut protease and protease A were higher than or comparable to that prepared with calf rennet.

Trypsin and pepsin-like proteases from fish guts have been isolated and characterized (4,5,6). Beside these proteases alkaline phosphatases, a different group of enzymes may be recovered from fisheries waste. Alkaline phosphatases have application in diagnostic kits. Frozen blocks of shrimps in water release an alkaline phosphatase. The enzyme is recovered by ultra filtration and an ion exchange column chromatography.

3. Proteins of Fish Origin

Wastes from seafood industry are a rich-source of many valuable by-products including rich protein source and other biochemicals listed in Table 7 which also includes traditional and new innovative technologies applicable in a profitable, cost-effective manner.

Converting fish and fishwaste into fish, protein concentrates is a good method of recovering valuable proteins. According to FAO Fish protein concentrate is suitable for human consumption, is prepared from whole or parts of fish and has high protein content'. For reasons of both palatability and nutrition the hydrolysed fish protein should not contain free amino acids and should consist of large polypeptides that are easily solubilized. Mechanical breakage or mincing, solvent extraction and hydrolysis are common features of the process involved. Hydrolysis of proteins may be accomplished by chemical or enzymatic processes. There are certain advantages of the enzymatic process over the chemical hydrolysis or physical treatments. The enzymatic method is independent of naturally occurring fish enzymes which are variable and often appear in insufficient quantities. The process is rapid and hence permits repeated cycles to handle large volumes of samples. Moreover the enzymatic process produces a consistent product.

The steps involved in enzymatic process are simple and include: (a) cutting sample into small pieces or grinding; (b) proper mixing with preselected enzyme at appropriate temperature; (c) allowing the hydrolysis to occur and then raising the temperature of the mixture to inactivate enzyme to prevent further unnecessary breakdown of proteins; higher temperatures may simultaneously serve to pasteurize the mixture; (d) screening may be necessary in some cases to remove fish bones and undigested large pieces; (e) oil recovery may require an addition centrifugation step; (f) stabilization of the hydrolysate either by drying or by acidification. In contrast to acidified hydrolysate, which cannot be completely dehydrated the enzymatic process yields a dried product. Although drying is expensive cheap storage and easy transportation costs of dried product can offset the drying costs. Moreover some markets will only accept dry materials. The important qualities sought in a fish protein concentrates are: (a) the product should be a light brown fine powder; (b) may have mildly fish odour; (c) should be readily soluble in water; (d) protein content should be about 90%.

Beside fish frames used to recover soluble proteins whole fish, capelin (Mallotus villosus, is a good source of protein. However, its protein content varies considerably depending on the season. The fish harvested in the Spring has much higher protein levels compared to that harvested during the Summer months. The work done at MUN using bacterial proteases and capelin suggested it is possible to obtain a dry end product with 3.3% moisture, 7.9% lipids, 77% protein and 7% ash. These values vary depending on the fish harvest time (Table 9).

The composition of fish protein concentrates made from different fishes tend to vary only slightly in their proximal composition (Table 9). The average value of protein and lipid in these products are 82.7% and 0.16% respectively.

Capelin is the most abundant fish in the North Atlantic. It is an important forage fish for marine mammals, marine birds and larger fish such as cod, haddock, flounder, salmon, and herring. In Newfoundland alone about 50,000 metric tonnes are caught each year to be used for bait, human food, dog food and fertilizer. Traditionally, capelin is harvested when the schools are near or on the spawning beaches. Only female capelin has high commercial value. The roe or fish containing roe are frozen and exported to Japan. The male capelin is not normally exported but has been used in the preparation of fish meal and as domestic food.

Capelin has a circumpolar distribution in the northern region of the Atlantic and the Pacific. In the Eastern Atlantic it is spread from western Norway to Russia and is widely encountered throughout the Barents Sea and around Iceland and Greenland. On the east coast of the North America, the stocks are found from Hudson Bay to Nova Scotia but are most abundant around Newfoundland and Labrador. Small stocks also exist on both sides of the North Pacific.

Most capelin harvested in Canada are landed on the east and southeast coast of Newfoundland. In addition to the above mentioned uses capelin in recent times has also been used in the preparation of fish sauces with high protein contents. Fish sauce is a fermented product well-known in South-East Asia under different names.

The process of making fish sauce is simple. Fish are mixed with salt in a large vat or cement tank and kept submerged under the brine which is formed. The ratio of fish to salt varies from 5:1 to 2:1. The fermentation may last from 6 to 36 months depending on the size of the fish. Longer time allowed for fermentation results in better quality of the sauce. At the end of fermentation, the liquid is drained off and left exposed to the sun for a period of 1-4 months before packing. The end color may vary from yellow straw to amber or dark reddish brown.

A study by Raksakulthai (9) demonstrated that capelin is an excellent raw material for fish sauce. Exogenous enzymes such as fungal protease, pronase, trypsin, chymotrypsin and squid protease when used as enzyme supplements resulted in accelerated protein hydrolysis during the early phases of fermentation. However the end product had lower yields of free amino acids.

The recovery of fish proteins by fermentation can be manipulated by added enzymes giving different end products such as fish paste, fish sauce, mam-chau (Cambodia), Kapi and pla ra (Thailand), funasushi (Japan), bagoong and buro (Phillipines), and belachan (Malaysia). In South East Asian countries fish sauce and products like it can provide 7.5% of the daily nitrogen intake. This fish sauce is also an important source of calcium for a population where diet may be low in this mineral.

Conclusions

There have been relatively few attempts to use fish proteases as industrial processing aids. Cold adapted fish are poikilothermic and as such their digestive enzymes will exhibit a great diversity with respect to temperature preferences. Their properties can be exploited for industrial applications specially proteases may be used in food industry where they are useful in brewing, dairy, cereal, feeds, fish, legumes, meats. etc. Thus there exists an enormous potential for the recovery and use of enzymes from fish processing wastes.

The problem of providing adequate protein for an ever-increasing world population is a growing concern of the world today. The aquatic habitat offers an enormous potential in supplying the world's protein needs. The proper management of fishing industry and the harvesting of underutilized fish is a major concern of fish-producing and fish-exporting countries. One of the consequences arising from this is the increased production of fish offal which represents a disposal and pollution problem. The utilization of such material as sources of industrial enzymes would serve to miminize the waste disposal problem and as well recover valuable by-products. Fish proteins in the form of fish meal, silage, fish protein concentrate, amino acid, peptones, carotenoid proteins and flavouring compounds have numerous applications. There is an urgent need for innovative biotechnological developments to increase recovery and profitability from fish industry.

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Table 1. Importance of proteases

Estimated value of annual sale 1.5 2.0 billion U.S. dollars.
Proteases make up 78% of total industrial enzymes.
Proteases represent 48% of total enzyme sales.
Types of proteases reported 490.

Source: Knorr and Sinsky, 1985.

Table 2. Industrial Enzymes of Importance

Enzyme	%
Hydrolases	44
Proteases	33
Carbohydrateases	17
Lipases	2
Specialized	4

Source: Priar, 1984

Table 3. Proteases from Gastric Mucosa of Greenland cod

Isoelectric points (pI) of 3 Zymogens Zymogen 1: pI 7.5 Zymogen 2: pI 6.2 Zymogen 3: pI 5.3 Optimum pH: Proteases 2 and 3: pH 2.0 Porcine pepsin: pH 2.0 Protease 1: pH 3.0

Source: Simpson, 1984

Table 4. Biochemical properties of Greenland cod protease

	CU/PU	Km-value	Stimulation by Nacl
Cod protease	High	Low	+
Porcine pepsin	Low	High	-

Source: Simpson, 1984

CU - clotting unit relates to milk clotting ability.

PUT - protease unit represents protein hydrolysing activity.

Stimulation, 25mM NaCl.

Table 5. Unique qualities of Greenland cod protease

Is trypsin-like
Is more effective catalyst:
- with high specific activity (low Km-value)
- with high turnover number
- with low free energy (G)
Is less sensitive to temperature changes:
- with lower Q_{10} – values
- with lower Ea and ΔH values
It's polypeptide is different from bovine trypsin

Table 6. Molecular Efficiency of Greenland cod trypsin.

	Turnover Number x 10^2 at 25°C		
Enzyme source	Amidase	Esterase	Amidas/Esterase
Greenland cod	2.10	1.40	0.18
Bovine	0.30	0.29	0.03

Source: Simpson and Haard, 1987

Table 7. Recovery-utilization of Fish Waste

1. Fish meal
2. Baits
3. Silage
4. Canned pet foods
5. Surimi
6. Fish Protein Concentrates (FPCs)
7. Natural pigments - carotenoides
8. Polyunsaturated fatty acids (fish oils)
9. Amino acids, peptones
10. Chitin/chitosans
11. Flavouring compounds
12. Nucleotides/Nucleosides

Table 8. Composition of capelin (Mallotus villosus)

	Summer	Spring
Protein	37.3	78.9
Lipids	54.0	11.6

Values are based on dry weights.

Source: J. Food Sci 47: 347 (1982).

Table 9. Composition of FPCs from different Fish Species

Species	Crude protein	Lipids	Ash
Red Hake	80.9	0.18	13.5
Atlantic Menhaden	78.5	0.18	19.4
Atlantic Herring	87.5	0.19	10.8
Northern Anchory	80.0	0.07	16.8
Ocean Pout	86.0	0.24	15.0
Alewik	86.0	0.09	15.7
Morocean Sardine	79.7	0.21	-

Source: FAO, 1969

Recovery of Valuable Organic Constituents by Membrane Separation Technology

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Abstract:

Recovery of organic species from process waters has implications in energy conservation, the environment and in economic terms. The use of membranes for these separations further enhances the separations since membranes present a rapid and non-degrading means of separation.

This paper discusses the history of some membrane applications that have been studied at the National Research Council (NRC) as well as some operational considerations of membranes in practical separation examples. Ultrafiltration (UF), Reverse Osmosis (RO) and Gas Permeation (GP) are specifically discussed and a new high temperature RO application for the recovery of valuable compounds from cook waters is introduced in more detail.

Introduction:

Membranes are barriers or physical interfaces across which specific species can migrate selectively under the influence of a chemical potential or other driving forces. Our experience has been mainly in pressure driven processes such as UF and RO, however we have more recently examined GP and gas diffusion through membranes for separation purposes. The main challenge has been to apply the membrane process at the same operating conditions as the target process for economic reasons or other constraints. This often requires operating at non optimal conditions from the membrane perspective, such as the use of the extremes of temperatures, pressures and concentrations of the desirable species to be recovered. The literature is not abundant in these high demand types of applications. It may be that commercial activities in this area precedes publications in the open literature.

Reverse osmosis may be the most commercially advanced of the processes, but only because of the large effort put into water purification by organizations such as OSW (Office of Saline Water) and by several companies. When it comes to operations in adverse environments, there are few membrane systems that will operate satisfactorily outside the traditional operating range for water purification, ie. above 75° C or above 1000 psi.

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Ultrafiltration and Nanofiltration are only now experiencing rapid commercial growth, while vapor separation is still mainly in the research stages. All of these unconventional membrane processes are highly dependent on availability of new materials that can perform under increasingly demanding conditions.

The history of food processing at NRC dates back to the late 60's when the pre-concentration of maple sap was performed with cellulosic membranes in rather crude stirred and flow through cells (1). The early work advanced the feasibility of concentrating maple sap from 1.5% to over 10% total dissolved solids. This prompted several companies to commercialize the process with spiral wound membranes. The arrival of thin film composite membranes led the way to widespread use of the RO process in the maple syrup industry.

A very successful early application was the fractionation of avidin from egg whites with cellulosic ultrafiltration membranes. The process of extracting this very valuable protein is still used today, presumably with improved membranes. Other significant applications explored in our laboratory included juice clarification(2-4), de-coloration of red wine as well as tea fractionation and concentration. (5)

Another early application in food, subject of an Industrial Research Assistance Program, was the concentration of hot gelatin solutions (6) from 2 to 12% using Ultrafiltration membranes. The membranes were made of relatively hydrophilic "Victrex" ™ Polyethersulfone, had to perform the separation at temperatures above 80° C with minimal gelatin losses in the permeate. Membranes were optimized to give permeate fluxes of 35 to 15 USGFD and separations of 98 to above 99% respectively. Initially it was thought that the separation was too low (losses were too high), but it was found that the losses were actually only low molecular weight material that did not add to the gel strength. It is conceivable that adjustments in the process could further increase the value of the gelatin to pharmaceutical grade from food grade. Pilot plant studies showed that the tubular membrane configuration was not appropriate, and alternative high temperature spiral or plate and frame modules were not available. The lack of commercial devices specifically designed for these conditions remains a challenge to this day. We consider this a technological limitation that will eventually be solved by a market pull for the development of appropriate devices (as opposed to a technology push).

NRC membrane development continued in the flat sheet (6) configuration to address the limitations of tubular type membranes and modules. Food applications in non-aqueous systems were pursued. The first was the removal of fine solids from hexane extracts of oat oils. Material compatibility with the solvents as well as the handling of large volumes of flammable solvents were some of the early hurdles that had to be crossed. Although the oat oil project proved uneconomical due to low oil yields, it was good proving ground for the more useful application of refining Canola oil with membranes.(7) Both first press and solvent extracted "micella" were processed. Membranes were demonstrated to provide many benefits to the oil refining process. Increased yields of oil, lower usage of bleaching clay, extraction of a non-degraded phospholipid, elimination of hydrolytic degumming steps and energy savings were some of the advantages of the

membrane process. Again limitations in the equipment for handling the high temperature, high pressure (200 psi) fluids proved to be the main obstacle. DDS equipment at that time (1979) was barely adequate to carry out pilot plant trials. NRC has partially met the challenge of equipment limitations by designing a high pressure, high temperature and high efficiency plate and frame module. Patents have been secured and demonstration modules have been tested. Licensees are presently being identified to take the equipment to market.

In the past two years there have been two other food-related trials where the integration of membranes into existing processes was investigated to improve energy efficiency and recover valuable products (8, 9). The first was the use of vapour permeation to extract volatile organic compounds from the headroom of a fermenter that was producing a natural volatile organic carbon product (10). In this case the study included not only developing the composite membrane, but the development of a spiral module that housed the membrane and contained the volotile organic stream. The other application, which will be discussed in more detail, was the high temperature membrane processing of shellfish and fish cook waters for the recovery of hot water for reuse and protein and flavour compounds as high value products. (11)

Experimental:

Figure 1 shows the experimental set up of 12 cells and feed tank in a thermostated enclosure, with the pressure regulator and pump on the outside. The temperature of the fluid to be processed could be controlled between room temperature and 90° Celsius. Table 1 lists the solutes used in characterizing the membranes at various temperatures. Table 2 lists the membranes tested in this study. The operating pressure did not exceed 375-400 psig to prevent excessive concentration polarization resulting from the high fluxes expected at higher temperatures. Analysis for salt content was performed using a conductivity cell and comparing the measured conductivity with that obtained for a series of standard salt solutions. Organic contents were determined with a total carbon analyzer.

Membrane performance with pure water was determined at the same operating conditions used for the test solutions before and after the separation tests. In this way, any changes in the membranes caused by exposure to the test solutions could be determined.

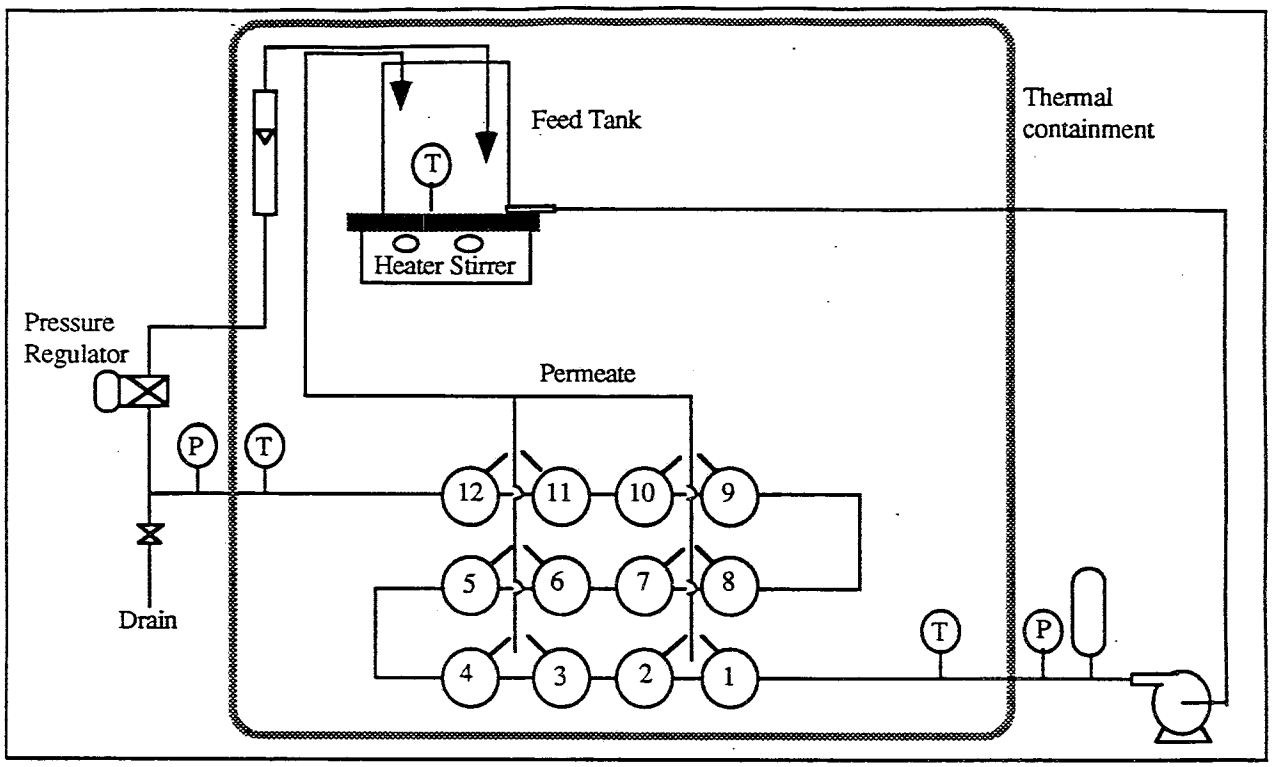

Fig. 1 Experimental set up

Solute	Concentration	Comments
Pure water		
NaCl	3500, 2000 ppm	
ß-alanine	200 ppm	
arginine (ARG)	200 ppm	Also 2000 ppm ARG with 3500 ppm NaCl
glutamic acid	200 ppm	
glycine (GLY)	200 ppm	also 2000 ppm GLY with 3500 ppm NaCl
Lobster Cook Water	~ 200 ppm organics/ 22000 ppm NaCl	

Table 1. Solutes Used in Membrane Characterization

#	Designation	Manufacturer	Nominal separation	Comments
1	DS 1	Desalination Systems Int.	97.5% NaCl	
2	DS 5	Desalination Systems Int.	70% NaCl	
3	DS	Desalination Systems Int.	98.5% NaCl	
4	R74	NRC Radel	1500 MWCO	
5	FT-37	Filmtech	99% NaCl	
6	SU	Torray	99% NaCl	
7	DU	Dupont	95% NaCl	
8	T7	NRC Polyether-imide	2000 MWCO	Destroyed
9	U35	NRC Udel	2500 MWCO	
10	BR-2.5	Biorecovery	2500 MWCO	
11	UC-33	NRC Carboxy-polysulfone	800 MWCO	
12	V36	NRC Victrex	4000 MWCO	
13	HPVD	Hydraunatics, PVA	92% NaCl	
14	CA316	NRC Cellulosic	90% NaCl	Collapsed

Table 2. Membranes tested in the study

Results and Discussion:

The flux of pure water through the membranes increased with temperature in an approximately linear fashion for the majority of membranes as can be seen in Figure 2. However the separation response of the membranes was better than expected in most cases. Figure 3 shows that with few exceptions, even though the flux in some membranes went up by a factor of four, the separation remained relatively steady. The separation and flux data for selected membranes appear in Tables 3 to 5 for a more complete impression of the performance of the various membranes. Surprisingly, the separation of amino acids occurred even using ultrafiltration membranes. The fluxes of these UF membranes however were far below what one would expect for open membranes operating at elevated pressures. It is obvious from the NaCl separation data on the Ultrafiltration membranes, that a dynamic layer on the membrane surface is responsible for the separation. Initial and final pure water permeability tests also confirm the presence of pore blocking substances in the UF membranes. The use of caustic (NaOH) did restore the flux of the UF membranes to virtually the original values.

The resultant data for individual amino acid and salt fluxes and separations can serve only as a preliminary indication of what will occur in a complex multicomponent broth emanating from the cooking and processing of fish or

shellfish. Data on the separation of amino acids in the presence of NaCl shows that salt has virtually no impact on organic separation, but does reduce the flux due to osmotic pressure effects.

Membrane processing of "real" lobster cook water, shows that the separation of organic species is higher. Organic species present in real lobster cook water includes amino acids as well as higher molecular weight proteins in various states of denaturation, which can account for the differences.

Two of the membrane separation values for the lobster cook water were taken in an attempt to calculate (simulate) the fate of some constituents if membrane permeates were recycled back to the cooking step several times. A Filmtec and a Desalination Systems membrane were chosen as representative of commercially available membranes. The differences in separation as well as the flux are important considerations in choosing any particular membrane. Figure 4 is a schematic diagram of this conceptual process.

Table 6 lists the assumed values for volume processed, initial concentrations of salt and organics as well as the separation factors for each constituent and upper limit of processability that was be set by considerations of osmotic pressure or other factors.

It can be seen in Figures 5 and 6 as well as Table 6 that even at low organic separation values, the recycling of the permeate allows the recovery of the target compound by membrane processing as a result of a build up in organic concentration in the cook water loop. It should be pointed out that in Case 1, Table 6, with the DS1 membrane, the low salt separation does not lead to an osmotic pressure limit to the process as there is in Case 2 with a FT-30 membrane. In the high salt separation mode we also do not know how much salt would be acceptable in the concentrate. Practically, it is easier to add sea salt if required than it is to take it away.

Comparing Figures 5 and 6 we can see graphically the advantage of using a more open membrane, such as the DS1, with a lower separation of NaCl. The use of more open membranes allows for higher organic concentrations to be built up in the concentrate, less water and lower salt levels in the concentrate with corresponding higher salt levels in the permeates. These facts may have a beneficial effect on the acceptability as well as the economics of the concentrate that would be sent to conventional spray dryers. Finally, there is the possibility of realizing higher energy savings with the use of a more open membrane, by reducing the heat load in the evaporative steps which have to follow.

Conclusions:

This study confirms the fact that membrane processing of shellfish cook waters has several advantages over conventional recovery of valuable species. In the case where the proteins or amino acids are of low value, and there is no

opportunity to recover sensible heat from an evaporative stage, membranes provide a means of recovering virtually all of the heat value.

It has been shown that the use of ultrafiltration membranes is not appropriate under the same operating conditions as reverse osmosis.

This preliminary investigation was only an attempt to test and demonstrate feasibility of the use of membranes at elevated temperatures, and was not a comprehensive study of the application of membrane technology to the shellfish processing industry. In addition to not considering typical concentrations and process conditions encountered in processing plants, this study did not address the problem of membrane life or containment devices. The tested membranes were taken from commercial spiral wound modules, which were not designed for and would not be expected to survive the demanding operating conditions investigated here.

Work on the design of a high temperature and high pressure containment device for membranes is continuing at NRC.

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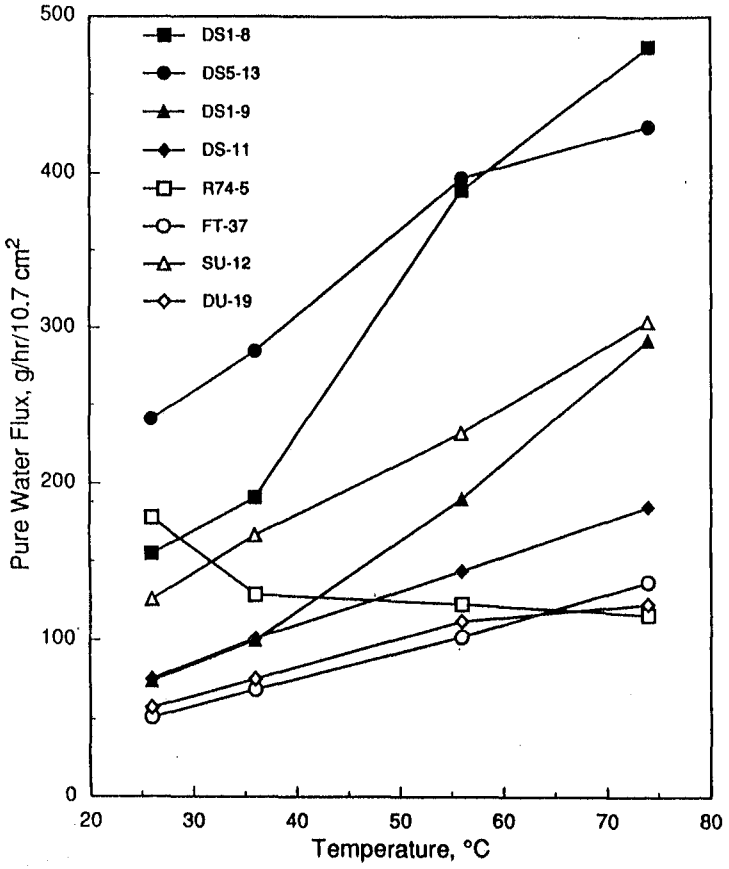

Figure 2. Pure Water Flux as a Function of Temperature

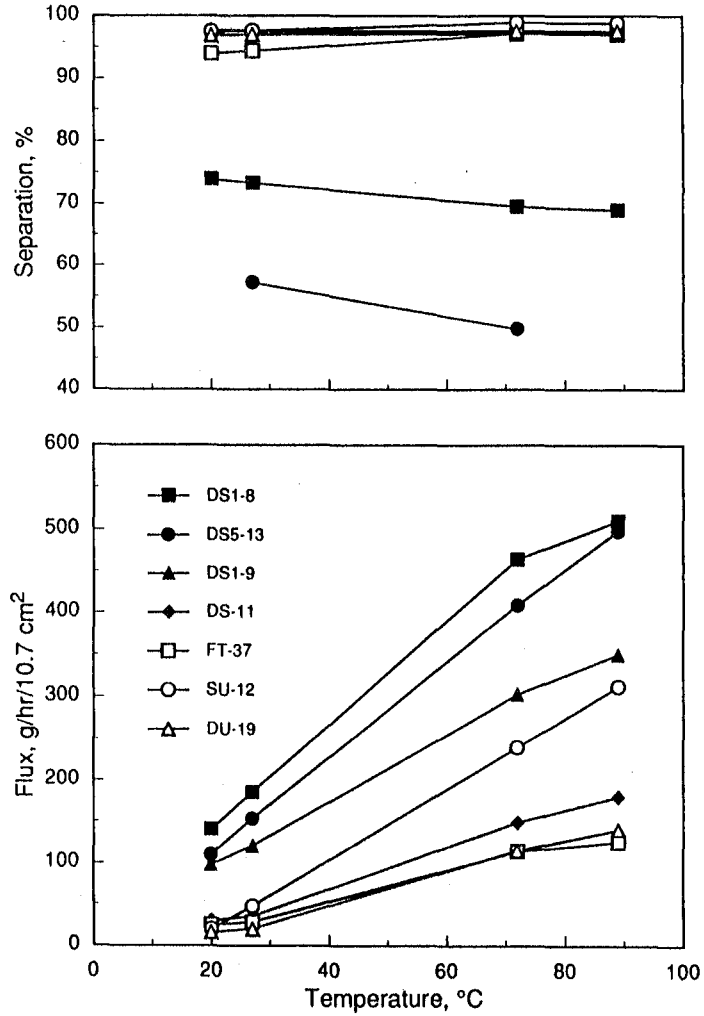

Figure 3. Flux-Separation Data as a Function of Temperature

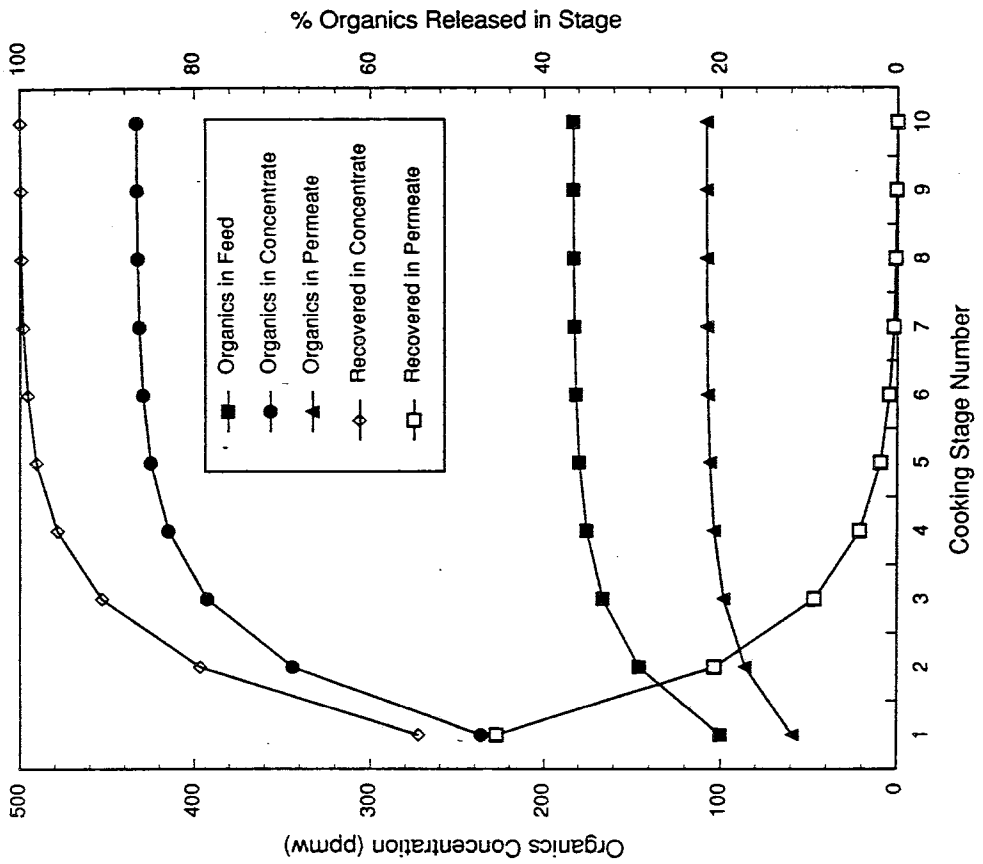

Figure 5. Fate of Organic Species as a Function of the Number of Times the Permeate is Reused as Cook Water. Membrane DS1-9

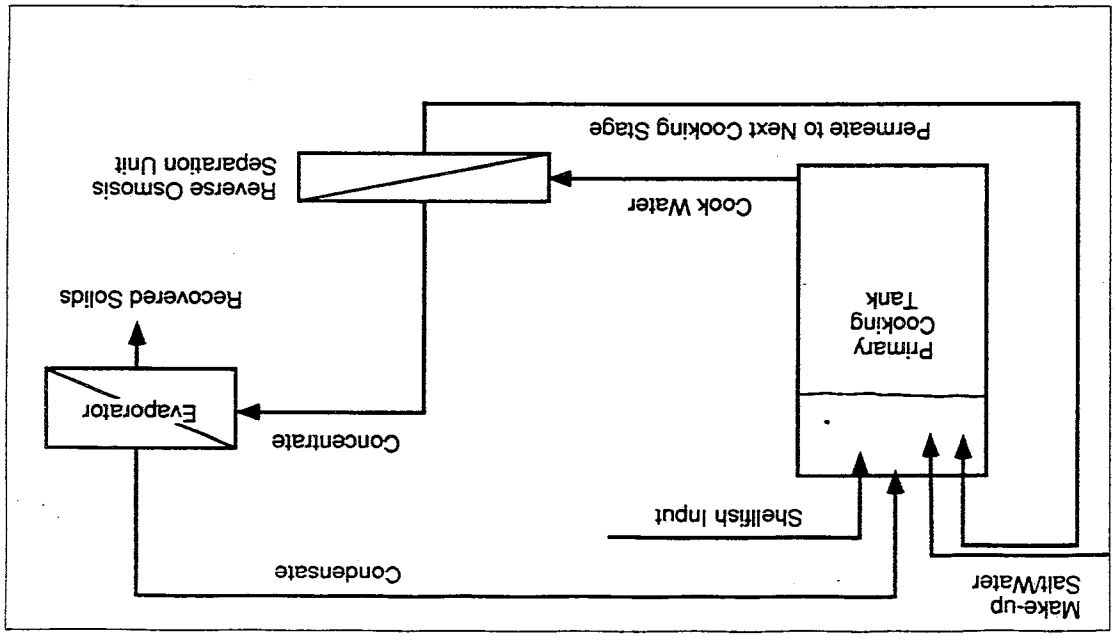

Figure 4. Schematic for Membrane Treatment of Cook Waters

2000 ppmw NaCl, 400 psig

Membrane	DS1-8	DS5-13	DS1-9	DS-11	FT-37	SU-12	DU-19	V36-1	BR-2.5	UC33-1
72 C										
Flux	463.6	408.8	302.6	148.6	114.4	240.4	115.3	47.5	36.9	
Separation	69.6	49.8	97.1	98.0	97.1	98.9	97.5	35.1	80.6	
27 C										
Flux	184.6	152.8	120.4	35.0	28.1	47.2	19.8	12.3	12.9	
Separation	73.3	57.2	96.9	97.6	94.4	97.6	97.0	58.7	81.7	
91 C										
Flux	276.0	288.4	556.6	186.0	175.4	304.4	105.8	27.2	52.3	314.4
Separation	47.0	53.5	65.4	95.6	92.7	93.9	95.1	62.4	72.1	12.4
26 C										
Flux	141.5	149.2	184.4	40.2	34.3	55.9	23.2	8.9	16.8	108.8
Separation	52.3	56.6	85.4	95.0	90.9	92.1	94.9	64.4	74.7	13.5

Table 3. NaCl Characterization Data (Flux, g/hr/10.7 cm2; Separation, %)

200 ppmw solute, 400 psig

Membrane	DS1-8	DS5-13	DS1-9	DS-11	FT-37	SU-12	DU-19	V36-1	BR-2.5	UC33-1
33 C, ß-Alanine										
Flux	94.1	99.5	181.2	47.0	43.7	70.9	25.0	9.7	17.1	114.3
Separation	20.1	82.2	38.2	91.2	90.5	90.4	78.9		9.5	6.2
91 C, ß-Alanine										
Flux	268.5	283.8	549.3	190.5	189.8	335.2	89.8	24.5	46.7	292.5
Separation	22.5	73.9	26.4	91.6	87.4	93.2	83.1	22.9	13.3	10.3
23 C, Arginine										
Flux	18.4	30.6	33.3	26.0	31.8	40.5	7.8	3.0	3.9	28.2
Separation	40.1	68.0	88.2	95.3	96.6	99.9	100.2	90.5	49.3	5.8
90 C, Arginine										
Flux	81.5	138.4	136.6	116.2	155.4	191.3	40.6	12.5	12.5	85.0
Separation	77.9	29.6	34.7	91.8	92.9	96.5	69.9	28.2	-2.3	-6.5
26 C, Glutamic Acid										
Flux	58.8	100.3	86.4	49.9	32.2	60.4	11.2	5.1	16.3	31.1
Separation	59.6	82.4	87.4	95.9	95.4	99.0	87.0	62.1	44.2	64.4
90 C, Glutamic Acid										
Flux	144.0	225.2	196.0	155.8	179.4	214.6	54.2	58.5	40.3	86.0
Separation	83.6	86.9	95.1	97.6	99.1	99.1	93.0	69.8	75.5	42.2
28 C, Glycine										
Flux	72.7	147.9	75.3	31.2	34.2	47.3	10.3	1.9	11.0	31.6
Separation	55.6	64.1	84.8	94.6	93.6	95.8	91.2	49.5	48.1	13.1
91 C, Glycine										
Flux	210.0	388.5	264.8	150.6	169.2	251.6	53.2	8.7	39.5	103.0
Separation	35.4	44.7	65.1	94.9	93.9	95.6	88.1	57.6	22.2	17.1

Table 4. Characterization Data Using Single Amino Acids (Flux, g/hr/10.7 cm2; Separation, %)

Membrane	~200 ppmw Total Organics, 22000 ppmw NaCl, 400 psig									
	DS1-8	DS5-13	DS1-9	DS-11	FT-37	SU-12	DU-19	V36-1	BR-2.5	UC33-1
23 C										
Flux	23.0	24.5	36.1	21.1	18.0	26.7	11.1	3.5	11.3	20.6
Organics Separation	83.3	97.9	96.3	96.9	97.6	96.7	96.6	90.7	81.4	74.2
NaCl Separation	33.6	35.0	55.2	97.6	96.2	97.9	98.1		44.1	12.8
91 C										
Flux	52.6	56.6	**96.6**	79.6	**68.8**	87.3	39.0	14.5	32.2	60.4
Organics Separation	59.4	79.7	**74.3**	91.1	**40.9**	94.6	91.8	64.4	41.6	24.2
NaCl Separation	17.7	16.8	**28.6**	93.1	**94.1**	94.0	90.4	42.3	24.5	-0.5

Values in bold, used in simulations

Table 5. Characterization Using Lobster Cook Water (Flux, g/hr/10.7 cm2; Separation, %)

Flowrate (liters)	100					
Salt Concentration (wt %)	1					
Stage Number		1	3	5	7	10
Case 1 Membrane DS1-9						
Salt Separation (%)	30					
Organics Separation (%)	75					
Salt in Concentrate (wt %)	1.3					
Salt in Permeate (wt %)	0.91					
Organics in Feed (ppmw)		100.0	166.1	179.8	182.6	183.3
Concentrate Flowrate	23.1					
Permeate Flowrate	76.9					
Organics in Concentrate (ppmw)		236.4	392.6	424.9	431.6	433.2
Organics in Permeate (ppmw)		59.1	98.2	106.2	107.9	108.3
% of Organics Released in Stage Recovered in Concentrate		54.5	90.6	98.1	99.6	100.0
% of Organics Released in Stage Lost in Permeate		45.5	9.4	1.9	0.4	0.0
Energy Savings (MJ /100 liters processed)	174					
Case 2 Membrane FT-37						
Salt Separation (%)	95					
Organics Separation (%)	40					
Salt in Concentrate (wt %)	2					
Salt in Permeate (wt %)	0.1					
Organics in Feed (ppmw)		100.0	156.0	165.0	166.4	166.6
Concentrate Flowrate	47.4					
Permeate Flowrate	52.6					
Organics in Concentrate (ppmw)		126.7	197.6	208.9	210.8	211.1
Organics in Permeate (ppmw)		76.0	118.6	125.4	126.5	126.7
% of Organics Released in Stage Recovered in Concentrate		60.0	93.6	99.0	99.8	100.0
% of Organics Released in Stage Lost in Permeate		40.0	6.4	1.0	0.2	0.0
Energy Savings (MJ /100 liters processed)	119					

Table 6. Assumptions and Calculations for Reuse of Permeate as Cook Water

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