

# *Bulletin*

The background of the cover is a photograph of a large, jagged iceberg floating in a deep blue body of water. In the distance, there are dark, forested mountains under a clear sky. The foreground shows some dark, out-of-focus branches and rocks.

of the Aquaculture Association of Canada

Proceedings of  
AQUATECH '96

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## **Message from the Chairman**

My thanks to all of you who took part in AQUATECH '96. Your participation in this focussed event is representative of a growing awareness of the aquatic environment: its riches and its limitations. We have heard about the opportunities in the culture of new species; advances in aquatic environmental and bioremediation technologies; integration of the infotech world with biotechnology; fish health and nutrition; investment; and new medical products from cold oceans.

But most of all, we have had an opportunity to meet with fellow scientists, business people and entrepreneurs to stimulate our minds as well as our taste buds. Many of us had taken full advantage of the venue and enjoyed the wonderful hospitality for which Newfoundlanders are noted.

I am pleased to acknowledge financial support for this workshop from the National Biotechnology Networks Secretariat, Canadian Institute of Biotechnology, Seabright Corporation, Canadian Centre for Fisheries Innovation, National Research Council — Institute for Marine Biosciences and IRAP, Industry Canada, Contact International Ltd. and others. I would also like to thank the Local Organizing Committee, for without their dedication and hard work, this workshop would not have been possible.

Sincerely,

J.M. (Monty) Little  
Chair, AQUATECH  
President, Syndel International Inc.  
9211 Shaughnessy St.  
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*Cover: The rugged coastline of St. John's, Newfoundland with Signal Hill and the historic Marconi Tower in the background. Photograph by Stephen Forgeron, Seabright Corporation Limited.*





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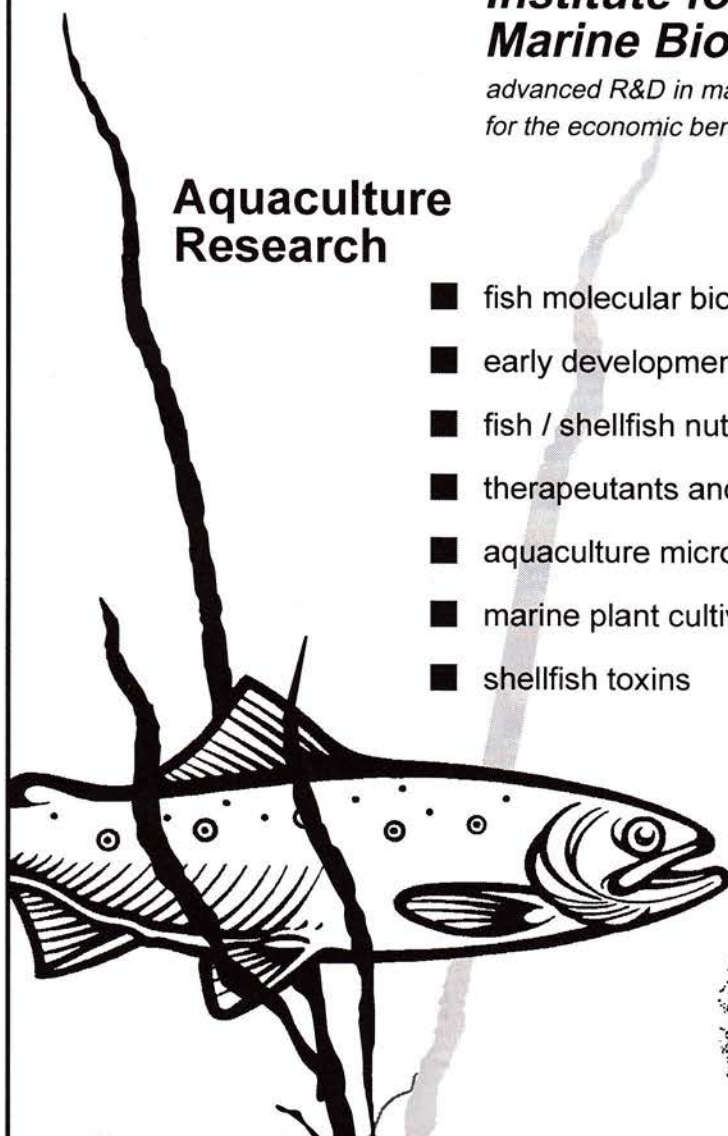
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G. Jay Parsons and Stephen Forgeron, guest editors

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# Introduction to AQUATECH '96

**"From research to the real world: alliances and  
opportunities in cold ocean biotechnology"  
May 5-7, 1996 — St. John's, Newfoundland**

A recent Industry Canada report by Arthur D. Little that looked at the commercialization of marine biotechnology concluded that despite the lack of a formal strategy, Canada is currently in a leadership position in this industry sector *vis à vis* other countries, including Japan, the United States and Norway. The report goes on to state that the "growth of these companies and the formation of new marine biotechnology companies will be limited and slow without more focused planning, guidance and investment". These are all potential roles for AQUATECH.

The philosophy underlying AQUATECH, a branch of the Canadian Biotechnology Networks, is an operational mechanism to enhance the interaction among universities, government and industry with the goal of increasing the commercialization of biotechnology opportunities. The networking function is designed to help industry identify promising technology being developed at university and government laboratories and to identify scientific expertise resident in those institutions that can assist industry in its R&D and commercialization activities. The latter should give rise to joint ventures, consortia and multi-disciplinary teams being assembled out of the three groups — industry, government and academia.

Under the leadership of the local organizing committee, the theme chosen for AQUATECH '96 was "From research to the real world: alli-

ances and opportunities in cold ocean biotechnology." The first section of the theme "from research to the real world" was selected because a need has been identified to emphasize how most biotechnology applications are derived from extensive periods of research which at some point become the solution to many of the problems identified in the industrial sector. The statement alludes to a partnering of researchers from universities, government institutions, and other research facilities with the industrial sector who are the end users of such technologies. The active partnering of aquatic biotechnologies and information technologies research has the potential to yield significant benefits in both fields. The middle section of the statement "alliances and opportunities" highlights the desire for networking that is the foundation upon which AQUATECH exists, and "cold oceans biotechnologies" reflects the need for the identification of potential opportunities from both fresh and marine waters from which the Canadian economy could benefit.

Six topics were selected around this theme and established the template for AQUATECH '96. Canadian, American and international speakers addressed more than 130 participants under sessions entitled:

- Culture of new species and evaluation of new products,
- Environmental and bioremediation technologies,

- Integration of information technology with biotechnology,
- Fish nutrition and health,
- Investment and biotechnology, and
- New medical products from cold oceans.

Ninety minutes were devoted to each topic in which a session chairman directed three 20-minute presentations representing industry, academic, and government perspectives. The speakers' views were then challenged and discussed during lively 30-minute question and answer sessions.

The event provided a dynamic forum and networking opportunity for participants representing R&D scientists from corporate, university and government laboratories, commercial and government managers, aquaculturists, policy makers and regulators, students, bankers and investment brokers.

AQUATECH is grateful to the Aquaculture Association of Canada for the opportunity to pub-

lish papers, excerpts, and abstracts of presentations in this special issue of the Bulletin. It seems appropriate to deliver proceedings of the AQUATECH conference to the aquaculture community, being members of a relatively new and rapidly developing industry poised to benefit from the interface of a widening band of business, research and government stakeholders. Special thanks to the AAC Bulletin editorial staff for their patience and diligence in creating this issue.

These proceedings would not have been possible without the contribution of manuscripts and abstracts from the conference speakers. Although lacking the animation and personality of the presenter, we hope that these written summaries of AQUATECH '96 will give the reader a useful insight into the range of topics covered at the conference.

—*Local Organizing Committee*

## Special Thanks

We gratefully acknowledge the financial contribution of the National Research Council — Industrial Research Assistance Program in the publication of this AQUATECH '96 proceedings.



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### Proceedings

National Research Council—Industrial Research Assistance Program  
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## **Status of commercial farming of cold-water marine fish in Norway, 1995-1996**

*Rolf Engelsen*

Halibut, cod and wolffish are the marine fish species being farmed in Norway. Turbot are also being produced, but the juveniles are exported to Spain for grow-out. Market volumes of marine fish were less than 50 metric tonnes in 1995 (halibut and cod only, no wolffish were sold). The prognosis for the year 2000 is 1-2,000 tonnes of halibut; no production of cod and wolffish are expected. Hence, the volume of farmed marine fish is still very modest in comparison to the 270,000 tonnes of salmon produced in Norway in 1995.

### **Halibut**

In 1992 a market study<sup>(1)</sup> on halibut indicated promising prospects for the species. The strategic summary of that study is presented in Table 1 and development of the industry has so far been consistent with expectations. Farmed halibut are being sold at prices (US\$10 per kilogram of live fish) well above those for wild-caught fish (approximately US\$13.50 per kilogram whole fish gutted CIF).

The competitive advantage of farmed halibut is related to freshness, availability of preferred sizes, reliability of supply, and quality. The market potential for farmed halibut in Europe is presently estimated at 20,000 t per year. The up-market restaurant segment demands a whole fish, 4-8 kg in size. In Norway, the catches (by volume) of wild halibut are composed predominantly of large fish and more than 90% of the fish are marketed as frozen product, so there is no regular supply of fresh wild halibut in Norway. The quality of wild halibut also varies more than cultured halibut because of greater handling.

The major factors determining profitability in halibut farming are sales price and growth rate of the fish. The mortality rate in halibut grown in culture tends to be very low, so production is a function of growth which is primarily depend-

ent upon temperature. The estimated optimum temperature ranges for various sizes of halibut are shown in Figure 1.

High summer temperatures can create problems, especially for large halibut. Long periods of low winter temperatures reduce growth to a minimum. The sexual maturation of males, typically occurring in the winter when the fish are 2.5-3.0 kg, must be postponed until the fish reach a weight of at least 6-7 kg. This can hopefully be achieved by manipulating the photoperiod cycle. Growth rates in land-based operations where temperatures vary seasonally from 6-12°C are fairly well known. Cage-based farming involves greater seasonal and geographic variation in temperatures. The water temperatures in the southwest of Norway can reach 18-20°C in the summer and winter temperatures, especially in the north of Norway, may fall to 2-4°C or lower.

Feed is a major cost in fish farming. Dry feed is becoming widely used and there is a tendency towards the use of feeds with reduced levels of protein and increased levels of fat, especially for larger fish.

The initial size of fish stocked into the cages is also an important factor in the economics of halibut farming. There is clearly a demand for a larger input fish, but to produce a one-year-old halibut (H1) of 200-500 g heated seawater is



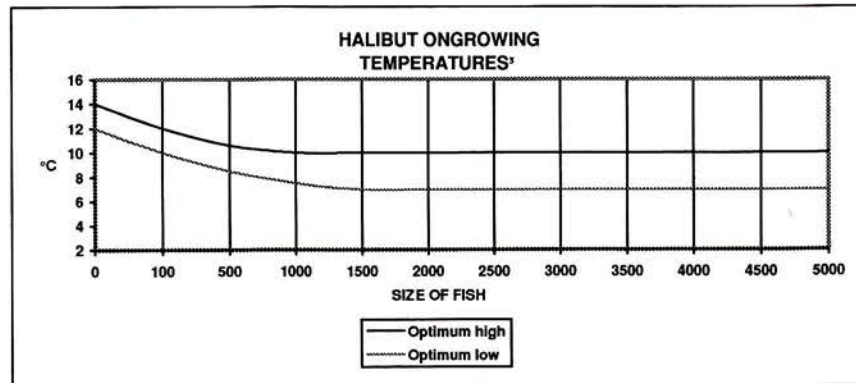


Figure 1. Estimated optimum on-growing temperatures for halibut. (2)

required. Compared to the traditional input of H0 fish in the autumn, cage farming requires a large H1 fish so that on-growing can be reduced by a year. Land-based operations without heating facilities would also benefit from using a large H1 fish as input.

The various farm concepts currently used for the on-growing of halibut are:

1. land-based — Aquahive (Halitek)-type cages (Fig. 2);
2. land-based — traditional separate circular tanks;

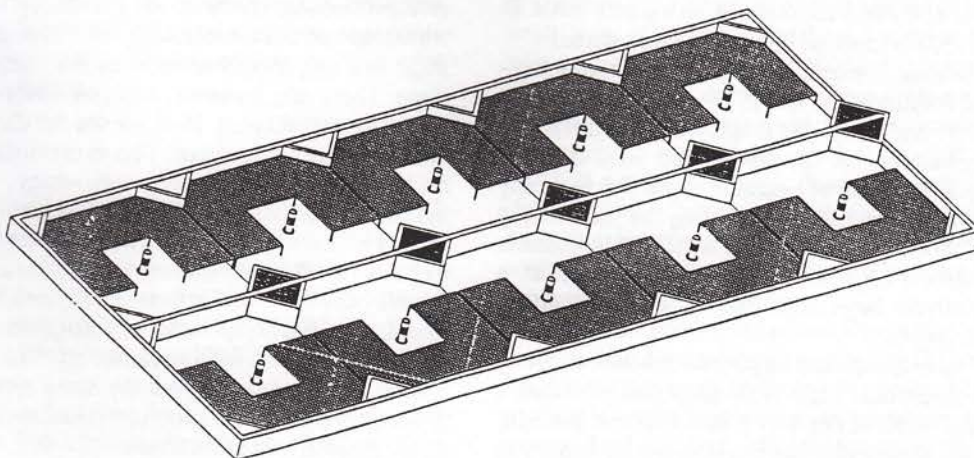
3. land-based — shallow raceways;
4. sea-based — cages with firm net bottoms (Fig. 3).

All modern land-based plants use photoperiod manipulation, and most oxygenate and pump relatively low volumes of water.

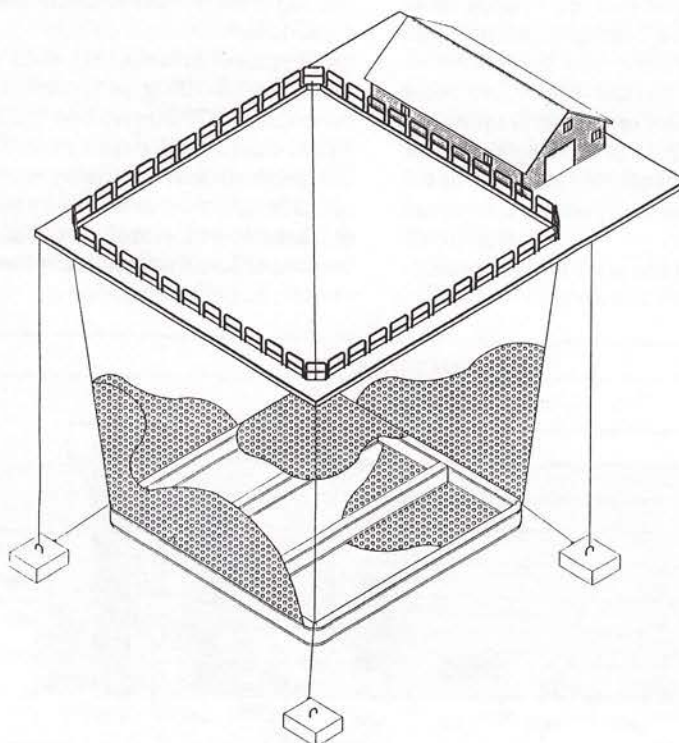
The major production plant, Stolt Sea Farm AS, is a forerunner of the Aquahive (Halitek)-type of operation. The Stolt Sea Farm plant has a market share of approximately 90%. There are currently experiments being conducted with other land-based systems, particu-

Table 1. Summary of a 1992 halibut marketing study. (1)

	Short term 1993-95	Long term 2000-2010
Volume (tonnes)	10-100	5 000-20 000
Goal	Market acceptance	Development of volume market
Market	Selected customers in UK & Scandinavia	Europe
Market segments	Up-market catering	Fragmented structure
Product	Whole fresh fish	Broad product range
Price (CIF/kg)	US\$11-14	US\$6.5-9
Turnover (US\$ million)	1	37-190
Distribution	Selected whole sales distribution	Diversified
Promotion	Direct marketing	Brand strategy



**Figure 2. Land-based Aquahive (Halitek) <sup>(3)</sup> farm system for on-growing halibut.**



**Figure 3. Cage-based farm system for on-growing halibut. <sup>(4)</sup>**



larly with shallow raceways. However, cage-based farming is currently attracting the most interest due to promising initial results and the low entry cost for farming on a small-scale. In 1995, 10-15 small operations were started.

When comparing these alternative technological solutions one has to focus on both productivity and entry cost (capital expenditure). The profitability of the land-based farm depends upon the scale of operations, but it is too early to draw conclusions regarding the profitability of cage-based on-growing of halibut. A possible solution may be land-based production of a relatively large input fish that is then grown in sea cages.

Halibut require a large area in which to grow. A traditional cage with deep nets provides a large volume but only a small area. It is calculated that production per unit can be five times greater for a volume-demanding fish such as salmon, compared to the area-demanding halibut. This fact has led to an interest in shelf systems for cage-based operations. Stolt Sea Farm AS has already installed a one-shelf, two-level system. The Halitek system has been tested with a two-shelf, three-level system. The goal is to include three shelves, in addition to the bottom area. Such a "halibut high rise" could expand available farming area by 100-200%. However, the shelf system introduces some challenges with respect to tank hydraulics and fish management. There is a strong economic driving force to obtain suitable solutions as the shelf system will dramatically reduce investment cost per square meter.

Considerable effort has gone into the produc-

tion of juvenile halibut, but progress has been hindered by problems related to up-scaling and shifting the technology from extensive to intensive production methods. The industry as a whole masters commercially the broodfish stage and egg supply as well as the yolk-sac stage. There are, however, still problems connected to start-feeding. Here we see the change to more intensive methods. This is mainly connected to feed and feeding technology. The industrial standards could be based upon an improved *Artemia*-based diet, a produced rather than harvested supply of zooplankton or, in the future, possibly even a formulated feed. The weaning stage still represents challenges in connection to feed and feeding technology. The first growth phase also involves the same type of questions. Up-scaling requires efficient solutions, especially in these fields.

There has been a lot of focus on disease problems in juvenile production, mirroring the fragile character of the very young halibut as well as, in some limited areas, an incomplete understanding of the required conditions for culture. Another factor is probably of substantial importance—under-investment in production plants making it impossible to control environmental conditions.

The price of juveniles has been very high. An input fish of 200 g or more has commanded more than US\$7.50 per fish. Small juveniles (5 g) have been sold at prices of US\$3.00-5.00. The production of halibut fry or small juveniles could be greatly intensified by producing several batches each year (Fig. 4). This would result in more efficient utilization of the facilities and

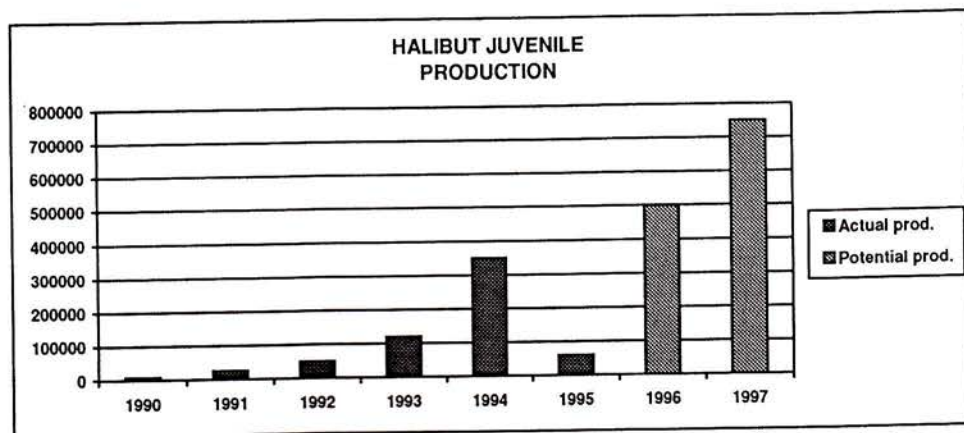


Figure 4. Juvenile halibut production.



lower production costs. Hence one can foresee a system of halibut farming involving production plants for fry (small juveniles), large juveniles (input fish for on-growing) and on-growing. The broodfish plant could be separate or integrated with the on-growing or fry production plant.

## Cod

Farmed cod have to be positioned for an off-season market or to special niches that require fresh fish. However, these markets do not pay a high premium compared to wild catches. Farmed cod also compete with wild-caught cod that have been kept and fed in cages for a period of time.

Farmed cod production reached a level of perhaps 500 t before declining in 1990. The setback was caused by market problems as rising wild catches reduced prices (even the higher prices were too low for cod-farming to be profitable). Catches in the North Sea and Barents Sea were at a minimum in 1990, with a total of 300,000 t, but rose to a level around 850,000 t a year in 1994-96.

Production costs for cod culture were high due to slow growth and early maturation, the disadvantages of culture on a small-scale, problems with small C0 fish (zero-year-old cod fingerlings are 10-20 g) and other factors.

The Ocean Research Institute in Bergen is now using photoperiod manipulation in the on-growing of cod. The results have been encouraging, producing both faster winter growth and fish that reach a marketable size without experiencing maturation. An additional effect of photop-

eriod manipulation is lower feed conversion rates and improved product quality.

An economic analysis has been undertaken<sup>(5)</sup> of a renewed cod farming concept located on the west coast of Norway and based upon a C1 (one-year-old cod juvenile) of 300 g, photoperiod manipulation, as well as large-scale modern farming techniques as in salmon farming. This analysis indicated a production cost of US\$2.75 per kg (live fish), financing costs excluded. A reduction potential of US\$0.30 in the short term seems to be realistic. Cod farming could be profitable today given a sales price of approximately US\$3 per kg.

The juvenile production of cod almost stopped due to falling demand from on-growers (Fig. 5). The Ocean Research Institute has continued to produce juveniles based upon an extensive culture method. In 1995, 100,000 juveniles were sold to commercial small-scale farmers.

The production of cod fry could be expanded along the same paths as halibut. It has been demonstrated that economics of scale are important in fry production. This is related both to the production of several batches per year and the size of each batch. The input fish could be a large juvenile, i.e. 300 g or more. This would again reduce on-growing time, as well as make life easier for the cod farmer as he would receive a more robust fish that demands less operational handling. Production of juveniles could be undertaken in land-based farms or closed systems, whereas on-growing has to be cage-based.

## Wolffish

The main interest is in the species *Anarhichas*

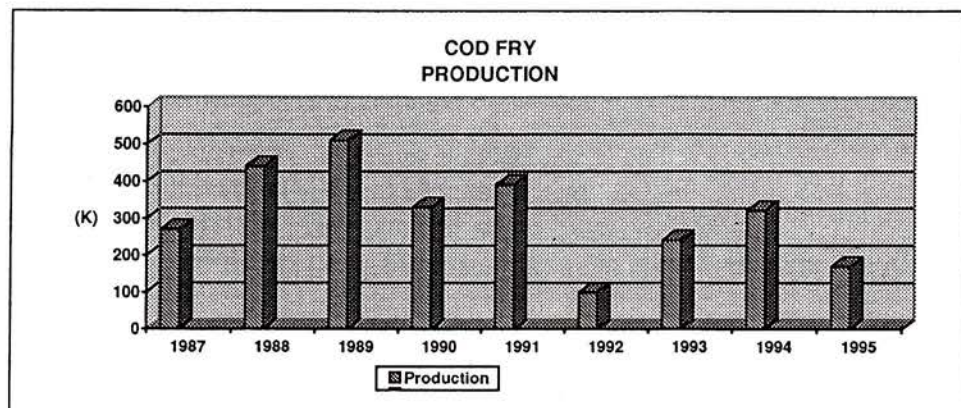


Figure 5. Juvenile cod production.



minor, the spotted wolffish, due to faster growth and higher filet yield than with *Anarhichas lupus* (the grey wolffish). The wolffish is another area-demanding fish and has to be grown on land-based farms or cage-based systems with a firm bottom or shelves.

There has been limited commercial interest in the wolffish, probably due to the low market price in Europe. There is also substantial competition from wild-caught fish. However, off-season prices and interest in up-market niches suggest some commercial potential in Europe.

The recognition in 1990 that fertilization occurs internally made juvenile production possible. About 5,000 juveniles are currently produced per year, but all activity is research-based. An industrial player could possibly increase juvenile production to a commercial level in a few years time.

There are indications of good growth at fairly low temperatures for the spotted wolffish. This is the most crucial question in addition to the market factor.

## Conclusion

Halibut could become an important species in Norwegian commercial aquaculture in the near future and has a potential of as much as 20,000 t in 15 years time. The increase will depend upon success of juvenile production and then market reaction.

Cod and wolffish have the potential to become new species in aquaculture. The crucial question is on the market side. Juvenile production for wolffish could possibly be expanded in the

short term. This could hopefully also be the case for cod. The solutions will however require a substantial investment from commercial players.

The central question in on-growing is verification of growth and productivity, ensuring profitability at realistic market prices.

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*Rolf Engelsen runs his own consultancy company in aquaculture/aquaeconomics. He was in charge of the Stolt Sea Farm AS marine fish operations from 1986-1992. He is engaged in commercial and development work in connection with halibut, i.e., juvenile production, use of heated water in production of big H1, land-based on-growing with inclusion of shelf systems and cage-based experiments. He has also undertaken a number of feasibility studies and economic analyses of halibut and cod farming. His company address is: Rolf Engelsen AS, Nordnesboder 3, P.B. 2031 Nordnes, 5024 Bergen, Norway.*

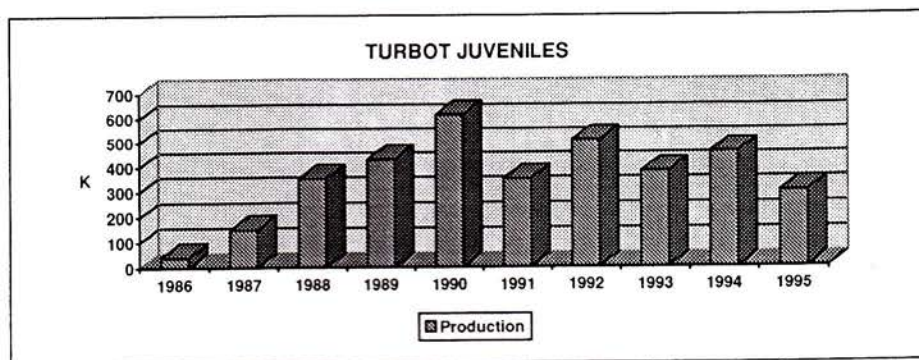


Figure 6. Juvenile turbot production.

**Aquabiotech:  
a blue revolution?**

*Elliot Entis*

Through the application of modern gene transfer technology, the aquaculture industry is closing in on the ability to produce fish and seafood products at a cost and at production levels which will make these protein resources available to much of the world's population. The ability to transfer desirable traits from one food source to another through biotechnology innovations allows aquaculturists to create beneficial changes much more rapidly than through traditional breeding techniques. What previously might have taken tens of generations of animal breeding to accomplish can now be done in three or four generations. This is the "blue revolution".

Aqua Bounty Farms, a division of A/F Protein, Inc. of the United States and Canada, is introducing the first "blue revolution" fish to the commercial world. Based on its decade of work with Atlantic salmon, Aqua Bounty Farms had demonstrated that it is possible to increase the growth rate of these fish by 400% to 600% over the norm. The company has begun to license its technology to salmon growers under the AquAdvantage label. Not surprisingly, the regulatory reaction to AquAdvantage parallels that of every other development from biotechnology. Some countries continue public wrestling matches over the very processes of biotechnology, while others have become more accustomed to simply regulating the products of biotechnology. In the United States, government agencies have been very supportive of agbiotech in general, and of the AquAdvantage breeding program specifically. In Canada, the governmental response is also generally supportive, but not yet well defined. The issue that requires the most attention is that of accidental escape. Aqua Bounty Farms believes that initial approvals to grow AquAdvantage fish commercially will be for land-based facilities where escape into the oceans is not an issue, or in seaside pens with fish that are sterilized, as is

often done now, thus rendering breeding issues mute. Growers, by and large, are excited by the tremendous enhancements in productivity which AquAdvantage and similar aquabiotech products can bring. To be sure, they need to be reassured that consumers will accept these products and are thus watching carefully the reactions of consumers to other biotech food applications.

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**Developing and evaluating  
seaweed diets to enhance the  
commercial gonad value of the  
green sea urchin,  
*Strongylocentrotus  
droebachiensis***

*Tom McKeever*

The green sea urchin, *Strongylocentrotus droebachiensis* supports a wild fishery in Newfoundland but the value of this fishery is limited by disappointing roe yield and quality, and a short harvesting season. The feasibility of feeding abundant seaweed species to sea urchins, to enhance yield and value, was investigated, in both aquarium culture and seabed cages. The kelp, *Laminaria digitata*, was found to be the most effective feed, while *L. longicuris* gave lower, but still good results. Ration size trials showed that feeding rates of 0.2% gave optimum results with no better growth at higher ration rates. Seasonal experiments showed greater growth in the summer and autumn, with slow growth rates in the winter and spring. Feeding rates were also lowest during the cold seasons, so assimilation was still high. Spawning, which terminates the wild fishing season, was suppressed in cold water aquaria, thus extending the harvestable season to any time of the year. Seabed trials gave less consistent results than tank culture.

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# The Canadian Institute of Biotechnology

## Aquaculture Initiatives

The Canadian Institute of Biotechnology (CIB) is a not-for profit organization dedicated to assisting in the human resource development, technology diffusion, networking, communication and promotion of biotechnology across Canada. CIB has three main activities: promoting partnerships and linkages between Canadian biotechnology companies and their international counterparts; supporting member-proposed biotechnology projects; and offering contracting services to the public and private biotechnology community. Membership in CIB is open to all not-for-profit professional and industrial associations, universities, research centres and regional groups with and involvement in biotechnology.

In the wake of dwindling fish stocks and a growing world population, aquaculture is becoming an increasingly important industrial sector. Applications of biotechnology are now being widely applied to improve the quality and health of many aquaculture species. Canada is a world leader in the research and development of biotechnology applications, products and processes that can serve the global aquaculture industry.

Canadian biotechnology companies and research organizations are developing leading edge aquaculture technologies in four main areas:

- **Health Management** disease diagnosis, vaccine development, health strategies
- **Broodstock Development** spawning induction, genetic analysis, monosex technologies
- **Environmental Management** microbial pond treatments, bioremediation, waste solutions
- **Quality Control** pathogen detection, processing to HCCAP, ISO and GMP standards

Canadian biotechnology organizations are in an excellent position to provide needed technical expertise and know-how in these areas to the global aquaculture community.

The CIB recognizes the impact that biotechnology can have on the aquaculture industry, and has begun concentrating on the international promotion of the Canadian aquaculture technology sector. Some selected activities include:

#### ***Aquaculture Technology in Canada Package***

In conjunction with Industry Canada, the CIB has developed a unique *Aquaculture Technology in Canada* marketing package. This package consists of a full colour brochure summarizing and illustrating Canadian biotechnologies in the aquaculture health management, broodstock development, environmental management and quality control areas. The package also contains brief descriptions of over seventy Canadian companies and research organizations offering biotechnology research, products and services to the aquaculture industry. This package is intended to showcase and highlight Canada's expertise in the aquaculture biotechnology sector, and is receiving wide international distribution at important aquaculture events.

#### ***Aquaculture Technology Round Table***

In October 1996, the CIB brings together the Canadian groups identified in the *Aquaculture Technology in Canada* package, at an inaugural aquaculture round table meeting in Fredericton, New Brunswick. The meeting has three goals:

- 1) To allow the Canadian aquaculture technology community to meet and speak together;
- 2) To present the Canadian aquaculture technology community with potential aquaculture business projects and market opportunities in Korea and Chile; and
- 3) To encourage the formation of strategic business alliances or "networks" between Canadian aquaculture technology organizations as a means of accessing these projects and markets.

The CIB feels that the formation of one or more strategic business networks between Canadian aquaculture technology companies and research organizations will enable them to pursue international aquaculture opportunities that they would be unable to approach on their own.

**For more information about CIB's aquaculture related activities please contact us at:**

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**Canada K1P 5G4**

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**E-mail: [cib@biotech.ca](mailto:cib@biotech.ca) Web Site: <http://www.biotech.ca/>**



### **Algal production in BIO-COIL for aquaculture: a business perspective**

*Lawrence C. Sherman*

Continuous production of algal species in the BIOCOIL was developed via a cooperative agreement between Biotechna Environmental Ltd. and Dr. Michael Borowitzka. Certain species of algae yielded production quantity results in an internally-funded development program. These algae are currently utilized in the aquaculture field in large quantities and by many users. The commercialization of the BIO-COIL for continuous algal production has been accomplished. Economies of scale result in cost-effective production at the end-user level. Service and maintenance requirements are minimal as are nutrient and carbon dioxide usage. Installation and operational procedures are easily followed to yield consistent results with regard to specific densities for individual species.

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### **Recirculation systems for cold water aquaculture**

*Ted White*

Aquaculture in British Columbia has traditionally required large supplies of high quality water. The quality of water is often degraded considerably after passing through an aquaculture facility and sometimes creates problems in

the receiving body of water. Additionally, many producers heat water to gain significant improvements in production levels throughout the year. Although heat recovery technology is used, much energy goes to waste in the effluent. Energy costs typically represent half of the production costs for Atlantic salmon smolts or approximately 3 to 4 million dollars per year in Canada on an industry-wide basis. Many sites are now water and heating capacity limited.

Recirculation or re-use of water is a method of reducing the amount of heated effluent produced and consequent energy wastage. A cost/benefit analysis indicates that potential savings with water re-use could be as high as 78% per day if a strategy of 80% re-use is adopted. This represents an industry-wide cost reduction of approximately 2 million dollars per year or 24% of estimated production costs. NovaCulture Inc. has developed a water re-use technology (US Patent #5,273,665, Canada Patent Pending), which has the potential to achieve the estimated cost benefits.

This technology is now in the early stages of commercialization after prototype trials at Malaspina University College. Two sizes of filter have been developed: 76 cm diameter and 122 cm diameter models, respectively, in response to identified industry needs. The main unit consists of a swirl separation chamber for primary solids removal and a packed media canister. The media pack provides a substrate for nitrifying bacteria colonization and aids in solids removal. A modified air-lift pump re-oxygenates the system water and returns it to the rearing unit. Operational data from four prototype systems and four commercial systems indicate that water usage may be reduced by as much as 95%. In addition, solids and ammonia removal have exceeded 95% efficiency and greater than 90% oxygen re-saturation has been achieved in a single pass. Advantages of this new system include minimum maintenance, no back-flushing and minimal cleaning, and simplicity of installation and operation. Both capital and maintenance costs are low with an estimated payback of less than two years.

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**Feeding, production and  
effluent waste in aquaculture:  
use of computer prediction  
models (BEPFEQ System)  
based on bioenergetics and  
MS-Excel**

*C. Y. Cho*

Feeding guides for salmonid fishes have been available from various sources for many years. These feeding guides originated, in one way or another, from earlier feeding charts in the 1950-60s. Few of the feeding guides available today are based on actual bioenergetics data collected at different water temperatures using high energy diets. A new feeding standard has been developed (Cho 1976-92) and this is based on the principle of nutritional energetics in which the digestible energy (DE) content of the diet and the amount of DE required to produce a unit of gain expressed as retained energy (RE) plus maintenance energy at different water temperatures are the main criteria. Series of models were developed using MS-Excel (ver. 5.0) on a PC computer to predict energy/N-P (nitrogen and phosphorus) gains, requirements and excretions to determine feeding standards, growth and effluent quality. Computer programs require initial body weight, water temperature, and apparent digestibility and retention coefficients (ADC and NRC) to estimate input and output. Accurate determinations of thermal-unit growth coefficients (TGC), ADC and NRC are essential and ADC and NRC are determined by biological experiments in the laboratory.

The software is available from the Ontario Ministry of Natural Resources (fax: 905-832-7177).

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**Quantifying the health  
of a benthic habitat  
using new acoustical  
techniques and  
fractal geometry methods**

*Jacques Guigné*

Information on the degree of activity or health of benthic organisms and how these activities are affected by environmental changes such as caused by aquaculture practices can be determined through close examination of the physical structure of marine sub-bottom sediments. New advances in very high resolution acoustic reconnaissance techniques allows, for the first time, the quantification of the spatial variations and heterogeneity pattern associated with bioactivity within the near surface zones of a seabed. The use of very broadband acoustics for benthic health estimations offers several advantages over current physical sampling techniques. Traditional grab and core sampling approaches disturb the structural integrity of the habitat and, therefore, key ecological information is lost in the process of retrieving and processing of the sediments. However, the non-destructive response from temporally and spatially precise coherent acoustics can reveal unique sediment characteristics. Images of biogenic structures and physical features of taxonomic groups of fauna can be acquired. Spatial variations found in the acoustical reflections are then handled by applying an extension of classical Euclidean geometry called fractal geometry. The physics for precise acoustic characterization of a marine habitat were presented whereby acoustical snapshots with millimetre scale resolution are generated. Actual data were used to illustrate how broad bandwidth signals are necessary for the development of unique acoustic fractal geometry classifiers which recognize the subtleties found in benthic fauna activity.

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## **Genomics and bioinformatics: new paradigms for biotechnology**

*Mark A. Ragan*

The international initiative to sequence the entire human genome (and genomes of other animals, plants, fungi, protists and bacteria) has hastened the development of automated technologies for chromosome mapping, gene localization, screening for mutations, library generation and high-throughput DNA sequencing. In combination with sophisticated analytical software and nightly updated on-line molecular-sequence databases, these technologies are nurturing genomics, the emerging science of whole-genome biology. Genomics promises to enable the understanding of entire high-level biological processes (development, disease resistance, evolution, immunogenesis, pathology, reproduction) by building on the complete inventory of genes present or active within a cell, tissue or organism.

The genomics revolution is already affecting many areas of biotechnology, from plant breeding to biomedicine. Virtually all major pharmaceutical companies, and growing numbers of companies in, e.g., agricultural biotechnology, now have active genomics programs. Two fish are proving valuable as animal models of the human genome — pufferfish (with one of the smallest known vertebrate genomes) and ze-

brafish (with well-established developmental genetics). In some cases, synteny and linkage are conserved between fish and human. For example, pufferfish homologs of human genes involved in familial Alzheimer and Huntington diseases have been characterized.

Pufferfish and zebrafish (or others yet to be identified) should be even better models for other fish. Indeed, a comprehensive understanding of the genome of any one fish species is likely to be broadly and immediately applicable to salmonids, flatfish, halibut, cod, bass, tilapia, etc., in the same way that genomic data from *Arabidopsis* are used in breeding programs for maize, wheat, canola and rice. The National Research Council of Canada is building expertise in key areas of genomics. Two collaborative genome-sequencing projects are underway at the Institute for Marine Biosciences (NRC-IMB) in Halifax. These projects are supported by a bioinformatics capability that includes computing infrastructure, an integrated Unix-based genomics environment, internet connectivity to remote facilities, and artificial intelligence-based tools. A distributed facility for leading-edge bioinformatics is under intensive development with substantial involvement of NRC-IMB. NRC is actively soliciting the involvement of companies and research groups in these initiatives.

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### **UPCOMING PROCEEDINGS FROM THE AQUACULTURE ASSOCIATION OF CANADA**

Sea urchin roe enhancement (workshop at Aquaculture Canada '96)

Cold water aquaculture to the year 2000 (Huntsman Marine Science Centre Symposium)

Critical issues in feed management (special session at World Aquaculture '97)

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## Vaccines and diagnostics for aquaculture: new approaches to old problems

Julian C. Thornton

The first step in the effective management of disease in fish is rapid diagnosis. The diagnosis of any disease usually follows a step by step procedure that should include visual observation of moribund fish for any obvious signs of specific diseases, and a necropsy of fresh mortalities for both gross- and microscopic pathological changes. In the case of infectious disease, the infectious agent(s) must be rapidly isolated and identified so that treatment can commence in a timely fashion. In many cases, vaccines are available for use in disease prevention programs. In aquaculture these vaccines range from simple monovalent bacterins delivered to fish by immersion, to polyvalent mixtures of vaccines in complex adjuvant emulsions. Recent advances in the way we examine disease causing agents have changed our understanding of many "old" fish diseases. These advances in vaccine development are discussed with respect to how highly technological scientific principles can be adapted to simple applications.

### Diagnosis of Infectious Diseases

The diagnostic process for infectious diseases in aquaculture has been well reviewed elsewhere,<sup>(1,2)</sup> so all that will be presented here are the general techniques used in diagnostic tool development.

For infectious diseases, the diagnostic process begins with the initial identification of a diseased state in an individual or population, and proceeds through a logical set of steps to the eventual isolation and/or identification of the infectious agent(s) that is (are) responsible for that disease. In aquaculture, important information that may aid the diagnostician typically includes farm location, water temperature, water salinity/hardness, affected species, age of the affected population, recent husbandry history (i.e., handling, grading, transport, etc.), and the symptoms and behavior of the affected population (i.e., changes in feeding, swimming, obvious gross pathology, etc.). Although these do not, in any way, result in a final diagnosis, they can influence the next steps in the diagnostic process.

The next steps in diagnosis are a complete histological examination of suspected target tissues, and the isolation of the pathogens from gross lesions and suspected target tissues. Either step on its own can lead to erroneous results, as fish succumbing to one infection are quite often at risk of developing severe secondary infections, and several types of histopathology are common to many of the infectious diseases.

The identity of infectious agents can be determined by a multi-stepped process involving the culture of suspected bacterial pathogens in differential media followed by specific biochemical tests. Some of the variables that affect the accuracy of the identity by culture include incubation time, (e.g., *Renibacterium salmoninarum* and *Vibrio salmonicida* are slow growing, and are therefore easily outgrown on media by co-cultured opportunistic bacteria and/or fungus) temperature (at 24°C, *Aeromonas salmonicida* loses the A-layer, thus the autoaggregating phenotype associated with virulence), and nutritional fastidiousness (e.g., *R. salmoninarum*, *V. salmonicida*, and many *Flexibacter*



spp. require specialized, rich media to support growth).

Typically, the identity of infectious agents is made by observing some special distinguishing characteristic, such as pigment production by *Aeromonas salmonicida*. But, as is seen with increasing frequency, many isolates of *A. salmonicida* are non-pigment producing strains (unpubl. obs. and Popoff<sup>(3)</sup>), and conversely, many non-aeromonads are capable of similar pigment production (unpubl. obs.). In these types of situations, secondary tests are required for accurate identification. These typically involve differential analysis of the biochemical profiles of the isolates such as those incorporated into the API20E system (bio Merieux). This level of diagnostic sophistication represents the first generation of diagnostic methods that were, and in some cases still are, used for disease diagnosis in aquaculture. In the case of viral pathogens, growth in tissue culture using different fish cell lines is the first step to identification. Isolation and characterization by these culture methods still represents the "gold standard" by which the specificity of all other methods are compared.

The second generation of diagnostic tools involves the use of specific polyclonal (pcAb) or monoclonal (mAb) antibodies, for identifying infectious agents by several different methods such as simple agglutination, direct or indirect fluorescent antibody tagging (DFAT and IFAT), enzyme linked immunosorbant assays (ELISA), or by Western and/or immuno-dot blots. Antibody based tests can be limited by the specificity and sensitivity of the antibodies used (mAb vs. pcAb), the choice of antigen used for the generation of said antibodies, accuracy in comparison to identity by culture, and the stability of the target epitope(s) during tissue processing. Of course, the ultimate utility of any test is limited by the required equipment, for example a sensitive test may fail in the field if ELISA plate readers are required. Despite these limitations, semiquantitative, antibody-based tests are now available for the detection of many fish pathogens that do not require costly plate readers. These tests have proven themselves as useful tools for the presumptive segregation of fish stocks into infected and "non-infected" groups, or for the rapid identification of the causes of mortalities.

The third generation of emerging diagnostic methods involves the detection of specific DNA

or RNA sequences in the infectious agent. These tests are typically based on either polymerase chain reaction (PCR) tests or nucleic acid hybridizations (typically in the form of dot blots), or a combination of both of these. These tests are extremely sensitive, with a detection limit approaching one cell or virus particle per sample. The availability of this type of sensitivity will no doubt impact on current regulations regarding notifiable diseases. For example, does the existence of one cell per gram of kidney tissue in a small percentage of a fish population mean that the entire population is at risk of contracting the disease in question, or are our tools becoming too sensitive to be of value for simple diagnostics? Due to their extreme sensitivity, these nucleic acid based tests are at risk of false positives due to contamination, unless stringent operating procedures are followed by highly trained laboratory personnel. Vaccination of the population can result in a false positive diagnosis if nucleic acids are present in vaccine preparations and are picked up by the test. Tests such as these are of no obvious use in a field situation, but they do open the door for extremely accurate screening of populations for the determination of carrier fish for certain diseases (e.g., furunculosis, BKD, and all viral pathogens) if they are used properly.

The difficulties in taking any diagnostic kit for aquaculture from the laboratory bench to full use in the field are numerous. All diagnostic methods, from the first generation to the fourth, require some degree of user expertise. All testing methods require sample stability, whether it is retaining viability and/or phenotype of infectious agents, retaining intact nucleic acids, or retaining the integrity of specific epitopes to which the specific detection antibodies bind. Also, the role of alternative diagnostic systems with respect to government regulations involving "notifiable" diseases is unclear. As sensitive diagnostic methods begin to rely less on the ability to culture a specific pathogen, the vaccination history of a population may obscure some of the new tests. Finally, the cost of a diagnostic test is of primary importance. It is a relatively simple task to develop a rapid and sensitive test for any infectious agent, but doing so in a way that results in a functional, yet affordable, test is a challenging project.



## Prevention of Infectious Diseases through Vaccination

Vaccination is the active process of inducing protective immunological responses against specific foreign substances. Thus, if the correct antigen (foreign target molecule) is presented in the correct manner, to the appropriate part of the immune system (either cell mediated, humoral, or both) an immune response may be elicited that will display a memory component. Unfortunately, it is not always evident which of the bacterial antigens are required for protective immunity, nor is the correct method of presentation of the antigens to the cells of the immune system always clear.

A factor that may contribute to the usefulness of an aquaculture vaccine is the method with which it may be administered. Some vaccines for aquacultural use must be injected, while others can be administered orally, or by direct contact (immersion, spraying, etc.).<sup>(4-6)</sup>

Effective immersion and injectable vaccines have been developed for most of the major bacterial diseases that affect cultured salmonids, such as vibriosis caused by *Vibrio anguillarum* or *V. ordalii*, cold water vibriosis (Hitra's disease) caused by *V. salmonicida*, furunculosis caused by *Aeromonas salmonicida*, and enteric redmouth disease caused by *Yersinia ruckeri*.<sup>(7,8)</sup> However, adjuvanted, injectable vaccines still remain as the only type of vaccine that have repeatedly been demonstrated to confer high levels of long lasting immunity. Due to the potential for vaccines as inexpensive prophylactic medicine, a substantial amount of research is being done on the development of increasingly effective vaccines for all finfish diseases.<sup>(5,9)</sup>

There are problems associated with the use of injection vaccines, these include physiological and psychological stress for the fish, a costly requirement for specialized equipment, and a need for skilled technicians to administer the vaccine. Problems also exist for immersion and orally delivered vaccines, these include poor duration of immunity for some diseases, and in the case of oral vaccines, they are too costly for larger fish. Bearing these factors in mind, vaccines designed for use in aquaculture must be at least as inexpensive and more effective than antibiotic therapy or pure economics will prohibit their use (for review see Ellis<sup>(9)</sup>). Many commercial vaccines are available for use in

disease prevention programs in aquaculture. These vaccines range from simple monovalent bacterins delivered by immersion, to polyvalent mixtures of vaccines in complex adjuvant emulsions for injection delivery.

During the past 50 years, furunculosis vaccines have probably received more attention than all other salmonid disease vaccines combined. The reasons for this are due, in part, to the economic importance of furunculosis in the farming of salmonid fish, and because safe, efficacious furunculosis vaccines are difficult to develop. Successes and failures of furunculosis vaccines have been reported using any or all routes of administration.<sup>(5)</sup> Thus, furunculosis vaccine development serves as a good model for demonstrating how improved scientific methods are changing the accepted dogmas surrounding a seemingly well characterized, specific disease.

Numerous studies have been carried out to elucidate which of the virulence associated factors of *A. salmonicida* (the causative agent of furunculosis) are important in inducing protective immunity to furunculosis. These studies have revealed that *A. salmonicida* possesses an extensive array of virulence factors including: a regular surface array (A-layer), lipopolysaccharide (LPS), high affinity iron sequestering systems and an overabundance of extracellular toxins and enzymes that are apparently associated with virulence.<sup>(5)</sup> Of these virulence factors, the A-layer has received the most attention by far, and although the reference list for research into the A-layer is very extensive, only a few will be given here.<sup>(10-12)</sup>

It has been proposed by several authors that in order to resist infection by *A. salmonicida*, the fish immune system needs to recognize and respond to A-protein (the subunit of the A-layer). Comparisons of the immunogenicity of various strains of *A. salmonicida*, demonstrated that A-layer negative strains were inferior as immunogens in both fish and rabbits.<sup>(12)</sup> In support of these observations, in comparisons of the vaccine potential of various strains of *A. salmonicida* by different methods, Olivier *et al.*<sup>(13)</sup> and McCarthy *et al.*<sup>(14)</sup> also found that A-layer negative strains were inferior as immunogens in both fish and rabbits. It was reported by these authors that of all bacterins tested, the only ones that conferred any significant level of immunity to fish were those bacterins made from a suspension of A-layer possessing *A. salmonicida*



cells. Assessments of the antigens recognized by immune sera from bacterin vaccinated salmonids revealed that the majority of antibodies produced by immunized fish are directed at A-protein and the repeating O-antigen of the LPS.<sup>(15)</sup> As far as antibodies to the extracellular components (ECP) produced by *A. salmonicida*, it has been demonstrated that both rabbits and fish produced antibodies to several distinct proteins in the ECP.<sup>(16)</sup>

Ellis et al.<sup>(17)</sup> developed a subunit vaccine based on two purified antigens that only weakly react with immune fish sera. Despite the fact that protection from other vaccines does not correlate well with antibody titre, these authors found a strong correlation between protection and mean antibody titre to these antigens in vaccinated fish. However, the mean antibody titre appeared to steadily decline throughout the trial (40 weeks) and subsequent boost apparently depressed the serum titre dramatically; coincidentally, the protection levels also fell. The authors suggested that possible explanations for this phenomenon may involve the development of tolerance to the antigens, or antigenic competition with other strong antigens in the vaccine preparation.<sup>(17)</sup> The actual antigens and precise formulation of the vaccine preparation were not disclosed by the authors. During all of this research, the ability of research groups to assess the importance of various antigens in a vaccine preparation has been limited to the identification and study of *A. salmonicida* antigens that are expressed under *in vitro* growth conditions (i.e., growth in synthetic media).

Our work on furunculosis vaccines has recently been focused on the development of live attenuated vaccines for furunculosis.<sup>(18)</sup> Live vaccine strains have been used in experiments to examine whether live attenuated strains of *A. salmonicida* could confer immunity to fish after administration by immersion. The vaccines are indeed effective as an immersion vaccines and are capable of providing a 35-fold increase in resistance with a single dose, to an approximate 1000-fold increase in resistance when administered with boosting doses.<sup>(19)</sup> These levels of protection can only be approached by bacterin mediated immunity when an adjuvanted bacterin is administered by injection (unpub. obs.).

Surprisingly, sera from fish immersion vaccinated live vaccines revealed that the majority of the humoral immunity generated by these vaccines is not directed at either A-layer or LPS. It

appears that the major immunogens on live furunculosis vaccines are a high, heterologous molecular weight, proteinase resistant fraction (possibly a carbohydrate), and a series of proteins with the major protein antigen being low molecular weight (~ 15 kDa).<sup>(20)</sup> This marked difference from the humoral response generated by standard bacterin vaccination is most likely indicative of the difference in antigens presented to the fish immune system by live vaccines. These antigenic differences are currently being addressed by us, and through this work it has become clear that there are several antigens expressed by *A. salmonicida* only during *in vivo* growth.

The growth of *A. salmonicida* in *in vivo* conditions using a surgically implanted growth chamber, has had some surprising results. Under these conditions *A. salmonicida* expressed a glycocalyx-like structure (possibly a capsule), that appears as a loosely associated coat external to, and apparently masking, the A-layer.<sup>(21,22)</sup> It is not yet known if the glycocalyx observed by TEM is the same as the putative carbohydrate antigen seen by Western immunoblotting, or if these structures are indeed the same as the capsular like material that has been reported for *A. salmonicida*.<sup>(23)</sup>

Further vaccination experiments using cells deficient in the A-layer and in the O-antigen of LPS have indicated that when using a live vaccine for furunculosis, the protective antigens are likely something other than these two cell wall components.<sup>(20)</sup> Interestingly, this protection may indicate that although A-protein and LPS appear to be strongly antigenic, they may not be the important protective immunogens.

These *in vivo* antigen studies are now being incorporated into full vaccine research for all of the major fish diseases. The results from this furunculosis work have reinforced the importance of keeping a clear view that what occurs in the laboratory and what happens during the real infection may have clear differences that directly impact vaccine and diagnostic kit development. Clearly, the development of the next generation of aquaculture vaccines in general should rely on the identification of important immunogens expressed by the pathogen *in vivo*, and the selection of *in vitro* conditions that maximize their expression. This strategy of research will undoubtedly result in the development of more effective vaccines for all diseases of finfish.



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## Efficacy and action of CME-134 used as an oral treatment for the control of sea lice, *Lepeophtheirus salmonis*

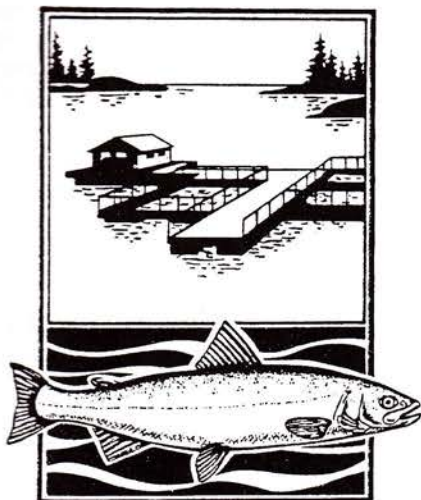
*Gordon Ritchie*

The efficacy of CME-134 (an acyl urea) used as an oral medicine for the treatment of sea lice (*Lepeophtheirus salmonis*) infestations on farmed Atlantic salmon (*Salmo salar*) under commercial farming conditions is reported. Results from two commercial sites showed that CME-134 was 95% efficacious toward certain stages of *L. salmonis*, when administered to fish at a dosage of 10 mg/kg body weight/day, for a period of 7 days. Attached chalimus and pre-adult stages were most susceptible. Reduced efficacy was observed toward adult *L. salmonis*.

Morphological examination of exposed lice showed the damage caused to the cuticle, which subsequently resulted in death. Egg strings of adult females were also damaged, suggesting CME-134 can influence egg production and viability in *L. salmonis*.

*Gordon Ritchie is project manager and researcher for Nutreco Aquaculture Research Centre, Forusbeen 35, PO Box 353, N4033, Forus, Norway.*

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## Role of big banks in financing biotechnology

*William Crawford*

The role the big six Canadian chartered banks are playing in financing Canada's biotechnology companies is evolving as banks attempt to provide non-traditional debt financing to knowledge-based businesses. Recognizing the important role of "patient equity capital" for the biotechnology industry, the equity investing activities of the chartered banks, with particular focus on the Bank of Montreal's commitment to equity investing in small businesses in Canada, is highlighted in this report.

### Introduction

In this paper, the role of the chartered banks in financing biotechnology companies is reviewed. Not long ago this would have been a very short report, for chartered banks weren't financing biotechnology companies, because according to traditional "tried and true" bank lending standards, there was nothing to finance — no plant or equipment, no inventory or receivables, and absolutely no assets as collateral that traditional bank lenders could liquidate if they had to. But over the last several years, given the changes occurring in Canada and the world, banks have come to recognize that a new way had to be found to finance the knowledge-based industries that were becoming the "engines of growth" in Canada.

### How Have the Banks Responded?

- Some of the banks have responded by creating new organizational structures to service so-called knowledge-based industry (KBI) customers;
- Some have specialized training programs for KBI account managers to help them better assess opportunities;

- A few have created new, more flexible lending products based on anticipated cash-flows;
- Most have rethought credit assessment, particularly as it relates to collateral security;
- Several are committing large amounts to equity and quasi-equity financing through specialized subsidiaries.

Most of the banks have responded in some of the ways mentioned in an attempt to provide non-traditional bank debt financing to knowledge-based companies.

In 1990, the Bank of Montreal made a strategic decision to target the small- and medium-sized enterprises market as a high priority. Two of the early successful initiatives were the *prime rate strategy* and the *small-business lending rate*. In 1993, the Bank of Montreal opened its first innovation and technology banking centre in Kitchener/Waterloo, which subsequently served as the prototype for the ten other innovation and technology banking centres presently operating. The account managers assigned to these centres have undergone specialized training to help them better forecast a company's potential future cash-flow based on an assess-

ment of management, product capability, marketability and competitive advantage.

After talking to knowledge-based companies to determine their requirements, the Bank of Montreal developed and standardized three new flexible lending products, namely:

1. Interim financing for R&D tax refund receivables;
2. Foreign accounts receivable financing; and
3. Contract financing.

These forms of lending are now possible due to our bank's assessment of the degree of certainty of specific future cashflows reviewed against a background of stable historical cashflows for the company. All of these Bank of Montreal initiatives and those of the other banks have been significant steps in the ongoing evolution of bank lending practices to adapt to the new business reality in Canada and the world. However, as commercial bankers, we remain challenged to find creative lending solutions for high-technology, knowledge-based companies when future prospects and cash-flows are nebulous and alternative sources of repayment are unavailable.

Bank lending by itself is not the entire answer to the funding problems of knowledge-based industries in general and biotechnology companies in particular. Most Canadian biotechnology companies have few revenues of any kind for many years, while they continue to "burn" cash for basic R&D, product development and the lengthy regulatory approval process. These characteristics do not suit debt financing but require "patient equity capital". It goes without saying that most Canadian biotechnology companies have a difficult time raising this patient equity capital, which is key to the most crucial stage in a company's development—the growth of the company through commercialization of its product. In these cases, again as commercial bankers, it is debatable to what extent, if at all, we should be placing depositor's funds at risk investing in companies with these characteristics. Our depositors, shareholders and regulators all share this concern, and for many years banks were not permitted to make equity-like investments. But over time, the Bank Act has been amended to permit banks to undertake a certain amount of equity and quasi-equity financing with limitations on:

1. The size of any individual equity investment;

2. The size of the overall portfolio of equity and quasi-equity investments in relation to a bank's regulatory capital (5% maximum);
3. The total amount of depositor's funds and bank equity capital which can be placed at risk in these types of investments.

While some banks (Royal, Toronto-Dominion and Canadian Imperial Bank of Commerce) have had subsidiaries making equity investments for several years, historically most of these equity funds have been directed towards "merchant banking" or venture capital investments in companies at later stages of development. The Bank of Montreal did not make equity investments until just this year. Some of the reasons Bank of Montreal launched the new equity financing initiative was because in recent years there has been a proliferation of labour-sponsored venture capital funds but these too have been directed towards larger-sized investments in companies at later stages of development. Based on feedback from our small- and medium-sized customers in all industry sectors from across Canada, the Bank of Montreal identified two specific opportunities for equity financing where there appeared to be a large "gap" in the supply of equity funds and the investment risk/reward relationship appeared reasonably attractive. First, equity investments of less than \$1 million in knowledge-based high growth businesses at all stages of development. And second, expansion equity capital in amounts of less than \$1 million for companies experiencing medium to high growth in a broad range of business sectors.

Bank of Montreal has responded to these gaps in availability of equity financing through our recently announced \$200 million commitment to equity investment in small and medium-sized enterprises, a large portion of which is dedicated to providing expansion equity financing for companies in amounts of less than \$1 million. Equity investments will be made across Canada by Bank of Montreal Capital Corporation under three separate programs.

First, a \$120 million small business capital program focused on providing expansion equity in the form of venture loans in amounts of less than \$1 million to finance growth opportunities for companies operating in all business sectors. These investments will be managed as "passive investments" by a small group of investment



managers in a wholly-owned subsidiary of the bank (Bank of Montreal Capital Corporation) based on opportunities referred to them by Bank of Montreal account managers, initially in our technology banking centres. We will invest in well-managed companies with demonstrated profitability that require a "passive equity investment" of up to \$1 million, on a temporary basis, to finance a significant growth opportunity. Our investment will be in the form of subordinated debt with a premium interest rate and a royalty on sales and equity participation. This structure will provide an overall equity-like return on our investment similar to that of the majority owner of the business. Specific terms will be tailored to suit each individual opportunity but we would expect to be quasi-equity investors for up to seven years if required, and would not be seeking to own a majority of the company but rather be a passive minority partner.

Our second major program is a \$60 million National Technology Investment Program focused on providing venture capital to high-growth knowledge-based companies at all stages of development in amounts from \$500,000 to \$5,000,000. This program will be managed by Ventures West a highly regarded experienced venture capital fund manager located in Vancouver and Toronto in conjunction with our technology banking centres. This program will definitely be involved in reviewing equity investments in biotechnology companies although they will probably tend to be at later stages of development. Our focus, first and foremost, will be on companies that are well managed and have excellent growth opportunities.

Our third program will be a portfolio of investments aggregating \$20 million invested in venture capital funds managed by others where the bank has a special interest, such as the intellectual property development funds being created and sponsored by various universities such as Queen's University and the University of Guelph (which include some biotechnology investments), and the Neuroscience Partners Fund backed by a consortium led by the Royal Bank. Altogether \$200 million (or 2% of regulatory capital) has been committed by Bank of Montreal to fund these three programs.

In addition, the Atlantic Investment Fund has recently been formed as a cooperative venture

between the four Atlantic Provinces, ACOA, and the private sector represented primarily by the chartered banks, and has created a \$30 million venture capital fund.

Together with the Bank of Montreal's recently announced merchant bank, which will operate in both Canada and the United States through our Nesbitt Burns investment banking subsidiary, these programs will utilize most of our present capacity under the Bank Act for equity and quasi-equity investments.

Several of the other banks have also made commitments over the past year or so to increased levels of merchant banking and venture capital equity investments. As a result, the amount of money committed by the banks to equity investing in general is at an all time high. In the near term this has not necessarily translated into increased equity investments in biotechnology companies given the difficulty of risk/reward assessment involved in these opportunities. However, as more positive investor experiences are realized from biotechnology equity investments, and banks become more comfortable with their ability to properly analyze these investments, either directly or through hired experts, the amount of bank equity investment in biotechnology companies should increase.

There is no denying that equity financing, which essentially means taking an ownership stake in a growing business, involves taking higher risks and, to compensate for those higher risks, owners expect and realize higher returns compared to bank lending. All of the banks involved with equity investments are confident that, with careful expert selection of opportunities and proper assessment of risks and rewards, the banks can provide this market with "patient equity capital" while realizing an appropriate return over time. And as we all learn from our individual equity investment experiences, I am sure that we will continue to evolve our financing capabilities to better serve the needs of the biotechnology industry over time.

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## **Financing biotechnology companies — changing myths and realities**

*Tony Going*

By the year 2004, the biotechnology industry has the potential to expand by over 130%. However, financing requirements will increase proportionately with growth and Canadian companies have to improve their ability to secure funding if they are to participate in this growth. There are fundamental issues that have to be addressed for companies seeking financing and a number of sources and strategies that can be used.

### **Financing: the Number One Issue in the Biotechnology Industry**

For many years, the major factor constraining the growth of Canadian biotechnology companies has been the inability to secure financing. The industry's perception of the causes of the difficulties include:

1. Banks do not understand biotechnology and usually invest in more traditional industries;
2. There is no interest in, or proponents of, the industry in the brokerage community;
3. There is little venture capital in Canada;
4. Other sources of funds are limited;
5. The public does not understand biotechnology.

In contrast, the financial industry's response to the problems faced by the biotechnology industry is often that there are few good companies in which to invest. The financial industry believes that the specific issues behind the financing problem include:

1. Executives of biotechnology companies have unrealistic expectations when negotiating financing;
2. Many biotechnology companies seeking financing lack good management expertise;

3. Biotechnology managers lack comprehensive plans.

There is little question that all of the above problems existed at the inception of the Canadian biotechnology industry in the early 1980s. However, the industry has matured significantly since then and presumably so has its ability to attract financing. One would also expect that the financial industry's knowledge and interest in this sector have also improved significantly. This is what I will explore in this presentation — What are the myths and realities of financing biotechnology companies in Canada?

### **A Complex Phenomenon**

The financing of biotechnology companies is a complex phenomenon. It is complicated by general investment patterns and regulatory considerations and by the different segments of the biotechnology industry and sizes of companies involved in the industry. Larger, more established, public companies in the biopharmaceutical sector, for example, face different financing challenges than smaller, start-up companies in aquaculture.

This report deals with simple financing issues that are more applicable to smaller and newly established companies and is not intended to minimize the effort required in the pursuit of

financing or to minimize the vagaries of the financing market that affect ability to secure funds. However, I believe the issues—while simple—are fundamental and not always adequately addressed by companies when they are seeking financing.

## **Perspective from the Biotechnology Industry**

### *Too many companies chasing too few dollars*

There has been the long-standing criticism that Canadian chartered banks are far too conservative and adverse to risk. This, according to small business in general, and knowledge-based companies in particular, results in the concentration of bank financing in relatively large companies in traditional sectors.

The knowledge-based industries (sometimes referred to as high technology industries) have historically complained that the banks do not understand their technologies or how to value them. In addition, traditional criteria for securing financing such as the pledging of assets is often not possible since much of the value of knowledge-based companies is in the ideas, skills and knowledge of scientific and technical personnel.

There is evidence, however, that banks are changing their attitudes. Most of the chartered banks have now established banking services for knowledge-based industries. Further, some banks have established joint ventures with government organizations specifically for the purpose of directing funds to small businesses and the knowledge-based sector. Examples of such arrangements include:

1. Royal Bank and the Business Development Bank of Canada provide financing to small- and medium-sized "new economy" businesses in southern Ontario, specifically including those involved in biotechnology.
2. ACF Equity Atlantic Inc. provides financing to business ventures in Atlantic Canada with capital provided by ACOA (33.3%), the four Atlantic provinces (33.3%) and private financial institutions, primarily the chartered banks (33.3%).

### *An uninterested brokerage industry*

Unlike many traditional industries, the biotechnology industry has for years not had brokers interested in their sector. The importance of this should not be overlooked. Brokers issue reports to the public and to institutional investors that encourage them to examine various growth industries and companies. These reports, and recommendations made through other channels, help educate the investment community and encourage investment flow into companies and industries. (Of course, they can also encourage outflows of investment.)

In this instance, the evidence suggests significant changes have taken place. Yorktown Securities, for example, has developed its own biotechnology index and publishes a biotechnology investment newsletter.

### *Scarce venture capital market*

The same phenomenon witnessed in the brokerage industry has happened in the venture capital industry. While few if any venture capitalists supported the biotechnology industry in its early days, the same can not be said today. A review of the 33 venture capital firms (about half of the industry) listed in the *Annual Statistical Review and Directory of the Association of Canadian Venture Capital Companies* shows that more than 50 percent invest in the biotechnology industry.

According to the MacDonald & Associates' survey of the venture capital industry, 9 percent of the \$669 million dollars (or \$58 million) invested in Canada by venture capitalists in 1995 went to biotechnology companies. This was more than double the amount of the previous year.

Nor does the venture capital market in Canada look disproportionately small when compared to that of the United States. The venture capital market in Canada is roughly one-tenth the size of the American venture capital market, similar in proportion to the size of the Canadian economy relative to the US economy. In fact, in Canada, there is five times more venture capital invested in the biotechnology industry on a proportional basis than in the United States.

An exciting recent development in the Canadian market is the introduction of new labour sponsored venture capital funds; one being the Medical Discoveries Fund that has just closed



its subscription for this year, raising \$185 million.

Susan Smith, Vice-President of the Royal Bank's Knowledge-based Industries Division suggests there is \$4 billion of venture capital that has not yet been invested.

#### *Other sources of financing are limited*

Thanks to Dr. Denys Cooper at NRC's Industrial Research Assistance Program (IRAP), we also have an indication of some of the other sources of financing accessed by biotechnology companies in Canada. As shown in Table 1, the Canadian biotechnology industry over the last five years has secured over \$1 billion in financing. As of the end of February 1996, the figures for 1996 are ahead of other years at the same time, even before the placement of \$251 million raised by Biochem Pharma on March 1<sup>st</sup>.

Private placements and public offers are only a few of the many other sources of funds that the biotechnology industry in Canada can access. There are, for example, many government programs that support the industry. One newly created program established by the federal government and focused on strategic enabling technologies, including biotechnology, is Technology Partnership Canada. By 1998-99, this program will invest \$250 million annually. The programs that provide assistance to biotechnology companies in Canada are:

1. Technology Partnership Program (TPP) emphasizes the business aspects of com-

mercializing university research. For information telephone (613) 996-4993.

2. Investment Prospect Project is an initiative sponsored by the Department of Foreign Affairs and International Trade (DFAIT). It focuses on attracting investment to Canadian biotechnology. For information telephone (613) 992-5339.
3. Industrial Assistance Program (IRAP) consists of a national network of technology advisors. IRAP offers financial support for promising research projects.
4. Business Development Program (BDP) is run by the Atlantic Canada Opportunities Agency (ACOA) to assist business set-up, expansion, or modernization. For information telephone (506) 452-3184.

Another source of financing is from companies in related traditional industries. While these companies have been touted for years as a potential source of financing, it now appears that they are beginning to invest significantly in biotechnology. This trend is being led by the giant pharmaceutical companies facing dwindling product pipelines and the adverse effects of managed health care. They had been frantically acquiring, aligning and merging with biotechnology companies and the pace of such activity seems unabated. Larger biotechnology companies are also aligning with their smaller counterparts in technology exchanges.

Major agricultural companies are also seeking ways to gain access to biotechnology's innovations. One of the biggest deals occurred in late

**Table 1. Investments in biotechnology in Canada (millions of dollars)**

Year	Private Placement/ Venture Capital	Initial Public Offering	Public Offering	Other	Total	Number of Placements
1991	64.4	131.3	66.3	0.0	262.0	40
1992	51.9	19.6	22.9	0.0	94.4	18
1993	80.9	87.8	53.5	19.5	241.6	34
1994	53.2	19.2	96.3	0.0	168.7	26
1995	140.9	12.5	74.1	6.7	234.2	43
Total	391.3	270.4	313.1	26.2	1,000.9	161
Number of Placements	109	23	22	7	161	



June of 1995 with Monsanto acquiring 49.9% of Calgene in an agreement that will be worth up to \$200 million to Calgene. In total, in the United States last year, the number of biotechnology alliances grew from 152 to 246, a 62% increase. The Canadian scene was also active and witnessed numerous strategic alliances and acquisitions.

I predict that this trend will continue and that, just as agricultural companies are beginning to see the benefits of investing in biotechnology, companies in other traditional sectors (forestry, food, chemicals) will be seeking alliances in the not too distant future.

### *The public doesn't understand biotechnology*

The biotechnology industry has many "publics" that it needs to inform and influence. However, it appears that the general public does not know much about biotechnology. In a 1993 survey of public attitudes on biotechnology, the Canadian Institute of Biotechnology found that attitudes were at an early stage of development, characterized by surprisingly little demographic differentiation of views regarding the risks and benefits associated with the technology.

The survey found that two-thirds of the public believes that biotechnology poses "some" or "a lot" of danger. Conversely, and in response to a different question, two-thirds also said that they believed biotechnology offers either "some" or "a lot" of benefit to society. Biotechnology was preferred over conventional technologies and the public was generally supportive of gene transfer involving plants. However, in terms of "trust of information sources", the industry scored relatively low.

As Rick Walter the Executive Director of the Canadian Institute of Biotechnology says, the position of the biotechnology industry is an enviable one when compared to other industries that have evolved in the past. Due to the paucity of vocal opposition and the early stage of market impact, the industry has the opportunity to attain a significant level of public acceptance by gaining consumer confidence and trust before vast numbers of products enter the market.

The public's perception of biotechnology is important because it is the public, as ultimate consumer, that has to be convinced of the merits of the products that the industry is selling. However, others such as the financial community are

no less important to the industry and it needs to build the trust and understanding of this community in much the same way as the Canadian Institute of Biotechnology is tackling the general public. In the case of the financial community, however, individual firms can be more active and affect the outcome more directly.

### **The Financial Industry Perspective**

#### *Not enough good companies in which to invest*

Attempting to validate the issues raised by the financial community concerning the problems faced by biotechnology companies in raising funds is difficult. In the first instance, the financial sector's attitude towards investing in knowledge-based industries will determine to a large degree the number of companies in which they are prepared to invest.

So, for example, to the extent that the financial community is still looking for companies with significant assets and revenues, and returns in a relatively short period of time, they will not find an overabundance of investment opportunities in biotechnology. There are indications, as shown above, that attitudes in the financial community are changing and more money is being invested in knowledge-based industries including biotechnology. But, the financial community is not yet backing the industry to the extent that it should.

Having said this, however, I believe that the biotechnology industry has in the past oversold itself. The 1980s and 1990s were supposed to be the decades of the biotechnology revolution. Now we are told (and I am one of those that support this notion) that the next decade will see biotechnology in full bloom. The "over-selling" of the industry has had an effect on the financial markets. In general, biotechnology companies have underestimated the time and money it takes to conduct and commercialize their research and have made some investors leery of investing more funds after the promised returns did not materialize.

In general, therefore, I believe that there have been unrealistic expectations on the part of owners and managers of biotechnology companies. The development of biotechnology products and services is a relatively high-risk business. Accordingly, investors expect to receive a high



level of ownership and/or high returns from their investments to compensate for the risk. I have seen a number of financing deals fall through because executives of biotechnology companies failed to appreciate the risks involved in their businesses and were not prepared to adequately compensate the investor for them.

### *Are there a lack of good managers?*

To borrow a quote from Wayne Schnarr of Yorktown Securities: "While good management cannot turn poor technology into a successful company, there are numerous examples of poor management ruining the potential of good technologies."

Many biotechnologies companies, as is the case with knowledge-based companies generally, are started by scientists and technicians who are experts in their field but sometimes lack the in-depth management skills and experience to secure financing and commercialize the results of their research and development efforts.

Recognizing that a gap might exist in management capabilities is the first important step towards resolving the problem. Once recognized, there are many ways that the gap can be overcome. For example, having experienced corporate executives on the Board of Directors is one means. Also, many communities offer mentoring programs where seasoned managers volunteer their time to coach managers of new companies. Many universities have support programs; venture capital companies will lend management support to companies in which they invest; and there are numerous "incubator" facilities that can be accessed.

Some places where managers of biotechnology companies can go to obtain management advice and support include: BCIT Entrepreneurial Centre (Burnaby, British Columbia); Advanced Technology Centre (Edmonton, Alberta); Inno-Centre (Montreal, Quebec); Canada Business Service Centres (sites across Canada, internet site <http://info.ic.gc.ca/cbsc>).

The fact is that if investors do not see a strong management capability in a biotechnology company, they are unlikely to make an investment even if the technology is attractive. So, how pervasive a problem is this in the industry? Suffice it to say that in many of the financing deals in which I have been involved (mainly start-up and early stage ventures), a major pre-occupation has been the identification of a sea-

soned executive who can be recruited to manage the company while the existing owners concentrate on managing the research and development programs.

### *Where are the comprehensive business plans?*

The amount of thought, time and energy that goes into planning a business usually reflects the degree to which investors can readily be convinced that investing in the business is an appropriate use of their funds. I have not found a better vehicle to ensure that such thought, time, etc., has been expended in planning than the process required to prepare a comprehensive business plan. Nor has the investment community found a better means of doing an initial assessment of an investment opportunity than reviewing such a plan. It is a key tool to securing financing.

A principal of a leading Canadian venture capital company has a favorite saying: "Show me an entrepreneur with a good marketing plan, and I'll show that entrepreneur some venture capital". The marketing section is usually the most important part of a business plan. All too often, though, it's the section given the least attention. Potential investors know that successful companies understand their customers' needs and use that knowledge to draft an appropriate strategy for the marketplace.

A good business plan should convincingly answer the following questions:

1. What is the specific need and market that the product is intended to target?
2. Who are the major competitors or potential competitors?
3. What are the competitors' strengths and weaknesses and how do your strengths and weaknesses compare?
4. What is the market position of your competitors, i.e. market share, pricing, distribution, financial resources, production capability, sales, marketing, and management strategy?
5. What does your distribution or potential distribution chain look like?
6. Who are the major players in the distribution chain and what strategies can be used to influence them?
7. What are the major barriers to your entering the market and how do you intend to overcome them?

8. What is the profitability, size, growth rate and rationale for each market segment that you intend to target?
9. What are your marketing objectives in terms of customer accounts, units of sales, market share, profitability, contribution margins, return on investments?
10. Does your marketing strategy deal with why a customer would buy your product instead of the competitors' product; what are you offering the customer; what will your customer pay for your product; how will the customer learn about your product; how will your product arrive at its target market?
11. Does your action plan contain a list of what activities are required, by what time, by whom, and at what cost; detailed revenue projections by product and target market segment; a summary of the annual marketing programs; and the anticipated cost of sales activities?

In general, it has been my experience that the industry tends to think more in terms of technology than markets. This imbalance needs to be addressed if Canadian biotechnology companies are to be successful in securing the financing they need for the future growth that we all believe will materialize.

*How important will financing be in the future?*

So where is the industry heading and what will

its financial needs be over the next five to ten years? I believe the industry has the opportunity to expand by over 130% by the year 2004. My forecast for the North American biotechnology industry, contained in Ernst & Young's third report on the Canadian biotechnology industry, *Canadian Biotech '94: Capitalizing On Potential*, is shown in Table 2. My review of the most recent data suggests that the industry is on target.

The financing requirements of the industry are going to increase proportionately with growth and Canadian biotechnology companies have to improve their ability to secure needed funding or they will not be able to participate in this growth.

Companies have to overcome the weaknesses discussed above that the financial community believes contribute to the financing problems faced by the industry. In particular, Canadian biotechnology companies need to elevate the importance they place on financing as a key business function and must devote more time, thought and energy to it. Finally, in the words of Wayne Schnarr, "Canadian biotechnology companies must never stop financing activities. The day after the last financing is complete is the day the next round should start."

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**Table 2. Forecast growth in North American biotechnology markets.**

Key Sector	Sales (CDN\$000,000)		
	Base Year	Forecast Year	1994-2004 Average Annual Growth Rate
Human Therapeutics	\$6,106	\$23,595	26%
Human Diagnostics	\$1,880	\$5,105	16%
Agriculture	\$300	\$2,131	55%
Others	\$299	\$2,002	70%
Suppliers	\$785	\$1,695	11%
Total	\$9,300	\$34,528	25%

Source: Canadian Biotech '94: Capitalizing on Potential, 3rd Report on the Canadian Biotechnology Industry, Ernst & Young, Ottawa.



## **Financing marine biotechnology: the Martek experience**

*Richard Radmer*

Since its inception in 1985, Martek has been engaged in the research and development, and manufacturing and sales of products derived from microalgae. In 1989, commercial production and sale of its products for drug design began. In 1992, Martek began to realize revenues from license fees related to Formulaid (an infant formula ingredient) and sales of sample quantities of Formulaid. The company received its first royalty revenue from sales of infant formula containing Formulaid in 1995.

Martek has financed its activities through a combination of research and development contracts [primarily Small Business Innovation Research contracts from US Government agencies], product sales, and equity investment. The company was able to attract equity investment in its formative years because it 1) had the ability, from its inception, to finance its R&D from external sources; 2) had unique premises and technologies; and 3) addressed large markets. As a result, early-stage investors could anticipate high payoff in the event of success. (Historically, a majority of these high-risk early-stage investments fail, and thus the occasional successes need to be very substantial.)

Since its inception, Martek has raised approximately US\$19.5 million from private sales of its equity securities. The company raised an additional US\$53.7 million in the public market as a result of its initial public offering in 1993 and its 1995 follow-on offering. Its current market capitalization is approximately US\$400 million. As Martek has grown, its character and skill base have changed substantially. In 1985 it had 8 scientist-employees and its financial and business matters were handled by its president, a scientist with little prior business experience. As the primary challenges evolved from the technological to the business and financial areas, the company hired a full-time chief executive officer in 1990 and a chief financial officer in 1992, and now also employs a controller, investor relations specialist, and sales and mar-

keting specialists. The ability of the company to attract highly-qualified individuals whose skills and interests matched the company's needs at critical times has been a major factor in Martek's continued growth and success.

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## ***Keynote address***

### **The potential of shark cartilage**

*William Lane*

Shark cartilage and its use with non-responsive solid cancerous tumors, generally stage IV cases, has become widely known in alternative medicine. The FDA has given phase 2 approval to a study on advanced, metastasized, and non-responsive prostate cancer and Kaposi's sarcoma for a unique specially processed shark cartilage product called Benefin. The clinical trial on prostate cancer began in Detroit at the Beaumont Hospital in January 1996. The FDA approved protocol, including tumor reduction, pSA tumor marker response, quality of life measurements including Karnofsky Index, blood work and other criteria, is being used. Early results were discussed as well as results from other countries (Chile, China and Japan) where advanced non-responsive liver, ovarian, uterine/cervical, breast, and brain tumors are being studied. The US trials and all clinical trials on all tumors mentioned have followed similar FDA-type protocols. The mechanism of action, believed to be angiogenesis inhibition, was discussed as was the Cuban study on 29 terminal patients representing 10 tumors. That study, though not peer reviewed, was well documented and is now in its 44th month with half the starting patients, all who had not responded to conventional therapy, alive and well with shark cartilage as the only therapy.

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## **Agricultural and medical applications of N,O-carboxymethylchitosan, a derivative of shrimp processing wastes**

*Clive M. Elson*

The polymer, N,O-Carboxymethylchitosan (NOCC) is synthesized from the natural polysaccharide, chitin, which is found in the exoskeletons of crustacea and insects, and in algae and the cell walls of certain fungi. NOCC consists of repeating monomer units of the amino-sugar, D-glucosamine, substituted with acetate groups on both nitrogen and oxygen centres within the polymer. NOCC is water soluble, biodegradable, biocompatible, viscoelastic and of high molecular weight. Because NOCC's chemical and physical properties mimic those of hyaluronic acid, NOCC has been evaluated as a drug delivery device, a device to prevent post-surgical adhesions and as an ophthalmic surgical aid. In addition, the efficacy of NOCC as a preservative coating for certain fruit and vegetables, and as a sustained delivery system for pheromones has been established.

### **Introduction to Chitin and Chitosan**

The protective shell of crustaceans such as crab, lobster and shrimp consists of 10 to 15 percent chitin, a polysaccharide. The other constituents of crustacean shells are inorganic salts, lipids, protein and pigment. Chitin is the second most plentiful natural polymer in the world, the first being cellulose (Fig. 1a). Chitin is also present in the cuticular or exoskeletal structure of most invertebrates, and is found in zooplankton and the cell walls of most fungi, molds and yeasts. As such it is small part of our normal diet.

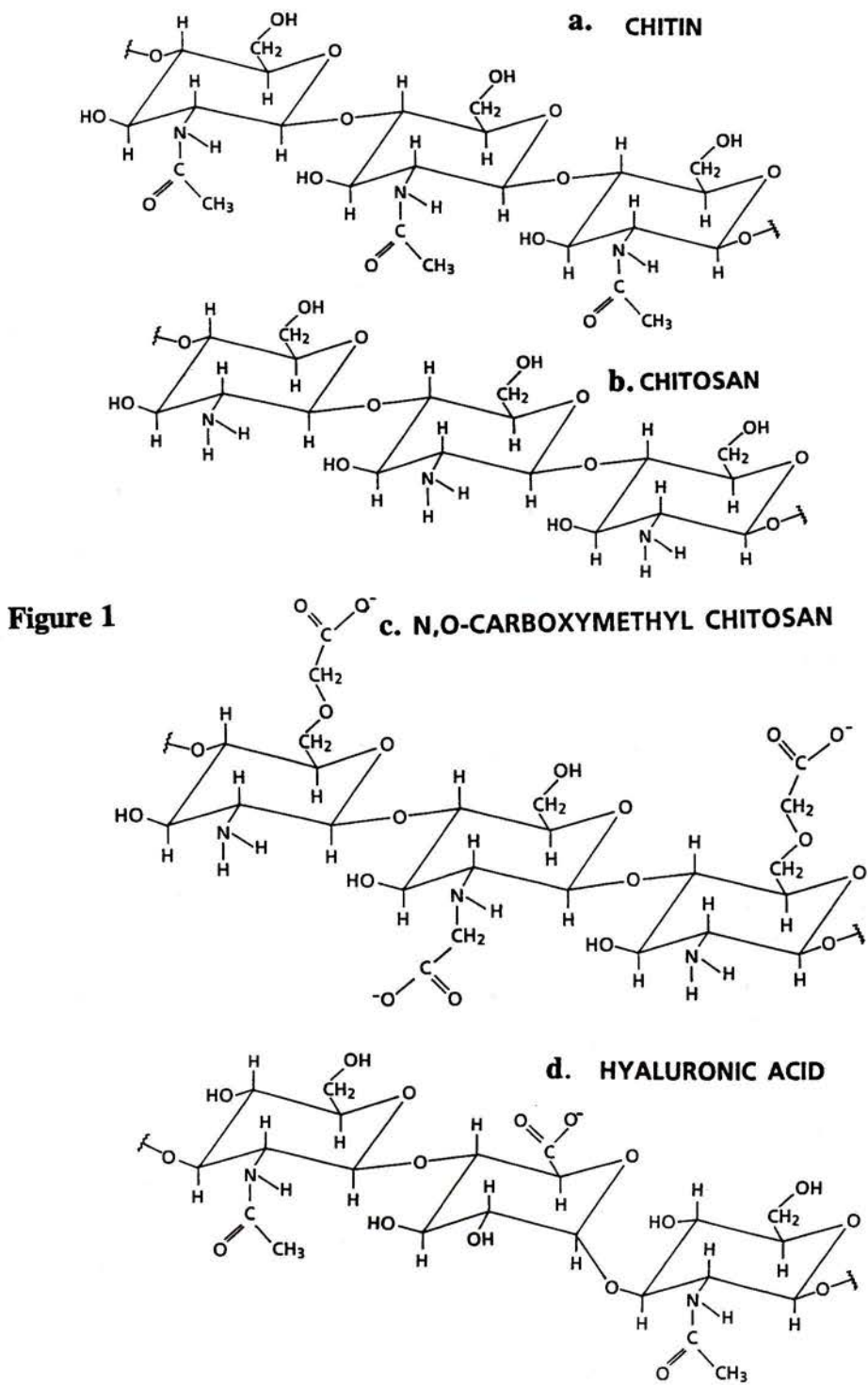
Chitin is water insoluble and soluble only in very special solvents, all of which are toxic. When chitin is treated with hot sodium hydroxide, it is deacetylated and yields "chitosan" which is soluble in dilute organic acids and in dilute hydrochloric acid (Fig. 1b). Lysozyme

enzyme systems found in intracellular lysosomes of mammalian tissues will degrade the polymer chain to the monomeric glucosamine sugars. Chitinases, widely distributed in bacteria, fungi and digestive glands of animals whose diets include chitin, will do the same.

Chitosan was first produced industrially in Japan in 1971 by Kyowa Yushi, Inc. By 1986, there were 15 Japanese companies manufacturing chitin, chitosan and chitosan derivatives. Today, about 4,000 tons of chitin and chitosan are produced yearly in Japan, primarily as a dewatering agent of sewage sludge as mandated by the Japanese government. Other manufacturing plants, with limited production, exist in Greenland, Poland, Ireland, Norway, Malaysia, China, Thailand, Canada and the United States.

Today, the primary commercial use of chitosan is as a flocculent in the clarification of





**Figure 1.** Structure of A) chitin, B) chitosan, C) N, O-Carboxymethylchitosan, and D) hyaluronic acid.

waste water. The positively charged amino groups of chitosan bind to the negatively charged proteins and other anionic contaminants. Since chitosan is a non-toxic, natural polymer, it is preferred in this type of application over synthetic polymers, which may contain leachable monomers or other toxic chemicals. The U.S. Food and Drug Administration (FDA) recently granted approval for the experimental use of chitosan to treat potable water. Chitosan is used to clarify beverages such as wine or fruit juice and is used in food processing plants to recover proteins. A limited amount of chitin and chitosan is allowed in animal feed and foodstuffs. Chitosan will also chelate toxic heavy and radioactive metals and is used to remove or recover uranium. Chitosan is a good moisturizer, adheres to skin or hair, and is currently being employed as an ingredient in skin creams and in hair sprays.

### N,O-carboxymethylchitosan

In the autumn of 1986, Nova Chem was awarded a composition of matter patent for a new polymeric compound, N, O-carboxymethylchitosan (NOCC) (Fig. 1c). The addition of negatively charged carboxymethyl or acetate groups to chitosan renders the biopolymer water soluble. However, the degree of carboxymethylation was limited leaving the structure of the new polymer basically the same as that of chitosan but with only half of the nitrogen centers unsubstituted. NOCC absorbs water to approximately 50% its own weight and this hydrophilic property, coupled with a long unbranched chain structure, accounts for the polymer's lubricity and viscoelasticity. The molecular weight of NOCC approaches three million Daltons. The material is benign.

The structure of NOCC has several features in common with the known viscoelastic substance, hyaluronic acid or sodium hyaluronate (HA) (Fig. 1d). HA is a co-polymer of N-acetylglucosamine (the monomer of chitin) and sodium D-glucuronate and is found in nearly all intercellular ground substances of vertebrae. It is the natural lubricating and shock-absorbing molecule of the musculoskeletal system and of the eye. HA is used to protect sensitive tissues during ophthalmic surgery and as a replacement for synovial fluid in joints such as knees. It is also

used in preventing post-operative adhesions and as a method to treat urinary incontinence. The medical applications of NOCC should parallel those of HA. Moreover, as a result of the presence of unsubstituted nitrogen centers, NOCC can be readily modified by simple chemical means, something that cannot be done to HA. One modification involves cross-linking NOCC to obtain higher molecular weight polymers that persist *in vivo* for longer periods of time.

### Biocompatibility of NOCC

A prerequisite for any new biomaterial is high purity. Purity is often defined in terms of the presence of zero or minimal adventitious protein since protein can be allergenic. We collaborated in the development of an amino acid - high performance liquid chromatographic method to assay the protein level of NOCC preparations and found levels close to the detection limit of the method at or near 0.1%. Furthermore, the fate of a biomaterial introduced into the bloodstream must be established. By labelling NOCC with the  $\gamma$ -emitter, technetium - 99m, it was found that NOCC that had been injected into the ear vein of rabbits was eliminated within 2-3 hours via the kidneys, bladder and urine. Moreover, there was no accumulation of NOCC in any organ or tissue other than the liver. It was assumed that the Kupffer cells of the liver were responsible for the degradation of NOCC.

The biocompatibility of NOCC (i.e., inability to elicit inflammatory or foreign-body reactions) has been established by *in vitro* and *in vivo* studies. Standard cell proliferation assays were performed on human peripheral blood lymphocytes; the proliferation of cells in response to a stimulus such as the addition of NOCC was treated as a direct measure of inflammatory response. The extent of cell proliferation was measured indirectly by estimating new DNA production via the incorporation of the radioactive nucleoside, tritiated-thymidine, ( $^3\text{H}$ )-thymidine, into cellular DNA. A stimulation index (S.I.) was calculated by dividing the radioactivity (counts per minute) of stimulated cells by the counts per minute of the unstimulated, control cells. The error associated with stimulation index values was of the order of 15-20%.



In experiments with human peripheral blood lymphocytes (PBLs) two different donors were studied. The PBLs from one donor were more active than the other and reflected the variability of this cell type. The concentration of NOCC was varied over three orders of magnitude from 1 µg/mL to 1 mg/mL and in addition positive and negative controls were included. The known PBL mitogen, phytohemagglutinin, demonstrated a stimulation index of 45, whereas concanavalin A which does not cause extensive proliferation of PBLs had a stimulation index of 11. The stimulation indices for all the NOCC treatments were less than 8 indicating that NOCC was non-inflammatory as well as non-inhibitory of normal cell growth.

In the *in vivo* study, samples of four different polymers were implanted or injected subcutaneously into pockets created in the ventral surface of the skin of rats. Two rats received three samples of each implant material. The materials studied were NOCC, carboxymethylcellulose, methyl cellulose, and fibrin sealant containing human fibrinogen (the control). The American Red Cross has documented that the fibrin sealant is non-inflammatory. After 7, 14 and 21 days, the rats were sacrificed and tissues were harvested for histological evaluation using haematoxylin and eosin stains. Staining enables leukocyte cells, particularly neutrophils, to be identified and their number estimated. The appearance of such cells in muscle tissue is a measure of inflammation. The results of the histological examination are presented in Table 1. Both of the cellulose derivatives were more inflammatory than the control whereas NOCC was comparable, if not better than, the fibrino-

gen control. Since the fibrin sealant was classed as a non-inflammatory material, these results placed NOCC in the same category.

### Sustained Release Device for Drugs

Nova Chem has developed an analgesic, slow release system initially designed for veterinary use but also applicable to humans. Morphine is the "gold standard" of analgesics and is readily available and inexpensive. However, it suffers from being highly controlled and, in the case of dogs, of being rapidly metabolized. Since many animal hospitals/clinics do not have staff certified to administer restricted drugs after hours there is a requirement for a sustained morphine release system which would be effective for 12-24 hours. A group of veterinarians headed by Dr. Andy Tasker at the Atlantic Veterinary School, University of Prince Edward Island, have been evaluating morphine-containing NOCC gels for sustained release. Preliminary results showed that with certain gel formulations the analgesic effect lasted for 12-16 hours (compared to 2-3 hours for a bolus, intramuscular injection). In addition, the rise in the blood morphine level immediately after injection with the NOCC gel was slower than that for straight morphine injections which reduced the side effects associated with morphine shock. This work was significant not only because it involved larger animals but because it confirmed the biocompatibility of NOCC.

Table 1. Evaluation of tissues harvested from 7-day implants

Response	Minimum	Mild	Moderate	Marked
Fibrin Sealant (Control)*	3	1		
NOCC	6			
Carboxymethylcellulose			3	3
Methylcellulose*		2		3

\* Slides on which only muscle tissue was present



## Prevention of Post-Surgical Adhesions

Adhesion formation, appearing as fibrous tissue joining normally separated surfaces, is a characteristic result of surgical intervention. This complication can lead to obstruction of the intestine following abdominal surgery, infertility following pelvic surgery, cardiac adhesions after heart surgery, and mortality. The formation of adhesions is essentially an inflammatory reaction with factors released from inflammatory cells increasing vascular permeability and creating a protein-rich exudate. Fibrinogen in the exudate is rapidly converted to fibrin and forms a thin, friable coating on local organs. Fibrin becomes organized into permanent fibrous adhesions as the ingrowth of fibroblasts and, subsequently, capillaries occurs.

For the past two years, two groups of surgeons have been studying the anti-adhesion properties of NOCC. In an initial study, Drs. T. Lee and V. McAlister of Dalhousie University's Medical School found that when NOCC was applied as a pre-operative lavage, followed by the application of a gel and a post-operative lavage, the incidence of fibrous tissue formation was reduced by 60-80% in kidney transplants in syngeneic and allogeneic rats. In all cases, the anastomosis healed properly and the renal artery and vein were patent. There was no evidence of inflammation caused by NOCC nor was there any evidence of NOCC 10 days following surgery.

These same workers have recently reported on an aortic anastomosis model in rats. This model was developed as a clinically relevant model for testing both the ability of NOCC to prevent abdominal adhesions and to determine its effect on the healing process in a high pressure anastomosis of the vasculature. During surgery, the aorta was exposed, separated from the vena cava, sectioned, and sutured back together. Fifty-six animals were involved in the study which was completed 7 days after the surgery. It was confirmed that the post-operative application of a NOCC-based hydrogel and a NOCC lavage to the anastomosis and surrounding area reduced the mean level of retroperitoneal adhesions by 66% and liver adhesions by 85%. The surgeons noted that, in all of the treated animals, the aortic anastomosis was in excellent shape

and that there was no evidence of either degradation of the suture site nor of any aneurysms or pseudo-aneurysms.

## Ophthalmic Surgical Aid

Currently, hyaluronic acid's main use is in ophthalmic surgery. In the implantation of intraocular lenses, viscoelastic agents are used to maintain the shape of the anterior chamber, to protect the corneal endothelium, to lubricate the eye as well as the intraocular lens and the surgical instruments, allowing all to move freely and to allow for manipulation of the iris.

T.J. Liesegang, MD, in a recent article, wrote the specific "chemical requirements are that the viscoelastic substance be inert, electrolyte balanced, at the same osmolality and colloid osmotic pressure as the cornea or aqueous fluid, pH buffered, soluble in water, highly purified, free of particles and transparent for use within the anterior chamber. It should be easy to instill and to remove, and should be removable from the eye biologically." As far as is known, NOCC and its derivatives meet all these requirements. In fact, NOCC (2% solution) and NOCC Acetate (1% solution) were evaluated as viscoelastic agents in rabbit eyes in comparison with hyaluronic acid (Healon, Pharmacia) at the Lindstrom Eye Research Laboratory in Minneapolis. The properties considered were ocular irritation and inflammation when injected into the anterior chamber, corneal thickness, intraocular pressure and inflammatory response when injected into the vitreous. The results were most promising, with NOCC solutions behaving comparably to the hyaluronic acid controls.

## Fruit Preservation

Another application of NOCC technology is as a food coating, designed to prevent senescence (aging) during storage of climacteric fruit (i.e., fruit that responds to controlled atmosphere storage). This product, trade-marked Nutri-Save, is a combination of NOCC solution plus a surfactant which is applied to freshly picked fruit prior to storage. The Nutri-Save coating forms a semi-permeable film several microns thick over the surface of the fruit. This film retains carbon dioxide within the fruit and acts as a barrier to the entry of oxygen; thus the



metabolic processes within the fruit are slowed which extends the fruit's storage or shelf life. The film will not support either bacterial or fungal growth and can be removed by washing.

The fruit preservative coating has been extensively tested in Canada, Chile, the United States and Australia. In collaboration with Agriculture Canada, a series of trials on apples were conducted with the results that coated fruit displayed improved retention of texture and titrable acids, showed reduced damage due to storage, and maintained post-storage fruit quality during shipping. These studies found that in some cases the application of Nutri-Save coating extended storage time by 67%. The Nutri-Save coating has been approved by Health and Welfare Canada as a "coating on apples to be washed, peeled and then further processed...the opinion is based on the premise of no human exposure."

Toxicological studies conducted at the Hazelton Laboratories America found that "50,000 ppm Nutri-Save polymer in the diet for 14 days had no observable effect on female rats and no observable adverse effect on male rats". These feeding levels were more than 200,000 times the levels expected to be ingested by the 90th percentile of "apple eaters". It has been demonstrated that washing and light brushing removes over 95% of the polymer with the remainder located at the calyx and stem ends.

### Pheromone Release System

For over a century it has been known that chemical communication is a part of insect mate-finding behaviour. The volatile chemicals that mediate mating are called pheromones (sex attractants). Pheromones are species-specific and over the past decade some 700 pheromones have been identified and synthesized. Sex pheromones are being used in the management of cropland pests in two different products. The first is a lure-based trap used to monitor the presence and number of insects. The second is a mating disruption device that blankets an area with pheromone reducing the probability of males finding and mating with females, thereby

controlling the population. The constant bombardment of the males' receptors with pheromones causes them to follow false trails or scramble messages to the brain resulting in temporary lack of response to any attractant.

Nova Chem has developed a novel, easy to use and inexpensive pheromone delivery system. This new system is based on NOCC hydrogels containing an amount of hydrophilic agent to maintain gel structure and moisture content. The pheromone, which is water insoluble, is dispersed in a lipid before incorporation into the gel. The product has a number of significant advantages over current commercial products. For example, the release rate and duration of release can be controlled by adjusting the level of lipid in the gel. In addition, the gel is easier to apply than the current plastic strips; a caulking gun device can apply the NOCC gel directly to a tree branch eliminating the tedious and expensive manual methods presently employed. Finally, the concentration of the pheromone in any particular tree's canopy can be easily controlled by altering the amount of gel applied. Field trials conducted in 1995 by Agriculture Canada were positive with injury to Macintosh apples being reduced by 60% compared to controls and *Isomate C Twist Ties*, a commercial product.

### Summary

The biopolymer, chitin, can be derivatized to N,O-carboxymethylchitosan which is a benign, bioerodible, biocompatible polysaccharide similar in structure and properties to hyaluronic acid. The polymer can be formulated as beads, sponges, films, hydrogels, foams and viscoelastic solutions. A wide range of applications have been identified extending from agricultural to cosmetic to medical.

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## **New strategies for drug discovery using marine organisms**

*Norman R. Wainwright* <sup>(1)</sup>

There is an increased need for isolation and characterization of new pharmaceutical compounds, particularly ones having antimicrobial activity. Reports of pathogenic microbes showing resistance to conventional drug therapy are increasing in both the popular press and in the scientific literature. Terrestrial sources of compounds with antimicrobial activity have been thoroughly exploited over the past several decades and the marine environment is now under intense scrutiny to provide new compounds. While past efforts have been made to extract novel compounds from marine organisms, new approaches are being taken to discover compounds. The interaction between marine microbes and marine invertebrates is one area of research that may yield promising new compounds for research and development.

### **Marine Diversity**

It is a well known biological phenomenon that species reach their greatest degree of diversity where competition between species or some physical stress is maximized. Such conditions often occur at boundaries between opposing forces. In the marine environment, some dramatic boundaries are clearly evident. For example, at hydrothermal vent sites extremes in temperature and pressure combined with competition for nutrients create powerful evolutionary forces for diversity. Also, the boundaries of air, land and water converging at intertidal zones and coral reefs yield visible evidence of divergence. Apart from these few exceptions, the marine environment does not appear outwardly to possess much variety. Compared to terrestrial examples of temperate and tropical species that are comparatively more accessible, the marine counterpart is mostly hidden beneath water far from shore. However, the number of genera found in the ocean is actually greater than in the terrestrial environment.<sup>(2)</sup>

It is not surprising that renewed attention is

focusing on the marine environment as a source of new molecules to be developed into products to raise human living standards. The depletion of the world's battery of effective antibiotics due to the spread of resistant bacterial strains alone merits extending the exploration of marine ecosystems. However, bioactive compounds with antiviral, antitumor and antihelminthic activity are also being actively pursued.

### **Marine Microbes**

Early work by Burkholder et al.,<sup>(3)</sup> demonstrating the production of pyrole antibiotics by marine bacteria, is being expanded to include the demonstration of novel, highly active molecules from marine bacteria,<sup>(4)</sup> dinoflagellates<sup>(5)</sup> and bryozoans, sponges, tunicates, crustaceans, fish and algae.<sup>(6,7)</sup> Most interesting is the realization that interaction between species, especially between marine invertebrates and microbes can be remarkably intimate. Symbiotic or commensal associations are common and, in fact, many bioactive compounds initially extracted from



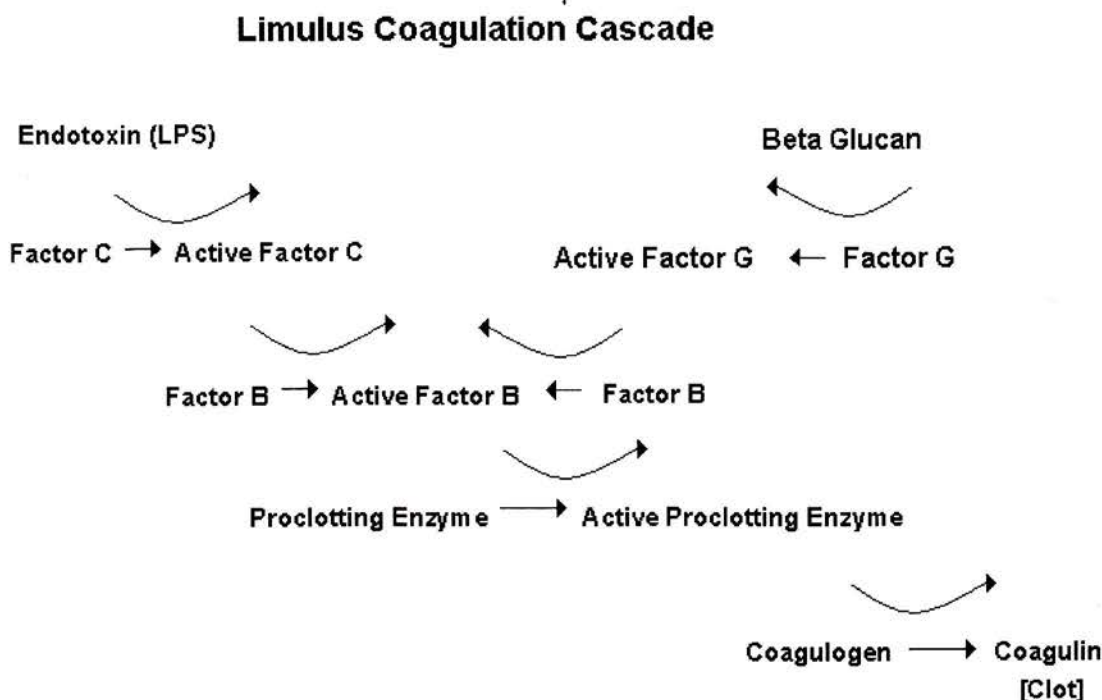
invertebrates have been found to be synthesized by a parasitic microbe.<sup>(5)</sup>

Lobster embryos were recently shown to possess some innate protective mechanism against pathogenic marine fungi. It has been shown that the eggs possess marine bacteria colonizing their surfaces, producing an antifungal compound.<sup>(8)</sup> Our laboratory has shown similar antifungal protective activity by bacteria on the surface of shrimp eggs and we are investigating the role of bacteria in the defense of a number of marine invertebrate eggs. We must emphasize the need to study the biology of marine organisms in order to discern the metabolic pathways responsible for synthesis of the active compounds.

### The Horseshoe Crab

Marine animals have evolved unique mechanisms to deal with microbial infections. Due to

the more primitive nature of their defense systems, these simple mechanisms may lend themselves to more convenient study. Horseshoe crabs also have been a valuable marine resource in medical research and pharmaceutical development. The discovery that horseshoe crabs, *Limulus polyphemus*, evolved unique mechanisms to cope with microbes present in their environment, was made at the Marine Biological Laboratory in Woods Hole during the 1950s.<sup>(9,10)</sup> The cells of the horseshoe crab work by attacking the outer cell wall of bacteria. An integral part of the outer cell wall of gram negative bacteria is comprised of lipopolysaccharide (LPS), also called endotoxin. Beta glucans serve a similar function in fungi. Lipopolysaccharides are ubiquitous and are known to be present in normal laboratory environments. LPS is also a potent activator of the immune system, stimulating cytokine release at very low concentrations; however, surprisingly small amounts can



**Figure 1.** Clotting reaction of horseshoe crab blood showing the cascade amplifying the detection of very low concentrations of endotoxin (LPS) from gram negative bacteria or beta glucan from fungi.

cause fever in humans and other mammalian systems, and in high doses, septic shock may result in death.

LSP has been shown to cause horseshoe crab blood to clot, acting as a primitive defense system.<sup>(9,10)</sup> Their blood cells, or amebocytes, initiate coagulation when exposed to endotoxin. The clotting cascade is composed of a series of serine proteases triggered by extremely small quantities of endotoxin. The result is entrapment of the invading bacteria or closure of the causative wound. This clotting reaction is now used as an assay for LPS (Fig. 1). Blood is collected into sterile, endotoxin-free glass containers by venepuncture. Cells are separated from the serum, or hemolymph, by centrifugation at low speed. The hemolymph can be stored or processed for extraction of other useful protein products. The amebocytes are lysed by addition of sterile water and mixed for several hours to cause the cells to osmotically lyse, releasing their contents. Broken cell debris is removed by another centrifugation and the final clarified lysate is stored under refrigeration or freeze-dried for long-term storage and shipment. The animals are not harmed by this process and are returned to the waters from which they were collected. Studies of the American horseshoe crab industry have shown greater than 95% of the animals recover fully when bled once per season. About one third of the available blood volume is collectable, so there is little impact on the animals' health.

All medical devices manufactured for human use and all injectable drugs and fluids must be tested for LPS prior to sale. Before the use of the horseshoe crab assay, it was necessary to sacrifice rabbits in order to perform the biological endotoxin assay. Today, sales of horseshoe crab blood products represent a US\$50 million market as a medical diagnostic. It is sold directly to manufacturers of medical products for quality control during manufacturing and release of injectable products. There is also a significant research market for the product. Since LPS has potent cell activation activity, cell culturists must be aware of the presence of LPS in the laboratory.

### Horseshoe Crab Antimicrobial Defense

Bacteria are abundant in seawater. Even seawater from non-polluted areas will typically

contain  $10^3$  to  $10^5$  bacteria/mL, and many of these are gram negative. It is not surprising then that high concentrations of bacterial LPS exist in seawater. An injury to the horseshoe crab, where there is contact of the blood with seawater, rapidly results in clot formation. This serves both as a physical barrier to blood loss and to influx of microbial pathogens, which are entrapped. Several lectins are present in the circulating hemolymph, including ones binding to specific microbial cell surface polysaccharides, such as N-acetyl-glucosamine. C Reactive Protein (CRP) is also present in high concentrations. Although there is no induction of specific immunity in horseshoe crabs, these lectins serve the general purpose of primitive "immunoglobulins" in which bacteria can be agglutinated.

In addition to releasing enzymes involved in the coagulation cascade, the blood cells, or amebocytes, also release antibacterial proteins and peptides. Tachyplesins and polyphemusins represent a family of low molecular weight peptides, originally isolated from the Japanese horseshoe crab, *Tachyplesus tridentatus* and *Limulus polyphemus*, respectively.<sup>(11)</sup> These typically contain 13 amino acids held in a planar ring-like structure by two disulfide bonds. They have broad antimicrobial activity against gram negative bacteria, gram positive bacteria, and fungi.

*Limulus* anti-lipopolysaccharide factor (LALF) is another low molecular weight protein isolated from the amebocytes of horseshoe crabs.<sup>(11)</sup> LALF has 103 amino acids that are arranged with a rich clustering of hydrophobic residues at the amino terminus and an array of basic amino acids in the central disulfide-bonded loop region. Its mode of action is thought to result in a very high affinity binding between the hydrophobic and cationic areas of the protein with the fatty acid and phosphate groups, respectively, of the core of LPS. This tight coupling results in a neutralization of the toxic effects of LPS *in vitro* and *in vivo*. Its antibiotic activity is hypothesized to be a result of the intercalation of the protein into the structure of the outer cell wall, disrupting its integrity. Other practical applications of the protein include eliminating LPS from cell culture medium or to fight bacterial sepsis in humans.<sup>(7,12,13)</sup>

Horseshoe crabs represent an example of a species that fit well into a program to develop



new products for the biotechnology industry and fill an important gap in a worldwide effort to discover novel sources of biodiversity.

## Synergism Between Antibiotics and LALF

We examined whether LALF would interact with conventional cell wall reactive antibiotics such as ampicillin in either a positive or negative way like the ability of LALF to bind to the surface of the bacterial cell wall. *E. coli* was grown in standard growth medium with dilutions of ampicillin, LALF or combinations of the two. As shown in Figure 6, concentrations of less than 2.5 g of ampicillin had no effect by itself on the normal growth curve of control cultures. Likewise, low concentrations of LALF had little effect. However, when mixed, LALF plus ampicillin had substantially more activity than the additive effect of the two.

## Summary

One of the most unique group interactions in the marine environment is the association of bacteria with invertebrates. In some cases, parasitic relationships exist where the microbe clearly adds to the defense of the host, as in the example of the lobster egg. What is yet to be understood is what advantage is gained by the microbe? Are there unique metabolites on the colonized surface essential to their growth? Are there specific receptors that capture the appropriate species? Is it possible that there is a mutual induction of specific metabolites in either the microbe or host that gives each an advantage? Symbiotic bacteria and fungi will continue to be isolated and explored as sources of novel chemicals that could have pharmaceutical potential.

The role of the invertebrate "immune" system in reacting to marine microbes demonstrates the power of simple mechanisms. The example of the horseshoe crab is representative of the presence of very successful strategies that have al-

lowed these ancient species to survive. By coupling the effects of agglutinating lectins, gel clotting and combining the antibiotic activities of small bioactive peptides, pathogenic bacteria can be effectively eliminated. We can benefit from understanding the mechanisms used to successfully compete with potentially pathogenic bacteria and potentially apply these mechanisms when dealing with human pathogens that are resistant to conventional drugs. The diversity of both the marine microbes and the marine invertebrates represents a rich source of possible future pharmaceutical compounds.

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## **Marine microbes: A novel source for new drug discovery**

*David Manyak*

Small organic molecules called "natural products," derived from terrestrial plants and from microorganisms found principally in the soil, have been responsible for nearly one-third of all drugs ever brought to market. For example, the antibiotics penicillin and streptomycin were isolated, respectively, from *Penicillium*, a terrestrial fungus, and *Streptomyces*, a soil bacterium, and the analgesic aspirin was derived from the bark of the willow tree, *Salix*. The marine environment, however, has remained relatively unexplored as a source of new drugs. With the pharmaceutical industry's ever-growing need for novel chemical structures, there has been a renewed interest in accessing unique sources of natural products. Marine organisms, given their enormous diversity and well-documented use of bioactive agents as a means of defense, communication and competition, represent a highly promising and underexploited source of novel compounds for drug discovery.

Oceanix Biosciences has established a drug discovery program based on three core components: 1) a library of marine microbes (currently exceeding 7500 isolates) selected for geographic, environmental, substrate, and species diversity — representing source material for drug discovery; 2) methods to grow culturable marine microbes based on fermentation technology and to derive otherwise unavailable compounds through selection/induction methods or by combining portions of the genomes of non-culturable microbes with those of culturable organisms (Combinatorial Genomics technology), representing the means to obtain a renewal supply of unique natural products for

drug development; and 3) a breadth of molecular-mechanism-of-disease screening assays (currently exceeding 100 different targets) based on *in vitro* receptor-binding or enzyme-inhibition assays or whole cell assays available through the Company's NOVASCREEN Division, representing the tools to perform high throughput screening of marine microbial extracts for potential pharmaceutical activities.

Therapeutic areas targeted by Oceanix include infectious diseases, central nervous system (CNS) disorders, and cancers. These targets were selected on the basis of market needs and commercial potential but also on a rationale related to the biochemical ecology of marine organisms. For example, marine organisms are known to produce growth-inhibitory substances as a means to ward off predators or as protection against being overgrown by sessile, colonizing organisms. Such growth-inhibiting compounds may hold promise as anti-infectives or anti-cancer drugs. In addition, marine organisms are known to communicate via small molecules that are synthesized and released, diffuse through the media (sea water), and are received by a receptor-mediated mechanism, thereby causing a functional or behavioural response. Such systems may yield new drugs for CNS disorders, many of which are modulated by receptor interactions. These premises appear to be supported by the company's screening results to date.

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### **Gene mapping in Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*)**

*C. McGowan and W. S. Davidson*

Many economically important animals are presently having their genomes mapped. The possibility of finding genetic markers that are linked to genes which contribute to quantitative characteristics can significantly improve the efficiency of breeding programs. For fish species such as Atlantic salmon or brown trout, a genomic map could be used to enhance the selection of health and population related traits as well as provide many new genetic markers for monitoring wild populations. We are currently using microsatellites, randomly amplified polymorphic DNAs (RAPDs) and expressed sequence tags (ESTs) as markers to construct a linkage map for brown trout and Atlantic salmon. Hybrid families, where the genetic contribution of each parent can be easily distinguished, are being screened for polymorphic loci and used to identify linkage disequilibrium. Microsatellites and RAPDs will provide many polymorphic markers that can be used to create a basic genetic map. ESTs are non-arbitrary markers that can be used in comparative gene mapping and supply reference points to the current composite gene map for salmonids.

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### **Flow dynamics in and around pearl nets of various mesh sizes**

*Floyd Cole, Jay Parsons and  
Cyr Couturier*

In this experiment, the effect of external current velocity (12.4 to 45.3 cm/s) on the internal current velocity in pearl nets was examined. The pearl nets had mesh sizes of 1x3, 4.5, 9, and 12

mm. The pearl nets significantly reduced the velocity of the incoming currents, but the reduction was inversely correlated with mesh size, and the percent reduction decreased with increasing external velocity. The inverse relationship between mesh size and percent reduction is due to the nature of the mesh. With small mesh nets, more of the face of the net is enclosed with the netting material, thereby limiting the amount of water passing through the net. However, as the mesh size increases, less of the net is enclosed, allowing more water to pass through. The phenomenon of decreased percent reduction at increased velocity is due to the increased momentum of the water. These results have implications for choice of grow-out methods for scallops.

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### **Effects of sperm longevity and gamete concentrations on fertilization success in the blue mussel**

*Lorelei A. Levy and Cyr Couturier*

Longevity of sperm after spawning and fertilization success as a function of sperm and egg concentration of the blue mussel, *Mytilus edulis*, were studied. Within four hours of spawning (at 18°C and a density of 10<sup>6</sup> sperm per mL), survival of sperm decreased by 24%. According to a regression model, the optimal sperm concentration was 10-100 sperm per egg at the optimal egg concentration of 60 eggs per millilitre. The optimal sperm density may be that at which sperm remain inactive until just prior to contact with the egg. Sperm concentration contributes more to the variability in fertilization success than either sperm age or egg concentration, unless these two factors are increased in fertilization practices. These factors are important considerations in practicing artificial fertilization where high rates of fertilization are desired.

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## Toxicity of un-ionized ammonia in juvenile giant scallops, *Placopecten magellanicus*

Allison Abraham, Cyr Couturier  
and Jay Parsons

The toxicity of un-ionized ammonia is a major concern to the operators of finfish hatcheries. Its importance to shellfish hatcheries and holding facilities is not as well documented, but could be a concern due to high stocking densities. Epifanio and Srna<sup>(1)</sup> found that un-ionized ammonia became toxic to the hard clam, *Mercentaria mercenaria*, at 3.3 mg/L and to the American oyster, *Crassostrea virginica*, at 6.0 mg/L. From these findings, they felt that 3.0-6.0 mg NH<sub>3</sub>/L was the lethal limits for all marine bivalves. To test this assumption, 30-40 mm juvenile giant scallops, *Placopecten magellanicus*, were exposed to four concentrations (plus control) of un-ionized ammonia (1, 2, 3, and 4 mg NH<sub>3</sub>/L) at two different temperatures. Juvenile giant scallops were found to be more sensitive to un-ionized ammonia than other bivalves and the sensitivity varied with temperature. The 96-hour LC50s were 1.8 mg NH<sub>3</sub>/L at 4°C and 1.0 mg NH<sub>3</sub>/L at 10°C.

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[1. Epifanio CE, Srna RF. 1975. Mar. Biol. 33:241-246.]

## Tolerance of the giant sea scallop to low salinity exposure

Craig Bergman, Jay Parsons and  
Cyr Couturier

Giant sea scallop (*Placopecten magellanicus*) culture has been hindered in Newfoundland by several episodes of mass mortality caused by low salinity. More data is needed to accurately determine the salinity tolerance of giant sea scallops. Salinity tests on juvenile scallops from Nova Scotia were done at Memorial University of Newfoundland. The first of two experiments consisted of two short term exposures to salini-

ties of 10, 13.5, 16.5, 20, and 31 ppt at an ambient winter seawater temperature of 1°C. The first part was a 2-hour exposure to low salinity before recovery in ambient flow-through seawater. This treatment resulted in nearly 100% survival in all test groups, although severe catatonic shock was exhibited by all groups except those at 31 ppt. The second part of the experiment was a 6-hour exposure in which similar results were obtained, except that all scallops in the 10 ppt group died. The second experiment involved long term exposure to salinities of 10, 13, 16, 18, 21, 24, 27, and 31 ppt. Salinities of 16 ppt and below were lethal; however catatonic shock was severe in all groups held at 21 ppt or less. These experiments were designed to mimic extreme environmental conditions on farms when a layer or lens of fresh water covers the site or the salinity of the entire site declines. Culturists and site selection advisors should be aware that long term exposures to approximately 18 ppt (and lower) and short exposures to 13.5 ppt or less can cause mortalities and that sites that tend to retain freshwater should not be chosen.

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## Kelp resource of Newfoundland and Labrador

Mohan Lal

In the most easterly Canadian province, Newfoundland and Labrador, there is a tremendous potential for the harvesting and processing of seaweeds. The most common species indigenous to the region are the brown seaweeds, collectively called "kelp". This includes *Fucus vesiculosus*, *Alaria esculenta*, *Laminaria longicruris*, *L. digitata*, *L. saccharina*, and *Sacchariza dermatodea*. *A. esculenta* has a midrib running down the middle of the main blade, whereas *L. digitata* has a robust blade divided into ten strips. *L. longicruris* has a long ribbon blade with frills on both sides. At present, kelp stands are of no commercial value except that they provide grazing fields for sea urchins and sea cucumbers. Traditionally, kelp has been used as fertilizer, food and medicine. The con-



stituents of kelp are a gelatinous substance called algin, mannitol, iodine, a volatile oil, beta-carotene, photosynthetic pigments, and various inorganic substances. *L. digitata* is a rich source of dietary iodine, an alternative to the iodized salt, and a fraction of a gram is sufficient to meet the daily recommended intake. Lipids are known to be hypolipidemic. As kelp is loaded with trace minerals, as such, it provides an excellent micronutrient source for

both humans and plants. Whole dried plants can be eaten as food and it has been found to be antiobesic and antihypothyroid. This multi-purpose health food can be used in soups, vegetables, pickles, and especially as a tickening agent.

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