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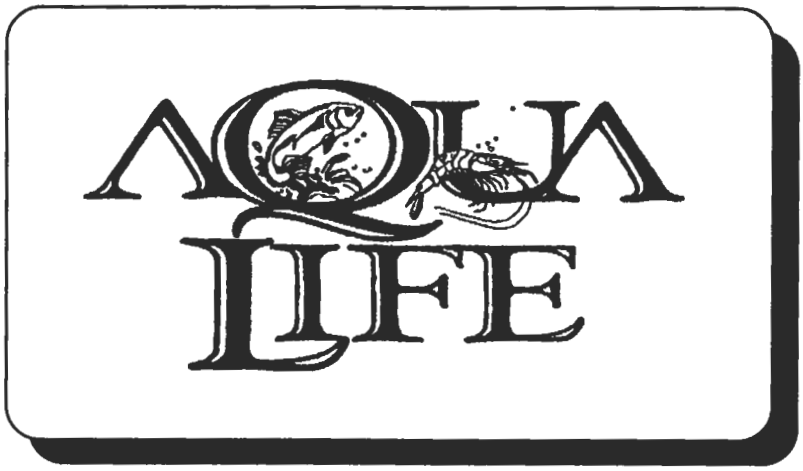

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Introduction to Aquatech '95

The 1995 workshop of the Canadian Aquatic Biotechnology Network — 28 January 1995, Vancouver, British Columbia

Kim Harrison

Aquatic biotechnology encompasses a wide range of topics. The onerous task of the program committee was to establish a focus and select topics relevant to the broad interests of the AQUATECH membership and the target workshop participants.

The first objective of the committee was to promote communication and partnerships among the scientific community, government, and industry; the second was to recognize and foster opportunities for research, commercialization, and development of international markets.

The future and opportunities for Canadian expertise applied outside Canadian borders was recognized as an important focal point. Thus the theme "Pacific Rim Opportunities in Aquatic Biotechnology" was selected to address opportunities for Canadian researchers and commercial enterprises in biotechnology to address the myriad problems (challenges) of the aquatic environments of Pacific Rim countries.

Owing to the academic and entrepreneurial strengths in Canada, and the limitations of a one day venue, this workshop addressed four session topics in aquaculture: nutrition, growth and reproductive technologies, health, and water quality. A fifth topic was presented by the luncheon speaker, Dr. Steve Pelech, whose Kinetek Biotechnology Corporation provided a Canadian success story in taking basic research in aquatic biotechnology from the laboratory to profitable commercialization of its applications.

Under the leadership of the Conference Coordinator, Devon Knight, a creative and successful program format was used. In each of the four sessions, the opening speaker "challenged" the subsequent speakers by reviewing problems,

crises, or bottlenecks that can be resolved through applications of aquatic biotechnology. The subsequent speakers presented approaches, techniques, or solutions, as opportunities to address those challenges.

The workshop was a unique gathering and networking opportunity of over 125 participants from a broad range of backgrounds, including R&D scientists from corporate, university and government laboratories, entrepreneurs, commercial and government managers, policy makers and regulators, to bankers, investment brokers, and patent lawyers.

AQUATECH is grateful to the Aquaculture Association of Canada for the opportunity to publish papers, excerpts, and abstracts of presentations in a special issue of the *Bulletin*. We hope, through this forum, to showcase Canadian contributions in aquatic biotechnology and to stimulate research and entrepreneurialism from presentations by our international colleagues.

AQUATECH wishes to thank those presenters who were able to submit manuscripts or summaries for this proceedings. Due to time constraints, other speakers have included only their abstracts so that we may present the full scope of topics from the workshop.

We hope this issue will entice you to participate in AQUATECH '96 in St. Johns, Newfoundland.

Aquatech '95 Workshop and Program Committees

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Workshop

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National Research Council, Institute for Marine Biosciences
Aquaculture Association of Canada

The Future of Aquatech in a Time of Change

John A. Spence

Since its founding in 1987 AQUATECH has functioned as a loose network of Canadian companies, university researchers and government laboratories and institutes. Each year AQUATECH, with support from the National Biotechnology Strategy, the Department of Fisheries and Oceans, provincial governments and industry, has hosted a conference. These annual conferences, rotating across the country, have enabled persons from across Canada to meet and present papers on the promotion and commercialization of biotechnology related to aquatic organisms. These conferences with their informal networking have been the core of AQUATECH. We know that they will continue with AQUATECH '96 being hosted by Newfoundland, starting a new cycle that will move westwards once again. Only once has AQUATECH moved to a more formal level of networking with the trial publication last year of a trial issue of AQUAFAX — a faxed newsletter modeled on the successful BIOFAX newsletter that has become the heart and soul of the British Columbia Biotechnology Alliance.

The successful completion of this most recent AQUATECH '95 in Vancouver, which focused on Pacific Rim themes in aquatic biotechnology, signals a critical point in planning the future of AQUATECH. Aquatic biotechnology is now an actual money making business with vaccines and other new technologies in support of aquaculture and valuable enzymes and other bioactive molecules being extracted from a wide range of marine organisms. Emerging from the wings are the many products of science with potential for commercialization, that have been presented at past AQUATECH meetings. A major factor to be considered in the future of AQUATECH is the structural changes that are occurring in Canada. They are signalled by the deficit cutting that will bring many reductions in federal laboratories and institutes across Canada, and probably reduced funding for research at a time when many new global market opportunities are emerging. In this uncertain and rapidly changing environment, communications among those involved in aquatic biotechnology in Canada will be of increasing importance, to create strong consortia for research and commercialization, overcome impediments, find answers, identify markets etc.

Other technology sectors are actively exploring and using more organized forms of networking to give themselves a competitive edge. A National Technological Network sponsored by the NRC is under discussion; among many new government initiatives to stimulate international partnerships is a scheme to increase the opportunities for Canadian companies to participate in international R&D. The information highway is happening and technological and business uses of the Internet are increasing rapidly.

Somewhere in this maelstrom there is a useful role for a renewed AQUATECH of the future with a strong industrial drive, while also enabling routine communications among our members. There would be no better way to start this process than by launching an AQUAFAX on a monthly or quarterly basis, to complement and reinforce our annual meetings. We could rotate the editorial and node responsibilities around the country each year. We all need better linkages and rapid communications on a host of emerging aquatic biotechnology issues. So lets do it. Full steam ahead to active participation in aquatic biotechnology around the world; ...and damn the torpedo's and outrageous slings of fortune.

The profitable application of starfish oocytes for cancer research

Steven Pelech

Kinetek is a Canadian biotech company striving to become a world leader in the mapping and manipulation of the molecular communication systems within living cells. The initial focus has been the development of unique antibody and enzyme reagents used in the search for kinase inhibitors that may be clinically useful for the treatment of cancer and other diseases.

Introduction

Government has always expected biomedical researchers to find better ways to diagnose and treat diseases in reciprocation for financial support. There are additional expectations for scientific research to directly benefit industry. Furthermore, economic pressures have made it very difficult for researchers to secure grant-funding from government and disease-related charities. But how can entrepreneurial scientists bring their promising findings from the laboratory into the international marketplace at a time when venture capital is troublesome to procure? In this article, I shall present our strategy to meet this challenge by recounting some of the critical factors that led to the inception and development of Kinetek Biotechnology Corporation. As shall become evident, marine organisms have played a prominent role in the success of the company

Kinetek was founded by myself and other researchers from my University of British Columbia (UBC) laboratory group in 1992. The mission of the company was to become a world front–runner in the mapping and manipulation of molecular communication systems in living cells. The malfunction of these signalling systems is at the root of cancer and many other degenerative diseases associated with aging. The initial focus of Kinetek's activities has been the development of unique enzymes and antibodies. While these were originally designed to facilitate our UBC research program, these reagents are also highly sought after by other scientists around the globe engaged in similar studies.

Protein kinases and antibodies

For the last 14 years, my research has been concerned with the characterization of enzymes called protein kinases, which are important for the dissemination of information inside of cells. Kinetek actually derived its name from the Greek root *"kine"*, which means "to move" and *"tek"*, which is abbreviated from "technology". Protein kinases are amongst the best studied enzymes in nature, with potentially thousands of variants. These enzymes work by tagging other proteins with phosphate. This may lead to the activation of certain recipient enzymes and the inhibition of others. Many of the phosphorylated enzymes are also protein kinases that are in turn recruited by this mechanism. Interconnected relays of sequentially activating protein kinases serve as the hardwiring in the molecular circuitry for amplifying and transmitting signals that arise from both the outside and inside of cells. The importance of these enzymes is underscored by the fact that nearly half of the hundred or so known oncoproteins responsible for causing cancer are defective protein kinases. The identification and characterization of these oncoproteins and our rudimentary understanding of their interrelationships is the culmination of nearly 2 decades of cancer research. Armed with a detailed knowledge of why and how cells become cancerous, we are now finally in an excellent position to better combat this dreaded disease.

Some of my research group's most important contributions to the cancer puzzle have arisen from experiments conducted with oocytes from

starfish. This work actually began in the early 1980s while I was a post-doctoral fellow in the laboratory of Nobel Prize winning laureate Dr. Edwin Krebs at the University of Washington in Seattle. At that time, I discovered that a series of protein kinases were markedly activated when starfish oocytes were induced to mature into fertilizable eggs. I was able to further show that these kinases were highly similar to kinases that controlled the proliferation of mammalian cells. Remarkably, starfish represent some of the most primitive animals that are still alive on the planet today. The strong conservation of protein kinase networks during evolution underlies their fundamental importance.

In 1987, I became an independent investigator at UBC as a faculty member of the Department of Medicine and senior scientist of the Biomedical Research Centre. Since then I have been privileged to receive generous and steady grant support from the Medical Research Council of Canada, the National Cancer Institute, the Heart and Stroke Foundation and the BC Health Research Foundation. This funding permitted me to form a highly productive team of pre- and post-doctoral trainees, research associates and technicians. During the course of our research, we developed highly specific immunological probes for certain protein kinases. These reagents were affinity-purified, anti-peptide polyclonal antibodies developed in rabbits to cross-react with protein kinases to facilitate their detection and isolation. In 1991, a newly formed US distributor of signal transduction reagents called Upstate Biotechnology Inc. (UBI) came to learn of our antibodies and approached us for marketing rights. As these reagents were developed at UBC, they were licenced for sale through UBI by the university. However, in a standard agreement with UBC, I was personally entitled to 50% of the royalties from the antibody sales. My intention was to return these proceeds back into the support of my university laboratory, but certain events transpired that made this impossible. Instead, I used this as seed funds to start Kinetek.

Several of the other senior scientists at the Biomedical Research Centre, where we were based, were uncomfortable with the fact that my UBC laboratory was involved with the production of antibodies that were sold. This was despite the fact that these reagents were originally designed to support my university-based research program. Due to this philosophical disagreement, I was forced to either cease this activity or conduct it elsewhere. I decided that it was important to continue making new antibodies and market the excess materials. The profits from the sales of these extra antibodies could cover the costs for the development of future antibodies and much needed scientific equipment for my research program. To achieve this, it was necessary to ultimately find a location off the UBC campus for this activity and conduct it as an independent business.

The formation of Kinetek Biotechnology Corporation

The decision to form Kinetek in 1992 was prompted in part by the requirement to produce antibodies independently of my UBC laboratory. But many other considerations also contributed to the establishment of this new company. Due to the reduced amount of financial support from government and disease-based charities, many of the talented graduate and post-graduate trainees in universities today have little prospect for becoming principal investigators. Furthermore, while the biopharmaceutical industry has provided some research career opportunities, the number of established pharmaceutical/biotechology companies in Canada is still small. While the outlook for my trainees to secure jobs in either academia or industry was not encouraging, even the security of my own "soft-money" university faculty position was tenuous. The principal source of my university salary has been from 5-year term scholarship awards awarded from the Medical Research Council of Canada.

Despite the declining support for academic-based research, there is tremendous growth potential for the biotechnology industry into the 21st century. Basic research has uncovered a wealth of new information about biological processes and this knowledge is ripe for application to solve important problems. Kinetek represented an opportunity to create innovative products that can have a very positive impact upon the diagnosis and treatment of human diseases. Then there was the profitability issue. The financial success of Kinetek would not only be personally rewarding, but it could also benefit the Canadian economy as most of Kinetek's products would be destined for export.

With the creation of Kinetek, I also had a mechanism by which I could share the proceeds of the original antibody sales with the personnel in my university laboratory. Their valuable contributions were taken into account in the issue of the company shares. As Kinetek's President and CEO, I retained controlling interest in the company, but the balance of issued shares in the company were distributed among the employees of Kinetek and the personnel in my university research group. New shares are provided annually on the basis of continued association with either Kinetek or my UBC laboratory. Approximately two–thirds of Kinetek's shares are still retained in reserve for future potential private or public placement.

Following the incorporation of Kinetek, UBC kindly permitted the company to temporarily sublease space at the Biomedical Research Centre. This allowed Kinetek valuable time to identify a site that would be suitable for at least five years. In the fall of 1993, the Kinetek R&D and production facility was relocated to a 5500 square feet laboratory on the top floor of the QLT Place building. This site is strategically located within half a kilometer from the Vancouver General Hospital complex and the BC Cancer Centre. The facility houses 23 individual (eight feet in length) laboratory benches in addition to shared areas designated for research and production activities. Kinetek currently possesses about $350,000 worth of scientific instrumentation and furniture at this site. My UBC laboratory group, which is also accomodated at the Kinetek site at company expense, has an additional $350,000 worth of scientific equipment. Over 30 university and company personnel work side by side at the facility.

Through various initiatives with granting agencies and BC educational institutions, Kinetek has been very active in supporting the training of promising young people for careers in biomedicine and biotechnology. Kinetek has provided desks, laboratory benches and equipment for the training of pre- and post-doctoral trainees in my UBC research group. Furthermore, about 12 undergraduate co–op education students from Simon Fraser University (SFU) are now hired annually at Kinetek. These students are employed full-time for four to eight month terms. Because of their high calibre, these students are often partially funded by industrial studentship awards from the Natural Sciences and Engineering Council of Canada. Our company has also hosted students for 6-week work experience terms from the BC Institute of Technology and from the West Vancouver Secondary School Co–op Education Program. Some of the best of these trainees will become future Kinetek employees as the company grows.

Kinetek products and marketing

Kinetek's partner for the distribution of its antibody and enzyme products is Upstate Biotechnology Incorporated (UBI), an affiliate of the W. Alton Jones Cell Science Center in Lake Placid, NY. It is a distributor of over 500 products for the investigation of cellular signal transduction. Nearly 100 of Kinetek's products are currently distributed through UBI. These reagents are heavily promoted by UBI in product literature that is directly mailed to over 40,000 scientists world-wide on a bimonthly basis. UBI's aggressive marketing strategies have propelled it into one of the top sales companies in the world for these products. UBI handles the marketing, quality control, packaging, shipping and collection of payments for all of Kinetek's current products. The alliance with UBI has allowed Kinetek to concentrate on the research, development and manufacture of new reagents.

While antibodies were the first products offered by Kinetek, we quickly recognized the strong market potential of highly purified preparations of protein kinases. Kinetek has exploited genetic engineering techniques to introduce human and mouse protein kinases into bacteria that highly express these enzymes. However, the mammalian kinases manufactured by the bacteria are generally inactive and have limited utility. By contrast, protein kinases purified from starfish oocytes are extremely active and are highly sought after by scientists. Last year in the late spring, we processed about 40 liters of starfish oocytes for protein kinase purification. The starfish were harvested by Kinetek employees from the beaches in the Vancouver area at low tide prior to their spawning. One of the reasons why the starfish is such a plentiful source of oocytes is that the fertilization of the gametes from starfish must occur in the open ocean. The overproduction of gametes is the strategy that this echinoderm has adopted to increase the probability that a starfish sperm will successfully

encounter a starfish egg in the water.

Kinetek's antibodies are sold in 100 µg amounts for approximately US$275 of which Kinetek receives about 25% in royalties. The starfish protein kinases are even more lucrative and retail at US$275 for 1 µg with royalty returns of about 30%. In the first year of its operation, Kinetek earned $109,000. in royalties from the sale of 20 products. By the end of its second year, Kinetek's royalties jumped to $360,000. from the sale of 50 products. As we approach the end of our third year, Kinetek now offers nearly 100 products. Kinetek has sufficient inventory of most of its currently marketed antibodies to meet the projected demands for these reagents for the next five years. However, we have not been able to keep up with the demand for starfish protein kinases. The sale of all of these reagents has been the principal financial support for Kinetek's growth and is anticipated to remain its main source of revenue over the next few years.

While Kinetek's reagents have been profitable, it should be appreciated that they have been a real boon to the international cancer research effort. For example, it may take 6 months and about $4000 to develop a useful antibody. In view of the highly competitive nature of biomedical research and simple economics, it makes much more sense for investigators to purchase the desired quantity of a proven antibody from UBI. By reducing the costs and accelerating the pace of cancer research, Kinetek is contributing to the discovery of new strategies for diagnosing and treating cancer and other diseases. Furthermore, Kinetek donates over a hundred thousand dollars worth of its products to researchers across Canada and internationally. This has allowed Kinetek to develop strategic liaisons with many of the best signal transduction researchers in the world.

New directions for Kinetek

In maligant tumour cells, certain protein kinases are often inappropriately activated and contribute to the development of cancer. These findings have two important implications. Firstly, measurement of the activities of these kinases in tissue biopsies from tumours could provide rapid diagnosis of whether the tumours are benign or malignant. This could yield quantitative data that would be extremely useful for

Male (right) and female *Pisaster ochraceus* spawning on a beach in Pacific Rim National Park, Vancouver Island, British Columbia. The oocytes from this echinoderm have been used as a source of activated protein kinases for cancer researchers. [Peter Thomas photo]

the early treatment of cancer. Secondly, inhibitors of relevant protein kinases should block the flow of proliferative signals in cancer cells and arrest their growth. This would provide extra time for the body's immune system to eliminate the cancerous growth. With the expertise that Kinetek has acquired in the study of these kinases, it is very well poised to exploit this knowledge for the development of new diagnostic kits and pharmacological agents for the detection and treatment of cancer. Through the pursuit of these objectives, Kinetek is undergoing the next stage of its evolution into an integrated biopharmaceutical research, development and manufacturing company.

Kinetek has completed preliminary studies that indicate that certain protein kinases are indeed activated in tumours of the lung. This work is currently being expanded for the examination of tumours derived from the breast and gastrointestinal tract. Kinetek has already developed protein kinase assays that are based upon the utilization of radioactive phosphate. Seven of the specific radioactive-based protein kinase assays designed by Kinetek are now offered in kit form by UBI. Kinetek is now trying to develop improved non-radioactive assays for protein kinases that are cheaper and faster to perform, and more amenable to automation. The kinase assay kits will be designed to use 96-well microtitre plates such that each plate can be used to test at least 6 distinct kinases from 12 different patients. This project is currently supported in part by a grant from the National Research Council of Canada Industrial Research Assistance Program and a loan from the Western Economic Diversification Fund.

Kinetek is most excited by the prospect that the protein kinase assays can be utilized for screening extracts from diverse organisms for inhibitors of selected protein kinases that are produced by the company. Novel specific inhibitors of any one of these kinases could have tremendous therapeutic potential for the treatment of diseases such as cancer and for facilitating organ transplantation by reducing immune rejection. These kinases represent some of the best rational targets for the development of new drugs that the international cancer research community has uncovered. With 1 out of 4 people in North America expected to develop cancer, the market for better cancer chemotherapy agents is immense. With the existing therapies, just under half of the people that develop cancer succumb to this disease.

The more cellular extracts that are probed for kinase inhibitors, the better the prospect that specific and potent inhibitors will be unearthed. Here again, the ocean has been extremely important as a source of biological material. Kinetek is currently collaborating with Dr. Raymond Andersen from the Department of Chemistry at UBC and the NRC Institute for Marine Biosciences in Halifax in the screening of several thousand cellular extracts from marine organisms. Eventually, this protein kinase inhibitor screening program will also be expanded to investigate extracts from microbes and medicinal herbs. The bioactive compounds that are responsible for the inhibitory activity towards protein kinases will be isolated and their structures elucidated in collaboration with the National Research Council of Canada. Novel compounds discovered by this route will be jointly patented and their therapeutic potential defined in collaboration with other pharmaceutical partners.

The screening program for protein kinase inhibitors has tremendous potential for the identification of new marketable compounds and their sources. No specific inhibitors of these kinases are available commercially despite the strong demand for such products by the research community. This is primarily because these kinases have only recently been discovered and few other companies have had access to active preparations of purified kinases. Therefore, even though the identification of novel therapeutic agents has always been a very risky affair, the prospects are excellent that Kinetek will identify commercially useful compounds.

It is evident that Kinetek is an overly ambitious company, perhaps from the naiveté of its youth. Nevertheless, our early successes have bolstered our self confidence that we will succeed in achieving many of our objectives. I am extremely pleased with the strides that our company has made so far without reliance upon outside private or public investment. This is a tribute to the energy and creativity of the talented associates that I have been privileged to work with at Kinetek.

Dr. Steven Pelech is CEO of Kinetek Biotechnology Corporation, Suite 500, 520 W. 6th Avenue, Vancouver, BC Canada V5Z 1A1

Brain regulation of feeding and growth in fish

Richard E. Peter[1]

Growth in fish is regulated by the brain neuroendocrine — growth hormone — insulin-like growth factor axis. The neuroendocrine regulation of growth hormone secretion in goldfish and other fish is multifactorial, with a balance of stimulatory and inhibitory neurohormones acting on the somatotrophs. Sex steroids, in particular estradiol, influence the responsiveness of the somatotrophs to neuroendocrine factors; the responsiveness to gonadotropin-releasing hormone and neuropeptide Y is increased by estradiol, whereas the responsiveness to dopamine and cholecystokinin is greatest in sexually regressed goldfish. Food intake is also regulated by the brain, and evidence indicates that cholecystokinin and bombesin provide a linkage between satiation and the neuroendocrine regulation of growth hormone secretion. Growth rates of goldfish can be stimulated by addition of selected neuroendocrine factors to food or by injection. Growth rates of farmed fish may be stimulated by addition of long lasting analogs of neuroendocrine factors to food to stimulate growth hormone secretion and appetite. Techniques for enhancing growth rates of farmed fish are in an experimental stage.

Introduction

Growth in fish is regulated by the brain neuroendocrine–growth hormone–insulin-like growth factor axis.[16] However, growth cannot be realized without adequate food intake. A relevant question then is whether there is any connection between the regulation of food intake and the brain neuroendocrine–growth hormone–insulin-like growth factor axis. A question of relevance to aquaculture is whether the brain neuroendocrine–growth hormone–insulin-like growth factor axis and food intake can be manipulated to stimulate faster growth rates of farmed fish.

Regulation of food intake

Brain electrical stimulation and brain lesioning studies on fish have demonstrated that food intake is regulated by the hypothalamic ventromedial-posterior and lateral lobes.[14] Given the availability of an adequate food supply, food intake is regulated to maintain growth in an orderly fashion, including variations in growth that may occur on a seasonal basis. Treatment of fish with growth hormone by repeated injection, intubation, pellet implantation or other means increases growth rates.[12]

Notably this chronic administration of growth hormone has been noted to cause increased feeding and food conversion efficiency in several teleosts.[3,15]

In a recent report, rainbow trout injected with growth hormone were reported to have increased appetite within 2 days.[9] This indicates that growth hormone not only regulates growth, but that it also influences food intake.

Is there a relationship between food intake and growth hormone secretion in fish? We have recently found that at 30 minutes following feeding a 2% wet body weight (bw) ration goldfish have an acute elevation in serum growth hormone levels.[7] This initial rise in serum growth hormone levels, which occurs independent of body weight, is followed by a sharp decrease and then a more gradual decrease to serum growth hormone levels significantly lower than in unfed control fish. This characteristic pattern in post-prandial serum growth hormone levels does not occur in fish on a maintenance diet.

Neuropeptide regulation of feeding in fish

The gut and brain peptides bombesin suppresses food intake within 45 minutes following intraperitoneal or brain intraventricular injection.[4] Likewise, intraperitoneal or brain intraventricular injection of the eight amino acid sulfated form of cholecystokinin also acutely suppresses food intake in goldfish.[5] At 30 minutes following suppression of food intake serum growth hormone levels increase.[5,6] Notably, immunocytochemistry studies demonstrate that bombesin and cholecystokinin are present in the brain hypothalamic feeding area of goldfish[5,6] and other teleosts.[7]

Preliminary receptor radioautography studies indicate the presence of bombesin and cholecystokinin receptors in the brain hypothalamic feeding area of goldfish.[8] Together these data provide strong evidence that bombesin and cholecystokinin are involved in satiation in goldfish and other fish.

Other neuropeptides are undoubtedly involved in regulation of food intake. Corticotropin-releasing factor, which has been reported to suppress food intake in goldfish following either intraperitoneal or brain injection.[2] Neuropeptide Y, which plays a prominent role in stimulating food intake in mammals is present in the goldfish ventromedial-posterior hypothalamus and hypothalamic inferior lobes.[17] Direct studies have not been done, to our knowledge, on neuropeptides that stimulate food intake in fish.

Neuroendocrine regulation of growth hormone secretion in fish

The neuroendocrine regulation of growth hormone secretion in goldfish and other fish is multifactorial, with a balance of stimulatory and inhibitory neurohormones acting on the somatotrophs.[7,15] Somatostatin is the primary inhibitor of basal and stimulated growth hormone secretion. Growth hormone secretion is stimulated by growth hormone-releasing factor, gonadotropin-releasing hormone, dopamine, neuropeptide Y, thyrotropin-releasing hormone, cholecystokinin, and bombesin. Sex steroids, in particular estradiol, influence the responsiveness of the somatotrophs to neuroendocrine factors; the responsiveness to gonadotropin-releasing hormone, neuropeptide Y, and thyrotropin-releasing hormone is increased by estradiol, whereas the responsiveness to dopamine and cholecystokinin is greatest in sexually regressed goldfish. The observations that intraperitoneal or brain intraventricular injection of cholecystokinin and bombesin goldfish have an inhibitory action on food intake and a stimulatory effect on serum growth hormone levels, and that both cholecystokinin and bombesin can stimulate growth hormone secretion by direct actions in the pituitary, together suggest that cholecystokinin and bombesin are involved in satiation and the post-prandial increase in growth hormone secretion in fish.

Neuroendocrine stimulation of growth rates of farmed fish

It has been demonstrated that at least some biologically active proteins and peptides can be absorbed intact from the gastrointestinal tract into the blood of fish.[11,18] The hypothesis to be tested is whether neuroendocrine factors can be added to fish food to stimulate growth hormone secretion, and perhaps food intake in fish, to stimulate growth rates. Since cholecystokinin and bombesin stimulate growth hormone secretion but inhibit food intake, these are clearly not neuroendocrine factors to be tested for growth stimulation.

Feeding or intraperitoneal injection treatment with apomorphine, a dopamine receptor agonist, stimulates growth rates of goldfish.[19,20] Also, treatment with a superactive agonist analog of gonadotropin-releasing hormone by multiple intraperitoneal injection[10] or by intramuscular injection of a slow release preparation of a superactive agonist analog of gonadotropin-releasing hormone[13] is also effective in stimulating growth rates of goldfish.

Using a food carrier vehicle developed by Syndel Laboratories, Ltd., feeding a combination of four neuroendocrine factors was tested for stimulation of growth rates of goldfish; the four neuroendocrine factors combination was found to have negative interactions on the somatotrophs, and therefore growth hormone secretion and growth was not stimulated.[15] However, the combination of two long lasting analogs of selected neuroendocrine factors added to the food carrier resulted in a highly significant increase in growth hormone secretion and growth rates of goldfish.[15] Since the fish were on a 2% wet body weight (bw) ration, the data

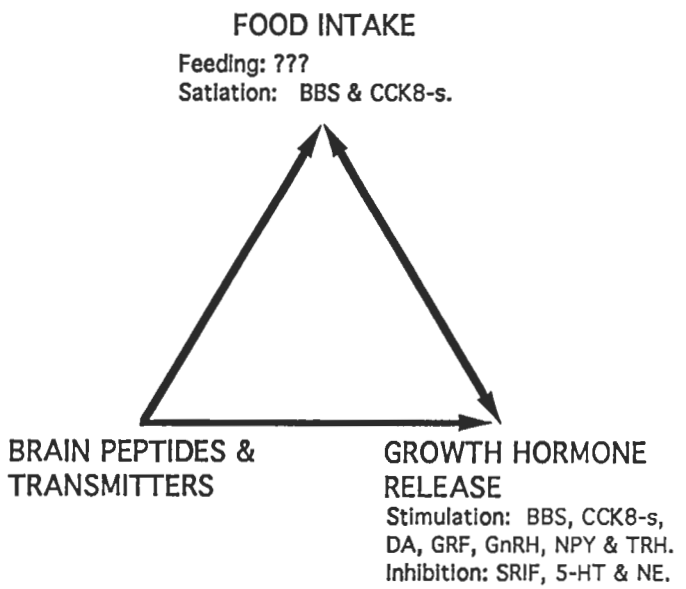

Figure 1. A diagrammatic summary of the relationship between brain regulation of food intake and growth hormone secretion. Abbreviations: bombesin, BSS; cholecystokinin (eight amino acid form – sulfated), CCK8-s; dopamine, DA; gonadotropin-releasing hormone, GnRH; growth hormone-releasing factor, GRF; neuropeptide Y, NPY; norepinephrine, NE; thyrotropin-releasing hormone, TRH; serotonin, 5-HT; somatostatin, SRIF.

suggest that food conversion was also increased. These results indicate that feeding selected neuroendocrine factors may be a highly effective means of stimulating growth rates of farmed fish.

Conclusion

Figure 1 provides a diagrammatic summary of the relationship between brain regulation of food intake and growth hormone secretion. Growth rates of farmed fish may be stimulated by addition of long lasting analogs of neuroendocrine factors to food to stimulate growth hormone secretion and appetite.

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Advanced diagnosis: You can't wage war if you don't know your enemy

John Reddington

One of the first lines of defence in controlling or eradicating diseases is the identification of the causative pathogen. Researchers over the last 5-10 years have been applying sophisticated technologies developed in the human and veterinary health care research field to the problems facing aquatic animal health. Significant advances have been made in diagnostics for some of the primary pathogens of salmonids; however, there is still a void in our knowledge about the disease causing agents of other farm raised species. The shrimp industry, producing the most significant aquaculture commodity in the Pacific Rim, is full of examples that amplify both the lack of understanding about the pathogens that affect shrimp and our ability to combat their spread.

Most people think of using diagnostic tests in the wake of a massive wave of morbidity or mortality. However, a more important use of diagnostics is as an integral component of a preventive medicine approach toward total herd health management.

Before you can create a vaccine and implement a war against a pathogen or group of pathogens, or even toxic agents, you must be able to tell what you are dealing with and where it is found. This is where diagnostic tests come into the picture. Since the focus of this workshop was on opportunities in the Pacific Rim, I would like to use the shrimp industry as a backdrop for this paper, primarily because shrimp are the "Number One" aquaculture commodity for that region of the world. Much of what is in this paper, however, also holds true for other aquaculture products such as salmon, carp, eels, tilapia, oysters, etc.

Shrimp are a non-domesticated species, although the industry is currently trying to develop High Health or Specific Pathogen Free broodstock. Many of the broodstock are wild caught, which is like having the cattle industry based on hunting. Broodstock are brought on shore and placed in hatcheries to spawn. Eggs are hatched and grown in the hatchery to the postlarval (pl) 10 to pl 32 stage. In many cases, they are then sold, transported, and placed in outdoor ponds. The grow-out production cycle is 120-180 days, depending on the species and climate. Losses can be 40% in hatcheries and up to 80% in ponds. Still, growers are able to make a profit because of strong world demand, and due to the fact that China (which up to 1993 was the world leader in production) saw its industry collapse due to a viral agent and environmental problems. This has lead to a shortage in supply, which in turn has kept the price of shrimp high.

One point that is important to make is that infection does not necessarily equate with disease, and events leading up to a disease outbreak are usually complex and multifactorial. A good example of the web of interacting factors can be seen in causative aspects of canine respiratory disease, or kennel cough as it is commonly called. First, you have predisposing events such as stress and/or primary viral infection. Stress can be physical or physiologic; the viruses can be multiple types. Stress, coupled with viral infection, leads to suppression of the immune system and cellular destruction. This, in turn, creates a perfect environment for secondary bacterial coloniza-

tion — which may ultimately kill the animal.

As a fish health specialist, one may be presented with a sick or dead animal and the first thing that is often done is take a sample for bacteriological analysis. The sample is positive for a bacterial infection and it is determined that *Aeromonas, Vibrio, Flexibacter,* etc., caused the disease — when in reality earlier events were the real culprits. This is an extremely important use of diagnostic methods or reagents — tearing apart and understanding the web of factors leading to the pathogenesis of disease.

What other functions or uses do diagnostics serve? One is in understanding the epidemiology of disease causing agents to help prevent their establishment and spread to new geographic locations or facilities. Also, if we know that an agent is capable of vertical transmission from parent to offspring, we can screen broodstock and eliminate positive animals from the production cycle. Another important use is before animals are sold or transported. It is wise to test the animals not only to help prevent the spread of pathogens, but also to protect the buyer from purchasing animals that have a poor chance of making it to market. In addition, the use of routine diagnostic testing during grow-out can serve as an early warning system and general indicator of how well your management practices are working. Finally, diagnostics are useful in monitoring the success of a vaccine program.

One of the most important aspects of diagnostic tests to keep in mind is that they are *tools* for the aquaculturist to use in making sound management decisions. For example, if growers are doing routine testing and see the pathogen load increase sharply they may decide to harvest early. They may not get $5 per pound, perhaps only $4.50 per pound, but at least they will be able to recoup some value from their crop. Another example is when the grower is farming a crop that is climate dependent (i.e., only two crops per year because of temperature). If they can detect a pathogen and elect to destroy the animals, disinfect, and restock, they still get two crops and prevent the spread of the pathogen throughout the facility. If it is a bacterial agent, they can implement *early* antibiotic treatment. Diagnostics can also be used to alert the aquaculturist to underlying or predisposing causes that they may be able to control. Some of these potential stressors that may initiate the progression of infection to disease are stocking densities, oxygen tension, salinity, nutrition, etc.

With regard to some of the specific diagnostic methods currently available in the shrimp industry, it should be noted that three quarters of the most widely used diagnostic methods entail the use of microscopy. The shortcoming here is that often you need a sophisticated histology laboratory to prepare the slides (unless a wet mount is used) and a highly trained diagnostician to interpret the slides. It should also be noted there are virtually no immunological reagents available to the industry, which are the predominant diagnostic tools in other health management sectors. There currently are no shrimp cell culture lines available, which is a common means of diagnosing viral diseases. However, there are a spectrum of DNA probes available, many of which we as a company are commercializing in the form of *in situ* and Dot Blot Hybridization kits.

If you want to develop useful diagnostic methods, what are some of the things to keep in mind? Obviously, you want to be able to specifically identify the agent of interest. Perhaps less obvious is the issue of sensitivity. How sensitive does a test need to be? Do you want or need to detect just one organism or is it more important to be able to detect 100 or 1000? Remember that infection that does necessarily mean disease. If a test is too sensitive and the pathogen is pandemic and does not cause disease at very low levels, you may wrongly elect to destroy all of the animals. Another aspect of a useful diagnostic is to have an appropriate format for the end user. If thousands of samples will be tested at once in a laboratory, a microtiter plate format is appropriate. However, if relatively few animals need to be screened pen-side, a tube or membrane test is more appropriate. Finally, you have to keep in mind that it must be cost effective for the grower to use. The grower has to gain more financial benefit from the results of the test than it costs to run the diagnostic method.

An important consideration in designing a useful diagnostic test is that, as technology moves from the laboratory to the field, the level of sophistication of the potential end user and/or the environment in which the method will be used often move in opposite directions. In the shrimp industry, for example, the test may be performed in a hut on a farm that does not have electricity or a good water source, or

Table 1. Penaeid shrimp viruses.

Virus	Nucleic acid	Type
DNA viruses		
IHHNV	ssDNA	parvo virus
HPV	ssDNA	parvo virus
LPV	dsDNA	parvo-like virus
BP	dsDNA	occlud baculovirus
MBV	dsDNA	occlud baculovirus
BMN	dsDNA	nonocclud baculovirus
PHRV	dsDNA?	baculo-like virus
YHV	dsDNA	baculo-like virus
IRIDO	dsDNA	iridovirus
RNA viruses		
REO-III	dsRNA	reo-like virus
REO-IV	ssRNA	reo-like virus
LOVV	ssRNA	togavirus
RPS	ssRNA	rhabdovirus

in a minimally equipped laboratory. Therefore, the diagnostic method must be designed such that nominal equipment and expertise is needed.

The goals of the end user are another important consideration to keep in mind when you are attempting to design and implement a diagnostic method or program. The aquaculturist's *primary* goal is to grow fish/shellfish and make a profit so that they can stay in business. Also, it is important to establish with the farmer or fish health specialist if they are trying to control or eradicate disease. More than likely they are trying to control disease. With eradication comes increased cost and it is extremely difficult when raising animals in an aqueous environment to totally eradicate a disease causing pathogen.

In terms of potential opportunities, Table 1 shows a list of viral agents that are wreaking various degrees of havoc in the shrimp industry. This is only a list of viral agents. In addition to these there are bacterial agents (such as fluorescent *Vibrio*), fungal, and parasitic, as well as toxin and environmental agents to consider. This list is less than one year old and there are already several other important viral agents to add, such as Taura virus and the China virus. Please keep in mind there are no immunological reagents available and only a handful of DNA probes available to help combat these pathogens. For researchers in this hemisphere to work on these agents, one needs to establish international cooperative research programs to gain access to research material in order to develop the necessary technologies.

Finally, the area of shrimp culture has a bright future in terms of supplying a continual stream of needed diagnostics. As we put more pressure on the animals and the environment in which they live we will see a continued evolution of new pathogens that seem to come out of nowhere that will require research and the development of new technologies to combat them.

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Interactions between feed and feeding behavior in larval fish

"Any feed is useless unless ingested"

Samuel Appelbaum

Providing a nutritionally adequate feed does not necessarily lead to success in larval culture. Fish larvae react instinctively to moving live food items but often ignore non-living, non-motile particles. Although the use of live plankton as initial feed promotes good growth and high survival, supplies of live food are subject to inconsistent availability and erratic quality. In addition, live zooplankton can introduce disease and the production of live feeds is costly. In order to develop manufactured feeds that fish larvae will accept, we need to understand the behavior and feeding patterns of the various larvae being cultured.

Introduction

Poor success in the mass production of seedlings of many fish species is a limiting factor in modern aquaculture. Although techniques for the induced reproduction of a number of fish including cyprinids, clarids, coregonids, sea breams, mahi mahi, red drum, and sea bass are in use, the feeding of early stages with artificial diets is far from satisfactory for most species. Nutritionally adequate feeds do not necessarily lead to success in larval culture.

On the other hand the use of live, planktonic organisms as an initial feed for larval fish, generally promotes good growth and high survival rates. Live feeds are palatable, acceptable to all species and when derived from a nutritious source they satisfy larval requirements. Furthermore, live feeds even when overdosed do not pollute the rearing water. This is a crucial factor for success.

Then why are manufactured dry feeds required? The reason is that natural, live feeds have several significant disadvantages:

1. Their availability may depend on uncontrollable weather conditions;
2. Their quality is not consistent and varies with environmental conditions:
3. They have to be sieved and harvested and selected at the proper size;
4. Various species of zooplankton found in cultures are predators or parasites, attacking larvae or causing mortality;
5. Live organisms may introduce diseases and parasites into the rearing system.
6. Live feeds have to be kept alive, requiring labor, facilities and expense.

The indoor production of nutritionally adequate, live organisms to replace natural plankton is laborious and costly. Therefore, fish culturists have a strong incentive to seek manufactured dry feeds to replace live feeds for larval fish. Manufactured dry feeds have several advantages:

1. Assuming raw materials can be obtained, they are always available and have consistent quality and size;
2. They are easy to store and dispense;

3. They are more cost-effective than most live foods;
4. Farmers appreciate new technologies that provide convenience and cost-savings.

Nevertheless, early stage manufactured dry feeds that are nutritionally adequate and palatable are not yet in use for most species. However, there has been much investment in research and development activities in this field which has resulted in the availability of a few commercial feeds in the market place.

What are the reasons for the slow progress?

Although there are many interesting challenges in the science of fish nutrition, we often do not know or understand enough of the specific behavior patterns of larval fish — particularly the feeding behavior — to determine the successful use of dry feeds. While the feeding of live organisms to larval fish is relatively simple, the use of dry feeds needs experience and a fish-rearing "green thumb" in order to guarantee success.

Larvae of most, if not all, fish species share the characteristics of predators. From the earliest feeding they instinctively respond to moving live items, but rarely respond to non-living, non-motile particles and usually ignore them. However, species not only differ in their rate of development, but also in their pattern of feeding behavior.

Larval fish possess inherited instincts to hunt for food, but at first are inexperienced and less successful at capturing food items. As the larvae develop, interact with, and confront their environment they become better able to face environmental challenges, including the acquisition of food in the form of non-living particles. In cultivation one of the critical roles of the culturist is to encourage the fish larvae to gain the experience to accept non-living food, shorten the larval stage and hasten the completion of metamorphosis.

As larvae react to external stimuli at a very early life stage, appropriate manipulation of the abiotic and biotic conditions by the fish culturist can enhance their learning and willingness to accept inert food particles, and accelerate their adaptation to dry food. For example:

Abiotic conditions

Temperature. Raising the water temperature accelerates the rate of larval development. Imitating the ambient temperature in the hatchery has not proved to be advantageous. Carp, for example used to be reared indoors in water temperatures of between 20-24°C. New investigations have revealed that the optimal temperature for both growth and survival of carp larvae indoors is higher than 30°C, irrespective of the feed used.[11]

Salinity. Salinities are known to be critical. However, the optimal salinities for larval culture are poorly understood. For example, investigations on lingcod *(Ophiodon elongatus)*, indicate that a better survival rate can be achieved when larvae are reared at salinities below ambient.[3]

Light. Conclusions in the literature such as: "The ability of fish to feed falls progressively during dusk or as illumination is artificially reduced"...or... "Feeding efficiency is dependent on light intensity which influences the performance of the eye" should not be interpreted to mean that full light for feeding is advantageous for all species. While in some species light enhances larval feeding, in others it has a negative effect on feeding and survival.

The eyes of the larval sharptooth catfish, *Clarias gariepinus*, have little apparent role in food location.[4,8] Eels are nocturnal feeders and avoid light. Glass eels in culture are less disturbed and feed very efficiently in the dark. In lingcod larvae, a reduction of light intensity and even darkness does not hinder larval feeding. Moreover, because of the lower energy expenditure in darkness, the survival rate of the larvae is improved.[3]

Water motion and depth. Larvae react in a rheotactic positive manner, thus water motion stimulates swimming against the current. Though the feeding instinct is strong, larvae will be hindered in capturing food when the water is too turbulent and their energy exhausted. Larvae generally feed pelagically and particles resting on the bottom are usually ignored. It is therefore essential to ensure proper water depth to guarantee sufficient opportunity for the larvae to capture floating food particles before they sink to the bottom.

Feeding regime. First feeding has to be carried out while endogenous feeding is still taking place, i.e., the yolk-sack has not been fully exhausted. Raising the water temperature increases the rate of metabolism so that larval fish can feed almost continuously around-the-clock, and food must be available accordingly. Though food has to be available constantly, providing it in pulses enhances feeding by the larvae.

However, intensive feeding with dry feed can easily cause pollution of the water. One way to minimize the risk of accumulating uneaten food is by increasing the number of larvae. A higher density of evenly distributed larvae will ingest many more of the food particles present in the water than will a lower density. A higher density of fish also stimulates feeding. For carp, densities as high as 200-250 per liter are acceptable.

Biotic conditions

Cannibalism. Cannibalism among sibling larvae can cause mortalities of over 50%. In some species, reducing light intensity can suppress cannibalism. The provision of sufficient, palatable and nutritious food also reduces cannibalism and there is evidence that feeding larvae on non-live food can reduce cannibalism.[9] Investigations on catfish, a voracious cannibal, have shown that providing feed on the right regime reduces cannibalism significantly.

Chemical stimulation. Regardless of the pattern of intake of a potential food item, the final decision on whether to accept or reject a particle is based on sensory information from the buccal cavity, which is equipped with taste and very likely tactile receptors.

Investigations have shown that larvae react to odor stimulation and recognize different types of taste.[2,5] Although in a number of species a tactile stimulus is required for the acceptance of food, some species will also accept appropriate non-motile particles. For example, copepod eggs are common in the stomachs of larvae and mackerel larvae accept and grow well on anchovy eggs. *Artemia* cysts from the culture medium have been found in larvae of several species.

Why do larvae accept non-motile and indigestible *Artemia* cysts? It is possible that in addition to their round shape, correct size and

shiny appearance, which attract the larvae, the larvae may also be stimulated through the leaching of aromatic constituents from the cyst. Inert particles may also mechanically stimulate the development and function of the gastrointestinal tract.

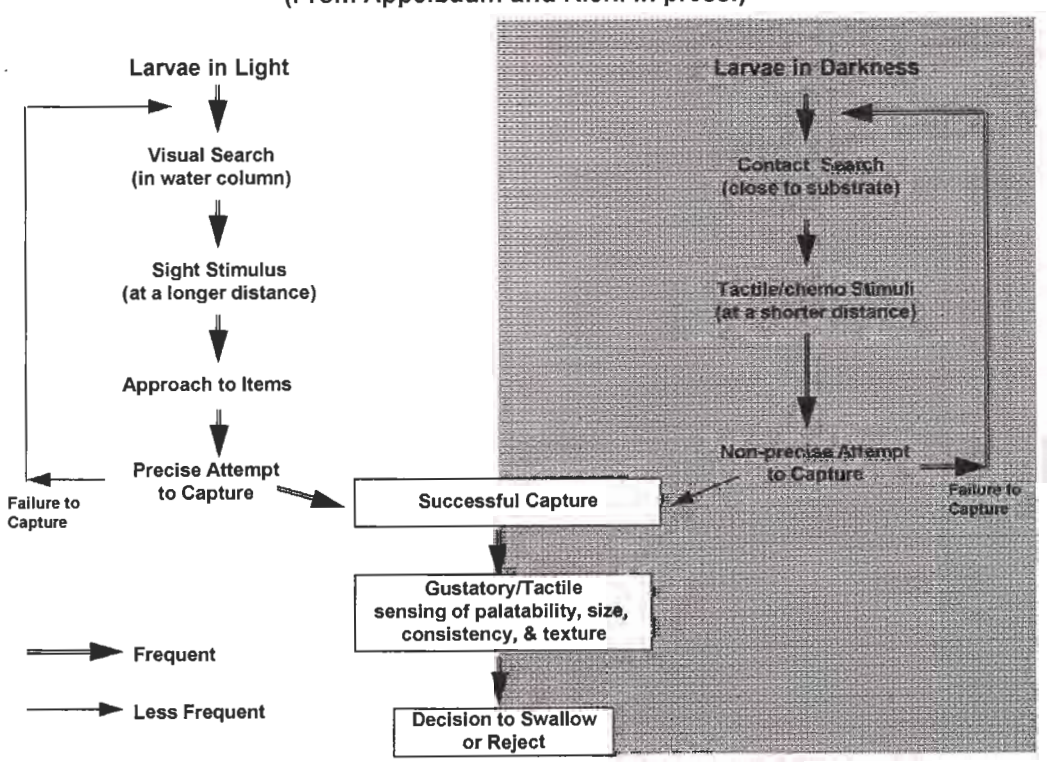
In spite of the use of dry feeds for some freshwater species, adapting most larval fish to artificial diets at the start of feeding remains a problem.

The fact that particles other than live organisms are eaten by some species during larval development, indicates the possible role of chemical stimuli in the decision to accept food. Certain aromatic substances improve palatability in fish. A mixture of amino acids stimulates feeding responses in fish, but not as strongly as the original tissue extract. Fish also respond to particular selections of amino acids, and not all amino acids play a role in the stimulatory activity. A mixture of betaine plus aspartic acid, glycine, glutamine, isoleucine and phenylalanine is virtually as effective as mixtures containing betaine plus all 21 amino acids present in the extract.[6,7] Early in their development, larval fish have a limited capability to produce digestive enzymes. Enzymes from prey organisms stimulate and assist proteolytic digestion in the larvae.

The introduction of processed products from planktonic organisms, in which the peptides, amino-acids and enzymatic activity are preserved will improve the adequacy of larval diets.

Carp, the most cultured fish in the world, demonstrates the history of the development of seedling production. Induced spawning of the common carp was successfully undertaken by the German scientist Probst more than a hundred years ago. Further advances in the incubation of carp eggs were achieved by the Hungarian scientist Woynarovich in the 1950s. Since then, carp aquaculture has developed into an industry, particularly in Eastern Europe and Asia. In spite of this, the production of carp fry has continued in the traditional way with larvae being reared in outdoor ponds on only natural food. Under these conditions, larval survival is dependent on external conditions and may re-

Figure 1: The Pattern of Feeding Behavior in Carp Larvae (Cyprinus carpio L.) in light and darkness
(From Appelbaum and Riehl in press.)



sult in almost total mortality through predation. Only in the late 1970s, did intensive research in several European countries lead to the development of commercial diets to fully replace live organisms in carp larval feeding. Rearing carp larvae exclusively on manufactured dry feeds, which is nowadays routine, had been thought impossible. The development of larval feeds, and success in the feeding of newly hatched carp larvae on artificial diets was derived from fundamental studies of the feeding behavior of the larvae, the key elements of which are:

1. Young carp larvae, still carrying a yolk-sac, start to actively search for food, mainly in the water column and to a lesser extent at the water surface and on the bottom;
2. Larvae are attracted by appropriately sized moving food particles, both living and non-living;
3. With an initial mouth opening of ca 500 µm, carp larvae can ingest relatively large items;
4. High light intensity improves feeding efficiency;
5. In the absence of light, carp larvae are attracted by moving particles and will feed. However non-motile particles will rarely be ingested in darkness (see Figure 1);
6. Particles resting on the bottom are usually ignored;
7. Larvae swim slowly but have a strong positive rheotaxis;
8. In the presence of sufficient feed, larvae require about 15 min to fill their gastrointestinal tract with live food, and about 20 hr to evacuate it;
9. Larvae attempt to ingest the largest particles they can cope with, usually neglecting smaller ones;
10. Elevation of the water temperature enhances their readiness to ingest non-motile particles;
11. Optimal densities of larvae enhance their feeding.

The development of adequate artificial feeds for carp larvae has been based on these findings. The following physical properties of these fed particles are relevant:

1. Particle stability;
2. Appropriate buoyancy to remain in the water column;
3. A compact, spherical shape;
4. Limited leaching of constituents;
5. A series of sizes to accommodate the needs of developing larvae.

These physical properties, crucial at the larval stage, are of secondary importance at later stages of development. When the carp become benthic, omnivorous feeders the sense of vision loses its primary position to the chemical and mechanical senses.

In conclusion, the development of techniques for the early rearing of fish larvae on dry diets are a critical prerequisite for efficient commercial cultivation of many species. The successful development of dry diets must be based on a sound understanding of the early development and feeding behavior of these species.

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Canola protein products in aquaculture

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Declining fish meal supplies and variability in fish meal quality and price have led nutritionists to seek alternative protein sources for finfish and crustacean diets. Primary among potential fish meal alternatives are plant proteins, including soybean and canola protein products, wheat flours and gluten, cottonseed meal, distiller's grains, and corn gluten meal. Canola meal, although relatively low in protein and high in crude fibre and anti-nutritive factors, can be upgraded to a canola protein concentrate that can be included in finfish diets at much higher levels than traditional canola meal. Efforts are underway to develop a commercially viable process for the large scale production of canola protein concentrate for the aquaculture industry and provide Canada with an export opportunity for alternative protein sources in the rapidly expanding Pacific Rim fish and shrimp markets.

The need for alternative protein sources

World aquaculture production has more than doubled in the last decade and continues to grow. Production exceeded 12 million tonnes[10] in 1990 and is expected to exceed 20 million tonnes by 2025.[40] This rate of growth for farmed species is necessary to maintain the world average of 19.1 kg of aquatic products consumed per person each year. Declining wild fish stocks and continued population growth require a concerted effort to insure the continued availability of high quality protein sources, and aquaculture is well-positioned to make a substantial contribution to this end.

Along with increased productivity is an increased need for high quality feed ingredients to insure optimum productivity of commercial aquaculture facilities. Profitability in aquaculture is marginal at best and novel approaches are required to enhance the market value of farmed products and to decrease production costs. Feed accounts for 40-60% of farm operating costs (Fig. 1) and protein sources comprise up to two-thirds of total feed expenses (Fig. 2). The high cost of the protein portion is due to extensive reliance on quality fish meal to meet the high dietary protein needs of farmed species. Fish meals are preferred protein sources because they have high palatability and usually contain high levels of digestible protein and energy.[11] Moreover, they have excellent levels and balances of essential amino acids required by salmonids and other finfish species.[22] Fish meals can, however, vary considerably in quality and price. Quality variation is mainly due to: i) dissimilar composition and freshness of raw material; ii) different proportions of whole fish, offal, and filleting residues leading to variable levels of collagen, ash, and other proximate constituents; iii) different processing conditions during meal manufacture (cooking and drying temperatures); iv) dissimilar ratios of solubles to presscake and differences in fish soluble quality; v) inappropriate antioxidant, moisture and chlorinated hydrocarbon levels, and vi) suboptimal meal storage and transportation conditions. Raw material spoilage coupled with excessive meal drying temperatures (>100°C) generally has the greatest influence on reducing the nutritive value of fish meal for salmonids.[12,35]

Fish meal prices are cyclical and depend primarily upon availability and the world production of soybean meal. For instance, warm El Niño currents off the coast of South America have, in certain years, decreased the catch of

some species used for meal production, while in other years, there have been increases. In 1988, the situation was exacerbated by a concurrent fall in soybean production in the United States.[19,52] Global fish meal production is expected to decline by about 5% between 1990 and 2000,[46] while the demand for fish meal for aquaculture feeds continues to increase. For example, in 1988 10% of the global production of fish meal (7 million tonnes[41]) was used in aquaculture, while by the year 2000, this will likely increase to 20-25%.[8] There will also be increased demands for fish meal in pet foods and speciality livestock feeds and more utilization of fish protein directly in the human diet owing to continuing population growth.

Consequently, fish meal prices and fish farming production costs will undoubtedly increase unless suitable inexpensive alternative protein sources of consistently high quality are identified and/or developed. We direct our attention in this report to the use of rapeseed/canola protein products in this regard.

Alternative protein sources

Assessment of the use of alternative protein sources includes consideration of supply, cost and nutritive value. In 1992-93, 226 million tonnes of oilseeds were produced worldwide[41] — soybeans, cottonseed, rapeseed/canola, sunflower seed and groundnuts accounted for 51.0, 14.4, 11.6, 9.3 and 7.4% respectively. Thus, rapeseed/canola ranks third in the global production of oilseed crops. The quantity of rapeseed/canola protein potentially available surpasses the global amount of fish meal protein produced each year. Canada is a major producer of canola and produced 14% of the 26.4 million tonnes of rapeseed and canola produced in 1992-93. Some of the rapeseed/canola protein is not, however, included in animal diets. For instance, in Asia, rapeseed rather than canola seed is grown and the meal is used as an organic fertilizer as well as a livestock feed supplement. In North America (primarily Canada) and in some European countries, canola is grown, and the meal is employed as a high protein dietary supplement for animal feeds.[15] Acreage dedicated to canola production is located primarily in the parkland and transition zones of Canada's Prairie Provinces, and in the Peace River area of British Columbia (over 5.6 million hectares in 1994).

The cost of canola meal is presently 56.2% of that of British Columbia herring meal (US$0.45 versus $0.80/kg protein). By contrast, the price of soybean meal is slightly higher, at approximately 61% of that of herring meal. Therefore, on the basis of availability and cost alone, rapeseed/canola meals are potential alternatives to fish meal and their use would reduce production costs of salmon farming.

Proximate composition and quality of canola protein

Canola meal contains considerably less protein, lipid and ash, and more fibre and nitrogen-free extract than do fish meals (Table 1). The percentage of crude fibre in canola meal (12%)

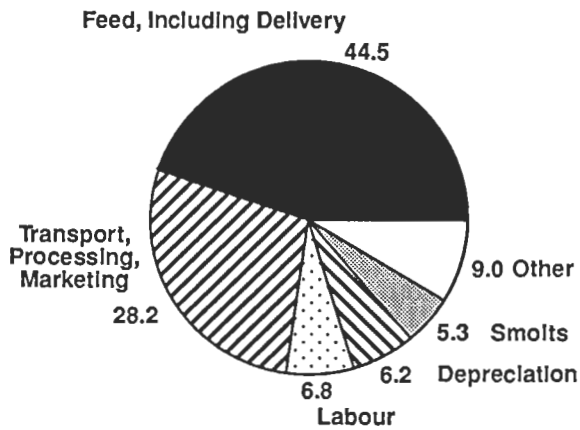

Figure 1. Relative operating expenses (%) of a chinook salmon farm in British Columbia producing 240 tonnes annually.

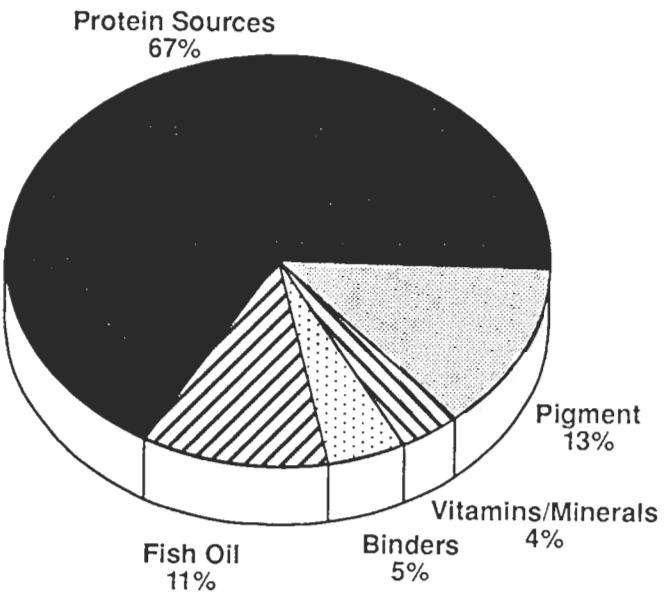

Figure 2. Percentage cost breakdown of a commercial extruded dry grower diet employed for Atlantic salmon in British Columbia.[14]

is not a true reflection of the total level of indigestible carbohydrate since other indigestible carbohydrates (e.g., raffinose and stachyose) are not measured as a part of the crude fibre determination. The high levels of hulls and indigestible carbohydrates in canola meal contribute to the reduced available energy content relative to fish meal in both salmonids and other animal species.[37] Thus, it is impractical to consider canola meal as a total replacement for fish meal, particularly in fish species with high dietary protein needs (e.g., salmon and shrimp).

Special processing of whole canola seed or of canola meal itself can, however, result in products that have protein and energy contents more comparable to those of fish meal.[31,34,38] Preparation involves either dehulling canola seed before further processing, or removal of hulls or fibre (cellulose, hemicellulose, and lignin) by air classification of defatted meal or milling moisture-adjusted meals followed by sieving. Dehulled seeds may be subjected to water and hexane washes to remove or decrease glucosinolates, problem sugars (e.g., raffinose and stachyose), phenolic compounds (e.g. sinapine), and lipids.[31] Alternatively the crushed and fibre-reduced seeds may be exposed to a 2-phase solvent system comprised of methanol, ammonia and water and then hexane to remove glucosinolates and decrease carbohydrate, phenolic compounds, nucleic acids, non-nitrogen compounds and lipids.[34,38,48] The further processing of fibre-reduced canola meal entails washing the product with selected solvents to decrease the levels of antinutritional factors (e.g. glucosinolates, sinapine, phytate, carbohydrates) and concurrently increase the protein content. The levels of protein, lipid and energy in a canola protein concentrate are similar to those found in fish meal (Table 1).

The quality of animal and plant protein sources is determined to a considerable degree by how well the available levels and balance of 10 essential animo acids conform to actual needs, assuming that there is appropriate dietary energy and an optimal balance between essential and nonessential amino acids.

Higgs et al.[22,25] examined the quality of canola protein by expressing each of the essential amino acids as a percent of the total weight of the essential amino acids (g/100g protein). Subsequently, these values were compared with respective percentages derived from the essential amino acid needs of rainbow trout and carp in the manner described by Oser[42] for calculation of an essential amino acid index (EAAI). The rating scale for EAAI ranges from 0 to 100, and this approach has proven to be worthwhile for predicting carp performance.[36] Fish whole body protein and fish muscle protein show the best correspondence to the pattern of amino acid needs of carp and rainbow trout.[22] Protein from canola meal and canola protein concentrate is

equal in quality to herring meal protein and superior to soybean meal protein. Thus, canola protein products have excellent protein quality for finfish provided that the amino acids are available and that there are no factors which restrict protein utilization.

Antinutritional factors and digestibility of canola protein products

The full expression of the high quality of canola protein sources is compromised by the presence of constituents common to all oilseeds (fibre, carbohydrates, phenolic compounds, and phytic acid) as well as by unique antinutritional factors (glucosinolates).[23]

Dietary levels of fibre above 10%, for instance, may depress growth, feed efficiency, transit time of intestinal contents, diet digestibility and mineral bioavailability in trout and tilapia.[6,30]

Tannins compromise protein and dry matter digestibility either by inhibiting proteases and possibly other enzymes, or by forming indigestible complexes with dietary proteins.[32] The bitterness of sinapine may decrease the palatability of canola meal; however, the treatment of canola products with solvents can dramatically reduce the levels of sinapine and other phenolic compounds.[31,34] Consequently, canola protein concentrates and upgraded meals can be anticipated to have improved nutritive value.

Phytic acid and glucosinolates probably have the greatest influence on reducing the nutritive value of canola protein products. The phytic acid content of canola meal is about 3.1-3.7% of dry weight, higher than in soybean meal (0.72-1.8%).[16,34] Phytic acid is strongly negatively charged at pH values normally encountered in food. Consequently, this compound may complex with proteins at acidic pH or polyvalent cations (especially zinc) at intestinal pH and thereby decrease their availability. The consequences include depressed growth, feed and protein utilization, survival and thyroid function.[18,45,47,50]

Phytic acid concentrations can be decreased by limiting the amount of phosphorus available to the oilseed during plant growth, removing the phytic acid from the oilseed during food processing, using the enzyme phytase from microbial sources (e.g. fungi, bacteria and yeasts) to dephosphorylate the phytic acid, and by allowing the seed to germinate.[13,55] The pretreatment of canola protein sources with microbial phytase or the direct addition of phytase to diets containing the foregoing protein source(s) may prove to be the most efficacious and economical means of eliminating the antinutritional effects of phytate. This approach has proven to be worthwhile in poultry, swine and fish fed phytate-rich diets.[39,46,49]

Glucosinolates and/or their hydrolytic products may account for impairment of homeotherm and fish performance by adversely affecting thyroid function.[21,26,33] This is particularly germane when commercially produced canola meals are employed. By contrast, up-

Table 1. Proximate composition of selcted fish meal and canola protein sources.

Proximate composition (% fed)	Herring meal	Anchovy meal	Canola meal	Canola meal fibre-reduced, ethanol and acid washed	Canola protein concentrate
Protein	72.0	65.5	37.0	48.8	63.0
Crude lipd	8.4	4.1	1.7	0.9	8.0
Fibre	0.7	1.0	12.0	11.4	4.7
Nitrogen-free extract	0.4	6.6	33.5	22.1	14.8
Ash	10.5	14.8	6.8	5.6	5.9
Moisture	8.0	8.0	9.0	11.2	3.6

graded canola meals or protein concentrates have significantly reduced glucosinolate levels, and in these products, phytic acid is more of a concern. Three studies on salmonids have provided conclusive evidence that glucosinolates are mainly responsible for the depression of growth and protein utilization with diets contain high levels of canola meal.[17,27,33] In each of these, simultaneous inclusion of T_3 in the diets reinstated normal circulating titres of thyroid hormone and, consequently, growth.

Assessment of the biological availability of nutrients and energy is essential for the screening of feedstuffs for their potential nutritive value in relation to the processing conditions used in their production. This statement is true because fecal energy losses from diets of salmonids represent the main reason for variation in their nutritional value.[11]

The percentage availabilities of protein and energy and the digestible energy values of canola protein products have been determined for trout and salmon, but not for warm water species. In trout, the apparent crude protein and gross energy digestibility coefficients and digestible energy values for rapeseed and canola meals are highly variable; crude protein digestibility coefficients range from 63.8 to 92.9% and gross energy digestibility coefficients extend from 21.4 to 82.7%. Part of the variation likely stems from differences in the methodology used to assess feedstuff digestibility. Other reasons for the poor and variable digestibility coefficients for protein and energy in rapeseed and canola meals are likely related to their levels of glucosinolates, phenolic compounds, fibre, and other indigestible carbohydrates.

The high availability of protein in canola protein concentrates for trout is probably a consequence of the diminished levels of the aforementioned anti-nutritive constituents in the concentrate versus the meals. Although the level of phytic acid is high in protein concentrates, and this constituent can depress protein availability, this dietary factor in native form probably exerts minimal influence on protein availability. Indeed, the protein digestibility coefficient for canola protein concentrate in trout was found to be almost identical to the average value determined for fish meal in *Oncorhynchus* species, namely 88.1%.[23] Further, Hajen et al.[20] observed high availability of protein (95.6%) and energy (80.5%) in canola protein concentrates for post-juvenile chinook salmon in seawater. Thus, canola protein concentrate appears to be promising as a complete replacement for fish meal in salmonid diets. Other canola products generally have not been found to have comparable availability of protein and energy for salmonid fish.

The apparent amino acid availabilities (%) in canola meal for rainbow trout in fresh water and Atlantic salmon in seawater appear to be similar in most cases.[7,29] Moreover, the values for trout generally parallel the range found for the availability of protein in canola meal. Unfortunately, there is no information regarding amino acid availabilities in upgraded canola meals and concentrates for finfish. Presumably, such values would be close to or higher than 90% for canola protein concentrate in trout and salmon based upon the high level of available protein that has been observed for this product.

Acceptable dietary levels of canola protein products for finfish

The criteria used to establish the acceptable levels of canola protein products in finfish diets were equivalent growth and feed utilization between test and control fish. On this basis, canola meal has a higher acceptance level in the diets of warm water species than in those of salmonids. Indeed, with the exception of one study, it would appear that rapeseed meal (regardless of glucosinolate content) and canola meal can comprise at least 28% of dietary dry matter or protein for common carp and tilapia.

In general, juvenile coho and chinook salmon tolerate more canola meal in their diet (16-23% of dry matter or 13-22% of dietary protein) than do rainbow trout (<13.5-20% of dry matter or <13.3-18% of dietary protein). Small rainbow trout (approximately 2 g) appear to be especially sensitive to the residual levels of glucosinolate compounds in canola meal, and total dietary glucosinolate levels of 172 µg of 3-butenyl isothiocyanate/g (158 µg/g air-dry basis) are deleterious to these fish.[29] By contrast, Higgs et al.[27] found that canola meal may comprise about 23% of the dietary protein for juvenile chinook salmon, provided that the dietary glucosinolate content was less than 300 µg/g as 3-butenyl isothiocyanate (<2.65 µmoles/g dry diet).

Supplementation of trout diets with arginine, lysine, zinc or energy (lipid) was not found to be efficacious in increasing the acceptable die-

tary level of canola meal.[29] Leatherland et al.,[33] working with trout, and Higgs et al.[27] and Fagerlund et al.,[17] with salmon, discovered that the acceptable dietary level of canola meal could be raised to 24-27% of dietary protein if triiodothyronine (T_3) was simultaneously provided in the diets to counteract impairment of thyroid function due to the presence of glucosinolates in the canola meal. Thus, glucosinolates appear to be the main reason for the poor acceptability of canola meal in trout and salmon diets. Trout are also far more sensitive than salmon to constituents within canola meal (e.g. glucosinolates and phenolic compounds) that adversely affect the palatability of canola meal and, for this reason, they often exhibit, unlike salmon, poor feed intake when the dietary canola meal level is high.[27,29] Upgraded canola meals appear to have high acceptability in trout (40% of dietary protein) and salmon (25% of dietary protein) diets (Fig. 3). McCurdy and March[34] attributed the improved performance of trout and salmon ingesting diets containing washed, as opposed to unprocessed, canola meal to the reduced levels of glucosinolates and sinapine in the former products.

Several years ago, Yurkowski et al.[54] and Higgs et al.[24] demonstrated that rapeseed protein concentrate (RPC) may comprise more than 24% of dietary protein in the diets of trout and juvenile chinook salmon by replacement of fish meal. The upper limit of fish meal replacement by RPC was not established in either study. Further, no attempt was made in these studies to assess whether the nutritive value of RPC could be improved by dietary zinc supplementation, or by removal of its phytic acid content. Also, the possible benefits of concurrently adding an appetite stimulant such as Finnstim™[53] to diets high in RPC but low in fish meal content to overcome appetite suppression had not been assessed. Consequently, three major studies on rainbow trout were conducted to address these issues. The first and second of these[28,51] found that: i) dietary zinc supplementation did not significantly influence trout performance regardless of the RPC level, ii) trout performance was not compromised when either undephytinized or dephytinized RPC comprised 39% of

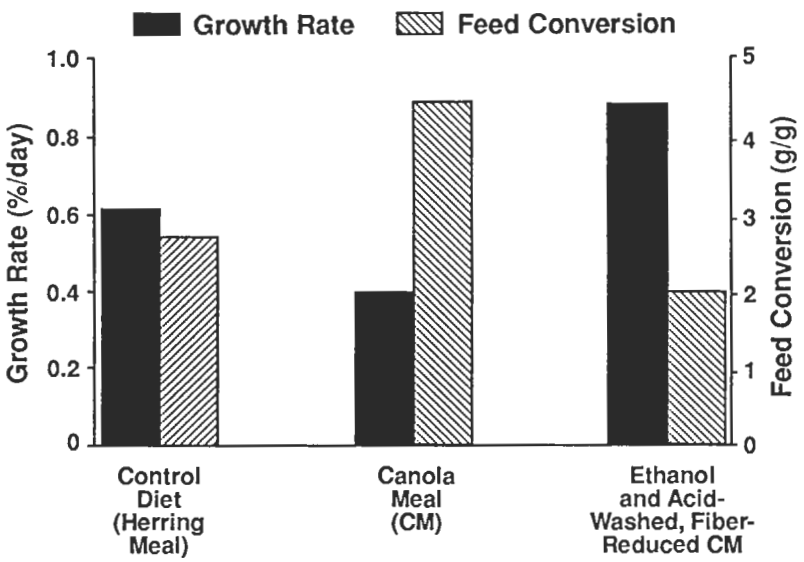

Figure 3 Specific growth rates (% gain in weight/day) and feed conversions (food intake on an air-dry basis/wet body weight gain) of post-juvenile chinook salmon over a 77-day period in relation to diet treatment. Fish (mean initial weight 23 g) held in 10-12.5°C seawater were fed to satiation once daily (6 days/week) with diets in which 25% of the protein (46%) originated from canola meal or ethanol and acid-washed, fibre-reduced canola meal by replacement of herring meal protein in the control diet.[34]

Table 2. Comparative costs of fish meals and canola protein products.[34,34a]

Protein Source	US$/kg protein	Relative %
British Columbia herring meal	$0.88	100
Menhaden fish meal	$0.72	81.8
Upgraded canola meal (concentrate)	$0.58[a]	65.9
Canola meal	$0.42	47.7

[a] FOB factory

the dietary protein by replacement of 66% of the herring meal protein in the basal diet, iii) the dephytinization procedure for RPC needed to be improved, iv) dietary Finnstim™ supplementation (1.5%) maintained normal feed intake in trout even when RPC totally replaced fish meal, and v) the mineral metabolism of trout ingesting diets with dephytinized RPC was normal. The third study, recently conducted[43,44] using an improved dephytinization procedure for RPC, showed that dephytinized RPC can entirely replace high quality fish meal without compromising trout performance provided that the diet is simultaneously supplemented with Finnstim™. Indeed, in this study, dephytinized RPC successfully comprised 59% of the dietary protein. Additional treatments involving RPC, such as adjustment of the dietary levels of essential amino acids or levels of cations (sodium and potassium) and anions (chloride and sulfate) to mimic the respective levels in the herring meal-based control diet did not further improve trout performance. Thus, dephytinized RPC appears to be comparable in nutritive value to fish meal for trout and possibly other salmonids and finfish species.

Future directions

The eventual widespread application of canola protein concentrate (CPC) in aquaculture diets will require the development of an economical procedure for its production on a large scale, identification of an economical and practical approach to dephytinize CPC, and assessment of the nutritive value of CPC in the diets of several species of commercial value.

In relation to the production cost of CPC (upgraded canola meal), Dr. Sandra McCurdy of the POS Corporation, Saskatoon, has estimated that the cost would be below that of fish meal on a per kilogram protein basis (Table 2). This statement is valid even if one adds shipping costs and profit, and the costs of supplemental Finnstim™, particularly if comparisons are made against the costs of fish meals from Scandinavian and Japanese/Chilean sources (prices vary between US$0.90 and 1.25/kg protein). Canola acreage in North America can be expanded whereas global fish meal supply is declining. Further, practical approaches to economically dephytinize CPC on a commercial scale are under development. Hence, the future for widespread use of dephytinized CPC in finfish diets appears to be highly promising, and this approach should significantly reduce the present production costs of aquaculture operations.

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Assessing the exposure of fish to pollutants: a sensitive molecular technique to quantitate CYP1A1 mRNA levels

P. M. Campbell and R. H. Devlin[1]

Although large amounts of pollutants are released into the aquatic environment, there is little understanding of the mode of action of these compounds in biological systems making it difficult to evaluate and predict the ecological effects. We have developed a technique for measurement of CYP1A1 mRNA levels in fish that should improve our ability to assess the degree of exposure to xenobiotics and determine the effects of exposure on their physiology.This technique has the potential to elucidate some of the mechanisms by which reproduction is disrupted in fish living in polluted environments.

Introduction

Halogenated aromatic compounds (e.g. polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls (PCBs)) and polynuclear aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants. Sources include herbicides, pesticides, industrial effluents and by-products of petroleum exploitation. The ultimate sink for many of these organic contaminants is the aquatic environment. Thus fish, including species of economic importance, are inescapably exposed. Exposure can result in acute lethality, but more commonly fish are exposed to sublethal levels resulting in subtle, often debilitating, physiological changes which can be used as early warning indicators of exposure. The bioaccummulation of these lipophilic organic compounds within edible fish species can pose a hazard to consumers. Hence, the ability to monitor exposure, as well as the biological responsiveness of populations to specific halogenated aromatic compounds and polynuclear aromatic hydrocarbons is critical.

Although these pollutants differ by orders of magnitude in potency, they evoke many similar physiological responses and are believed to share common mechanisms of action. The more potent compounds elicit a broad spectrum of effects, including changes in enzyme activity, endocrine and reproductive dysfunction, and the production of birth defects and cancer. Although our understanding of the mechanisms of toxicity is limited, one response that occurs across species, including humans, rodents, birds and fish,[28] and in many tissues, is the induction of CYP1A1 (P4501A1), a xenobiotic-metabolizing enzyme. The increased synthesis of CYP1A1 is mediated through the binding of organic compounds to a cognate receptor, the Ah receptor (AhR), which then directly upregulates transcription of the *CYP1A1* gene resulting in elevated levels of CYP1A1 specific mRNA and the substrate-binding portion of the catalytically active enzyme (P4501A1) (Fig. 1). Although it is not clear whether the induction of this gene, and the production of the enzyme has a causal relationship with the toxic effects of inducing compounds, the induction of CYP1A1 is a sensitive marker for the exposure and responsiveness of an animal to these contaminants.

Previous studies have shown that fish exposed to dioxins, PCBs, PAHs, and complex industrial effluents like bleached kraft mill effluent (BKME)[2,16,27,37] have elevated hepatic CYP1A1 levels correlated with sublethal effects, including the disruption of physiological processes. Deleterious effects on reproduction extend from reduced plasma sex steroid levels and depressed

gonadal growth to reduced reproductive success and even sterility.[17,18,27,31]

Over the past decade, concern has focused on the depletion of Pacific salmon stocks due to factors such as climatic change, destruction of juvenile habitat and exposure to pollution.[5,21-23,42] The prized chinook salmon (*Oncorhynchus tshawytscha*) has become one of the most threatened species of salmon in Western Canada. Stream-type chinook, the life-history type predominant in the Fraser River, are at risk of exposure to pollutants such as BKME throughout their first year of life and when they return to freshwater to spawn. The induction of CYP1A1 observed in several studies on marine fish species indicates that much of the marine environment is also contaminated,[39] possibly representing a continued threat during their years at sea. Sublethal effects of pollutants, particularly on early sexual development and final maturation and spawning, could play a pivotal role in the survival of some salmon stocks. Such insidious physiological changes could go unnoticed unless techniques are available to detect them. Previous studies[22,33] which examined the effects of exposure to BKME on early life history stages of salmon in the Fraser River system, were limited by the lack of sensitivity of techniques to measure CYP1A1 induction.[35] In order to measure CYP1A1 induction in single embryos, alevins and specific tissues of juvenile chinook, a more sensitive assay is required.

In this paper, we report the development of a reliable, sensitive, and quantitative RT-cPCR (reverse transcriptase-competitive polymerase chain reaction) assay for CYP1A1 mRNA levels.

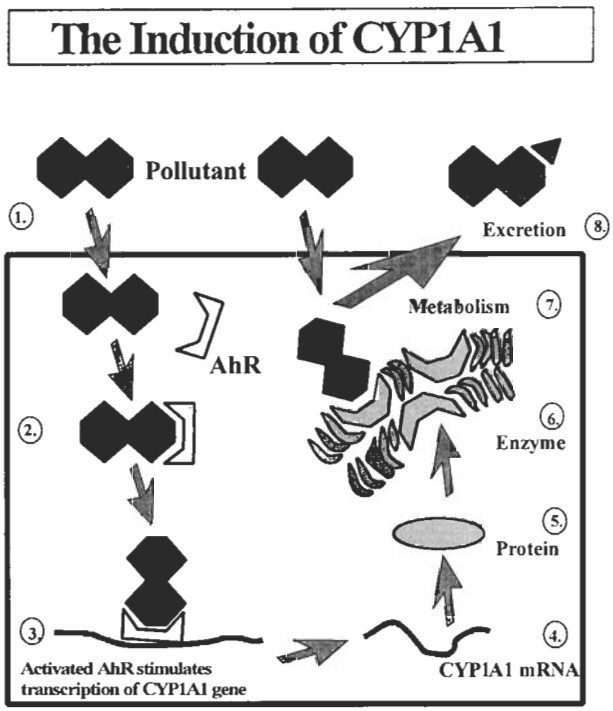

Figure 1. Simplified schematic diagram illustrating the induction of CYP1A1. The box respresents a cell (e.g. a liver cell of a pollutant-exposed fish. 1) The organic pollutant, e.g., dioxin, diffuses into the cell. 2) There it binds to the Ah receptor (AhR), which is consequently converted into a DNA–binding protein. 3) The activated AhR binds to a region of the genome which regulates transcription of the CYP1A1 gene, resulting in increased gene transcription (induction). 4) CYP1A1 mRNA is produced and subsequently translated into protein. 5) The protein has haem inserted into it, becomes associated with various cofactors and embedded in the endoplasmic reticulum membrane. 6) Here it becomes an active enzyme and can then metabolize the pollutant that induced its synthesis.

Materials and methods

Treatment and sampling method

An all female stock of juvenile chinook (n=30; mean weight 21.3 ± 0.5 g) were maintained outdoors in fibreglass tanks supplied with well water (10.0 ± 0.5°C). Fish were anaesthetized with MS-222 (100 mg/L, buffered with 100 mg/L sodium bicarbonate) and injected intraperitoneally with 50 mg/kg body weight ß-naphthoflavone (BNF: Sigma Chemicals) dissolved by sonication in canola oil (5 mg/mL). Control fish were injected with canola oil only. Fish

were sampled at time zero and 48 hr after injection — at each sample, 3 fish were taken from both treated and control groups and weights and lengths were recorded. Fish were killed with a sharp blow to the head, livers and gonads were removed and weighed and a few gill filaments were removed. Tissues were placed in ice cold RNA isolation buffer (typically 10-25 mg tissue in 500 µL buffer) and homogenized using two 5 sec bursts of a motor-driven homogenizer (Brinkman). Total RNA was isolated from these homogenates (details in RNA isolation section).

Design of oligonucleotides

Oligonucleotides (primers) were prepared on an Applied Biosystems PCR Mate DNA Synthesizer at the Oligonucleotide Synthesis Laboratory at UBC. Oligonucleotides were designed to anneal to highly conserved regions of published CYP1A1 sequences. The CYP1A1 sequences were obtained from Genbank for trout (Accession #M21310), mouse (#K02588), rat (#K02246), rabbit (#X05685), guinea-pig (#D11043) and human (#X04300). They were aligned and highly conserved regions identified using the CLUSTAL alignment program within the "PC/GENE" software package (Intelligenetics Inc., Mtn View, CA). Two 3′ primers, 1A1-3 and 1A1-4, and one 5′ primer, 1A1-1, were used in this study. Primer sequences are published in detail elsewhere.[8]

RNA isolation and RT-PCR

Total RNA was isolated using the method of Chomczynski and Sacchi.[10] Pieces of liver and gonads were homogenized in the presence of guanidine isothiocyanate buffer on ice and the A_{260}/A_{280} ratios were between 1.8 and 2.0. Total RNA was diluted to 0.5 µg/µL or 1.0 µg/µL in RNase-free water, mixed with 1 µL primer 1A1-3 (10 pmol) and 8 µL RNase-free water, incubated at 70°C for 10 min and then quick-chilled on ice. The CYP1A1 mRNA in these samples was converted to cDNA in a final reaction volume of 20 µL containing first strand buffer (50 mM Tris (pH 8.3), 40 mM KCl, 1 mM DTT, 6 mM $MgCl_2$), "Superscript" reverse-transcriptase enzyme (200µ; Gibco BRL). These reactions were incubated at 37°C for 1 hr. The cDNA produced was then amplified by polymerase chain reaction (PCR) after the addition of 70 µL PCR premix, primer 1A1-1 (10 pmol) and Taq DNA polymerase (0.05 u/µL). Reactions were run for 30 cycles with a 65°C annealing cycle (1.0 min), 72°C extension cycle (2.0 min), and a 95°C denaturing cycle (1.0 min) in a Perkin Elmer Cetus DNA thermal cycler. A final incubation was done at 72°C for 10 min. Amplification from CYP1A1 cDNA using primers 1A1-1 and 1A1-3 produces a fragment 270-bp in length.

Adaptation to competitive PCR: RT-cPCR

The competitive internal standard, a DNA fragment 149-bp in length with the same primer recognition sites as the 270 bp fragment amplified from chinook CYP1A1 mRNA, was designed using the method of Celi et al.,[9] and produced using primer 1A1-4. Amplification from CYP1A1 cDNA using the same reaction conditions above, apart from the replacement of 1A1-3 with 1A1-4, yields a fragment 149-bp in length. This fragment was run on a 1% low melting point agarose gel (Ultrapure, Gibco BRL), the 149-bp band excised and purified using the freeze/thaw method of Qian and Wilkinson.[32] After quantitation using a spectrophotometer, serial dilutions of this 149-bp competitor were prepared ranging from 20 pg/µL to 0.016 pg/µL.

For each sample of RNA to be quantitated, a series of assay tubes received 0.5 or 1.0 µg of total RNA. The CYP1A1 mRNA in these samples was converted to cDNA using the conditions described above. These tubes, containing CYP1A1 cDNA, then received a further 80 µL containing reagents required for the subsequent PCR. The PCR conditions were similar to those described above, but with the addition of 10 µL of a known, variable amount of the 149-bp competitor. Following PCR amplification the reaction products were run on a 1% agarose gel with 0.5 µg/mL ethidium bromide staining at 80V for 5-6 hr. Both positive and negative photographic images were obtained of the gel under UV light using Polaroid 665 film.Negatives were scanned using an LKB Image Master DTS densitometer desk top scanner 100 and analyzed using Image Master software (Pharmacia). Total band volume (OD x mm) of both amplified products were calculated by the software (1D analysis program, Image Master). The 270/149 ratio was calculated for each sample and plotted against the amount of competitor added per tube and linear regression analysis was used to define

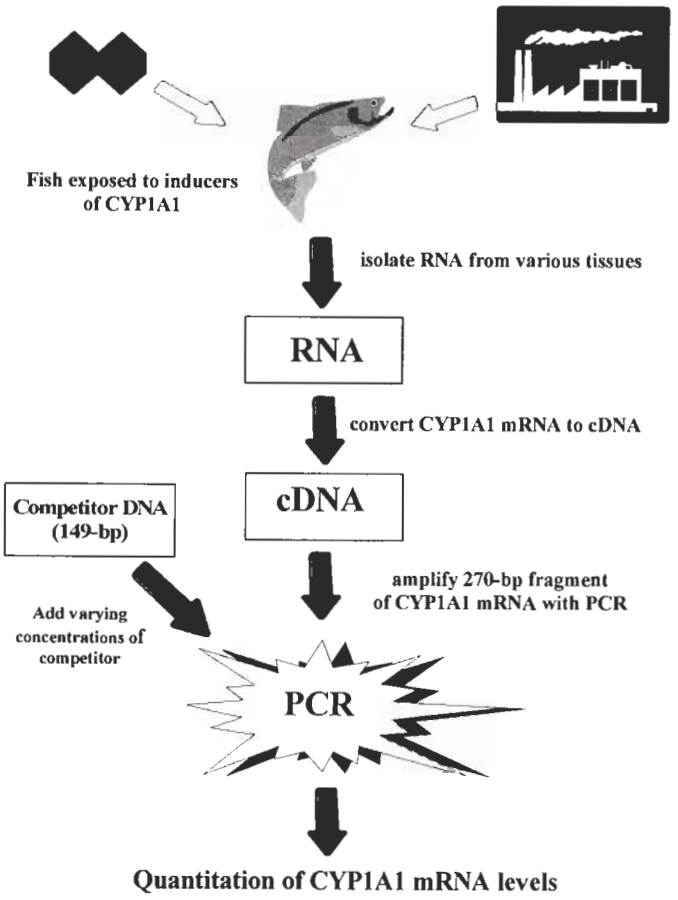

Figure 2. Summary of the protocol of the RT-cPCR assay for CYP1A1 induction in fish.

the equation for the line through the data points (Sigmaplot). The fmols of CYP1A1 cDNA (270-bp fragment), and hence the amount of CYP1A1 mRNA present in the original sample, could then be calculated from the expression 270/149 =1. A summary of the protocol of the RT-cPCR assay is shown schematically in Figure 2.

Results

Detection of CYP1A1 expression: RT-PCR

CYP1A1 mRNA levels were detected using reverse-transcriptase-polymerase-chain-reaction (RT-PCR). An example of RT-PCR analysis using RNA isolated from chinook salmon, injected 48 hr previously with ß-naphthoflavone or the canola oil carrier, and primers 1A1-1 and 1A1-3 is shown in Figure 3. When CYP1A1 mRNA is present in the samples, RT-PCR amplifies a 270-bp fragment of CYP1A1 cDNA. The outside lanes of the gel contain molecular size markers. The band at 270-bp shows the induction of CYP1A1 mRNA in the gill of the treated fish (see lane 3), whereas no detectable CYP1A1 mRNA were observed in the gill of control fish (lane 2). Lane 4 shows the results from the liver of a control fish, and lane 5 the results from the liver of a treated fish 48 hr post-injection. The band at 270-bp indicates increased expression of CYP1A1 in the liver of fish treated with BNF compared to controls. Lane 6 of the gel shows the results from a control fish ovary, where no detectable levels of CYP1A1 mRNA are present, and lane 7 the results from a treated fish showing CYP1A1 induction in the ovary.

Quantitation of CYP1A1 expression RT-cPCR

The qualitative results displayed in Figure 3 were refined to allow accurate quantitation of the observed induction of CYP1A1 mRNA in various tissues of chinook salmon. Figure 4a shows the results of a typical RT-cPCR. In this case, 4 equal aliquots of RNA were prepared from the gill, liver and ovary of a juvenile chinook which had been injected 48 hr pre-

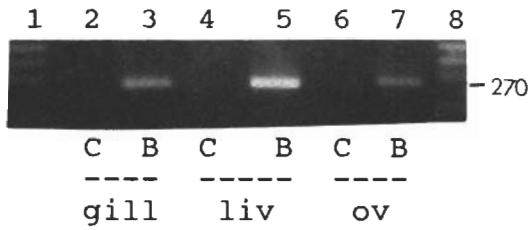

Figure 3. An agarose gel showing an example of the results obtained using the RT-PCR assay for CYP1A1 induction. When CYP1A1 mRNA is present in the sample a band at 270-bp is evident on the gel. In this case, CYP1A1 mRNA was detectable in the gill, liver and ovary of a BNF-treated fish (present in the sample "B" lanes, 3, 5, and 7 respectively), but not in the same tissues taken from a control fish ("C" lanes 2, 4, and 6). Lanes 1 and 8 show molecular size markers.

viously with BNF. The CYP1A1 mRNA within these samples was converted to cDNA, and 2-fold dilutions of the competitor DNA ranging from 10 pg to 0.16 pg were added to each tube. The exact range of competitor used depended on the intensity of the 270-bp band following initial qualitative RT-PCR. In this case the range of competitor used was 2.5-0.31pg for the gill sample, 5.0-0.63 pg for the liver sample and 1.25-0.16 pg for the ovary. The 2 fragments, the 270-bp fragment from the CYP1A1 mRNA and the competitive 149-bp fragment, then competed for amplification within the PCR. As the amount of competitor added decreases, the amount of CYP1A1 cDNA being amplified increases (Fig. 4); hence the ratio of the two bands changes across the gel for each sample. A negative of the gel was prepared and scanned and the ratio of the volume of the competitive-DNA/CYP1A1-cDNA PCR products was plotted against the amount of competitor added. Linear regression analyses of the data from the liver and ovary are presented in Figure 5. The results of an RT-cPCR assay using RNA isolated from the liver and ovary of a control fish at the same time point indicated no detectable levels of CYP1A1 mRNA. In these examples, the amount of CYP1A1 cDNA in the sample from a treated fish liver was equivalent to 2.06 pg of competitor compared to <0.16 pg in the control fish liver; an induction of over 13-fold. The amount of CYP1A1 mRNA in the sample from a treated fish ovary was equivalent to 0.619 pg of competitor, whereas undetectable levels were observed in the ovary of control fish, an induction of over 4-fold 48 hr post-injection.

CYP1A1 expression in juvenile chinook livers, gills and gonads

The RT-cPCR assay was used to quantify CYP1A1 induction in gill, liver and ovary (Fig. 6) of fish 48 hr after injection with BNF. All fish sampled on the day of injection had undetectable levels of CYP1A1 mRNA in their livers,

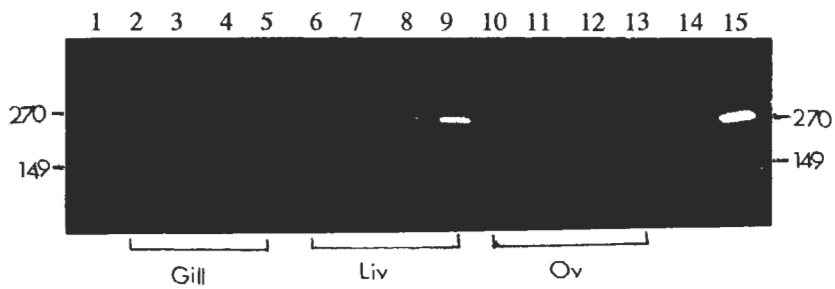

Figure 4. An example of quantitation of CYP1A1 expression using RT–cPCR. The gel (a) shows RT–cPCR results from the gill (lanes 2-5), liver (lanes 6-9), and ovary (lanes 10-13) of a fish 48 hours post–injection with BNF. Lane 1 shows molecular size markers, lanes 14 and 15 assay control tubes.

gills and ovaries. There was a 7.4-fold mean increase in CYP1A1 expression in the gills of treated fish 48 h following injection (Fig. 6), whereas no detectable levels of CYP1A1 mRNA were found in gill filaments from controls. Similarly, there was a mean 8-fold increase in hepatic CYP1A1 expression in the fish injected with BNF after 48 hr, whereas no CYP1A1 mRNA was detected in the livers of control fish. A mean 5-fold increase in CYP1A1 expression was noted in the ovaries of fish injected with BNF, whereas no detectable levels of CYP1A1 mRNA were observed in the ovaries of control fish.

Discussion

Results indicate that the RT-cPCR assay can be used to detect and quantify CYP1A1 induction in fish tissues as a measure of exposure to xenobiotics. In previous studies, catalytic assays for CYP1A1 induction have also proved useful for assessing exposure to xenobiotics. This assay has some advantages over the traditional enzyme assays, the ethoxyresorufin-*O*-deethylase or EROD assay[7] and the aryl hydrocarbon hydroxylase or AHH assay,[11,12] used to measure 1A1 induction. The quantitation of increases in mRNA levels is a direct indicator of CYP1A1 gene expression and is not as subject to interference from endogenous and exogenous factors that can interfere with enzyme activity. The production of catalytically active enzyme is at the end of a chain of cellular events initiated by the production of CYP1A1 mRNA and each stage of this process is susceptable to disruption by a number of factors, i.e., 1A1 enzyme activity can be inhibited by common environmental contaminants such as cadmium,[13] halogenated aromatic hydrocarbons and PAHs.[16,26] In addition, enzyme activity can be modulated by endogenous factors such as steroids and exogenous influences such as temperature and dietary factors.[3,15,20,29,43] It is important to note that general protein production and function is disrupted in highly polluted environments, often resulting in obvious hepatotoxicity, and inevitably affecting production of catalytically active enzymes.[14,17] These factors may also have an impact on gene transcription, but a number are known to inhibit enzyme activity directly. The RT-cPCR assay is up to 1000x more sensitive than some traditional enzyme assays, although enzyme assays are becoming increasingly sensitive and less time consuming.[19]

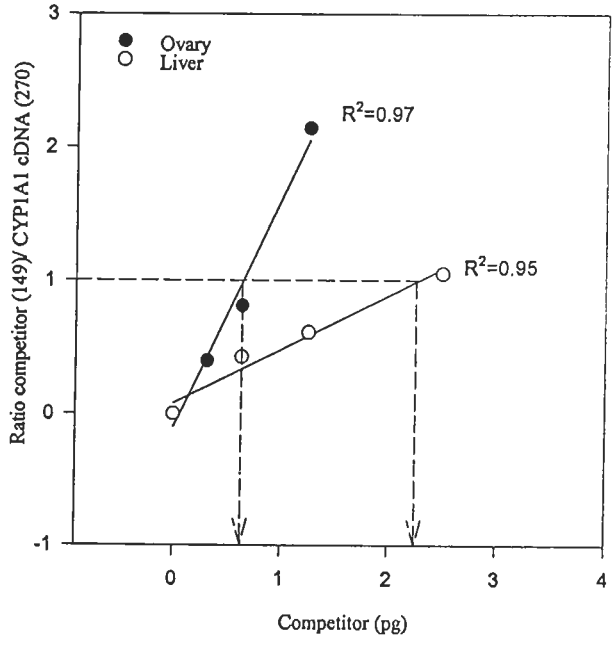

Figure 5. Linear regression analyses of the RT–cPCR results for the liver and ovary in Figure 4, following densitometric scanning of a negative image of the gel.

The sensitivity of this assay has enabled us to quantitate the induction of CYP1A1 when the sample size is extremely small — in the thread-like gonads of juvenile fish and in just 1-2 gill filaments.

Although CYP1A1 is primarily induced in the liver, consistent with this organ's major role in detoxification of xenobiotics, it is also induced in tissues important in metabolism and excretion, e.g. gill, kidney and intestine.[39] The results of this study show that CYP1A1 is induced in the gonads of chinook exposed to xenobiotics.

In several studies, a relationship between increased CYP1A1 expression and reproductive disturbances has been found. Fish sampled from polluted areas, with elevated P4501A1 (CYP1A1) enzyme activity, have a higher incidence of disrupted gonadal devel-

opment.[18] Fish treated with potent CYP1A1 inducers, such as PCBs, have depressed circulating steroid levels and reduced ovarian growth.[36,40] White suckers sampled from sites downstream of bleached kraft mills had elevated CYP1A1 enzyme activity, reduced plasma sex steroid levels, reduced gonad size and delayed sexual maturation.[27] The mechanisms underlying these reproductive disturbances are to a large extent unknown.

P450 enzymes, particularly 1A1, play an important role in facilitating the excretion of xenobiotics, by inserting molecular oxygen and increasing their water solubility. Other P450 enzymes are involved in the synthesis and metabolism of endogenous compounds such as steroids, fatty acids and prostaglandins. It is possible that increased induction of specific xenobiotic metabolizing enzymes could contribute to the deleterious effects on reproduction through mechanisms involving increased sex steroid metabolism, or through alterations in the induction of other P450 enzymes important in sexual development. The role of 1A1 in sex steroid metabolism is uncertain, although there is evidence from mammalian studies that enzymes of this family can play a significant role in the metabolism of estradiol.[4,38] Support for the latter speculation comes from some studies in which exposure of rats to tetrachlorodibenzo-p-dioxin, the most potent inducer of CYP1A1, resulted in depressions in P450 enzymes essential in the steroid biosynthetic cascade.[25] Studies on fish have indicated similar effects — isolated ovarian follicles exposed to BKME have reduced steroid biosynthetic activity.[41]

Studies in fish, using immunochemical techniques, have located P4501A1 in the gonads of zebrafish and scup, though not within steroidogenic tissue.[6,37] In contrast, preliminary evidence from studies using rat cell cultures, has indicated that *CYP1A1* is expressed in Leydig cells exposed to the PAH, 20-methylcholanthrene[30] — an in-

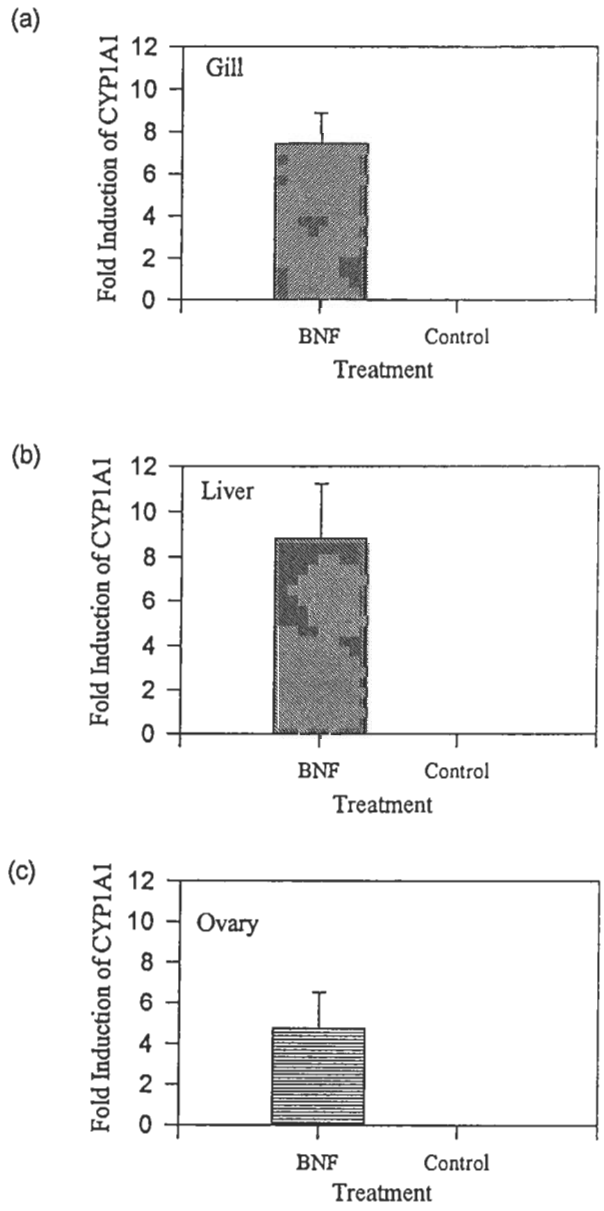

Figure 6. CYP1A1 induction in gill (a), liver (b) and ovary (c), 48 hours post-injection with BNF.

teresting result since the Leydig cells constitute the androgen-synthesizing tissue of the testes. In mice treated with PAHs, induction of *CYP1A1* within the ovary is associated with destruction of primordial oocytes, ovarian failure and subsequent tumour development.[24] The results of this study indicate a significant and rapid induction of *CYP1A1* within the ovaries of juvenile chinook exposed to xenobiotics, but does not identify exactly within which ovarian cell types *CYP1A1* is expressed. On present evidence, it is unclear whether the induction of *CYP1A1* within the gonads of fish has a causal relationship with the toxic effects of inducing compounds on reproductive processes.

In conclusion, we have developed a sensitive molecular tool which can be used, not only as an early warning indicator to assess exposure of fish to xenobiotics in the field, but also for fundamental research into the mechanisms of *CYP1A1* induction and the ultimate effects of this on fish physiology.

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Role of vitamins and β-glucans on immune response and disease resistance in Atlantic salmon

Santosh P. Lall and Gilles Olivier [1]

Our recent work on the effects of vitamins E, ascorbic acid and commercially available glucans (β-1,3 glucan) on immune response and resistance to two common infectious diseases, *Vibrio anguillarum* and *Aeromonas salmonicida*, in Atlantic salmon is reviewed. No significant differences in disease resistance against *V. anguillarum* and *A. salmonicida* were observed among Atlantic salmon smolts fed 100 to 2000 mg of ascorbic acid per kilogram of diet for 16 weeks. Although limited protection from *A. salmonicida* was observed with incorporation of some commercial β-glucans in the diets (0.05 and 0.1%), the protection was not consistent.

Introduction

The importance of a proper diet in preserving the health of fish is widely recognized. Unfortunately, information on the nutrient requirements of Atlantic salmon is limited[2] and the relationship between infection, nutrition and immunological parameters are poorly understood.[3] The National Research Council's nutrient requirement data[2] do not provide additional allowances for feed processing and storage losses, bioavailability of nutrients from feeds, economic considerations of feed manufacturer and the distribution of contaminants in feed ingredients. When fish are fed marginally vitamin deficient diets, they succumb to infection and the underlying deficiency is never diagnosed as the cause of death.

The immune system of fish may be also compromised due to high stocking density, adverse environmental conditions, handing stress, poor water quality, etc. Some evidence now exists that certain micronutrients and immunostimulants may potentiate non-specific defense mechanisms in fish and thus minimize the use of vaccines and chemotherapeutants. Several β-glucans as immunostimulants are now marketed as effective feed supplements to control disease losses in commercial farms. This paper summarizes our recent studies on the effects of selected antioxidant nutrients (vitamin E, ascorbic acid, iron) and commercially available glucans (β-1.3 glucan) on immune response and resistance to three common infectious diseases, *Vibrio anguillarum, Vibrio salmonicida* and *Aeromonas salmonicida,* in Atlantic salmon.

Vitamins

Vitamins are essential micronutrients required for normal physiological functions and health. Their deficiencies or excesses may have profound effects on disease development. Gross morphological and functional changes often develop in fish deprived of vitamins.[2] A low intake of vitamins affects many biochemical functions and consequently cellular and organ dysfunction, which may gradually be manifested as a clinical deficiency.

Vitamin deficiencies influence the integrity of skin and epithelial tissues, the composition of tissues and body fluids, and reduce mucous secretions, consequently predisposing the fish to infections. Generally, dietary vitamin levels that can prevent deficiency symptoms and maintain a reserve body pool are considered adequate. However, recent work with terrestrial animals and fish suggest that recommended levels of vitamins based on these cri-

teria may not have been sufficient to provide optimal health benefits under all conditions and different stages of development.[3]

Although a minimum requirement which will prevent signs of deficiency has been established,[2] the reports on the amount of vitamins required to prevent common infectious diseases are limited and also conflicting.[3] Most recent studies on the role of micronutrients on immune response and disease resistance are confined to vitamins C and E. The major interest in vitamin C in salmonids is not in deficiency but in the use of mega doses to improve disease resistance. Our recent unpublished work on Atlantic salmon fails to support the view that high doses of vitamin C are beneficial. No significant differences in disease resistance against virulent *V. anguillarum* and *A. salmonicida* strains were observed among Atlantic salmon fed 100 to 2000 mg ascorbic acid/kg of diet for 16 weeks (Fig. 1).

The antioxidant functions of vitamins C, E and β-carotene are now being widely investigated in laboratory animals because there may be a connection between the amounts of these micronutrients consumed and the amount of oxidative damage leading to degenerative diseases. For optimum activity in the body, these vitamins may be required in higher amounts (but not megadoses) than those needed to prevent deficiency symptoms. We have observed a nutrient interaction between ascorbic acid, vitamin E and iron and the pathogenesis of ascorbic acid deficiency is affected by the concentration of vitamin E and iron in the diet.

β-glucans

The parenteral administration of immunostimulants including glucans has been shown to increase non-specific protection in several fish species including salmonids (reviewed by Anderson[4]). However, published information on the efficacy of immunostimulants, including glucans, administered in the feed are limited. Atlantic salmon fed for five weeks with glucans (0.1% Macrogard®) showed increased resistance to *V. anguillarum* and *V. salmonicida*.[5] In chinook salmon, Nikl et al.[6] have tested the incorporation of VitaStim® in the feed of concentrations of 0, 0.01, 0.1 and 1%. Salmon were fed for 7 days and triplicate groups were bath challenged with *A. salmonicida*, the best protection was achieved with the diet containing 0.1% VitaStim®. Siwicki et al.[7] have tested the dietary intake of six immunostimulants in adult rainbow trout for one week. When groups

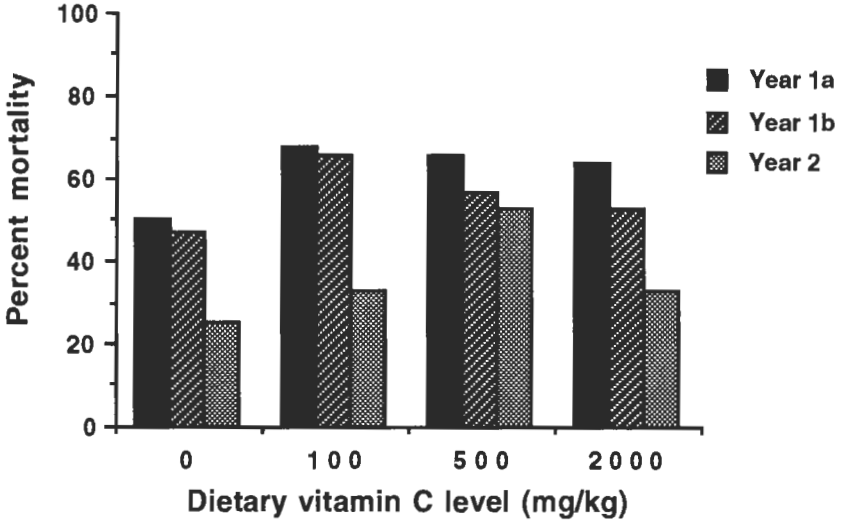

Figure 1. Percent mortality of Atlantic salmon smolts fed for 16 weeks with various levels of vitamin C and challenged with *A. salmoncidida* by cohabitation (groups of 15 fish were used for each experiment). Two challenges were performed in the first year of the study (1,ab) and one challenge the second year. Fish were fed and challenged at 12° C in heated sea water.

of 10 fish were challenged with *A. salmonicida* by an ip injection of 1 x 10^7 cfu, one week after feeding, the best protection (60% mortality) was observed with Macrogard® and cells of *Saccharomyces cerevisiae*, compared to 100% mortality in the control group.

Two experiments were conducted to determine the effect of commercially available glucans (β-1,3 glucan) on the immune response of Atlantic salmon. Fish were also challenged with *V. anguillarum* and *A. salmonicida*.[8] In the first feeding trial, four β-1,3 glucans (0.1% of the diet) commercially available as immunostimulants (Macrogard®, VitaStim®, Curdan and Alpha-Beta Technology glucan) and brewers dried yeast (5% of the diet) were fed to Atlantic salmon parr (30 ± 1.5 g initial weight) which were subsequently challenged with *V. anguillarum* and *A. salmonicida*, 6 and 12 weeks post-feeding. During the experiment, fish were maintained in a flow through system of 100 L circular tanks. Water was supplied to each tank at a flow rate of 2 L per minute and maintained at a temperature of 12 ± 1°C. The composition of the experimental diet was very similar to the practical diet regularly used in our Atlantic salmon nutrition studies and Department of Fisheries and Oceans hatcheries in the Maritimes.[9] There was no significant effects of these immunostimulants in protection against these diseases. However, glucans delivered by intraperitoneal injection provided increased resistance against vibriosis and furunculosis.

In the second feeding experiment, non-specific resistance to furunculosis was tested after feeding diets containing glucans to juvenile Atlantic salmon, the practical diets tested contained 0.05 and 0.1% of either Macrogard® or VitaStim®. One group was fed for two weeks with all diets then challenged by cohabitation, another group also fed for two weeks with glucans, returned to normal feed for 6 weeks followed by two more weeks of feeding with glucans.

Non-specific protection was verified by cohabitation challenge using *A. salmonicida*. Groups of three control fish were lethally injected intraperitoneally with approximately 1 x 10^6 cfu of the virulent *A. salmonicida* strain 80204 grown as previously described.[8] Injected fish were added to tanks containing 15 fish from each diet in the first challenge and to groups of 12 fish from each diet in the second challenge. All injected fish died within 2 to 3 days following injection, mortality in the re-

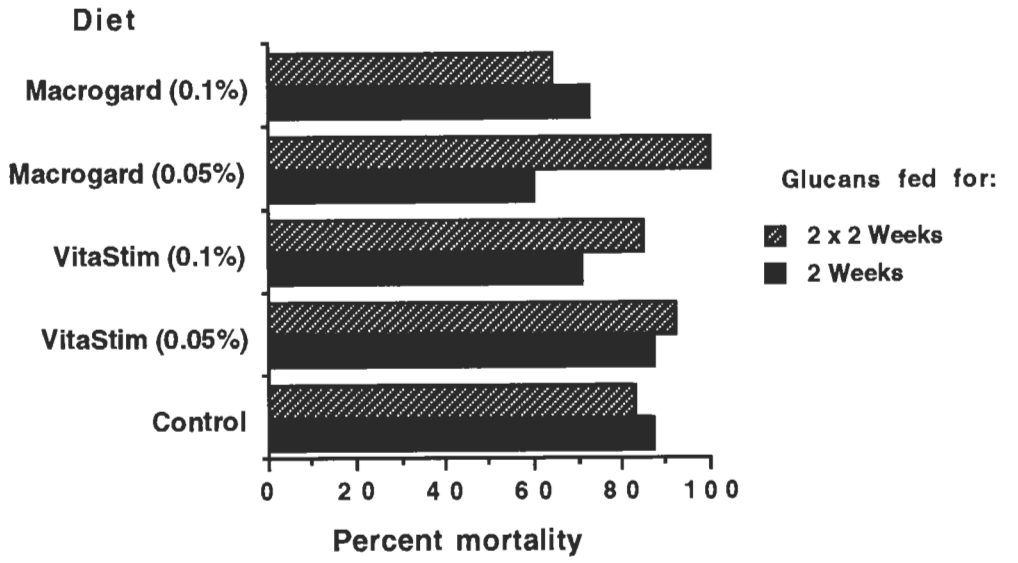

Figure 2. Precent mortality of juvenile Atlantic salmon fed two concentrations of glucans for different time periods. All experiments were performed at 12° C and groups of 15 fish were used for the cohabitation challenges with *A. salmonicida*.

maining fish was monitored daily for 14 days and each mortality was confirmed by streaking kidney material on TSA plates.

Results of these cohabitation challenges with *A. salmonicida* indicated that a low level of protection was conferred after two weeks of feeding with three of the treatments tested. After two cycles of feeding only Macrogard® (0.05% of the diet) provided minimal protection against furunculosis (Fig. 2).

Although some protection was observed with the incorporation of glucans in the diets, the protection was not consistent and it seems that the concentration of glucans fed is important. These results are preliminary and suggest as others that glucans could be used in the feed but more work is needed on the concentration of immunostimulants and length of feeding. Finally, standardization of protocols to assay their influence on immune parameters and disease resistance needs to be assessed further.

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Chromosome manipulation begins to pay off

Gary Thorgaard

Performance of triploids and inbred lines have generally not lived up to expectations, but new applications for chromosomally-manipulated fish may prove significant. Sterilization by induced triploidy appears the best alternative for addressing environmental and conservation concerns and interspecific triploid hybrids are of interest for aquaculture and the aquarium trade. The utility of clonal lines developed by gynogenesis and androgenesis for biomedical research and as control lines in selective breeding programs is becoming evident.

Fishes are tolerant of a variety of types of chromosome manipulations which are not possible in mammals. These include the induction of triploid indviduals having three sets of chromosomes, of tetraploids having four sets, and androgenetic or gynogenetic individuals having all their chromsomes from the male and female parent, respectively. These manipulations involve the disruption of early cell divisions in all cases (typically by heat or pressure treatments) and the inactivation of the chromosomes from one parent (typically with radiation) in androgenesis and gynogenesis. The principal applications of these manipulations are sterility and improved hybrid survival in triploids, the use of tetraploids to generate triploids, the production of all-female populations by gynogenesis, and the generation of clonal populations by androgenesis and gynogenesis.

There were initially very high hopes that these methods would generate high-performing fishes for use in aquaculture. Because female triploid fishes generally do not show substantial gonad development or develop secondary sexual characteristics, triploids were thought to potentially be faster-growing than diploids and likely would have higher quality than diploids at the time of sexual maturation. Clonal populations generated by gynogenesis were proposed to have potential value for generating high-performing production fish by line crosses, as has been done in corn breeding.

Unfortunately, the realized performance of chromosomally manipulated fish has not lived up to the early hopes. The immature performance of triploids has been mediocre; most studies of juvenile salmonid triploid performance have shown either superior survival and growth in diploids or equal survival and growth in the two groups. Survival and growth have almost never been superior in the triploids.

Similarly, homozygous fish have been found to have very poor viability and female fertility. The performance of these fish may simply be too poor for them to be widely utilized as production animals in commercial aquaculture.

These observations might seem to imply that chromosome manipulation does not have any significant role in aquaculture. However, I argue that developments during the last decade have in fact increased the need for these types of manipulations in aquaculture. The needs, however, have shifted from performance-focused needs to alternative missions. For example, the central role for triploidy may prove to be as a means to sterilize fish and prevent them from reproducing. The central role for clonal fishes may prove to be as experimental controls in breeding and research programs.

The increased recognition of the importance of sterilizing some aquacultured fish has come about from two major developments. First, there has been an increased recognition that selectively-bred fish are substantially altered genetically from the wild stocks from which

they are derived, and the escape of such fish from farms and their interbreeding with wild stocks could represent a threat to the genetic integrity of the wild stocks. Such concerns have been most widely expressed in Norway and also raised in Scotland, Ireland, eastern Canada and the USA for Atlantic salmon. There are also concerns about the unwanted establishment of species outside their native ranges, e.g., Atlantic salmon on the Pacific coast of North America. A second major development has been the recognition of the potential of transgenic technologies for fishes. It is clear that the capability exists to produce fast-growing fishes by this method (e.g., by growth hormone gene insertion) and this may soon extend to more disease resistant strains. However, there are reservations about possible escape and establishment of transgenic fishes in nature. Selectively-bred and transgenic fishes clearly should have a role in aquaculture, but it needs to be done so that they do not become established in nature or interbreed with wild stocks.

Induced triploidy could present an effective means to address these concerns. An even more assured way to genetically contain domesticated and transgenic fishes could be the use of sterile triploid hybrids. Triploid hybrid fishes frequently show improved survival relative to diploids and potentially could combine the best attributes of the two parent species. In our laboratory, Peter Galbreath has shown that Atlantic salmon X brown trout triploid hybrids appear very similar to Atlantic salmon and also show similar growth. Triploid Atlantic salmon X brown trout hybrids could allow an increased assurance of sterility because even if triploidy is not 100% effective, the diploid hybrid is impaired in reproduction relative to diploid Atlantic salmon. The triploid brown trout X brook trout hybrid (tiger trout) is another example of a good-performing triploid hybrid.

Although the reproductive performance of homozygous female fishes produced by gynogenesis and androgenesis has been disappointing, such fishes have now been produced in several laboratories. Homozygous females can be used to generate clonal lines by gynogenesis and homozygous males can be used to generate clonal lines by androgenesis; we have produced clonal rainbow trout by these approaches in our laboratory. Crosses between clonal lines can generate hybrid clonal lines which are both vigorous and genetically uniform (isogenic). Biomedical research using laboratory mice has largely been built around the use of homozygous and hybrid isogenic lines.

In spite of their low reproductive capacity, which likely would limit their direct commercial application, isogenic lines of fishes are likely to have substantial applications in both fundamental and applied fish research. Fish are increasingly being recognized as significant research models for cancer, immunology and toxicology studies, and genetically uniform animals can greatly facilitate such work. Similarly, in aquaculture research, genetically uniform fishes could be very appropriate control animals in selective breeding programs. Improvements are constantly being made in husbandry, diet and disease control, and it is difficult to document the source of improvement in cultured fishes over time without using appropriate control lines. Such lines can be very expensive to rear. However, isogenic hybrid crosses may provide suitable benchmarks against which the performance of selected lines can be compared. These lines, because of their identity and method of propagation, will not change over time and will thus provide excellent controls for a long period of time at a modest cost. Isogenic hybrids could also be used in quantitative genetic studies to estimate environmental variance because all variance within the group must be environmental rather than genetic in origin.

In summary, at first glance it appears that chromosome manipulation has not lived up to our expectations for aquaculture. However, our requirements in aquaculture are changing and these methods may help address these changing needs. Triploidization, by allowing assured sterilization of some cultured stocks, may allow fish to be raised where they otherwise could not while not risking interbreeding with wild stocks or the establishment of unwanted new species. Triploid hybrids could provide sterile, new forms for aquaculture and the aquarium fish industry with desirable attributes. Isogenic fish lines may serve as biomedical research animals, providing alternatives to the widely used mammalian biomedical models, as well as serve as highly suitable controls for selective breeding programs.

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What the farmer needs

Ted Needham

Canadian production of farmed salmon has tripled in the last 6 years while Chile's production has grown 15-fold. The difference is largely due to the British Columbia government moratorium on the expansion of salmon farming. Out-produced by Chile, Canada is also becoming uncompetitive because we are denied the fast growing strains of Atlantic salmon available to Chile from Norway. Through selective breeding, the Norwegians have improved farm stock by 10-15% per generation over 5 generations. They now grow fish to market size in 12 months, instead of the 18 months it takes in British Columbia. Instead of being supported by a similar breeding program, farmers here are being encouraged to use triploids, which have failed in Scotland, and all-females that will under perform unless fast growing strains are used. Biotechnology could actually increase the competitive disadvantage of our industry, particularly if consumers perceive our product to have been genetically engineered. Instead, farmers in BC should be helped in the following critical areas: development of year round smolting and reproduction, vaccine production, and selective breeding.

We have two challenges in aquatic biotechnology in the Pacific Rim of the United States and Canada:

- develop a sustainable aquaculture industry;
- safeguard and effectively enhance wild stocks.

These two challenges are not mutually exclusive — in fact they are complementary, despite the posturing of the players involved. It is tragic that there is so little crossover and cross fertilization between biologists, health experts, economists and managers working for government in stock enhancement and those in private sector aquaculture.

It is ridiculous, for example, that furunculosis vaccines developed with huge success by the private sector for commercial salmon farming are not being applied wholesale in the publicly funded Pacific salmon enhancement program. Indeed, they should be pressing for vaccines against kidney disease in chinook and coho salmon, and for IHN vaccines to protect wild sockeye. Almost all wild sockeye salmon in British Columbia is infected with the IHN virus. This not only increases the mortality of sockeye, but its presence in wild fish poses a dangerous threat to farmed salmon in the area.

We must not forget that salmon farming in BC is more valuable than the sockeye salmon fishery. Not only does it employ more people full time, but it earns more dollars for BC. Also, as sockeye are an endangered species in the United States, one would think that vaccines would be one of the many tools being used to safeguard the species.

For the present, I regret the move to naturalness that is being widely applied in salmonid enhancement programs. Instead of overreliance on vague concepts of survival of the fittest, government hatchery operators should be putting to good use what we have learned in commercial aquaculture where fish have to survive.

We have a challenge to develop a sustainable aquaculture industry. In a world context, the recent change in salmon supplies has been nothing short of revolutionary. One in every three salmon consumed is farmed. Over 400,000 tonnes of salmon were raised in 1993, with production in North and South America representing roughly 25% of the total production.

Chile, with 63,000 tonnes, is comfortably in second place behind Norway in the world salmon farming league. Canada, with 33,000 tonnes, is a modest 4th after Scotland. This is a

dramatic change — six years ago Chile lagged behind Canada, raising an insignificant 4,400 tonnes in 1988. Canada's total that same year was 10,000 tonnes and although Canada's production has tripled in the past six years, Chile's has grown 15-fold.

This year, 1995, we expect Chile to dominate salmon production in the Americas with 85,000 tonnes. Chile will be close to displacing Canada as the main supplier of farmed salmon to US markets. It is not surprising that four of the major salmon farming companies in British Columbia now have substantial interests in Chile.

Canada has fallen behind because of the provincial government's 2-year moratorium on additional sea sites, and a 5-year moratorium on lake sites. Furthermore, unlike Chile, Canada has been denied the best available strains of Atlantic salmon.

So, we have two problems — political and technical. Politically, we have to moderate the orchestrated hysteria that breaks loose every time farmed Atlantic salmon escape. Technically, we need to advance with a selective breeding program for our farmed salmon. In Norway, T. Gjedrem and co-workers have shown an improvement in the growth rate of Atlantic salmon of 10-15% per generation over 5 generations of selective breeding. This is a 2.5% increase in growth per year, and a cumulative improvement since the program began in 1971 of 50-60%. In Norway, most of the farmed Atlantics are now harvested after 12 months in the sea, instead of 18 months in Canada and the US.

In Washington State, W.K. Hershberger has achieved comparable success with coho. He claims, as a result of his family breeding program, a 60% improvement in growth rate in 10 years.

Instead of helping us develop a selective breeding program here in BC, our sponsoring Ministries are telling the industry to use biotechnology to develop single sex or sterile Atlantics. This is despite the total lack of any evidence that escaped farmed Atlantics do any harm. Administrators appear not to have learned from the failure of the very closely related brown trout to compete effectively with native salmonids on the West Coast. And in their native environment in Europe, the brown trout easily outcompetes Atlantics. Atlantic salmon are very much at the bottom of the heap.

The danger is that by being forced to go single sex the international competitiveness of our salmon farming industry could be eroded further. All female triploids have failed in Scotland. From a high of 5 million eggs in 1990, few if any were produced in 1994. This is because triploid Atlantics have underperformed. They are extremely susceptible to oxygen shortage in the water and are the first to die in plankton blooms and in routine handling operations. According to N. Bromage of Stirling University in Scotland, this is because the red blood cells are enlarged. Each cell has proportionately less surface area to absorb oxygen.

Single sex diploids might work provided we use fast growing strains. The concern is that all females, like neutered coho, will lack testosterone which induces the growth spurt essential in the run-up to harvest. The chinook farming industry in BC has been dragged down by sex reversed females of an extremely narrow genetic base. Worse still, these sex reversed fish have not survived any rigorous seawater testing and selection process. It is not surprising that more Atlantics are now grown in BC than single sex chinook.

The technology itself is not foolproof. Populations of so-called "all female" chinook contain some males. The danger of incomplete feminization is greater in Atlantics because attempts so far to develop a gene probe have failed. This probe is necessary to determine the genetic sex of the masculinized precursors necessary for all female production stock.

There is a further problem. Consumer outlets in the US have already threatened to stop buying farmed salmon from Canada if they are genetically engineered. It will be ironical, then, if biotechnology ends up destroying our industry, rather than helping it. The danger is all the greater if the possible move to transgenics is not handled carefully.

Instead, we should be using aquatic biotechnology to move forward:

- First in vaccine production. In France, there is an experimental recombinant vaccine against VHS virus. It is a glycoprotein and protects rainbow trout. As this virus is widespread in herring in the Pacific Northwest, our farmed Atlantics need to be protected against it.
- Second, we need to have year-round spawning and smolt production. In British Columbia we have the right sea temperatures for successful smolt entry every month of the year. This is essential to give a flow of

even-sized fish year-round to the market. It could be achieved by light manipulation combined with the use of hormone analogues so successfully developed by Syndel here in Vancouver.

- Lastly, we have to have a meaningful family-based selective breeding program. Moves to single sex culture will take us even further backwards without it.

To conclude, we have yet to apply aquatic biotechnology in any meaningful sense to safeguarding our wild stocks. Many of the biofundamentalists involved in Pacific salmon enhancement would be appalled at the thought. In commercial aquaculture there is considerable scope for aquatic biotechnology provided we are able to develop our industry unharassed. If present trends continue, the outlook for both commercial salmon farming and aquatic biotechnology in the Pacific Northwest is not promising.

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Preliminary Agenda
Aquatech '96 —
from research to the real world

Alliances and opportunities in cold ocean biotechnology

Location	Hotel Newfoundland, St. John's
Date	May 5-7, 1996 (two full days) In conjunction with the Canadian Society of Zoologists May 7-11, 1996
Agenda	6 sessions with the following major topics: 1) Evaluation and culture of new products and species 2) Environmental and bioremediation technologies 3) Integration of information technologies 4) Fish nutrition and health 5) New medical products from cold oceans
Social events	Guided tour of historical sites Adventure walk Seafood gala buffet with entertainment Traditional Newfoundland "screech ins" Bird and whale watching Spousal program

Detecting stress in fish

George K. Iwama[1]

Biological, chemical and physiological factors all impose stress on fish that can impair growth, reproductive success, and resistance to pathogens. Field-based and molecular approaches are being used to identify stress in fish. Preliminary results indicate that stress proteins are correlated with physiologically stressed states in fish and that antibodies against these proteins may prove useful as probes for determination of stress-states in fish.

The homeostatic mechanisms of fish, like those of other organisms, are constantly challenged by a variety of biological, chemical and physiological factors. These factors, individually or collectively, can impose considerable stress on the animal. The responses of fish to stressors are divided into three categories: *primary,* the neuro-endocrine and possibly the expression of novel proteins; *secondary,* the physiological response caused by stress hormones produced in the primary response such as ventilatory, cardiovascular, immune, or metabolic changes; and *tertiary,* or the individual and population responses to the sum of the primary and secondary responses. It is well known that stress impairs growth, reproductive success and resistance to pathogens in individual fish, and that if the stress is severe and long-lasting, succeeding higher levels of biological organizations become affected and eventually cause populations to decline.[2,4] My paper constituted a brief review of potential methods for detecting stress in fish. It first provided a basic description of the stress response in fish, and then highlighted some of the field-based and molecular approaches to detecting stressed states in fish.

The need for a probe for stressed states in aquatic animals can be found in at least two areas: intensive aquaculture and monitoring of environmental quality. Whether the fish or shellfish is in its natural environment, or under conditions of intensive aquaculture such as in government or commerical fish culture facilities, the detection of stressed states would aid in the mitigation or prevention of undesirable environmental conditions. The correlation between stressed states and compromised physiological condition, such as immune function in fish, has been shown repeatedly.[2] In addition to the health of the animals themselves, the health of the animals in the aquatic environment reflect the quality of the water in which they live. This has implications to the quality of human life in communities that draw domestic water from natural sources. As organisms that live in intimate association with their aquatic environment, fish have been used as indicators of the aquatic habitat. Reliable indicators of health or stress in fish and shellfish are generally lacking. Many of the indicators of health at the population level, such as growth and reproductive potential, take time to measure and may not be useful in monitoring acute or transient effects. On an individual level, many of the reliable indicators of stress in fish, such as plasma cortisol or glucose concentrations, require sophisticated and dedicated instruments which do not lend themselves to routine monitoring programmes of fish in the wild.

The methods and technologies for detecting stress in fish rely on the responses of the fish to stress, as described above. Although the description of stressed states in fish are conducted through the more classical methods of determining the concentration of the stress hormones, adrenaline and cortisol, the detection of stress in fish under field conditions must rely on simpler, and more portable instruments. In my talk at the AQUATECH '95 Workshop, I described a set of experiments where we compared the measurements of selected physiologi-

cal variables with laboratory and portable instruments.

The first set of experiments were conducted in the laboratory, where a standard handling stressor was applied to juvenile coho salmon, *Oncorhynchus kisutch* (Walbaum). Results of blood samples analyzed by both laboratory-based and portable methods were in good agreement. The tested portable instruments included: the Exactech® glucometer (Medisense, Cambridge, MA) the Ames™ Minilab (Miles Canada Inc., Etobicoke, Ontario) and the BMS® Haemoglobinometer (Buffalo Medical Specialties Inc., Clearwater, Florida). The Minilab is a fixed wavelength (546 nm) mini-photometer that is pre-programmed to perform a series of blood tests using pre-packaged reagents. The BMS® Haemoglobinometer is a portable photometer (540 nm) for the evaluation of haemoglobin using the oxyhaemoglobin method. Haematological variables including haematocrit (% red blood cells, RBC), leucocrit (% white blood cells) and lymphocyte ratios (number per 10^3 RBC) were determined using methods described by Houston.[4] Plasma chloride and sodium ion concentrations were determined with a Haake-Buchler chloridometer and a Corning flame photometer, respectively.

The second set of experiments focused on field-testing the most promising methods based on the laboratory tests. Production lots of Atlantic salmon, *Salmo salar* L., at commercial fish farms were sampled to assess stress and health status with the selected field techniques, from the swim-up to smolting stages. The measured variables included: blood glucose, protein, haemoglobin concentrations; haematocrit; erythrocyte and lymphocyte numbers; and an autopsy-based health profile, which involved the internal and external examination of the body for texture and colour. In addition to establishing resting, or normal, values for each variable under field conditions, blood glucose levels were observed to increase significantly at 4 hr after a stressful event (e.g., grading, handling) using these field methods. Some of these field techniques were found to be reliable indicators of stress in fish and have potential for use in aquaculture facilities and field monitoring programs.

The second part of my talk summarized our work to date on describing the relationship between stress protein (SP) expression and physiological stress in fish. SPs are a group of highly conserved proteins that are expressed in response to a variety of stressors in all organisms studied to date, from bacteria to humans. This response has been shown mainly in cell lines, but there is growing evidence that tissues of intact animals also express SPs when stressed.

We have purified a number of stress proteins from fish cell lines (SP70, SP30) and oysters (SP60) and have developed polyclonal antibodies against SP70 and SP30, and a monoclonal antibody against SP30.

Four fish cell lines (rainbow trout gonadal, chinook salmon embryonic, rainbow trout hepatoma, and carp epithelial) exposed to stressors such as temperature increase, Bleached Kraft Mill Effluent (BKME) as a toxicant, pH change, and L-azetidine (a proline analogue that causes denatured protein structure), showed expression of a new 50 kDa and a 70 kDa protein in all the cell types in response to all stressors. A 100 kDa protein was specifically expressed by all cell lines exposed to the toxicant. Some proteins therefore, might serve as indicators of general stress, and others might be indicators of specific stressors. Our preliminary evidence from experiments with whole animals showed that the elevation in plasma cortisol concentration, which is caused by stress in the whole animal, does not correlate with stress protein expression in the tissues of major organs. SP70 is expressed in the liver and head kidney tissue of coho salmon infected with Bacterial Kidney Disease, the most important disease of farmed salmon. Various stress proteins were expressed in the mantle tissue of oysters exposed to various stressors. Increased water temperature for 26 hr induced the expression of new proteins of 30-40 kDa, 60 kDa, and 65 kDa. New proteins of 15, 40, 90, and 200 kDa were expressed in response to BKME exposure. We are encouraged by our preliminary results that stress proteins are correlated with physiologically stressed states in the whole animal, and that antibodies against those proteins might be useful one day as probes for the health of the animals that live in our aquatic environments.

In aquaculture, there is no substitute for knowing how your fish are doing on a daily basis through personal observation. These tools may at best aid the monitoring of the possible unseen physiologically stressed states in your

fish. They may also be useful in monitoring the health of a new group of fish or a group of fish at a new site, or on a new feed. In the long term, we are hoping that the application of this technology to the monitoring of environmental quality through the health of the animals that live there might become a practical reality.

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Water quality challenges for aquaculture around the Pacific Rim

David A. Levy and Malcolm Winsby[1]

Water quality degradation is spatially correlated with human population concentrations and in several instances previously profitable aquaculture industries have collapsed due to water quality degradation and/or eutrophication from adjacent urban areas. In future, Pacific Rim countries will need to pay more attention to the maintenance of suitable water quality so that aquaculture productivity can be maximized.

Water quality degradation around the Pacific Rim is spatially correlated with human population concentrations, occurring in the vicinity of most cities and towns situated adjacent to rivers, estuaries, and in the coastal zone. Pollution conflicts with aquaculture reflect the localization of the aquaculture industry in these same aquatic environments. In contrast with wild fish stocks, where it is inherently difficult to dissagregate the quantitative effects of water pollution from the effects of fishing (e.g. Sindermann[5]), localized pollution impacts can be extremely damaging for aquaculture. In several instances (e.g. shrimp farms in the northern Gulf of Thailand), previously profitable aquaculture industries have collapsed due to self-induced water quality degradation and/or eutrophication from adjacent urban areas.

Coastal/estuarine pollution can affect any life stage of fish or invertebrate, but it is during their first few months of life that aquatic organisms are particularly sensitive to contaminants and water quality degradation. In addition to causing mortality, contaminants can also interfere with an organism's ability to reproduce, thereby affecting production.

Tropical aquatic environments around the Pacific Rim may be especially susceptible to water quality degradation due to physical conditions (e.g. higher temperatures and lower oxygen solubility). For example, a March 1992 spill of 700 m^3 of molasses into the Chee River in Thailand had a drastic effect on freshwater fish populations, creating lethal conditions and a severe fish kill for 420 km downstream of the spill site.[4] The fish kill stopped at an area of rapids in the Mun River, suggesting that the

fish mortality was related to hypoxic conditions (biological oxygen demand — BOD) induced by the molasses spill. The spill occurred at the end of the dry season under extremely low water levels, contributing to the severity of the fish kill.

From an aquaculture perspective, the most significant water quality problems are associated with organic inputs, causing high BOD, and inorganic nutrient loading. Inputs of toxic contaminants into waters of the Pacific Rim (e.g. PCBs, dibenzofurans, dioxins, PAHs) tend to be highly localized adjacent to industrial sites. Most of these substances are input via liquid effluents, and are therefore easily identified where they occur in close proximity to aquaculture facilities.

A key parameter which regulates the susceptibility of aquaculture to pollution impacts is water flushing efficiency. Poorly-flushed coastal environments tend to suffer from water pollution impacts to a much greater degree than well-flushed environments. Where flushing characteristics are poorly understood, it is prudent to undertake water current delineation studies prior to siting aquaculture facilities.

Although not definitively documented, a side effect of increased eutrophication of the coastal zone may be an increase in the frequency of toxic algal blooms (red tides).

Dissolved oxygen is a critical water quality parameter which can greatly influence aquaculture production success. Fish may respond to reduced oxygen availability in one of several ways[2] such as reducing their activity level, increasing aquatic surface respiration, or undertaking vertical or horizontal habitat shifts (e.g., Levy et al.[3]). All of these responses to hypoxic environmental conditions result in decreased aquaculture production.

In general, water quality requirements for aquaculture of finfish and shellfish are well known, and environmental analyses can be undertaken to predict potential water quality problems and mitigation requirements. It is critical to the future success of aquaculture enterprises that such analyses be undertaken at the project feasibility stage, before investment decisions are made. Water quality analyses should be undertaken as a key activity during project site assessment and can be also designed to formulate ongoing monitoring programs at aquaculture facilities.

Water quality issues in aquaculture can be divided into three categories:

- the effects of external pollution sources on aquaculture operations;
- the effects of aquaculture operations on aquatic receiving environments; and,
- the effects of aquaculture "self-pollution".

A Pacific Rim example under the first category is shrimp farm losses near the Guayas River in Ecuador which have been attributed to fungicides applied in upland banana plantations. Fish farm waste by-products in the form of nutrients, suspended solids, BOD, and other chemical substances provide examples under the second category. Self-pollution at an aquaculture site usually is related to poor site selection, site design, or site management.

In summary, major water quality challenges around the Pacific Rim, insofar as they affect aquaculture operations, are generally associated with:

- rapidly expanding human population and economic development;
- inadequate water treatment facilities;
- increased organic loading to aquatic environments;
- localized hypoxic conditions in strategically important aquatic environments; and,
- inadequate zoning and meaningful planning procedures.

In the future, Pacific Rim countries will need to pay more attention to the maintenance of suitable water quality conditions so that aquaculture productivity can be maximized.

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New vaccines: the regulatory and economic reality

Robert A. Busch

The application of new and emerging biotechnology based solutions to aquatic issues, such as protective immunization of fish and shellfish, presents tremendous opportunities and challenges compared to similar applications in higher vertebrates. The successful immunization of aquatic animals was first reported in 1942. Since that time, over 14 commercially important species of finfish and shellfish have been successfully immunized against over 20 viral, bacterial and parasitic diseases using experimental, autogenous, and fully licensed commercial products developed by universities, government laboratories, and at least 15 different manufacturers worldwide. In several aquaculture industries, immunization has 1) become a well established, proven, and cost effective method of controlling certain infectious diseases; 2) significantly reduced the use of antibiotics and overall cost of production; 3) increased the overall survival and health of the animal; and 4) improved the quality of the food product and the environment it is grown in. There can be no question that vaccines have become an important tool in a comprehensive and integrated program of aquatic animal health management for many producers.

The first fish vaccines that were commercialized in the early 1970s against some of the marine vibrios and *Yersinia ruckeri* were essentially crude monovalent formalin killed bacterins administered by various immersion techniques. However, modern products are more often multivalent formulations 1) produced and processed to provide a high level and critical balance of specific protective antigens, 2) coupled with various adjuvant systems for sustained release and enhanced response, 3) administered by immersion, oral and injection techniques that are becoming more automated and less stressful, and 4) with use patterns better adapted to the epizootiology of the disease and integrated into the production protocols of the cultured host species.

Biotechnologists often approach research on new vaccines as a basic science leading toward an eventual applied solution. However, commercialization of a new product or technology does not just end with a demonstrated biological effect under controlled conditions in the laboratory. Elements for successful commercialization of a new product must also include: 1) consistent field efficacy and safety under uncontrolled and highly variable conditions, 2) effective scale-up of an appropriate manufacturing process to commercial volumes, 3) acceptability for licensing to a variety of standards by multiple regulatory agencies worldwide, 4) an application method and use pattern that integrates into the normal production protocols for the animal and is relevant to the typical ecology and epidemiology of the disease, 5) cost effective to the producer and 6) profitable to the manufacturer.

Many promising new fish vaccines have been developed using biotechnology-based approaches, but few if any have been successfully commercialized to date due to various constraints and failures of the commercialization process.

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Biotechnology— where are the tools for effective disease prevention and diagnosis?

Gerry Johnson

The current situation

The changes in the last two decades of aquaculture development have been profound: new tests, innovative procedures and exciting research abound. Many of the same disease problems still exist and in some cases have become worse. The products of aquaculture are approaching true commodity status so perhaps it is time to evaluate our progress against the backdrop of competing species. Aquaculture, like agriculture, intensively cultures a diverse array of animals. Regardless of whether the farmed animal lives in water or on land, application of technology in diagnosis and disease prevention demonstrates a close relationship with the characteristics of the culture system utilised. If we adjust our expectations to suit, our attempts to deal with problems may have more success than anticipated.

All aquaculture animals are ectothermic and so are their pathogens. Scientists have utilized this for years to deal with the pathogens — things as simple as storage by refrigeration or freeze drying. However, the fish, the recipient of all these developments in aquaculture, does not respond as reliably or as specifically as mammalian or avian farmed animals. We are just now beginning to utilize this variation to our advantage.

The major problems

Aquaculture deals with sizable animal populations that one cannot readily see to evaluate. Mortality and lost production may be the first clinical signs. Health care requires focus on prevention of pathogen development in the population. When the culture system does not allow that, there needs to be a form of continuous stimulation of the fish's protective systems that compensate for ectothermia and for the less specific immune capability.

Populations need to be monitored to determine health status. Using large numbers of tests increases economic constraints and demands rigorous reliability, low unit costs and noninvasive test sampling. This is not an easy request to balance and has no precedent.

Diseases constantly emerge and always will. Agents of disease will change continuously— compounding and confounding with a variety of insults, infectious and noninfectious. In dealing with some of the common problems, we have made surprisingly little headway. Part of the problem is the infrastructure to evaluate, deliver and interpret new technologies.

Addressing the problems

No one has invented a silver bullet for disease diagnosis, treatment or prevention in fish. Problems remain complex, often interacting with the ecology of the fish, occur concurrently, and involve all aspects of farm management. Products, both preventions and diagnostics, that provide production enhancements should not be confused with cures. All the necessary pieces are coming into being, but progress must be cost-effective. New avenues are still needed.

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High energy diets and product quality

Clive Talbot

The past 25 years have seen a remarkable increase in the production of cultivated salmonids. Developments in genetic selection, nutrition, disease control, husbandry and farm equipment have resulted in marked improvements in growth rates and feed conversion ratio (FCR). The reduction in the overall economic FCR in Atlantic salmon farming in the Norwegian industry during the last two decades, from 4.5 to 1.2 kg feed per kg fish produced, demonstrates that the industry can now produce 3-4 times more fish per unit of feed used, and for the same level of discharges to the environment, compared to 20 years ago. The time required to produce fish of market size has been reduced significantly. Even more important for the farmer are the cost savings brought about by the higher yield and lower feed costs of production. Amongst these developments, the use of "high energy" diets (digestible protein (DP) to digestible energy (DE) ratios of around 19 g DP/MJ DE and DE levels of approximately 21 MJ/kg), in combination with improved feeding regimes which prevent both over-, and under-feeding, are perhaps the most significant.

While the application of best farming practice can have a dramatic effect on fish production, many questions remain regarding the effects of such production strategies on the quality of the farmed fish. The presentation reviewed the development of diet formulations and feeding regimes used in salmonid aquaculture today, and the interactions between nutrition, feeding, and carcass composition, including fish texture and pigmentation, which ultimately determines fish quality, and the productivity and economy of salmon farming.

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Improving vaccines through controlled release

Julian Thornton

The stimulation of long lasting immunity to several bacterial diseases of fish has been accomplished recently by the incorporation of oil based adjuvants into polyvalent, injectable vaccines. These vaccines, while quite effective, may produce a series of undesirable side effects such as tissue adhesions, granulomas, and sterile abscess formation in the injected fish. The encapsulation of antigens into microscopic beads of biodegradable polymers enables the design of vaccines which deliver several doses of antigen from a single injection. These vac-

cines can be tailor-made to allow for the controlled release of antigens only at times when the immune system of the fish is physiologically prepared to mount an effective response.

Efficacious oral vaccines have long been considered as the ultimate product for aquaculture. The delivery of antigens to the hindgut of fish is required for the protection of vaccine antigens from degradation by the acidic conditions of the stomach. The ability to protect antigens from the acid hydrolysis that occurs in the stomach in crucial to the development of cost effective oral vaccines, as many of the required antigens are rendered useless after partial acid hydrolysis. The encapsulation of vaccines into a pH sensitive polymer matrix is a viable technology for use in the development of oral vaccines for aquaculture.

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Fish farm effluents: cyanobacterial treatment and biomass recycling for fish feeding

Joël de la Noüe

Increasing environmental concern and regulations will force fish farms to treat their effluents before their discharge into natural water bodies. A solar biotechnological system using the cyanobacterium *Phormidium bohneri* has been tested. *P. bohneri*, a self-flocculating species, was cultivated on simulated and undiluted genuine fish farm effluents in completely mixed 70 L bioreactors using three hydraulic retention times of 8, 12 and 24 hours. On simulated effluents inorganic nitrogen (NH^{4+}) and phosphorus removal (PO_43-) were 73-94% and 69-75%, respectively. On effluent from tanks in which rainbow trout were reared, removal of ammonium nitrogen reached 82% and removal of soluble inorganic phosphorus was 85%.

In order to test the recycling of *P. bohneri* biomasses into fish feeds, rainbow trout juveniles (average mass 0.56 g) were fed diets containing 15% (dry matter basis) freeze-dried *P. bohneri*. After 15 weeks of feeding, no differences were found between fish groups in survival, body moisture, protein or lipid contents. Fish fed 15% cyanobacteria did not differ in growth performance nor condition factor versus control fish. Fish fed 30% *P. bohneri* had a final body mass 9.6% lower (12.4% lower when 5% cod liver oil was also added) than control. No flesh coloration could be detected in any of the treatment groups.

The conclusion is that biotreatment of fish-farm effluents with *P. bohneri* is feasible and that their incorporation at a level of 15% of the diet leads to good zootechnical performance of rainbow trout juveniles.

Dr. Joël de la Noüe is Director and Professor, Université Laval, Sainte-Foy, Québec G1K 7P4 (Supported by CORPAQ and by Fonds FCAR grants to Joël de la Noüe).

Calendar

•**2nd International Conference on Ecological Engineering,** 18-22 September 1995, Wardenswil, Switzerland. Conference will focus on ecological technologies for the reuse and recycling of resources in wastewater such as wastewater aquaculture, composting and urine-separating toilets and greywater reuse technologies. Information: Conference Board c/o Jean-Bernard Bachtiger and Regula Treicher, P.O. Box 335, Ingenieurschule Waedenswil, CH-8820, Waedenswil, Switzerland (tel 41 1 798 99 25; fax 41 1 789 99 50).

•**50th Anniversary of the Food and Agriculture Organization (FAO),** 11-16 October 1995, Quebec City. Meeting is composed of an International Symposium (11-13 Oct), Exhibition (11-14 Oct), and 50th Anniversary Celebration (16 Oct), and World Food Day (16 Oct). Theme: People at the heart of development: food security through knowledge. Sub-themes: Managing natural resources, Managing markets and Managing know-how and technology. Contact: Forum Québec, 30 Grand Allée Ouest, Québec (Québec) Canada G1R 2C6 (tel 418 524-8093; fax 418 529-1172).

•**Fourth Asian Fisheries Forum,** 16-20 October 1995, Beijing Convention Center. Special symposia on aquaculture, pollution and environment; development trends and scientific issues in capture fisheries and aquaculture; fishery policy and investment; and shrimp culture. Contact: The China Society of Fisheries, 31 Min Feng Lane, Xidan, Beijing (tel 861 6020794; fax 861 6062346).

•**Pollution Processes in Coastal Environments,** 6-10 November 1995, Mar del Plata, Argentina. Aim: a global view of the occurrence, distribution, accumulation, transference and circulation of pollutants in coastal environments; characterization of the health status of coastal areas as well as an assessment of transference routes. Information: Dr. J. E. Marcovecchio, President, Organizing Committee, Lab. de Contaminacion, INIDEP, Casilla de Correo 175, 7600 Mar del Plata, Argentina [fax 54 23 51-7442].

•**ECOSET 95**, International Conference on Ecological System Enhancement Technology for Aquatic Environments, 6-10 November 1995, Nihon University, Tokyo. Forum for the exchange of information on all aspects of aquatic ecological systems associated with artificial reefs, estuaries, wetlands, tidal flats, rivers, lakes and other natural and man-made habitats. Information: Japan International Marine Science & Technology Federation, Kyodo Bldg, Room 65, 1-3-5 Nihonbashi-Kakigara-Cho, Chuo-Ku, Tokyo 103 Japan (fax 81-3-3667-7174).

•**The VIIth Industrial Biotechnology Conference,** 4-6 December 1995, Sheraton Centre, Montreal. The National Research Council of Canada holds conferences on industrial biotechnology every second year. Highlights of the 1995 conference include including an exhibition of biotechnology research organizations and biotechnology in Canada. Contact: Doris Ruest, Conference Services, National Research Council Canada, Montreal Road, Ottawa, Canada K1A OR6 (tel 613 993-9228; fax 613 956-9828).

• **Biochemistry in Marine Technology**, 11-16 December 1995, Univ. Havana, Cuba. Topics: purification and characterization of polypeptides from marine sources, genetics of marine species, hormones, metabolism of marine animals, biochemistry teaching. Language: Spanish and English. Information: Dra Olimpia Carrillo Farnes, President Comite Organizador, Departamento de Bioquimica, Facultad de Biologia, Universidad de la Habana, Calle 25e/JeI, Vedado, C. Habana, Cuba (fax 53-7-321321).

•**Canadian Conference for Fisheries Research (CCFFR)**, 4-6 January, 1996. The major themes of the conference are 1) behavioural ecology and fisheries science (Invited speakers: M. Abrahams, M. Gross, M. Keenleyside, D. Kramer, D. Swain); 2) Recent advances in the science of aquaculture (In vited speakers: J. Brown, M. Litvak); and 3) a joint CCFFR/SCL Session the land-water

interface. Papers on these major themes are particularly encouraged, but submissions on other areas related to Fisheries and Aquatic Habitat are also welcome. Deadline for abstracts is 15 September 1995. Abstracts submitted to Dr. Jim Grant, Dept. Biology, Concordia University, 1455 de Maisonneuve Blvd. West, Montreal, Quebec H3G 1M8 (tel 514 848-2431; fax 514 848-2882; e-mail grant@vax2.concordia.ca)

•**World Aquaculture 96 & the Bangkok Seafood Show,** 30 January– 2 February 1996, Queen Sirikit National Convention Center, Bangkok, Thailand. The annual conference and exposition of the World Aquaculture Society is hosted by the Thailand Department of Fisheries and the Chulabhorn Research Institute. Information: Sea Fare Expositions, 850 NW 45th Street, Seattle, WA USA 98107 (tel 206 547-6030; fax 206 548-9346).

• **11th Indian Seafood Fair,** 9-11 February, Bombay. Organized by the Marine Products Export Development Authority (MPEDA) and the Seafood Exporters Association of India (SEAI). Information: R. Ganapathy, MPEDA, 17 Battery Place, Suite 227, New York, NY 10004 (tel 212 425-9437; fax 212 363-3456).

•**Aquaculture America 96** Conference and Exposition, 14-17 February 1996, Arlington Convention Center, Arlington, Texas, USA. Sponsored by the US Chapter of the World Aquaculture Society and hosted by the Texas Aquaculture Association. Technical sessions, producer seminars, and trade show. Tours to aquaculture facilities, reception at the Circle R Ranch. Information: Sea Fare Expositions, 850 NW 45th Street, Seattle, Washington 98107 (tel 206 547-6030; fax 206 548-9346).

•**Refrigeration and Aquaculture Colloquium,** 20-22 March 1996, Bordeaux Convention Centre, France. Will provide the opportunity to review the applications and consequences of refrigeration in aquaculture, from the scientific and technological viewpoint, as well as economic aspects. This colloquium is one part of the major scientific, professional and commercial forum of Bordeaux Aquaculture. Information: Bordeaux Congrès Service, Palais des Congrès, Quartier du Lac, 33 3000 Bordeaux Lac, France (tel 33 56 11 88 88; fax 33 56 43 17 76).

•**Aquaculture Canada 96** — the Annual Meeting of the Aquaculture Association of Canada, June 1996, Ottawa. Information: Cyr Couturier, Aquaculture Unit, Fisheries and Marine Institute, Memorial University, P.O. Box 4920, St. John's, Newfoundland (tel 709 778-0609; fax 709 778-0661).

•**International Congress on the Biology of Fishes,** 14-18 July, 1996, San Francisco State University. Meeting combines several established meetings (GUTSHOP, Amazonian Fishes, High Performance Fish, Pacific Biotech, Smolt Workshop, Fish Larvae/Eggs, Anadromous and Catadromous Fish, Fish Stress) into one venue. Themes: Metabolic Performance, Tropical Fish Biology, Biotechnology Applications, Functional Anatomy, Feeding Ecology & Diet, Contaminant Impacts, Environmental Adaptation, Species Specific Symposia. Deadlines: Session proposals: 15 September 1995; Paper titles 15 November 1995; Abstracts 15 February 1996; Papers 15 April 1996. Contact: Don MacKinlay, Fisheries and Oceans, 555 West Hastings Street, Vancouver, BC V6B 5G3 (tel 604 666-3520; fax 604 666-3540).

•**Second World Fisheries Congress,** 28 July – 2 August 1996, Brisbane Convention and Exhibition Centre, Brisbane, Australia. Theme: Developing and sustaining World Fisheries Resources, the state of science and management. Congress is being hosted by the Australian Society for Fish Biology. Abstract deadline: 31 August 1995. Information: Second World Fisheries Congress, P.O. Box 1280, Milton, Queensland 4064, Australia [tel 617 369 0477; fax 617 369 1512].

•**International Astacology Association,** 11th Symposium, 11-16 August 1996, Lakehead University, Thunder Bay, Ontario, Canada. Includes paper and poster sessions on all aspects of crayfish science — culture, physiology, management, taxonomy, zoogeography, and ecology. There will be field trips to visit crayfish habitats in the Thunder Bay region. These will involve travels through northern Ontario's coniferous forest adjacent to Lake Superior. For information contact: Dr. Walter Momot, Dept. Biology, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario P7B 5E1 Canada (tel 807 343-8277; fax 807 343 8023; e-mail Walter.Momot@lakeheadu.ca).