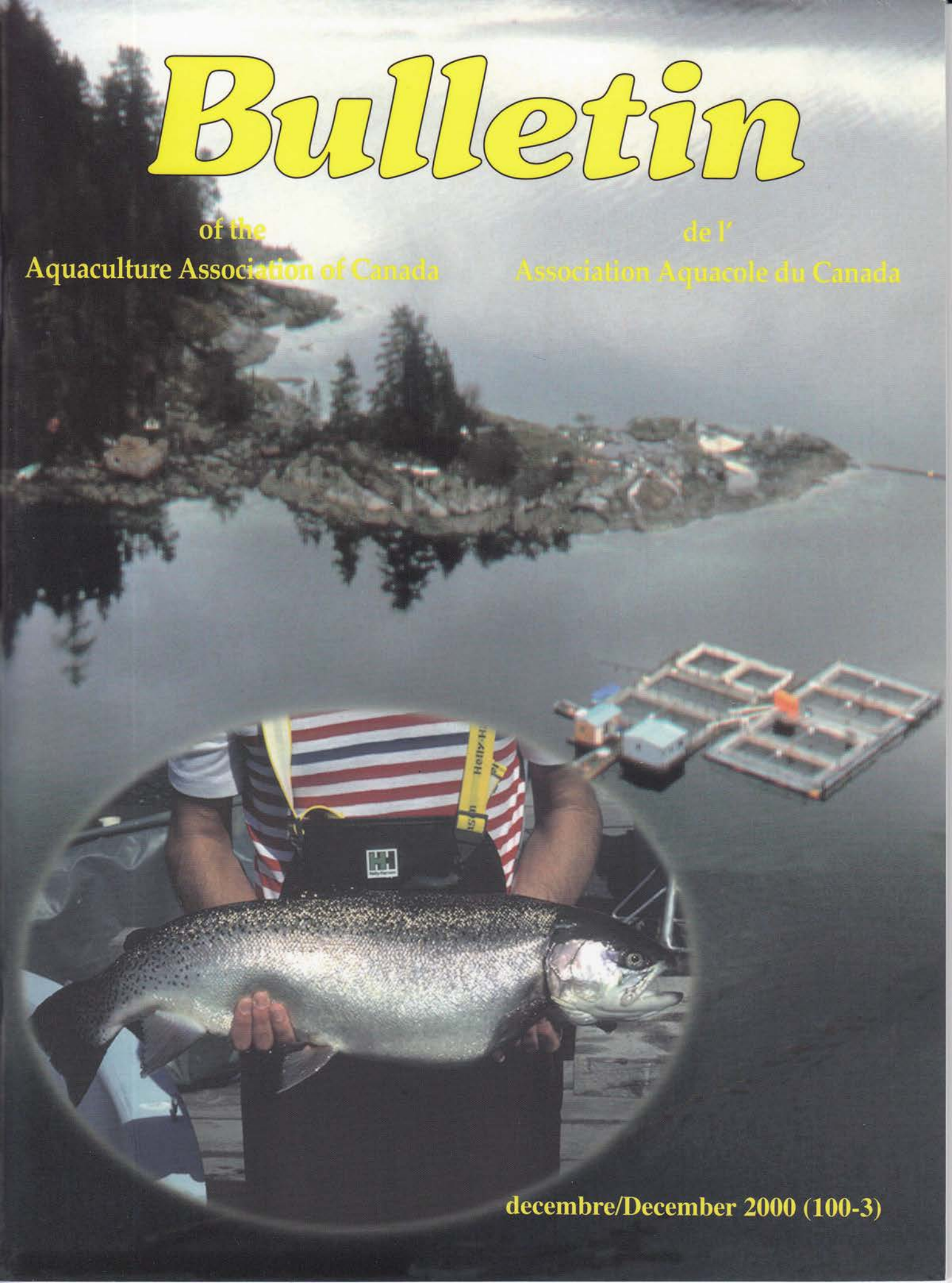
Bulletin

of the
Aquaculture Association of Canada

de l'
Association Aquacole du Canada



decembre/December 2000 (100-3)

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ISSN 0840-5417

Imprimé par Print Atlantic, Moncton (N-B)

Bulletin of the Aquaculture Association of Canada

December 2000 (100-3)

The *Bulletin* is available through subscription ($40 per year) or as a benefit of membership in the Aquaculture Association of Canada, a nonprofit charitable organization. For membership information contact: Aquaculture Association of Canada, 16 Lobster Lane, St. Andrews, N.B., Canada E5B 3T6 [telephone 506 529-4766; fax 506 529-4609; e-mail aac@mar.dfo-mpo.gc.ca; website http://www.mi.mun.ca/mi/aac]. Annual dues are $50 for individuals ($40 for students and seniors) and $85 for companies; 25 percent of dues is designated for *Bulletin* subscription. The *Bulletin* is indexed in Aquatic Sciences and Fisheries Abstracts (ASFA) and the Zoological Record. Mailed under Canada Post Publications Mail Commercial Sales Agreement No. 525375. Change of address notices and undelivered copies should be mailed to AAC. Return postage guaranteed.

ISSN 0840-5417

Printed by Print Atlantic, Moncton, NB

Cover: Insert—Three-year-old female triploid rainbow trout grown at DFO's West Vancouver Laboratory in the 1980s [Tillmann Benfey photo]. Background — BC salmon farm [William Pennell photo].

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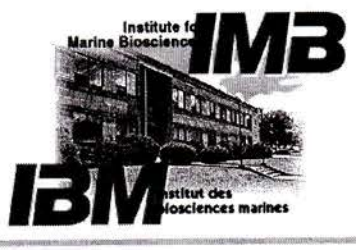
Funding for this issue of the *Bulletin*
was provided by the
Institute for Marine Biosciences
National Research Council, Halifax, NS



From the Guest Editor

Biotechnology: What Does it Mean to the Aquaculture Producer?

Laura Brown

From May 28 to 31, 2000, the Aquaculture Association of Canada held its annual general meeting, conference, and trade show at the Delta Beauséjour Hotel in Moncton, in New Brunswick. There were a number of varied presentations and speakers from industry, academia, government and community groups. I was very pleased to be asked to organize one of the special sessions, entitled "Biotechnology: What Does it Mean to the Aquaculture Producer?"

Once I had agreed to organize the session, and as I was thinking about potential speakers, I realized that the question above had to be preceded by the question "Biotechnology: What Does it Mean?"

The Casell Dictionary of Science defines biotechnology as "the use of living organisms in the large-scale industrial manufacture of foods, drugs, and other products".(1) By this definition, all agriculture and aquaculture can be classified as biotechnology. Current interpretation of the word leans more to the idea that "biotechnology" involves manipulation of DNA, genes, and proteins. Advances in biotechnology have led to the development of improved crops and vaccines, and advances in our understanding of genes and gene expression. One example of a large, high-profile biotechnology project is, of course, the Human Genome Project.

Unfortunately, this explosion in biotechnology has also resulted in misconceptions and controversy, and heightened public concern about food and environmental safety. Genetically modified organisms, "GMOs", is now a term laden with potential for conflict and arguments. Articles abound in the popular press about "frankenfoods" and various advocacy groups around the world question the validity, ethics and safety of biotechnology products. While some researchers and industry personnel may view this as more of a nuisance factor than anything else, it should be recognized that public concerns are a fact of life and it is the responsibility of all those involved in biotechnology to acknowledge and address them. Furthermore, questions raised by such advocacy groups can be used to help gauge the social relevance of our work.

Taking "biotechnology" in the very broad sense of the word can mean different things to diffentent people, and this special session featured several invited speakers whose presentations ran the gamut from vaccines, diagnostic techniques, new aquaculture species, and chromosome manipulation, to environmental issues.

Dr. Julian Thornton is a researcher in fish immunology and also is the Vice-President of Research and Development at Microtek International Ltd. in Victoria, BC. Dr. Thornton addressed the question "Biotechnology: How is it Improving Vaccines for Aquaculture?"

Dr. Diane Elliott is an internationally renowned bacteriologist and Fish Health Research Scientist at the Western Fisheries Research Center, Biological Resources Division of the US Federal Government. She spoke on "The Role of Biotechnology in the Detection and Control of *Renibacterium salmoninarum* Infections in Salmonid Fishes — Promises and Pitfalls".

Dr. Reginald Blaylock is a parasitologist and fish health researcher. He is a faculty member at the Gulf Coast Research Laboratory, University of Southern Mississippi, and one of the researchers involved in a project to investigate the culture of red snapper for stock enhancement. The title of Dr. Blaylock's talk was: "The US Gulf of Mexico Marine Stock Enhancement Program (USGMMSEP): The use of Aquaculture Technology in 'Responsible' Stock Enhancement".

Dr. Tillmann Benfey is a professor at the University of New Brunswick and is one of the pioneers in the development of chromosome manipulation techniques for salmon stocks. Dr. Benfey spoke on "Production of All-Female Populations of Fish for Aquaculture".

Dr. Barry Glickman is a Professor at the University of Victoria and has been investigating the interactions between aquaculture, the environment, and human health at the University's Center for Environmental Health. Dr. Glickman had been scheduled to speak on "Human Health Considerations of Consuming Farmed Salmon". Unfortunately, at the last minute, Dr. Glickman was unavoidably detained and was unable to attend the AAC meeting. Dr. Julian Thornton, who is a colleague of Dr. Glickman's, very kindly stepped in and presented his talk in an abridged format. However, we are not able to provide a manuscript from that talk.

I would like to thank all of the authors who took the time to come to the meeting and present their research in the oral presentations and in these manuscripts. I also thank the organizers of the AAC meeting for inviting me to organize and chair this very interesting and stimulating session, and I am grateful to the National Research Council of Canada for their financial support of the session.

1. Harrison P, Waites G. 1997. *Cassell Dictionary of Science*, Cassell, London, UK. 503 p.

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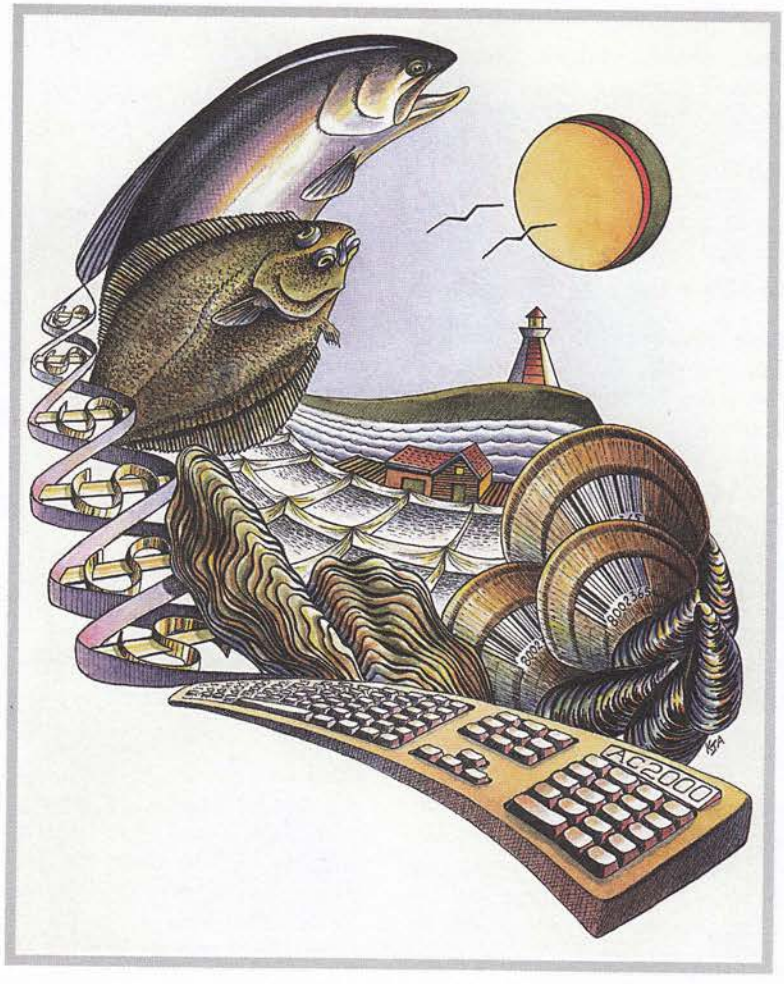

The Role of Biotechnology in the Detection of *Renibacterium salmoninarum* Infections in Salmonid Fishes — Promises and Pitfalls

Diane G. Elliott

The slow growth and fastidious nature of *Renibacterium salmoninarum* in culture has made detection of the causative agent of bacterial kidney disease (BKD) difficult. The development of immunological methods such as the fluorescent antibody test (FAT) and the enzyme-linked immunosorbent assay (ELISA) have facilitated the rapid detection of *R. salmoninarum* in fish. More recently, methods to detect DNA or RNA specific to the pathogen have been developed; these have included a variety of nucleotide probes and polymerase chain reaction (PCR) techniques. Each detection procedure has strengths and weaknesses relative to speed, cost, sensitivity, specificity, quantification of infection severity, detection of infections in tissues other than those sampled, and discrimination of live from dead bacteria.

Introduction

Bacterial kidney disease (BKD) caused by the gram-positive bacterium *Renibacterium salmoninarum* (*Rs*) causes significant problems in salmonid aquaculture, and is among the most difficult of salmonid bacterial diseases to control.[1-3] Chemotherapy has been only partially effective, and no fully licensed vaccines are available for prevention of the disease.

Several factors have contributed to difficulties in controlling BKD. For example, *Rs* is transmitted by two routes: vertically from the female parent to the progeny within the ovum,[4-7] and horizontally from fish to fish.[8-11] The pathogen also can survive and apparently multiply within the host fish's phagocytic macrophages,[12-15] thus enabling evasion of certain normal host defenses. Finally, *Rs* multiplies slowly and is fastidious in its requirement for growth media,[3,16,17] making detection of the pathogen by conventional culture methods difficult. A lack of rapid and sensitive techniques for detecting *Rs* infections has made the development of effective control methods problematic.

Because of such difficulties, *Rs* has been considered an ideal candidate for the testing of biotechnological procedures, such as immunological or nucleic-acid-based techniques, for disease diagnosis. Following is a brief review of some of the most popular or promising biotechnological solutions that are being used or are being considered for diagnosing *Rs* infections.

Criteria for Evaluating Diagnostic Tests

Because of slow growth, *Rs* colonies may not become visible until 6-19 weeks after inoculation of plate cultures with fish tissue samples.[17] For this reason, diagnosticians have long been interested in alternative methods for more rapid and reliable detection of *Rs* infections. As each new test has been developed, there has been a tendency to reject older techniques. Nevertheless, no single ideal diagnostic test has yet been developed. A number of criteria have been used to evaluate diagnostic tests. Some of the more important criteria include: 1) specificity for *Rs*, 2) sensitivity (ability to detect subclinical infections), 3) ability to quantify infection levels, 4) ability to distinguish live from dead bacteria, 5) ability to detect infections in tissues remote from those sampled, and 6) provision of time and cost savings for evaluation of multiple samples. These factors will be considered for the principal diagnostic tests discussed.

Immunological Methods

The first *Rs* diagnostic techniques developed via the application of biotechnology were immunological methods. The premise on which all the immunological methods are based is that antigens unique to the bacterium can be identified, and that antibodies that will specifically bind to these antigens can be produced. Among the many immunological tests developed for *Rs* detection, two — the fluorescent antibody technique (FAT) and the enzyme-linked immunosorbent

assay (ELISA) — have become most widely used for screening fish in aquaculture facilities.

FAT

The FAT, first developed for *Rs* in the mid-1970s, is performed as either a direct[18] or indirect[19] test. In the direct FAT (DFAT), antibody that is conjugated directly to a fluorescent dye is used to detect *Rs* in tissue smears that are examined by UV light with a fluorescence microscope. The indirect FAT (IFAT) first uses unlabeled antibody produced against the antigen of interest. After the smears are incubated with the primary unlabeled antibody, they are incubated with a second fluorescent-labeled antibody that was prepared against the primary unlabeled antibody.

Immunoglobulins used for the FAT and other immunological tests are of two general types, either polyclonal or monoclonal antibodies.[20] Polyclonal antibodies are usually raised in mammals. They are a mixture of immunoglobulins that represent the products of several different clones of antibody-forming cells that responded to different epitopes (binding sites) on the antigen molecule. Monoclonal antibodies are produced by a single clone of antibody-producing cells responding to a single epitope of an antigen. These antibodies are produced in quantity by fusing the desired antibody-producing B lymphocyte with a mutant myeloma cell that does not secrete antibody but proliferates rapidly. The B lymphocyte product provides specificity to a single antigenic epitope, and the myeloma cell confers immortality to the hybridoma. Development of monoclonal antibody production represents a major breakthrough in obtaining a constant source of antibody with the same specificity.

A few specialized FATs have been developed, including a procedure for detection of *Rs* in ovarian fluid or water samples.[21,22] In this test, the membrane filtration-FAT (MF-FAT), bacteria in a fluid sample are first concentrated on a membrane filter by forcing the fluid through the filter, then the bacteria are stained by DFAT or IFAT and observed by fluorescence microscopy. A variation of the MF-FAT has been used for enumeration of bacteria in homogenized kidney tissue.[23,24]

The specificity of the FAT is determined in part by the uniqueness of the epitopes against which the monoclonal or polyclonal antibodies are directed, and the stringency of the antibody screening and purification procedures used.[20] Cross-reaction of non-*Rs* bacterial species has been reported with some antisera used for FATs.[18,24-29] The number of layers in the FAT can also affect specificity. Because an IFAT has more layers than a DFAT, it can provide more opportunities for nonspecific reactions to occur.[30]

Researchers have estimated that about 10^3 *Rs* cells per gram of kidney tissue[31] or 10^4 cells per kidney smear[18] are needed for a positive IFAT or DFAT, respectively. The MF-FAT is more sensitive, allowing the detection of fewer than 10^2 bacteria/mL in ovarian fluid.[21,22] Sensitivity may vary depending on the quality of reagents and the procedures used,[32] and the number of layers in the test. Increasing the number of layers often increases the number of specific binding sites, so that an IFAT may yield brighter fluorescence and therefore increased sensitivity compared to a DFAT.[30] The sensitivity of the FAT also depends on the suitability of samples; whole bacteria must be present for a positive result. Thus, examination of kidney smears by the FAT will not provide a sensitive means of detecting the bacterium in fish with focal infections elsewhere in the body.[33]

Smear FATs of material from homogenized kidney tissues can provide qualitative or semi-quantitative measures of *Rs* infection levels.[32] Although smears of material pelleted from ovarian fluid are not a reliable method for quantification of *Rs* cells,[22,34] the MF-FAT is highly quantitative.[21,22] Counts of bacteria by the MF-FAT correlate well with culture results but are usually slightly higher because the MF-FAT can detect intact dead bacteria as well as live bacteria.[21] A disadvantage of all FATs is that they cannot distinguish live from dead bacteria.

All FATs are labor-intensive and require a fluorescence microscope and sufficient skills for interpretation of results. Although large numbers of field samples can be processed and analyzed by the FAT, the tests have not been highly automated because of the interpretation required.

ELISA

Besides the FAT, the ELISA is the immunological test that has gained the widest use for *Rs* detection in aquaculture. The ELISA for *Rs* is an immunoassay in which soluble *Rs* antigen in tissue or fluid samples binds to specific antibody that has been coated onto a solid substrate such as a plastic tube, microtiter plate well, or bead. After the antigen-antibody reaction and a wash step to remove unbound material, an antibody-enzyme conjugate, which will react specifically with any bound test antigen, is added. After washing, the appropriate enzyme substrate is added. The reaction is usually read as a color change, often by use of a spectrophotometer.

Both monoclonal-antibody-based and polyclonal-antibody-based ELISAs have been developed for *Rs* detection.[35-42] Several of these ELISAs are based on the detection of various epitopes of the major surface-associated protein of *Rs*, a 57-kDa molecule known as p57, which is believed to be a viru-

lence factor.[43-45] Virulent strains of *Rs* produce copious amounts of p57,[46-49] which is released into tissues and circulates in the blood.[37,39,50]

The principles and concerns regarding the specificity of antibodies for the FAT also apply to ELISA procedures. Cross-reactivity with non-*Rs* bacteria has been reported with some antisera used in ELISAs.[28,29,35,37,40] Although such cross-reactivity is more common with polyclonal antisera, cross-reactions with other bacterial species has been noted with certain monoclonal antibodies as well.[51-53]

Some researchers[54,55] have suggested that the p57 protein may be a cross-reactive antigen in immunoassays, based on results from immunoblot (Western blot) analyses. The immunoblot separates antigens by molecular mass as well as reactivity with specific antibody, and has sometimes been used to confirm positive ELISA results.[37,39] The immunoblot results of Bandín et al.[54] and Toranzo et al.[55] demonstrated that polyclonal antisera raised against whole cells of *Rs* cross-reacted with similarly sized proteins from two gram-positive opportunistic fish pathogens, *Carnobacterium piscicola* and *Corynebacterium aquaticum*. However, further experiments[53] showed that a 60-kDa heat shock protein (hsp60), which is structurally and functionally conserved across all phylogenetic groups, comigrated with p57 in electrophoresis gels and was a likely candidate for the previously reported cross-reactivity with p57. These experiments[53] supported the hypothesis that p57 protein is unique to *Rs* and suggested that the reported cross-reactivity with p57[54,55] resulted from a failure to remove problematic nonspecific antibodies from polyclonal antisera by immunoadsorption procedures prior to experimentation. Although it is unknown whether similar cross-reactivity would affect the results of immunoassays other than the Western blot, which requires relatively high concentrations of antigen for a positive test,[29,56,57] these results emphasize the necessity for careful affinity purification and screening procedures for any antibodies to be used in immunoassays.

A principal reason for the development of ELISAs for *Rs* detection was to obtain rapid diagnostic tests that were more sensitive than the FAT. Testing of tissue or blood samples has usually shown greater sensitivity of ELISAs compared with FATs and several other immunological procedures.[38,58-61] Greater sensitivity of ELISAs compared to bacteriological culture also has been reported,[40-42,59] although exceptions have been noted.[57,62] In tissue samples, p57 antigen concentrations as low as 3 ng/g have been detected by ELISA testing.[39]

Several factors can affect the sensitivity of an ELISA. One factor is the type of ELISA system used. Laboratory ELISAs, in which samples are inoculated into microtiter plates and results are analyzed by use of a spectrophotometer, are more sensitive than ELISA field kits, in which samples are placed in test tubes and results are read by visual comparison of test samples to standards.[63] Some comparisons have indicated that field ELISA kits may only be able to detect the most highly infected fish.[64] A second factor that can affect the sensitivity of an ELISA is the type of antibody used. One study suggested that a monoclonal antibody ELISA was less sensitive than a polyclonal antibody ELISA for detecting fish that were positive for *Rs* by culture.[42] A likely explanation for this result is that additional epitopes of *Rs* antigen were recognized by the polyclonal antiserum but not the monoclonal antibody. A third factor that can affect the sensitivity of the ELISA is the type of sample analyzed. Whereas an ELISA can be very sensitive for detection of *Rs* antigen in tissue samples and in blood, some polyclonal antibody ELISAs[65,66] and monoclonal antibody ELISAs[57] lack sensitivity for detecting *Rs* in ovarian fluid of spawning salmonids. Studies demonstrated that a polyclonal antibody ELISA did not reliably detect *Rs* antigen in ovarian fluid samples until MF-FAT counts exceeded about 10^4 to 10^5 bacteria/mL,[65,66] although the reason for this discrepancy was not determined.

Because ELISA absorbance values increase with increasing *Rs* infection levels,[37,39,59,65] laboratory ELISAs are useful for monitoring infection levels in salmonid populations. Nevertheless, the instability of the p57 protein both *in vitro* and *in vivo*[67] makes it impossible to precisely determine the number of *Rs* cells corresponding to a given amount of p57 protein. Thus, laboratory ELISAs are considered to be semi-quantitative tests. Because field ELISA kits reliably detect *Rs* antigen only in severely infected fish,[64] they are best used to rapidly confirm the cause of suspected BKD outbreaks rather than for monitoring changes in infection levels in populations of subclinically infected fish.

No ELISA can distinguish live from dead *Rs*. The persistence of *Rs* antigen can cause problems in the interpretation of ELISA results when management practices such as antibiotic chemotherapy or vaccination are evaluated. For example, in a study of rainbow trout *Oncorhynchus mykiss* injected intraperitoneally with killed *Rs* cells with and without adjuvant, *Rs* antigen persisted at high levels in the fish for more than 110 days after vaccination.[68] This phenomenon made it impossible to distinguish by ELISA the live bacteria of the challenge strain from dead bacteria of the bacterin strain.

One advantage of the ELISA over many diagnostic tests is that it can detect *Rs* infections in tissues other than the one sampled.[59] This is possible because soluble antigen released by the bacterium circulates

throughout the body.[37,39,50] Because localized *Rs* infections can occur in areas such as the head and skin,[69-71] which are not usually sampled for *Rs* detection, the ELISA can be a particularly useful population screening tool.[33]

Another attractive feature of laboratory ELISAs is that they can be used for rapid testing of large numbers of samples, because many of the steps of the procedures have been automated. However, technical expertise and costly, specialized equipment are required for these assays. Field ELISAs do not use costly equipment and require less expertise, but these ELISAs yield less information than the laboratory assays about the *Rs* status of a fish population.

Nucleic-acid-based Detection Methods

The most recent advances in *Rs* diagnostics have come from the field of molecular biology. Nucleic-acid-based diagnostic tests are now gaining favor for the detection of fish pathogens.[72] Among the early tests developed were nucleic acid probes designed to detect specific segments of genomic DNA or ribosomal RNA of *Rs*.[73-75] These techniques have been largely supplanted by a plethora of molecular tools known collectively under the name polymerase chain reaction (PCR, see Erlich[76]). The PCR procedures are considered to have a greater potential for improving both the sensitivity and specificity of *Rs* detection because of the amplification steps incorporated in the tests.[77]

The basis of the PCR technique is the enzymatic amplification of a specific unique DNA fragment or target DNA. The DNA sample is put into a reaction tube with oligonucleotide primers (short synthetic pieces of single-stranded DNA) that exactly match and flank the target DNA. Also in the reaction mixture are dinucleotide triphosphates (the building blocks of DNA), buffers, and a heat-resistant DNA polymerase enzyme. Heating the mixture separates the template strands of DNA. When the mixture is cooled, the primers bind to the complementary strands of the target DNA sequence. At a slightly warmer temperature, the DNA polymerase extends the bound primers in one direction, using the original target DNA as a template. At the end of one such cycle, the DNA count has doubled. At the end of 30 cycles only a few hours later, there will be about a billion copies of the target DNA sequence.[78] The PCR products are analyzed by gel electrophoresis and ethidium bromide staining, and gels are observed with UV light for the presence of the appropriate products.

Various PCR procedures have been developed for detection of *Rs* in kidney tissue,[77,79-83] ovarian fluid,[66,81-84] leukocytes,[80] blood,[82] whole fry,[82] and eggs.[28,82] Some of these methods have included modifications to the basic PCR technique to improve performance.

For example, improved sensitivity has been obtained by a 2-step or nested amplification of specific nucleic acid sequences.[77,81] For nested PCR, the first set of primers is used to amplify a nucleic acid sequence that includes an internal target DNA sequence. The PCR product from the first round reaction is used as a template with a second set of primers that will amplify only the target DNA sequence. In addition to increasing the sensitivity of the PCR, the internal primers used in the second round of amplification act as an internal control by confirming the presumptive product of the initial amplification.[77]

Several PCR techniques, called reverse transcription PCR (RT-PCR), are designed to detect the transcriptional products of a gene (RNA) rather than DNA. Certain of these techniques have been developed to detect specific unique sequences of the 16s subunit of ribosomal RNA.[82,84] Primers targeting variable regions of 16s rRNA are used in phylogenetic studies to discriminate and identify bacterial species,[85,86] but they can also be useful for diagnostic tests. The high copy numbers (10^3 to 10^4 copies per cell) of the rRNA target sequences increases the sensitivity of this PCR.[82] For RT-PCR, a DNA copy complementary to the target rRNA is produced enzymatically by reverse transcription. This DNA copy is then amplified by PCR; nested procedures can be used.

A nested RT-PCR technique has been developed to detect specific sequences of messenger RNA of *Rs*.[83] Because mRNA has a very short half-life often measured in minutes, its detection decreases relatively quickly after the loss of bacterial cell viability. Thus, the RT-PCR for mRNA is designed to detect viable (or recently killed) cells of *Rs*.

If a unique nucleic acid sequence has been chosen for amplification, PCR procedures have shown high specificity for *Rs*.[29,77,79,80,82-84] Nevertheless, because PCR can detect very few molecules of target sequence, and because a typical PCR reaction generates 10^{12} molecules of DNA in a 0.1-mL volume, contamination of samples resulting in false positive results can be a serious problem.[87] Strict precautions must be exercised to prevent the physical transfer of DNA between amplified samples, and between positive and negative experimental controls.

Most PCR techniques developed for *Rs* are very sensitive for detecting the pathogen in a variety of sample types. Direct comparisons of samples from naturally infected fish by ELISA, FAT, and PCR testing indicated that the PCR was more sensitive than both of the other tests for detection of *Rs* in kidney tissue[77] and ovarian fluid.[66] The sensitivity of PCR for *Rs* also has been estimated to be equal or greater to that of bacteriological culture.[80,81,83] Estimated detection limits for

Table 1. Summary of criteria for evaluation of some diagnostic tests for *R. salmoninarum*.

Criterion	Diagnostic Test					
	Culture	FAT	MF-FAT	Field ELISA	Laboratory ELISA	PCR
Specificity	Low/High[1]	Moderate	High	Low	Moderate/High[2]	High
Quantitative	No/Yes[3]	Semi-quantitative	Yes	No	Semi-quantitative	No
Distinguish live/dead bacteria	Yes	No	No	No	No	No/Yes[4]
Detect remote infections	No	No	No	No/Yes[5]	Yes	No
Nonlethal samples	Yes[6]	No	Yes[6]	No	Yes[7]	Yes[6,7]
Rapid test (≤ 2 days)	No	Yes	Yes	Yes	Yes	Yes
Time/cost savings for multiple samples	No	No	No	No	Yes	No
Specialized equipment	No	Yes[8]	Yes[8]	No	Yes[9]	Yes[10]
Technical expertise	Low	Moderate	Moderate	Low	High	High
Commercial reagents	Yes	Yes	Yes	Yes	Yes	Custom

[1] The presence of other organisms in samples can reduce the detection of *R. salmoninarum*.
[2] Limited experimentation has shown higher sensitivity for a polyclonal antibody ELISA compared to a monoclonal antibody ELISA.
[3] Spread plate cultures can be quantitative.
[4] Only the RT-PCR for mRNA can distinguish live from dead bacteria.
[5] The ability of field ELISAs to detect remote infections has not been determined; high antigen levels in infected tissue would be required for a positive result.
[6] Ovarian fluid can be used as a nonlethal sample.
[7] Blood can be used as a nonlethal sample.
[8] A fluorescence microscope is required.
[9] Specialized equipment includes a microtiter plate reader (spectrophotometer, often attached to a computer). A reagent dispenser and microtiter plate washer are essential for ELISA analyses involving large numbers of samples.
[10] Specialized equipment for basic PCR includes a thermal cycler, gel electrophoresis system including power supply, and a UV gel viewer and gel documentation system.

PCR procedures vary depending on the PCR procedure and the method used for quantification of bacteria in samples.[82] Rhodes et al.[82] calculated that their RT-PCR for 16s rRNA could detect 5 *Rs* cells/mg (wet weight) of whole fry, and 10 cells/mg (wet weight) in kidney, blood, or egg samples. These authors calculated the sensitivity to be between 190 and 1000 *Rs* cells/mg of kidney tissue for non-nested PCR procedures for genomic DNA reported by other authors [80, 81] Cook and Lynch[83] reported the *Rs* detection limits for a non-nested PCR for genomic DNA and a nested RT-PCR for mRNA to be between 4 and 40 cell equivalents/mg and 7 and 70 cell equivalents/mg, respectively, in seeded kidney tissue. For some PCR assays, the presence of kidney tissue (but not ovarian fluid) may reduce the sensitivity of the test.[81,84]

Current PCR techniques for *Rs* detection are not considered quantitative.[66] However, quantitative PCR methods have been developed for human health applications. Perhaps the most promising techniques are real-time quantitative PCR procedures (see Heid et al.,[88] Gibson et al.,[89] and Haugland et al.[90]). These procedures use a non-extendable oligonucleotide hybridization probe labeled with a reporter fluorescent dye at one end and a quencher fluorescent dye at the other end. The probe hybridizes to the target template at a site between the two primer recognition sequences. When the probe is intact, the reporter dye emission is quenched because of the physical proximity of the reporter and quencher dyes. During the extension phase of a PCR cycle, however, the hybridization probe is cleaved by the nucleolytic activity of the DNA polymerase, resulting in the release of the reporter dye from the probe. The use of an automated sequence detector allows real-time monitoring of reporter dye fluorescence emission and accurate quanti-

fication of target nucleic acid sequences. An additional benefit of real-time quantitative PCR is that it eliminates post-PCR manipulations of samples and thus reduces the potential for contamination.[88]

With the exception of the RT-PCR for mRNA,[83] no PCR for *Rs* detection can distinguish viable from nonviable bacteria.[81-83] Research has demonstrated that target DNA of nonviable or nonculturable *Rs* cells can be detected by PCR.[83] Nonviable bacterial cells can be detected by PCR as long as intact target DNA or rRNA sequences are available.[91,92] The detection of nucleic acid from nonviable *Rs* cells may cause false-positive results when populations of fish undergoing antibiotic chemotherapy are evaluated for BKD status.[81]

The ability of PCR to detect *Rs* infections localized in sites remote from the tissue sampled has not been demonstrated. Evidence suggests that differences in detection rates by PCR in different sample types (e.g., kidney and ovarian fluid) reflect the unequal distribution of *Rs* cells in those samples.[81] Both DNA and RNA have limited chemical stability.[93] Even though target DNA may be detectable by PCR for several weeks after bacterial cells are killed,[91,94] one study showed that target DNA sequences from nonviable cells of several gram-negative human pathogens were detectable by PCR only as long as whole bacterial cells were detectable by acridine orange staining.[91]

Despite the automation of portions of PCR procedures, equipment capacity limitations and the number of manipulations required make PCR impractical for assaying large numbers of samples.[82] In addition, PCR is unsuitable as a field test because of the expertise necessary and the extreme care that is crucial for avoidance of contamination. Nevertheless, the sensitivity of PCR makes it useful as a confirmatory test for large-scale screening procedures such as ELISA.

Summary

Although the development of immunoassays and nucleic-acid-based tests has provided rapid and sensitive methods for *Rs* detection, these techniques have not completely replaced conventional culture methods. Each detection procedure has strengths or weaknesses relative to specificity, sensitivity, quantification of infection levels, ability to distinguish live from dead bacteria, detection of infections in sites remote from tissues sampled, and provision of time and cost savings for testing multiple samples (Table 1). Other criteria, such as specialized equipment needs, availability of commercial reagents, technical expertise required, and the time required to complete a test, can be important in a particular diagnostic situation (Table 1). Because no single ideal diagnostic test has been developed, a combination of tests may be necessary to obtain the desired information about the *Rs* infection status of a fish population.

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Production of All-Female Populations of Fish for Aquaculture

T. J. Benfey, D. J. Martin-Robichaud, C. I. Hendry, C. Sacobie, H. Tvedt and M. Reith

Females of many of the fish species used or proposed for aquaculture in Atlantic Canada have the potential to be more valuable than males because, depending upon the species in question, they have faster growth rates as juveniles, are older at sexual maturity, reach a larger ultimate size, and/or are a source of roe or caviar. In comparison to traditional terrestrial livestock species, it is relatively simple to produce all-female populations of fish through genetic (gynogenesis), endocrine (steroidal), and/or environmental (thermal) manipulations. Recent advances in molecular biology, such as microsatellite markers and sex-specific genetic markers, have facilitated the application of these technologies to aquaculture. This paper reviews the methods, and updates the status, of sex control research and development in local (Atlantic Canada) aquaculture species.

Introduction

Females have clear advantages over males for the commercial culture of many fish species utilized or proposed for aquaculture in Canada. The reasons for this include faster growth as juveniles (flatfish), delayed sexual maturation (flatfish and salmonids), larger ultimate size (flatfish, salmonids, and eels), and roe/caviar production (sturgeon, lumpfish, and salmonids). Compared to terrestrial livestock species, fish are remarkably amenable to genetic, endocrine or environmental manipulations leading to the production of single-sex populations.[1] The production of all-female salmonid populations was pioneered in Canada,[2] but little research has been conducted on producing all-female populations of non-salmonid species for aquaculture in Canada. This paper presents a brief overview of the methods used for controlling sex ratios of cultured fish populations, with an emphasis on species relevant to aquaculture in Atlantic Canada.

Gynogenesis

The term 'gynogenesis' describes a process whereby embryonic development is initiated without the incorporation of a functional paternal genome. In fish, gynogenesis is generally induced by treating spermatozoa in such a way as to destroy or inactivate their genomic DNA without affecting their ability to swim and penetrate the egg in order to activate development. Both gamma (γ) and ultraviolet (UV) radiation can be used effectively to inactivate fish spermatozoan DNA:[3] γ-radiation by physically destroying chromosomes, and UV-radiation by causing conformational changes in DNA molecules that prevent their duplication. Of the two, UV-radiation is generally preferred because it is much safer, and therefore easier, to work with. However, because of UV-radiation's weak penetrating power, sperm must generally be diluted, spread in a thin layer, and mixed during UV-exposure. Optimum γ- or UV-exposures must be sufficiently high to completely inactivate the paternal genome carried within the spermatozoa, but no so high as to affect spermatozoan swimming ability. For UV-treatments, optimum exposures are in the range of 103 to 104 ergs/mm^2; for instance, we have found that gynogenetic Atlantic halibut, *Hippoglossus hippoglossus*, can be produced from eggs activated by sperm diluted 1:80 in halibut seminal plasma and exposed to 6.5 - 8.6 x 10^3 ergs/mm^2 of UV-radiation.[4]

Gynogens produced by egg activation with radiation-inactivated spermatozoa will be haploid, carrying a single set of maternal chromosomes. Haploid gynogens generally survive through most of embryonic development, but show characteristic abnormalities ('haploid syndrome') and usually die before yolk absorption is completed. Viable gynogens can be obtained by making diploids of such haploids, either by retaining the haploid second polar body which is normally extruded from the egg shortly after fertilization, or by blocking the first mitotic cell division of the zygote.[3] Extrusion of the second polar body represents the completion of meiosis, so these two types of diploid gynogens are referred to as 'meiogyns' and

'mitogyns', respectively. Temperature and hydrostatic pressure treatments can be used effectively to produce meiogyns and mitogyns, using treatments identical to those which would yield triploids and tetraploids, respectively, after normal fertilization with genetically-intact spermatozoa.[3] Hydrostatic pressure treatments are preferred because it is easier to ensure a uniform treatment of all eggs. Optimum pressure treatments are in the range of 7-10 x 10^3 psi for 5 minutes; for instance, we have produced triploid and gynogenetic diploid (meiogyn) Atlantic halibut using pressure treatments of 8.5 x 10^3 psi for 5 minutes.[4]

The sex ratio of gynogens depends on the genetic mechanism of sex determination. Salmonids have the typical mammalian system of homogametic females (XX) and heterogametic males (XY); in such a system, gynogens are always female, as has been demonstrated with Atlantic salmon, *Salmo salar*.[5] Other species have the typical avian system of heterogametic females (WZ) and homogametic males (ZZ); in such a system, gynogens will be either 'super-females' (WW) or normal females and males. All-female populations can then be produced by crossing super-females with normal males.

Distinguishing normal diploids from triploids and gynogenetic diploids at early stages of development can be difficult. Recent advances in molecular biology have, however, addressed this problem. Specifically, parent-specific genetic markers (microsatellites) can be used to confirm the presence or absence of the paternal genome in individual fish. Such microsatellite markers have been developed for Atlantic halibut,[6] and have proven to be very useful for the early determination of success in producing gynogens.[4]

Sex Steroids

Independent of the genetic mechanism of sex determination, the actual differentiation of gonads into ovaries or testes is mediated by endogenous sex steroids, with estrogens and androgens having feminizing and masculinizing effects, respectively.[7] The process of gonadal differentiation can easily be overridden by the exogenous application of sex steroids at the appropriate developmental stage, i.e., before differentiation has been completed.[1,2] Steroids are inexpensive and easy to administer either by immersion or feeding. For immersion, steroids are first dissolved in ethanol and then diluted in a water bath for static immersion. Immersions are generally of short duration (one to a few hours), and may be repeated several times. In fish that will accept an artificial diet during the treatment period, steroids can be dissolved in ethanol and sprayed on the diet, leaving the ethanol to evaporate before the food is used. If fish require a live diet, it is also possible to treat the prey with steroids, and then feed these steroid-laden prey to the fish. Estrogens have been used to produce all-female populations in several species of fish, including Atlantic salmon using estrogen-treated artificial diets[8] and lumpfish, *Cyclopterus lumpus*, using estrogen-enriched brine shrimp.[9] We have recently produced Atlantic halibut populations with a 3:1 female bias by feeding them an artificial diet containing 10 ppm 17β-estradiol for 45 days.[10]

Sex steroids must be handled with care to protect both the user and the environment. Not surprisingly, there is public concern over the sale of steroid-treated fish for human consumption. To circumvent this problem, 'indirect feminization' can be used, whereby hormonally-masculinized genetic females ('neo-males') are used as broodstock to yield all-female populations when crossed to normal females. Indirect feminization works well in salmonid aquaculture[11-13] because of the XX/XY mechanism of sex determination in these species.The male-determining genes are thus excluded in crosses between normal females and neo-males. Although broodstock fish have been treated with steroids, the actual all-female populations destined for human consumption are one generation removed from steroid treatment. Among the salmonids, charrs (*Salvelinus* spp.) have proven to be most difficult to masculinize.[14] However, we have recently produced male-biassed brook charr (*S. fontinalis*) populations by immersion in 17α-methyltestosterone (MT; 3 hours of 100 or 800 μg/L once per week for 5 weeks).[15] We have also produced all-male Atlantic halibut populations by feeding them a diet containing 1 or 5 ppm 17α-methyldihydrotestosterone (MDHT) for 45 days.[10] However, we do not yet know whether Atlantic halibut have the XX/XY sex-determining mechanism. In the case of the WZ/ZZ system, neo-males will produce equal proportions of W-chromosome- and Z-chromosome-bearing spermatozoa; a cross between a normal female and a neo-male will thus yield a population of 25% superfemales, 50% normal females and 25% normal males. As is the case with gynogenesis, such superfemales can be crossed with normal males to yield all-female populations, which in this case are two generations removed from steroid treatment.

The separation of normal and sex-reversed fish is often difficult, and is also greatly facilitated by the use of molecular techniques. In this case, the development of sex-specific genetic markers has been invaluable for the separation of neo-males from normal males in some salmonid species.[16] However, in spite of considerable effort, such markers have remained elusive in Atlantic salmon and Atlantic halibut.

Steroidogenic Enzyme Inhibitors

The endogenous production of the sex steroids involved in gonadal differentiation in fish is dependent upon the actions of key steroidogenic enzymes.[7] Rather than applying sex steroids to override these natural steroidal signals, it is thus also possible to interfere with steroid production through the use of steroidogenic enzyme inhibitors. The administration of aromatase inhibitors, for instance, can be used to create all-male populations.[17] Aromatase is the enzyme responsible for the *in vivo* conversion of testosterone to 17β-estradiol, a potent feminizing steroid during natural gonadal differentiation. By inhibiting 17β-estradiol synthesis, aromatase inhibitors block ovarian differentiation with the result that such fish develop testes. Such neo-males can then be incorporated into breeding programs for the production of all-female populations, as already described. Although still making use of chemical treatments, the use of steroidogenic enzyme inhibitors gets around concerns over the use of sex steroids in aquaculture.

Temperature

All enzymes are temperature sensitive, although the range of temperatures at which given enzymes are functional varies greatly. In many species of reptiles, steroidogenic enzymes are temperature sensitive within the range of temperatures at which eggs are incubated, with the result that such species have temperature-dependent sex determination (i.e., the sex ratio of a given batch of offspring is dependent upon the egg incubation temperature).[18] Temperature-dependent sex determination also occurs in a variety of fish species,[19] suggesting that steroidogenic enzymes may also be influenced by incubation temperature during gonadal differentiation in such species. The use of thermal manipulations to alter sex ratios, if effective, would represent the most 'environmentally-friendly' technique available for the production of all-female populations for aquaculture, since it involves no genetic or endocrine manipulations. Initial attempts to produce all-female American eel, *Anguilla rostrata*, populations by thermal manipulations were, however, unsuccessful.[20]

The research conducted by Chris Hendry and Harald Tvedt on halibut sex control is funded through an NSERC Strategic Project Grant, while that of Charles Sacobie on brook charr sex control is funded by the Woodstock First Nation.

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The US Gulf of Mexico Marine Stock Enhancement Program (USGMSEP): The Use of Aquaculture Technology in "Responsible" Stock Enhancement

R. B. Blaylock, K. M. Leber, J. M. Lotz, K. C. Stuck, and D. A. Ziemann

The USGMSEP is investigating the use of aquaculture technology as an additional tool for the restoration of depleted marine stocks, particularly the red snapper, *Lutjanus campechanus*. Molecular genetics, feed production technology, and larval production technology are used to address three critical stock enhancement issues: 1) understanding the nature of the system to be enhanced, 2) producing robust, compatible individuals for release, and 3) critically evaluating the effects of releases. The program has developed 1) microsatellite and mitochondrial DNA markers that allow population analysis, broodstock analysis, and offspring analysis; 2) a method of copepod culture that allows for mass production of red snapper; and 3) a manageable, repeatable procedure for larval rearing of large numbers of red snapper, a species heretofore unculturable in large numbers.

Introduction

Worldwide, marine fish populations are in decline. Some predict that the current capture fisheries (while relatively stable over the last 15 years) are unsustainable past the year 2040.[1] Population trends for the 15 most commercially important species in the US indicate that about half of those are declining.[2, 3] Five fish species in the Gulf of Mexico, including the red snapper (*Lutjanus campechanus*), are listed as overfished by the National Marine Fisheries Service (NMFS).

The Magnuson-Stevens and Sustainable Fisheries Act require that plans for restoration of overfished species be put in place. Historically, there have been four approaches to deal with the problem. Two methods (fishing regulation and habitat protection) form the basis for most of the current approaches. Production aquaculture is a third approach. Stock enhancement constitutes a fourth approach. Fishing regulation has had limited success.[4] Habitat management/restoration has shown some promise,[5, 6] but is yet unproven as a long-term, large-scale solution. Production aquaculture has been hugely successful on a worldwide basis and now accounts for about 25% of the world's food fish production.[1] Stock enhancement, a combination of aquaculture technology and release of cultured fish into the wild, was, in fact, the technique of choice in marine fisheries management in the nineteenth and early twentieth centuries. However, a century of enhancement activities produced little evidence of effectiveness.[7] Further, concerns over maladaptive behaviors, artificial genetic selection, and disease problems in cultured fish created skepticism about the desirability of stock enhancement.

In the face of growing concern over the continued decline of managed stocks, Blankenship and Leber,[7] citing studies in Japan,[8, 9] Norway,[10] and Hawaii,[11] revived the idea that marine stock enhancement was possible through carefully planned research. Their paper outlined ten essential components of a "responsible" enhancement program that, once the species in question and management goals are determined, can be distilled into three critical issues: 1) understanding the nature of the system, 2) producing robust, compatible individuals for release, and 3) evaluating the effects of releases.

The U.S. Gulf of Mexico Marine Stock Enhancement Program (USGMSEP) is a research consortium consisting of the Gulf Coast Research Laboratory, Ocean Springs, MS; Mote Marine Laboratory, Sarasota, FL; and the Oceanic Institute, Waimanalo, HI. It is investigating the use of aquaculture technology as a tool that could operate in conjunction with traditional fisheries management techniques to restore depleted marine stocks, particularly the red snapper (*Lutjanus campechanus*), the primary foodfish in the Gulf of Mexico.[12] In this paper, we will focus on how our program uses aquaculture technology, specifically molecular genetics, feed production, and larval production to produce red snapper that

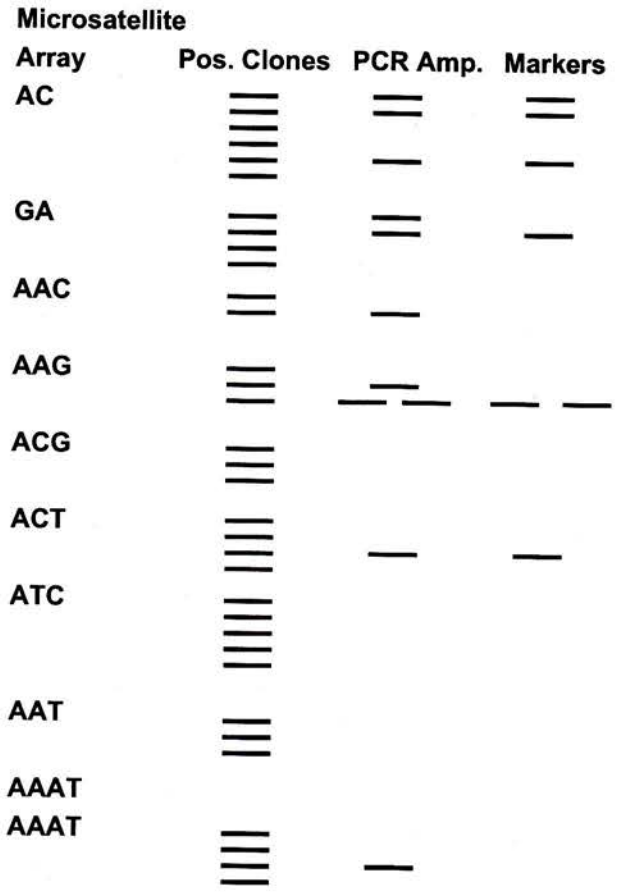

Figure 1. Microsatellite DNA arrays in red snapper, *Lutjanus campechanus*. Clones positive for the various arrays are represented in the first column, successfully amplified arrays are represented in the second column, and clones with polymorphisms sufficient to serve as population markers are represented in the third column.

will be used to address Blankenship and Leber's three critical stock enhancement issues.

The Program

Molecular genetics

Enhancement programs have come under increasing scrutiny during the last 10-15 years due to concerns over artificial or domestication selection in cultured animals and the resulting genetic risks to wild stocks.[13] Busack and Currens[14] concluded that some degree of selection is inevitable in cultured animals; therefore, potential impacts must be identified and, if possible, mitigated through proper selection of broodstock, robust mating protocols, natural rearing conditions, and "wild-fish- friendly" release strategies.[15] Our genetics program is designed to develop genetically sound breeding and release procedures to minimize insofar as possible the genetic impacts on receiving populations. This can be accomplished through the development of molecular markers that are tracked in both donor and recipient populations. We have developed two kinds of molecular markers that will allow us to acquire baseline data on the wild population, characterize broodstock, and conduct parental analysis of offspring.

Microsatellite DNA

Microsatellite markers consist of repeated arrays of non-coding nucleotides that exist as distinct alleles at a single locus in the nuclear genome and are inherited in a Mendelian fashion.[16] A size-selected genomic li-

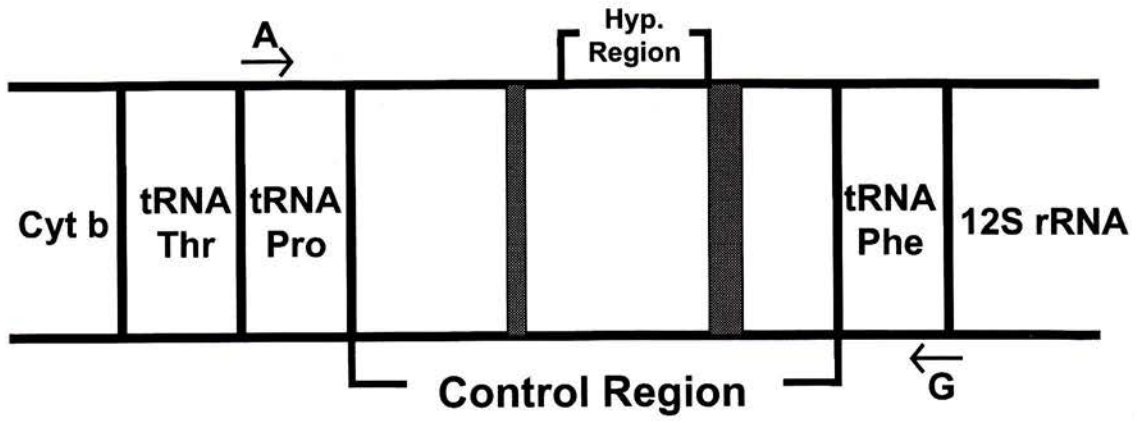

Figure 2. Representation of the mitochondrial DNA control region in red snapper, *Lutjanus campechanus*. Previously published primers used to amplify the region are shown. The hypervariable region, which is sufficiently variable to serve as a population marker, is indicated.

brary was screened for the presence of ten di-, tri-, and tetra-nucleotide microsatellite arrays. The most frequently occurring clones were those that contained the di-nucleotide repeat AC (6 clones) and the tri-nucleotide repeat ATC (5 clones) (Fig. 1). Thirty four clones were sequenced, 10 of which (1 clone contained 2 microsatellites) yielded sufficient flanking sequence to design PCR primers that successfully amplified the entire array (Fig. 1). The primers were then used to amplify each of the 11 microsatellites from 20 fish collected from several locations in the northern Gulf and Atlantic Florida coast. Six of the 11 loci examined displayed polymorphisms at a level to be considered useful as a genetic marker. Using the combination of the microsatellite markers and recently published PCR primer sequences,[17] we now have a sufficient number of single-locus microsatellites available to use for population structure analysis, broodstock characterization, and parental analysis of offspring.

Mitochondrial DNA

The mtDNA genome of fish is a 16.5 kb, closed-circular piece of DNA containing 13 genes coding for proteins, 2 genes coding for ribosomal RNAs (small 12s and large 16s RNA), 22 genes coding for tRNAs, and a major non-coding AT-rich region that contains the initiation sites for mtDNA replication and RNA transcription. This region, often called the control region or D-loop, has a high mutation rate compared to the nuclear genome as well as other mtDNA regions, and it has proven suitable for many population genetics studies with fish.[18]

The control region is immediately flanked by sequences encoding two tRNAs (threonine-proline) and cytochrome b, and by additional sequences encoding tRNA (phenylalanine) and the small 12s rRNA (Fig. 2). The entire control region was amplified using primers located in tRNA-Pro and tRNA-Phe, respectively. The appropriate PCR product was gel-purified, quantified, and cloned. Blue/white selection was employed to screen for inserts. Plasmids from white colonies were purified and screened for inserts by EcoRI-digestion followed by agarose gel electrophoresis. Clones were quantified and sequenced. To obtain flanking tRNA sequences, species-specific primers were designed in the control region and coupled with other published primers in adjacent genes[19] to amplify DNA fragments that contained either the tRNA-Phe or the tRNA-Thr and tRNA-Pro. Appropriate PCR products were electrophoresed, purified, cloned, and sequenced. Sequencing was conducted either at the University of Maine DNA Sequencing Facility or at the Gulf Coast Research Laboratory using automated sequencers. To verify that specific target mtDNA was being amplified rather than nuclear pseudogenes, nested PCR with mtDNA primers published in a previous study[20] were also used to successfully amplify a portion of the control region. Secondary structures of the tRNAs Thr, Pro, and Phe were elucidated and DNA sequences were imported into a multiple sequence editor and aligned.

Using the control region sequence obtained from 27 red snapper collected from several geographically separated sites from the northern Gulf and Atlantic coast of Florida, we determined a consensus sequence for the control region. From this information, a ~300

Table 1. The effect of the inoculation of filtered copepodids and adult copepods into ponds as they are refilled. Treatments consisted of either a ½ water change using ambient "brown" water or a ½ water change with the inoculation.

Container / Experiment	Treatment	Lag Time (days)	Nauplius Density (#/L)
Pond 2	Water change	15	140
Pond 4	Water change	12	245
Pond 3	Water change, inoculation	5	424
Pond 1	Water change, inoculation	3	263
Pond 1 (2nd exp.)	Water change, inoculation	2-5	126-140
Pond 2 (2nd exp.)	Water change, inoculation	4	209

base-pair segment of the control region, which contains a high proportion of variable sites (hypervariable region), was identified. PCR primers flanking this hypervariable site were constructed and used to produce a PCR fragment that could be easily direct sequenced. Preliminary results on more than 100 fish from the target population have shown that the hypervariable region contains sufficient polymorphisms to be useful as a genetic tag.

Feed production

Culture of members of Lutjanidae (more generally known locally as snappers) has been limited by the ability to determine and supply appropriate larval foods. Doi and Singhagraiwan[21] showed that copepod nauplii were important for the culture of lutjanid species. Schipp et al.[22] reported 40% survival (at day 21) of *L. argentimaculatus* fed on cultured copepod nauplii. Bootes[23] successfully grew 283 red snapper juveniles only after rearing in a tank of bloomed zooplankton supplemented with wild zooplankton. Therefore, it seems that lutjanids, including the red snapper, require copepod nauplii rather than easily cultured brine shrimp and rotifers as an initial food.[22,24] Copepod mass culture technology, unfortunately, is a young and inexact science. Copepod culture systems previously had required complicated, labor intensive tank systems, which included separate algal culture facilities or fertilization.[22,25] Further, many systems required terminal harvesting[22,26] and were susceptible to population crashes.[25] Ogle[27,28] showed that copepods could be cultured in our facility using a simple "brown-water" method; therefore, we investigated how to "scale-up" production of nauplii to accommodate continuous mass production of fish while minimizing cost and labor using modifications of the "brown-water" method. We investigated closed system culture as well.

Table 2. Closed system production of copepod nauplii compared to pond production.

Container	Maximum Density (#/L)	Mean Density (#/L)
Raceway – aerated	94	47
Raceway – not aerated	123	48
Pond 2	209	69
Pond 4	339	168
Pond 3	424	183
Pond 1	268	143

We showed that inoculating the ponds with the filtered adults and copepodids (from the nauplius harvest) as the pond is refilled with ambient bay water increased the nauplius density in a considerably shorter time than natural blooming (Table 1). We also showed that by filtering only about half the pond in any given harvest, we could maintain continuous production presumably by maintaining a variety of life history stages.

Our refined technique pumps ambient "brown" water from Davis Bayou, Mississippi Sound, through a 300-µm mesh into pairs of 75 m^3 tanks. After being allowed to settle for 1 day,

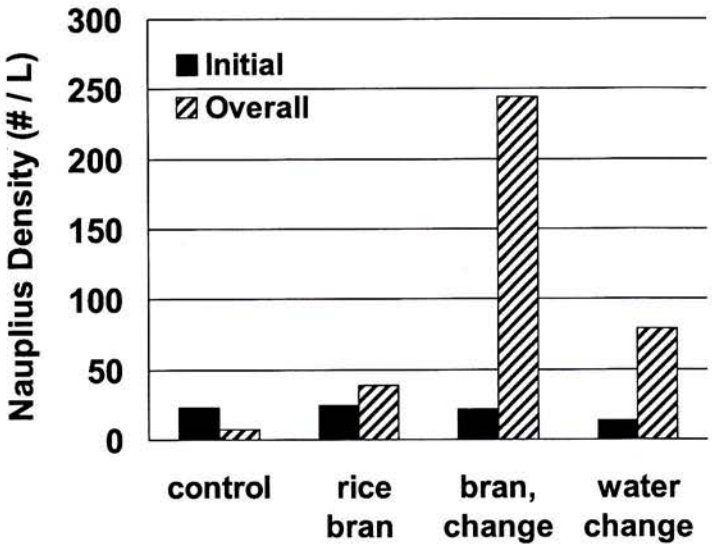

Figure 3. Closed system copepod nauplius production under different feeding regimes.

half of each tank is filtered through a mesh designed to retain adult and copepodid stages of copepods. That filtrate is subsequently filtered through a variably sized mesh to retain nauplii of the desired size fraction. Adults and copepodids are returned to the production tanks and the tanks are refilled with ambient "brown" water. Harvested nauplii are fed to red snapper larvae. Four tanks (2 pairs) provide continuous production on alternate days. Production tanks (75 m^3) produced an average of 3.7 million nauplii/day (range 616 000 – 12.5 million) with an average concentration of 139/L (range 4.5 – 424). Typically, the system produces either *Acartia tonsa* or *Pseudodiaptomus pelagicus*. This system produces enough nauplii to provide 22 500 red snapper larvae with 2000 copepod nauplii/L each day.[29]

Closed system culture (raceways) produced copepod nauplii but at a lower density than the ponds (Table 2). Experience suggests that raceways may not support the nauplius exploitation rate supported by the ponds. Ponds can support as much as 25% daily exploitation. Based on a limited experiment, aeration appears to make no difference in raceway production. Data suggest that water exchange is a critical component of production. Moreover, the data suggest that production might be greatly improved by fertilization with inexpensive rice bran (perhaps in ponds as well) (Fig. 3). So far, the closed system has not worked well, but it deserves further consideration because of potential benefits such as predictable, year-round production and isolation from disease organisms in the wild.

Larval culture

Production of a new aquaculture species such as the red snapper is constrained by lack of knowledge of larval biology, developmental processes, water quality tolerances, and disease issues. In general, culture of lutjanids has developed in the last 10-15 years only. Previous attempts at culturing other lutjanid species

Table 3. Larval red snapper rearing conditions (1998 data). Recirculation, filtration, and siphoning treatments were fed at the 5 nauplii/mL rate.

Treatment	Number Stocked*	Percent Survival at 26 Days
Recirculation	1000	2.2
Filtration	1000	7.5
Siphoning	1000	7.6
Fed 5 nauplii/mL	1000	12.5
Fed 20 nauplii/mL	1000	30.4

* Stocked into a 200-L tank. Density of larvae had no effect.

Table 4. Mortality and survival of red snapper in 1999 production.

	Discrete Mortality (%)	Cumulative Survival (%)
Hatchery	93.3	6.7
Handling	7.8	5.4
Nursery	51.3	3.4

have provided mixed results. Lim et al.[29] (using *L. johnii*) and Emata et al.[31] (using *L. argentimaculatus*) reported 1-5% survival with 2 major periods of mortality, whereas Watanabe et al.[32] reported 10-20% survival, with chronic mortality occurring throughout culture of *L. analis*. Watanabe et al.[32] hypothesized that the differences in survival rates among the studies may be related to the different feeding regimes in the studies. They also noted that innate species-specific differences probably were involved. The only previous successful attempt at red snapper culture[23] produced 283 fish (at 30 days) with an average survival of 1.8%. Our program is working to identify and solve some of the technical problems constraining production of red snapper.

Briefly, we capture wild adult fish, inject them with HCG, and strip the resulting gametes about 24 hr post-injection. Eggs and milt are mixed, and larvae are hatched in a hatching chamber. To date, larvae have been produced at the Claude Poteet Mariculture Center, Alabama Department of Natural Resources, then transported to the Gulf Coast Research Laboratory. Upon arrival, following the methods of Ogle et al.,[33] larvae are stocked into 1000-L rearing tanks containing 250 L of settled, chlorinated, dechlorinated, and salinity-adjusted (35 ppt) water at a density of 40/L. Copepod nauplii (typically *Acartia tonsa* or *Pseudodiaptomus pelagicus*) (28-68 μm in size) are titrated to a density of 2.5/mL (avg. 1.8/mL) by day 3. Beginning on day 5, aliquots of water are added daily to result in a total volume of 1000 L and a larval density of 10/L by day 12. Copepod density is maintained but with increasing size fractions over time (28-125 μm from days 5-7, 68-125 μm from days 7-9, and 68-200 μm from days 9-23). *Artemia* nauplii are offered to the larvae beginning on day 12, titrated to a final density of 10-20/mL by day 14, and maintained until day 35. On day 23, larvae are harvested, counted, and transferred into the nursery where they are maintained until release (3-6 months). In the nursery, they are offered commercial pellets (Moore-Clark mahimahi diet, Moore-Clark, Vancouver, BC) and weaned off *Artemia*.

The primary rule that we have determined is that you must feed the larvae and LEAVE THEM ALONE. Rearing condition experiments indicate greater survival in undisturbed tanks (Table 3). Experience also suggests that larvae do not do well in tanks smaller than 200 liters. We now routinely use 1000-L tanks. The data suggest that high food density may be important (Table 3), but we have never reached the highest densities achieved in the experiments in the mass production program. The lower density of food in the mass production program may partly explain our pattern in mortalities. Some larvae fail to initiate feeding and die early, but because the larvae are so small in such a large volume (and because we have been unwilling to terminate the experiments for sake of production) we can not exactly quantify this mortality event. In general though, larvae do exceptionally well until about day 19, at which point they begin dying. By day 23, over 90% of the larvae are dead (Table 4). We cannot explain this event. Histologically, this appears to be a time of major organogenesis; thus perhaps this is a normal mortality event. On the other hand, perhaps this is related to improper or inadequate nutrition in the early developmental stages. At day 23, the larvae are harvested, counted, and transferred to the nursery. Aggressive behavior then becomes a significant source of mortality. In fact, about half of the larvae entering the nursery will die due to aggressive behavior (Table 4). So far, grading is ineffective in controlling this mortality because the grading process itself produces mortality. So, by the end of 3 months, there is only 3.4% survival (Table 4), but at least we have identified the major problems. Experiments planned for this year may elucidate the cause of the 19-day mortality. We continue to develop an effective grading procedure. This year we will try to reduce the aggressive behavior in the nursery by overcrowding the fish. Some research suggests that overcrowding may reduce aggression.

Other constraints include susceptibility to infection with the parasitic dinoflagellate *Amyloodinium ocellatum* and water quality. In 1998, we lost virtually all our fish to *Amyloodinium* (or the inability to tolerate treatment for *Amyloodinium*). In general, fish pro-

duced in 1998 were intolerant of handling. Although we cannot demonstrate it scientifically, we suspect that the intolerance may have been due to stress resulting from improper nutrition. In 1999, we had problems with *Amyloodinium* as well, but the fish were able to tolerate the treatment (perhaps due to better nutritional status), allowing us to control the outbreak. We had some water quality problems in 1999 due to filter failures, which resulted in some mortality. Generally, however, the routine sampling that is part of our health management program detects problems quickly. Rapid detection allows us the opportunity to deal with the problems. We plan to expand biosecurity to prevent infections.

We sincerely thank graduate students Jason Lemus (copepod culture) and Amber Garber (genetics) for providing information related to their theses. Dr. Shiao Wang provided the microsatellite data. We thank Walter Grater and Dr. Robin Overstreet for their comments. This work was funded by NOAA, NMFS Awards NA76FL0446, NA86FL0476, and NA96FL0358.

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Calendar

conferences, workshops, courses and trade shows

✓ **The Cultivation of Salmon II**, 7-11 May 2001, Bergen, Norway. Contact: Cultivation of Salmon, Institute of Marine Research, Bergen (tel 47 55 23 85 00, e-mail salmon@imr.no, website http://www5.imr.no:591/salmon/)

✓ **International Workshop on *Artemia*,** 12-15 May 2001, *Artemia* and Aquatic Animals Research Center, Urmia University, Urmia, Iran. Prominent scientists will give special oral sessions on the most crucial issues on *Artemia*, while other participants will present some of their research on culture, genetics, ecology and resource assessment, enrichment and use of *Artemia* in larviculture of fish and shrimp. Contact: *Artemia* workshop, Urmia University, PO Box No. 165, Urmia 57153, Iran (e-mail artemiaworkshop@urmia.ac.ir).

✓ **Diseases of Warmwater Fish** (2-week course), 14-25 May 2001, University of Florida, Tropical Aquaculture Laboratory, Ruskin, FL, USA and Whitney Laboratory, St. Augustine, FL, USA. This is an intensive two-week class designed to provide instruction in the methodology of diagnosis and treatment of parasitic, bacterial, viral, nutritional, and environmental diseases of warmwater food fish and aquarium species. Also advanced procedures in fish anaesthesia and surgery have been included in this year's schedule. The course is open to students, veterinarians, fish biologists, aquaculturists, and professional aquarists. Enrollment is limited and registration will be accepted on a first-come first-served basis. Contact: IFAS Office of Conferences & Institutes, University of Florida, PO Box 110750, Gainesville, FL 32611-0750 (tel 532-392-5930, fax 352-392-9734, website http://www.ifas.ufl.edu/~conferweb/wwf)

✓ **Seafood China Expo 2001**, 14-17 June 2001, Dalian Xinghai Convention and Exhibition Centre, China. Opportunity to explore the China seafood market. Information: Ms. Ling Chan, Business and Industrial Trade Fairs Ltd., Unit 1223, HITEC, 1 Trademart Drive, Kowloon Bay, Kowloon, Hong Kong (tel (852) 2865 2633, fax (852) 2866 1770 or 2866 2076, e-mail enquiry@bitf.com.hk).

✓ **Open Ocean Aquaculture IV**, 17-20 June 2001, St. Andrews, New Brunswick, Canada. Theme sessions: Marine Policy, Ocean Engineering, Ocean Environment, Candidate Species and Integrated Open Aquaculture. Information: Open Ocean Aquaculture IV Symposium, 703 East Beach Drive, PO Box 7000, Ocean Springs, Mississippi 39566-7000, (tel 228 875-9341, fax 228 875-0528, email ooa@usm. edu, website: http://www-org. usm.edu/~ooa/ooa_iv.html).

✓ **Symposium on Microalgae and Seaweed Products in Plant/Soil Systems**, 20-22 June 2001, Faculty of Agricultural Sciences, University of West Hungary, Mosonmagyaróvár, Hungary. The main topics of the Symposium will cover the following areas: (1) synthetic and natural plant growth regulators in plant production, (2) antimicrobial compounds of algal origin in plant protection, (3) algae as soil conditioners and their use in soil bioremediation, (4) plant nutrition by seaweed products, cyanobacteria, and microalgae, and (5) microalgal and seaweed products for plant or soil treatments. Information: Vince Ördög or Zoltán Molnár, Faculty of Agricultural Sciences, University of West Hungary, H-9000 Mosonmagyaróvár, Kolbai K. Str. 8, Hungary (tel +36 96 578 637, fax +36 96 215 931, e-mail plantph@mtk.nyme.hu, website http://mtk.nyme.hu/~plantph/symp2001.htm).

✓ **Atlantic Aquaculture Conference, Trade Show and Fair,** 21-24 June 2001, St. Andrews, NB, Canada. The 14th annual fair will explore the concept of "smart farming", and will include a trade show, industry sessions, and workshops on both freshwater and marine topics. Iinformation: Atlantic Aquaculture Exposition Conference & Fair, 157 Water Street, Unit G, St. Andrews, NB E5B 1A7 (tel 506 5294578, fax 506 5294284, email aquafair @nbnet.nb.ca, website http://www.aquafair.com).

✓ **4th International Symposium on Sturgeon**, 8-13 July 2001, Park Plaza International Hotel and Convention Center, Oshkosh, Wisconsin, USA. Symposium objectives are to provide a forum for exchange of information and knowledge

on the biology, culture and management of Acipenseriformes, and to provide an opportunity for scientists, biologists, enforcement specialists and commercial interests working with sturgeon around the world to network, share experiences and develop new research and management initiatives for the benefit of sturgeon populations and their users. Info: 4th ISS, PO Box 109, Oshkosh, WI USA 54903-0109 (tel 920 424-3059, fax 920 424-4404, e-mail bruchr@dnr.state.wi.us, website: http://www.sturgeonsymposium.org/).

✓ **Aquaculture Europe 2001**, 4-7 August, Trondheim, Norway. Biennial meeting of the European Aquaculture Society. Conference program: New Species (juvenile production, optimum production, feed/flesh quality, marketing, economics, impact and positioning of new aquaculture products), and New Technologies (re-circulation, polyculture, feed technology, offshore technology, feed management, waste management). Special workshop on Aquaculture Chain Management. Information: European Aquaculture Society (tel + 32 59 32 38 59, fax +32 59 32 10 05, e-mail ae2001@aquaculture.cc, website http://www.easonline.org).

✓ **Larvi 2001**, 3-6 September 2001, Ghent University, Belgium. Aim is to evaluate progress, identify problems and stimulate cooperation in research and the industrial production of fish and shellfish larvae. Sessions: Session 1 — broodstock, egg and larval quality epigenetics, broodstock feeding and offspring quality, fish and shrimp maturation, wild vs. domestic strains, evaluation methods, etc.; Session 2 — genetics, biotechnology and developmental biology; Session 3 — nutrition, feeding and growth, nutritional physiology, feeds and feeding strategies (live food optimisation, live food substitution/ supplementation diets, formulated feeds, dietary requirements), quantification of food uptake, behavioural interactions; Session 4 — larviculture zootechniques and economics, extensive vs intensive culture techniques, backyard hatcheries, interaction with the environment, cost effectiveness, zootechnical aspects, automation, upscaling methodology, etc.; Session 5 —microbiology and disease control, bacteriology: probionts and pathogens, virology, chemotherapeutics, immunostimulants, immunology, etc. Information: Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium (tel +32-9-2643754, fax +32-9-2644193, e-mail larvi@rug.ac.be, website: http://www.rug.ac.be /larvi/).

✓ **International Commemorative Symposium: 70th Anniversary of the Japanese Fisheries Society,** 1-5 October 2001, Yokohama, Japan. Many of the topics weill deal with aquaculture. Information: Dr. Toshiaki Ohshima (tel +81 3 5463 0613, e-mail symp70yr@tokyo-u-fish.ac.jp, website http://www.symp70yr.or.jp).

✓ **2nd International Conference on Marine Ornamentals**, 27 November - December 1 2001, Wyndham Palace Resort and Spa, Walt Disney World® Resort, Lake Buena Vista, Florida. The aquarium hobby is second only to photography in popularity in the United States, and is rapidly becoming popular in many countries worldwide. The long-term goal is to develop culture protocols that can be used by industry to continue the growth of an important economic activity, while at the same time reduce harvest pressure from worldwide reef ecosystems. Contact: Dr. James C. Cato, Director, Florida Sea Grant College Program, University of Florida, State University System of Florida, PO Box 110400, Gainesville, FL 32611-0400 (tel 352 392-5870, fax 352 392-5113, e-mail: jcc@gnv.ifas.ufl.edu, website: http://www.ifas.ufl.edu/~conferweb/MO/).

✓ **Aquaculture America 2002,** January 2002, Town and Country Hotel, San Diego. The US National Annual Conference and Exposition of the US Chapter of the World Aquaculture Society, the National Aquaculture Association, and the US Aquaculture Suppliers Association. Contact: Director of Conferences (tel 760 432-4270, fax 760 432-4275, e-mail: worldaqua@aol.com).

✓ **Tenth International Congress of Parasitology,** 4-10 August, Vancouver Conference and Exhibition Centre, Vancouver, British Columbia, Canada. Sponsored by the Canadian Society of Zoologists (Parasitology Section) and the American Society of Parasitologists. Program: plenary sessions, invited lectures and submitted posters and oral presentations. Tentative sessions: immunology, molecular biology, morphology and ultrastructure, biochemistry and physiology, systematics and evolution, ecology and epidemiology. Information: Conference Secretariat, Venue West Conference Services Ltd., #645-375 Water Street, Vancouver, BC (tel 604 681-5226, fax 604 681 2503, e-mail congress@venuewest.com, website http://www.venuewest.com).

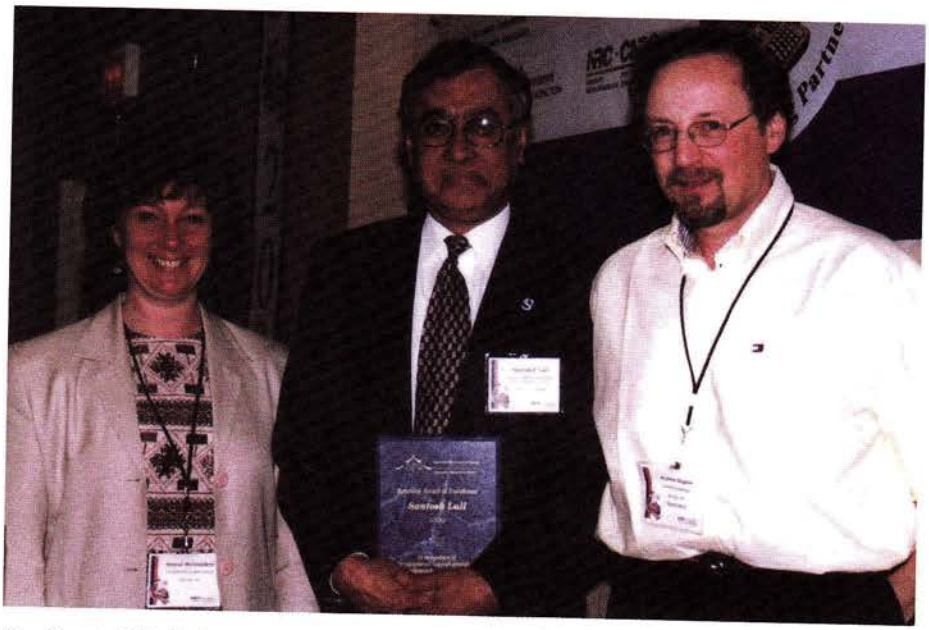
Association News

Awards

The Aquaculture Association of Canada recently honored two scientists, both of whom are longstanding AAC members. Dr. Neil F. Bourne was given the association's Lifetime Achievement Award while Dr. Santosh Lall received the Excellence in Research Award. Both these scientists have been members of AAC since its inception and Dr. Bourne served as President in 1987.

Dr. Santosh Lall is currently Group Leader and Senior Research Officer at the Institute for Marine Biosciences, National Research Council of Canada in Halifax and is an adjunct professor at both Dalhousie University and the University of Prince Edward Island. From 1974 to 1996, Dr. Lall was a research scientist with the Department of Fisheries and Oceans (DFO) at the Halifax Fisheries Research Laboratory and served as Head of the Fish Health and Nutrition Section (Aquaculture Division). During his time with DFO, he forged strong ties between the government research community and the aquaculture industry, bringing fish nutrition to the forefront of applied research in the Department. Dr. Lall's own approach to research is reflected in the dedication and enthusiasm for fish nutrition shown by his many graduate and post-graduate students working with salmonids, marine fish, eels, sea urchins and various warm-water fish species. Dr. Lall and his students work closely with the aquafeed industry in Canada, the United States, Europe and Asia, which has certainly given his menus an international flavor! Dr. Lall has published extensively throughout his career, has been the recipient of numerous honors (not the least of which is this one!) and is considered by both industry and the scientific community as a true pioneer in the field of fish nutrition. If you are what you eat, and you enjoy cultured fish, you are linked gastronomically to Dr. Lall's research achievements.

Dr. Neil F. Bourne has dedicated his working career to the pursuit of both mollusc culture and sustainable mollusc fisheries. He started his research career on the



Dr. Santosh Lall (centre) receiving AAC's Excellence in Research Award from Dr. Sharon McGladdery (left), a director of the Aquaculture Association of Canada, and Dr. Andrew Boghen, President of the AAC, at the Aquaculture Canada 2000 conference in Moncton, NB.

Atlantic Coast with the federal Department of Fisheries and Oceans (then the Fisheries Research Board) at the Biological Station in St. Andrews. In 1965 he moved to the Pacific Biological Station, in Nanaimo, BC, where he put down his roots and can still be found today. While at PBS, Dr. Bourne pioneered scallop aquaculture as well as the culture and harvesting of several other commercially important species, including Pacific oysters, abalone, butter clams and manila clams. Dr. Bourne's productivity and experience in this field soon captured the attention of other fisheries-related and developmental organisations. In addition to his prolific work with the mollusc industry on the West Coast, Dr. Bourne hosted the 9th International Pectinid Workshop in Nanaimo in 1993, was the only Canadian ever to be elected to the position of President of the National Shellfisheries Association (NSA) (1981-82), was named an Honorary Life Member by NSA, and was awarded NSA's Wallace Award for dedicated service in promoting research, understanding and cooperation among shellfisheries scientists, managers, producers and regulators. Dr. Bourne has served as assistant editor of the *Journal of Shellfish Research* — a journal that is one of the most important in shellfisheries research. His productivity won the attention of the Canadian International Development Agency (CIDA) which seconded Dr. Bourne to assist in fisheries training in Fiji, and oyster culture and processing in Brazil. He has also led an FAO project to improve oyster production in the People's Republic of China. His so-called "retirement" from the Department of Fisheries and Oceans occurred in 1994, but he has continued to work as a Scientist Emeritus at the Pacific Biological Station, as well as a volunteer advisor to CESO (a CIDA-affiliated organisation). In nominating Dr. Bourne for the Lifetime Achievement Award, the Board of Directors recognized that he is so productive he may accomplish enough to qualify for a second such award in the future!

Charlottetown Chosen as Site of 2002 Conference

At its mid-year meeting in January 2001, the Board of Directors selected Charlottetown, Prince Edward Island, as the site of the 2002 Aquaculture Canada conference. The meeting is tentatively scheduled for the third week of September and will be held in conjunction with the PEI International Shellfish Festival. The meeting will be co-hosted by the PEI Aquaculture Alliance and the PEI Department of Fisheries, Aquaculture and Environment. Other sponsors include the University of Prince Edward Island's Atlantic Veterinary College, the Marine Institute of Memorial University, and National Research Council (IRAP).

Dr. Neil Bourne (centre) receiving AAC's Lifetime Achievement Award from Dr. Sharon McGladdery (left) and Ms. Linda Hiemstra, President-Elect of the AAC, at the Aquaculture Canada 2000 conference in Moncton, NB.

Student Endowment Fund

Each year, AAC awards travel bursaries to students who want to present papers at the Aquaculture Canada conference. Last year, 17 students received partial travel support. To be eligible, students must be members of AAC and be giving an oral or poster presentation.

Anyone interested in making a donation to support student travel should contact Theresia at the AAC office by telephone (506 529-4766) or e-mail (aac@mar.dfo-mpo.gc.ca). AAC is a registered charity and issues official tax receipts for all donations.

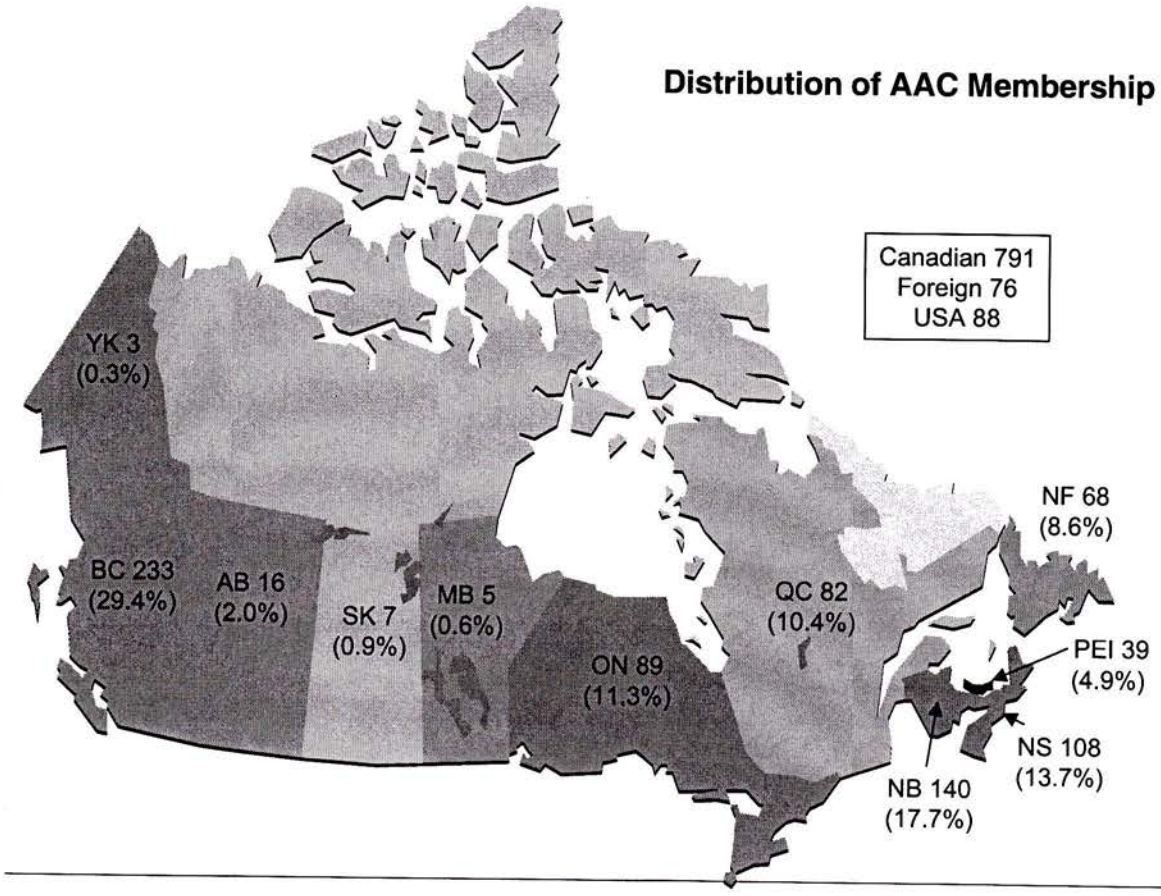
Membership

Membership is currently just under 1000. Although most of the members are from Canada, over 17% are from other countries (Australia, Belgium, Brazil, the United States, England, France, Germany, Greece, Iceland, Ireland, Italy, Japan, Korea, Kuwait, Monaco, New Zealand, Nigeria, Norway, the Philippines, Scotland, South Africa, South Korea, Sri Lanka, The Netherlands, and Mozambique).

Within Canada, over 60% of the members are from British Columbia (29%), New Brunswick (18%) or Nova Scotia (14%).

Aqua-L, the AAC Electronic Discussion List

Aqua-L is thought to be the oldest electronic discussion list in the world devoted to aquaculture. Established in the mid-1980s, the list currently has over 1200 subscribers from more than 20 countries. The list is owned and operated by the Aquaculture Association of Canada, and is housed and maintained at the Marine Institute of Memorial University. Subscription to the list is open to anyone with an interest in aquaculture. The list is archived at http://www.ifmt.nf.ca/aqua-l.archive. Additional information on the list, including subscription to the list or digest, can be found at the AAC website: http://www.ifmt.nf.ca/mi/aac.



Changes to AAC's Publications

AAC's publications are one of its most important products. The goal is to publish as many papers as possible from the annual meeting (and occasionally the proceedings of workshops hosted by other groups). The greatest difficulty in attaining that goal is cost — publishing is expensive. The approach taken by AAC has been to use volunteer editorial and production staff. By eliminating the cost of labor for editing and layout, the entire publication budget can be spent on printing and postage. This publication "policy" allows AAC to publish many more pages each year (currently 200 to 300) than would otherwise be possible.

In an effort to improve the quality of the publications and deal with the challenges of publishing using volunteer staff, the Board of Directors has reduced the number of *Bulletin* issues from 4 per year to 3. The fourth issue—the proceedings of the contributed papers from the annual meeting—will be published in AAC's special publication series under new editorship.

The spin-off of the proceedings issue of the *Bulletin* was approved by the Board of Directors at the Aquaculture Canada 2000 conference in Moncton. The first challenge was to find an editor who was willing to begin work immediately. Dr. Sharon McGladdery (DFO, Gulf Fisheries Centre, Moncton) generously volunteered to serve as editor and find someone to do the desktop publishing. Chris Hendry had been involved with production of the *Bulletin* and quickly offered to assist Sharon.

The transfer of the proceedings to the special publication series provides an opportunity for more AAC members to become involved in the publications. It is still undecided whether the editorship of the proceedings will change each year or if someone can be found who is willing to take on the job for several years. Anyone with an interest in the position should contact the AAC President or the Chair of the Publications Committee.

Another advantage to publishing the proceedings as a special publication is that the new editor will have more flexibility in the format and style of the proceedings than if it continued to appear as an issue of the *Bulletin*. Because the *Bulletin* is a periodical, it must be published according to a regular schedule (or at least it is supposed to be!) and each issue must have the same design and format. The special publications, in contrast, are published irregularly as monographs and can vary in format. This provides the new editor with the opportunity to improve the format or alter editorial policy.

The expectation is that both the proceedings and the *Bulletin* will be positively affected by this change. The involvement of more volunteers will reduce the individual workload so that more time can be spent improving the publications.

Almost a decade ago, the same desire to involve more AAC members in the publications and improve the *Bulletin* led the Board of Directors to develop and implement the concept of "guest editors". The involvement of guest editors led to a dramatic improvement in the *Bulletin* (if anyone doubts that, just compare recent issues with those from 1990). The guest editors develop a *Bulletin* issue on a specific theme, invite authors to submit manuscripts, ensure papers are received on time, and edit the papers. In the past year, six people have served as guest editors of the *Bulletin*: Dr. William Heath, Dr. Joanne Constantine, Dr. Laura Brown, Dr. Simon Courtenay, Mr. Cyr Couturier, and Dr. Gilles Miron.

The AAC publications are a good example of what can be accomplished with a team of dedicated volunteers. AAC could not be a publisher of Canadian aquaculture science and technology if authors were not willing to write papers, guest editors were not willing to conceive and develop thematic issues, and editorial and production staff were not available to edit papers and photos, do the desktop publishing, and arrange for printing and mailing.

There has been one final change to AAC'S publications. The invited and contributed abstracts from the annual Aquaculture Canada conference are now being published on the AAC website (http://www.ifmt. nf.ca/mi/aac). Those from the 1999 meeting have been posted and the abstracts from Aquaculture Canada 2000 and 2001 will appear soon.

S. L. Waddy, editor

***[aac@mar.dfo-mpo.gc.ca** to comment on material appearing in the Bulletin]*

AQUACULTURE CANADA 2001

Sur la bonne voie grâce aux partenariats

18ième Rencontre Annuelle de

l'Association Aquacole du Canada

le 6 au 9 mai, 2001

The Westin Nova Scotian, Nouvelle-Écosse, Canada

Aquaculture Canada 2001 concentrera son attention sur les progrès réalisés par l'industrie aquacole et les défis auxquels elle doit faire face à l'aube du nouveau millénaire. Le programme inclus une revue détaillée des principaux aspects liés aux espèces cultivées au Canada et abordera les problèmes clés ayant un impact sur l'industrie. Des experts renommés s'adresseront aux délégués et aux exposants durant les 3 jours de l'événement et les participants auront l'opportunité de discuter avec les conférenciers, en plus d'exprimer leurs points de vue sur les sujets abordés. Des kiosques seront installés à l'entrée des salles de conférence afin de faciliter l'interaction entre les exposants et les utilisateurs. Des visites guidées après la conférence seront possibles chez les entreprises de Lunenberg Shellfish, Scotia Halibut, l'Institut des biosciences marines du CNR, l'Institut océanographique Bedford, et dans les installations de recherche d'omble chevalier et de tilapia.

Les thèmes inclus au programme sont:

- Développements récents dans l'élevage de la morue
- Séances vidéo
- Gestion de l'environnement et les Meilleures Pratiques de Gestion
- Innovations dans l'élevage des mollusques
- Progrès dans la gestion des géniteurs
- Contrôle des algues toxiques
- Nouvelles technologies
- Poissons présentant un potentiel aquacole
- Enjeux nationaux pour le développement de l'aquaculture : perspectives d'Ottawa
- Défis des communications en aquaculture : perspectives d'entreprise
- Génétique en aquaculture
- Aspects socio-économiques en aquaculture
- Progrès dans la production de crustacés en écloserie : aspects nutritionnels
- Enjeux en formation aquacole et en éducation
- Rôle de l'aide technique dans le développement de l'aquaculture
- Gestion sanitaire des crustacés
- Nutrition et développement des premiers stades de poissons marins
- Espèces sous-exploitées pour l'élevage des mollusques
- Plan d'action pour l'aquaculture : permettre à l'aquaculture d'atteindre son plein potentiel
- Physiologie et aquaculture

Profitez de la diversité des conférences, du bal folklorique écossais des étudiants, et faites l'expérience de la tradition hospitalière de la Nouvelle-Écosse lors de leur *NOVA SCOTIA KITCHEN PARTY.*

Veuillez contacter: Linda Hiemstra, Tel: 250-741-8708, hiemstra@mala.bc.ca.
Exposition: Gary Scott, Trade Show Manager, Tel: 902-424-0344, scottg@gov.ns.ca.
Visites guidées: Darrell Harris, Tel: 902-426-3231, harrisd@mar.dfo-mpo.gc.ca.
Étudiant: Sharon McGladdery, Tel: 506-851-2018, mcgladderys@dfo-mpo.gc.ca
Logement: The Westin Nova Scotian, Halifax NS: 1-888-NS WESTIN

Vous voulez en savoir plus, visitez notre site WEB: gov.ns.ca/nsaf/aac2001

AQUACULTURE CANADA 2001

Moving forward through partnerships

The 18th Annual Meeting of the

Aquaculture Association of Canada

May 6-9, 2001

The Westin Nova Scotian Hotel, Halifax, Nova Scotia

Aquaculture Canada 2001 will focus on the progress made by the aquaculture industry and the challenges faced at the onset of the new millennium. An all-inclusive program will examine many of the species cultured in Canada and address key issues impacting on the industry. Well-known experts will address delegates and exhibitors during this three-day event and participants will have opportunity to interact with the presenters and express their views. Trade show booths are placed next to the conference halls in the coffee area providing lots of traffic to showcase products. Post conference tours include Lunenberg Shellfish, Scotian Halibut, NRC Institute of Marine Biosciences, Bedford Institute of Oceanography and tilapia and Arctic char facilities.

Program Highlights

- **Progress in Cod Aquaculture**
- **Challenges in Aquaculture Communications: Corporate Perspectives**
- **Aquaculture Video Session**
- **Innovations in Shellfish Culture**
- **Broodstock Developments**
- **Harmful Algal Blooms**
- **New Technologies, Suppliers Session**
- **Alternate Finfish Cultures**
- **National Issues in Aquaculture Development: An Ottawa Perspective**
- **Physiology and Aquaculture**
- **Socioeconomic Aspects of Aquaculture**
- **Advances in Shellfish Hatchery Production: Nutritional Aspects**
- **Issues in Aquaculture Training and Education**
- **Role of Extension Services in Aquaculture Development**
- **Shellfish Health – Risks and Management**
- **Nutrition and Early Marine Fish Development**
- **Aquaculture Action Plan: Enabling Aquaculture to Achieve Full Potential**
- **Alternate Shellfish Species**
- **Aquaculture Action Plan: Enabling Aquaculture to Achieve Full Potential**
- **Environmental Assessment & BMPs**

Enjoy the sessions, the Student Ceilidh and experience the Nova Scotian tradition of hospitality at the ***NOVA SCOTIAN KITCHEN PARTY.***

Conference Information: Linda Hiemstra, Tel: 250-741-8708, hiemstra@mala.bc.ca.
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Visit our Conference website: gov.ns.ca/nsaf/aac2001