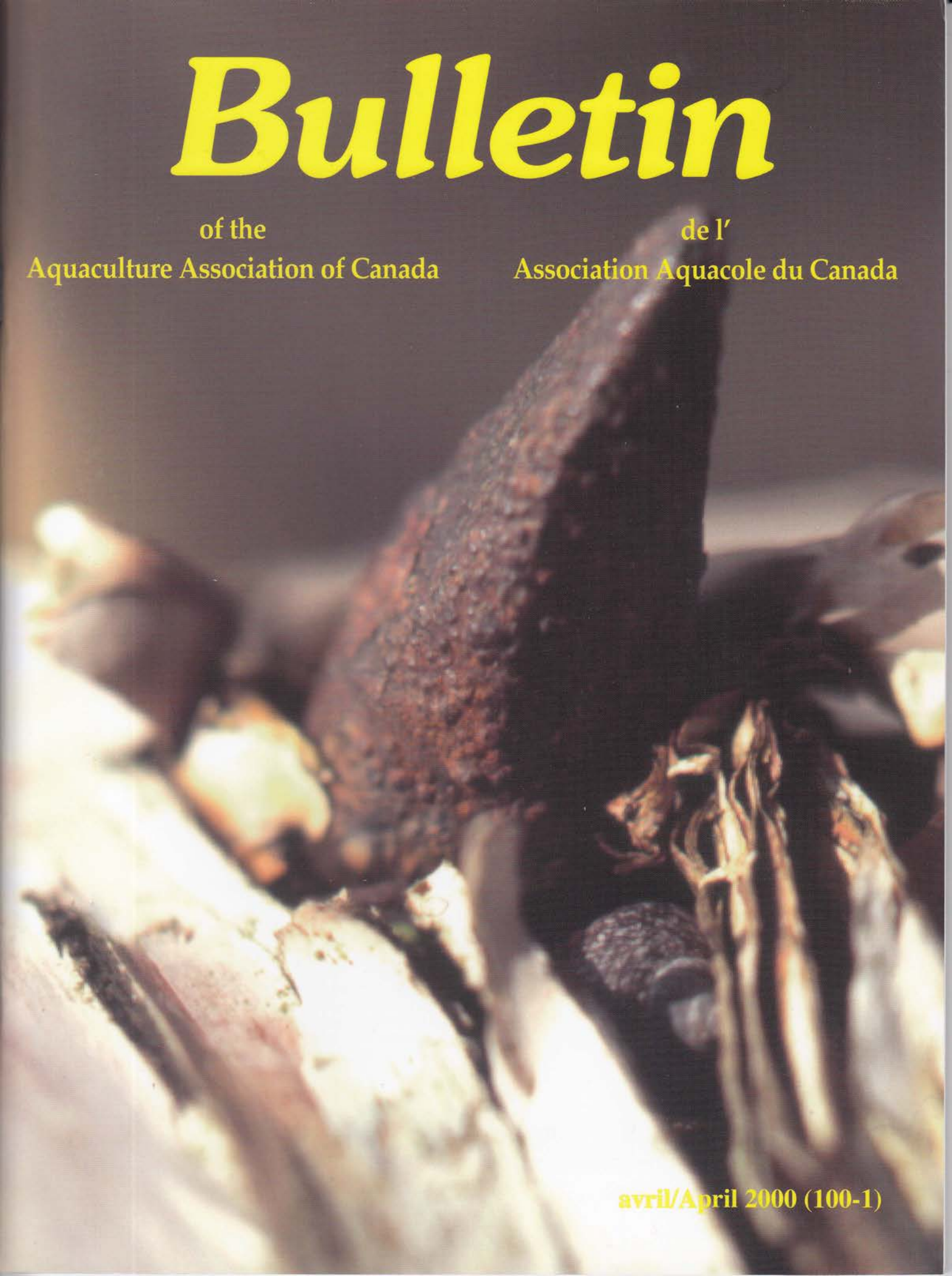
Bulletin

of the
Aquaculture Association of Canada

de l'
Association Aquacole du Canada



avril/April 2000 (100-1)

Bulletin de l'Association aquacole du Canada

avril 2000 (100-1)

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ISSN 0840-5417

Imprimé par Print Atlantic, Moncton (Nouveau-Brunswick)

Bulletin of the Aquaculture Association of Canada

April 2000 (100-1)

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ISSN 0840-5417

Printed by Print Atlantic, Moncton, NB

Front cover: Rusty nail protruding from flotsam on a Bay of Fundy beach. In the early days of the Bay of Fundy Atlantic salmon farming industry, growers held their salmon in circular wooden cages designed by pioneer salmon farmer John Malloch [D.E. Aiken photo]

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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
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- 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

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Development of a Vaccine Against Infectious Salmon Anaemia Virus (ISAV)

Laura L. Brown, Sandra A. Sperker, Sharon Clouthier and Julian C. Thornton

Development of a vaccine against infectious salmon anaemia virus (ISAV) would provide salmon farmers with a method for protecting their fish stocks against this devastating pathogen. The ISA virus used to develop the vaccine used in this study was isolated from ISA moribund farmed Atlantic salmon from New Brunswick. The virus was cultured, inactivated and formulated as an oil-based vaccine with a titre of $1x10^7$ $TCID_{50}$/mL. The formulation also contained antigens for *Aeromonas salmonicida*, *Vibrio anguillarum* serotypes 01 and 02, and *V. salmonicida*. Atlantic salmon in duplicate tanks were each given 200-µL intra-peritoneal (i.p.) injections of the vaccine formulation. Control fish were injected with 200 µL of the vaccine formulation without the inactivated virus. The fish were held in freshwater at 4 to 8°C. Five weeks after vaccination, each fish was challenged by i.p. injection with $5 \times 10^{3.5}$ $TCID_{50}$ live ISAV. Mortalities were monitored daily until 99 days post-infection. All mortalities were confirmed to be due to ISAV by indirect fluorescent antibody test on tissue imprints, virus culture from tissue, and RT-PCR. The vaccinates had a relative percent survival (RPS) of 54% compared to controls ($P < 0.01$). In addition, neutralizing activity in convalescent sera was demonstrated by incubating ISAV with sera from fish that had survived, and then inoculating the ISAV onto salmon head kidney (SHK-1) cells in tissue culture. Sera from vaccinated and non-vaccinated fish neutralized the virus when diluted 1/20 whereas sera from vaccinated fish when diluted further to 1/40. The data from this study indicate that the vaccine formulation provides significant protection against ISA.

Introduction

Infectious salmon anaemia (ISA) caused by infectious salmon anaemia virus (ISAV) was first described in Norwegian aquaculture operations in the mid 1980s. In 1996, Dr. D. MacPhee identified haemorrhagic kidney syndrome (HKS) in sea cages of Atlantic salmon in the Bay of Fundy, New Brunswick, Canada.[1] Signs of HKS include interstitial hemorrhage and necrosis of kidney tubules, but the large areas of hepatic necrosis and hemorrhage normally associated with ISA were not present.[1] However, ISAV was recovered from fish showing signs of HKS.[4] More recently, Canadian salmon with ISAV infection have displayed the same severe liver lesions characteristic of ISAV infections as those reported in Norway, while subsequent Norwegian infections with ISAV have been reported to show renal lesions characteristic of HKS.[4] Now it is accepted that HKS is caused by a Canadian isolate of ISAV.[8] ISAV has also been detected in broodstock and yearlings through normal screening techniques at an aquaculture operation in Cape Breton, Nova Scotia, Canada. However, there have been no disease outbreaks among stocks where the virus was detected.[2] In 1998, ISAV was also detected in farmed Atlantic salmon in Scotland and the ISA outbreaks there have caused serious economic losses.

Because of the potential for severe economic losses due to ISA, the present study was undertaken to develop a vaccine against ISAV. An experimental ISAV vaccine has also been reported by Jones et al.[6] We report here the preliminary results of an experimental vaccine to protect Atlantic salmon (*Salmo salar* L.) against ISAV.

Materials and Methods

Fish

Atlantic salmon smolts (mean weight 80 g) were transported from a commercial hatchery in Nova Scotia to the quarantine aquarium facilities on the Dalhousie University campus, Halifax, Nova Scotia. Fish were placed in tanks with flow-through freshwater with seawater trickled in to maintain low water temperature during the summer months (5 ppt). Ambient

water temperature ranged from 4–8°C during the experiment. The animals were distributed into 4 tanks, 30 animals per tank.

Vaccine Formulation

ISAV was inoculated into 500-cm^2 tissue culture flasks containing confluent monolayers of salmon head kidney (SHK-1) cells, (donated by Dr. B. Dannevig, Norwegian Veterinary Institute). The SHK-1 cells were grown according to a modification of the method of Dannevig et al.[3] The infected cells were incubated at 15°C and were periodically checked for signs of cytopathic effect (CPE). At 14 days post-inoculation, The cell culture supernatant containing ISAV was removed from the flasks and the virus was inactivated by a proprietary method. Immediately prior to and following inactivation, aliquots of the virus culture were serially diluted (ten fold) in SHK medium and inoculated onto 96 well plates containing confluent monolayers of SHK-1 cells. These infected cells were incubated at 15°C, and the number of wells showing CPE were counted. Virus particles were enumerated by the Spearman-Kärber method to determine the titre of the virus used in the vaccine formulation and to verify that the ISAV was indeed inactivated.

After inactivation the virus was mixed with Microtek/Bayotek's commercial vaccine formulation, MultiVacc4®. This vaccine is an oil-in-water emulsion which contains bacterins that protect against *Vibrio anguillarum* serotypes 1 and 2, *V. salmonicida*, and *Aeromonas salmonicida*. Inactivated ISAV was added to a final titre of $1x10^7$ $TCID_{50}$/mL. The control treatment consisted on Multivacc4® only.

Vaccination and ISAV Challenge

Duplicate groups of 30 fish were injected intraperitoneally (i.p.) with either MultiVacc4® or MultiVacc4® containing inactivated ISAV (0.2 mL per fish). Five weeks after vaccination, all fish in each group received an i.p. injection of 5×10^3 $TCID_{50}$ live ISAV. For the live challenge, the virus was grown as described above, harvested at 14 days post-inoculum, and diluted in SHK-1 medium. Mortalities were monitored daily and all moribund or dead fish were assayed to verify ISAV as the cause of mortality.

Verification of Cause of Mortality

All mortalities or moribund fish were removed from tanks daily. Kidney, spleen, pyloric caecae, and gill were taken aseptically for virus culture. The tissues were homogenized with Hank's balanced salt solution (HBSS) to a final dilution of 1:50 (w/v). Homogenized tissues were centrifuged at 1070 x*g* for 15 minutes and then the supernatant was passed through a 0.45-µm filter. The filtrate was inoculated onto triplicate wells in a 96-well plate seeded with confluent monolayers of SHK-1 cells. The plate was incubated at 15°C for at least 21 days and the monolayers were monitored for evidence of CPE.

The content of those wells displaying ISAV-specific CPE were further assayed by reverse transcriptase polymerase chain reaction (RT-PCR) to confirm the presence of ISAV. RT-PCR was performed using the protocol described in Mjaaland et al.[9] with the exception that the RNA was extracted with QIAamp (Qiagen, Mississauga, ON) and reverse transcription was performed with a commercially available kit according to the manufacturer's instructions (Invitrogen, Carlsbad, CA).

Examination of Survivors

At 100 days post-challenge all surviving fish were euthanized with an overdose of tricaine methane sulfonate (TMS, Syndel Laboratories, Vancouver, BC). Blood was taken from the caudal vein and the sera were frozen at –80°C until needed. Tissues (as above) were extracted for virus culture, which was performed as described above.

Serum Neutralization

Sera from fish in each tank were pooled, serially diluted (two-fold from 1/10 to 1/40) and incubated (1:1 v:v) for 30 minutes at 15°C with $1x10^4$ $TCID_{50}$/mL live ISAV (grown on SHK-1 cells as above for 14 days at 15°C). The virus:sera mixtures were inoculated onto triplicate wells of SHK-1 cell monolayers in a 96-well plate. Positive control wells were inoculated with virus only, and negative control wells were inoculated with serial dilutions of sera only. The cell monolayers were monitored for CPE. At 14 days post-inoculum the cells were treated according to the method of Secombes et al.[10] to quantitate the CPE produced by the virus. Briefly, the cells were fixed with buffered formalin (6.5 g Na_2HPO_4 + 4 g NaH_2PO_4 in 1L 10% formalin) for 1h, and then washed with PBS. The cells were stained with 1% crystal violet in PBS, washed and then the crystal violet was eluted from the cells with 70% ethanol. Colour absorbance was read in a microplate reader at 590 nm.

Results

Mortalities due to ISAV started at approximately 40 days post-infection. At 99 days post-challenge, the average mortality of the fish in the two control tanks was 58% compared to only 25% within the vaccinated group (Fig. 1). The relative percent survival (RPS) within the vaccinated group was calculated to be 54%

and was shown to be statistically significant ($P < 0.01$).

ISAV was recovered by tissue culture from all but one of the moribund and dead fish during the challenge. All of the tissue culture samples showing positive CPE were examined by RT-PCR. ISAV was recovered (and verified) from 60% of the survivors within the vaccinated group and from 100% of the control group.

A difference was observed in the colour absorbance obtained from those wells containing cells infected with virus only compared to those wells containing cells infected with virus previously incubated with sera from vaccinated fish (Table 1). A high absorbance indicated increased survival of the SHK-1 cells and therefore inactivation of the ISAV. Pooled sera from the vaccinated group and the control group were able to neutralize ISAV to a dilution of 1/20 whereas sera from the vaccinated group were able to neutralize the virus at a 1/40 dilution (further dilutions were not done). This difference in the degree of ISAV neutralization obtained with the two groups of sera was shown to be statistically significant ($P < 0.01$).

Discussion

The vaccine formulation conferred 54% RPS on the vaccinated fish. It should be noted that the control used in this study were fish injected with the same commercial formulation (Multi-Vacc4®), without the addition of the inactivated virus. This is the only appropriate control for a study of this type. The adjuvant effect of the oil-in-water emulsion does confer some slight, non-specific protection to fish. Therefore, using saline-injected animals as a control could result in a higher RPS value than is truly reflective of the protection afforded by the inactivated virus. Our study avoided this possible inaccuracy.

The results obtained in this study are encouraging, particularly in light of the fact that the water temperatures were so low. At lower temperatures the immune responses of salmonids are reduced. This applies to the humoral as well as the cellular components of the immune system[5,7] and therefore we anticipate that the RPS would be higher at increased water temperatures. At this point we do not know whether the protection conferred by the vaccine is due to antibody (humoral) production or cellular immunity or a combination of both.

ISAV was recovered by tissue culture from a higher number of survivors within the control group than the vaccinate group. These data, combined with the data from the serum neutralization assays, indicate that the vaccine is not only able to confer protection, but can also induce an immune response to eliminate the virus from the host. Attempts were made to determine the titre of antibodies against ISAV within sera from survivors, using immunoblots or enzyme-linked immunosorbent assays (ELISA). However, this was not possible (data not shown) and it may indeed be that the protective immune response observed within this trial is cell- rather than antibody-mediated.

The study described here is preliminary and further trials are underway to improve the efficacy of the vaccine and to further elucidate the nature of the salmon immune response against ISAV. However, it is clear that the vaccine does confer significant protection against ISAV.

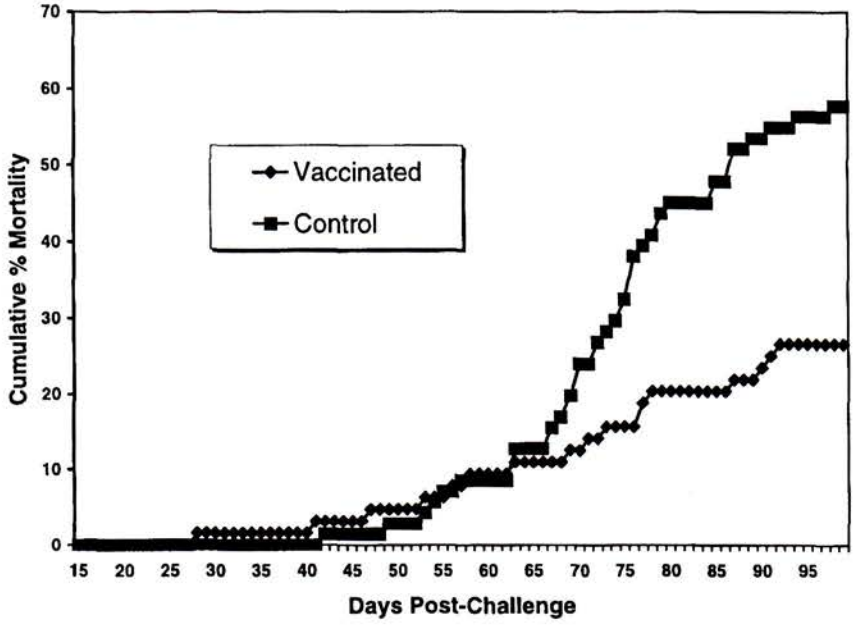

Figure 1. Cumulative mortalities due to infectious salmon anaemia virus (ISAV). Control fish (duplicate tanks, n=30 per tank) were injected i.p. with MultiVacc4®. Vaccinated fish (duplicate tanks n=30 per tank) were injected i.p. with MultiVacc4® + ISAV ($1x10^7$ $TCID_{50}$/mL). 5 weeks post-vaccination fish were challenged with $5x10^{3.5}$ $TCID_{50}$ ISAV. Mortalities were confirmed as due to ISAV by tissue culture and RT-PCR. Water temperature was 4-8°C.

We thank Dr. Birgit Dannevig of the Norwegian Veterinary Institute, Oslo, Norway, for her generous donation of SHK-1 cells. We are grateful to Mr. M. Greenwell (NRC) and Mr. S. Carlos, Ms. P. Byrne and Mr. D. Machander (Microtek) for their technical assistance.

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***Dr. Laura Brown** and **Ms. Sandra Sperker** are at the National Research Council Canada, Institute for Marine Biosciences (IMB), 1411 Oxford Street, Halifax, NS, B3H 3Z1, Canada. Laura Brown is a Research Officer and Group Leader, Cell and Molecular Biology; Sandra Sperker is a Technical Officer. **Dr. Sharon Clouthier** and **Dr. Julian Thornton** are at Microtek International Limited, 6761 Kirkpatrick Crescent, Saanichton, BC, V8M 1Z8, Canada. Sharon Clouthier is a Research Scientist and Julian Thornton is Vice-President of Research and Development.*

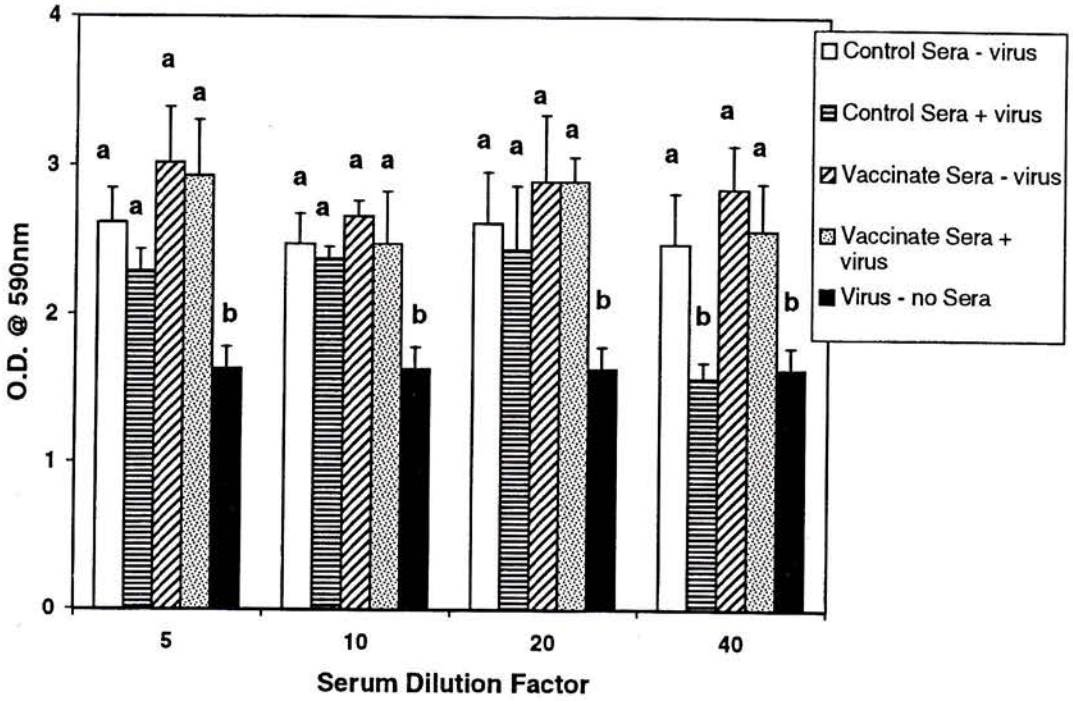

Figure 2. Serum neutralization of infectious salmon anaemia virus (ISAV). Sera from vaccinated and non-vaccinated survivors of an ISAV challenge were diluted and incubated with ISAV for 30 min. Equal numbers of salmon head kidney (SHK-1) cells were inoculated with sera/virus mixtures and incubated at 15°C. CPE was monitored and cells fixed and stained with crystal violet. Colour absorbance was read at 590 nm. Values are means ± standard error ($n = 4$ wells). Different superscript letters indicate a significant difference ($P < 0.01$). A high absorbance reading indicates viable SHK-1 cells and therefore neutralization of ISAV by the sera. A low absorbance reading indicates active infection of SHK-1 cells by ISAV and therefore little or no neutralization by the sera.

Expressed Sequence Tags (ESTs) of the Salmon Louse, *Lepeophtheirus salmonis*

S.C. Johnson, K.V. Ewart , J.A. Osborne,
S.E. McIntosh, L. Stratton and N.W. Ross

Since the early 1970s, infection by the salmon louse, *Lepeophtheirus salmonis,* has been recognized as a major disease problem in marine farmed salmonids. Research into the biology and control of the salmon louse is ongoing at numerous institutions in Canada and abroad. In our laboratory, biochemical and molecular biological techniques are being used to investigate interactions between *L. salmonis* and Atlantic salmon and to identify targets against which new control measures such as vaccines and chemotherapeutants may be directed. We have shown that mucus of infected salmon with *L. salmonis* has elevated protease and alkaline phosphatase activity. The protease activity is due to *L. salmonis*-derived trypsin-like enzymes, which we have isolated, purified and are now sequencing. These sequences will be compared to sea lice enzyme sequences deduced from cDNAs identified among expressed sequence tags (ESTs) from a cDNA library of preadult *L. salmonis* constructed in our laboratory. Partial cDNA sequencing to produce ESTs is an effective approach for gene identification. For pathogens, this may allow the identification of targets for chemotherapy and vaccine development. To date we have sequenced 392 ESTs, of which 7 have significant matches to other trypsin-like proteases. We are now determining the full length sequence for these proteases and will compare them to proteases present in the mucus of *L. salmonis* infected Atlantic salmon.

Introduction

Since the early 1970s, infection by the salmon louse, *Lepeophtheirus salmonis,* has been recognized as a major disease problem in marine farmed salmonids. Over the past 10 years, a good understanding of the basic biology and ecology of *L. salmonis* has been developed, as well as a variety of methods for its control.[1] At present, the control of *L. salmonis* is based on management strategies that rely in part on the use of either chemical or pharmaceutical treatments. Of great interest to the salmon farming industry is the possibility of developing a vaccine against *L. salmonis* and research on vaccines has been undertaken.[2-4] Unfortunately, such research activities to date have not resulted in the identification of antigens, which when used, will provide an effective immune response.

Numerous biological molecules have been identified as important virulence factors in a wide variety of parasite groups, especially the arthropods.[5] These substances have anti-hemostatic, vasodilatory, anti-inflammatory and/or immunosuppressive properties and include enzymes, enzyme inhibitors and eicosanoids (such as prostaglandins).[5-7] It is likely that such substances are also important in the biology of *L. salmonis* and they might therefore serve as targets for vaccine or new chemotherapeutant development.

Increased alkaline phosphatase and protease activity in the mucus of Atlantic salmon infected with *L. salmonis* has been reported.[8] It was thought that *L. salmonis* produced these enzymes, which were secreted or excreted onto the host's surface to aid in parasite establishment and feeding. Based on molecular weight, inhibition studies, affinity chromatography and Western blotting with an antibody raised against Atlantic salmon trypsin, these proteases were proven to be a series of low molecular weight (17-22 kDa) trypsins produced by *L. salmonis*.[9] It is believed that these trypsins function to aid in feeding activities and possibly to interfere with host defence mechanisms. The copepodid stage of *L. salmonis* has been grown on Atlantic salmon epidermal tissue cultures.[10] This study reported reduced chemotaxis and phagocytosis activities and an increase in fluid phase endocytosis of Atlantic salmon head kidney macrophages when in-

cubated with supernatants obtained from these cultures. Based on these studies, there is good evidence for secretion of biologically active substances by *L. salmonis*.

In our laboratory, we are using biochemical and molecular biological techniques to investigate interactions between *L. salmonis* and Atlantic salmon and to identify substances produced by sea lice that may serve as targets against which control measures such as vaccines and chemotherapeutants may be directed. Partial cDNA sequencing of clones from a sea lice cDNA library allows identification of the clones that represent expressed genes. Expressed sequence tags (ESTs) are an effective approach for gene identification and ESTs for pathogens may result in the identification of targets for chemotherapy and vaccine development. We have developed an EST library for the preadult stages of *L. salmonis* and are presently randomly sequencing clones from this library. Using this approach, we will be able to identify genes that encode virulence factors of *L. salmonis* based upon their similarity to published sequences from other parasites.

Materials and Methods

Preadult male and female *L. salmonis* were removed from laboratory infected Atlantic salmon, rinsed briefly in fresh seawater, placed in sterile containers, flash frozen in liquid nitrogen and stored at -80°C. The general procedure followed for the production of sea lice ESTs is shown in Figure 1. Briefly, poly(A)$^+$ RNA was isolated directly from the frozen samples using the FastTrack7 kit (Invitrogen). A DNA copy of the RNA (cDNA) was synthesized using oligo(dT) as a primer and then directionally cloned into the Lambda ZapII7 vector using kit-supplied reagents (Stratagene). A representative portion of the cDNA library was converted from phage to pBluescript plasmid form by *in vitro* excision using reagents and protocols from Stratagene. The resulting clones (which harbour individual sea lice cDNAs) were then grown in duplicate mini-cultures in microtitre plates and frozen with 15% added glycerol. Master reference plates were frozen at -80°C and plates for frequent use in sequencing were frozen at -20°C. Individual clones were grown from the frequent use plates, plasmid DNA extracted and sequencing performed on an ABI 373 Automated Sequencer using the SK primer and PRISMJ Big Dye Terminator (PE Applied Biosystems) as described previously.[11] The data were analyzed using Sequencher (Gene Codes, Inc.) and submitted for database searching at the National Center for Biotechnology and Information.[11]

Results and Discussion

Over the past several years, major advances in our knowledge of the biology of parasites have been achieved through the use of molecular biological techniques such as EST sequencing. Sequencing of EST libraries is a powerful tool to look at genes that are being expressed at the time of the RNA isolation. Large EST projects have been undertaken for a variety of parasites, most of which are important with respect

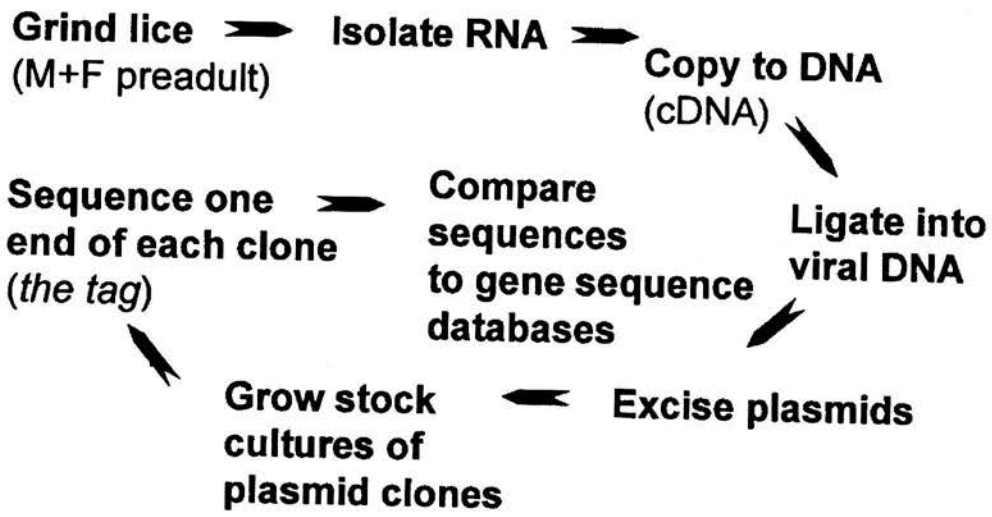

Figure 1. Summary of method used to prepare sea lice ESTs.

Figure 2. Comparsion of a deduced partial amino acid sequence for a *Lepeophtheirus salmonis* trypsin-like protease (clone 15) with other sequences of high similarity. Sequences aligned using Genomatix DiAlign Progam. The catalytic triad residues are denoted in bold and marked with an asterisk above the alignment.

Lepeoph	*Lepeophtheirus salmonis* clone 15
Penaeus	*Penaeus vannamei* trypsin 61% AA identity
Astacus	*Astacus astacus* trypsin 61% AA identity
Rattus	*Rattus norvegicus* trypsinogen 60% AA identity
Hypoderma	*Hypoderma lineatum* hypodermins 60% AA identity

Note: only uppercase letters are considered to be aligned.

```
Lepeoph          1   dtdsveex-- ---------- ---------- -VGGEEVEPN SIPFQISFQT
Penaeus          1   -S--LVLCLL LAGAFAApsr kptfrrglNK IVGGSDATPG ELPYQLSFQD
Astacus          1   ---------- ---------- ---------- IVGGTDAVLG EFPYQLSFQE
Rattus           1   mS--ALLILA LVGAAVAfpl ed-----DDK IVGGYTCPEH SVPYQVSLNS
Hypoderma        1   mlkfVILVCS VACVFGAvvp ggmlpqlDGR IVGGFETDIE DFPWQVSIQr

                                                   *
Lepeoph         28   TGG---FHFC GASVMDKDTI ITAAHCCDSF Y---PSEVQV VAGEHDLFST
Penaeus         48   VSFGFAFHFC GASIYNENWA ICAGHCVQGE DMNNPDYLQV VAGEHNRDVD
Astacus         21   TFLGFSFHFC GASIYNENYA ITAGHCVYGD DYENPSGLQI VAGELDMSVN
Rattus          44   --G---YHFC GGSLINDQWV VSAAHC---- Y---KSRIQV RLGEHNINVL
Hypoderma       51   -GG---YHFC GGSIYSPEII VTAAHCLEKI D---ASQLRV RVGssywde-

                                                   *
Lepeoph         72   SGDEQKIAVS DITYHEKFAS HGTNYDVCLL KLKSSLHFNE KVKPIALPEk
Penaeus         98   EGNEQTVVLS KIIQHEDYNG FTISNDISLL QLSQPLSFND FVAPIALPEA
Astacus         71   EGSEQTITVS KIILHENFDY DLLDNDISLL KLSGSLTFNN NVAPIALPAQ
Rattus          82   EGDEQFINAA KIIKHPNYSS WTLNNDIMLI KLSSPVKLNA RVAPVALPSA
Hypoderma       93   EGSll--TVS NFKIHEKYDP MIMWYDVALL KLSSKLTYGP TVKNIELAKE


Lepeoph        122   dqefiGDVVV SGWGTISSSG PPSP-VLKAV TVQVVSDEDC SDAYYG---S
Penaeus        148   GHAASGDCIV SGWGTTSEGG STPS-VLQKV SVPIVSDDEC RDAYGQ--ND
Astacus        121   GHTATGNVIV TGWGTTSEGG NTPD-VLQKV TVPLVSDAEC RDDYGA--DE
Rattus         132   CAPAGTQCLI SGWGNTLSNG VNNPDLLQCV DAPVLSQADC EAAYPG---E
Hypoderma      141   TPPdnaDAVV SGWGTIYENY PYMPVQLRSV DVKMVSREVC RSEEYGygNA

                                                  *
Lepeoph        168   IDETMICAAA P--GKDSCQG DSGGPLAQDG T----LVGIG SWGYGCAAPG
Penaeus        195   IDDSMICAGM PEGGKDSCQG DSGGPLACSD TGSTYLVGIV SWGYGCARPN
Astacus        168   IFDSMICAGV PEGGKDSCQG DSGGPLAASD TGSTYLAGIV SWGYGCARPG
Rattus         179   ITSSMICVGF LEGGKDSCQG DSGGPVVCNG Q----LQGIV SWGYGCALPD
Hypoderma      191   IGPTMICAYA V--GKDACQG DSGGPLVVGE A----LVGVV SWGEGCAYPG


Lepeoph        212   YPGVY----- ---------- --
Penaeus        245   YPGVYAEVSY HVDWIKANA- --
Astacus        218   YPGVYTEVSY HVDWIKANAv --
Rattus         225   NPGVYTKVCN FVGWIQdtia an
Hypoderma      235   FPGVYTDVSV VRSWITENAk sf
```

to human health.[12] Our EST project on *L. salmonis* is the first that has been conducted on a disease-causing agent of fish. We created our cDNA library from multiple preadult male and preadult female *L. salmonis*. The library titre of approximately 10^6 plaque-forming units was sufficient to provide a representative sample of expressed genes in the preadult stages. We chose to do our EST study on the preadult stages because when there are high numbers of *L. salmonis* on salmon, high levels of mortality commonly occur immediately after the molt from the fourth chalimus to first preadult stage.[8,13,14] The use of multiple individuals to construct the library should allow the detection of genes, that may only be expressed for short periods within either of the preadult stages. Unfortunately there are several important aspects of the biology of *L. salmonis* that cannot be addressed in this study. These include the production of the frontal filament, which occurs only in the copepodid and chalimus stages, and those aspects of reproduction that occur only during the adult stage.

To date, we have sequenced the 5′ ends of 424 clones to generate ESTs. Of these clones, 32 (7.5 %) contained only vector sequences and were removed from our EST database. Of the remaining 392 clones, 184 (46.9%) did not match any known DNA sequences in the database and 208 (53.1%) had significant matches to genes from other organisms (Table 1).

Proteases have been implicated in the pathogenesis of numerous parasitic diseases.[5] They assist in the establishment and the maintenance of parasites in or on hosts by facilitating the invasion of host tissues and the evasion of host immune responses. They are also important for parasite feeding by digestion of host tissues or in the case of blood feeding parasites acting as anticoagulants. It has been postulated that the secretion or excretion of proteases from *L. salmonis* into the mucus of its hosts may play an important role in this parasite's virulence.[8,9] Of the 208 ESTs which produced significant alignments with genes from other species, 9 encoded extracellular secreted proteins such as enzymes. Of these, 7 were homologous to serine proteases (trypsin or trypsin-like enzymes). Five ESTs (clones 15, 58, 154, 132, 133) were found to be es-

Table 1. Summary of ESTs obtained from a preadult male and preadult female *Lepeophtheirus salmonis* cDNA library. The category has been determined by comparison of the EST sequence against known sequences in databases.

Category	Number of ESTs	Percentage of Library
Ribosomal Proteins	43	11.0
Mitochondrial	20	5.1
Nuclear (e.g., transcription factors, DNA replication, RNA splicing)	8	2.0
Cellular Components (e.g., cell membrane, endoplasmic reticulum, lysosomes, secretory vessicles)	4	1.0
Cytoplasmic Proteins (e.g., cytoskeleton, housekeeping, kinases, phosphatases)	54	13.8
PROCESS-RELATED AND STRUCTURAL PROTEINS		
Metabolism	6	1.5
Extracellular Secreted Proteins (e.g., proteases, protease inhibitors)	9	2.3
Development and Tissue Repair	8	2.0
Extracellular Matrix (e.g., collagen, elastin)	4	1.0
Cuticle and Molting	6	1.5
Reproduction	1	0.3
OTHER		
rRNA	27	6.9
Miscellaneous	3	0.8
Hypothetical/unknown	15	3.8
No Data Base Match	184	46.9
TOTAL	**392**	**100.0**

sentially identical and clone 15 was selected as representative of these clones (Fig. 2). The catalytic triad residues which are characteristic of trypsin are present within the AA sequence of clone 15. Comparison of the deduced amino acid sequence of clone 15 with published sequences revealed close similarities to the serine proteases (trypsin and hypodermins) having 61% amino acid (AA) identity with the shrimp (*Penaeus vannamei*) and the broad-fingered crayfish (*Astacus astacus*) trypsin; 60% AA identity with Norwegian rat (*Rattus norvegicus*) trypsinogen; and 60% AA identity to the warble fly (*Hypoderma lineatum*) hypodermins. The presence of 5 copies of this EST in our database suggests that this gene is expressed at high levels in the preadult stage of the salmon louse.

The ESTs derived from clones 13 and 189 did not share a similar nucleotide sequence. These ESTs are highly similar to other known serine proteases. Comparison of the EST sequence of clone 13 with published sequences revealed close similarities to chymotrypsin and other serine proteases, having 49% AA identity with African clawed frog (*Xenopus laevis*) chymotrypsin, 46% AA identity with the yellow fever mosquito (*Aedes aegypti*) blood meal induced trypsin, and 52% AA identity to a serine protease involved in development of the fruit fly (*Drosophila melanogaster*). Comparison of this EST sequence of clone 189 with published sequences revealed close similarities to hypodermins and chymotrypsin. This sequence had 55% AA identity to two of the hypodermins of *H. lineatum*, 55% AA identity with the *A. aegypti* blood meal induced trypsin, and 53% AA identity with chymotrypsin of *P. vannamei*. Hypodermins are proteolytic enzymes, which belong to the serine proteases.

By EST sequencing, we have been able to identity 7 clones with inserts that code for serine proteases. In addition to being similar to proteases involved in routine digestion in a variety of animal species, all of these inserts are also highly similar to related proteases that are considered to play important roles in the host parasite relationships of other parasite species. For example, the hypodermins are proteolytic enzymes, which belong to the serine proteases. These enzymes are secreted by the first-stage larva of *H. lineatum* into the host and are thought to play an important role in the host-parasite relationship.[15] The blood-meal induced trypsin of *A. aegypti* is necessary to break down proteins obtained during blood feeding.[16] All of these enzymes have been proposed as candidates from which vaccines against these parasites can be devised.

The data we have obtained from our EST sequencing has formed the basis for our ongoing studies of the serine proteases of *L. salmonis*. We will be obtaining the full-length sequences for these inserts. These inserts will also be used as probes to screen the cDNA library for other related serine proteases. We are also isolating and purifying trypsin from mucus of *L. salmonis* infected salmon. The identity of this trypsin will be determined using mass spectrometry. This sequence will be compared to the deduced amino acid sequences of the trypsin-like proteases from our EST project and screening of the cDNA library. The isolation and characterization of cDNAs encoding mature proteases makes possible the production of relatively large amounts of these proteins through the use of recombinant technology. This opens up the possibility of investigating possible roles of these proteases in the host-parasite relationship between *L. salmonis* and its salmon hosts.

This research was supported by an NRC/NSERC partnership grant, the Salmon Health Consortium and the Institute for Marine Biosciences, National Research Council, Canada. We are grateful to Sue Douglas and Mike Reith for helpful advice.

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The authors are researchers at the Institute for Marine Biosciences, National Research Council Canada, 1411 Oxford St., Halifax, Nova Scotia, Canada, B3H 3Z1.

Antibiotic Use in the British Columbia Aquaculture Industry (1996-1998): Is the Comparison with Norway Realistic?

M.E. Sheppard

Prescribing antibiotics to control infections in food-animal populations has been debated for decades and one should not anticipate the debate to end in the future. Yet contrary to most media reports, the judicious use of antibiotics by the aquaculture industry has become a standard code of practice over the past decade and the monitoring programme surrounding antibiotic use may be considered an appropriate model for other food-animal industries in Canada. Reducing the need for antibiotics by aquaculture companies remains one of the mutual goals of all parties involved. On an annual basis all medicated feeds manufactured for food animals are reported to the British Columbia Ministry of Agriculture and Food by the feed mills. A quantitative summary of antimicrobial usage in aquaculture can then be generated and analysed with assistance from prescribing veterinarians. The salmon industry data and statistics from 1996 to 1998 are reviewed in this paper. Similar databases maintained by the Norwegian government have been available for many years, yet any direct comparison of those data with that from British Columbia has been largely misleading for a number of reasons. An attempt to make equivalent and relevant comparisons between these two aquaculture industries will be outlined.

Antibiotic use in food-animal production is always controversial. The latest concerns of Canadians have been highlighted in parallel with the development of salmon production in marine cages. In the future there may be opportunities to compare antibiotic use in aquaculture to usage in other industries, but currently aquaculture appears to be the only food-animal industry in Canada that monitors its antibiotic use. Consequently, comparisons are generally limited to fish species internationally, and Norway is widely considered to offer the "gold standard" in this matter.

The initial data on antibiotic use in British Columbia (BC) comes from veterinary prescriptions manufactured by the BC feed mills. The prescriptions are manufactured to specification by the feed mill and dispensed to the designated fish farm. The feed mills annually submit both their antibiotic purchase and milling records to the British Columbia Ministry of Agriculture and Food (BCMAF) in accordance to the Canada Feeds Act,[1] and the provincial Pharmacist, Pharmacy Operations and Drug Scheduling Act.[2] BCMAF undertakes to compile and analyse the aquaculture data with assistance from each feed mill and the prescribing veterinarians. Additional records of antibiotic use can readily be referenced within the files of each aquaculture farming company, and within the confidential files maintained by each prescribing veterinarian as per the provincial Veterinarians Act.[3] In addition, the Ministry of Environment, Lands and Parks (MELP) monitors antibiotic and chemical usage data annually from each fish farm in accordance with the provincial Waste Management Act.[4]

Each BC farming company applies considerable effort to minimise its need for, and its use of, medicated feeds. The decision to apply antibiotics to animals is made with diligence and deliberation by the owner and the attending veterinarian. A negative public perception, the effect of restricting fish growth, and the high cost of medications are each examples of the considerations a farmer must make prior to weighing the benefits of a proposed treatment. Some farm sites are able to produce fish efficiently without the need for antibiotics. Other companies have a self-imposed "no medication" period of six or ten months before harvest dates. Yet other farm sites find that antibiotic medications are essential to mitigate bacterial diseases of the caged fish population that is subjected to specific stressors.

In 1998, seven veterinarians acted as the prescribing veterinarians to the BC aquaculture industry, and one hundred percent (100%) of the manufactured medicated feed for salmon was prescribed. In other words,

no manufactured medicated feed was applied to salmon in a BC fish farm under the allowance of the Compendium of Medicating Ingredient Brochures (CMIB) of the Canada Feeds Act. CMIB feeds are legal and commonly used in other food-animal industries according to the licensed drug label. As such, CMIB feeds do not require a veterinary prescription. In aquaculture, the fact that 100% was prescribed in 1998 is significant in that it represents an immediate control point within this food-animal industry. It serves as a centralization of responsibility, activity, and record collection. As part of the ongoing development of Codes of Practice in fish farming in BC, the use of only prescribed medications is already one of the marine aquacultural standards.

In 1998, the BC aquaculture industry used, and continues to use, three basic antibiotic compounds: oxytetracycline, two potentiated sulfonamides, and florfenicol. When one considers each of the antibiotic products applied to fish in 1998, 99.7% were approved for use in fish (99.6 % in 1997 and 99.7 % in 1996). The remaining 0.3 % of the antibiotics applied to fish were in fact licensed for use in food animals and was prescribed to fish under field experimental protocols, or prescribed to fish not destined to be food for humans (i.e., broodstock). Each of the prescribed antibiotic medications for farmed salmon was applied with a therapeutic intent to minimise and control specific bacterial infections within a population. None of the oral antibiotics used in BC farmed fish have been used for the purpose of growth promotion of the animals.

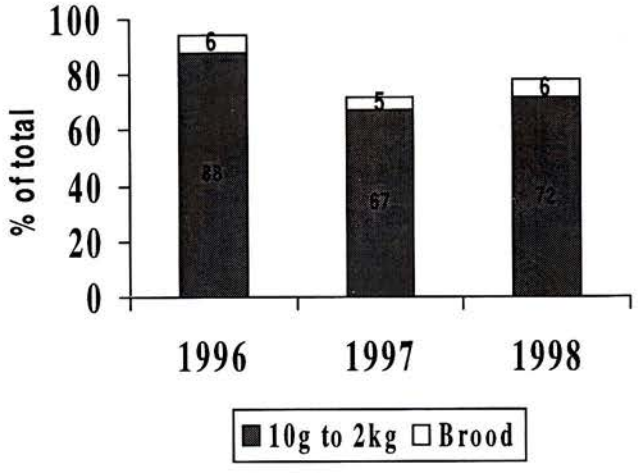

Figure 1. Antibiotics fed to BC salmon of various sizes.

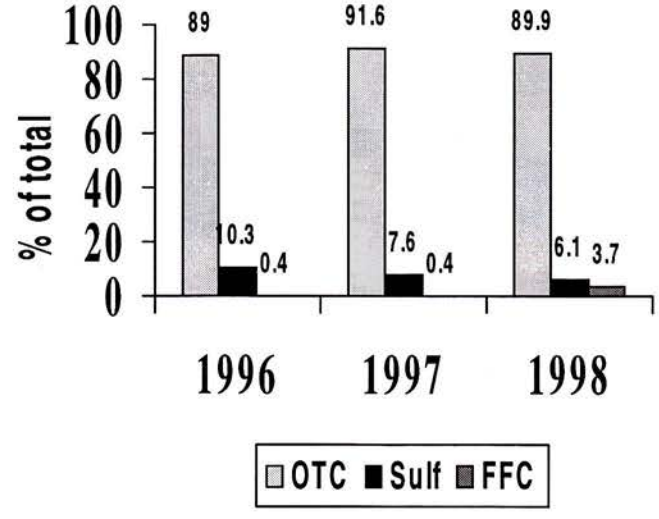

Figure 2. Relative percentage of antibiotic products fed to BC salmon.

For reasons unknown to the author, most discussions about antibiotics seem to be accompanied by mention of hormones, so the opportunity will be taken here to reiterate that hormones are not antibiotics. In addition, absolutely no medicated fish feed was manufactured to contain hormones. The legal use of hormones in fish is extremely low and is strictly designed to manipulate the sex of broodstock (when they 0.5 g in size). The hormone does not genetically modify the fish, rather it causes the female broodfish to develop testes and become a phenotypic male for future matings. Hormones are not used in production food fish.

As illustrated in Figure 1, the majority of antibiotic feed used in BC aquaculture was applied at a time when the fish were juveniles (i.e., smaller than 2 kg). Generally, 72% to 94% of the antibiotics applied to BC salmon were fed to small fish. This suggests a concerted effort by the farmers and veterinarians to minimise infectious bacterial problems early in the production cycle. The treatment of juvenile salmon also creates a long drug-free clearance period of 4 to 12 months before the fish are considered for harvest. Oxytetracycline represents the greatest percentage of antibiotic applied to salmon when compared with the other two antibiotic types shown in Figure 2. Figure 3 illustrates the variety of antibiotic products used in the Norwegian fish farm industry.[5] Oxolinic acid is the antibiotic in greatest use in Norway.

Unlike oxolinic acid, oxytetracycline is a relatively inefficient oral drug due to its strong predisposition to bind with divalent cations of magnesium and calcium, rendering the oxytetracycline not bio-available or no longer pharmaceutically active. Yet despite its inefficiencies, oxytetracycline remains the only drug choice to control bacterial kidney disease and rickettsial infections in Canada. Other antibiotic compounds may prove to be more effective than oxytetracycline but they are not licensed in this country.

There are many differences between the salmon farming industries of Norway and British Columbia. Norway enjoys a much larger aquaculture industry where Atlantic salmon constitutes the main production tonnage. Trout are also farmed in marine cages.[6] Atlantic salmon production also comprises the main market tonnage in BC.[7] However, approximately 15% of the farm-raised tonnage is Pacific salmon, a fish that is more susceptible to chronic bacterial infections and also shows the highest mortality rates toward the end of its marine production period.

Two more relevant and noteworthy distinctions occur between Norway and British Columbia in terms of fish health and antibiotic use. Firstly, the Norwegian industry currently has very few bacterial infections that require antibiotic chemotherapy. Rather, the diseases of greatest economic concern are viral or parasitic in nature[8,9] and thereby do not benefit from antibacterial medications. To date, this is not the case in BC fish farming which still endures endemic bacterial infections. Hence the appropriate application of antibiotics. Figure 4 shows the relative amount of anti-parasitic compounds versus the antibiotic compounds used in Norway. Fifteen anti-parasitic products are used in Norway,[6] whereas the BC aquaculture industry has had the very limited need for only one (ivermectin).

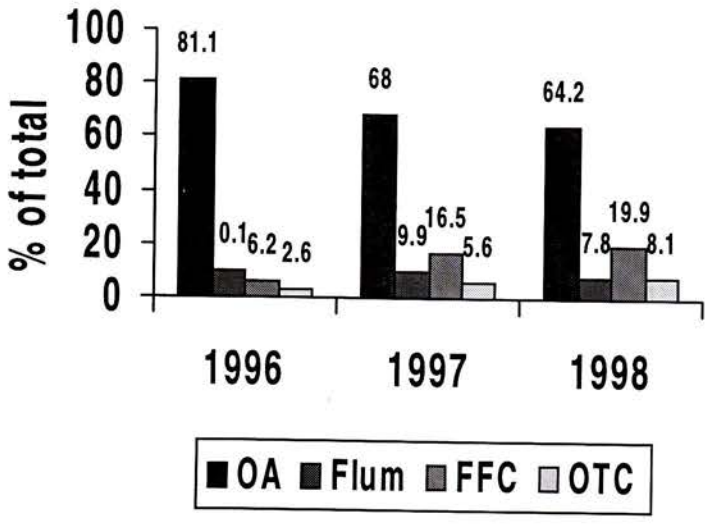

Figure 3. Relative percentage of antibiotic products fed to Norwegian salmon.

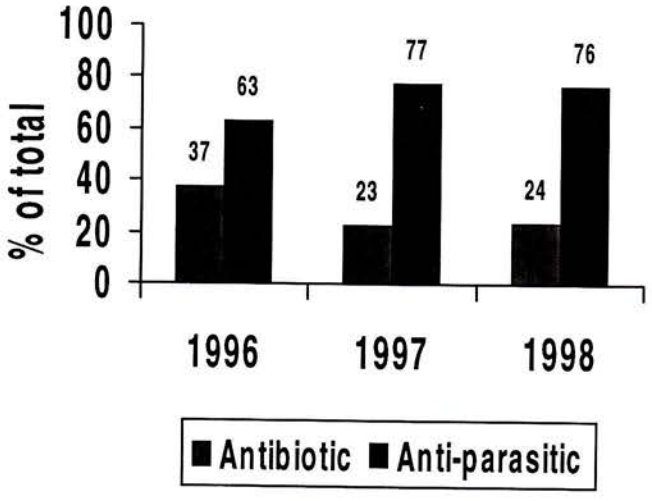

Figure 4. Relative percentage of antibiotic vs. anti-parasitic products fed to Norwegian salmon.

Secondly, Norway makes legal use of very effective quinalone antibiotics (i.e., oxolinic acid and flumequine) that are neither licensed nor available in Canada. The quinalone drugs are more available for absorption and distribution throughout the fish tissues than is oxytetracycline, and the daily dosage recommendation of quinalone antibiotics is a mere 10% of that of oxytetracycline. These two differences between the Norwegian and BC aquaculture industries, in large part, help to account for some of the differences in antibiotic usage in the two countries. It must be recognised, however, that Norway does use much less antibiotic in the production of Atlantic salmon than does British Columbia. As shown in Figure 5, the average production of a metric ton of Atlantic salmon in BC required 151 g of active antibiotic whereas the same quantity of fish produced in Norway required only 1.9 g of antibiotic in 1998.

When one compares the familiar historical graph of Norway's antibiotic use since 1981[5] to British Columbia's use of 151 g / MT salmon in 1998, the superimposed line in Figure 6 suggests that the BC fish farm industry exists as the Norwegian industry did in 1990. Relatively new

Canadian industries such as aquaculture will continue to be the focus of attention and inquiry, but Figure 6 may predict one aspect of the industry's future. With ever increasing vaccine efficiencies, continued improvements to farming codes of practice and natural genetic stock selection, the overall use of antibiotics in the production of salmon in British Columbia is likely to decline in the years to come. That remains the goal of all parties involved.

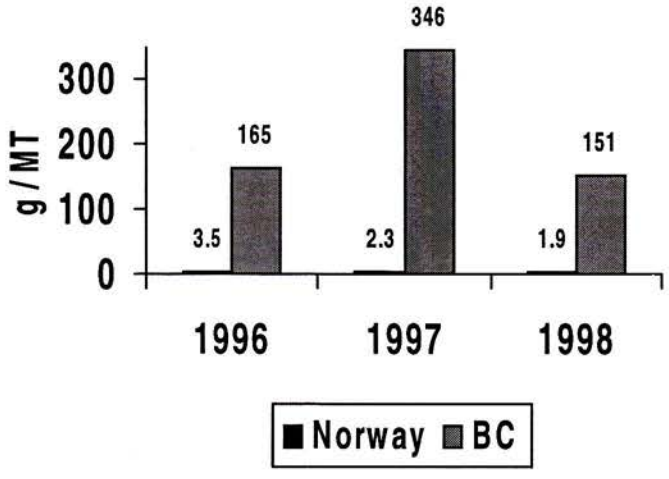

Figure 5. Active antibiotic (g) used per tonne of Atlantic salmon produced in Norway vs. British Columbia.

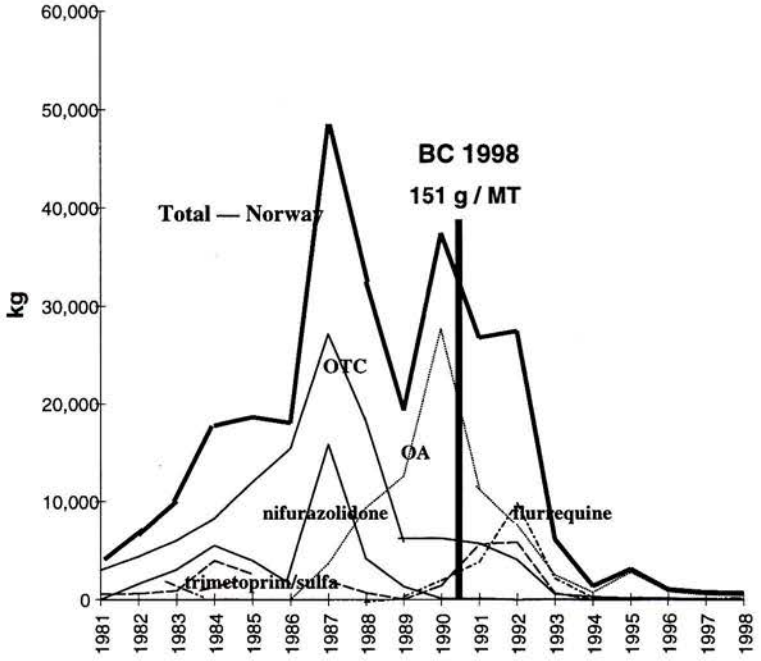

Figure 6. Historical antibiotic use in Norway, 1981 – 1998.

The data contained within this document were compiled with the assistance of Drs. Henry Lange, Joanne Constantine, Trygve Poppe, Asgeir Ostvik, Tor Atle Mo, Tor Horsberg, Rich Moccia and Anne Mette Onarheim. I thank them for their interest, participation and support.

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***Mark Sheppard** is a private veterinary consultant within BC's aquaculture industry. He can be reached at Sakana Veterinary Services Ltd., Campbell River, BC Canada V9H 1J4. E-mail: lvl@oberon.ark.com*

Trends in Aquaculture Diagnostic Service Delivery: Comparisons with Other Sectors of Canadian Livestock Agribusiness

David J. Speare

Producers of terrestrial livestock in Canada benefit from an integrated regional, provincial, and federal health service and extension delivery system which has evolved over the years in line with growth and changes within the livestock industry. The veterinary practitioner forms the vital function of plugging the producer into the extensive menu of services provided within this public-funded infrastructure. The system is equally available to a dairy producer in Nova Scotia and a beef producer in northern Alberta. The emphasis of this diagnostic infrastructure is to enhance farm production and sustain rural development, and provides the template through which new diagnostic tests are conceived, funded, tested, and implemented. Tests with different, but known, sensitivities and specificities are used cost-effectively, with a reasonable balance of turn-around-time and accuracy. Attention is also paid to identifying the risks that wildlife impose as reservoirs of infectious agents which may become transferred to farmed animals. In contrast to the well-defined diagnostic network available to livestock producers, aquaculturists currently find themselves in a less desirable position. The available infrastructure network is more nebulous, the paradigms less easily defined, the range of diagnostic tests limited, and the centers of expertise relatively fragmented. A bias in the intensity of testing will likely result in the detection of infectious agents in farmed fish prior to their identification in wild fish, leading to results that may erroneously suggest farmed fish pose a risk to wild fish. With these differences in infrastructure in mind, this paper will examine some of the important implications.

The marked growth phase of the fish farming industry in Canada has created a situation where its needs for a rapid, integrated, accurate response to disease situations can often not be met. Closing this gap is an important priority, particularly in order to appropriately capitalize on the potential pipeline of new diagnostic tests brought about by advances in molecular biology.

For most diseases of humans, animals and fish there are several ways to make a diagnosis. Based on cost, availability, and time-to completion, different approaches are appropriate at different times or for different conditions. For example it would not be cost-effective within human medicine for virus culture to be attempted on every case of the common cold. It would provide some fascinating data, but in many cases, the results would only become available once a person had recovered. The virus testing activity would consume a considerable portion of the medical budget (from taxpayers) and thus weaken dollar allocation to other areas. Additionally, it would likely reveal the presence of many viruses, and a great deal of time would need to be spent in trying to determine which, if any, were actually causing disease.

Equally, in veterinary medicine, the vast majority of clinical cases are recognized through an interpretation of clinical signs. Thus, only a fraction of cases in human and veterinary medicine require that samples be forwarded to laboratory networks for confirmation or diagnosis. If sample numbers increase, so does the public burden of paying for those tests.

These two broad examples are intended to highlight the relevance of the highly-trained clinician, and the value of the clinical exam as the first step in any disease problem-solving exercise. Diagnostic tests, no matter how accurate, inexpensive, or fast they are, are part of the secondary process of confirming or ruling-out a problem. Focusing on diagnostic test development, and ignoring the issue of how clinical services are delivered, and how diagnostic test results

are interpreted and utilized for the fish farming community will lead to a very unbalanced approach to disease management. Bypassing the clinician, and sending diagnostic materials directly to a diagnostic laboratory, may in some isolated cases be a reasonable approach, but in most instances it is a bad choice.

The client-clinician relationship, with the clinician being the access point to the laboratory network system has several key attributes that make it an ideal system around which to model the service network for fish farm disease diagnosis. The clinician is able to act as an information broker. For any particular problem they can identify which tests are likely to be most informative, and select them on a cost-effective basis. Technical data emerging from these tests is not likely to be overly meaningful to most clients, and the clinician performs the valuable task of interpreting the results in the context of the problem at hand and clinical observations made at the time of the farm-visit. Furthermore, establishing an early relationship with a clinician, ensures that if medications are needed, that delays in starting treatment will be minimal. An aquaculturist seeking a prescription, from a clinician who has not been involved in the diagnostic process, is likely to be met with considerable delay since the clinician is ethically and legally obligated to thoroughly review the situation first.

The client-clinician relationship is also a key ingredient to ensure client confidentiality. This is particularly important today, as molecular biological tests are becoming the norm. The potential high sensitivity of these tests may trigger positive results indicating the presence of a viral agent, or residual genetic material from a virus. The virus may or may not be pathogenic. In any case, does the fish farmer want to have these results kept confidential — and if so, how can this be done once the samples have already been submitted and the farm location identified?

An issue which is as equally pressing as the need to understand the role of the clinician, is to understand that ***the laboratory diagnostic infrastructure servicing the needs of fish farmers in Canada is underevolved.*** It falls far short of what has been developed to support other livestock industries, and it has deficiencies even when compared to several other countries where farmed salmon are produced. For example, a cattle farmer in Canada, through his veterinary practitioner, is able to have diagnostic samples sent to a regional diagnostic laboratory. In most cases, this lab would be able to complete histopathological, bacterial, virus, parasitic, clinical chemistry, and toxicological testing. Staff are highly trained, and many of them are certified specialists in their fields. Certification usually requires on-going formal continuing education to ensure that the professional remains current in their respective fields. The regional lab is connected to the provincial laboratory system where more specialized testing may need to be carried out. The provincial lab in turn is connected with federal reference centres, which in turn are connected to international reference centres. Tests with different, but known, sensitivities and specificities would be used at different levels within this hierarchal specialization network. Attempts are made to have quick turnaround for results. The paradigm is to use the laboratory testing to provide results which are geared towards rapid understanding of the problem such that the farmer is able to limit economic impacts of disease.

The fish farming industry in Canada is not new, but there is likely to be lag time before the diagnostic infrastructure develops such that it resembles the systems that are already in place for other livestock producers. The current lack of organized infrastructure is problematic in itself. We need to have reference centres, and we need to have established links between different laboratories. However, an additional problem arises because of the growing availability of new diagnostic tests and how they will be used, and how the results may be interpreted and used. This is particularly relevant since the development of new tests will likely to directed towards problems which are first detected on fish farms. Thus novel infectious agents will first be identified on fish farms, although the availability of test reagents will later show the same agents to be present within feral fish as well. The question of whether wild fish serve as reservoirs for infections that affect farmed fish, or vice versa, is thus likely to broaden and deepen as new data are created. The aquaculture industry may find itself in a position that it needs to support disease investigations and/or surveys for the presence of pathogens in wild fish in order that the risks which wild fish impose on farming activity are more fully understood and appreciated.

The aquaculture industry is in a unique position to influence the direction that diagnostic service infrastructure development takes in Canada. ***However, it is critical that the different service models be understood and fully evaluated for strengths and weaknesses. Demands for new tests are unlikely to be adequately fulfilled until the service network that will provide these tests is solidified.*** The advantages of a strong service network would include:

1) staff who are trained to use new tests once they are developed;
2) provision of a mechanism through which research and development of new tests can be developed and evaluated;
3) a focus on certification and quality control of staff and new tests;

4) a means of data storage that could be used to help support R&D or grant proposals aimed at new test and new technology development;
5) mandated rapid turnaround of test results, and uninterrupted service;
6) client confidentiality;
7) cost-recovery mechanisms that would ensure that fish farmers receive benefits similar to those enjoyed by other livestock farmers in Canada.

Paradoxically, much may be gained if the focus on the diagnostic activity is also aimed towards regular intensive investigation of diseases and pathogens as they occur in feral salmonids and non-salmonid species which inhabit the vicinity of seacage sites.

***Dr. David J. Speare** is an Associate Professor of Fish Pathology at the Atlantic Veterinary College Charlottetown, Prince Edward Island (speare@upei.ca), and is an aquatic animal veterinary histopathologist with AVC's Aquaculture Diagnostic Services.*

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Alaskan Field Trials of MIST™ Diagnostic Kits for Detecting Paralytic Shellfish Poisoning

A.L. Burbidge, J.F. Jellett, R.L. Roberts, E.R. Belland, and A. Russell-Tattrie

Jellett Biotek Ltd. recently completed an extensive validation trial of its cell-based diagnostic test kits for paralytic shellfish poisoning (PSP) in Alaska. Over 1300 tests were performed on 617 different shellfish samples, using the Maritime In Vitro Shellfish Test (MIST™, both quantitative and qualitative versions), the mouse bioassay (MBA) and high performance liquid chromatography (HPLC). The kits were shipped by air courier to trial participants in Alaska which included a regulatory laboratory, hospital, college, research institute, community health agency and an aquaculture operation. The trial results clearly demonstrated that the MIST™ kits are as effective as the mouse bioassay in detecting PSP toxins in shellfish in the regulatory environment and are an accurate, cost-effective screening method for these toxins. The cell kits were also effectively used in regional testing centres and field applications, demonstrating the potential of user-friendly MIST™ kits for harvest management at aquaculture sites and for fishery and beach monitoring.

Introduction

Toxic algal blooms occur all over the world and appear to be increasing in number, severity and diversity. Of major concern are the toxic forms that can cause human illness ranging from mild discomfort to paralysis and death, but the problem also includes species that can affect our utilization of shellfish resources. The economic loss from shellfish that cause human illness can be catastrophic, but if harvesters, managers, government officials and the media can work together to develop monitoring systems and ensure responsible press coverage, it should be possible to protect the interests of all concerned.[1] With a reliable PSP pre-screening test, shellfish growers and harvesters will be able to test a sample of their product before harvesting and processing to ensure the product is safe. If the product is toxic, it may be left in the water for a period of time before it is tested again prior to harvest. Harvest management will save the time and expense of harvesting contaminated products, many of which have to be destroyed if they test positive at the regulatory lab. The costs involved in shipping samples and paying for a mouse bioassay in the regulatory lab, which can be quite high for remote locations, will be avoided on positive samples. This should result in lower operating costs to the producers and state regulatory laboratories as well as less wasted product. Shellfish products will be safer by having another level of testing.

Alaska is a state with a long standing history of PSP, thousands of miles of coastline, and an enormous shellfish resource. Efforts to develop this resource have been hampered by the recurrent and unpredictable PSP problem, the logistics of the long and in many areas remote coastline, and the economic and workload constraints placed on the state regulatory laboratory, the Alaskan Department of Environmental Conservation (DEC). This laboratory, centrally located in Palmer, supports the entire biotoxins monitoring program, which is primarily mandated for testing and certification of commercial fisheries. Although some testing of non-commercial shellfisheries, such as some of the aboriginal subsistence harvests, is performed at the DEC laboratory, the technical, logistical and economic constraints of a single state mouse bioassay laboratory has been problematic. The inability to have more broadly-based monitoring under the current system is thought by some to be hampering the expansion of commercial shellfish activity and continues to put some beach harvesters at risk for illness or death from PSP. The potential to screen for PSP and monitor beaches in southeast Alaska will be enhanced by having regional testing available, which currently does not exist. Subsistence shellfisheries and areas not covered by the State testing program could use such a toxin screening service to help avoid the chance of harvesting contaminated product. Potential exists to open new shellfish areas (subject to final regulatory tests). During the trial it was shown that the MIST™ technology could reduce current MBA testing costs by up to 28%. This could

Table 1. Comparative cost of mouse bioassay and pre-screening using the MIST™

	Cost
DEC Mouse Bioassay	
6000 samples @ $125/sample	$750 000
MIST™ Screen and MIST™ Quanti	
6000 samples @ $70.24	$421 440
1200 samples (20% positives) @ $99.77	$119 724
Total	$541 164
Savings	$208 836 or 27.8%
Prescreen using MIST followed by mouse on positives (20%)	
Savings	$178 560 or 23.8%

have a direct impact on the State of over $200 000 per year (Table 1).

Methods

Two types of kits were used in the trial, the MIST™ Quanti and a qualitative (yes/no) version of the cell-based test called Mini-MIST™. The MIST™ Quanti, which is a fully quantitative test, uses mouse cells rather than live mice in the test. Saxitoxin, the parent compound in the family of closely related toxin analogues that cause PSP, is a sodium channel blocker. If saxitoxin is present in the shellfish being tested, the sodium channel is blocked and the mouse cells are protected against competitive reagents, which are added to the test. The competitive reagents destroy cells unprotected by saxitoxin and, when stain is later added, the cells appear clear. Cells protected by PSP will stain purple, the intensity of the staining indicating the amount of cells remaining and therefore the amount of toxin present. A microplate reader is required to quantify the saxitoxin present in the shellfish tissue. The cost of MIST™ Quanti test is $50 per sample and four samples can be tested per plate; a kit consists of five plates, saxitoxin standard, and the competitive reagents, and costs $1000. The other kit used in the trial was the Mini-MIST™, which was used for qualitative screening of the shellfish samples. This kit also uses mouse cells but does not require a microplate reader as the results are determined visually after staining the 96-well plate. The cost of the Mini-MIST ™ test is $35 per sample and 8 samples can be tested per plate; a kit consist of three plates, saxitoxin standard and competitive reagents, and costs $840. Trial partners (Fig. 1) were selected to ensure the MIST™ kits were used in a variety of applications. The DEC laboratory in Palmer demonstrated the kits in a regulatory food safety application by performing the fully quantitative MIST kits in parallel with the mouse bioassay. The DEC lab also did some Mini-MIST™ tests. The Ketchikan General Hospital (Ketchikan), University of Alaska Fisheries Industrial Technology Centre (Kodiak) and Sheldon Jackson College (Sitka) used the Mini-MIST™ qualitative kits as a screen for PSP, and demonstrated the potential of the kits in a regional PSP screening application. The Bristol Bay Health Corporation (Dillingham) demonstrated the kits as a beach monitoring tool, and Elfin Cove Oysters,

1. Department of Environmental Conservation, Palmer
2. Sheldon Jackson College, Sitka
3. University of Alaska Fisheries Technology Centre, Kodiak
4. Bristol Bay Health Corp., Dillingham
5. Elfin Cove Oysters, Elfin Cove
6. Ketchikan General Hospital, Ketchikan

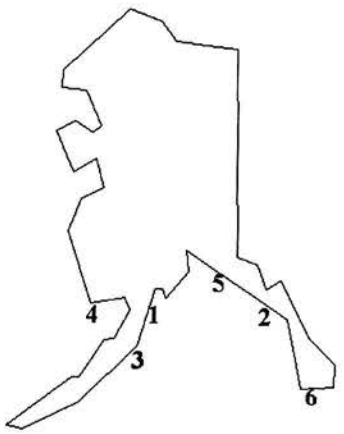

Figure 1. Location of field sites that participated in the Alaska trial.

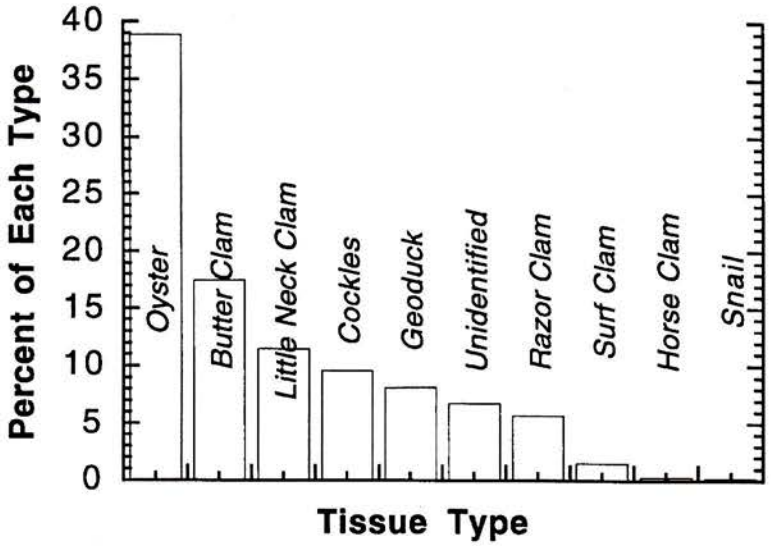

Figure 2. Broad range of tissue types tested using the MIST™ technology.

(Elfin Cove), demonstrated the kits as a harvest management tool for the aquaculture industry. The Fisheries Industrial Technology Centre (Kodiak) also performed some fully quantitative MIST™ kits to demonstrate regional testing for PSP.

A training session for all Alaskan participants was held by Jellett Biotek staff at the DEC laboratory in Palmer, where the Association of Official Analytical Chemist (AOAC) PSP toxin extraction method [2] and fully quantitative MIST™ and Mini-MIST™ kits were demonstrated and practiced by all participants. During the trial, shellfish samples were collected by trial participants, the tissue homogenized, then split, with half the sample being sent to the DEC laboratory for a regulatory AOAC toxin extraction, a fully quantitative MIST test, MBA and Mini-MIST™ test. An acid extraction was performed by the trial participants on the other half of the sample, and the MIST™ assay was performed. The DEC lab also tested shellfish samples submitted under the ongoing, normal regulatory program using the fully quantitative MIST™ kits with the mouse bioassay as control. The trial ran from June 1998 to January 1999. Shipping the MIST™ test kits to the trial partners in remote areas proved challenging, and the packaging was upgraded with the inclusion of phase-change gels designed to keep the kits at 20 to 25°C and the addition of a thicker polystyrene shipping container, which offered more insulating value. Levels of agreement with the MBA went up after implementation of the upgraded packaging (Table 2).

Results

There was a broad range of tissue types tested using the MIST technology (Fig. 2). Of the 617 tests performed, the majority of samples were oysters and butter clams, followed by littleneck clams, geoducks, blue mussels, cockles, surf clams, horse clams and snails. No differences in the results of any particular species were noticed.

In the 617 comparative tests of the MIST™ Quanti and the MBA, the MIST™ Quanti detected toxicity 99% of the time where the MBA detected toxicity, demonstrating the efficacy of the MIST™ kits for screening. It is important to note that of the 6 samples (1%) where toxicity was not detected, none were at a level likely to cause sickness in a human. Because the MBA and MIST technologies are biological tests, they are both subject to variabilities in results, estimated at ± 20%.[3,4]

Table 2. Overall agreement of MIST™ bioassay with mouse bioassay results, with and without ± 20% variability of each technology

Type of MIST™ Tests	± 20% Error	% Agree	%Disagree
All Quanti	no	82	18
	yes	88	12
Original Packaging	no	81	19
	yes	87	13
Improved Packaging	no	86	14
All Quanti + Mini	no	85	15
Excluding Site 4	yes	90	10

A third technology, the HPLC (a chemical analytical method) was used to corroborate the results between the MBA and MIST kits when they disagreed. It was found that all three technologies — the MBA, MIST™ Quanti and HPLC — each missed detecting toxins on some samples and in effect could have provided a false negative result, demonstrating that no detection system is perfect. Because of the variability in the mouse and MIST testing systems that produced differences in quantitation, and because there is a set cut off in toxicity (i.e. 80 µg per 100 grams of tissue), care must be used in comparing the MIST™ Quanti and MBA test results. For example if the MBA result was 81 µg/100 g of toxicity and the MIST™ technology result was 70 µg/100 g of toxicity, these results are statistically the same, given the natural variability of each method. With the 80 µg cut off point, however, this MIST™ result would be reported as a false negative. Table 2 summarizes comparisons of overall agreement between the MBA and the MIST tests. Given a ± 20% variability factor for both MIST™ Quanti and MBA, the MIST results agreed with the mouse results 86.8% of the time within the 0 to 80 µg/100 g toxicity level, 75.4% at the 80 to 400 µg/100 g level, and 70% at the 400+ µg/100 g level, with an overall agreement level of 88%. Because the Mini-MIST™ is qualitative, agreement falls on either side of the detection limit and data cannot be compared using ± 20% variability as with MIST™ Quanti. However, ± 20% variability is still ascribed to the MBA results when comparing with the Mini-MIST™ results.

There was more variability from site to site with the Mini-MIST qualitative (yes/no) test, which we found later to be caused by shipping problems to remote locations and a matrix effect in the shellfish extract that provided erroneous results. If we remove the Bristol Bay site from the data, (where we had the greatest matrix effect and shipping difficulties), the agreement of the Mini-MIST™ results from the other 5 field sites with the MBA was 96.4%; the overall agreement of the Mini-MIST™ and MIST™ Quanti to the MBA is 90%. Identification of a matrix effect on the Mini-MIST™ technology led us to redesign this product to eliminate this problem. Consequently, Mini-MIST™ has been replaced by the MIST™ Screen, which eliminated the potential problem caused by matrix effects on the Mini-MIST™.

Discussion

More extensive quantitative and statistical comparisons of the results from this trial will appear elsewhere, along with the results of an AOAC intercollaborative trial involving 15 international laboratories.[5] As shown here and in Jellet et al.,[5] the cell based MIST™ Quanti has been proven to be as effective as the MBA in screening for PSP. Quantitatively, levels of overall agreement were also high, but were even better when toxicity levels were below 300 µg/100 g.[5] Aside from the identified problem of a matrix effect on the Mini-MIST™, the qualitative screening kits performed well at all sites. The replacement MIST™ Screen kit appeared to eliminate the matrix problem, the origin of which remains unknown at this time.

Limitations were experienced in shipping these temperature-sensitive MIST™ kits to remote areas, and feedback from field participants indicated that they would prefer single use, faster tests with no temperature sensitivity or equipment requirements. These limitations in the application of the MIST™ screening technology to remote field sites has resulted in the development of a rapid diagnostic test kit for PSP by Jellett Biotek Ltd. The MIST Alert™ for PSP is a qualitative test that is simple to use, inexpensive, rugged, single use, and provides visual results of PSP in less than 20 minutes. It has a detection limit of approximately 5 µg/100g, although the tests are currently configured to detect PSP 40 µg/100g. The MIST Alert™ for PSP detects all important analogues. Applications of this test include: as a harvest management tool to determine a safe time to harvest, to screen samples in a regulatory laboratory and as a quality control tool for processing plants. The MIST Alert™ test is currently in validation trials and is expected to be on the market in the spring of 2000.

We acknowledge Bob Chaney and Jim Palin and the Alaska Science and Technology Foundation (ASTF) for funding the trial, Dick Barrett, Chris Allison and staff at the Department of Environmental Conservation (DEC) and Ray Ralonde at the University of Alaska for the continued support.

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The authors are with Jellett Biotek Ltd., 101 Research Drive, PO Box 790, Dartmouth, NS, Canada B2Y 3Z7.

Value Added by the Canadian Aquaculture Industry

Mark Elward

As part of its mandate to provide Canadians with relevant, reliable, and timely statistics about Canadians and their activities, Statistics Canada is strengthening the aquaculture data set for this increasingly important industry. Last year, the first Value Added Account for the Canadian aquaculture industry, referring to 1997, was released to the public. 1998 has now been released and the intention was to produce data for 1999 in August 2000. These data that result from the Unified Enterprise Survey represent the first comprehensive attempt to collect aquaculture financial data on an annual basis. This paper will briefly explain the survey and the aquaculture statistics program before presenting the value added data.

Statistics Canada

Statistics Canada's mandate, derived from the Statistics Act, requires the Agency to collect and disseminate statistical information on the socioeconomic and general conditions of the country and its citizens. Statistics Canada is the "core" of a centralized statistical system frequently cited as a model by observers of the international statistical community and by members of the international business press. There are several guiding principles that influence much of what the organization does. It is important that the statistics be:

- relevant — that they are used to make decisions
- accurate
- objective and scientific (removed from politics)
- timely
- available to the public in a clear, "friendly" way
- confidential, if they pertain to an individual or a business
- historic (there are exceptions)

In summary, it is Statistics Canada's role to provide data, to provide information on the quality of the data, and to provide advice on the use of the data if asked. The quality of data required is a function of the decisions that will be based on the data. Although Statistics Canada will provide advice on the use of the data, it cannot really control its use. Most often, the data are used appropriately. However, this is not always the case and for aquaculture, where the industry itself is controversial, users have been known to inappropriately use the data to support their own perspective.

As part of its mandate, Statistics Canada produces, through the Canadian System of National Accounts (CSNA), a myriad of integrated economic information. This includes official estimates such as the gross domestic product. The value-added data for aquaculture were the result of a larger project known as PIPES, or the Project to Improve the Provincial Economic Accounts.

The Survey

One of the ways that Statistics Canada is improving the provincial economic accounts is by the development of the Unified Enterprise Survey (UES). This new survey is designed to collect financial data about virtually any type of business, incorporating several business surveys into an integrated framework. The survey will produce consistent and accurate data for different industries operating in different ways with different levels of complexity with sufficient quality to produce accurate provincial statistics. For more information on the survey itself, see Appendix 1.

Statistics Canada has responded to the increasing demand for aquaculture statistics by including this growth industry in the first year of the Unified Enterprise Survey. There were several reasons for this decision. Aquaculture has been recognized for some time as a data gap. Recently, it was identified under the North American Industrial Classification System (NAICS) as an independent industry. Even though its relevance is expected to increase, it is still a relatively small industry which, from a statistical perspective, makes it well suited and manageable for a pilot survey.

The importance of aquaculture as a Canadian industry is increasing because this country does have some competitive advantages, including a long, protected, and relatively uninhabited coastline that is ideal for aquaculture production. Globally, the industry is expected to expand and play an important role in the supply of food, as the demand for protein expands exponentially. Aquaculture is a very efficient converter of feed.

In addition, an important factor in the inclusion of aquaculture in the United Enterprise Survey was the fact that the industry is supportive and cooperative. The industry, which is somewhat controversial because of a perceived threat to the wild fishery and because of environmental concerns, has been requesting statistical recognition. The importance of the cooperation of the industry cannot be overstated and Statistics Canada greatly appreciates the support it has had in the development of the program.

Aquaculture

Statistics Canada defines aquaculture as the managed production of fish. In Canada, the industry is dominated by the production of finfish, primarily salmon off the coasts of British Columbia and New Brunswick. Production of shellfish is smaller, with Prince Edward Island and British Columbia being the major producers.

Under NAICS, the aquaculture industry comprises establishments primarily engaged in farm-raising finfish, shellfish, or any other kind of aquatic animal. These establishments use some form of intervention in the rearing process to enhance production, such as keeping animals in captivity, regular stocking and feeding of animals, and protecting them from predators.

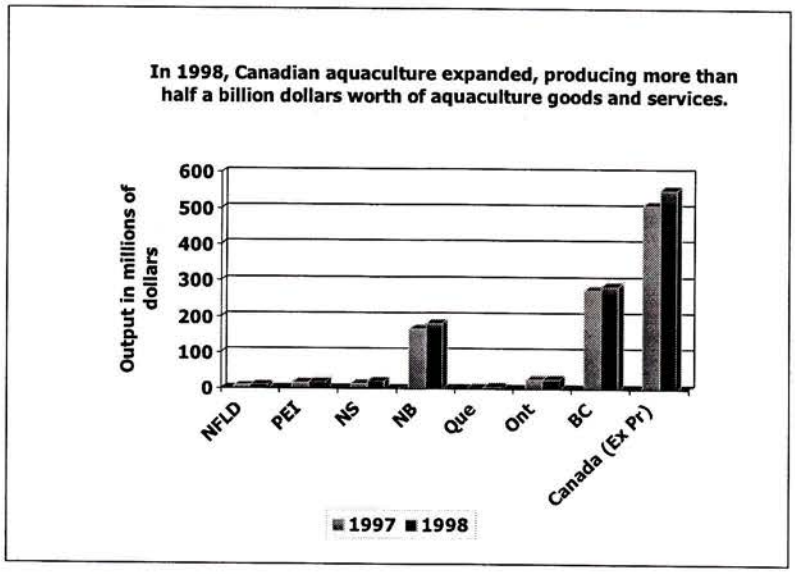
Statistics Canada basically uses a 50% rule to classify a business. If more than 50% of an establishment's revenue is derived from the activity defined, then that establishment is classified to that industry and all of its activities are accounted for as part of that industry. For aquaculture, this means that processing of fish is a part of the industry when the processing is carried out by a business where the primary activity is "farm-raising of finfish or shellfish." Businesses that only process aquaculture products would be classified to the food processing sector. Also, suppliers of goods or services to the aquaculture sector are classified to industries other than aquaculture, unless their primary business is aquaculture production.

The aquaculture industry includes hatcheries and sales within the industry; for example, sales from a hatchery to a grow-out operation are included. The aquaculture industry does not include sport fishing or the wild fishery.

Aquaculture is very similar to agriculture — the only real difference is that aquaculture by definition is in the water and agriculture is on land. In fact, parallels can be made between the transition that is occurring now in the oceans and the transition that occurred on land several thousand years ago as man progressed from a hunter/gatherer to a producer.

Aquaculture Statistics

Statistics Canada's Livestock Section, which is part of the Agriculture Division, is responsible for



aquaculture statistics. The Section publishes three data sets related to aquaculture.

The first is based on provincial regulatory data and is activity based. Data are presented for production and value by species and province. The time series starts in 1991 and the data were first published in March 1997 in the Livestock Statistics Binder (catalogue 23-603). As the price is a farm gate price, these data do not account for all the value added activities that occur in any particular aquaculture business.

The second data set relates to the export of aquaculture products. The data are displayed by country of destination in tonnes and dollars for mussels, chinook salmon, and Atlantic salmon. These data are available in the Livestock Statistics Binder as of September 1999.

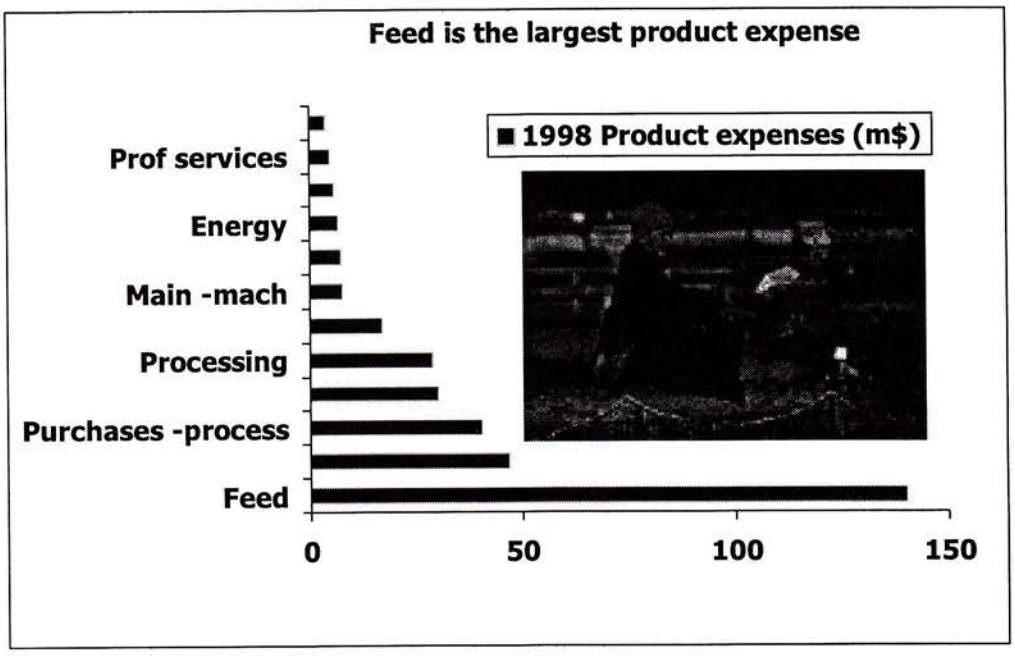
The third data set is the Value Added Account for Aquaculture, which measures the value added by the Canadian aquaculture industry. There were three components that contributed to these accounts. The administrative production and value data were used as check data for the survey results. Tax data were used for a second check, to provide data for smaller operations as well as provide information for imputation purposes. And, most importantly, the primary source of the data resulted from the United Enterprise Survey, a probability sample survey that focuses on larger operations.

Out of the population of some 637 identified aquaculture producers, 209 were included in the 1998 sample, accounting for over 77% of industry sales. Respondent cooperation was excellent; there were few refusals to provide data. However, the number of positive responses was less than the total number of responses because of out-of-scope operations and because responses were not received before the data collection cut-off.

Value Added

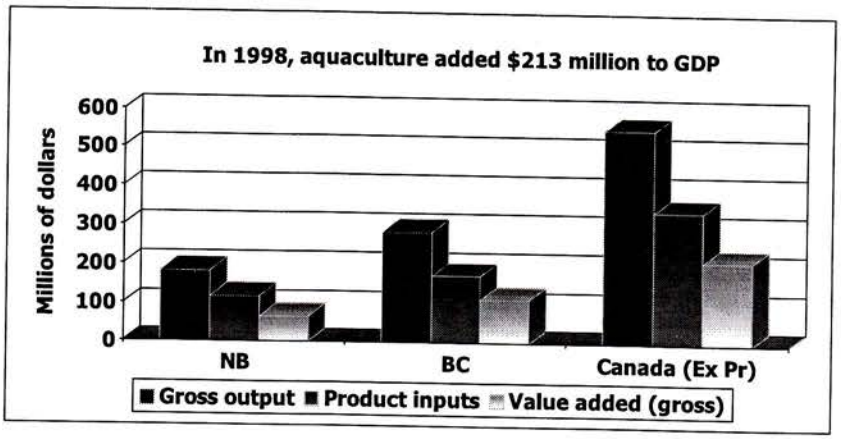
The aquaculture value added account measures the value of the economic production of goods and services directly from aquaculture establishments. Economic production can be defined as any process that creates value or adds value to existing goods. Consistent with this definition, the Canadian System of National Accounts defines economic production as the production of goods or services that are exchanged for money in the market economy. Value added in aquaculture is then a function of the gross output (total operating revenue plus change in inventory value for goods) less product expenses.

In 1998, over 600 operations were involved in fish farming. These businesses produced output worth $553 million, climbing 8.4% from the previous year. Of this figure, sales represented $507 million, a 14% increase from 1997, while rising inventories repre-



sented $25 million. By far, the single most important product was salmon. Appendix 2 displays the detailed value added account for the aquaculture industry.

The sales of whole fish dressed, fresh, or chilled contributed 61% ($308 million) to the sales of aquaculture products and services, while the sale of fish eggs and live fish for grow-out was worth $46 million (9%). Fish fillets, fresh or frozen, earned $53 million (11%).



Revenues from British Columbia and New Brunswick alone accounted for 86% of all aquaculture sales in 1998. Fish farmers in British Columbia had sales of $264 million in 1998, over half of the national total. In New Brunswick, fish farming was worth $173 million, about 34% of national sales. There are aquaculture operations in every province, although estimates are not provided for the Prairie Provinces because there are only a handful of operations in this region.

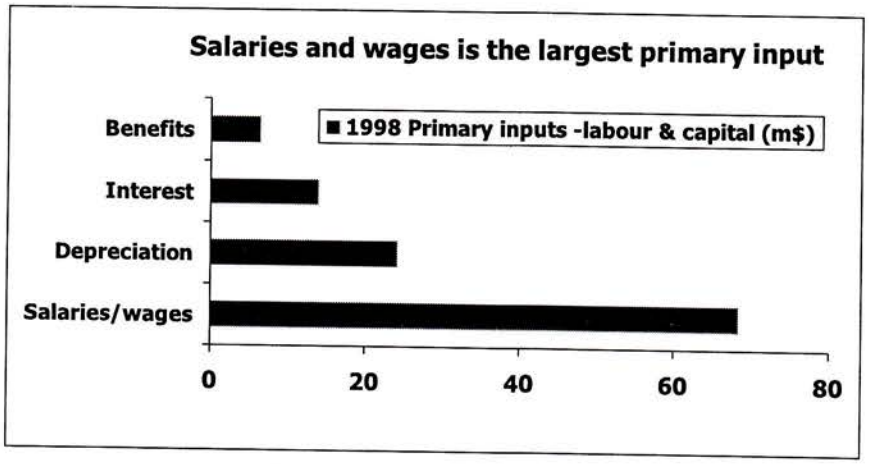
Shellfish contributed $35 million (7%) to the 1998 sales, rising 13% from the previous year. Over half the revenues from shellfish are generated by Prince Edward Island, while British Columbia is responsible for 28%.

As aquaculture is an expanding industry, increasing inventories of goods in process and finished goods also made an important contribution (+$25 million) to the overall output. It should be noted that government subsidies amounted to $6.6 million, or 1.2% of the gross output.

In contrast, fish farming operations incurred product expenses (refers to products and services purchased from other businesses but does not include capital and labour costs) totalling $342 million in 1998. Feed, the single largest expense at $140 million, rose 16% from 1997 as production expanded. Other large expenses included purchases for grow-out ($47 million), purchases for processing ($40 million) and processing services ($29 million).

The industry's net contribution to the economy (its value added) in 1998 amounted to $213 million, a 17% increase from 1997, which is the difference between gross output (revenues and inventories) and product inputs. British Columbia contributed $112 million to the total value added, while New Brunswick accounted for $65 million.

The gross value added for an industry is conceptually the same as the gross domestic product. The mea-



count is on a factor cost basis. Factor cost valuation represents the sum of incomes of factors of production as measured by the cost of labour and capital inputs in the production process. Certain selected primary inputs (labour and capital) are displayed in the account.

Aquaculture producers paid out $75 million in salaries and benefits during 1998, while depreciation charges stood at $24 million. Interest costs were another $14 million.

Data on 1998 aquaculture production released in August 1999 showed that the industry has expanded during the 1990s. Between 1991 and 1998, production climbed from 49 600 tonnes to 92 000 tonnes. The 1998 level represented a 12% increase from 1997.

Meanwhile, exports have skyrocketed, particularly for Atlantic salmon. In 1992, farmed Atlantic salmon exports stood at $114 million. By 1999, they had almost tripled to reach $339 million. The vast majority of the exports are shipped to the United States, primarily to Washington, California, and Massachusetts.

Comparison with Other Industries

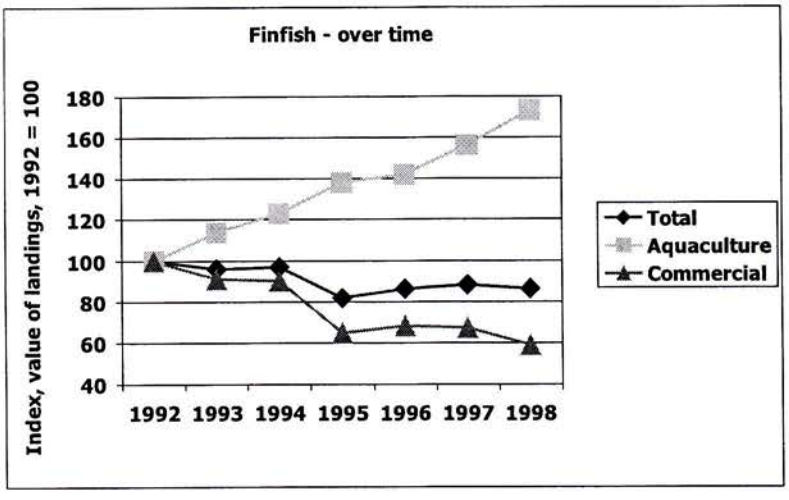
The aquaculture industry is relatively small (1.5%) when compared to agriculture at the national level; however, in some provinces it is increasingly significant. For instance, in New Brunswick, aquaculture is just over half the size of agriculture and in British Columbia it is about one-seventh the size of the farming sector.

Compared to fishing, aquaculture represented 22% of all landings in 1998. For finfish, aquaculture contributed almost half (48%) of the landings. Finfish dominates aquaculture, while shellfish is increasingly more important to the wild fishery.

Summary

Aquaculture is an industry in transition that contributed $213 million in value added to the Canadian economy during 1998. It is now recognized as an independent industry by Statistics Canada. The current statistical program, which includes production and value by species and province, exports and value added, represents the beginning of more and better information from Statistics Canada. The program was developed because aquaculture is a growth industry that is expected to continue to expand. Even so, the importance of the impressive industry support and cooperation in the search for objective information cannot be overstated. Statistics Canada looks forward to working with industry and government alike as it continues to develop the aquaculture information set.

Mark Elward *is Chief of the Livestock and Animal Products Section, Agriculture Division, Statistics Canada, Jean Talon Building, 12-B2, Tunney's Pasture, Ottawa ON Canada K1A 0T6. This report is updated as of June 2000. For more information please contact Mark Elward (Tel. (613) 951-8715, Fax (613) 951-3868, e-mail elwamar@statcan.ca) or Bernadette Alain (Tel. (902) 893-7251, e-mail Bernadette.Alain@statcan.ca.*



Appendix 1

Concepts, Methods, and Data Quality — Aquaculture Financial Statistics

Concepts

The Aquaculture Financial Statistics represent calendar-year estimates of revenue and expenses for the aquaculture industry. The aquaculture farms manage the production of fish.

Under the North American Industrial Classification System, this industry comprises establishments primarily engaged in farm-raising finfish, shellfish, or any other kind of aquatic animal. These establishments use some form of intervention in the rearing process to enhance production, such as keeping animals in captivity, regular stocking and feeding of animals, and protecting them from predators.

The aquaculture industry includes hatcheries and sales within the industry (sales from a hatchery to a grow-out operation are included). The aquaculture industry does not include sport fishing and the wild fishery.

The estimates include the costs and revenues derived from processing where it is an integral part of the establishment but not the main activity or source of revenue.

The estimates provide the revenues and selected expense items at the national and provincial level. Estimates are not produced for the Prairie Provinces where aquaculture is a relatively small industry.

Definitions

Business entity

A business entity is an economic transactor having the responsibility and the authority to allocate resources in the production of goods and services.

Establishment

A statistical establishment is one production entity or the smallest grouping of production entities that produces as homogeneous a set of goods and/or services as possible; that does not cross provincial boundaries; and for which records provide data on the value of output together with the cost of principal intermediate inputs used and cost and quantity of labour resources used to produce the output.

Population of interest

The population of interest is all establishments classified to aquaculture under NAICS 112510 and operating for at least one day during the reference year.

Financial variables

Operating revenues are generated from the sale of: whole fish (fresh or chilled); fish eggs or live fish for grow-out; live fish; whole fish dressed and frozen; fish fillets; fish that are dried, smoked or in brine; molluscs (oysters, mussels, clams, scallops) and, seed or larvae for grow-out. Operating revenue may also include revenue from other sources such as real estate rental, consulting or government subsidies.

Non-operating revenues include income from interest or dividends.

Salaries and benefits include wages, salaries, and benefits such as vacation pay, commissions or bonuses paid to employees as defined by Revenue Canada and requiring a T4 supplementary form. This item includes the employer portion of employee benefits for items such as health care insurance plans, Canada Pension Plan contributions, or Employment Insurance premiums.

The cost of materials is primarily the cost of feed and therapeutants (pesticides, drugs, and vaccinations).

The processing services are the costs incurred when another company provides services related to gutting, cleaning, slitting, or shelling.

Other operating expenses include a long list of items such as: energy (electricity, gasoline, diesel, propane), water, transportation, rental and leasing, maintenance and repair, legal, accounting, consulting, veterinary, financial services, insurance, advertising, travel, property taxes, licenses, permits, office, management, and depreciation.

Non-operating expenses relate to interest expenses on loans or the interest component of a capital lease.

Methods

The data were produced as part of Statistics Canada's Unified Enterprise Survey conducted in 1997 for the first time. The survey incorporates several annual business surveys into an integrated survey. It aims to ensure Statistics Canada receives consistent and integrated data from many types of surveys and sizes of businesses, with enough detail to produce accurate provincial statistics.

The target population for this survey is: all establishments classified to aquaculture under the NAICS 112510 that operated for at least one day during the reference year.

Frame and sample design

Two sources of data were used to derive the estimates:

- a probability sample survey of aquaculture establishments with a gross business revenue greater than or equal to a cut-off that varied by province from $45 844 to $250 000 (for 1998);
- taxation data to estimate for businesses with gross business revenue less than a cut-off that varied by province from $45 844 to $250 000 (for 1998), and to assist with the imputation of specific records.

The frame for the selection of the probability sample is Statistics Canada's Business Register. This list frame was updated and verified prior to sample selection. For 1998, in this frame 637 establishments were classified to aquaculture.

Before a sample was taken, the 637 records were stratified by province. Within each province, to improve the efficiency of the sample design, strata were defined using the gross revenue variable on the Business Register.

- The "must-take" stratum contains the enterprises (with all its associated establishments) with revenue greater than or equal to $25 000 000. These establishments were sent a questionnaire. However, no businesses from aquaculture were above the threshold of $25 000 000.
- The "take-none" stratum contains the establishments with gross business revenue less than a cut-off that varied by province from $45 844 to $250 000 (for 1998). Data for these businesses were obtained from taxation data.
- For the establishments not selected in the "must-take" nor in the "take none" three strata were defined to improve the efficiency of the sample design. There were a "take-all" stratum (all establishments were sent a questionnaire) and two "take-some" strata (a sample of establishments was selected and sent a questionnaire).

The overall sample size was 209 establishments (sent a questionnaire).

Data collection

In the Spring, respondents selected in the questionnaire part of the sample were asked to report their fiscal-year transactions. Later in the process, the fiscal-year data were adjusted to align with the calendar year using provincial level industry indicators.

The survey was conducted by mail along with computer-assisted telephone interviews. The data were examined for inconsistencies and errors using automated edits coupled with an analytical review. Data for non-respondents and no-contacts were imputed, partially with the assistance of tax data.

Estimation design

The sampling weights derived from the sample design were modified and improved using post stratification. This was possible because, during the passage of time since the sample was selected, the Business Register was updated further with more complete information.

Analysis of the estimates

The last step of the process was analytical. The financial picture for aquaculture was assessed within the context of other related production statistics available from provincial regulatory sources. Although the two sources measure different things, the provincial administrative data proved valuable to assist in the reduction of error and confirming the accuracy of the estimates.

Data Quality

All surveys are subject to sampling and non-sampling errors. Statistics Canada uses a variety of methods to minimize all types of errors. Measures of sampling error along with other indicators of quality are provided.

The coefficients of variation, a measure of sampling error, were computed. The quality of the estimates are classified as: excellent (CV is 0.01 to 4.99%), very good (CV is 5.00% to 9.99%), good (CV is 10.00% to 14.99%), acceptable (CV is 15.00% to 24.99%), use with caution (CV is 25.00% to 34.99%), and unreliable (> 35.00%).

Using these ratings at the national level, the 1998 estimates were judged to be very good. At the provincial level the estimates ranged from excellent to acceptable. The estimates for New Brunswick and British Columbia, accounting for 85% of the revenue of aquaculture, were judged to be very good and good (respectively). Between 1997 and 1998 there was a notable improvement in this rating. In 1997, the ratings for New Brunswick and British Columbia were judged to be acceptable.

Every effort was made to minimize the non-sampling error of omission, duplication, reporting and processing. When necessary, some records were imputed using information from tax files where possible.
The response rates of the 209 sampled establishments receiving a questionnaire were: completed 59%, partially completed 1%, refusal 2%, no response before the survey deadline 14%, other (inactive, out of business, change of ownership, amalgamation) 9%, not able to contact 4%, out-of-scope to aquaculture 11%.

These response rates are considered normal for a business survey. The out-of-scope rate of 11% reflects the quality of the Business Register at the time of sampling. This is not unexpected because, until the recent introduction of the NAICS, there was no classification for aquaculture.

Of the original sample of 209 establishments, 18% required imputation to complete the data. Reasons for imputation include partial response, failure to respond before the survey deadline, refusals, and inability to contact the respondent. This rate is considered normal for a business survey.

The sample of aquaculture producers (after edits and imputation) represented 77% of the estimated industry revenues. Complete responses represented 73% of the industry revenues, as those requiring imputation tended to be smaller operations. Small businesses that were not included in the sample and where tax data were used to provide an estimate represented 1% of the industry revenues.

Finally, the aquaculture estimates were compared to and found to be consistent with administrative data sources obtained from the provinces, reinforcing confidence in the quality of the aquaculture statistics. All of the data were reviewed for accuracy and consistency and are viewed as providing a reliable portrait of the aquaculture industry.

Appendix 2. Value Added Account — Aquaculture Industry[1]

1998	Thousands of dollars							
	NF	PEI	NS	NB	QC	ON	BC	Canada[2]
A. Sources of output								
Sales of aqua products/services	8100	19 100	16 950	173 150	6200	19 400	263 700	506 600
Whole fish dressed, fresh or chilled			5000	122 000	2000		178 000	308 000
Fish eggs and live fish for growout			5000	16 000			24 000	45 800
Whole fish live (ex for growout)					2000			25 350
Whole fish dressed and frozen								8000
Fish fillets, fresh or frozen							26 000	53 400
Fish, dried, smoked or in brine								300
Total finfish	7300	900	13 350	166 000	5700	19 000	248 500	460 750
Total molluscs	800	18 000	3500	3000			10 000	35 450
Other goods and services NES[3]		200	100	4150		400	5200	10 400
Subsidies				3700				6600
Other operating revenue				1950				15 080
Total operating revenue	**9780**	**19 200**	**17 350**	**178 800**	**6400**	**26 650**	**270 100**	**528 280**
Change in inventory value — goods	750	600	4500	3000	200	700	15 000	24 750
Gross output	**10 530**	**19 800**	**21 850**	**181 800**	**6600**	**27 350**	**285 100**	**553 030**
B. Product inputs								
Product expenses	8200	8220	12 990	118 250	3390	17 250	174 140	342 440
Feed	4100	300	5300	39 000	1500	6800	83 000	140 000
Therapeutants	300		350	1300	50	100	3700	5800
Purchases, eggs/fish — growout	500	6200	4000	16 000	100	5800	14 000	46 600
Purchases, fish - processing/resale			200	36 000	150		4000	40 350
Insurance premiums	50	100	380	2050	60	300	4300	7240
Energy (electricity, fuel, etc.)	200		700	1500	400	800	3200	6800
Goods transportation and storage	300		220	4100	80	200	12 000	16 900
Processing services	1400		300	3600		300	23 000	28 850
Rental and leasing expenses	200		200	500		200	2200	3350
Maintenance/repairs, buildings	200	400	100	1000	100	400	1600	3800
Maintenance/repairs, machinery	100	150	320	2200	100	400	4500	7770
Professional services	390	330	130	1550	120		2410	4930
Other operating expenses NES[3]	460	540	790	9450	630	1950	16 230	30 050
Change in inventory value — raw materials	-100		100	1500	100	200	1000	2800
Total of product inputs	**8300**	**8220**	**12 890**	**116 750**	**3290**	**17 050**	**173 140**	**339 640**
C. Gross value added (factor cost)	2230	11 580	8960	65 050	3310	10 300	111 960	213 390
D. Selected primary inputs								
Salaries and wages	2500	4000	5400	20 000	1500	6000	29 000	68 400
Employer portion of employee benefits	300	300	400	1700	150	500	3000	6350
Depreciation	700	1000	1300	5800	400	1050	14 000	24 250
Interest paid	600	200	600	5000	300	1200	6000	13 900

Notes: [1] Data and account structure are subject to revision.
[2] Sum of estimated provinces excludes Manitoba, Saskatchewan, and Alberta
[3] NES = not elsewhere specified.
* Empty cells indicate figures not available.

Effluent Treatment Facilities and Methods in Fish Farming: A Review

A. Dumas and A. Bergheim

Intensification in aquaculture has contributed to a deterioration in the quality of the water in fish farm effluents. Consequently, treatment facilities have been developed for the removal of suspended solids, ammonia and particulate phosphorus. The main sources of pollution and the resulting effluent loadings are briefly described in this paper, as are the treatment devices used (single flow-through and recirculating systems). Some alternatives are proposed for removing dissolved nutrients, particularly $P\text{-}PO_4^{-3}$.

Introduction

The trend toward intensification in aquaculture has contributed to a deterioration in the quality of the water in fish farm effluents.[1] Suspended solids, ammonia nitrogen ($N\text{-}NH_3$) and phosphate phosphorus ($P\text{-}PO_4^{-3}$) are considered to be the main pollutants.[2,3]

In order to reduce the pollution from hatcheries, some studies have focussed on improving the digestibility of fish feed and varying feeding strategies.[4-6] Other studies tried to determine the carrying capacity of a given area, or focussed on the removal of pollutants using treatment facilities.[1,7] Most of these studies were conducted on single flow-through systems and did not consider the dissolved nutrients.

Since recirculating systems are becoming more widespread with the intensification in fish farming and the dissolved fraction of nutrients can have an immediate impact on the growth of phytoplankton, this paper investigates the specificity and the efficiency of treatment devices used until now in aquaculture, and proposes some alternatives to removing the dissolved $N\text{-}NH_3$ and $P\text{-}PO_4^{-3}$ discharged by land-based fish farms.

Pollutants in Fish Farming and Their Characteristics

The different waste sources and the range of effluent concentrations and loadings from salmonid farms were reviewed by Beveridge et al.,[8] Pillay,[9] and Cripps and Bergheim.[10] All reported that effluent characteristics and loadings of feed-derived wastes are carried out at land-based tank and pond systems with distinct inlet and outlet points. The waste loadings from cage farms (the almost universal system for ongrowing of salmon) usually must be estimated from mass balances based on input of feed and chemicals less the retained fraction in the fish stock. The main sources of pollution and the resulting effluent loadings are briefly described below.

Suspended Solids (SS)

Particle concentration peaks in the outlet water from smolt tanks are usually related to manual cleaning-flushing operations.[11] In modern land-based ongrowing farms using tanks with good self-cleaning properties, the outlet is generally characterized by low and stable particle concentration below 5 mg SDM/L, where SDM corresponds to the solid dry matter.[12]

When employing so-called high-energy feed (HEF), i.e. enriched feed containing up to 35% fat, an outlet loading less than 150 g SDM per kg of produced fish is a realistic objective (Table 1). However, a waste loading as low as this is only achieved with high rates of feed utilization and negligible feed loss.

In aquaculture wastes, the number of particles appears to be high. Cripps[13] measured 1.83×10^3 to 1.88×10^6 particles/L within the size range of 9 to 269 µm in hatchery effluent. A knowledge of the particle size distribution of the effluent is most relevant for the optimization of mechanical treatment attempts. With a stepwise increasing filtration effort within the pore size range of 200 to 5 µm, Cripps[14] found a total decrease in SDM concentration of 74%, a 48% decrease in TP (total phosphorus) concentration and a 33% decrease in TN (total nitrogen) concentration.

Nutrients

In 1990, approximately 70% of nitrogen (N), phosphorus (P) and organic matter (as energy) from commercial feeds in Norwegian salmon farming were found to load the environment.[19] Use of a high energy diet (assuming low feed loss) reduces the envi-

Table 1. Survey of reported feed-derived wastes compared with estimated low waste production in salmonid culture (estimated low waste production assumes the use of high-energy diet with no feed loss).

Compound	Reported/ Estimated	Effluent concentration range (mg/L)	Specific effluent loading (g/kg fish produced)	Percent lost of supplied	References
Suspended solids (SDM)	Reported	1.6 - 50	191 - 1350	20 - 80	Effluent concentrations: Cripps;[15] UMA Engineering[16]
	Estimated	–	< 150	< 20	
Biochemical oxygen demand (BOD)	Reported	3 - 20	200 - 400	–	Effluent loadings: Hennessy et al.[17]
	Estimated	–	< 200	–	
Total nitrogen (TN)	Reported	0.4 - 5	83 - 104	> 60	Estimated loadings: mainly based on Cowey & Cho[18]
	Estimated	–	< 40	< 60	
Total ammonia (TA)	Reported	0 - 1.6	20 - 56	–	
	Estimated	–	< 30	–	
Total phosphorus (TP)	Reported	0.05 - 0.27	9 - 27	> 70	
	Estimated	-	< 6	< 50	

ronmental load of N and P to 51% and 64% of supplied load, respectively.[19] The P excretion can be further reduced by substituting dietary fish meal for low P protein sources.[21]

Since part of the nutrients are particle-based, the hatchery effluent concentrations of TN and TP fluctuate correspondingly to the solids concentrations.[10] The diluted excretory product ammonia (TAN) constitutes a significant portion of the effluent, normally composing between 25% and 75% of the TN load from salmonid cultures.[20] In practice, TAN cannot be removed in flow-through tanks and is potentially harmful to salmonids, especially in seawater at low water exchange rates.[22] For phosphorus, a dissolved fraction of 10% to 60% of TP is reported (Table 2).

Reported nutrient waste loads are relatively high and fluctuating, and should be reduced to less than 40 g TN and 6 g TP per kg of fish produced at an up-to-date farm (Table 1).

Biodegradable Organics

The biochemical oxygen demand (BOD) of fish farm effluents is mainly considered to be linked to the suspended solids content of the flow.[23] Therefore, the effluent BOD concentrations fluctuate with particle concentrations in both a diurnal and seasonal fashion.

Usually, the solid fraction accounts for at least 50% of the total BOD, although the relative proportion of solid and soluble components varies.[24] By contrast, however, Muzigwa and Muir[25] found that only approximately 30% of the total 168-h BOD uptake of hatchery effluent was due to oxidation of coarse and fine solids.

Pathogens

A wide range of pathogens can cause severe disease problems in salmonid culture. In this context, the most relevant concern is the potential risk of spreading pathogens from farmed fish to wild stocks with consequent harm to the latter.[24] An example is the significant role of marine fish farming in spreading furunculosis along the Norwegian coast.[26] Reported studies have shown that there is a significant contribution of microorganisms from the effluent flow of salmonid farms,[27] notably aerobic heterotrophic bacteria that are generally non-pathogenic.

Chemotherapeutants and Antibiotics

Most chemicals employed in salmonid cultures are used to control diseases (e.g., bactericides, fungicides, parasiticides). Some compounds are used to

Table 2. Total phosphorus (TP) and dissolved phosphorus (DP) observed in fish farm effluents.

Concentration	TP	DP	References
Average (mg/L) Range (in parentheses)	0.125 (0.05 - 0.27)	0.06 (0.001 - 0.100)	Cripps and Kelly;[30] Dumas;[28] UMA Engineering Ltd.[16]
Percent Associated to Particulate Matter	20 - 84	–	Cripps & Bergheim;[10] Hennessy et al.;[17] Cripps and Kelly;[30] Bergheim and Kelly;[49] Stechey and Trudell[36]
Percentage of TP	–	10 - 60	

improve water quality (e.g., lime for pH regulation). Braaten[29] has presented an overview of commonly used therapeutic agents and chemicals applied in salmonid culture, comprising five types of antibiotics, parasiticides (such as organophosphates), fungicides (such as formalin and malachite green), disinfectants (such as chloramine and formalin) and antiseptics (such as chlorobutanol).

Existing Treatment Facilities

Suspended Solids Removal

There exists at present, a relatively wide variety of treatment facilities to remove the suspended particles from fish farm effluents. This paper will focus on commercial devices that have to the best of our knowledge been the object of scientific publications.

For economic and technical reasons, sedimentation and screening are the two main physical treatment facilities employed in aquaculture. Since the SS contents in effluent vary between fish farms (2 to 50 mg/L in European Countries),[30] the need for physical treatment facilities merits careful consideration. The choice of such a facility should take into account rearing conditions (flow rate, SS concentrations in the effluent, land availability, design of the fish tanks, capital and operational costs), and environmental regulations.

High flow rates and relatively low SS content observed in fish farm effluents limit the efficacy of sedimentation units. Preconcentration of solid wastes seems to offer a more effective means of treating effluent and can be achieved by means of self-cleaning tanks that incorporate a flushing device or sediment trap.[3,30,31] However, fish must be prevented from coming into contact with the deposited solids in order to avoid resuspension. Two devices are recommended to remove the SS once pre-concentration has occurred: the swirl concentrator and the sedimentation basin. The former has the advantage of requiring smaller areas of land, but contributes to the splitting up of particles. Both have low operational costs in comparison to other methods such as centrifuges and pressurized hydrocyclones. Where land availability is not restricted, sedimentation basins are much more widespread, and have been studied more extensively over time than any other treatment facility used in aquaculture. On the other hand, their effectiveness is limited to particles exceeding 100 µm diameter[10] and they do not usually incorporate a rapid sludge removal system, which can result in the release of phosphorus in the water column under anoxic conditions.[32]

The terms "screening" and "microscreening" refer in this paper to the sieving of particulate matter as a primary treatment. Unlike the sedimentation units, screening methods are more suitable for the treatment of fish farm effluents. Also, the land area requirement is reduced comparatively with the sedimentation basin, and the effluent does not require a pre-concentration device to improve the SS removal. The screening units can be stationary or rotary.[33] The latter device is gaining in popularity, presumably because its rotation speed can be adjusted according to the effluent loadings. Even though the efficiency of a screening unit does not for the most part depend on flow rate, it can be affected by the quantity and the size of particles. Hence, it is important to characterize the latter variables and their ranges over a period of time. Indeed, the concentration and the size of particulate matter will fluctuate according to the hydrodynamics in the hatchery system,[34] and the developmental stage of the fish being raised. Particles tend to be finer when the water turbulence increases and the fish are immature. It becomes more appropriate to use devices that include two screens in series (200 µm and 60 µm for example). The risk of clogging and the need for backwashing represent two disadvantages of the screening methods. Moreover, the frequency of backwashing has to be adjusted to the concentration of SS, which, for example, increases during raceway cleaning.[35]

Removal of Nutrients

Treatment devices used in aquaculture to remove nutrients are classified as to whether they employ physical or biological methods. Chemical methods will not be considered in this section since their application is unusual in fish farming.

Since a certain percentage of nitrogen and phosphorus is bound to particulate matter, the use of physical treatment units as employed in the removal of SS is generally recommended in order to limit freshwater eutrophication. The proportion of nutrients bound to SS varies considerably, however. From 10 to 30% of the total nitrogen (TN) and 20 to 84% of the total phosphorus (TP) is likely to be associated with the SS > 45 µm.[10,30,36] Also, the reported concentrations of DP (dissolved phosphorus) in fish farm effluent seem to vary considerably (Table 2). Both of these factors influence the DP content of treated effluents which is generally held responsible for eutrophication of freshwater. The DP concentration (mg/L) was not considered so much by the researchers, even though it is the form of phosphorus that is most likely to cause immediate impacts on the receiving bodies of freshwater.

Although optimally functioning physical treatment devices are capable of removing a fair percentage of TP, they are less effective in removing DP. Bergheim et al.[37] reported an orthophosphate removal efficiency of 25% to 50% when using rotating microsieves (mesh size of 60 µm). Orthophosphate concentrations were over 30 µg P/L in the treated fish farm effluent. Assuming a DP content of 100 µg P/L in a given farm effluent and a removal efficiency of 25%, the treated wastewater is likely to contain as much as 75 µg P/L when using a microscreening treatment unit. Removal efficiency would be lower when using a sedimentation basin. Indeed, the release of phosphorus is likely to occur if the settled matter is not removed frequently. Therefore, the problem of eutrophication is not necessarily solved when the SS are removed efficiently.

According to Wetzel,[38] a DP level above 10 µg P/L is likely to stimulate the development of nuisance populations of phytoplankton in oligotrophic or mesotrophic waters. Algal growth will be enhanced when the following favourable conditions prevail:

- high DP content in the non-treated effluent;
- low removal of DP;
- water temperature over 10°C;
- light intensity and duration (over 2 mmol photon·m^{-2}·s^{-1} depending on photolithotrophic species);
- poor fish farm effluent dilution by the receiving stream (during a low-flow period for example);
- receiving bodies of water are oligotrophic (TP < 50 µg/L).[38-42]

The potential of fish farm effluent for producing eutrophication of receiving saltwater bodies is directly related to its nitrogen content. Rotating microsieves attain only minimal levels efficiency with regard to the removal of both TN and TAN (total ammonia nitrogen): 7 to 32% and 0 to 5%, respectively.[37]

Biological filters that incorporate nitrifying bacteria are commonly used to eliminate the ammonia nitrogen in recirculating systems.[43-45] Nitrification contributes, however, to a decrease in pH and in the dissolved oxygen content of the water. In some situations (during a warm water period for example), aerators would be required to maintain a desirable level of dissolved oxygen in the effluent or recirculating waters, implying significant increases in operational costs. Also, biofilters are generally ineffective in removing phosphorus (only 10 to 30 % of TP removed by nitrifying bacteria).[46]

Removal of Pathogens

A variety of methods and products (UV radiation, ozone, antibiotics, vaccines, disinfectants, etc.) are used in aquaculture to reduce the risks associated with pathogens.

Ultraviolet light and ozone are effective bactericidal and virucidal agents. UV radiation and ozone are also harmful to higher forms of life, but by-products of ozone do not normally attain toxic levels in the receiving ecosystems.[46] Both these systems (particularly ozonation) imply high energy costs. Also, the removal of particulate matter is a prerequisite to maintaining the efficiency and optimizing the lifetime of the UV disinfection lamps. Use of UV radiation and ozone is obviously limited to land-based fish farms, and their applications may be attractive for intensive recirculating water systems.

Cage aquaculture systems and land-based fish farms employ mainly antibiotics and vaccines to eliminate pathogens. Efficient solids removal facilities would likely reduce the environmental impacts of chemicals and antibiotics that are administered in the feed.

Chlorine, which is a widespread chemical disinfectant used for municipal wastewater, is expensive and must be maintained at high concentrations.[47] Moreover, dechlorination with its attendant costs becomes necessary to reduce toxic effects on aquatic life. Hence, the application of chlorination is unsuitable to aquaculture.

Need for Alternative Methods of Phosphorus Removal

The risk of eutrophication of freshwater bodies increases proportionately from 10 µg P/L and becomes

unavoidable at 100 µg P/L.[48] Maintaining phosphorus concentrations at their lowest possible level may be achieved either by reducing input or by removing it using mechanical, chemical or biological methods. The former option is much more cost effective in the long term and can be achieved by improving the digestibility of phosphorus and modifying the feeding strategy.[49] However, the use of various treatment options may become unavoidable in situations where the phosphorus concentration in the fish farm effluent or in receiving waters must be maintained below critical levels.

Among the various technologies used for the removal of phosphates from fish farm effluent, mechanical and biological treatments have proven to be the most practical. The choice of a given method should take into consideration the following points:

- the concentration of nutrients in the effluent;
- the duration of high discharge periods;
- type of system (single flow-through vs recirculating);
- removal efficiency of other nutrients such as ammonia;
- cost effectiveness.

Given that the periods when phosphorus concentrations attain critical levels are relatively short in the course of a year, treatment devices should not require significant investments in equipment or land and ideally should be adaptable for periodic use.

Biological removal of orthophosphate is achieved using reactors containing bacteria or photosynthetic organisms (algae, macrophytes) which also remove dissolved ammonia. This represents a definite advantage over mechanical methods whose effectiveness is limited mainly to particulate phosphorus.

Biological phosphorus removal using bacteria is, however, a complex system to operate, requiring both aerobic and anaerobic or anoxic conditions.[46,50] Moreover, the oxygen consumption that occurs during treatment may require the addition of an aeration unit in order to maintain a certain level of dissolved oxygen in the water being recirculated or discharged.

Algae and certain cyanobacteria offer an interesting alternative to bacteria since their application only requires one reactor combined with a settling device.[51] The use of photosynthetic cells contributes to the oxygenation of the treated waters. Thus, there is no need for aerators during the light period. Biomass must to be removed before reusing or discharging the treated waters. This represents a disadvantage of utilizing unicellular microalgae, the harvesting of which is expensive.[52] The problem can be avoided by selecting filamentous organisms (such as *Phormidium* spp.) that have the capacity to form flocs several millimeters in diameter when grown in suspension.[53] Biomass harvesting by sedimentation can then be readily achieved by stopping agitation of the cultures.[54]

Very few technologies using photosynthetic organisms have been applied on a large scale to fish farm effluents. The application of such methods in this context requires further investigation.

Conclusion

Although good arguments can be made for the use of either physical and biological methods of treating effluent, it would seem from this overview of the subject that physical methods are generally effective in reducing to acceptable levels the environmental impact of discharged nutrients from single flow-through facilities. In recirculating systems, however, higher concentrations of dissolved phosphorus make the use of biological methods employing photosynthetic organisms the most appropriate means of removing dissolved nutrients. The removal of orthophosphate becomes particularly important when environmental conditions favour the development of nuisance populations of phytoplankton. These biological methods are technically achievable, but still need to be developed and marketed.

Efforts at reducing pollution at the source are much more cost effective than removing pollutants.[39,48,55] Although observable progress in this area has been made, the sustained development of the fish farming industry will continue to depend on its response to the challenge of developing cost-effective techniques of raising fish and treating effluent.

Special thanks to Dr. Brian Mathieu for the English revision of this document.

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***A. Dumas** is affiliated with the ABA (Aquatic Biotechnology & Aquaculture), 542 St-Cyrille, Normandin (Québec, Canada) G8M 4H4. **A. Bergheim** is affiliated with Aquaculture and Water Research, Rogaland Research, PO Box 2503, Ullandhaug, N-4004 Stavanger, Norway.*

Calendar

conferences, workshops, courses and trade shows

• **13th International Pectinid Workshop**, 18-24 April 2001, Coquimbo, Chile. This biennial workshop provides a unique opportunity for scallop researchers from around the world to meet and interact on both a scientific and social level. The scientific program will include thematic sessions, keynote speakers, working groups and plenary discussions. Contact: Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile (fax (56) 51 209782, email pectinid2001@nevados. ucn.cl, website http://www.geocities.com/pectinid 2001).

• **International Workshop on Aquaculture and Its Role in Integrated Coastal Zone Management,** 19-21 April 2001, Oostende, Belgium. Jointly organized by the European Aquaculture Society and the Flanders Marine Institute. Information: EAS Office (e-mail oost2001@aquaculture.cc, website http://www.vliz.be/eas/index.htm).

• **Aquaculture Canada 2001 — 18th Annual Meeting of the Aquaculture Association of Canada**, 6-9 May 2001, Westin Nova Scotian Hotel, Halifax, Nova Scotia, Canada. Theme: Moving Forward Through Partnerships. An all-inclusive program will examine the species cultured in Canada and address key issues affecting the industry. Well-known experts will address delegates and exhibitors during this 3-day event. Sessions: Progress in Cod Culture, Aquaculture Action Plan: Enabling Aquaculture to Achieve Full Potential, Environmental Assessment of Aquaculture Sites, Advances in Fish and Shellfish Health, Nutrition, Biotechnology, Broodstock Management, Suppliers and Vendors Session, National Issues in Aquaculture Development, Challenges in Aquaculture Communications, Video Session, Aquaculture and Integrated Coastal Zone Management. General conference information: Linda Hiemstra (tel 250 741-8708, e-mail hiemstra@mala.bc.ca). Program information: Cyr Couturier (e-mail cyr@mi.mun.ca). Trade show information: Gary Scott (tel 902 424-0344, e-mail scottg@gov.ns.ca). Conference web site: http://www.gov.ns.ca/nsaf/ aac2001.

• **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, P.O. Box No. 165, Urmia 57153, Iran (e-mail artemiaworkshop@urmia.ac.ir).

• **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 by-the-Sea, 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).

• **Atlantic Aquaculture Conference, Trade Show and Fair,** 21-24 June 2001, St. Andrews, New Brunswick, Canada. For information, telephone 506 658-0018.

• **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 of the world, and to provide an opportunity for scientists, biologists, enforcement specialists and com-

mercial 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, 54903-0109, USA (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. The aim of Larvi 2001 is to bring researchers and professionals together to evaluate recent progress, identify problem areas and stimulate future cooperation in research and industrial production of freshwater as well as marine fish and shellfish larvae. Tentative sessions: Session 1 (broodstock, egg and larval quality epigenetics, broodstock feeding and offspring quality, fish and shrimp maturation, wild versus domestic strains, evaluation methods, etc.), Session 2 (genetics, biotechnology and developmental biology), Session 3 (nutrition, feeding and growth, nutritional physiology (functional effects of various compounds), feeds and feeding strategies (live food optimisation, live food substitution/ supplementation diets, formulated feeds, dietary requirements), quantification of food uptake, behavioural interactions (vision/predation in relation to nutritional status)), 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, Rozier 44, B-9000 Ghent, 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).