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of the Aquaculture Association of Canada

Fish Health

106-3

Contents

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décembre 2006 (106-3)

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Cover photo by Bill Pennell — Adult male and female sea lice *(Lepeophtherius salmonis)* on a wild chinook salmon (ca 15 kg) caught on the west coast of Vancouver Island in the summer of 2007. The female lice are the ones with the long egg strings. Virtually all adult wild salmon carry this species of sea louse which they bring back from the high seas. These lice do not appear to cause significant harm to adult fish and the different species of Pacific salmon appear to have different degrees of resistance to the lice. There is controversy over the role sea lice might play in the survival of juvenile salmon which are also infected by sea lice. Much remains to be learned about these interesting parasites.

Viral Hemorrhagic Septicemia Virus, Type IV in the Great Lakes

J.S. Lumsden, L. Al-Hussinee, S. Russell, K. Young, A. Yazdanpanah, P. Huber, S. Lord, and R.M.W. Stevenson

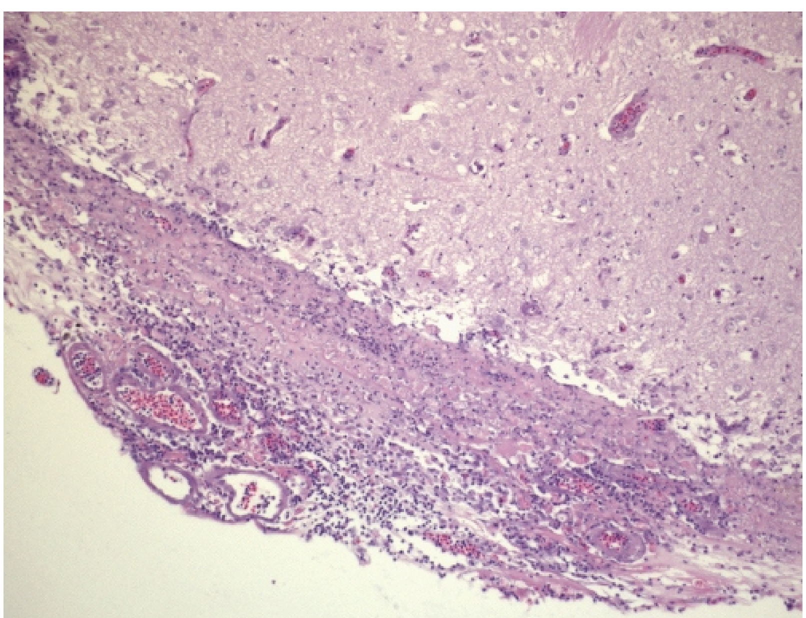
A genotypically distinct variant of viral hemorrhagic septicemia virus (VHSV) genotype IV was identified in association with ongoing mortality events in 2005 and 2006 that affected multiple free-ranging species of fish in the lower Great Lakes. This discovery, and the resulting regulatory response, once again highlights the need for the rapid implementation of national aquatic animal health programs in Canada and the USA, and for funding to support diagnostic work. Potential management options in stocked species are considered.

VHSV in the Great Lakes: Review of events in 2005 and 2006

VHSV was first identified following mortalities in freshwater drum (*Aplodinotus grunniens*) that occurred in the Bay of Quinte in Eastern Lake Ontario during April and May 2005. A conservative estimate was that 30,000 drum had died, although net surveys conducted in the Bay of Quinte later in the year did not note a decrease in the sampled population. The majority of the fish affected were adults and those submitted for laboratory study averaged 3 kg. During this time, round goby (*Neogobius melanostomus*) were also dying in the same area, while increased numbers of dead muskellunge (*Esox masquinongy*) were noted in the mouth of the St. Lawrence River (approximately 75 mature fish).

Lesions in the drum were dramatic, with extensive hemorrhage grossly typical of a viremia or bacteremia (Fig. 1). Internal lesions were less remarkable but included serosanguinous ceolomic fluid, mild serosal hemorrhage, mildly enlarged spleens and marked segmental intestinal congestion/hemorrhage. Histologically, the lesions were also striking, with vasculitis, often fibrinoid, and marked necrotizing myocarditis, meningoencephalitis (Fig. 2) and enteritis. In particular, the severity and pattern of the myocarditis was a warning that the causal agent was likely to be exotic to the Great Lakes. Lesions most commonly associated with VHSV in salmonids are multifocal to confluent necrotizing interstitial nephritis and splenitis; however these were inconsistently present and subtle. The gobies had minimal

Figure 1
Florid diffuse severe fibrinous meningitis from a drum infected with VHSV (80x H&E).



gross lesions including mild hemorrhage and an enteritis. All the muskellunge submitted for examination had been frozen. A myocarditis was present histologically in the muskellunge; however preservation was often very poor.[1] Initial attempts to grow a virus on chinook salmon embryo-214 and rainbow trout gonad cell lines from both frozen and fresh drum and goby tissue were unsuccessful; however numerous bullet-shaped viral particles were noted with transmission electron microscopy in affected drum heart. A virus was subsequently isolated on fathead minnow cells at the Atlantic Veterinary College, University of Prince Edward Island by Dr. Carmencita Yason. Dr. David Stone at the CEFAS Weymouth Laboratory in the United Kingdom confirmed the isolate to be VHSV by enzyme immunoassay and polymerase chain reaction (RT-PCR). G-glycoprotein sequencing demonstrated that it was most similar to genogroup IV isolates.[2]

Information about the work in progress was included in an oral presentation at the Eastern Fish Health Workshop in June 2005 Shepherdstown, WV, to alert other fish health investigators to the suspected presence of a novel agent. Subsequently, VHSV was identified from a frozen muskellunge collected in Lake St. Clair in 2003.[3] The World Organization for Animal Health (OIE), was duly notified by Canadian and then US authorities in 2005 (and in 2006 as required). During the spring of 2006, several mortality events in the Great Lakes region were identified in Canada and the USA, with the most dramatic events being observed in the states of New York, Ohio, and Michigan, and affecting fish in the St. Lawrence River, Lake Ontario, Lake Erie and Lake St. Clair.

Events of the past calendar year dramatically illustrate how, in Canada, the lack of funds and personnel available and able to submit suitable material hampers the ability to conduct disease surveillance in wild fish, compared with the USA. Diagnostic services in both countries experience challenges in recognition of mortality events by field personnel and submission of material in suitable condition for proper diagnosis. The majority of states bordering the Great Lakes have comparatively more robust networks for recognition and collection of material, with laboratories specifically funded for surveillance of disease in wild fish, not just primarily for hatcheries, as is the case in Ontario. The few fish (drum and a few smallmouth bass (*Micropterus dolomieu*), black crappie (*Pomoxis nigromaculatus*), pumpkinseed (*Lepomis gibbosus*) and muskellunge) collected during 2006 on the Canadian side of the border had minimal or no gross lesions; however microscopic lesions, including a vasculitis, were present and virus was isolated in cell culture and detected by nested PCR.[1] These few fish were difficult to obtain, as biologists in the area were not accustomed to making such submissions — nor, indeed, have they had funding to do so.

An important need apparent from these 2006 outbreaks is for a detailed description of le-

Figure 2
Adult freshwater drum from spring 2005 with multifocal hyperemia and hemorrhage.

sions associated with the VHSV infections in Canada and the USA. Even in the very limited number of species examined in our laboratory, there were two distinct patterns of histological lesions.[1] For a virus that infects such a broad range of species, this isn't completely surprising and species-specific lesion patterns have been described previously for VHSV. [4] There are over a dozen species from the lower Great Lakes now recognized to be clinically affected by VHSV, with several additional species known to be infected (virus isolated) without apparent clinical signs. This list of species will continue to expand thanks to ongoing work, primarily by Drs. Faisal and Bowser at Michigan State and Cornell universities, respectively.

At this point it is difficult to properly assess the real impacts on any one species. The drum, goby, muskellunge and yellow perch (*Perca flavescens*) seem to be highly sensitive, with the impact on the muskellunge presently of greatest concern. While relatively smaller numbers of muskellunge have been examined, a good portion of the fish identified from Lake Ontario to date have been gravid females that have died before spawning.[5] The scope of wildlife mortality events, particularly aquatic ones, can be unclear and often are impossible to accurately quantify.

All isolates of VHSV collected from fish in the Great Lakes are genetically very similar, if not identical, based on the limited sequencing of G- and N-genes performed to date.[6] The few isolates examined are also genetically distinct from other genotype IV 'North American' strains.[2,3] The behavior in cell culture (cell line susceptibility and cytopathic effects) is also not typical of known genotype IV VHSV strains and work is ongoing in a number of laboratories to better characterize the growth requirements.

Management Options

The well-publicized mortality events of spring 2006, months after initial notification of the OIE of the presence of the virus in Lake Ontario, sparked a belated regulatory reaction that culminated briefly in a total movement ban of a range of live fish from eight states and two provinces (Ontario and Quebec) bordering the Great Lakes and St. Lawrence River in October 2006. This ban was subsequently, and correctly, altered within weeks to allow interstate movement of VHS-susceptible species for slaughter and cross-border shipment of VHSV-susceptible salmonids from Ontario and Quebec having met USFW Title 50 certification. This should also be applied (and may in the future) to lots of baitfish or other fish that have been certified to OIE or equivalent (Title 50; Canadian Fish Health Protection Regulations) standards.

Additional practical management strategies to reduce the impact of VHSV are needed and some have already been implemented on a region-to-region basis. These should include: a) re-examining the collection of broodstock and fry for enhancement, at least from certain sites; b) limiting use of surface water, particularly contiguous with lakes where VHSV is known to be present, c) reviewing or upgrading facility biosecurity, and d) re-visiting a certification process for inland aquaculture operations in Ontario. Most of these practices are reasonable within existing frameworks and programs, or can be put in place with relatively low effort or funding.

However, the reality is that the bodies of water in which the virus is already present will continue to be infected and, likely within a short time frame, the virus will be endemic within the Great Lakes basin. Therefore, strategies should also be pursued that will blunt the impact in endemic areas, particularly for sensitive species. A management strategy that deserves further investigation would be to delay spring stocking and likely exposure of naïve young-of-the-year until the water temperatures are high enough to allow infection yet prevent prolific viral replication, limiting clinical illness. VHSV replicates best between 4^{o} and 14^{o}C,[7] although the temperature range of the Great Lakes strain may be slightly different. Limited exposure would best stimulate development of an anamnestic immune response, potentially providing immunity to VHSV. The pertinent temperature range for the Great Lakes VHSV strain and for the target fish species would require additional experimental work to assess the expected benefit. In species such as lake trout (*Salvelinus namaycush*) where stocking presently takes place at a time of year when the water temperatures are approaching the upper limit of its range, it might be possible to alter stocking. Given the substantial cost and practical considerations of workers, transportation, etc. involved in altering stocking practice, the expected benefit would need to be significant. While the precise preferred temperature range of the Great Lakes strain hasn't been determined, the outbreaks of VHSV in the spring of 2005 and 2006 have occurred when the water temperatures were approximately within the above-mentioned range. Hitting this window of opportunity may not be practical for all of the fish stocked but may provide a worthwhile increase in survival given the lack of readily available and inexpensive alternatives.

Vaccination and experimental exposure to VHSV can produce immunity,[8-10] suggesting that the above-mentioned strategy could be successfully employed. Environmental exposure to a pathogen followed by prolonged non-permissive temperatures for pathogen replication has been used for proliferative kidney disease in Europe.[11] Experimental trials would be needed to match the viral infection to likely environmental doses and estimate the expected benefit. Another, less palatable and more wasteful approach would be to simply stock greater numbers of fish in a body of water once it is confirmed to be infected. The success of either strategy would have to be evaluated based on recruitment survey data.

Experimental trials, similar to those underway in Ontario outbred rainbow trout, would use graded waterborne doses of the Great Lakes strain of VHSV, initially compared to intraperitoneally (i.p.) injected fish and horizontal transmission from i.p.–injected cohabitants. Morbidity, mortality, antibody titres and viral carriage (virus isolation and PCR) would be determined and fish that survived waterborne infection would be re-infected one month after the last mortalities had occurred. After an initial trial with a unique species to establish the model, the temperature of exposure would be varied from the optimum (e.g., 12^{o}C to a potentially non-permissive one of ~17^{o}C).

Selection for rainbow trout that are resistant to VHSV genotype I in Europe has been demonstrated.[12] Direct selection for resistance to an infectious disease is the fastest method to potentially reduce population susceptibility; however this approach is fraught with pitfalls, including increased susceptibility to other agents[13] or unusual variants of the same agent.[14] As a crude example, selection of muskellunge for resistance to VHSV may result in fish more susceptible to *Renibacterium salmoninarum*. The agent is always more mutable than the host and this is particularly true for RNA viruses[15] such as VHSV. Direct selection would require infection of fish with an OIE listed agent in a hatchery environment, which is not desirable. A more practical (and less expensive) immediate approach would be to identify relatively 'resistant' extant strains of fish maintained for stocking programs (i.e., experimental infection of lake trout strains in an isolation facility with preferential stocking of 'resistant' strains to infected waters). In the longer term, indirect selection for mechanisms of resistance (e.g., complement activation to block primary viremia in naïve young of the year fish) should take place simultaneously with selection for a panel of innate and acquired immune response parameters. This approach should reduce the likelihood of a resultant increased susceptibility to a given class of pathogen; however it is expensive.(16) Additional mechanisms of innate resistance to viral spread between cells, specifically Mx protein(17) and interferon,(18) should also be studied in high priority species.

Impact on Development and Implementation of NAAHP

The discovery of VHSV in 2005/06 and the recent discovery of spring viremia of carp (SVC) virus in the summer of 2006 in Lake Ontario have readily illuminated the deficiencies of disease surveillance in wild fish in the Great Lakes. This is particularly true on the Canadian side of the border, with its relatively reduced manpower and funding. SVC was identified(19) in carp held awaiting export that otherwise wouldn't have been examined. These fish were unexpectedly held for a prolonged period, likely resulting in stress and viremia. Fish caught and sampled in previous years from the same collection site, but without being held, were negative by virus isolation. SVC has likely been present for a period of time. It is unlikely that SVC will have an impact similar to that of VHSV in Great Lakes fish. Nevertheless, it is an OIE list disease and within the context of developing national aquatic animal health plans (NAAHP) in both the USA and Canada, these recent discoveries should be timely reminders of the need for critical components within these programs.

The following comments are directed towards the Canadian program as it is more relevant to the authors and is at a more advanced implementation stage. Given the reality that funding constraints will force restriction or elimination of portions of developing programs, it is paramount that a passive surveillance component be given sufficient priority. Passive surveillance networks are an integral component of Canadian Food Inspection Agency (CFIA) administered terrestrial surveillance programs and they must also be included within NAAHP. Ontario Ministry of Natural Resources (OMNR) funded active surveillance of Ontario hatchery fish understandably did not detect the presence of this agent and no other surveillance was effectively in place! The danger of relying solely on an active surveillance program is particularly evident when a novel variant such as a virus that has altered growth characteristics in cell culture, or is genotypically and/or antigenicly distinct, is 'newly' present. The Great Lakes strain of VHSV was detected in the spring of 2005 because a mortality event of sufficient size occurred and a diagnosis was actively sought by Bruce Morrison, a concerned biologist with the Lake Ontario Management Unit of OMNR (RR # 4, Picton, Ontario, KOK 2Y0). Subsequent mortality events in spring 2006 would have ensured the detection of VHSV; at this point, however, a major consideration should now be ensuring that NAAHP has adequate resources allocated for detection of novel or altered agents that may not otherwise be detected by routine active surveillance for listed pathogens. The emergence of the Great Lakes strain of VHSV genotype IV should be adequate warning to ensure that diagnostic programs responsible for investigation of unusual morbidity/mortality occurrences are funded alongside active surveillance for listed pathogens within NAAHP.

The emergence of the Great Lakes strain of VHSV genotype IV should be adequate warning to ensure that diagnostic programs responsible for investigation of unusual morbidity/ mortality occurrences are funded alongside active surveillance for listed pathogens within NAAHP.

As CFIA develops the risk analysis required for VHSV in the Great Lakes region and works in coordination with the USDA-APHIS and state/provincial agencies to develop a surveillance protocol for sampling for spring 2007, the potential impact of this virus is starting to be appreciated. While it remains to be seen if the greatest impact of VHSV in the Great lakes will be on fish health or on alterations and improvements in how fish health and movements are managed. What is certain is that VHSV will continue to present a challenge to wildlife agencies, aquaculturists and commercial fishing operations in the region, at least in the near future.

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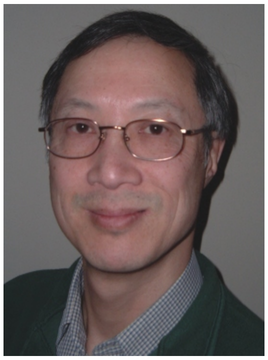


Blythe Chang

Phytoplankton Early Warning Approaches for Salmon Farmers in Southwestern New Brunswick: Project Summary

B.D. Chang, J.L. Martin, F.H. Page, G. Harrison, L.E. Burridge, M.M. LeGresley, A.R. Hanke, E.P. McCurdy, R.J. Losier, E. Horne, and M.C. Lyons

This project investigated the usefulness of several potential early warning approaches for predicting harmful phytoplankton blooms at salmon farms in southwestern New Brunswick. The components of this project included: training farm personnel on the sampling, identification, and counting of harmful phytoplankton species; implementation of daily phytoplankton monitoring by workers at selected salmon farms; retrospective analyses of existing phytoplankton monitoring data; laboratory experiments to determine threshold concentrations of selected harmful phytoplankton species that can cause problems for farmed salmon; use of a tidal circulation model to predict movements of phytoplankton blooms in the vicinity of salmon farms; evaluation of the effectiveness of a light sensor array for bloom detection; and evaluation of the usefulness of satellite imagery for bloom detection. The field and laboratory programs were completed in 2004 and 2005. Data analysis and report preparation are nearly completed and the project will end in March 2007. This presentation provides a summary of the project's activities and results.

Introduction

When phytoplankton blooms occur in areas where salmon farming is conducted, severe economic losses can result, due to decreased fish growth or mortalities. This has happened several times in southwestern New Brunswick (SWNB) within the past decade, especially within the Passamaquoddy Bay area[1] (Fig. 1). Blooms occur less frequently in other areas of SWNB, but a bloom in 2003 caused elevated mortalities at several farms in eastern Grand Manan Island.[2]

As a result of the economic consequences, salmon farmers would like to have a monitoring approach that warns them of a potentially harmful phytoplankton event. Warnings of hours to days are useful since farmers could act on the information by adjusting harvesting schedules, delaying the entry of smolts, and/or adjusting feeding schedules and medication treatments. The purpose of this project was to investigate the feasibility and cost-effectiveness of several potential early-warning approaches and to estimate concentration thresholds (for causing losses in farmed salmon production) of some of the dominant harmful phytoplankton species in SWNB. The project started in June 2004 and the major field and laboratory work was completed in 2004 and 2005. The project terminates in March 2007. A preliminary report from this project was previously published.(3)

Discussions among participating scientists and fish farmers in SWNB resulted in the development of a project with the following components:

- Training farm staff in the sampling, identification and counting of phytoplankton, and implementing a means of rapidly communicating information between Fisheries and Oceans Canada (DFO) and farms;
- Enhanced (daily) phytoplankton sampling by farm staff at critical locations and at critical times of the year;
- Retrospective analyses of historical time series data for individual phytoplankton species to determine the potential for forecasting bloom events;
- Laboratory experiments to determine the critical threshold levels of some phytoplankton species that cause harm to farmed salmon;
- Use of a water circulation model to predict movements of phytoplankton blooms;
- Testing the usefulness of a light sensor array to detect phytoplankton blooms;
- Examining the usefulness of satellite imagery for detecting phytoplankton blooms.

This project was a collaborative effort involving scientists from DFO and salmon farmers in SWNB including Aqua Fish Farms Ltd., Cooke Aquaculture Inc., Heritage Salmon Ltd., Stolt Sea Farm Inc., Admiral Fish Farms Ltd., and the New Brunswick Salmon Growers' Association. Funding was provided by the DFO Aquaculture Collaborative Research and Development Program (ACRDP), DFO Science, and the participating companies.

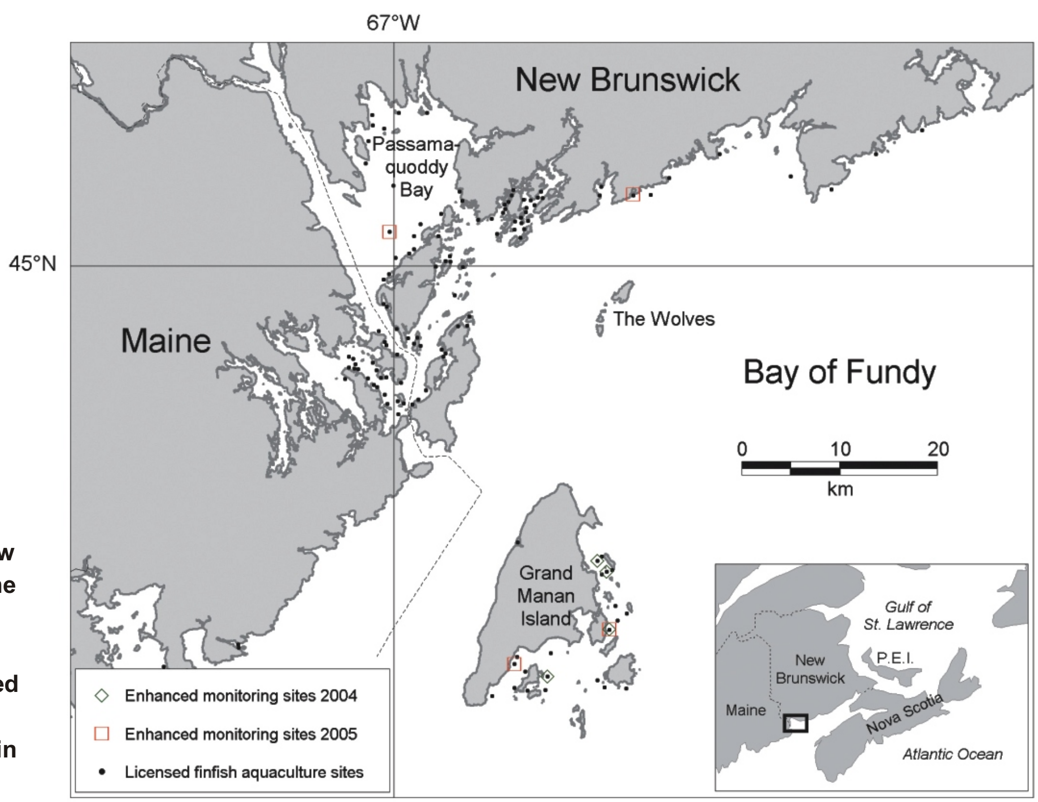


Figure 1
Map of southwestern New Brunswick and the adjacent eastern coast of Maine showing enhanced phytoplankton monitoring sites in 2004 and 2005.

Project Components

Following are summaries of activities and findings for each project component. Details on each component will be published in separate reports.

1. Training and communication of information on harmful phytoplankton blooms between DFO Science and salmon farmers

Farm workers were trained in phytoplankton sampling techniques, species identification, and counting methods. The initial training session was held in July 2004 and further training was provided in May and June 2005.

An efficient mechanism for rapidly and frequently communicating results was established. This allowed industry partners to report results to DFO via telephone or email and allowed scientists to provide farmers with up-to-date information on phytoplankton bloom occurrences, as well as key findings and progress of the project.

2. Enhanced phytoplankton sampling at fish farms

The goal of this component was to implement daily sampling of phytoplankton by workers at participating salmon farms, following training of staff (see component 1). When combined with statistical time series analysis approaches, this sampling was meant to test the hypothesis that the onset of a bloom in an area could be detected and forecasted by daily phytoplankton monitoring at the farms.

It was originally proposed that daily phytoplankton sampling would be conducted at 4 to 6 locations in the main areas of concern (Passamaquoddy Bay and Grand Manan Island). Unfortunately, due to an outbreak of infectious salmon anemia (ISA) in Passamaquoddy Bay in 2004, it was not possible for farms in that area to participate. As a result, in 2004 the project was limited to 4 farms in the Grand Manan Island area (Fig. 1). In 2005, enhanced monitoring was also conducted at 4 farms, two in the Grand Manan Island area (including one of the farms monitored in 2004), one in Passamaquoddy Bay, and one on the mainland coast of New Brunswick (Fig. 1).

Farm workers at each participating site were asked to collect a 250 mL surface sample and a 10 m vertical plankton net haul each day, if possible. Sample bottles with preservative were supplied by DFO. Farm workers were asked to count the numbers of key phytoplankton species in subsamples using a Sedgewick-Rafter

Table 1. Numbers of samples collected at four enhanced monitoring sites in 2004 and 2005. Numbers are ranges for the 4 sites in each year; numbers in parentheses are means. For the percent of potential samples, 100% would indicate that samples were collected every day from the first sampling date to the last sampling date in the year.

	Surface Samples		Vertical Hauls	
2004 (July-September)				
Number of samples collected	21	66 (48.3)	15	64 (46.5)
% of potential number of samples	26.6	97.1 (63.6)	19.0	94.1 (61.2)
2005 (May-September)				
Number of samples collected	50	93 (77.0)	45	93 (76.0)
% of potential number of samples	56.8	93.9 (76.0)	51.1	93.9 (75.0)

slide. The data and preserved samples were forwarded to DFO's St. Andrews Biological Station, where confirmation analyses were done on selected samples.

The species chosen for identification and counting included 4 species which have been associated with problems at salmon farms in SWNB (*Alexandrium fundyense, Chaetoceros socialis, Ditylum brightwellii,* and *Eucampia zodiacus)* and other species, known to cause problems for salmon farms elsewhere, that have been observed in the Bay of Fundy.

Data on the numbers of samples collected by farms are shown in Table 1. The numbers of samples collected averaged over 60% of the possible numbers in 2004 (100% represented one sample collected every day from the first day sampled until the last day sampled in the year). In 2005, the average improved to over 75% of the potential number.

Data on the numbers of samples for which phytoplankton were identified and counted are shown in Table 2. The numbers of reports received on phytoplankton species counts were much lower than the numbers of samples collected. In 2004, less than 10% of the possible daily counts were submitted, while in 2005, less than 15% of the possible counts were submitted. Among the reasons for these low numbers were: workers were busy with other tasks, phytoplankton identification and counting required much more time than sample collection, there were no large blooms at the project sites, and in at least one case the company moved the microscope to another site which was experiencing blooms of concern. The small numbers of counts submitted were insufficient to allow statistical analysis to determine if daily sampling would allow forecasting of blooms.

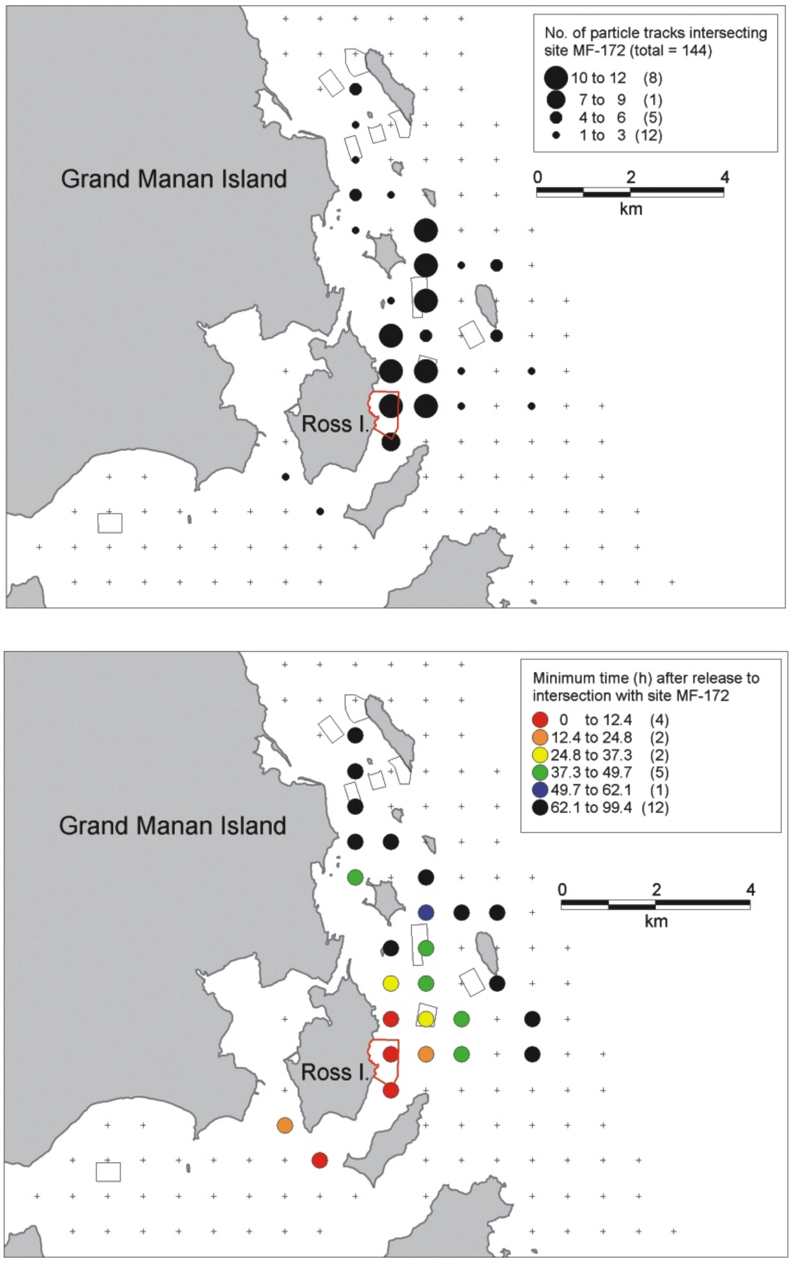
A. fundyense was the main species of concern in both years. During the summer of 2004, *A. fundyense* counts reached 190,000 cells L^{-1} at one Grand Manan Island site in July, but were low at the other enhanced monitoring sites. There were no elevated mortalities or reduced growth rates of salmon reported from these farms. However, elevated salmon mortalities, believed to be due to elevated *A. fundyense* levels (which reached >3,000,000 cells L^{-1}), were reported from at least 5 farms in other areas of SWNB (Lime Kiln Bay, Bliss Harbour and Beaver Harbour) in July 2004. In 2005, *A. fundyense* levels up to 160,000 cells L^{-1} were reported by the farm on the mainland coast in July, but numbers were low at the other sites. Other species that showed some elevated counts at the project sites included *Pseudo-nitzschia* spp., *Mesodinium rubrum,* and *Membraneis challengeri.* Additional data on phytoplankton abundance from this component are reported in a separate paper in this issue.[4]

Table 2. Numbers of samples for which phytoplankton species counts were submitted from enhanced monitoring sites. Numbers are ranges for the 4 sites sampled each year; number in parentheses are means. For the percent of potential samples, 100% would indicate that sample counts were done every day from the first sampling date to the last sampling date in the year.

	Surface Samples	Vertical Hauls
2004 (July-September)		
Number of samples collected	0 9 (3.3)	0
% of potential number of samples	0 12.0 (4.3)	0 20.7 (8.3)
2005 (May-September)		
Number of samples collected	0 36 (11.3)	0 23 (13.3)
% of potential number of samples	0 40.9 (11.2)	0 26.1 (13.1)

3. Retrospective analyses of abundance patterns in harmful phytoplankton species

This component analysed existing phytoplankton monitoring data to estimate bloom characteristics of harmful species. The phytoplankton community in SWNB has been monitored by DFO at several locations at weekly to monthly intervals since 1988.[5,6] The data were analyzed for bloom characteristics such as the time of onset, duration, maximum cell concentration, frequency per year, spatial extent and inter-annual time trends. Some results on *Alexandrium* have been published.[7,8] Data analyses are being completed on other species (*Chaetoceros convolutus, Chaetoceros socialis, Corethron criophilum, Ditylum brightwellii, Eucampia zodiacus, Mesodinium rubrum, Pseudo-nitzschia delicatissima* group, *Pseudo-nitzschia seriata* group, *and Pseudo-nitzschia* sp.). Additional information on this component is reported in a separate paper.[4]



4. Determination of the threshold concentrations of phytoplankton harmful to farmed salmon

This component examined the concentrations (threshold levels) at which harmful phytoplankton will cause mortalities in farmed salmon. This work can help determine trigger points for farmers to initiate husbandry strategies to mitigate the impacts of the harmful phytoplankton. Laboratory experiments were conducted with monocultures of: *Alexandrium fundyense, Chaetoceros socialis, Ditylum brightwellii,* and two clones of *Pseudo-nitzschia*

Figure 2
Maps showing release points of model particles that intersected the target fish farm (red outline; other farms are shown in black outline). Top: the largest circles indicate that particles released throughout most of the tidal cycle intersect the target site, while the smallest circles indicate that this occurs only during a small part of the tidal cycle. Bottom: the circle colors indicate the shortest time between particle release from this point and intersection with the target farm (red indicates the shortest time, less than one tidal cycle; black the longest, 5 to 8 tidal cycles). Small crosses are release points of particles which did not intersect the target farm.

delicatissima. Salmon smolts (supplied by participating farms) were tested for lethality during 24 h exposure to a range of concentrations of the cultures. The LC50 for *A. fundyense* was estimated to be 614,000 cells L^{-1}. Behavioral responses were noted in salmon exposed to 4 x 106 cells L^{-1} of *C. socialis*, but only one fish died during the experiments. Cultures of *D. brighwellii* as high as 106 cells L^{-1} had no deleterious effects. No mortalities were observed at concentrations up to 128 x 106 cells L^{-1} of the two clones of *P. delicatissima.*

5. Prediction of bloom movements using a water circulation model

The spatial and temporal origins of water that flows through a farm were estimated on tidal time scales using an existing numerical model of the mean tidal circulation in the SWNB area. This information can be useful for estimating the likelihood of a bloom being transported to a farm.

In a previous ACRDP project on fish health, we calculated the tidal excursion areas around salmon farms in SWNB using a tidal circulation model.[9-13] This data can be used to predict if a bloom that is infecting one farm is likely to affect neighboring farms within a tidal excursion (12.4 h).

For this project, we used the same model to release particles in a grid around Grand Manan Island to estimate the probable movements of blooms originating over a broader area around the island (i.e., not just blooms originating at other farms). For this component, we also ran the model for a longer period (8 tidal cycles, or approximately 4 days). Example results are shown in Figure 2. This methodology can also be applied to other salmon farming areas of SWNB.

6. Testing the usefulness of a real-time moored light sensor to detect phytoplankton blooms

A passive light sensor array can be moored at a fish farm and will provide continuous data collection on light extinction throughout the upper water column. This can provide an indicator of gross phytoplankton abundance, but cannot identify individual species. The sensor array recorded the intensity of the 490 nm wavelength light at different depths in the upper 16 m of the water column (the 490 nm wavelength is in the middle of the phytoplankton chlorophyll absorption range).

We planned on starting this work in the fall of 2004, but the project was delayed due to difficulties in obtaining and deploying equipment components. The equipment was successfully deployed at a site in Passamaquoddy Bay between July and September 2005. The data are still being analyzed, but preliminary analyses indicate that the equipment can measure light extinction with depth. The data needs to be calibrated with actual chlorophyll concentration data collected at the same time. Unfortunately, during the time of deployment, no high concentrations of phytoplankton were observed, so there was insufficient data for calibration. We also experienced some difficulties with instrument performance during the trial. Nevertheless, we feel that this equipment, with further development, may be useful in predicting gross phytoplankton abundance.

7. Evaluation of the usefulness of satellite imagery as a tool for detecting phytoplankton blooms in the Bay of Fundy

Historical Sea-viewing Wide Field-of-view Sensor (SeaWiFS) satellite images were examined for evidence of being able to detect and track phytoplankton blooms within the lower Bay of Fundy. This component required reprocessing of archived satellite images and extraction of intensity data for this area. For the period 1998-2004, there are potentially more than 5,000 satellite images (2 satellite passes per day). However, due to the number of days when the SWNB area is cloudy or foggy, the actual number of usable images was much lower. For the area around The Wolves, there were only 705 usable images for this time period.

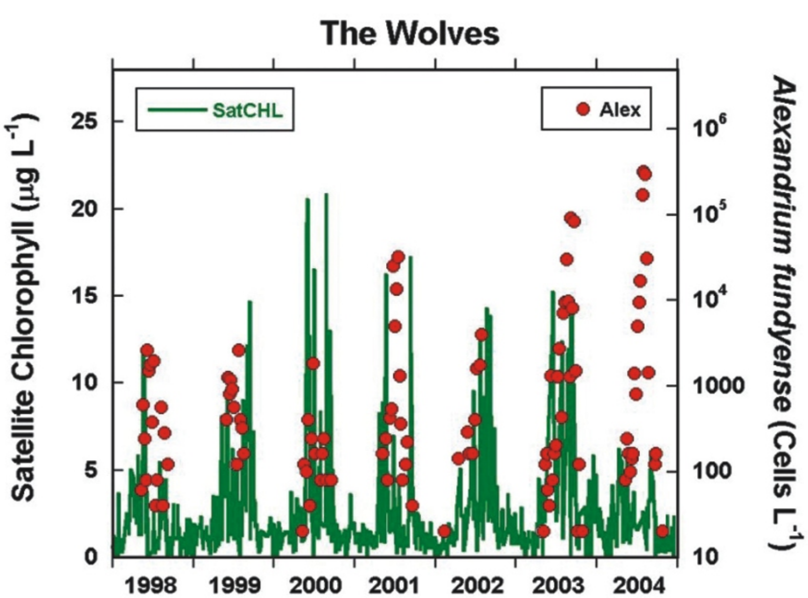
The chlorophyll signal extracted from the usable images must be corrected due to interference from colored dissolved organic matter, suspended sediments, and bottom reflection in very shallow waters. This is done by calibrating the satellite chlorophyll signal with actual chlorophyll data collected in the same area within a few hours of the satellite pass. The corrected satellite chlorophyll signal can then be compared to data on actual phytoplankton species abundance in the area. At The Wolves, peaks in chlorophyll concentration estimated from the satellite signal roughly coincided with peaks in *Alexandrium fundyense* abundance in actual samples during the years 1998 to 2003 (Fig. 3). However, in 2004, when there was a large bloom of this species, there was no peak in the satellite chlorophyll signal. When the data in 1998 and 2001 were examined in closer detail, the peaks in cell abundance were seen to occur after the peaks in the satellite chlorophyll signal. This data suggests that the satellite imagery can be used to track bulk chlorophyll, but not blooms of specific species such as *A. fundyense.*

The spatial resolution of these satellite images (1.5 1.5 km) allows for characterization of only very large scale blooms. Also, because of high background chlorophyll levels in SWNB, only very high intensity blooms will be detected. Although the satellite imagery products currently available may not serve as a practical early warning for phytoplankton blooms, they may be useful, together with phytoplankton monitoring programs, in better understanding the development of blooms. Future developments in satellite imagery products may improve their usefulness.

Summary

The participating farms felt that the project was useful, especially the enhanced monitoring component. Unfortunately, the frequency of sample identification by farms was not sufficient to permit forecasting. The retrospective data analyses provided data on general trends for blooms of several species. The threshold component provided information on cell concentrations that may be harmful to farmed fish. The water circulation model provided predictions of how blooms may move toward or from farms. The light sensor array and satellite imagery showed some promise for detecting blooms, but both require additional development.

Figure 3
Satellite chlorophyll levels (green line) and *Alexandrium fundyense* abundance (red circles) at The Wolves, 1998-2004.



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Jennifer Martin

Industry Enumeration, Early Warning and Hindcasting of Harmful Phytoplankton Blooms in Southwest New Brunswick

J.L. Martin, M.M. LeGresley, F.H. Page, B.D. Chang, and A. Hanke

Within the past decade, harmful phytoplankton blooms have compromised the health of fish at a number of salmon farms in southwest New Brunswick. An ACRDP collaborative project between Fisheries and Oceans Canada and the aquaculture industry was undertaken in 2004 to look at a monitoring approach that would provide an early warning of a potentially harmful algal event. The objectives were to determine the feasibility and need for high-frequency sampling and to design a cost-effective operational approach for phytoplankton monitoring. Industry partners were trained in the collection, identification, and enumeration of potentially harmful phytoplankton species that might cause problems for the salmon. Phytoplankon samples collected from 4 sites during 2004-05 were analysed by trained personnel at salmon farms and a comparison with results from analyses done by staff at the Biological Station is presented, as well as analyses of spatial and temporal characteristics of existing phytoplankton monitoring data. In 2004, a number of salmon farms were affected when *Alexandrium fundyense*, the organism responsible for producing paralytic shellfish poisoning (PSP), bloomed at densities exceeding 3 million cells L^{-1}. The ability of the industry to analyse additional water samples provided an opportunity to mitigate some of the bloom effects and reduce the number of fish mortalities.

Introduction

Phytoplankton blooms can cause problems for the salmon aquaculture industry throughout the world. Blooms have been increasingly implicated in recent years in stress and mortality to Atlantic salmon (*Salmo salar*) in the southwest New Brunswick region of the Bay of Fundy. The area most affected prior to 2003 has been the Passamaquoddy Bay region where blooms of *Chaetoceros* spp., *Mesodinium rubrum*, and *Eucampia zodiacus* have caused concern.[(1)] However, in September 2003, high concentrations of *Alexandrium fundyense* (8.8×10^5 cells L^{-1}), the organism responsible for producing paralytic shellfish poisoning (PS) toxins, were observed off eastern Grand Manan Island (Figure 1) and resulted in greater than "normal" levels of shellfish toxicity and salmon mortalities.[(2)]

Following the 2003 event, an ACRDP (Aquaculture Collaborative Research Development Program) project between the salmon culture industry and Fisheries and Oceans Canada (DFO) was initiated in 2004[3,4] and continued through 2005. Industry personnel were trained in the collection and identification of plankton species that might be cause for concern, have caused problems to the salmonid industry in the Bay of Fundyin the past, or were implicated in fish mortalities elsewhere in the world.

During 2004, high concentrations (up to and including 3 million cells L^{-1}) of *A. fundyense* at a number of locations along the mainland of New Brunswick between Letang and Lepreau affected the behaviour of caged salmon and resulted in fish stress and mortalities. The ability of the industry to pre-screen and analyse additional samples reduced mortalities and activities in close proximity to cage sites. Results from this collaborative project between the industry and DFO are presented.

Materials and Methods

Industry samples

Workshops were held in early July 2004, and beginner and refresher courses in May and June 2005, where personnel from participating salmon aquaculture sites were trained in the use of microscopes and the collection, preservation, identification, and counting of key phytoplankton species (*Alexandrium fundyense*, *Chaetoceros* spp., *Corethron criophilum*, *Ditylum brightwellii*, *Eucampia zodiacus*, *Leptocylindrus minimus*, *Pseudo-nitzschia delicatissima* group, and *Mesodinium rubrum*). Locations of participating sites in 2004 and 2005 are indicated on Figure 1. Field sampling protocols included daily recording on a field data sheet of available sample information such as location/site, date, dissolved oxygen, name of sampler, time, surface water temperature, weather, wind and air temperature, fish behaviour, water clarity/visibility, and the site personnel's phytoplankton counts. Whole water samples (250 mL) were collected as frequently as once a day (but often less frequently) from the surface and preserved with 5 mL formaldehyde:acetic acid (1:1) (FAA) solution. Vertical 20 m mesh net hauls (23 cm diameter) from a depth of 10 m were used for concentrating 45 mL phytoplankton, which was preserved with 5

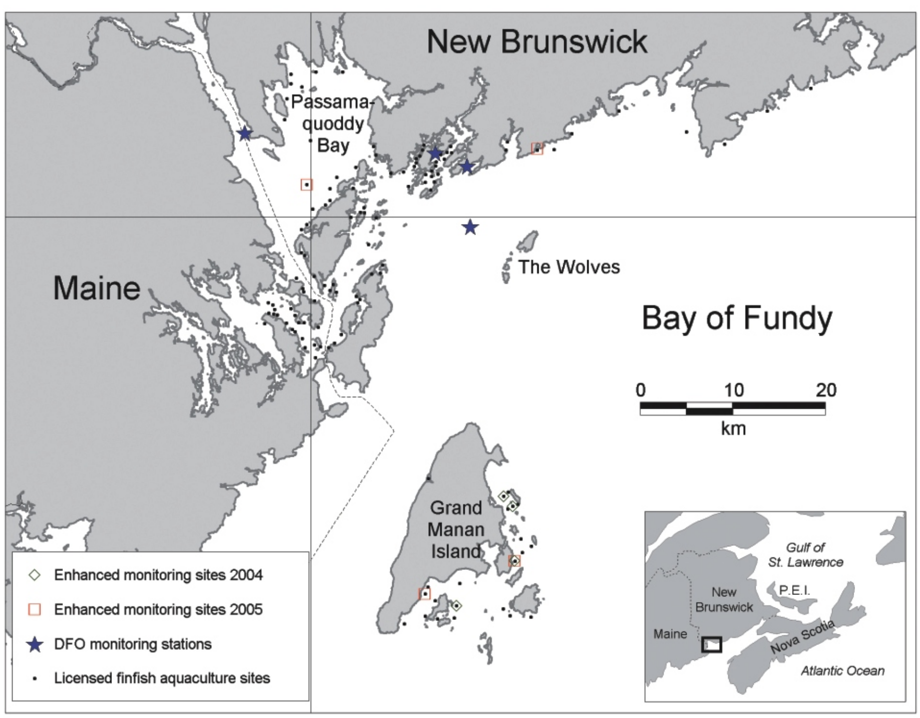


Figure 1
DFO phytoplankton monitoring stations and industry sampling sites in 2004 and 2005.

mL FAA. Whole water and net samples were counted by industry personnel using a Sedgewick-Rafter counting slide(3) and at least one full column of the slide or 20 grid squares were counted.

Industry samples and datasheets from participating sites were collected by DFO personnel once a week and returned to the laboratory at the St. Andrews Biological Station where data was entered into an Microsoft Excel spreadsheet. Some samples were counted for quality control by DFO personnel using either a Sedgewick Rafter or a Palmer Maloney(5) counting slide.

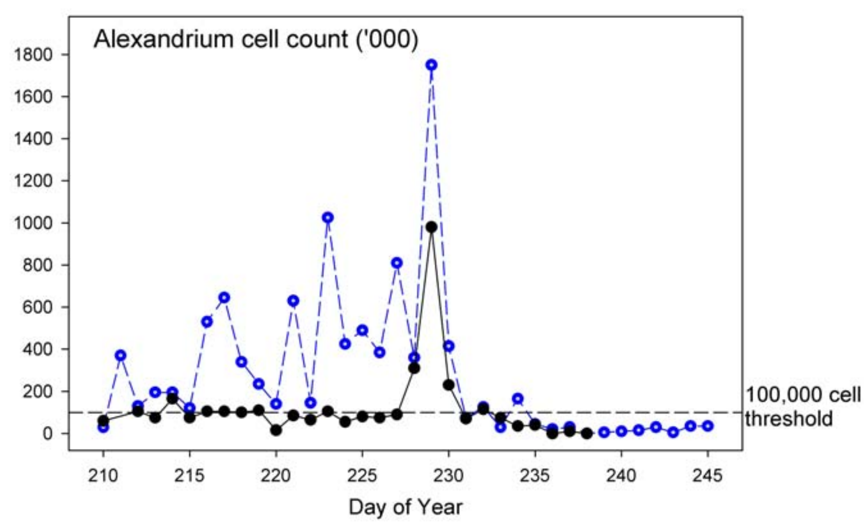
DFO samples

Phytoplankton samples were enumerated and identified as part of a DFO phytoplankton monitoring programme that was initiated in 1988.(6) As part of this programme, water samples (250 mL) have been collected weekly from May through October, biweekly during April and November, and monthly during the winter months from four locations (Brandy Cove, Lime Kiln Bay, Deadmans Harbour and The Wolves (Fig. 1)). Samples were immediately preserved with 5 mL FAA. Later, 50-mL subsamples were settled in Zeiss counting chambers for 16 h. Phytoplankton greater than 5 µm were identified and enumerated (as cells L^{-1}) with the Utermöhl technique using a Nikon inverted microscope.(7) Results were stored in an Microsoft Access database from which time series of the near-surface abundance of species such as *A. fundyense*, *M. rubrum* and *E. zodiacus* were analysed to determine bloom features.

Results and Discussion

Although there were varying levels of participation and numbers of samples counted among industry partners, when the results from samples counted by industry personnel were compared with those from DFO staff there was good agreement between the numbers for *A. fundyense* and *Membraneis challengeri* (formerly *Tropidoneis antarctica* var. *polyplasta),* especially at higher concentrations. Counts for other species such as *Pseudo-nitzschia* spp., *Ditylum brightwelli*, *Chaetoceros socialis*, *Chaetoceros convolutus*, *Mesodinium rubrum*, and *Leptocylindrus minimus* did not agree as well. This may have been due to the difficulty in determining cell concentrations when the numbers of a species were low, and species such as *Pseudo-nitzschia* and *Leptocylindrus* can be very thin and thus difficult to distinguish. It appeared that industry personnel counted fewer samples when the fish were not experiencing difficulty, although they collected and counted more samples in 2005 (n = 98) than in 2004 (n = 38).

Figure 2
Industry counts of *Alexandrium fundyense* from two locations along the mainland coast of New Brunswick in 2004.



In 2004 all the participating sites were located in the Grand Manan area (Fig. 1). Although these sites did not experience problems as a result of harmful algal blooms, a number of samples were collected as part of the programme. DFO counts at one site during 2004 between July 8 and September 27 indicated that the highest concentrations of *A. fundyense* observed were 1.9×10^5 cells L^{-1} and concentrations of *Pseudo-nitzschia* spp. reached 3.0×10^6 cells L^{-1}. Eighteen of the collected samples were analysed at DFO and although the industry partner collected samples, they were not able to perform counts on the samples because fish on this farm on Grand Manan Island were not experiencing abnormal behaviour so the microscope was deployed to another farm that was experiencing difficulties. Industry counts from two sites that were experiencing problems (Figure 2) indicate that the peak concentrations of *A. fundyense* occurred on day 228 (August 15). Initially it was suspected that fish behaviour was affected at concentrations of around 1.0×10^5 cells L^{-1} but as the industry became more confident with the counts, they began to understand the patchiness of the bloom, and the behaviour of this particular bloom, and they determined that fish were not affected until levels reached around 3.0×10^5 cells L^{-1}. This allowed them to continue feeding the fish at concentrations higher than 1.0×10^5 cells L^{-1}.

During 2005, industry participating sites were located off Grand Manan Island, Passamaquoddy Bay, and along the New Brunswick mainland coast at a location affected by a bloom in 2004 (Fig 1). In that year, phytoplankton blooms did not appear to impact any of the aquaculture operations. However, a number of samples were collected and analysed by both DFO and the industry. Results from one site where 36 samples were analysed by industry and 10 by DFO indicate that the highest concentrations of *A. fundyense* observed by industry were 1.6×10^5 cells L^{-1}, and a new phytoplankton species for our area, *Membraneis challengeri,* was detected in Bay of Fundy waters.

Results from the DFO phytoplankton study initiated in 1988 indicated that most species tend to occur annually, although there is significant inter-annual and within species variability. Figure 3 shows the annual time series for *A. fundyense* at The Wolves station plotted on a log transformed scale. From 1988 through 2005, *A. fundyense* occurred every year with the abundance in each year focused in one or more bloom events. Only during 1989, 2003, and 2004 did the maximum observed concentration of *A. fundyense* at The Wolves exceed 7.0×10^4 cells L^{-1}. Close inspection of the *A. fundyense* time series indicated that each year there was a characteristic "day of first appearance" that varied inter-annually in a synchronous fashion throughout the lower Bay of

Murielle LeGresley sampling aboard the Pandalus III in Lime Kiln Bay

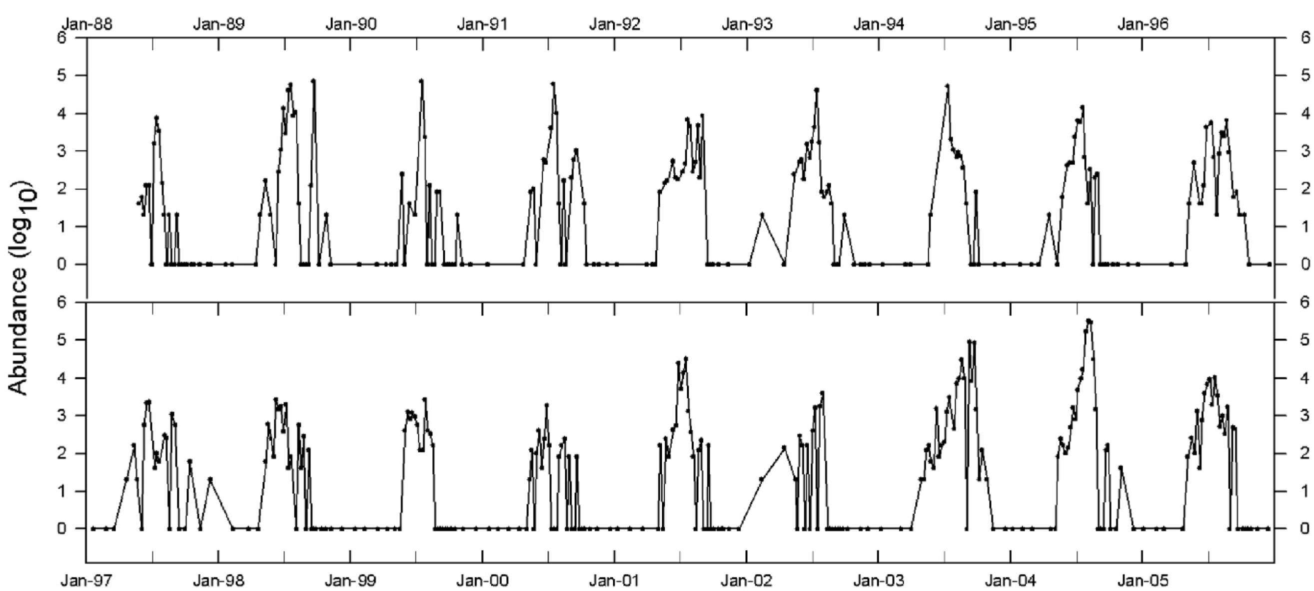
Fundy area.[8,9] The median "day of first appearance" occurred earlier in the offshore and coastal sites (~ day 135) than in sheltered inshore sites (~ day 145). The maximum cell abundance for a given year also varied annually as did the date on which it occurred.

It should be noted that all phytoplankton species behave differently. For example, whereas *A. fundyense* tends to have greatest cell concentrations in the offshore, *M. rubrum* cell densities were highest inshore, in the Passamaquoddy Bay region during the years 1988 to 2006. Another species, *E. zodiacus* was not observed every year; it tended to be more abundant in the Passamaquoddy Bay area and had major bloom events in 1999 and 2002.

Conclusions

Following 2 years of study, it was determined that the level of collection and analyses of samples by industry was dependent on: 1) the level of the phytoplankton problem, 2) availability of personnel on the site to do the work, and 3) the ease of recognizing the species (the easier to reognize, the more apt it was to be counted). Participation improved with time. Through the project, the industry was able to have trained personnel collect and analyse samples as required, as well as train additional personnel when problems were occurring. Samples could be prioritized and analysed rapidly at each site during an event providing site managers with the information needed to make rapid management decisions on mitigation. Through frequent monitoring it was determined that it is possible to provide an early warning of harmful algal blooms as well as allow for the monitoring of the decline of the bloom so that the industry could determine when to resume normal activities. However, the low frequency of sample analyses by the participating farms meant that bloom "prediction" was not possible during this project although farm participation was useful in confirming the presence of

Figure 3
***Alexandrium fundyense* cell densities since 1988 at The Wolves.**



blooms inferred from observations (such as coloured water, abnormal fish behaviour, or presence of nearby blooms) and determining when threshold levels of a particular species are reached that require management actions. Additionally, a more cohesive network was established both within industry and with DFO that provided advice and sharing of data pertaining to bloom events within the Bay of Fundy in the southwest area of New Brunswick.

Acknowledgements

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Meghan Mills

The Marine Harvest Canada Strategy for Effective Fish Health Management

Meghan Mills, Diane Morrison, Brad Boyce, Cilka LaTrace, and Jean Veal

Fish health is vital for successful salmonid aquaculture. In fact, without undermining other aspects of the industry, fish health is the key issue upon which everything else depends. A poor and neglected fish health program may lead to reduced production, increased labor costs and increased expenses due to treatments. In addition, sites with compromised fish health management may lead to increased risk of disease transmission between wild populations and farm stocks. Marine Harvest Canada recognizes the importance of an effective fish health management program. Our strategy is a holistic approach and involves all aspects of our business such as: surveillance and investigation of fish health issues, medical care, treatment when necessary, selective breeding, fish health and biosecurity training for all staff, environmental analysis, clinical/field research, and development and delivery of policies. Our goal is to comply with and exceed the requirements of current fish health regulations, and ultimately protect our stocks.

Introduction

To be commercially viable in aquaculture it is imperative to maintain a successful fish health program. The fish health department at Marine Harvest Canada provides total fish health management to all freshwater and saltwater facilities. Our goal is to improve overall fish productivity (survival, growth and food conversion (FCR)) through proactive and reactive fish health management. Our team consists of five fish health and quality assurance professionals: Manager/Veterinarian, Lab Manager, Quality Control Manager, and two Fish Health Technicians. We focus on surveillance and investigation, biosecurity, vaccination and treatment, sea lice monitoring,

Figure 1
Marine Harvest Canada operated 27 active sites on and around Vancouver Island in 2006. The remoteness of several sites in the Broughton and Klemtu makes regular site visits challenging.

clinical/field research, education and training, quality assurance, communication and support. Effective management of all these elements is crucial in order to reduce the economic, social and environmental impact of health events in aquaculture.

Surveillance for Potential Salmonid Pathogens

All marine and freshwater facilities are visited monthly for routine surveillance for potential salmonid pathogens. Our examinations and tests generally include: gross pathology, bacteriology (lesions and/or kidney plated on standard TSA and blood agar), virology (pooled tissue from gill, pyloric caeca, spleen and kidney cultured on CHSE, SSN-1, EPC, RTG and Fat Head Minnow; PCR for IHN & VHS), histopathology (gill, brain, heart, liver, spleen, kidney, pyloric caeca and muscle) and ELISA for *Renibacterium salmoninarum* (positive $n = 0.010$, negative $n = 0.005$, suspect $0.005 < n < 0.010$ corrected value). At Marine Harvest Canada, we know the health status of our stocks and we routinely monitor for changes. Biosecurity is key as we try to limit risk of pathogen introduction and transfer in an uncontrolled environment.

Figures 2 and 3
Regular site visits and staff training are key aspects of our fish health program.

The extensive sample collection and testing by the Fish Health Department is recorded and maintained on our in-house fish health report database. It is also vital that sites collect and report accurate mortality data to alert the Fish Health Department to potential or developing problems, for timely treatment and management, for production analysis and for use in future research and decision making.

In addition to our in-house surveillance program, the BC Ministry of Agriculture and Lands (BCMAL) conducts a fish health surveillance program involving random farm inspections and specimen collection for health evaluation. Selection of farms is weighted based on the number of farms in a zone as a percentage of the total number of farms in the province. Results of these audits are recorded in the fish health database and posted quarterly on the BCMAL website.

Education and Training

Our in-house fish health training program encourages site staff to be proactive about fish health and teaches them to recognize potential or developing problems and to report these observations in a timely manner to the fish health department for investigation and support. All employees at marine and freshwater sites are provided with annual training in fish health and biosecurity. In addition, all sea site employees receive ongoing training in sea lice monitoring. This training is a condition of advancement to managerial positions. All marine site staff also participate in the Harmful Algae Monitoring Program offered by Nicky Haigh, a faculty member at Malaspina University-College in Nanaimo, BC. Dissolved oxygen (DO), temperature, salinity and plankton are monitored daily at every site. Currently, plankton and low DO events account for greater losses than all disease-related mortality.

Disease

The diseases of most concern to our Atlantic salmon stocks are infectious hematopoietic necrosis (IHN), bacterial kidney disease (BKD), fungus *(Saprolegnia* sp.), and mouth rot. The most recent IHN outbreak in British Columbia was in 2000-2003. This outbreak accounted for between 30 and 100% mortality on farms industry-wide. All smolts entered in traditionally high risk areas now receive the Apex™ (Novartis, PEI, Canada) IHN vaccine. Vaccination against common bacterial infections (i.e., furunculosis and vibriosis) is very effective.

Marine Harvest Canada places high priority on thorough disease screening of broodstock at spawning. All broodstock undergo viral screening. Females are tested for BKD and all positive and suspect egg lots are rejected. We want to ensure that the next generation gets off to a healthy start and to date we haven't found any clinical BKD or viral infection in freshwater.

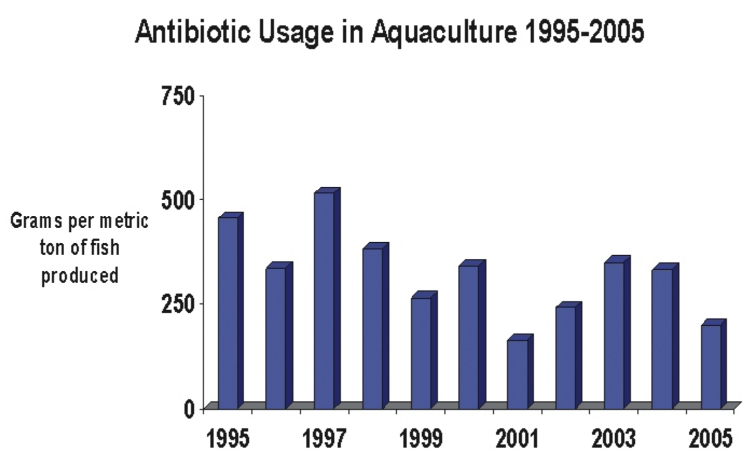
Fungus is an ongoing problem in freshwater rearing. Because we are results based, we have looked at vaccination practices, handling, husbandry, water quality and treatments in trying to reduce fungal infection in our hatcheries and lake sites.

Myxobacterial stomatitis, more commonly known as mouth rot, can potentially cause significant mortality four to eight weeks post-saltwater entry. Occurrence of this disease is typically site specific and highly dependent on salinity and smolt quality. Studies have shown that lower salinity entry, full smoltification, and large good quality fish are key to avoiding a potential problem and costly treatment.

Sea Lice

Controversy surrounding sea lice on BC salmon farms started in the Broughton Archipelago where Marine Harvest, as well as other companies, operate a number of sites. In 2002 a reduction in pink salmon returns led to concerns that sea lice may have impacted pink salmon out-migration. In 2003 the Province of BC initiated mandatory monthly monitoring and reporting of sea lice on all Atlantic salmon farms. Industry counts are reported to the British Columbia Salmon Farmers Association Fish Health Database and can be accessed at www.agf.gov.bc.ca/fisheries/health/Sealice_monitoring_results.htm. During periods of juvenile salmon out-migration, sites must treat or harvest if the farm average reaches the BCMAL action trigger of three motile lice per fish.

Figure 4
Antibiotic use in chinook and Atlantic salmon aquaculture in British Columbia, 1995 to 2005. Chinook salmon were prescribed significantly more antibiotic than Atlantic salmon due to on-going BKD throughout production. At Marine Harvest Canada, antibiotic use is currently one-tenth of the industry reported level.



As part of the BCMAL sea lice action plan, 25% of active salmon farms are audited quarterly by provincial fish health biotechnicians. Auditing increases to 50% of active farms during the peak smolt out-migration period (April to June inclusive). The results of these audits confirm the validity of farm reported data.

Currently, Slice™ (emamectin benzoate) is the only commercial sea lice treatment available for use in BC. It is highly efficacious and usage is minimized by optimizing treatment timing based on historic data. Marine Harvest is successfully managing sea lice on our farms.

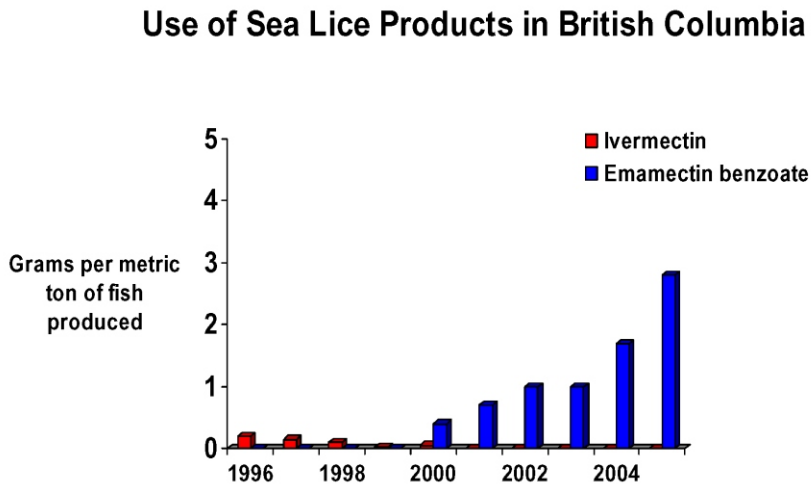


Figure 5
Use of sea lice products (ivermectrin and emamectin benzoate) in British Columbia from 1996 to 2005.

Food Safety and Quality Assurance

Our comprehensive quality assurance program is a testament to Marine Harvest Canada's commitment to providing the highest quality farmed fresh salmon at harvest. Fish are sampled quarterly for contaminants (e.g., heavy metals, pesticides, PCBs) and to date all substances tested for have been far below Canadian Food Inspection Agency (CFIA), Health Canada, and country of export acceptable levels. Prior to harvest, fish are also tested for any therapeutant they received during their production cycle. These residues have always been below CFIA, Health Canada, and country of export acceptable levels.

Summary

Marine Harvest is a results-oriented, production-driven company. The Fish Health Department identifies potential or developing problems and we investigate and analyze, make recommendations and guide staff in implementing solutions. We visit our sites, train our staff, and establish protocols—all to ensure our fish are productive. At Marine Harvest our focus is our fish. We are farmers producing food and to do so successfully we must have healthy stocks. Our goal is to meet and exceed current fish health regulations and ultimately to minimize potential losses due to fish health events.

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Survival of a Deformed “Miracle Fish” *Oreochromis Niloticus* in an Intensive Water Recirculating System

Thomas T. George

The “miracle fish” *Oreochromis niloticus* is the world’s most important warmwater cultured food fish, and is farmed under both extensive conditions and in super-intensive recirculating systems. This is possible because the fish is hardy and tolerates water conditions with a wide range of physical and chemical characteristics. It can survive even when it is deformed. This paper reports on how an *Oreochromis niloticus* developed its dorsal and anal fins to perform the action of an atrophied caudal fin.

Introduction

The “miracle fish” *Oreochromis niloticus* is one of 77 tilapia species that belong to the Family Cichlidae of the Tribe Tilapiini.[1,2] It originated in the African continent and evolved in the River Nile.[3,4] It has colonized widely different habitats due to its efficient grazing habits, relatively low position on the aquatic food web, rapid growth rates, and large sizes at first reproduction. Also, it has a high degree of maternal brooding and care of the young. It is now one of the world’s most important warmwater cultured food fish. It is farmed in both extensive conditions and in super intensive (> 100 kg/m^3) recirculating and integrated hydroponic systems in tropical, subtropical, and even temperate countries.[4,5] The species has an amazing ability to adapt to its environment and has survived in an intensive water recirculating system with an atrophied caudal fin. Hereinafter, are six topics that provide an account of these adaptations and describe its survival even when deformed.

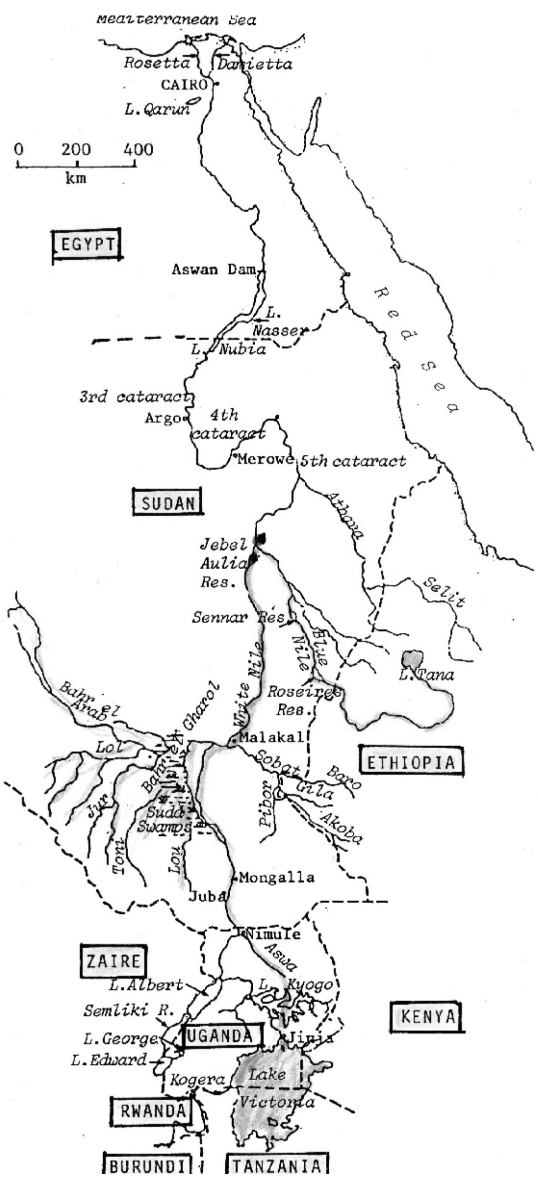

Figure 1
Rivers and Lakes of the Nile System.
(from Welcomme 1972)[6]

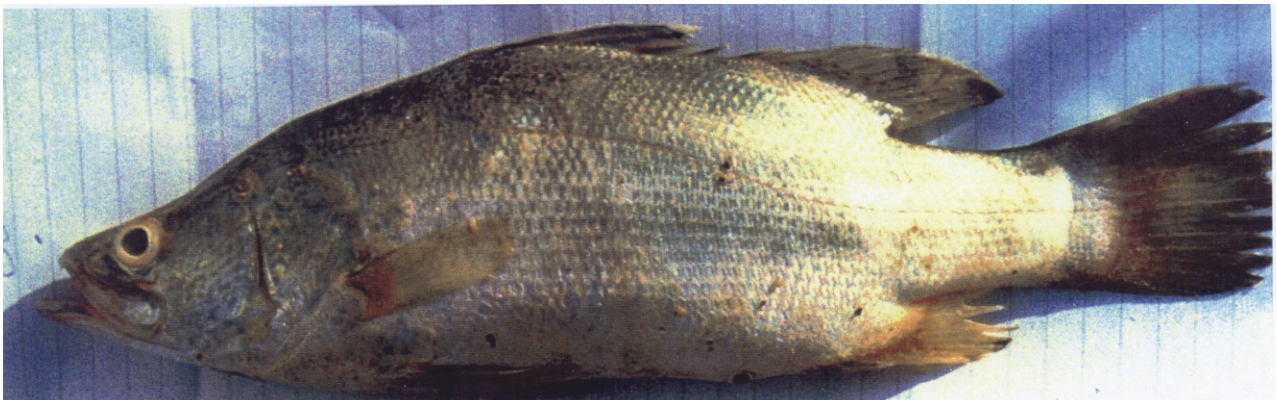

Figure 2
The exotic, carnivorous Nile perch, *Lates niloticus.* Joseph George photo

Ecological and Economic Domination in Lake Victoria

Natural selection is known to be the survival of the fittest, which means the most reproductively successful.[7] Of all the tilapiine species, *O. niloticus* in Lake Victoria—the largest lake in Africa with an area of 68,000 sq km (26,828 sq miles) and a depth of 95 m (Fig. 1)—could be one of the best examples to demonstrate natural selection. Although *O. niloticus* is not native to this lake, it has become an ecologically and economically dominant species, second only to another introduced species, the carnivorous Nile perch, *Lates niloticus* (Fig. 2), with which it co-exists and avoids direct competition for available resources. The Nile tilapia is the only tilapia species that could thrive in the presence of the predator Nile perch, a species that has had great impacts on the indigenous fishes of the lake. This is mainly because the Nile tilapia, unlike other tilapia species with primitive substratum habits, is a highly adapted mouth brooder (Fig. 3). The females carry the fertilized eggs in their mouths (Fig. 4) and can migrate long distances, allowing the species to disperse quickly, establishing and overpopulating favorable environments. Although it does not produce numerous progeny at each spawning, it provides its young with a high level of maternal care. Coupled with several extended reproductive periods each year, maternal care minimizes the risk of predation and enhances survival of the offspring.[8]

Figure 3
The Nile tilapia, *Oreochromis niloticus*—a colourful male before mating. Gary Chapman photo

Reproductive Behavior in Confined and Intensive Culture Conditions

Under confined conditions, *O. niloticus* has a great ability to switch from somatic to reproductive growth. In ponds or resevoirs, it matures at smaller sizes and younger ages and spawns more frequently than under natural conditions. Early maturation is coupled with a shorter life span and the production of a greater number of smaller eggs. This is homeostatic response to the environment and is not due a misunderstood 'stunting' problem that is thought to occur in cultured tilapia. Surprisingly, *O. niloticus* behaves quite differently in intensive recirculating systems than in pond conditions. Highly intensive stocking in a recirculating system eliminates reproduction and the fish resort to growing rapidly, which results in a desirable fish with a large body size within a short period of time. These adaptive abilities are responsible for the widespread distribution, plasticity, and success of *O. niloticus* as a colonizer.[4,8]

Structural Adaptations for Feeding and Reproduction

O. niloticus has the ability to solve problems related to feeding, incubation of eggs, etc. It has structural adaptations to being an omnivore, including its small pharyngeal teeth which are used to grind the coarse particles of its diet, a stomach with low pH (2.0) that helps to dissolve walls and membranes of cells, and a long intestine (up to 14 times the body length) that allows additional time for digestion and absorption. As a mouth brooder, it has a wider head than that of the primitive substrate spawner, which increases the capacity of the mouth for egg incubation. Furthermore, changes in the structure and behavior of very young fish also provide definitive morphological evidence of such an evolutionary sequence. The very young of substrate spawners are helpless at an early stage of development and have specialized larval organs called 'head glands' which enable them to anchor themselves to the substratum. These glands disappear as the larvae become sufficiently strong to swim effectively. Such glands are not required by larvae of mouth brooders because the larvae are provided with maternal care. However, it is very interesting to note that sections of the head of several mouth brooders have revealed non-functional rudiments of these organs, which clearly indicate that mouth brooders were derived from substrate spawners.[4,8]

Figure 4
A female *Oreochromis niloticus* taking its eggs into her mouth immediately after being fertilized by the male.
Gary Chapman photo

Reaction to Physical and Chemical Environmental Parameters

O. niloticus has adaptations that allow it to live in an extraordinary range of physical parameters, including temperature, dissolved oxygen, salinity, pH, ammonia, and other gases. It tolerates temperatures from 16 to 42 C, becomes inactive below 16 C, and does not survive below 9 C. Growth is poor at 20 C, optimum between 25 to 30 C, and greatest at 26 C. It reproduces at temperatures above 22 C . This adaptation to a stable temperature regime has limited its natural distribution to tropical areas. It can also tolerate a pH of 5 to 9 [3,4] Moreover, it inhabits areas where most other fish genera are unable to live, even under favorable food conditions, due to the fact that it tolerates dissolved oxygen levels as low as 0.1 ppm.[9] It also tolerates brackish water of 10 to 14 ppm and very high salinity (42 ppt) seawater. That is why it is assumed that tilapias evolved from a marine ancestor and that penetration to fresh water is secondary.[3,8,10]

Positive Reaction to Ammonia Toxicity Treatment in Intensive Recirculating Systems

With respect to ammonia, *O. niloticus,* as other fishes, excretes most of its nitrogenous waste through the gills in the form of ammonia. The toxicity of un-ionized ammonia depends on the amount of dissolved oxygen, with toxicity being higher when DO concentration is low. Therefore, controlling ammonia levels using bio-filters has revolutionized the aquaculture of *O. niloticus* in intensive recirculating systems due to its very positive reaction to higher oxygen levels. This fact, and other similar cases, refute the theory of the so-called ‘living space factor effect’ on fish growth, once believed to be responsible for fish not growing in limited or confined small areas.[11]

Ability to Survive in an Intensive Water Recirculating System even when Deformed

O. niloticus has survived in an intensive recirculating system even when with an atrophied caudal fin. Propulsion in this cichlid fish is achieved by the propagation of waves of muscular contractions along the body, of which the tail or caudal fin is a paddle-like extremity that contributes markedly to the propulsive effect. Such movements drive the fish forward. The conspicuous dorsal and anal fins are important in preventing rolling during swimming. They function like the keel of a boat as they can be raised or lowered according to the demands placed upon them. Both fins are supported by skeletal elements, simple spines and soft rays. Spines are restricted to the anterior portion of the dorsal and anal fins; they are stout and sharply pointed, while their flexible soft rays splay out towards their free end. The spines when erect present a set of spikes that can be used for defense. Besides the functions already described, fins have been utilized by *O. niloticus* for a variety of other purposes, some of which are mechanical.[8] The flexible rays of both the dorsal and anal fins, in the absence of an atrophied caudal fin, got extended beyond the posterior of the body and performed the action of a normal caudal fin (Fig. 5). This induced adaptation makes *O. niloticus* one of the most hardy and adaptive species in the aquatic environment.

Figure 5
A 13-cm *Oreochromis niloticus* with an atrophied caudal fin and the flexible rays of both the dorsal and anal fins extending beyond the posterior end of the body. Joseph George photo

Conclusion

There is a global consensus that *O. niloticus* is the most suitable species for aquaculture development. In fact, because all the above mentioned attributes are not shared with any other cultured species, it has become the most important aquaculture species of the 21st century.[5]

Recommendations

1) Fisheries and aquaculture scientists should record their observations of any fish abnormalities they encounter.
2) To overcome the so-called problem of 'stunting' in tilapia pond culture due to excessive reproduction, which is actually a homeostatic response to confined pond conditions, aquaculturists are advised to use high stocking densities and enough aeration and feed to control reproduction and encourage somatic growth, as is the case in confined intensive recirculating systems.

Acknowledgments

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Growth, Body Morphology and Muscle Metabolism Patterns in Newly-hatched Salmonid Species (*S. alpinus and O. mykiss*): Effects of Water Velocity

Nathalie Le François

N.R. Le François, T. Grünbaum, K. Chu, A. Savoie, and R. Cloutier

Increased growth rate at higher water velocities is commonly observed in salmonids. The improvement in swimming performance (maximum sustainable speed, rates of recovery, endurance), however, remains poorly documented. We propose to use indicators of enhanced swimming performance and muscular growth of newly-hatched Arctic charr and rainbow trout, two species with different degrees of polymorphism. The impact of water velocity on body morphology, growth, and temporal changes in white muscle metabolic capacities (citrate synthase, lactate dehydrogenase, and nucleoside diphosphokinase) were measured. Newly-hatched fish were reared for 100 days at four water velocities: A = 3.2 cm/s, B = 1.6 cm/s, C = 0.8 cm/s, and D = 0.4 cm/s. At the end of the growth trial on Arctic charr, the velocity treatments were reversed (i.e., high velocity (HV) fish were transferred to the low velocity (LV) rearing units and vice versa) and adjustments in enzyme activity level of LDH, CS, and NDPK were monitored for 67 days. Preliminary results show that the high velocity treatment had a more pronounced impact on the growth rate of Arctic charr than on rainbow trout. We suggest that adjustments in muscular energy metabolism and muscle synthesis should be observable and positively related to water velocity.

Introduction

Environmental inputs such as temperature, oxygen, salinity, light, or water velocity can affect fish physiological systems, including skeletal musculature[7] and body morphology.[13] Phenotypic plasticity is defined as the ability of an organism to respond to an environmental cue with a change of form, state, movement, or rate of activity.[16] It is well known that moderate and high water velocities enhance skeletal muscle growth in salmonids. However, it is unclear whether this is a result of decreased antigonistic activity, better access to food,[1] or enhanced swimming capacity.[6]

Activity in fish requires the expenditure of energy for movement. Muscular adaptations to enhanced activity often involve structural changes in cellular organelles or supporting structures such as capillaries.[8] These muscular adaptations have been thoroughly studied via enzymatic activities.[9] Lactate dehydrogenase (LDH), a glycolytic enzyme, has been associated with burst swimming capacity.[10,11] Citrate synthase (CS) is a mitochondrial enzyme associated with sustained swimming capacity.[14] Nucleoside diphosphate kinase (NDPK) is implicated in biosynthesis and its activity is therefore indicative of the scope for growth.[2,5]

Species can differ in their response to environmental variation since strategies for cost reduction and energetics may diverge according to their different natural habitat.[12] Salmonid species in nature encounter major variations in water flow which favour phenotypic plasticity. Furthermore, variation in morphological traits related to body size and shape may be adaptative to hydrodynamic conditions.[12,13] Water velocity is therefore suggested to be an environmental parameter able to induce different interspecific phenotypic responses in terms of external morphology.[3]

The present study was conducted on two salmonid species—Arctic charr and rainbow trout—exposed to different water velocities. Sustained exercise and/or water velocity may be relevant to husbandry practices in a commercial facility to improve larval or juvenile fish performance and/or quality.

Material and Methods

Newly hatched larvae (approximately 1200 alevins per group) were stocked in 8 velocity-channels (recirculating system designed by Aquabiotech Inc. (Coaticook, Québec) at 10°C and exposed to 4 different water velocities: A = 3.2 cm/s, B = 1.6 cm/s, C = 0.8 cm/s, D = 0.4 cm/s, in replicate. After yolk-sac resorption (approximately 20 days post-hatching), four specimens of each species were sampled from each tank every 10 days until 100 days post-hatching. Fish were measured and weighed and, in the case of rainbow trout, were stored at –80°C for enzymatic analysis. Digital images of eight specimens of Arctic charr sampled every other day starting from hatching to day 100 in each treatment (i.e., 408 specimens per treatments) were used in two ways: 1) for measurements of ten morphometric traits related to body size and shape (head length, head height, yolk-sac height, body height, dorsal fin base, anal fin base, caudal peduncle height, caudal fin height, standard length, total length; Figure 1) and 2) for observations of external deformities (i.e., strong body flexure characterized by the caudal part of the body bent upwards).

Enzymatic analyses

LDH and CS assays were conducted as described by Thibeault et al.[15] NDPK

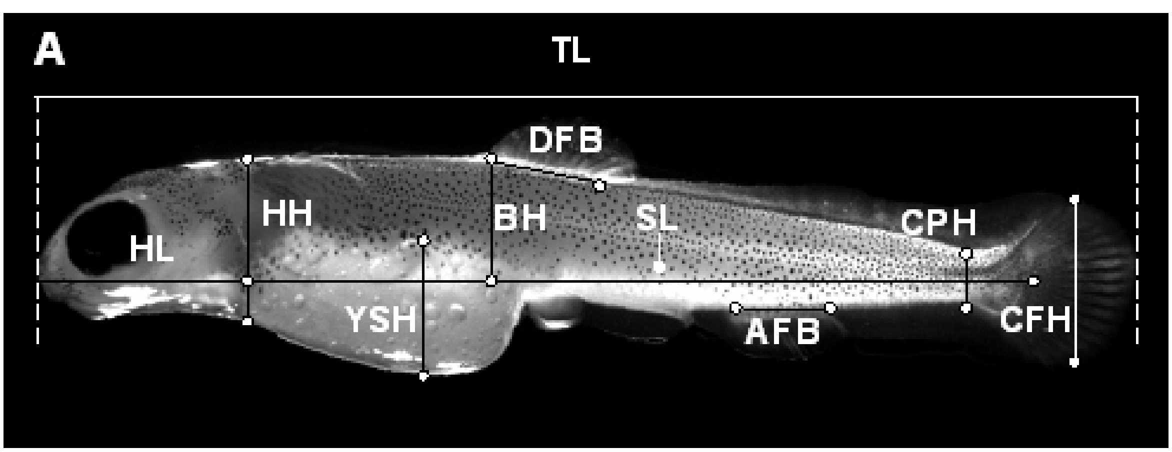

Figure 1
Arctic charr used for morphometric analyses. Modified from Grünbaum et al.[3]

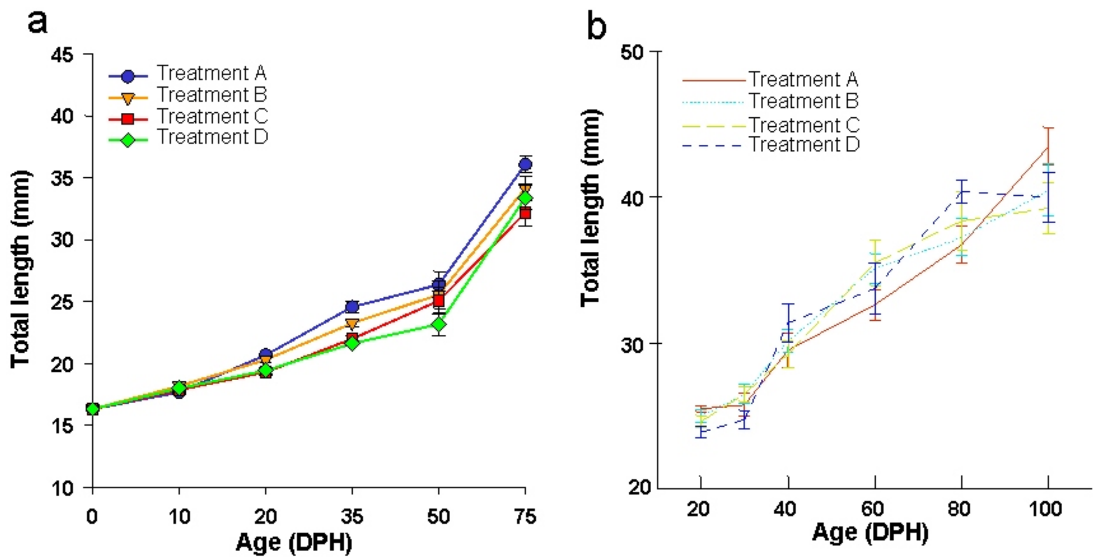

Figure 2
Growth rate of a) Arctic charr and b) rainbow trout in length (mm) from hatching to 75 dph and 100 dph respectively in four water velocities treatments (Grünbaum et al.[4]).

was conducted as described by Couture et al.[2] These assays were adapted for rainbow trout.

Results

Arctic charr

Water velocity had a significant positive effect on growth rate in length (Figure 2a). Mean specific growth rate (SGR) calculated for 75 days was 2.26 %/day, 2.21 %/day, 2.40 %/day and 2.62 %/day respectively from still (D) to fast (A) treatments. For all morphometric traits, with the exception of the yolk-sac height (YSI), differences in means were significantly different among treatments in relation with age (Grünbaum et al.[3]). No external deformities were found. The enzymatic analyses have not been conducted yet.

Rainbow trout

Growth tended to be higher at the highest velocity treatment, although the differences were not significant (Figure 2b). No significant differences were detected in enzymatic activities among the fish in the different treatments.

Discussion

The positive growth response of Arctic charr to increased velocity appeared sooner than in rainbow trout (Figure 2). From 35 days post-hatch, Arctic charr displayed a greater length at a velocity of 3.2 cm/s than at the other velocities. Comparatively, rainbow trout had a greater length at higher velocity only at 100 DPH. This may reflect the greater plasticity of Arctic charr to environmental variations. In their comparative study on brook charr (*Salvelinus fontinalis*) and Arctic charr (*S. alpinus*), Peres-Neto and Magnan[13] showed that the phenotypic plasticity of the two species differed and was probably linked to their degree of phenotypic divergence. Pakkasmaa et al.[12] also concluded that relative habitat heterogeneity or homogeneity may be reflected in the amount of variation in body morphology.

Our results also indicate that higher performances can be achieved through a moderate increase in water velocity early in the development of salmonids. Growth differences between velocities became more pronounced with time.

Acknowledgement

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