

# ***Bulletin***

of the

Aquaculture Association of Canada

de l'

Association Aquacole du Canada



2016-1

## AQUACULTURE – LEADING SUSTAINABLE FOOD PRODUCTION

Aquaculture is recognized globally as a sustainable food production activity that can adapt to climate change. Canada has a role and responsibility to play in growing more seafood, and this year's theme reflects that necessity. The conference will highlight advances in science and technology of Canadian aquaculture, in preparing for the sustainable food systems of the future.

## AQUACULTURE CANADA AND COLD HARVEST 2016 CONFERENCE AND TRADESHOW

SEPT 18-21, 2016, ST. JOHNS, NEWFOUNDLAND

### LIST OF SESSIONS BEING PLANNED

- Aquatic Animal Health and Integrated Pest Management
- Integrated Waste Management
- Aquaculture and Coastal Management
- Benthic Monitoring
- Nutrition and Feeding
- Genomics and Breeding
- Physiology
- Integrated Multitrophic Aquaculture and Aquaponics
- Labour Market
- Land-based Aquaculture
- Advances in Shellfish Aquaculture
- Advances in Finfish Aquaculture
- Innovative Technologies

For further information and updates to the program sessions please visit our website:

[WWW.AQUACULTUREASSOCIATION.CA](http://WWW.AQUACULTUREASSOCIATION.CA)

AQUACULTURE  
ASSOCIATION  
OF CANADA



ASSOCIATION  
AQUACOLE  
DU CANADA



Conference Manager: Joanne Burry | Email : [jmburry@nl.rogers.com](mailto:jmburry@nl.rogers.com) | 506-529-4766

# **Bulletin**

## **de l'Association aquacole du Canada**

### **2016-1**

Vous pouvez recevoir le *Bulletin* en vous y abonnant pour la somme de 60\$ par année ou en devenant membre de l'Association aquacole du Canada (AAC), organisme à but non lucratif. Pour de plus amples renseignements, communiquez avec l'Association aquacole du Canada, 16 Lobster Lane, St-Andrews (Nouveau-Brunswick), Canada E5B 3T6 [tél.: 506 529-4766; téléc.: 506 529-4609; courriel.: [info@aquacultureassociation.ca](mailto:info@aquacultureassociation.ca); site Internet: <http://www.aquacultureassociation.ca>]. La cotisation s'élève à 100\$ par personne (50\$ pour les étudiants et les retraités) et 300\$ pour les sociétés. Le *Bulletin* est répertorié dans l'Aquatic Sciences and Fisheries Abstracts (ASFA), et le CAB Abstracts.

ISSN 2369-1603

#### **Dirigeants**

Cyr Couturier, Président  
Tillmann Benfey, Président désigné  
Tom Taylor, Vice-présidente  
Stefanie Hixson, Secrétaire  
Matthew Liutkus, Trésorier

#### **Membres du conseil d'administration**

Kathy Dalton, Betty House, Tim Jackson, Tara Daggett, Joanne Liutkus, Bruno Gianasi

#### **Rédactions du Bulletin**

Tara Daggett and Betty House

---

# **Bulletin**

## **of the Aquaculture Association of Canada**

### **2016-1**

The *Bulletin* is available through subscription (\$60 per year) or as a benefit of membership in the Aquaculture Association of Canada, a non-profit charitable organization. For membership information contact: Aquaculture Association of Canada, 16 Lobster Lane, St. Andrews, N.B., Canada E5B 3T6 [telephone 506 529-4766; fax 506 529-4609; e-mail [info@aquacultureassociation.ca](mailto:info@aquacultureassociation.ca); website <http://www.aquacultureassociation.ca>]. Annual membership dues are \$100 for individuals (\$50 for students and seniors) and \$300 for companies and associations. The *Bulletin* is indexed in Aquatic Sciences and Fisheries Abstracts (ASFA), and CAB International.

ISSN 2369-1603

#### **Officers**

Cyr Couturier, President  
Tillmann Benfey, President-elect  
Tom Taylor, Vice-president  
Stefanie Hixson, Secretary  
Matthew Liutkus, Treasurer

#### **Directors**

Kathy Dalton, Betty House, Tim Jackson, Tara Daggett, Joanne Liutkus, Bruno Gianasi

#### **Editors**

Tara Daggett and Betty House

# Contents

|   |    |
|---|----|
| Advanced aquaponics in action .....   | 1  |
| Jason Oziel   |    |
| Meet the Cat Cove Marine Laboratory, Salem State University.....  | 5  |
| Joseph K. Buttner, Mark Fregeau, Edward Maney, Scott Weston   |    |
| A new Huntsman for the new millenium: linking industry with research innovation via a new NBIF research chair in aquatic biosciences.....     | 14 |
| Duane Barker  |    |
| Reduction of total suspended solids and phosphorus concentrations in salmon-smolt hatchery effluent using chemical treatment .....            | 18 |
| Lionel T. Hayter, Marc Sorensen, Michel Couturier   |    |
| Macroalgal and sulfur denitrification as efficient biofilters of dissolved inorganic nutrients? A case study at the BioDôme de Montréal ..... | 26 |
| A. Tremblay-Gratton, E. Tamigneaux, J-C. Boussin, G.W. Vandenberg and N.R. Le François  |    |
| The development of a numerical water circulation model of Shelburne, Nova Scotia for aquaculture support .....                                | 34 |
| S.P. Haigh, D.G. Pratomo, F.H. Page, R.J. Losier, D.A. Greenberg and B.D. Chang   |    |
| Modelling aquaculture waste dispersal in Jordan Bay .....   | 43 |
| Adam Drozdowski, Brent Law and Fred Page  |    |
| Oxygen measurement using a needle-type microsensor .....  | 51 |
| K.G. MacKeigan, M.C. Lyons and F.H. Page  |    |
| Development of a rapid, high throughput microplate method for the quantification of sulfide in marine sediment pore water .....               | 55 |
| D. K. H. Wong   |    |
| Review of different opinions about using the grass carp in aquaculture production and aquatic weed management in Canada and Sudan .....       | 59 |
| Prof. Thomas T. George  |    |

## ADVANCED AQUAPONICS IN ACTION

Jason Oziel<sup>1</sup>

<sup>1</sup> NOA Fisheries Inc.  
www.noafisheries.com  
416-546-6623



As a prime example of applied science in action, NOA Fisheries provides a rare learning opportunity through their comprehensive aquaponics courses. The courses, based equally on both the science and business of aquaponics, was originally conceived to increase the knowledge base of company founders, Jason and Tamar Oziel. After a brief study under the Godfather of Aquaponics, Dr. James Rakocy in the US Virgin Islands, the couple returned to Canada to begin food production, but found a void of available advanced aquaponics courses for a temperate climate.

“Of the many aquaponics courses offered in North America, we found that few were taught from a scientific perspective by qualified speakers,” said Tamar, “We were impressed with the research of Dr. Nick Savidov, but could not find a location where he was presenting, so we began to create the courses that we would want to attend.”

The first aquaponics course was held at the Double Tree Hilton in Toronto in December 2012 and attracted delegates from across North America. The speakers included Dr. Nick Savidov, Dr. Michael Timmons, Dr. Kevin Fitzsimmons, Donald Bailey, and Charlie Shultz.

“We brought in an aquaponic system and live tilapia for the hands-on portion,” explains Jason. Participants were able to “harvest eggs” and “sex tilapia”, a hands-on approach that continues in the courses they now offer from their Whitby area farm.



**Figure 1**  
**Demo aquaponic system**





**Figure 2**  
**Broodstock and hatchery room**

After the first course, they ran eight courses across the country with Dr. Savidov, who is known for running his system with unconventional low pH, and other leading aquaponics experts. In the summer of 2015, NOA Fisheries began to offer courses on a regular basis. The next one is scheduled for Aug. 22-24, 2016 and features sessions from Dr. Nick Savidov, Dr. Jason Danaher, John Derksen, Fred Rutman, Steve Naylor, Cara McCreary, and Sarah Zeldman.

“Our focus is more on creating a flourishing, marketable business than on introducing new research to the field,” says Jason, “but the research and understanding of it is vital to creating a viable business.”

“Research was critical to becoming the top source for guaranteed hormone-free all-male tilapia in Canada,” says Tamar, explaining that it was critical from the start that they would only deal with hormone-free fish and the process of finding the sources necessary to start carrying Natural Male Tilapia; the stock is produced with YY males and hormone-free through all generations.

Research continues to be critical as the company continues to contribute to the field with current projects that include working with Lethbridge College to develop an organic feed geared towards aquaponics and with The Guelph Food and Technology Centre to create a new line of fish snack products.

Everything they've learned is condensed into the courses they now offer. Over the course of three days, students are introduced to the concepts of commercial aquaponics and basic business operations through the expert guests at the NOA Fisheries location.

Dr. Jason Danaher presents the UVI (University of the Virgin Islands) system. This system is the design base of most successful commercial aquaponic systems today, developed by the same Dr. James Rakocy who first inspired the Oziels. It is a recirculating aquaculture system with a hydroponic component. The large effluents are separated and discharged, the water is then pumped through a series of raft beds where floating plants use the nitrates for growth thus filtering the water and improving water quality before it is returned to the fish.

Dr. Danaher presents on alternative uses for fish effluent discharge and demonstrates his research using bags to store and dehydrate the discharge to be used later to grow several different species of plants.

John Derksen is a professor at Lethbridge College. He has spent over 30 years in the fisheries and aquatic field, in roles for provincial and federal governments, academia, and the private sector. Currently a full time instructor/researcher at Lethbridge College (18 years) he teaches courses in Aquatic Ecology, Fish Habitat, Fisheries Techniques, Fisheries Science, Water Quality, and Fish Culture. Derksen is also the Head of Research for the Lethbridge College Aquaculture Centre of Excellence, with a research focus on aquaponics, fish culture, and aquatic ecology. His presentations at NOA Fisheries focus on Water Quality, Fish Health, Fish Feed and Nutrition and he also leads the cool water aquaponics discussions.

Sarah Zeldman is a Social Media trainer, speaker and trusted consultant for a wide variety of businesses and organizations. She educates and empowers companies to handle the social media in-house, as part of their regular business routine. She specializes in on-boarding professionals who think they may be too old to master social media, or who might describe themselves as “technophobic”. In one 3-hour session, she gets them posting, tweeting, and engaging like a pro! Zeldman shows individuals and organizations how to implement her 3-step social media formula, which allows them to build brand awareness, connect to their target customers, generate leads, and ultimately increase sales. Her 50+ LinkedIn recommendations demonstrate her proven track record for results: expanding customers’ bases, traffic, revenues, and retention. Her presentation shows the participants how to save time and use social media to effectively market their business.

Fred Rutman is a former professor of Marketing and Finance with an MBA in Marketing and Finance and certifications in Adult Education, Marketing Professional and Quality Assurance. He has written business and marketing plans for a variety of start-ups, small businesses and national organizations. He walks students through the writing of business plans and cash flow projections.

Cara McCreary is a Greenhouse Vegetable IPM Specialist with OMAFRA. Cara is working out of the AAFC Greenhouse and Processing Crops Research Centre in



**Figure 3**  
**Nursery-grow out**  
**room**

Harrow. McCreary has several years of experience as a greenhouse scout and supervisor and as a horticultural advisor. She has a Master of Science in Environmental Biology from the University of Guelph, a Bachelor of Commerce in Business Administration from the University of Windsor, and an Associate Diploma in Horticulture from the University of Guelph. McCreary presents greenhouse production, heating, lighting, and integrated pest management.

Steve Naylor has been working with the aquaculture industry in Ontario for 25 years. For the past 15 years, he has been with the Ministry of Agriculture, Food & Rural Affairs (OMAFRA) as the provincial aquaculture specialist working with the industry and other government agencies on the many issues that have evolved as the aquaculture industry has grown in Ontario. He presents Aquaculture and Aquaponics in Ontario, regulations, and permits.

**Figure 4**  
**Retrofit**





## MEET THE CAT COVE MARINE LABORATORY, SALEM STATE UNIVERSITY

Joseph K. Buttner<sup>\*1</sup>, Mark Fregeau<sup>1</sup>, Edward Maney<sup>1</sup>, Scott Weston<sup>1</sup>

<sup>1</sup> Department of Biology  
Salem State University  
Salem, MA 01970 USA

\*Corresponding author



### Abstract

The Cat Cove Marine Laboratory, Salem, MA was established in 1997 and officially opened in 1999. While labeled a marine laboratory, activities target fresh, brackish and marine waters. Invertebrates, vertebrates, and algae are studied in flow-through, static, and natural systems, the latter facilitated by Smith Pool, a 3.3-ha tidal pond with gates that permit environmental manipulation. Initiatives pursued and insight gained has involved education, research, and outreach efforts of local and broad application.

### Introduction

The Cat Cove Marine Laboratory (CCML) is housed within the Department of Biology at Salem State University (SSU), Salem, MA. Since its establishment in 1997, it has been home to the NorthEastern Massachusetts Aquaculture Center (NEMAC). CCML officially opened in 1999, after two years of construction and renovation, processes that continue today as facilities and resources are expanded, improved, or acquired (e.g. bivalve mollusk, nursery building and Floating Upweller Systems - FLUPSYs; emergency natural gas generator that automatically provides electricity sufficient to power the entire building during electrical disruptions; boats and trailers to facilitate offshore projects; dive locker to support scientific diving).

CCML is located on a 6.6-ha site adjacent to Salem Sound (Fig. 1). Arguably the most unique asset is Smith Pool, a 3.3-ha tidal pond where water flow and environmental conditions can be controlled, largely through opening or

closing tidal gates as needed. On-site structures include an environmentally controlled 510-m<sup>2</sup> wet laboratory with two fish rooms, one shellfish room, an algal culture room, analytical room, utility room, and classroom. Additional on-site facilities include a boat garage/work area/dive locker (79 m<sup>2</sup>), mollusk nursery (35 m<sup>2</sup>) and storage shed (22.5 m<sup>2</sup>).

**Figure 1**  
**The Cat Cove**  
**Marine**  
**Laboratory is**  
**located adjacent**  
**to, and draws**  
**filtered water**  
**from, Smith Pool,**  
**a tidal pond**  
**where water flow**  
**is controlled by**  
**gates.**



CCML investigates freshwater, marine, and brackish environments; biology and culture of finfish, shellfish, and algae; and open, semi-closed, and closed-system aquaculture through education, research and outreach initiatives. Efforts target opportunities and challenges faced by aquaculture and aquatic environments in Massachusetts, the Northeastern United States and southern Canada. As Salem Sound connects with the Gulf of Maine and the Atlantic Ocean, which bathes Europe and Africa and communicates with oceans globally, impact and application of CCML initiatives extend well beyond the North Shore of Massachusetts.

## **Education**

SSU originated as a teachers' college, Salem Normal School, established in 1854 under the guidance of renowned educator, Horace Mann. SSU possesses a strong tradition of education. The Department of Biology offers a Bachelor's Degree in multiple concentrations including aquaculture. No graduate degree is offered; although, two graduate courses exist that complement the graduate program in the School of Education. Students in the Aquaculture Concentration take Fish Biology (BIO 323), Introduction to Aquaculture (BIO 343), Advanced Aquaculture (BIO 403), an additional chemistry course Quantitative Analysis (CHE 321), and Introduction to

Business (BUS 170), beyond their core courses required in biology. Students are encouraged to participate in an Independent Study (BIO 408) or internships (Biology Internship, BIO 416; Aquaculture Internship, BIO 417; Marine Biology Internship, BIO 418) and Underwater Research Methods (BIO 344). Two graduate courses that target and attract primarily K-12 teachers are instructed by CCML faculty: Topics in Aquaculture (BIO 705) and Estuarine Ecology (BIO 706).



**Figure 2**

**The Cat Cove Team, 2015-2016 (left to right: Louis Logan, Alexa Fiorillo, Curtis Fahey, Mitch Tepe, Dr. Mark Fregeau, Devan Nichols, Scott Weston, Eliza Kessler, Dr. Joe Buttner, Jimmy Elliott). Missing from image is Dive Instructor, Ted Maney.**

While traditional coursework introduces students to the aquatic world; most students supplement these experiences with personalized, hands-on pursuits. Typically, 4-6 students are employed at CCML (Fig. 2). Many join CCML as freshmen or sophomores doing essential tasks that include washing glassware, feeding fish, cleaning tanks, and monitoring and managing water quality. By their junior and senior year, students become involved in externally funded projects and/or conduct personal research as Independent Studies or Internships. A wealth of projects have been pursued such as working with zebra fish at Children's Hospital, Boston, MA; investigating behavior of cephalopod mollusks at the Marine Biological Laboratory, Woods Hole, MA; developing more effective lobster baits; and characterization of sea cucumber toxins at the Institut de Recherche sur les Zones Côtières, Inc., Shippagan, NB. Five undergraduate students have published their findings (Buttner & Miller, 2001; Buttner & Cifuni, 2002; Buttner & Best, 2004; Buttner et al., 2007; Monteiro & Buttner, 2013), three have received best student presentation awards at professional meetings (Fig. 3), and several have written and received grants. Application of classroom experiences provides students with invaluable skills, maturity, and insight as they navigate their projects from conception through implementation to dissemination.



**Figure 3**

**Ms. Emily Crescenzi (with Drs. Brad Hubeny, Geology; Lt; Joe Buttner, Rt) by her award winning poster presentation Grain Size Characterization and Soft Shell Clam (*Mya arenaria*) Survival Rates in Selected Boston Harbor Tidal Flats at the 2009 New England Estuarine Research Society spring meeting.**

## Research

A variety of research initiatives are pursued by staff and students at CCML. Recent projects range from monitoring and enhancing eel grass (*Zostera marina*) and characterizing green crab populations (*Carcinus maenas*) on the North Shore of Massachusetts to expansion of pond aquaculture in Liberia and securing a dependable supply of potable water for orphanages in Morocco. While a spectrum of activities is pursued, three major aquaculture initiatives have been particularly important: production of softshell clams (*Mya arenaria*), longline culture of blue mussels (*Mytilus edulis*) in waters off Massachusetts, and development of modest-size aquaponics appropriate for the Northeastern United States and southern Canada.

**Figure 4**  
**Softshell clams**  
**produced by CCML**  
**facilitate sustainable**  
**aquaculture in urban**  
**areas, creating jobs,**  
**generating revenue,**  
**perpetuating a fishing**  
**tradition and working**  
**water front while**  
**improving water**  
**quality. As the tide**  
**returns, predator-**  
**exclusion nets that**  
**protect clams**  
**disappear beneath the**  
**waves within the**  
**shadow of Boston.**



Softshell clams are important recreational and commercial bivalve mollusks in Massachusetts, throughout New England, and the Canadian Maritimes (Fig. 4). To provide a dependable supply of juvenile clams for aquaculture, personnel at CCML initiated production of clams in 1999. Since then, culture protocols for softshell clams in nursery, hatchery, and grow out phases have been refined and made broadly available (Buttner et al., 2007, 2007a; Buttner et al., 2010; Weston & Buttner, 2010; Weston et al., 2010). Nearly 38 million clams have been produced and provided to over three dozen communities in Massachusetts and to people in other states and provinces (Table 1). Aquaculturists prefer pumpkin seed size spat (20 mm shell length, SL) though squash seed size is acceptable (10-15 mm SL) for grow out.



**Table 1. Number of clams produced annually by CCML since 2000.**

| <b>Year</b>  | <b>Number of Clams</b> |
|--------------|------------------------|
| 2000         | 30,000                 |
| 2001         | 200,000                |
| 2002         | 1,040,000              |
| 2003         | 1,540,000              |
| 2004         | 1,103,000              |
| 2005         | 2,900,000              |
| 2006         | 3,345,000              |
| 2007         | 3,799,500              |
| 2008         | 2,374,000              |
| 2009         | 3,357,500              |
| 2010         | 2,909,000              |
| 2011         | 4,080,000              |
| 2012         | 3,905,000              |
| 2013         | 2,947,000              |
| 2014         | 2,326,000              |
| 2015         | 2,011,000              |
| <b>Total</b> | <b>37,872,000</b>      |

Blue mussels are a commercially important aquaculture species in eastern Canada and Europe (FAO, 2014; Chopin, 2015). Production in the United States has been limited, due to social, regulatory, and other issues. Since 2007, CCML researchers have worked with industry and agencies to nurture sustainable blue mussel aquaculture in waters off Massachusetts. Recently, NEMAC secured an US Army Corps of Engineers (USACE) permit (NAE-2012-1598 NEMAC Mussel Culture) to establish a 13.4-ha commercial scale mussel farm site 13.7 km off the coast of Cape Ann, Massachusetts. Permitting conditions require gear be deployed coupled with extensive monitoring of environmental conditions. The site will serve as a fixed station to assess the feasibility of shellfish culture along the New England coast, to explore mussel vulnerability to climate change, to monitor interactions with protected pelagic species, to investigate fishery and habitat enhancement effects attributed to shellfish aquaculture. Mussel condition will be assessed using a physiological model that predicts mussel shell and meat growth along with reproductive output based on environmental input parameters of temperature and food availability (Sarà et al., 2011; Matzelle et al., 2014). Findings will be evaluated by USACE and NOAA to assess environmental impacts of aquaculture in offshore areas and to determine the potential for



site expansion/commercialization. This study will hopefully generate a protocol to assess and pursue other aquaculture projects in federal waters.

**Figure 5**  
**Greenhouse and solar array used to operate aquaponics system successfully into January 2016. The collaborative project involves 3 NGOs: *The Food Project, Full Circle Earth, The Trustees of Reservations*, the Massachusetts Department of Agricultural Resources and Division of Fisheries and Wildlife, CCML, and participants from high school students to pensioners.**



Aquaponics is a form of integrated multi-trophic aquaculture where wastes generated by fish in a recirculating aquaculture system are modified by bacteria and removed as nutrients by plants grown in a soilless medium. While pursued widely, aquaponics isn't well established locally. Acknowledging its potential, in 2014 a collaborative project initiated that constructed a greenhouse and employed solar power to support an aquaponics initiative (Fig. 5). The goal was to assemble a system with materials procurable from Walmart and Home Depot that can be used to train others, leading to the formation of small- to modest-scale operations that could link to form cooperatives. To date, the growing season has been extended by several months using bubble wrap insulation and primarily solar-powered DC systems. In late fall, batteries were recharged with a generator as daylight hours waned and overcast, rainy days became nested.

### **Outreach**

Our charge as scientists and a functional hatchery is to understand the needs of our clientele and provide them the means to succeed and prosper. As illustrated by the projects described in this paper, networking and outreach are essential pursuits at CCML. Dissemination of information through workshops, fact sheets, and hands-on training is facilitated by links with growers, industry, state and federal agencies, and the general public.

Since 2009, over 1,800 people toured the CCML and nearly 1,750 requests for aquatic assistance were received and addressed (Table 2). The vast majority of requests originated from Massachusetts, but queries came from across the United States. Over 130 K-12 school teachers have taken graduate courses instructed at CCML. These efforts provide taxpayers and citizens with factual, timely information so they can assess and pursue aquaculture with increased competence and confidence.

**Table 2. Tours of CCML and response to unsolicited queries of assistance are important charges. Requests for assistance originated primarily from Massachusetts but, queries arrived from across the United States CT, DC, DE, FL, GA, HI, MD, ME, MI, NC, NH, NJ, NY, OH, PA, RI, VA, WA), and other countries (Canada—Quebec, PEI, Ontario, New Brunswick, Alberta; Croatia, Germany, India, Israel, Liberia, Kenya, UK).**

| Academic Year and Subsequent<br>Summer | Individuals<br>Touring CCML | Responses to Requests<br>for Professional Assistance |
|--|-----------------------------|--|
| 2009-2010                              | 218                         | 221  |
| 2010-2011                              | 164                         | 273  |
| 2011-2012                              | 328                         | 305  |
| 2012-2013                              | 292                         | 216  |
| 2013-2014                              | 319                         | 274  |
| 2014-2015                              | 526                         | 356  |
| 2015-present                           | 108                         | 78   |

## Discussion

In less than two decades, CCML has positively impacted aquaculture and the aquatic environment locally and broadly. Alumni are working with finfish and shellfish in Massachusetts and beyond (e.g. DC, CA, CT, FL, NY, OR, VA, VT). Industries have been created and expanded. Knowledge and understanding have been generated, disseminated, and applied. The foundation for future efforts and contributions has been forged.

## Acknowledgements

Support from state and federal entities including Salem State University, Massachusetts Department of Agricultural Resources, Massachusetts Division of Marine Fisheries, Massachusetts Department of Fisheries and Wildlife, U.S. Department of Agriculture, and National Oceanic and Atmospheric Administration is acknowledged. Industry and educational institutions, both local and distant, have collaborated with projects and employed our graduates. Current students and successful alumni make our efforts possible and relevant.

## References

- Buttner, J.K. and J.T. Best, 2004. Fish handling in the classroom. *Aquaculture Magazine* 30(5): 8-15.
- Buttner, J.K. and R. Cifuni, 2002. Polyculture of striped bass and summer flounder in RASs. *Aquaculture Magazine* 28 (6): 36-38.
- Buttner, J.K., Fregeau, M., Weston, S. and McAneney, B., 2007. Overwintering and suspension growout of softshell clams (*Mya arenaria*) in Northeastern Massachusetts, pages 18-19 in *Atelier de Travail sur l'Elevage de la Mye Commune, Institut des Science de la Mer de Rimouski, 20 au 22 Avril 2005, I. Calderon (ed). Les Publications de la Direction de l'Innovation et des Technologies, Compte Rendu No. 32, Ministere de l'Agriculture, des Pecheries et de l'Alimentation du Quebec, Gaspé.*
- Buttner, J.K., Fregeau, M., Weston, S. and McAneney, B., 2007a. How to seed softshell clams: what works in Massachusetts, pages 28-29 in *Atelier de Travail sur l'Elevage de la Mye Commune, Institut des Science de la Mer de Rimouski, 20 au 22 Avril 2005, I. Calderon (ed). Les Publications de la Direction de l'Innovation et des Technologies, Compte Rendu No. 32, Ministere de l'Agriculture, des Pecheries et de l'Alimentation du Quebec, Gaspé.*
- Buttner, J.K. and Miller, M.S., 2001. Red hake: an alternative species for mariculture. *Aquaculture Magazine* 27(1): 45-48.
- Buttner, J.K., Pikora, K.G. and Leavitt, D., 2007. Augmenting the lobster catch: oyster aquaculture in modified lobster traps. *World Aquaculture* 38 (3) 26-29.
- Buttner, J.K., Weston, S. and Beal, B., 2010. Softshell clam culture: hatchery phase, broodstock care through seed production. NRAC Fact Sheet No. 202-2010. Northeastern Regional Aquaculture Center, University of Maryland, College Park, MD.
- Chopin, T., 2015. Marine aquaculture in Canada: well-established monocultures of finfish and shellfish and an emerging integrated multi-tropic aquaculture (IMTA) approach including seaweeds, other invertebrates, and microbial communities. *Fisheries* 40(1); 28-31.

FAO (Food and Agriculture Organization of the United Nations), 2014. The state of world fisheries and aquaculture: opportunities and challenges. Rome, Italy.

Matzelle, A., Montalto, V., Sarà, G., Zippay, M.L. and Helmuth, B., 2014. Dynamic energy budget model parameter estimation for the bivalve *Mytilus californianus*: application of the covariation method. *Journal of Sea Research* S1:105-110.

Monteiro, D. and Buttner, J.K., 2013. Maintenance and culture of paralarval longfin squid (*Doryteuthis (Loligo) Pealeii*) in a laboratory. *Journal of Shellfish Research*. 32(2): 347-350.

Sarà, G., Kearney, M. and Helmuth, B., 2011. Combining heat-transfer and energy budget models to predict thermal stress in Mediterranean intertidal mussels. *Chemistry and Ecology* 27:135-145.

Weston S. and Buttner, J.K., 2010. Softshell clam culture: nursery and growout phases through harvest. NRAC Fact Sheet No. 203-2010, Northeastern Regional Aquaculture Center, University of Maryland, College Park, MD. 8pp.

Weston, S., Buttner, J.K. and Beal, B., 2010. Softshell clam culture: basic biology and general culture considerations. NRAC Fact Sheet No. 201-2010, Northeastern Regional Aquaculture Center, University of Maryland, College Park, MD. 6pp.



## **A NEW HUNTSMAN FOR THE NEW MILLENNIUM: LINKING INDUSTRY WITH RESEARCH INNOVATION VIA A NEW NBIF RESEARCH CHAIR IN AQUATIC BIOSCIENCES**

**Duane Barker**

New Brunswick Innovation Research Chair in Aquatic  
Biosciences

Huntsman Marine Science Centre

St. Andrews, NB, Canada

E5B 2L7

(506)-529-1207 Fax (506)-529-1212

[Duane.Barker@huntsmanmarine.ca](mailto:Duane.Barker@huntsmanmarine.ca)

[www.huntsmanmarine.ca](http://www.huntsmanmarine.ca)

### **Huntsman in the beginning**

Founded in 1969, the Huntsman Marine Science Centre (Huntsman) is a private, not-for-profit, research and education facility located on the shores of picturesque St. Andrews, New Brunswick. In 1969, a consortium of 20 universities and several government departments came together to form the Huntsman Marine Laboratory. Drs. A.G. Huntsman and J. Anderson, two of Canada's most distinguished marine biologists, were the catalysts of this research and education facility. From the early beginning came the idea that a completely separate, federally incorporated, non-profit, registered, charitable organization should be established to become a "co-operative venture in learning" between governments and universities. The (now named) federal Department of Fisheries and Oceans provided the site at Brandy Cove, consisting of 20 acres of land and two residential buildings (the Ambridge Estate), which was contiguous with the Biological Station. The provincial government assisted with the purchase of another 50 acres (the Sir Thomas Tait Estate) including a large home, Anderson House, which now provides accommodation for visiting students.

Today, Huntsman's mission includes inspiring stewardship through engagement of academic, public, and commercial communities in the advancement of marine sciences. This is achieved through collaborative research and the development of innovative technical solutions for its public and private sector partners. The



Huntsman relies entirely on fee-for-service research and education grants and contracts to meet its operational budgetary requirements; it does not receive any core operational funds.

## **New Investment and New Opportunities**

The *Innovation Research Chair Initiative*, through the New Brunswick Innovation Foundation (**NBIF**), was established with the goal of promoting economic development in New Brunswick by combining researchers with industry to foster collaborative innovation and technology transfer. Each Chair receives \$1 million over 5 years (\$200,000 yr<sup>-1</sup>) which contributes to salary expenses, lab setup and consumables, plus enables leveraging of additional funds. On May 7, 2015, the NBIF officially announced that the Huntsman Marine Science Centre was awarded a prestigious New Brunswick Innovation Research Chair. The award to the Huntsman was one of four in 2015 and completes a group of seven awarded over the past two years. Obtaining the Chair was a highly competitive process for research institutes and universities, which included stages of a letter of intent, a full proposal, and a final presentation before a selection committee. The Huntsman has appointed Dr. Duane Barker (Fig. 1) to serve as the *New Brunswick Innovation Research Chair in Aquatic Biosciences*.

Dr. Barker, a native of Newfoundland and Labrador, has a long history with the Huntsman, beginning with his postdoctoral studies on diseases of flatfishes that were completed at Huntsman during 2000-2001. Dr. Barker returns to the East Coast from his most recent 10-year appointment at Vancouver Island University (VIU), bringing his hands-on research and teaching experience in aquaculture, aquatic animal health, and environmental assessment. Dr. Barker has over 25 years of experience in research and teaching in academia (Dalhousie University, Saint Mary's University, Marine Institute of Memorial University of Newfoundland, Vancouver Island University) and has supervised over 90 students during that time. As a researcher, he has been involved with numerous fish and shellfish species as part of work in marine



**Figure 1**  
**Dr. Duane Barker, a *New Brunswick Innovative Research Chair in Aquatic Biosciences*, is the newest addition to the research core at the modern Huntsman Marine Sciences Centre.**

and freshwater ecosystem health assessments, aquatic animal health surveys, and various fish health projects including pathogen challenges, disease modelling, plus vaccine, immunostimulant and therapeutic testing. Most recently, his research at VIU investigated the potential of sea lice as vectors of bacteria and viruses, plus examined the cellular and genetic immunity of various salmon species to sea lice. At Huntsman, Dr. Barker works directly with the *Aquatic Services* group, together with its existing staff of scientists, technicians, and associate scientists. He also provides support to Huntsman's *Education and Outreach* group to develop and deliver school, university, and continuing education programs. He will use the NBIF funding to support the sustainable development of industries and communities in New Brunswick that rely on our valuable aquatic resources. With the existing programs of environmental monitoring, broodstock development, and eco-toxicology at the Huntsman, the addition of this new fish health capacity gives the facility a broad breadth of customizable services to excel in a new role as a contract research organization (CRO).

More specifically, as a result of the new NBIF Chair, plans are to expand the research and development portfolio and corporate client base of Huntsman, encourage foreign direct investment (FDI), and augment the number of highly qualified personnel (HQP) and infrastructure in New Brunswick. Ultimately, the plan is to continue a legacy of research, development, and innovation at Huntsman and support a knowledge-based economy serving a global clientele.

### **If you build it...**

The Huntsman (Fig. 2) has a proven track record of implementing and managing significant scientific endeavours that meet client and funding objectives and budget/expenditure audits. The Huntsman salmon broodstock program has just completed a four-year research endeavour with new spin-offs such as commercialization of broodstock services and a proprietary sea-lice infection model. Additionally, as part of a four-year \$1.5M funding by the Environmental Science Research Fund (**ESRF**), Huntsman (with the new Chair) is engaged in various toxicology assessments evaluating the impact of oil (and dispersant) on early life stages of lobster, Atlantic cod, herring, and northern shrimp. On a similar theme, the Huntsman (with the new Chair) has secured federal funds from DFO's National Contaminants and Advisory Group (**NCAG**) to study the effects of diluted bitumen (or "dilbit" in the form it is used for transport via pipeline and train) on salmon eggs and alevins.

Currently, several projects are underway which are serving the research developmental needs of large international pharmaceutical companies as well as

emerging new entrepreneurs requiring proof of concept studies (names withheld due to confidentiality agreements). In the final stages of work are a sea-lice taxonomy project and a sea-urchin gonad evaluation and health assessment project. Huntsman has also recently begun innovative product testing that includes development work on new plant-based therapeutants for sea lice and *Saprolegnia*. In addition, the Research Chair has collaborated with DFO (St. Andrews Biological Station) and industry to seek funds for pre-commercialization studies on physical removal of sea lice using water sprayers. In the initial months of the NBIF Research Chair appointment at Huntsman, over \$100,000 in funds have been leveraged and new projects (> \$450,000) are forecast for 2016.

### New Research Initiatives

To complement his industry contract research, Dr. Barker is currently seeking collaborators to engage in potential multi-year aquaculture research projects including:

- Ecology and epidemiology of *Tenacibaculum* and *Moritella* (causative agents of mouth rot & skin ulcers)
- Risk assessment of pathogens of cleaner fish (cunners, lumpfish)
- Role of sea lice in potential transfer of ISAV and *Renibacterium*
- Pathogen exchange in integrated multi-trophic aquaculture (IMTA) systems
- Climate change impacts on fish health

As with all Huntsman activities, Dr. Barker will collaborate extensively with the world-class cluster of expertise that exists in New Brunswick and the regional universities, government departments and agencies, industries, and communities. All aquatic sector industries, key to the prosperity of New Brunswick, have development needs that can now be met through the controlled laboratory exposure research provided by the Huntsman and its NBIF Research Chair. *Come visit us and see what we can do for you.* [www.huntsmanmarine.ca](http://www.huntsmanmarine.ca)



**Figure 2**

Some of Huntsman's facilities for holding aquatic animals (with saltwater or freshwater capabilities). All tank designs are customizable to the client's research design needs and eight buildings are dedicated to research projects.



## REDUCTION OF TOTAL SUSPENDED SOLIDS AND PHOSPHORUS CONCENTRATIONS IN SALMON-SMOLT HATCHERY EFFLUENT USING CHEMICAL TREATMENT

Lionel T. Hayter<sup>\*1</sup>, Marc Sorensen<sup>2</sup>, Michel Couturier<sup>3</sup>

<sup>1</sup> Chemical Engineering Department, University of New Brunswick, Fredericton, NB; [lionel@soreng.ca](mailto:lionel@soreng.ca)

<sup>2</sup> Sorensen Engineering, St. Andrews, NB; [marc@soreng.ca](mailto:marc@soreng.ca)

<sup>3</sup> Chemical Engineering Department, University of New Brunswick, Fredericton, NB; [cout@unb.ca](mailto:cout@unb.ca)

\* Corresponding Author

### Abstract

The use of coagulation and flocculation in the treatment of the effluent of a salmon-smolt producing site, containing multiple recirculating aquaculture systems was found to be a viable option to reduce total suspended solids and phosphorus concentrations to within governmental regulations for freshwater discharge. Using a ferric sulphate coagulant and anionic polyacrylamide polymer flocculant, solid and phosphorus removal efficiencies of 98% and 81% were achieved, respectively.

### Introduction

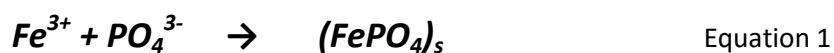
Intensive recirculating aquaculture systems (RAS) produce a low volume, concentrated effluent that requires treatment to remove contaminants prior to discharge into receiving waters. Increasingly stringent environmental regulations have necessitated more intensive effluent treatment at some locations. The key to successful effluent treatment is selection of a combination of methods that best reduces environmental impacts while minimizing capital and operating costs. Two important parameters of effluent discharge to fresh water are solids and phosphorus, as they can lead to eutrophication of receiving waters.

A significant portion of effluent solids from RAS can be smaller than 60  $\mu\text{m}$ . These fine particles are negatively charged causing repulsion leading to long settling times. This charge can be reduced or eliminated using coagulation and



flocculation. The coagulant destabilizes the electrostatic charge, allowing the particles to agglomerate, and the flocculant fosters floc formation, increasing sedimentation velocity (Tzoupanos and Zouboulis, 2008).

When multivalent metal salts are used as coagulants, phosphorus concentrations are also reduced. Phosphorus is precipitated with metal salts such as ferric/ferrous sulphate and aluminum sulphate via equation 1.



A specific coagulant and flocculant may behave differently depending on the water quality with special consideration given to pH, solids concentration, salinity, and temperature (Ebeling et al., 2005; Rich, 2005). The combined dosage of the coagulant and flocculant must be optimized for effective effluent treatment.

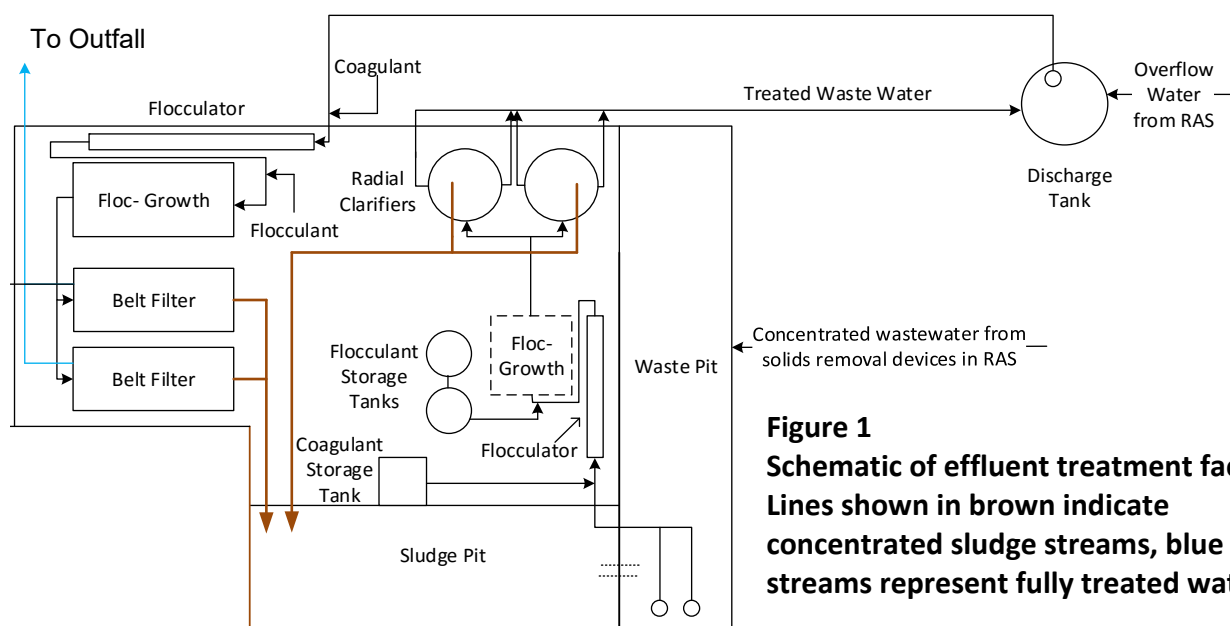
The objective of this study was to determine the viability of using chemical treatment to reduce fine solids and phosphorus concentrations in the effluent of a salmon-rearing hatchery.

## Methods

Testing was conducted on the effluent treatment system of a salmon-smolt production site in Charlotte County, NB. A schematic of the effluent treatment facility can be seen in Figure 1. The facility was designed to reduce the effluent concentrations of TSS and phosphorus to within freshwater governmental limits of 35 µg/L of phosphorus, 100 m downstream, with in this case a stream dilution of 5. A ferric sulphate blend coagulant, Eclipse 803, and a high molecular weight anionic polyacrylamide flocculant, Eclipse 864, from Buckman Laboratories International were used.

Concentrated wastewater from solids removal equipment in the RAS is sent to a waste pit acting as a flow dampener. The wastewater entering the pit has a total suspended solids (TSS) concentration between 100 and 4000 mg/L, phosphorus concentration of  $2.0 \pm 0.9$  mg/L, and a variable flow depending on site activity. The pit, except around the transfer pumps, is aerated to prevent sedimentation and anoxic conditions. Transfer pumps meter water from the waste pit to a flocculator where the ferric sulphate blend is injected. The flocculator is a long tortuous pipe which serves as a rapid mixing zone to ensure good coagulant/particle interaction. The flocculator flow velocity ranges between 0.6 and 0.9 m/s, with retention times of 120 to 90 s.





Polymeric flocculant is added to the water leaving the flocculator before it enters a floc growth chamber containing a slow mixer intended to allow particles to agglomerate. The water is then split between two radial flow separators (RFS), each with a surface load up to  $0.0008 \text{ m}^3/\text{s}$  per  $\text{m}^2$ . From the RFS, this stream joins the overflow water. Solids collected in the RFS are periodically discharged into a sludge pit and transported by truck for final disposal.

The ratio of treated concentrated effluent to overflow water entering the cylindrical discharge tank is up to 1:2.3. The combined stream has a TSS concentration less than 10 mg/L and a dissolved phosphorus concentration of  $0.82 \pm 0.36$  mg/L. Transfer pumps meter water from the discharge tank through a flocculator and floc growth chamber combination similar to that used to treat the concentrated effluent with a total residence time of 12.6 minutes. The water then passes through two 200-300  $\mu\text{m}$  belt filters before discharging into a nearby stream. The solids collected by the belt filters are sent to the sludge pit.

Coagulant and flocculant addition is controlled using metering pumps, modulated by the transfer pumps' variable frequency drives. Optimal doses of ferric sulphate and polymer for the concentrated waste water was determined by adjusting the dosage and waiting approximately 30 minutes for the system to equilibrate. If the outlet of the RFS was visibly clear, the dose was recorded as an effective solids removal dose. Periodically, 0.5-L samples of the RFS' discharge were analyzed to quantify solids and phosphorus removal efficiencies.

TSS and phosphorus removal efficiencies were determined using methods adapted from jar testing standards ASTM D-2035 (Ebeling et al., 2003). A series of untreated waste samples was placed in 1-L glass beakers. Magnetic stirrers were added to the samples and placed on mixing plates and the effluent treatment process was simulated. TSS and phosphorus concentrations were determined following EPA method 160.2 (EPA, 1971) and Hach Tests for Reactive Phosphorus (Hach, 2014), respectively. The TSS and phosphorus concentrations of the initial samples were used as baselines to determine solids and phosphorus removal efficiencies.

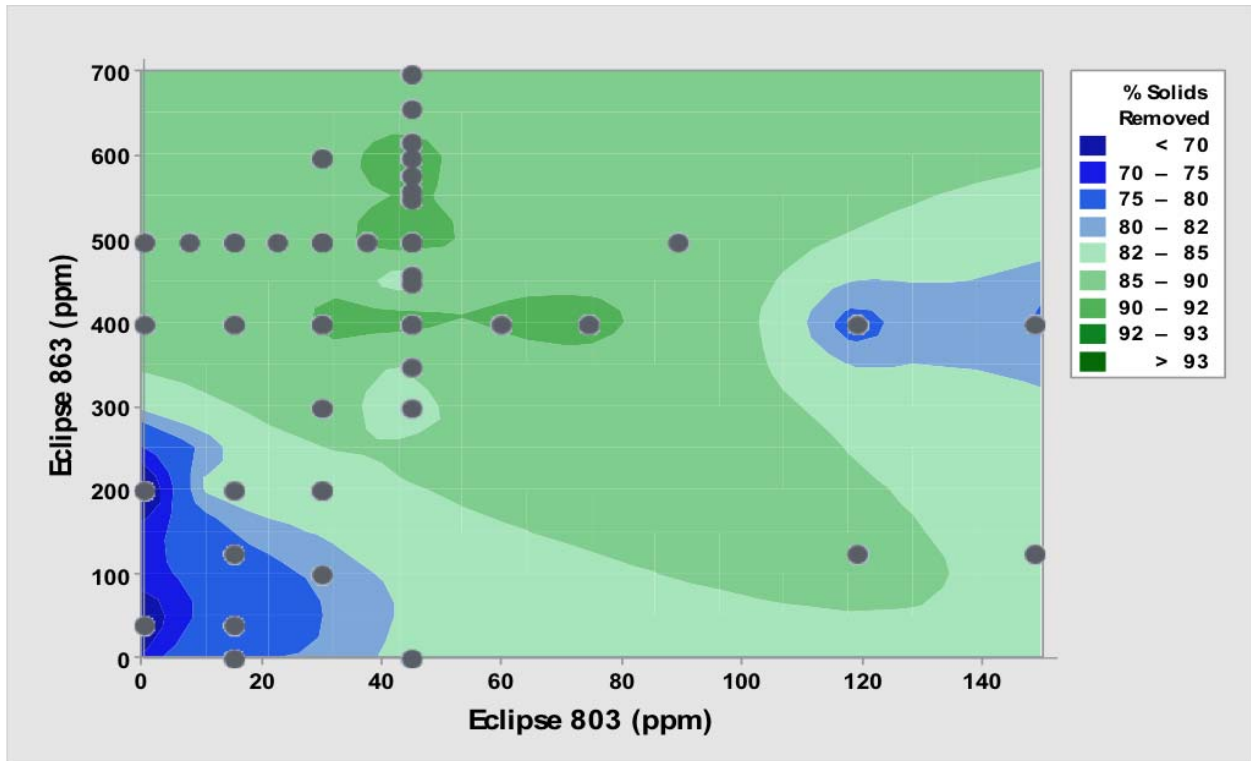
## Results

Jar tests produced a contour map of solids removal efficiency as a function of Eclipse 864 (polymer flocculant) and Eclipse 803 (ferric sulphate coagulant) dosage for samples with TSS concentrations of 200-350 mg/L (Fig. 2). This indicates that there is a “sweet spot” for chemical dosage. Too little or too much chemical addition results in decreased solids removal efficiency. The contour plot was generated using an interpolation technique in Minitab with data points overlaid; the areas of the contour plot without points are approximations from the software. Small changes, as low as 22 ppm, from the optimal dose of coagulant resulted in significantly reduced water quality. The results suggest that coagulant dose is most influential on solids removal efficiency.

Optimal dosing on the concentrated waste stream resulted in solids removal efficiencies of 89-98% and outlet TSS concentrations of 6.7-30 mg/L for the full scale system (Table 1) with similar chemical doses to those obtained from jar tests. Since the coagulant is adsorbed by the waste particles to facilitate charge neutralization or charge reversal (Dentel 1988), increased TSS concentrations should require higher doses of coagulant. This was observed as treatment of concentrated wastewater (4000 mg/L) required 150 ppm coagulant whereas more dilute wastewater (200-350 mg/L) required 30-60 ppm coagulant.

Significant phosphorus removal was observed in both concentrated waste and discharge streams, removing  $86\pm 7\%$  of the phosphorus from the concentrated waste stream and  $79\pm 7\%$  from the discharge stream, yielding an overall phosphorus removal efficiency of  $81\pm 7\%$  (Table 2).

Phosphorus removal was apparently affected by the pH of the water. The highest phosphorus removal efficiencies of 95% were observed when the pH was 7.4, which is in agreement with research conducted by Rich (2005) for ferric sulphate. Proper maintenance of pH levels in the effluent treatment facility would improve phosphorus removal efficiency.



**Figure 2**

Contour map from jar testing depicting solids removal efficiency as a function of Eclipse 864 (flocculant) and Eclipse 803 (coagulant) dosage. Samples had TSS concentrations ranging from 200-350 mg/L.

**Table 1. Solids removal efficiencies and outlet concentrations for the concentrated waste stream. Samples denoted with an asterisk (\*) represent those taken after swirl separator cleanings.**

| Initial TSS of Effluent (mg/L) | TSS after Treatment (mg/L) | Solids Removal Efficiency |
|--------------------------------|----------------------------|---------------------------|
| 506.7                          | 30.0                       | 94%                       |
| 126.7                          | 14.1                       | 89%                       |
| 205.7                          | 18.3                       | 91%                       |
| 245.0                          | 6.7                        | 97%                       |
| 1160.0*                        | 26.7                       | 98%                       |
| 632.0*                         | 12.0                       | 98%                       |
| 106.7                          | 8.0                        | 93%                       |

**Table 2. Phosphorus removal efficiencies and outlet concentrations.**

| Date   | Concentrated Waste          |                 | Discharge                   |                 | Overall         |
|--------|-----------------------------|-----------------|-----------------------------|-----------------|-----------------|
|        | Outlet Concentration (mg/L) | Percent Removal | Outlet Concentration (mg/L) | Percent Removal | Percent Removal |
| 01-Oct | 1.07                        | 74%             | 0.21                        | 75%             | 75%             |
| 14-Oct | 0.19                        | 83%             | 0.23                        | 72%             | 76%             |
| 06-Nov | 0.56                        | 94%             | 0.17                        | 88%             | 90%             |
| 18-Nov | 0.33                        | 82%             | 0.2                         | 82%             | 82%             |

## Discussion

Chemical treatment was found to be a viable option for bringing hatchery effluent TSS and phosphorus concentrations to within governmental regulations. Maintaining the proper chemical dosage is complicated by the varying effluent flows and TSS concentrations from the salmon-smolt production site. Varying phosphorus and TSS concentrations and pH were found to affect the optimal chemical dose such that three different chemical doses were found necessary to maintain optimal efficiency: the first for typical conditions (drum filter backwash), the second for use during swirl separator cleaning, and the third for use during static filter cleaning. These different chemical doses are managed manually by site staff.

To minimize operating costs, the flow of wastewater should be minimized and flow fluctuations eliminated. Reductions of flow can be achieved by optimizing drum filter backwashing time and frequency and by minimizing the volume of water lost during swirl separator and static filter cleanings. Flow fluctuation can be managed using a buffer tank (e.g. the waste pit in this study). Pump selection is also important, the original waste transfer and chemical dosing pumps had to be changed to different models. The original waste transfer pumps (Goulds GT Self-priming) were not able to handle larger solids such as biocarrier media and so were replaced with Barnes submersible pumps.

A similar effluent treatment facility was described by Wolters et al. (2009) with several important differences. In Wolters' effluent treatment facility, alum was used as the flocculant, a flocculation chamber with a mixer was used to mix the coagulant, belt filters were used to capture flocculated solids in the concentrated stream, and the facility handled a lower flow. Replacing the mechanical mixer and floc growth chamber with a flocculator and RFS was found

to be advantageous. Neither unit has moving parts, simplifying maintenance and eliminating solids settling during floc formation.

During testing, it was observed that the transfer from floc-growth chamber to RFS reduced floc size. The chamber in the concentrated waste stream treatment section was removed as it was suspected that the RFS could provide the gentle mixing zone and eliminate this problem. This simplified process maintained removal efficiencies. The floc-growth chamber for discharge water was left in place to prevent floc formation after belt filtration.

Establishing and implementing best practices is an intensive process which, based on the studied site, requires more than a year of continual involvement. Further work is being completed to optimize the volume of sludge produced. This includes testing RFS with 60° base cones (instead of 45°) and using diaphragm pumps, rather than gravity, for sludge removal. Preliminary results for both are promising.

### **Acknowledgements**

We wish to thank Cooke Aquaculture Inc. for their support and funding for this research as well as the Cooke Aquaculture staff with special thanks to Mitchell Dickie. We also wish to thank the MITACS Accelerate program for funding.

### **References**

Dentel, S.K., 1988. Application of the precipitation-charge neutralization of coagulation. *Environmental Science Technology* 22 (7): 825-832.

Ebeling, J.M., Rishel, K.L. and Sibrell, P.L., 2005. Screening and evaluation of polymers as flocculation aids for the treatment of aquaculture effluents. *Aquaculture Engineering*, February 2: 235-249.

Ebeling, J.M., Sibrell, P.L., Ogden, S.R. and Summerfelt, S.T., 2003. Evaluation of chemical coagulation-flocculation aids for the removal of suspended solids and phosphorus from intensive recirculating aquaculture effluent discharge. *Aquaculture Engineering*, April 28: 23-42.

EPA, 1971. EPA Method 160.2. caslab.com. Accessed August 21, 2015. <http://www.caslab.com/EPA-Methods/PDF/EPA-Method-160-2.pdf>.



Government of New Brunswick, 2013. Environmental management program for land based finfish aquaculture in New Brunswick. Government of New Brunswick. October. Accessed February 2015. <http://www2.gnb.ca/content/dam/gnb/Departments/env/pdf/MarineAquaculture-AquacoleMarin/EnvironmentalManagementProgramLandBasedFinfish.pdf>.

Hach, 2014. Phosphorus (reactive) TNT reagent set, low range. Hach. January. Accessed October 24, 2015. <http://www.hach.com/phosphorus-reactive-tnt-reagent-set-lowrange/product?id=7640196016>.

Rich, D., 2005. The removal of total phosphorus from natural waters by precipitation. Northwestern International University.

Tzoupanos, N.D. and Zouboulis, A.I., 2008. Coagulation-flocculation processes in water/wastewater treatment: the application of new generation of chemical reagents. IASME/WSEAS. Rhodes. 309-317.

Wolters, W., Masters, A., Vinci, B. and Summerfelt, S., 2009. Design, loading, and water quality in recirculating systems for Atlantic Salmon (*Salmo salar*) at the USDA ARS National Cold Water Marine Aquaculture Center (Franklin, Maine). Aquaculture Engineering 60-70.



## MACROALGAL AND SULFUR DENITRIFICATION AS EFFICIENT BIOFILTERS OF DISSOLVED INORGANIC NUTRIENTS? A CASE STUDY AT THE BIODÔME DE MONTRÉAL

A. Tremblay-Gratton<sup>1,2</sup>, E. Tamigneaux<sup>3</sup>, J-C. Boussin<sup>1</sup>, G.W. Vandenberg<sup>2</sup> and N.R. Le François<sup>1\*</sup>

<sup>1</sup> Division des collections vivantes et de la recherche, Biodôme de Montréal, 4777 Avenue Pierre-de Coubertin, Montréal, QC, H1V 1B3

<sup>2</sup> Département des sciences animales, Université Laval, 2425 Rue de l'Agriculture, Québec, QC, G1V 0A6

<sup>3</sup> École des pêches et de l'aquaculture du Québec, 167 La Grande-Allée Est, Grande-Rivière, QC, G0C 1V0

\*Corresponding author: Nathalie Rose Le François

### Introduction

In closed aquatic systems, such as the Biodôme de Montréal, accumulation of organic wastes such as feces and uneaten food is a major factor affecting water quality. Water quality is assured by controlling different parameters; adequate level of total suspended solids concentration (TSS) is obtained using mechanical filtration as well as ozonation, while classical nitrification explains the significant reduction of ammonia concentration within the ecosystem. The control of high nutrient concentrations such as nitrate ( $\text{NO}_3^-$ ), and phosphate ( $\text{PO}_4^{3-}$ ) is of great importance since high  $\text{NO}_3^-$  concentration, in particular, can become a major source of stress to aquatic organisms, while high  $\text{PO}_4^{3-}$  concentration will favor unwanted phytoplanktonic blooms (Vidal et al., 2002). Since water renewal for a 2 500-m<sup>3</sup> tank is a costly operation, denitrification by bacteria or plants is the only other option to remove excess  $\text{NO}_3^-$ . With the aim to control  $\text{NO}_3^-$  concentration, heterotrophic denitrification was evaluated at the Biodôme de Montréal from 2000-2008, but abandoned after technology transfer failed, due to complexity of operation and unstable operation conditions. Therefore, there was a need to implement a more user-friendly technology. The Biodôme is currently operating a pilot-scale autotrophic sulfur denitrification (**ASD**) system, based on the activity of the bacteria *Thiobacillus denitrificans*. Many

configurations have been tested that successfully improved the efficiency of  $\text{NO}_3^-$  removal and will be discussed here. Furthermore, more in line with the Biodôme de Montréal's mission, EDUCATION, CONSERVATION & RESEARCH, an alternative strategy, macroalgal biofiltration (MAB), is currently being evaluated (M.Sc. project supported by MITACS and Société des Amis du Biodôme de Montréal) and will also be presented along with preliminary results. Seaweeds have the power to remove both  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ , in addition to carbon dioxide ( $\text{CO}_2$ ) while producing oxygen ( $\text{O}_2$ ) (Neori et al., 2004).

### Nitrate concentrations issues in the Gulf of Saint-Lawrence Ecosystem

The aquatic exhibits at the Biodôme de Montréal include a 2 500- $\text{m}^3$  marine aquarium representing the Gulf of Saint-Lawrence ecosystem, referred to as La Baie, and a smaller habitat of 25  $\text{m}^3$ , the Rocky Coastline, both held at 24 PSU (practical salinity units). While the first one is home to numerous species of fish (sturgeon, cod, wolffish, skate, etc.) and birds and maintained at a temperature of 9-10°C, the second contains mostly invertebrates like anemones, sea urchins, and sea cucumbers and is kept at a colder temperature (i.e. 5-6°C).

Historically, before 2009,  $\text{N-NO}_3$  concentrations in La Baie and the Rocky coastline regularly reached 150 mg/L and 100 mg/L, respectively. In natural marine habitats,  $\text{N-NO}_3$  level rarely reach 1 mg/L (Yeats, 1990) and a maximum concentration of 20 mg/L should not be exceeded to ensure the marine fauna's health and well-being (Camargo et al., 2005). To solve this issue, the Biodôme de Montréal now performs 30-40% water renewal more frequently. Currently,  $\text{N-NO}_3$  concentrations are kept under the threshold value of 60 mg/L. However, the costs related to the use of artificial saltwater formulation are prohibitive and there is still a need to find an alternative solution in order to reach  $\text{N-NO}_3$  concentrations under 30 mg/L.

In 2012, the Biodôme research staff connected a pilot scale **ASD** on the rocky coastline exhibit. Between 2012 and 2015, a series of tests were required to improve nitrate removal efficiency of the technology under our specific environmental conditions (i.e. cold seawater at 24 PSU). The optimum operating conditions for **ASD** are known to be 25-35°C and pH 7.0-8.0, and it is more efficient in fresh water. Indeed, denitrification quality starts to decline when salinity rises (Koenig & Liu, 2004). Claus & Kutzner (1985) mentioned that the inhibitory effect of salinity on denitrification rate start to show at a concentration of 20 PSU. Our efforts were concentrated on the maximization of the flow rate and the stratification patterns (sulfur-limestone) that would translate in increased removal efficiency.

## Autotrophic sulfur denitrification

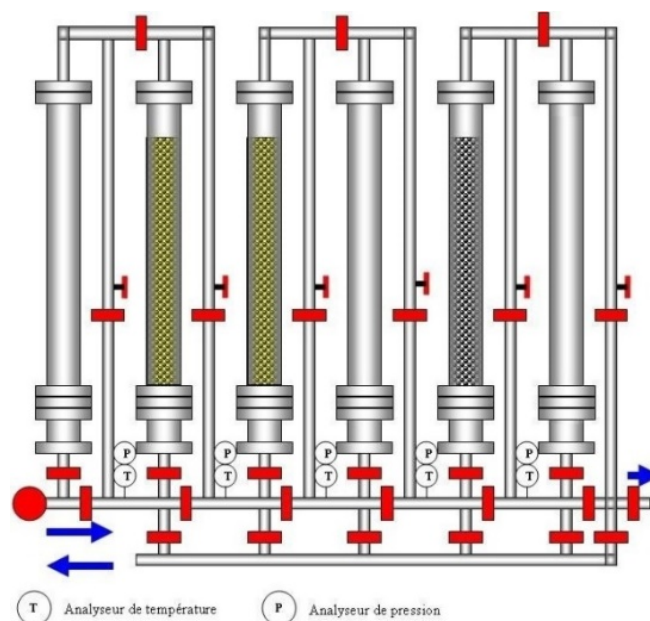
Autotrophic sulphur denitrification is based on the denitrifying activity of *Thiobacillus denitrificans*, an autotrophic bacteria that requires an anaerobic environment to remove nitrate from its habitat. The bacteria will bind to a sulfur-containing substrate and use it as a source of electron acceptor to achieve an oxidation-reduction reaction. From there, nitrate will be reduced to nitrite ( $\text{NO}_2$ ), which will be reduced to gaseous nitrogen ( $\text{N}_2$ ). As a result, sulfur will be oxidized to sulfate ( $\text{SO}_4^{2-}$ ), leading to water acidification that will impair the bacteria's effectiveness. The addition of a calcareous substrate (e.g. limestone) in the system is used to increase the water pH after the reaction to near neutrality.

Autotrophic sulphur denitrification has been used in some public aquaria mainly the Musée des Arts d'Afrique et d'Océanie (MAAO), now known as the Aquarium public de la Porte Dorée in Paris, France. This aquarium proposed an elaborate overview of the system operations in Hignette et al. (1997). A high yield of  $1.5 \text{ kg N-NO}_3 \text{ m}^{-3} \text{ d}^{-1}$  has been observed but environmental conditions were quite different of those of the Biodôme de Montréal (i.e. hot temperature of 25-30°C and low  $\text{N-NO}_3$  concentrations of 2.5 mg /L). The Aquarium du Québec also uses this technology to treat the water of a 350- $\text{m}^3$  exhibit, characterized by a salinity of 28 PSU and a temperature of 12°C. It has been demonstrated that almost all of the nitrates (25 to 30 mg  $\text{N-NO}_3 \text{ L}^{-1}$ ) could be removed from the water after a hydraulic retention time of 16-19 hours (Simard et al., 2015).

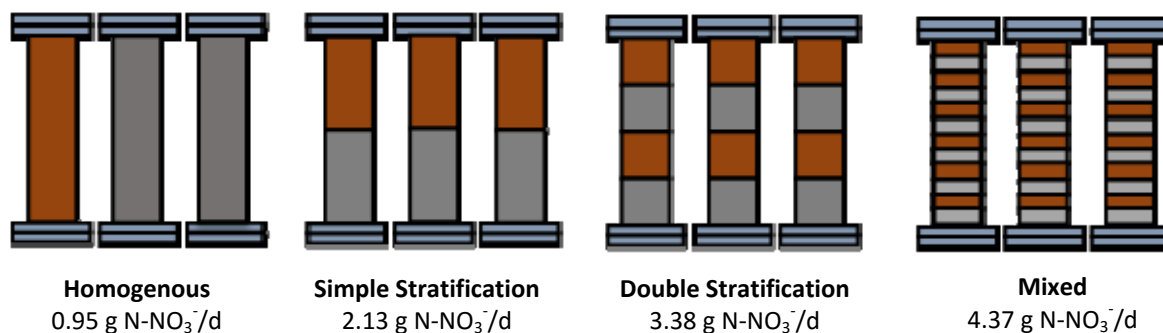
Since the conditions to favour **ASD** were clearly not met in the Rocky Coastline habitat (too salty and too cold), the following improvements were considered and evaluated: water flow rate, water pre-treatment (microfiltration, oxygen removal) quality and size of the substrate and stratification of the different substrates.

The pilot-scale ASD on the Rocky Coastline holds six 47.1-L columns offering flexibility of use. Our first trials have been to operate three 47.1-L columns, filled with homogenous substrate. The first two filled with elemental sulphur 99.5% (Tiger-sul company, particle size 260 SGN), the third with a source of substrate buffer, oyster shells (Fig. 1). The water, loaded with nitrates, passed through each column and left the denitrification loop at significantly reduced concentration of nitrates. Nitrate removal efficiency varied depending on the way the system was operated but noticeably, pressure build-up in the columns provided better operation temperature since incoming water was at 5-6°C and left the system at 13-14°C.

A smaller ASD unit (experimental scale) with three 3.7-L columns (12.7 × less volume than the pilot-scale ASD) was built to accelerate the stratification and substrate evaluations (Fig. 2). After optimizing the size and the quality of sulfur and calcareous substrates employed (identification of best substrate), stratification sequence in the system was next evaluated. First, homogenous substrate columns were evaluated (configuration no.1) then simple stratification, allowing both sulfur and calcareous substrate to be present in the same column (50:50). This new organization of the columns was based on the hypothesis that water pH would be more stable over the pathway, leading to better nitrate removal efficiency. Indeed, the nitrate removal efficiency more than doubled using simple stratification. Configuration no. 3, double stratification, led to configuration no. 4, mixed stratification (12 layers), which is now currently being evaluated at a smaller scale and should be implemented at the pilot-scale. Preliminary data is promising and plans to build a full-scale ASD connected to the La Baie exhibit are underway.



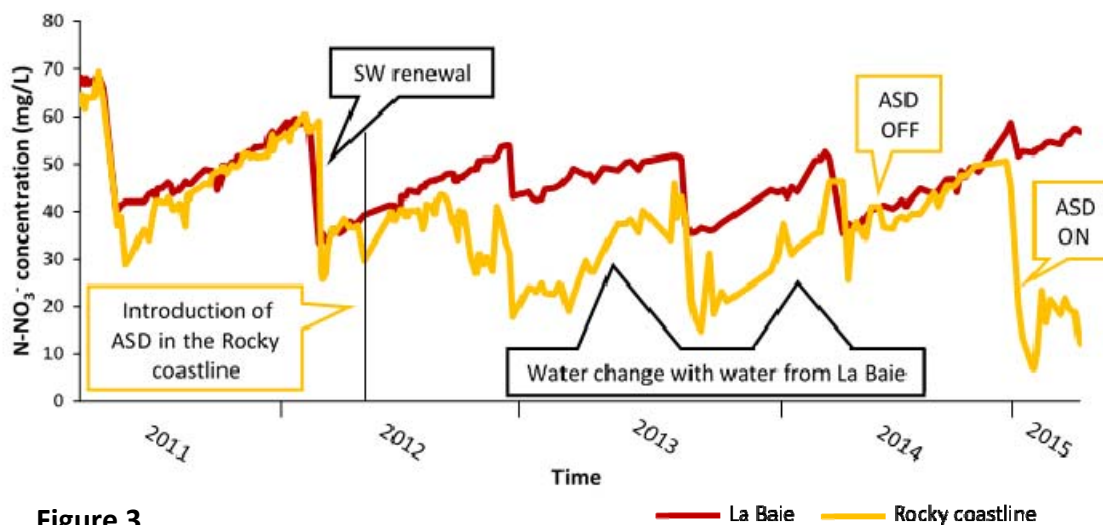
**Figure 1**  
Pilot-scale autotrophic sulfur denitrification unit: Configuration no. 1: 2 sulfur and 1 oyster shell, uniform packed columns.



**Figure 2**  
Maximum removal rate observed for each stratification test with the experimental denitrator. Grey: limestone, brown: sulfur.

Figure 3 highlights the impact of ASD technology implementation and our progress in improving its efficiency on the Rocky Coastline exhibit in comparison with the La Baie exhibit (no ASD).





**Figure 3**  
Variation of N-NO<sub>3</sub><sup>-</sup> concentrations in La Baie and the Rocky Coastline between 2011 and 2015.

### Macroalgal biofiltration project

Seaweeds unlike **ASD**, may contribute to remove both NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup> from the aquatic environment, as they use dissolved nutrients for the production of added-value plant biomass. A macroalgal biofiltration (**MAB**) unit would be an interesting complementary approach to the water treatment loop of the seawater aquatic exhibits. These marine plants produce oxygen through photosynthesis and might also have antimicrobial properties that could contribute naturally to the health of the aquatic exhibits.

**Phase 1.** The first phase of this project is to evaluate an effective macroalgal biofilter adapted to the conditions characterizing the marine ecosystem of the Biodôme de Montréal. A lead project entitled “Evaluation of the denitrification and dephosphatation performance of two native species of macroalgae, *Ulva lactuca* and *Palmaria palmata*”, is currently being conducted under the following operating conditions: salinity of 30 PSU at respective densities of 3 g/L for *U. lactuca* and 7 g/L for *P. palmata*, in replicates at two temperatures 5-6°C and 9-10°C and supplemented at 3 different N-NO<sub>3</sub><sup>-</sup>: P-PO<sub>4</sub><sup>3-</sup> concentrations: 40:6, 50:7.5, 60:9 mg/L.

*P. palmata*, or dulse, is an elegant red seaweed, interesting for its large surface to volume ratio and its ability to grow rapidly in cold water. Its biofiltration capacities are recognized in many scientific studies (e.g. Corey et al. 2014; Titlyanov & Titlyanova, 2010). *U. lactuca*, or sea lettuce, is part of Ulvaceae, a family of fast growing green seaweeds responsible for large green tides in China

and which thrives in nutrient-rich environments (Fletcher, 1996). Experimental phases of the project were performed at École des Pêches et de l'Aquaculture du Québec in Grande-Rivière, Quebec. Based on preliminary results of summer 2014, 225 kg of *P. palmata* could remove 50 g NO<sub>3</sub><sup>-</sup> d<sup>-1</sup>. This efficiency is being met with 100 kg of sulfur substrate based on our adaptation of the **ASD** technology. Recommendations based on the pros and cons of using MAB vs ASD in a biofiltration system will be made (Table 1 and 2).

**Table 1 Pros and cons of autotrophic sulfur denitrification.**

| Pros to ASD   | Cons to ASD   |
|---|---|
| <ul style="list-style-type: none"> <li>• Relatively easy to operate</li> <li>• Important yield of 1.5 kg NO<sub>3</sub> m<sup>-3</sup> d<sup>-1</sup> (Hignette et al., 1996)</li> <li>• Have shown its effectiveness in the Rocky Coastline exhibit</li> </ul> | <ul style="list-style-type: none"> <li>• No reusable by-product</li> <li>• 100% bioengineering approach</li> <li>• Production of sulphates (unknown effects on aquatic life)</li> <li>• No PO<sub>4</sub><sup>3-</sup> removal</li> </ul> |

**Table 2 Pros and cons of macroalgal biofiltration.**

| Pros to MAB  | Cons to MAB  |
|--|--|
| <ul style="list-style-type: none"> <li>• Removal of NO<sub>3</sub><sup>-</sup> as well as PO<sub>4</sub><sup>3-</sup></li> <li>• Net oxygen production</li> <li>• Production of reusable biomass to be used as fertilizer in horticulture</li> <li>• Can be used as a complementary approach to <b>ASD</b></li> <li>• Potential antimicrobial activities</li> <li>• Interest of the public for sustainable-green technologies</li> </ul> | <ul style="list-style-type: none"> <li>• Need more space than <b>ASD</b></li> <li>• Frequency of gamete or spore release unknown in stable environmental conditions (long-term)</li> <li>• Requires more manpower to operate than <b>ASD</b> (epiphyte removal, supplementation with micronutrient-rich solution is needed in RAS with ozonation and/or artificial saltwater)</li> </ul> |

**Phase 2:** Enrichment of the ecosystem of the Rocky Coastline by adding live seaweeds in the exhibit. Trials with rope-cultured and wild-collected macroalgae are actually being conducted at the Biodôme de Montréal. Trials with two brown algae, sugar kelp (*Laminaria latissima*) and sea colander (*Agarum clathratum*) are also planned. Sugar kelp was selected for its large and majestic frond and colander for its known resistance to urchin grazing activities.

**Phase 3:** In collaboration with the Jardin Botanique de Montréal (<http://espacepourlavie.ca/en/botanical-garden>) and OrganicOcean Inc. ([www.organicocean.ca](http://www.organicocean.ca)), a private company specialized in the development and manufacturing of innovative marine products for plant nutrition. Trials are being conducted to assess the stimulation effect of different macroalgal extracts on

the root growth stimulation of fast growing species (*Salix sp.* and *Medicago sp.*) used in phytoremediation.

## References

Camargo, J.A., Alonso, A. and Salamanca, A., 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere* 58: 1255 – 1267.

Corey, P., Kim, J.K., Duston, J. and Garbary, D.J., 2014. Growth and nutrient uptake by *Palmaria palmata* integrated with Atlantic halibut in a land-based aquaculture system. *Algae* 29: 35 – 45.

Claus, G. and Kutzner, H.J., 1985. Physiology and kinetics of autotrophic denitrification by *Thiobacillus denitrificans*. *Appl. Microbiol. Biotechnol* 22: 283 – 288.

Fletcher, R.L., 1996. The occurrence of “green tides” – a review. In Schramm, W. and Nienhuis, P.H., eds. *Marine benthic vegetation in Europe: recent changes and the effect of eutrophication*. Springer, Heidelberg, Germany. pp. 7 – 43.

Hignette, M., Lamort, B., Langouet, M., Leroy, S. and Martin, G., 1997. Élimination des nitrates par filtration biologique autotrophe sur soufre en aquariologie marine. Congrès E.U.A.C., Munich, Salzbourg & Innsbruck 1996. *Mém. Inst. Océano. P. Ricard* 1997, pp: 7 – 13.

Koenig, A. and Liu, L., 2004. Autotrophic denitrification of high-salinity wastewater using elemental sulfur: batch tests. *Water Environment Research* 76: 37 – 46.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Jraemer, G.P., Halling, C., Shpigel, M. and Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture* 231: 361 – 391.

Simard, M., Masson, S., Mercier, G., Benmoussa, H., Blais, J., Asce, M. and Coudert, L., 2015. Autotrophic denitrification using elemental sulfur to remove nitrate from saline aquarium waters. *Journal of Environmental Engineering* 141: 04015037.

Tremblay-Gratton, A., Tamigneaux, E., Vandenberg, G. and Rose Le François, N., 2015. Évaluation de l'efficacité d'un biofiltre à algues marines pour l'enlèvement de  $\text{NO}_3^-$  et  $\text{PO}_4^{3-}$  de l'eau de l'écosystème caractérisant le Golfe Saint-Laurent au Biodôme de Montréal. MSc project Département des sciences animales, Université Laval.

Titlyanov, E.A., and Titlyanova, T.V., 2010. Seaweed cultivation: methods and problems. *Russian Journal of Marine Biology* 36: 227 – 242.

Vidal, S., Rocha, C. and Galvão, H., 2002. A comparison of organic and inorganic carbon controls over biological denitrification in aquaria. *Chemosphere* 48: 445 – 451.

Yeats, P.A., 1990. Reactivity and transport of nutrients and metals in the St. Lawrence estuary. M El-Sabh, N. Silverberg (eds.) *Coastal and Estuarine Studies*, vol 39. Springer-Verlag, New York, 153 – 169.



## THE DEVELOPMENT OF A NUMERICAL WATER CIRCULATION MODEL OF SHELBURNE, NOVA SCOTIA FOR AQUACULTURE SUPPORT

S.P. Haigh<sup>1\*</sup>, D.G. Pratomo<sup>2</sup>, F.H. Page<sup>1</sup>, R.J. Losier<sup>1</sup>, D.A. Greenberg<sup>3</sup> and B.D. Chang<sup>1</sup>

<sup>1</sup>St Andrews Biological Station, Fisheries and Oceans Canada, 531 Brandy Cove Road, St Andrews NB, Canada

<sup>2</sup>Department of Geodesy and Geomatics Engineering, University of New Brunswick, 15 Dineen Drive, Fredericton NB, Canada

<sup>3</sup>Bedford Institute of Oceanography, Fisheries and Oceans Canada, Dartmouth NS, Canada

\*corresponding author

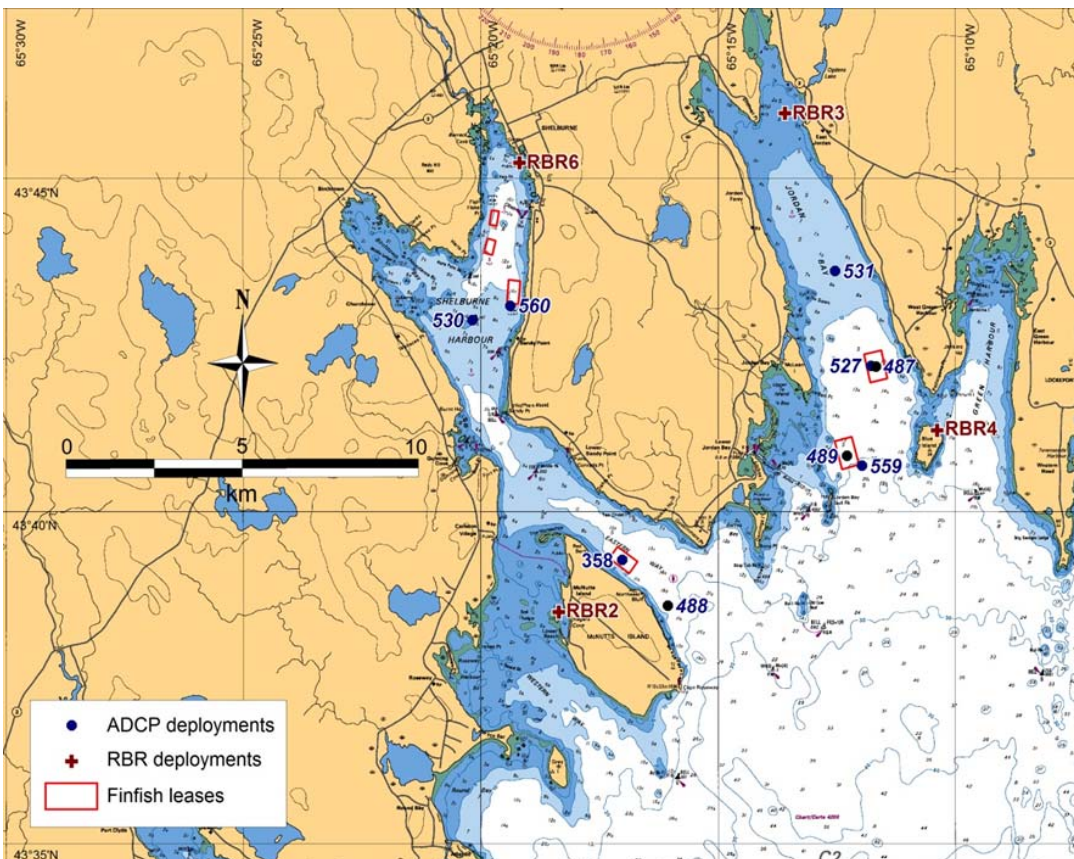
### Abstract

Shelburne, Nova Scotia is an area with an active aquaculture industry. With the presence of fish farms comes the need to map the transport and deposition of organic waste and the transport and dispersal of pesticides and drugs, all of which require knowledge of the water circulation in the area. To this end, the Finite Volume Community Model (FVCOM) was implemented for Shelburne, Nova Scotia. The model was run in barotropic mode, forced with tidal variations of sea level at the open boundaries, and forced with temporally and spatially varying 2013 seasonal winds at the surface. The model is validated against measured sea surface height and current data. The model is capable of adequately reproducing the sea surface heights for which the tidal components accounts for at least 88% of the variation. The observed currents in the area were determined to have tidal and non-tidal variances of similar amplitudes. The amplitude of the M2 current along the major axis is reasonably well predicted by the model but the model does not capture the vertical variation of the minor amplitude, inclination, or phase. A barotropic model forced with tides and wind was found to be insufficient to predict the non-tidal component of the current. These results indicate that the model can be used for transport and dispersion in the near field but should not be used to predict far field effects.



## Introduction

The coast of Shelburne County, Nova Scotia is an active aquaculture area (Fig. 1) with fish farms in Shelburne Harbour, near McNutts Island and in Jordan Bay. With the presence of fish farms comes the need to map the transport and deposition of organic waste and the transport and dispersal of pesticides and drugs, all of which require knowledge of the water circulation in the area. To meet this requirement, implementation of the Finite Volume Community Ocean Model (FVCOM; Chen et al., 2006) was initiated for the study area. As a first step, a barotropic model forced with seasonal winds for 2013 was developed.

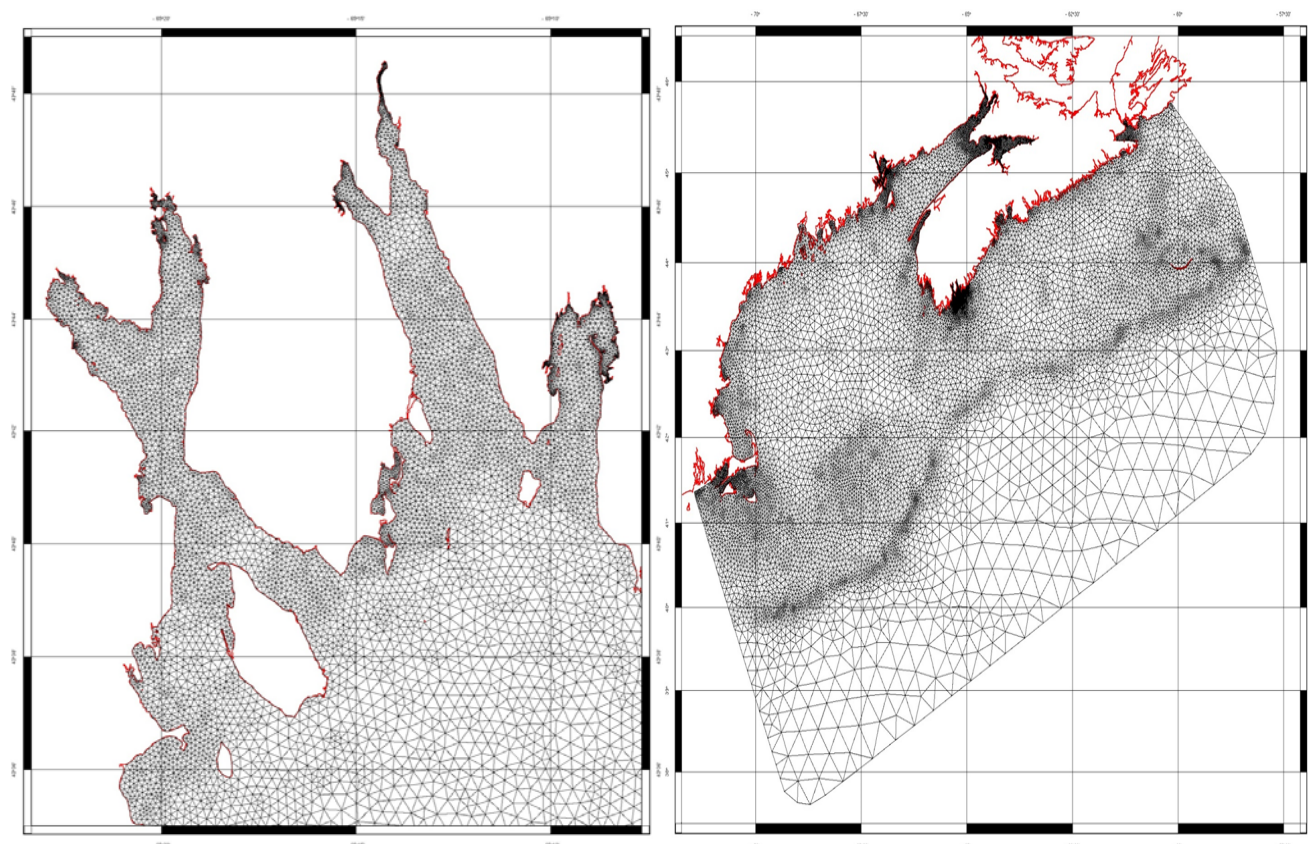


**Figure 1**  
Map of the Shelburne area. The background map is part of Canadian Hydrographic Service chart 4241 (Lockeport to Cape Sable, November 2002 edition); depths are in metres below lowest normal tide.

## Methods

FVCOM uses an unstructured mesh which allows for accurate representation of the coastline and bathymetry and capturing of small scale features in areas of

interest without requiring higher horizontal grid resolution throughout the model domain. FVCOM version 3.2.1 was used. The grid used for this project (Fig. 2) encompasses the Bay of Fundy, Gulf of Maine and Scotian Shelf. The grid has 24,151 nodes (vertices of triangles) and 44,526 cells (triangles) with horizontal length scales ranging from 53 kilometers off the Scotian Shelf to 15 meters with the highest resolution in the area of interest, the Shelburne area. In the vertical, FVCOM uses terrain following coordinates. The model used 21 vertical levels with higher resolutions near the surface and the bottom. The



**Figure 2**  
**FVCOM mesh for**  
**Shelburne area:**  
**24,151 nodes; 44,526**  
**cells, horizontal**  
**resolution of 15 m to**  
**53 km. a) entire**  
**model domain; b)**  
**enlargement of grid**  
**for Shelburne**  
**Harbour and Jordan**  
**Bay.**

model was run in barotropic mode with wetting and drying, allowing for representation of inter-tidal areas. The model was forced with tidal variation in sea level at the lateral open boundaries and with spatially and temporally variable seasonal winds for 2013 at the surface. For all runs, the model was started from rest and spun-up for 6 days, including a 12-hour ramp-up time.

Observations on current speeds and sea level were used to calibrate and validate the FVCOM model. The current data was obtained using acoustic Doppler current profilers (ADCP) equipped with a pressure sensor and coastal sea level was obtained from pressure data loggers (RBR). The locations of the equipment deployments are shown in Figure 1 and the data are described in Chang et al. (2016). Analysis of the sea surface height records in the Shelburne region revealed that the tide accounts for 88% to 97% of the total sea surface

height variation (Chang et al., 2016) indicating the importance of ensuring that the model correctly predicted the tidal sea surface height. The open boundary of the FVCOM model consists of all nodes along the outer edge of the model domain which are not coastline. For the Shelburne implementation, the open boundary was forced with five tidal constituents: M2, N2, S2, K1, and O1. The tidal amplitudes and phases of these constituents along the open boundary were initially interpolated from WebTide's (WebTide Tidal Prediction Model) Scotian Shelf data set, but were then adjusted so that the model gave good agreement with the Shelburne field data. Results of the model calibration for the M2 tidal constituent, the dominant constituent, are given in Table 1 and indicate that, in the Shelburne region, the model, on average, predicted the M2 tidal amplitudes and phases of the sea surface height very well. Calibration results of the other four constituents used to force the model were similar.

In addition to the calibrated tidal forcing at the open boundary, the model was forced with spatially and temporally variable seasonal winds from 2013. The wind data are from the Canadian Meteorological Centre's Global Deterministic Prediction System ( $0.6^\circ \times 0.6^\circ$ , approximately 48 km x 67 km). The weather model provided twice daily forecasts at 0:00 and 12:00 with output predictions at 0, 3, 6, and 9 hours from the forecast time. The wind data was spatially interpolated to the FVCOM grid. Each season was modelled by a representative 30-day simulation (Table 2). There are seasonal variations in the winds, but there is little spatial variation in the wind fields in the Shelburne area. This is a result of the coarse horizontal grid resolution ( $0.6^\circ$ ) used by the global weather model. As a first approximation of the wind fields, this is acceptable since the Shelburne area has little topographic structure and hence there is likely little topographic steering of the winds. Comparison of modelled winds with observed wind data collected by Environment Canada at Baccaro Point, Nova Scotia show that the modelled wind fields capture the low frequency variation of the observed wind, but as the wind fields are only provided at three hour intervals, the high frequency variation is not represented.

## Results

The model forcing was calibrated against the tidal sea surface heights, but for aquaculture regulatory purposes it is the predicted current fields which are of primary interest. The results of the tidal analysis of the currents for six ADCP deployments (358, 527, 530, 531, 559, and 560) and the tidal analysis of the FVCOM model currents at the same locations were compared. Results show that the model's ability to correctly predict the tidal currents varies from location to location but there are features that are evident at most locations. The M2 tidal parameters for deployment 531, which is located in Jordan Bay (Fig. 1), are

shown in Figure 3. M2 is the dominant constituent with amplitudes that are 4-5 times the next most important constituent, N2. Deployment 531 is one of two ADCP records, deployment 530 being the other, whose deployment dates have an overlap with the model run dates.

In general, the model provided a reasonable prediction of the major amplitude, though there was some indication that the current decays too quickly in the bottom boundary layer. Minor amplitudes were generally over-predicted by the model and the vertical structure was not captured but it should be noted that the differences between observed and modelled values are in the range of

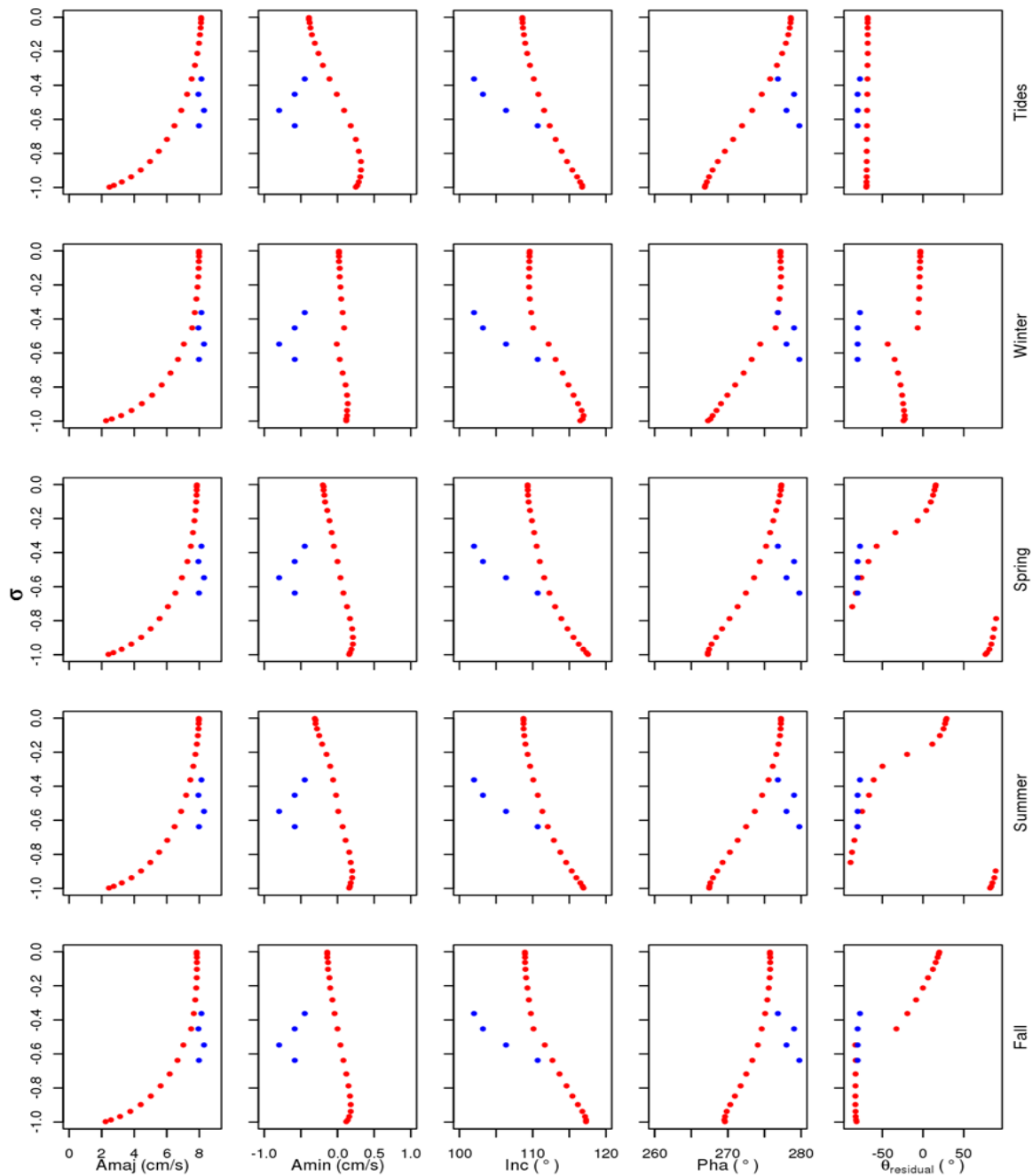
**Table 1 The M2 amplitudes and phases at the locations used to calibrate the open boundary forcing. The M2 amplitudes from the analysis of the observed and modelled sea surface heights are given as well as the ratio of the modelled amplitude over the observed amplitude. The phase from the tidal analysis of the observed and modelled sea surface heights are given as well as the difference between the modelled phase and the observed phase. The distance is the length of the error vector between the modelled and observed amplitudes and phases plotted in polar coordinates.**

| M2   | Amplitudes |       |       | Phases |       |          | Distance |
|------|------------|-------|-------|--------|-------|----------|----------|
|      | OBS        | FVCOM | Ratio | OBS    | FVCOM | $\Delta$ |          |
| RBR2 | 0.728      | 0.727 | 0.999 | 3.9    | 2.0   | -1.9     | 0.023    |
| RBR3 | 0.722      | 0.720 | 0.997 | 3.0    | 1.0   | -2.0     | 0.025    |
| RBR4 | 0.713      | 0.712 | 0.999 | 2.7    | 0.6   | -2.1     | 0.026    |
| RBR6 | 0.739      | 0.739 | 1.001 | 3.9    | 2.2   | -1.7     | 0.021    |
| 358  | 0.743      | 0.719 | 0.968 | -0.6   | 1.3   | 1.9      | 0.034    |
| 488  | 0.697      | 0.715 | 1.025 | -2.6   | 1.2   | 3.8      | 0.049    |
| 489  | 0.680      | 0.713 | 1.048 | 0.3    | 0.8   | 0.5      | 0.033    |
| 527  | 0.717      | 0.714 | 0.996 | -2.6   | 0.8   | 3.4      | 0.042    |
| 530  | 0.750      | 0.738 | 0.984 | 2.9    | 2.3   | -0.6     | 0.015    |
| 531  | 0.728      | 0.717 | 0.986 | 2.1    | 0.8   | -1.3     | 0.020    |
| Mean |            |       | 1.000 |        |       | 0.0      | 0.029    |
| S.D. |            |       | 0.022 |        |       | 2.3      | 0.011    |

**Table 2 Simulation dates for FVCOM seasonal runs.**

| Season | Run Start Date  | Run End Date    |
|--------|-----------------|-----------------|
| Winter | 1 February 2013 | 3 March 2013    |
| Spring | 1 May 2013      | 31 May 2013     |
| Summer | 1 August 2013   | 31 August 2013  |
| Fall   | 1 November 2013 | 1 December 2013 |





**Figure 3**

Comparison between the M2 constituent results from the tidal analysis of currents from ADCP deployment 531 (blue) and the FVCOM model (red). Shown are the amplitudes of the M2 tide along the major and minor axis of the current, the inclination ( $Inc$ ) of the major axis, the phase ( $Pha$ ) and the direction from the principal component analysis of the residual currents ( $\theta_{residual}$ ). The vertical coordinate,  $\sigma$ , varies from 0 at the surface to -1 at the seabed.



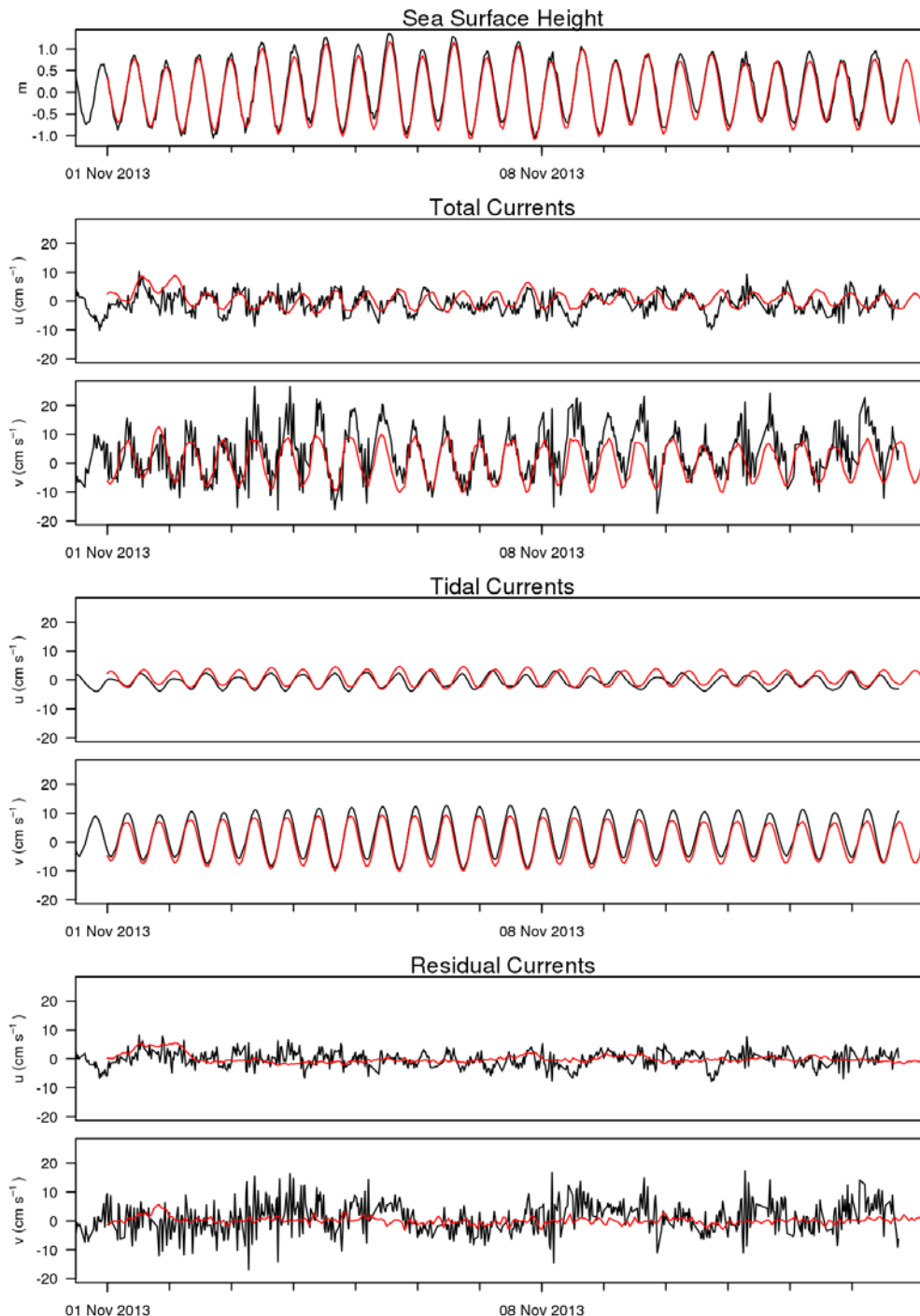
uncertainty in both data measurement and analysis. The model's inability to capture the vertical variation is evident in most of the parameters. There are two rivers which discharge into the area of interest: the Roseway River into Shelburne Harbour and the Jordan River into Jordan Bay. From CTD measurements (Chang et al., 2016), there is evidence that these rivers do affect the vertical temperature and salinity structure at certain times of year and so it is possible that baroclinic effects caused the observed vertical variation in the currents. As the FVCOM model was run in barotropic mode, these effects were not taken into account. Additionally, the modelled vertical structure is dependent on the vertical turbulence closure scheme used. The FVCOM implementation of the Mellor and Yamada (1982) level 2.5 turbulent closure model (Chen et al., 2006) was used for this study. Although other turbulence closure schemes are available, their effects have not been investigated.

For the phase and inclination, the differences between modelled and observed phases and inclinations are as great as  $20^\circ$ , where  $20^\circ$  represents a 41-minute difference in the timing of observed and predicted currents. Typically, the values agree quite well at one vertical level but diverge moving away from that level. It should be noted that adding wind had little effect on the tidal parameters.

Unlike sea surface height for which the five tidal constituents used to force the model account for 88% to 97% of the variation, the same five constituents account for a smaller amount of the currents variation, between 30% and 80% (Chang et al, 2016). For this reason, non-tidal contribution to the currents must be examined. Time series decompositions for ADCP deployment 531 are shown in Figure 4. Forcing the barotropic FVCOM model with tides and wind alone was insufficient to capture the range of the observed currents. The measured non-tidal residual currents are of the same order of magnitude as the measured tidal currents but the model did not capture this important feature. Ongoing work concerning implementation of a baroclinic model will hopefully address this shortcoming.

## **Discussion**

A FVCOM model for the Shelburne, Nova Scotia region was developed to examine the effects of seasonal winds on the circulation and to assess its usefulness for aquaculture regulatory purposes. The model was run in barotropic mode and included wetting and drying of inter-tidal areas. The open boundaries were forced by the tidal constituents M2, N2, S2, K1, and O1. Surface winds were also included in four 30-day runs, one during each season of 2013.



**Figure 4**  
Time series comparison between current data for ADCP deployment 531 (black) and modelled currents from FVCOM fall 2013 simulation (red) at vertical level  $\sigma = -0.4525$  where  $\sigma = 0$  is the surface and  $\sigma = -1$  is the seabed. Total currents have been decomposed into tidal and residual components where the residual current is the difference between the total current and the tidal current. Tidal currents include contributions from all constituents included in the tidal analysis.

It was demonstrated that the model was capable of reproducing sea surface heights. For the currents, the model was able to predict the tidal amplitude in the major direction reasonably well. This is encouraging as the major amplitude has the biggest impact on the tidally driven distance travelled by agents which are advected by the currents. There was some indication that current amplitudes decayed too quickly towards the seabed. The model did not capture

the observed vertical variation in the minor amplitude, inclination, or phase. Additionally, winds alone were insufficient to capture the non-tidal variation of the currents.

One of the limitations of this study was the lack of overlapping time series for model and observations. Additionally, the ADCP were bottom mounted facing upward resulting in a lack of current observations near the sea bed. Work is continuing on improving the Shelburne model. Including baroclinicity and waves are under investigation.

### **Acknowledgements**

This study would not have been possible without the involvement of many people in the collection of the field data including Sarah Scouten and Kenneth MacKeigan of Fisheries and Oceans Canada (DFO) and crew members Captain Perry Smith, Danny Loveless and Michael Dow of Canadian Coast Guard (CCG) vessel Viola M. Davidson, and Captain Richard Starr and Charles Hamilton of CCG vessel Sigma T. We would like to thank Brendan DeTracey (DFO) for kindly providing the wind data and Sheila Gidney (DFO) for administrative support. This project was funded by the Program for Aquaculture Regulatory Research (PARR), DFO.

### **References**

Chang, B., Haigh, S., Losier, R. and Page, F., 2016. Oceanographic observations in coastal waters of Shelburne County, Nova Scotia, 2008-2014. Canadian Technical Report of Fisheries and Aquatic Sciences 3xxx, in preparation.

Chen, C., Beardsley, R. and Cowles, G., 2006. An unstructured grid, finite-volume coastal ocean model (FVCOM) system. *Oceanography* 19(1): 78-89.

Foreman, M., Cherniawsky, J. and Ballantyne, V., 2009. Versatile harmonic tidal analysis: improvements and applications. *J. of Atmos. and Oceanic Technol.* 26:806-817.

Mellor, G.L. and Yamada, T., 1982. Development of a turbulence closure model for geophysical fluid problems. *Rev. Geophys. Space. Phys.*, 20, 851-875.

WebTide Tidal Prediction Model (V0.7.1), 2015. Available at: <http://www.bio.gc.ca/science/research-recherche/ocean/webtide/index-en.php>

# MODELLING AQUACULTURE WASTE DISPERSAL IN JORDAN BAY

Adam Drozdowski<sup>\*1</sup>, Brent Law<sup>1</sup> and Fred Page<sup>2</sup>

<sup>1</sup> Bedford Institute of Oceanography, Fisheries and Oceans Canada,  
1 Challenger Drive, Dartmouth, NS, B2Y 4A2

<sup>2</sup> St. Andrews Biological Station, 531 Brandy Cove Road, St. Andrews,  
NB E5B 2L9

\* Corresponding author: [adam.drozdowski@dfo-mpo.gc.ca](mailto:adam.drozdowski@dfo-mpo.gc.ca)



## Abstract

Waste dispersal from a fin-fish aquaculture facility in Jordan Bay N.S. was simulated using the benthic boundary layer model (BBLT). The currents, waves, and bottom stress were measured during the fall of 2014 and used as inputs. Four waste classes were considered: fines, flocs, fecal waste, and feed pellets and modelled with a 46-day continuous release scenario. The mass of each class entering the cage each day was fixed to provide a normalized concentration of 1 g/m<sup>3</sup> if all the material settled in the bottom 10 cm of a 50-meter diameter fish cage. The resulting concentration varied by as much as 5 orders of magnitude. The four classes peaked at 0.004, 0.2, 7.5, and 8.8 g/m<sup>3</sup> inside the cage. Spatial patterns reveal the concentration of the fines and flocs to be relatively uniform inside and near the cage, and to fall by 1 to 2 orders of magnitude a few kilometers away; fecal and pellet waste tend to be deposited near the cage. The higher concentrations of fines and flocs in the far field (as compared to fecal material and pellets) represent a possible transport mechanism for constituents such as trace metals, pesticides and organics which have an affinity for large surface area fine-grain sediments.

## Introduction

Fin fish aquaculture in marine coastal waters is a growing industry worldwide as well as in Atlantic Canada. Farmed fish are typically cultivated in circular cages suspended in the upper layer of the water column. The fish are nourished by feed pellets. Any uneaten pellets, as well the fecal waste, are deposited into the benthic environment generally beneath the cage. The removal of this waste is dependent on physical advection and dispersion processes. The modelling of

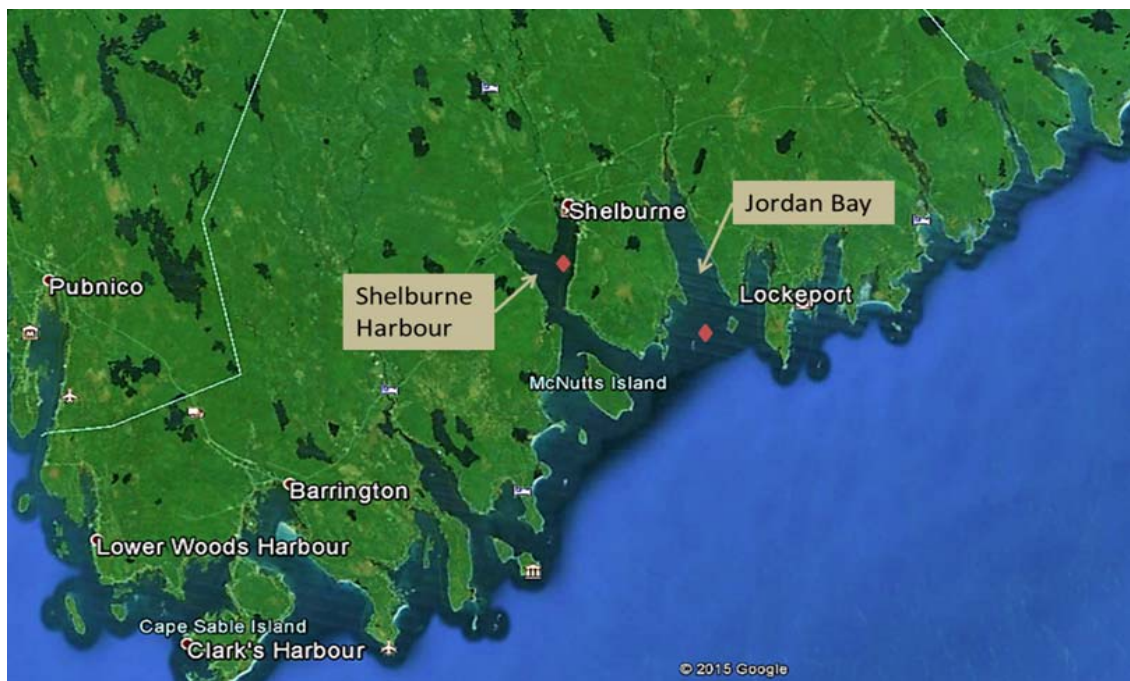
these processes can aid operators and regulators in determining environmental impacts and optimal carrying capacities.

The work presented here was completed using the benthic boundary layer model (BBLT; Drozdowski et al., 2004), originally developed and used to study suspended particulate drilling waste in the offshore environment (Hannah & Drozdowski, 2005), where the fate and sub-lethal biological impacts of drilling mud released during the drilling phase of oil and gas platforms, such as Hibernia on the Grand Banks and North Triumph on the Scotian Shelf, was of primary concern. More recently AMEC E&E division has used BBLT to conduct environmental assessments of the discharge of effluent solid waste in Long Harbour (AMEC E&E Division, 2007). Sediment dynamics in BBLT are modelled with the modified Rouse profile (Rouse, 1937) and include a wave boundary layer (Grant & Madsen, 1986).

## Methods

BBLT requires time series water column currents and bottom stress. In the fall of 2014, three acoustic instruments were deployed in Jordan Bay for this purpose (Fig. 1). An upward-facing RDI 600-KHz acoustic Doppler current meter (ADCP) measured flow in the bulk of the water column as well as surface waves. An Aquadopp (8 Hz) measured near-bottom currents and bottom stress. A downward-facing RDI 1200-KHz ADCP was deployed for 2 days to help resolve near bottom currents.

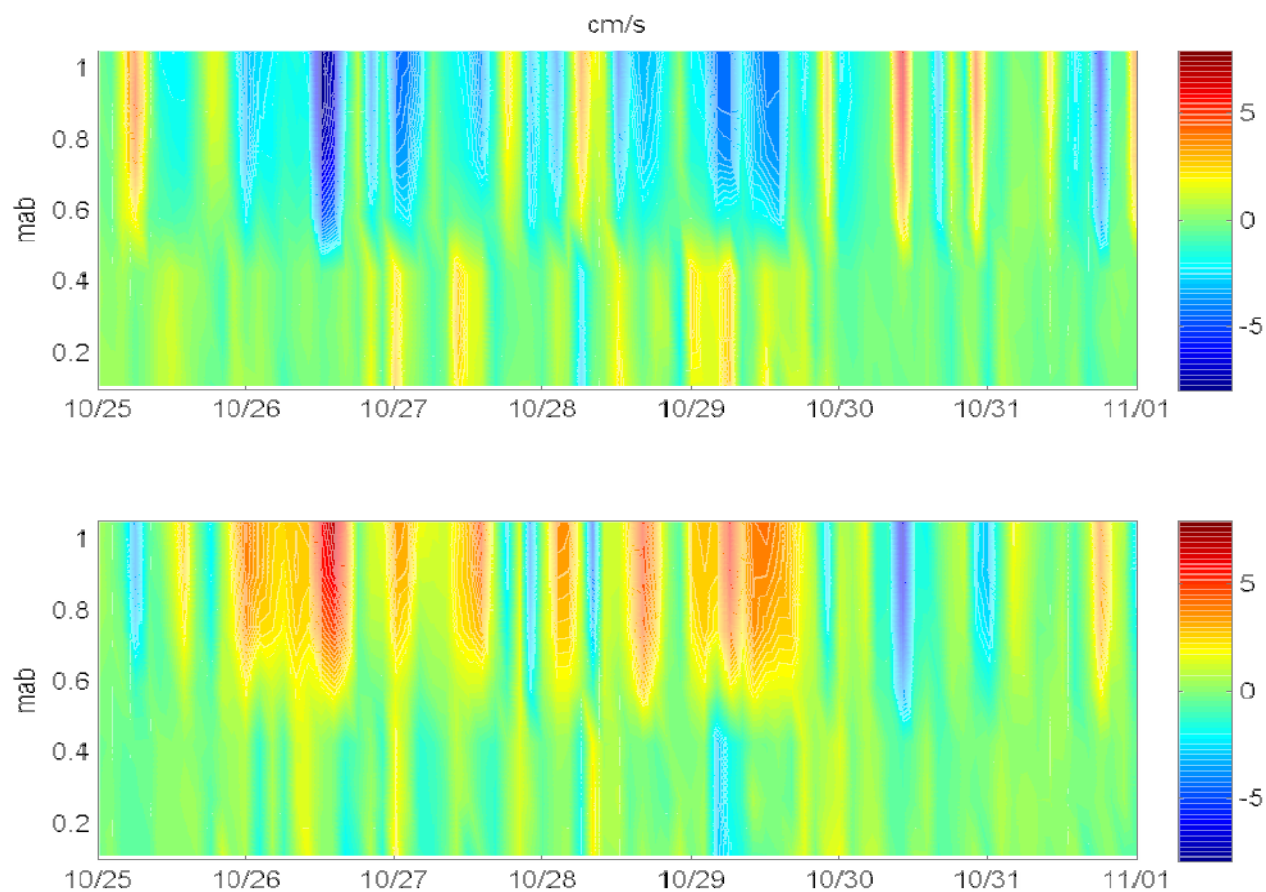
**Figure 1**  
Red diamonds  
show locations of  
2014 current  
meter  
deployments.





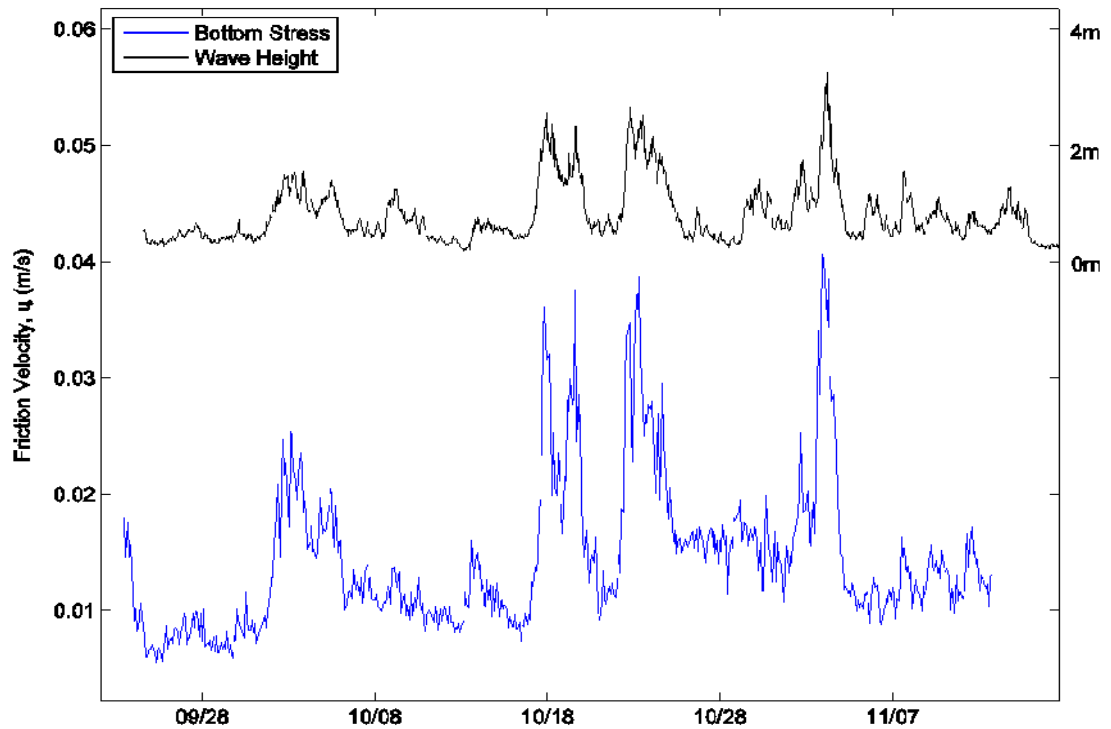
The current data reveals that maximum speeds generally don't exceed 30 cm/s in the area. Semi-diurnal tides have an underlying presence but account for only 20-40% of the variance. A fragment of near bottom currents from the Aquadopp is shown in Figure 2. The combined waves and currents bottom stress (or friction velocity) from the Aquadopp is shown in Figure 3. Values usually exceed 0.01 m/s and can reach 0.05 during large wave events.

Four separate sediment classes were modelled: background fines, fish fecal waste, and uneaten feed pellets (See Table 4; Law et al., 2014). Deposition occurred when the bottom stress fell below the critical stress. The average critical stress values were used. Bottom roughness was set to 1.0 mm based on grain size analysis ( $=5 \times$  grain size of 200  $\mu\text{m}$ ).



**Figure 2**  
**Aquadopp east (top) and north (bottom) velocity profile.**

**Figure 3**  
Bottom  
stress and  
wave  
heights  
from Jordan  
Bay.



**Table 1 Waste class parameters used in BBLT simulations.**

| Sediment Waste Class     | Fines  | Flocs  | Fecal | Pellets |
|--------------------------|--------|--------|-------|---------|
| Settling velocity (mm/s) | 0.1    | 1      | 40    | 100     |
| Critical Stress (m/s)    | 0.0015 | 0.0015 | 0.009 | 0.015   |
| Average Diameter (mm)    | -      | -      | 0.5   | 10      |

For each simulation, a mass of waste equalling 196.4 g, represented by 10 000 particles, was dumped into the fish cage every 24 hours (starting 00:00Z 25-Sep-2014) for 46 days. This mass

gives a concentration of  $1 \text{ g/m}^3$  (can be scaled when more realistic inputs are available) if all the material settles in the bottom 10 cm of the fish cage with 50-meter diameter. The actual decent of sediment to the bottom was not modelled. All sediment input is immediately redistributed vertically based on the Rouse profile.

## Results

Time series of concentration inside the cage is shown in Figure 4. The fines and flocs disperse rapidly in most cases leaving a near zero concentration most of the time. Fecal waste begins to accumulate over days 0 to 7 but the arrival of a wave event clears the back log and no further buildup occurs. For the feed

pellets, early buildup occurs and clears just as for fecal waste but reappears again for days 11-22 and somewhat for days 40-45. The buildup finally clears by a wave event towards the end of the simulation. Summary statistics are presented in Table 4. Of note is the fact that the fecal and pellet waste are within a few millimeters of the bottom (large Rouse numbers) for the whole simulation and spend a significant fraction of time deposited in the bed-load.

**Table 2 Summary of Results. Values derived from time series output inside the cage. Heights are the Mean/Maximum of the center of mass of all sediment.**

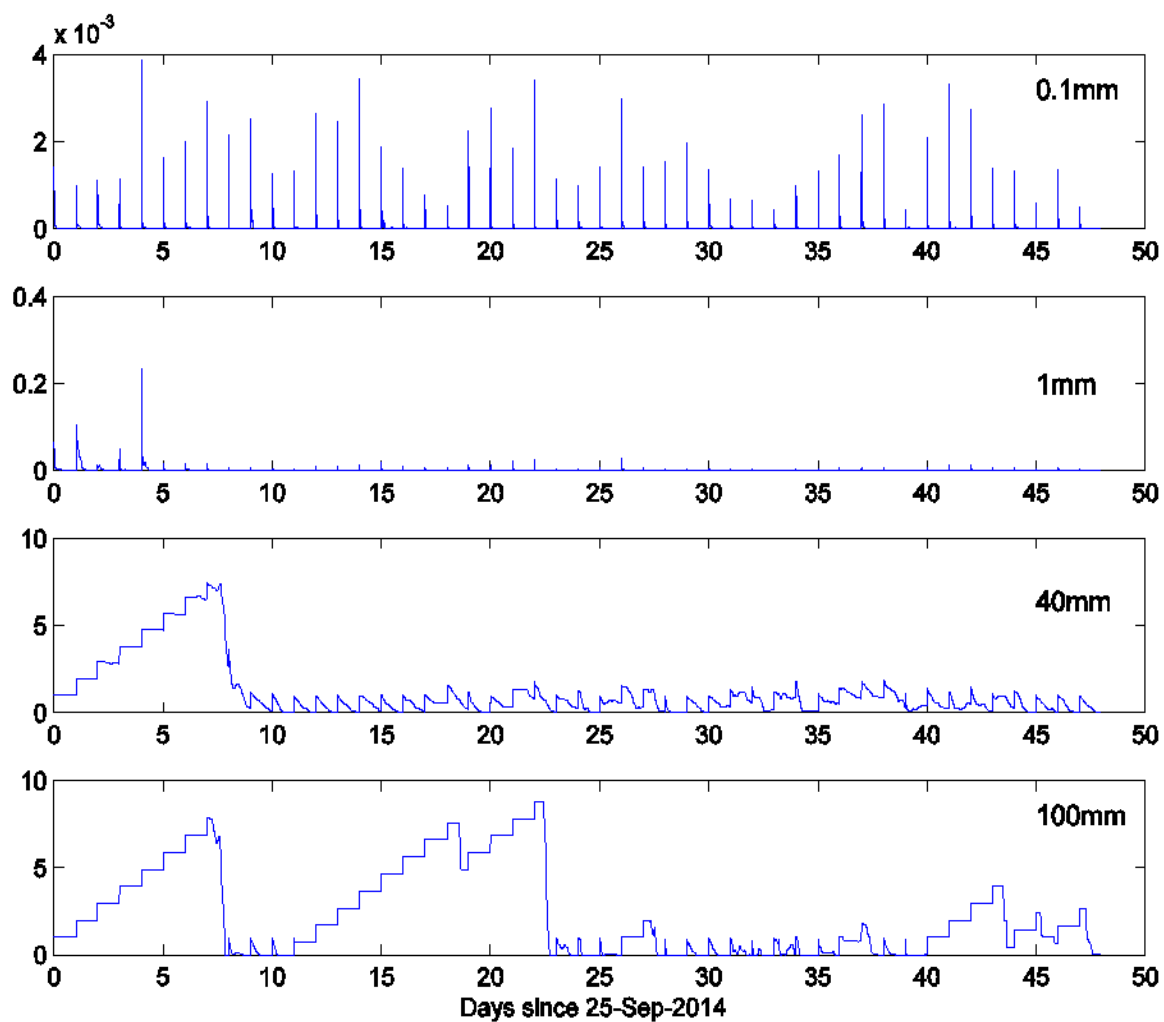
| Settling Velocity (mm/s) | Mean  | Maximum | Mean/Maximum Height Above Bottom (meters) | Mean/Maximum Rouse Number | % of time in bed-load |
|--------------------------|-------|---------|---|---------------------------|-----------------------|
| 0.1                      | 3 e-5 | 4 e-3   | 8.9 / 9.0                                 | 1.5e-2 / 3.5 e-2          | 0                     |
| 1                        | 8 e-4 | 0.2     | 7.2 / 8.5                                 | 0.15 / 0.35               | 0                     |
| 40                       | 1.2   | 7.5     | 1.8e-3 / 6.5e-3                           | 5.9 / 14                  | 15                    |
| 100                      | 2.3   | 8.8     | 5.3e-3 / 7.0e-3                           | 14.8 / 34.9               | 62                    |

Figure 5 shows the spatial distribution of mean. Peak values are typically inside the cage and, except for the fines, rapidly decrease outside. For fines, the concentrations stay relatively uniform within a few hundred meters of and inside the cage, and fall by an order of magnitude a few kilometers away. Floc concentrations fall to 1/10 of peak values within a 100 m of the cage and to 1/1000 within a few kilometers. Fecal waste concentrations decrease tenfold directly outside the cage and should be undetectable (i.e.  $< 1\text{e-}6 \text{ g/m}^3$ ) within a kilometer (faster along East-west axis, particularly eastward). Pellets disperse very slowly and experience episodic deposition and advection of the sediment patch which appear as cage-sized artifacts with increased concentration. Concentration drops by at least an order of magnitude directly outside the cage and by as much as 3 orders of magnitude within a few hundred meters. The general direction of travel is to the south.

## Discussion

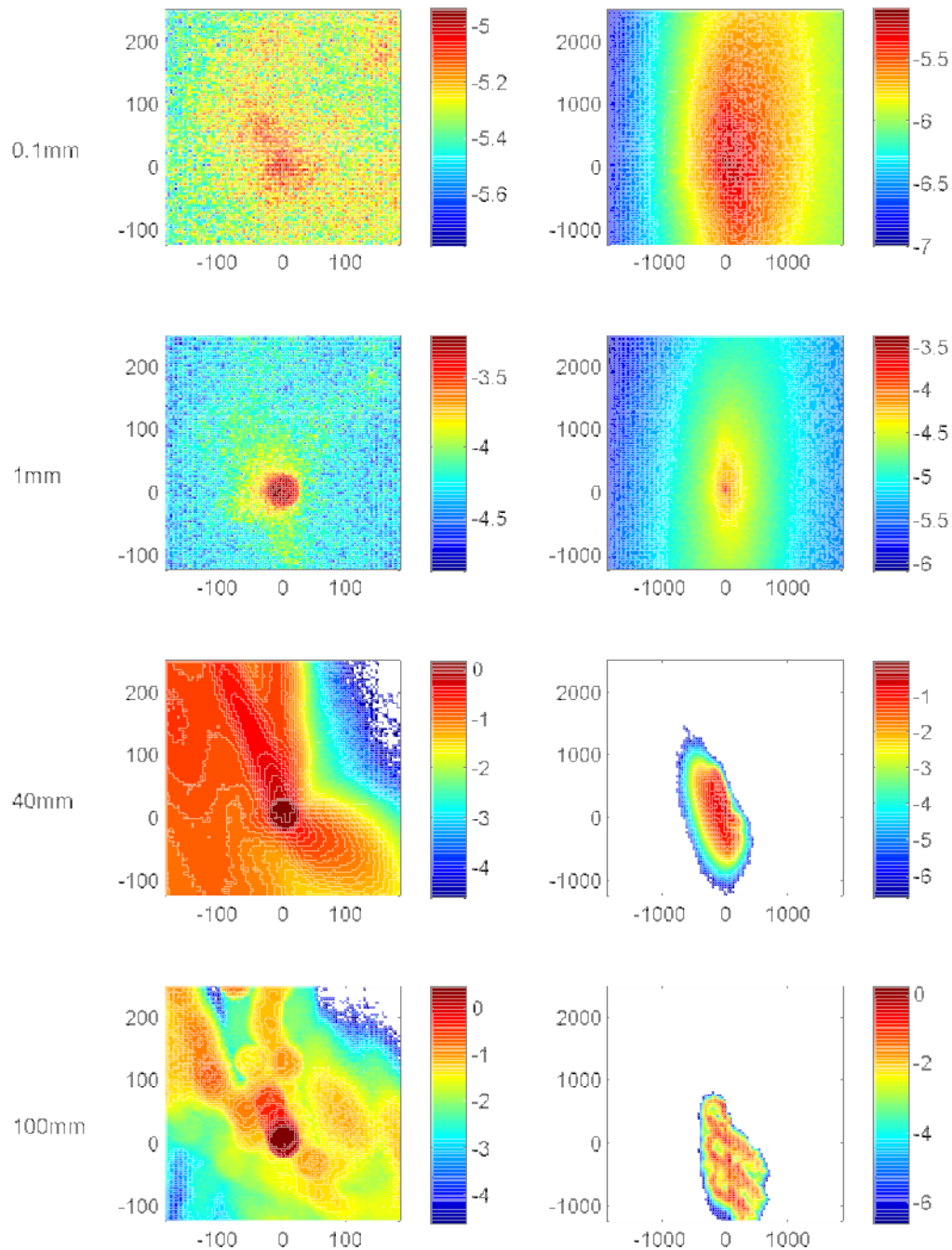
The results suggest that while fecal and pellet waste is deposited in the proximity of the cage, the fines and flocs can be transported to the far-field (i.e. kms from the site). The concentration of these materials does decrease but can provide a mechanism for organics, contaminants, other chemicals (e.g. pesticides) that are particle reactive to be transported. The predictive capacity of this model will be limited until there is better knowledge of input parameters and validations with field data are carried out. It is a recommendation of this

study to continue to validate these findings and use models to increase our predictive capacity of aquaculture operations.



**Figure 4**

Bottom concentration (g/m<sup>3</sup>) time series at fish cage from the 4 full forcing scenarios. Panels from top to bottom are 0.1,1,40,100 mm/s settling velocity.



**Figure 5**  
Near field (left)  
and Far field  
(Right) mean  
bottom  
concentrations  
[Log10 (g/m3)].

## Acknowledgments

We would like to thank Vanessa Zions, Casey O’Laughlin, Sarah Scouten and Randy Losier for field support. The captain and crew of the CCGS Viola Davidson are thanked for a great working platform and their help with instrument deployment. Funding was supplied by PARR, the Program for Aquaculture Regulatory Research.

## References

- AMEC E&E Division, 2007. Physical oceanography input to the effluent modelling study for the Long Harbour commercial processing plant environment assessment. Oceanographic component: effluent solid waste dispersion modelling, short and long term fate. Report prepared for Voisey Bay Nickel Ltd.
- Drozdowski, A., Hannah, C. and Tedford, T., 2004. BBLT Version 7.0 user's manual. Can. Tech. Rep. Hydrogr. Ocean Sci. 240: vi + 69 pp.
- Grant, W.D. and Madsen, O.S., 1986. The continental shelf bottom boundary layer. Annual Review of Fluid Mechanics 18: 265-305.
- Hannah, C.G. and Drozdowski, A., 2005. Characterization of drift and dispersion of drilling mud on three offshore banks. Marine Pollution Bulletin 50: 1433-1443.
- Law, B.A, Hill, P.S., Maier, I., Milligan, T.G. and Page, F., 2014. Size, settling velocity and density of small suspended particles at an active salmon aquaculture site. Aquaculture Environment Interactions, 6: 29-42, doi:10.3354/aei00116.
- Rouse, H., 1937. Modern concepts of the mechanics of turbulence. Trans. Am. Soc. Civ. Eng. 102: 463-543.



# OXYGEN MEASUREMENT USING A NEEDLE-TYPE MICROSENSOR

K.G. MacKeigan<sup>1</sup>, M.C. Lyons<sup>\*1</sup> and F.H. Page<sup>1</sup>

<sup>1</sup> St. Andrews Biological Station  
531 Brandy Cove Road  
St. Andrews, NB E5B 2L9 Canada

\* Corresponding author [Monica.lyons@dfo-mpo.gc.ca](mailto:Monica.lyons@dfo-mpo.gc.ca)



## Introduction

Impacts on benthic communities from organic enrichment result from a combination of sulfide (*S*) toxicity and dissolved oxygen (*DO*) depletion. *DO* measurement methods available a decade ago suffered from numerous technical and practical issues. New methodologies for rapidly and directly measuring *DO* at low cost and effort, and under field conditions, are currently available. Sediment organic enrichment classifications based on *DO* have already been suggested to support similar *S* and redox potential (*Eh*) classifications (Hargrave et al., 2008b). Both *S* and *Eh* measurement methods have issues and are at times problematic. Consequently, *DO* measurements may provide a potential additional or alternative impact indicator to *S* for aquaculture monitoring purposes to support decisions based on *S* classifications.

The purpose of the following work was to gain some experience with and evaluate some of the new technologies. Our initial developmental work used dipping probes, sensor spots, and needle-type microsensors along with either the Fibox or Microx oxygen meters purchased from Hoskin Scientific (manufacturer- PreSens- Precision Sensing GmbH, Germany). The work was conducted at the Canadian Department of Fisheries and Oceans' St. Andrews Biological Station (SABS), with preliminary field tests conducted at aquaculture sites in both Nova Scotia and New Brunswick (Fig. 1). The work was subsequently refined to include laboratory and field comparisons of methodologies at SABS and field work at salmon aquaculture sites in Passamaquoddy Bay, New Brunswick and Shelburne, Nova Scotia.

Needle-type oxygen micro-sensors are described by the manufacturer as being perfectly suited for measuring oxygen in sediment with a high spatial

**Figure 1**  
**Needle-type**  
**microsensor used**  
**to measure DO in**  
**bottom sediment**  
**collected from a**  
**grab sample using**  
**a 5-cc syringe.**



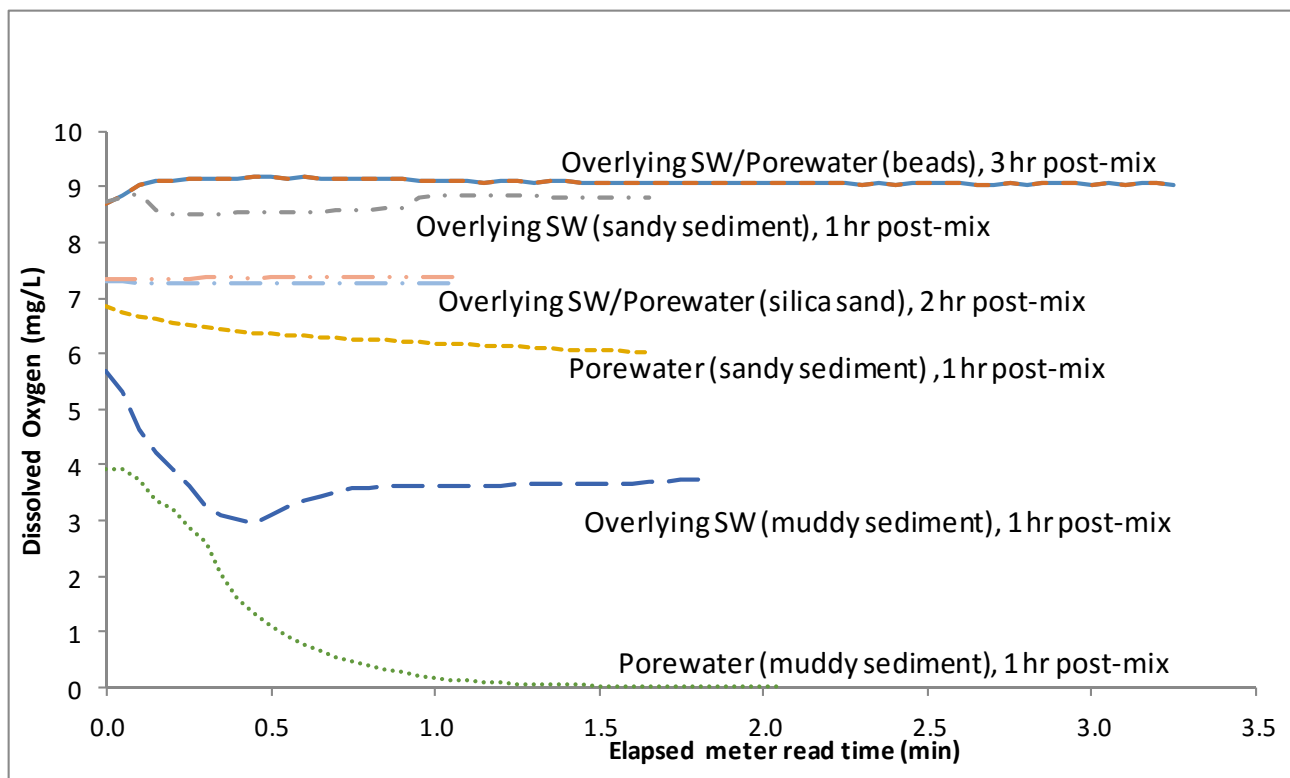
resolution of less than 50  $\mu\text{m}$ . The oxygen-sensitive tip of an optical fiber is protected inside a stainless steel needle. This design is optimal for easy penetration of sediment. After penetration the sensor tip is extended for measurement. The needle-type micro-sensors have fast response times. A laboratory experiment was set up at SABS to examine needle-type oxygen micro-sensors for rapidly measuring *DO* in pore and overlying water.

### **Materials and Methods**

The interaction between different sediment types and oxygenated overlying seawater (SW) was examined. Filtered SW was saturated with oxygen (*DO* ~9 mg/L) by bubbling air through it before adding it to beakers containing four different sediments. The SW was added to the sediment in a 50:50 proportion. The *DO* was first measured in the seawater overlying the sediment and then the seawater and sediment were mixed and the *DO* was followed over 3 minutes in the mixture. The *DO* was measured again in the overlying seawater and in the sediment, approximately 1 to 3 hours later. Two materials, silica sand (#00, Shaw Brick, Saint John, NB) and glass beads (0.400 to 0.520 mm, Canadawide Scientific, Ottawa, ON), were used as clean sediment substrates (containing no organic matter) along with two bottom sediment samples collected in the field, a muddy sediment from Shelburne Harbour, NS and a sandy sediment from Jordan Bay, NS.

## Results

The 3-hour post-mix measurements (Fig. 2) show that the *DO* in the overlying SW and the *DO* in the glass beads/ SW mix are both well oxygenated (*DO* ~9 mg/L). The same result occurred at 2-hour post-mix when clean lab (silica) sand was the substrate used, with a slightly lower *DO* in the sediment/ SW mix. The 1-hour post-mix measurements using the sandy sediment collected at Jordan Bay, NS showed a larger difference (~ 2 mg/L) between the overlying SW (*DO* ~9 mg/L) and the pore water sand/SW mix (*DO* 6-7 mg/L). The muddy sediment from Shelburne Harbour showed an even greater difference (~4 mg/L), which we attribute to oxygen consumption by the sediment. The 1-hour post-mix *DO* measurement in the overlying SW was ~4 mg/L and ~ 0 mg/L in the pore water mud/ SW mix.



## Discussion

Hypoxic and anoxic conditions are created in surface sediments if rates of oxygen consumption exceed supply (Hargrave et al., 2008). Benthic oxygen consumption results from aerobic heterotrophic activity of fauna and bacteria and from the reoxidation of reduced inorganic products released during the anaerobic heterotrophic degradation (Glud, 2008). The field-collected natural

**Figure 2**  
**Comparison of DO**  
**in the pore water**  
**and overlying**  
**seawater of four**  
**sediments.**

sediments used in this experiment consumed oxygen from the overlying SW while the glass beads and silica sand did not. The sandy sediment collected in the field showed less consumption of oxygen when compared to the muddy sediment. Sand generally holds little organic material (Glud, 2008). The *DO* in the overlying SW dropped from the initial measurement of ~9 mg/L to ~4 mg/L after mixing with the muddy sediment and the sediment was anoxic indicating the rate of oxygen consumption exceeded supply during the mix.

### **Acknowledgements**

This project was funded by Fisheries and Oceans, Program for Aquaculture Regulatory Research (PARR).

### **References**

Glud, R.G., 2008. Oxygen dynamics in marine sediments. *Marine Biology Research* 4: 243-289.

Hargrave, B.T., Holmer, M. and Newcombe, C.P., 2008. Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. *Marine Pollution Bulletin* 56: 810-824.

# DEVELOPMENT OF A RAPID, HIGH THROUGHPUT MICROPLATE METHOD FOR THE QUANTIFICATION OF SULFIDE IN MARINE SEDIMENT PORE WATER

D. K. H. Wong<sup>1</sup>

<sup>1</sup> St. Andrews Biological Station  
531 Brandy Cove Road  
St. Andrews, NB E5B 2L9



## Abstract

Quantification of sulfide in sediment is used as a regulatory indicator of environmental impact around marine finfish aquaculture sites in Canada. The current method employs a  $\text{Ag}^+/\text{S}^{2-}$  (silver/sulfide) ion selective electrode (ISE) coupled to a meter that displays the determined concentration when the electrode is immersed in a basified sediment slurry sample. An alternative method has been developed which determines soluble sulfide in sediment pore water instead of slurry. It is based on the classic methylene blue method which determines sulfide by colorimetric detection. However, unlike the traditional methylene blue method applications, the newly developed method can analyse a large number of samples simultaneously and uses smaller volumes of sample and reagents.

## Introduction

Market demand for affordable seafood has fuelled the global aquaculture industry. It has grown exponentially over the past 30 years to such an extent that demand for farmed fish (which includes but not limited to finfish, molluscs and crustaceans) has overtaken fish sourced from wild fisheries. Intensive finfish aquaculture using open net pens can result in adverse environmental impacts due to excess feeding and faecal matter being deposited on the sea floor. Bacterial decomposition of this settled organic matter depletes the surrounding oxygen resulting in anoxic conditions which promote the production of, amongst other things, hydrogen sulfide. Hydrogen sulfide is highly toxic to organisms and adversely impacts the benthic communities under and in the vicinity of cage sites.

Determination of sulfide by the currently accepted regulatory method using ion selective electrodes (ISE) is time consuming since samples have to be analysed

sequentially. The method also has limitations (Chang et al., 2014) in that the electrode calibration is not robust and needs recalibrating on a frequent basis if there are lots of samples to be analysed. The new microplate method described here has no calibration stability issues since a calibration curve is run and analysed simultaneously with the test samples. Assay performance is also checked by analysing quality control (QC) samples along with the calibration and test samples.

The stability of collected sediment samples is also a concern due to the volatility of sulfide. Standard operating procedures (SOPs) differ from the west coast and east coast of Canada in terms of prescribed storage times prior to analyses of collected samples. British Columbia states sample analysis must be performed within 1 hour after collection whereas New Brunswick and Nova Scotia SOPs allow samples (chilled with no headspace) to be stored for up to 72 hours before analysis. The new Federal *Aquaculture Activities Regulations* for owners and operators state that samples should be analysed within 5 minutes of collection or stored at  $3^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and analysed within 36 hours after collection. Samples from the UV microplate method have been shown to be stable for up to 7 days when stored at  $ca +4^{\circ}\text{C}$ .

The new microplate method differs from the ISE method in that sediment pore water is analysed instead of sediment slurry. Sulfide detection is based on the methylene blue reaction whereby the added reagents react with sulfide in the sample to form a blue colour which is directly proportional to the concentration of sulfide present in the sample. Unlike the traditional methylene blue method, the microplate method uses smaller volumes of sample and reagents and allows for many samples to be analysed simultaneously within a 96-well microplate configuration.

## Materials and methods

Sodium sulfide nonahydrate ( $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ , formula weight = 240.18) was used as the source of sulfide for this method development. It was used to prepare spiking solutions in Milli-Q water containing known concentrations of sulfide. The prepared solutions were used to spike raw seawater to give calibration and quality control (QC) samples for method validation purposes. Aliquots of the calibration and QC samples were pipetted into a fixing solution of alkaline zinc acetate solution containing EDTA which precipitated the soluble sulfide as the insoluble zinc sulfide salt. The samples were vortex mixed then pipetted into the wells of the 96 well microplate followed by addition of Milli-Q water to dilute the samples. Colour development was achieved by adding a hydrochloric acid solution containing *N,N*-Dimethyl-*p*-phenylenediamine dihydrochloride and iron

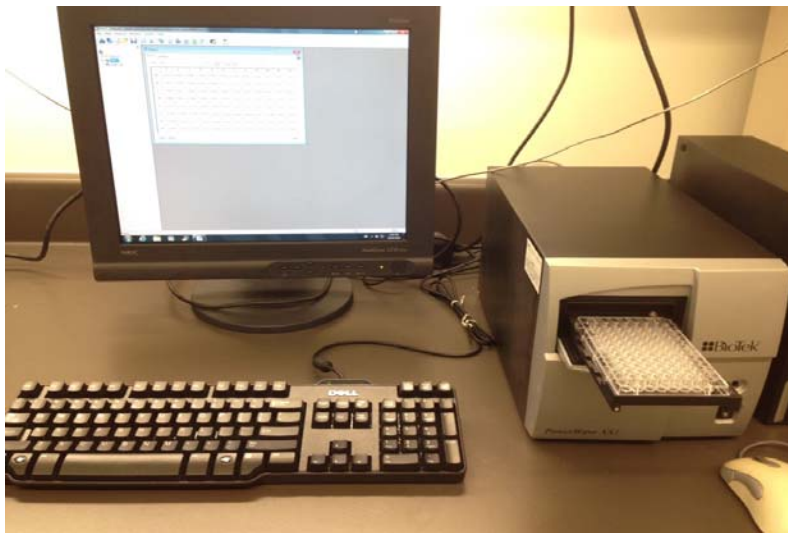


(III) trichloride hexahydrate. Analysis was performed using a BioTek Powerwave XS microplate spectrophotometer (Fig. 1) employing Gen5 version 1.06 software. Microplates were loaded into the plate reader, incubated at ambient temperature for 5 minutes then the colour change quantified by absorbance at 660 nm.

## Results and discussion

The developed method was successfully validated for limit of quantification, linearity, accuracy and precision, and storage stability. The lower limit of quantification (LLOQ) was established at 200  $\mu\text{M}$  with linearity achieved over the range 200 to 10,000  $\mu\text{M}$  (typically  $R^2 = 0.999$ ,  $1/x^2$  weighting factor employed). Accuracy and precision of the method was determined at 300  $\mu\text{M}$  (+1.3% of actual, CV = 3.9%), 1000  $\mu\text{M}$  (+1.3% of actual, CV = 2.9%) and 6000  $\mu\text{M}$  (-1.7% of actual, CV = 2.5%). The stability of sulfide in seawater when fixed in alkaline zinc acetate with EDTA and stored at  $ca +4^\circ\text{C}$  was assessed at 300, 1000, and 6000  $\mu\text{M}$ . Results showed that the samples were stable for up to 7 days at the three concentrations examined. For 300  $\mu\text{M}$ , the overall stability ( $n=3$ ) was  $94.5\% \pm 1.5\%$  (mean  $\pm$  SEM), for 1000  $\mu\text{M}$  it was  $96.0\% \pm 1.1\%$  and for 6000  $\mu\text{M}$ , it was  $99.8\% \pm 0.7\%$ .

The linear range of the developed UV microplate method has been established from 200 to 10,000  $\mu\text{M}$  compared to 100 to 10,000  $\mu\text{M}$  for the ISE method. The method is accurate and precise over the analytical range with stability for up to 7 days when stored at  $ca +4^\circ\text{C}$ . It also utilises sediment pore water which is a less complicated matrix than sediment. Due to the complex geochemical properties of sediment, it has been shown that using the ISE method to quantify sulfide gives higher sulfide concentrations than with corresponding pore water samples (Brown et al., 2011). Brown *et al.* (2011) postulated that the reaction of the highly alkaline sulfide antioxidant buffer (SAOB), which is used to convert all sulfide species to the sulfide ion ( $\text{S}^{2-}$ ), with metal sulfides in the sediment released further sulfide ions thus increasing the overall sulfide concentration. Since this method quantifies soluble sulfide in sediment pore water, no secondary reactions can occur which could affect the overall sulfide concentration of the sample.



**Figure 1**  
**BioTek PowerWave XS**  
**microplate**  
**spectrophotometer.**

Further work will be conducted to assess this method against the ISE method and another newly developed direct UV method along with feasible methods for pore water extraction from sediment samples.

## References

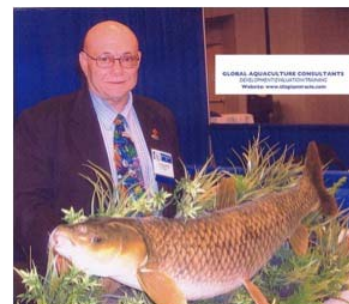
Chang, B.D., Lewis-McCrea, L.M., Wong D.K.H., MacKeigan, K.G., Page, F.H., Cameron, S.L., Sweeney, R.H. and Smith, A.E., 2014. Interlaboratory research on factors affecting the calibration of electrodes used for sediment sulfide analyses at fish farms. Can. Tech. Rep. Fish. Aquat. Sci. 3090: v + 66 p.

Brown, K.A., McGreer, E.R., Taekema, B. and Cullen, J.T., 2011. Determination of total free sulphides in sediment pore water and artefacts related to the mobility of mineral sulphides. Aquat. Geochem. 17: 821-839.

# REVIEW OF DIFFERENT OPINIONS ABOUT USING THE GRASS CARP IN AQUACULTURE PRODUCTION AND AQUATIC WEED MANAGEMENT IN CANADA AND SUDAN

Prof. Thomas T. George<sup>1</sup>

<sup>1</sup>Global Aquaculture Consultants  
Toronto, Canada.  
[www.tilapiamiracle.com](http://www.tilapiamiracle.com)  
+ 416 297-6045



## Introduction

The Chinese grass carp or white amur (*Ctenopharyngodon idella* (Cuvier & Valenciennes, 1844) inhabits the Amur River that forms the border between China and Russia and flows into the Pacific Ocean. It belongs to the Order Cyprinoformes, Family Cyprinidae and is the sole member of the genus *Ctenopharyngodon*. Unlike other carps, it has no barbells at the edge of its mouth. It tolerates a wide range of temperature 0-38°C, salinity up to 10 ppt, and very low oxygen level, and that is why it has been introduced in more than 50 countries for aquaculture production and aquatic vegetation management. Now, it is one of the top four cultured species in the world (Shireman & Smith, 1983; Zonneveld & Zon, 1985; George, 2011; FAO, 2006).

Grass carp is an excellent food fish and a biological control agent for floating aquatic plants. Pond production increases significantly when it is cultured in polyculture with other omnivorous species and/or bottom feeders such as the common carp. It eats weeds up to 3 times its body weight because it has a short digestive tract (2-3 times the body length), no enzymes for cellulose digestion, a toothless mouth and “pharyngeal teeth” in the throat that grind food against a hard, horny pad beneath the lower skull. It has to eat 48 g vegetation to produce 1 g gain in weight. Feeding is limited below water temperature 13°C, moderate between 13-18°C and optimum between 18-26°C (Shireman & Smith, 1983; Zonneveld & Zon, 1985; George, 2011).

Grass carp males mature at two years while females a year later. Fecundity is very high, about 730,000 eggs in a fish weighing 3.5 kg. A female spawns naturally only in long, fast flowing rivers with fluctuating water levels but do not breed in captivity. This problem had been solved by induced breeding

(hyophysation technique) producing fertile diploid carps (Jingran & Pullin, 1988).

In 1983, USA breeders produced sterile triploid grass carp and used it very extensively in lakes to control aquatic weeds. Normal fertile grass carp has 48 chromosomes. This is known as the diploid or 2N chromosome number. Triploid grass carp are produced in hatcheries by physically shocking fertilized eggs with temperature (hot or cold) or hydrostatic pressure. The resulting fish are triploid (3N) because each cell has an extra set of chromosomes and are sterile (Bailey, 1972; Allen & Wattendorf, 1987; Cassani & Caton, 1986; Clugston & Shireman, 1987).

## Discussion

The above information has triggered different opinions about using the grass carp in different countries. In Canada, Duncan Lloyde introduced the grass carp into the Province of Alberta from Florida and they were tested from 1994 until 1999. Triploid grass carp, like in US hatcheries, were produced to control aquatic weeds in ponds as an alternative to herbicides. The Province owns all adult broodstock and spawning and rearing of young are only done by the Aquaculture Center of Excellence (ACE). All fish for stocking are individually triploid tested with Coulter counter and are nasal tagged before being used for weed control operations (Hatchings, 2004; Derksen, 2006).

However, the Province of Ontario totally prohibited the culture of the grass carp and considered it as an invasive species. Furthermore, in 2005 the Ontario Government banned the transport, sale and purchase of live grass carp. Subsequently, Fisheries and Oceans Canada began a quantitative and qualitative risk analysis to determine the ecological risk this species may pose in Canada on the local environment (Gudmore & Mandrak, 2004). By all means, the Ontario Government is trying its level best to keep the grass carp and other Asian carps out of The Great Lakes so they do not eat the food supply of native fish and damage sport and commercial fishing, which brings millions of dollars a year into the province's economy. Moreover, Ontario has partnered with the Ontario Federation of anglers and Hunters since 1992 on programs to fight invasive species including education, outreach and training province-wide monitoring, and early detection.

In Sudan, ten thousand fingerlings of grass carp were introduced in 1975 from India by T.T. George, bred by induced breeding for the first time in Africa and used in a large-scale project funded by the International Development Research Center of Canada (IDRC) for controlling the floating aquatic weeds (*Potamogeton*

spp.) and the snails, *Bulinus* and *Biomphalaria*, the intermediate hosts of Bilharzia, in the irrigation canals of the Gezira Scheme for cotton plantation and also, for promoting polyculture in fish ponds (Fig. 1) (George, 1982). Based on the significant results of this project and the field visit in 1990 of the Director-General, World Bank, a large-scale project to control aquatic weeds in the Gezira irrigation canals by the grass carp had been approved by the World Bank for two million US Dollars. As Project Manager of this project, George absolutely refused the decision of the Agricultural Research Corporation (ARC) Management to allocate one million dollars for the biological control of aquatic weeds in the irrigation canals by the grass carp and one million dollars for the use of chemical pesticides to control harmful insects which damage the cotton plantation, resigned his job and in October 1990 settled as a landed-immigrant in Toronto, Canada!

## Conclusion

Grass carp have been used in polyculture and as a biological control agent. The use of sterile or mono-sex fish seems to offer a desirable margin of safety. Also, it may provide the only

control method for water bodies which are used as drinking supplies and where herbicides cannot be used. Therefore, proper scientific evaluation should be considered before introducing the grass carp and proper research should be conducted after its introduction. This is because once established, exotic species can have undesirable ecological, genetic, economic, and human health impacts; they are now considered to be the most important threat to native biodiversity and habitat destruction (George & Sinha, 2008).



**Figure 1**  
**Heavy weed**  
**(*Potamogeton* sp.)**  
**infestation in the Gezira**  
**Canal, Sudan.**

## References

Allen, S.K. Jr. and Wattendorf R.J., 1987. "Triploid grass carp: status and management implications," Fisheries, Vol. 12, No. 4 pp20-24.

Bailey, W.M., 1972. Arkansas's evaluation of the desirability of introducing the white amur (*Cenopharyngodon idella*) for control of aquatic weeds. Arkansas Game & Fish Commission memo rep. 59 pp.

- Cassani, J.R. and Caton, W.E., 1986. "Efficient production of triploid grass carp (*Ctenopharyngodon idella*) utilizing hydrostatic pressure, "Aquaculture, Vol 56, pp 43-50.
- Clugston, J.P. and Shireman, J., 1987. "Triploid grass carp for aquatic plant control, Fish and Wildlife Service, US Department of the Interior, Washington, DC.
- Derksen, J., 2006. Control of grass carp in Alberta. Bull. Aquacul. Assoc. Canada 106-1.2, pp.82-87.
- FAO, 2006. Aquaculture production statistics 1988-2004, FAO, Rome.
- George, T.T., 1982. The Chinese grass carp *Ctenopharyngodon idella*, its biology, introduction, control of aquatic macrophytes and breeding in Sudan. The Netherlands, Aquaculture 27 pp: 317-327.
- George, T.T. and Sinha, V.R.P., 2008. Aquaculture: public concerns and sustainable practices, pp. 137-149. In: New Frontiers of Zoology by Pandey BN and Pandey PN, Narendra Publishing House, New Delhi, India.
- George, T.T., 2011. The grass carp and tilapias as biological control agents and their role in aquaculture for food security. AAC Spec. Publ. No. 13 pp: 26-29.
- Gudmore, B. and Mandrak, N.E., 2004. Biological synopsis of grass carp (*Ctenopharyngodon idella*). Fisheries and Oceans Canada.
- Hatchings, E., 2004. Aquaculture development in Alberta. AAC Special Publication No. 11.
- Jingran, V.G. and Pullin, R.S.V., 1988. A hatchery manual for the common, Chinese and Indian major carps. Asian Development Bank. International Center for Living Aquatic Resources Management.
- Shireman, J.V. and Smith, C.R., 1983. Synopsis of biological data on the grass carp, *Ctenopharyngodon idella*. FAO Synopsis no. 135. FAO Rome.
- Zonneveld, N. and Zon, H.V., 1985. The biology and culture of grass carp (*Ctenopharyngodon idella*) with special reference to their utilization for weed control. In J.F. Muir and R. J. Roberts, editors. Recent Advances in Aquaculture. Croom Helm, London. pp. 119-191.



