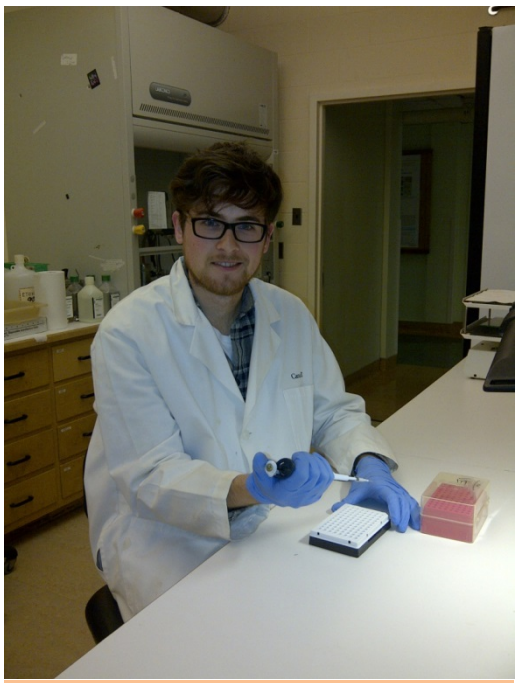
NSERC Canadian Integrated Multi-Trophic Aquaculture Network
Réseau canadien d'aquaculture multitrophique intégrée du CRSNG

CIMTAN Snippets

Promising results of an IMTA product of interest in Parkinson's disease

James Giffin, an MSc student in Biology at Dalhousie University, presented a poster at the World Parkinson Congress, which took place October 1-4, 2013, in Montreal, gathering 3,300 delegates from 70 countries.

James has been working under the supervision of **Vanya Ewart**, a research scientist from the Aquatic and Crop Resource Development Portfolio (ACRD) at the National Research Council of Canada (NRC) in Halifax, and in collaboration with **Shawna MacKinnon** (also a ACRD scientist in Halifax) and **Thierry Chopin** (CIMTAN-University of New Brunswick) on the potential bioactive properties of IMTA species and products.



James Giffin preparing samples in the laboratory (photo credit: Vanya Ewart).

The poster is entitled **"Modulation of alpha-synuclein protein folding by a marine-sourced extract"**.

Besides James, who was lead author, the authors included **Robert Richards, Cheryl Craft, Nusrat Jahan, Cindy Leggiadro,** Shawna MacKinnon, and Vanya Ewart from the NRC, Thierry Chopin from CIMTAN-UNB and **Michael Szemerda** from Cooke Aquaculture Inc. (Blacks Harbour, New Brunswick).

This has been a remarkable collaboration between the National Research Council of Canada, the University of New Brunswick, Dalhousie University and Cooke Aquaculture Inc. The Natural Sciences and Engineering Research Council of Canada has funded much of this research through Discovery Grants to Vanya Ewart and Thierry Chopin, and the CIMTAN Strategic Network and the Synergy Award for Innovation to Thierry Chopin.

One could wonder if this is a case of serendipity or, in fact, educated intuition! Protein misfolding triggers several human neurological diseases. Alpha-synuclein (α-synuclein) is a protein that is abundant in the brain. When misfolded into an amyloid form, it is implicated in Parkinson's disease. That is one situation in which marine species become very interesting. Because of variable and extreme conditions in some marine environments (such as in the Bay of Fundy), species must adapt to protect their own proteins. Therefore, they may have ways of protecting proteins that work in other situations, such as in our aging brains. With this in mind, we evaluated the effects of extracts from marine species used in IMTA on the folding stability of α-synuclein.

Activities that increased and decreased the α-synuclein fold stability were identified within a single extract. Components with these contrasting activities were separated both by precipitation and size fractionation, providing insight into the distinct sources of these activities. The effects on amyloid formation and protein structure were also assessed. Compounds identified in this way may provide sustainable marine-sourced functional foods, nutraceuticals or pharmaceuticals that directly address the protein misfolding that appears to be causative in Parkinson's disease.

Project D1P5, under the leadership of **David Speare**, at the Atlantic Veterinary College at the University of Prince Edward Island, has been working on **developing a laboratory model of Microsporidial Gill Disease of Salmon (MGDS)**, as a tool to better understand pathogen transmission between trophic levels. The group now has 4 published papers on the topic and David Speare has summarized their findings below.

MGDS is a severe seasonal disease of farmed Pacific salmon and rainbow trout (*Oncorhynchus mykiss*) caused by host inflammatory response to spore-filled cysts of the microsporidian "parasite" *Loma salmonae*. Due to its infectious stage, an environmentally resistant spore, this parasite may have the capacity to exploit aquaculture settings where the spore stage is retained. Evidence of this can be found in recirculating aquaculture systems (RAS). Whether or not an IMTA setting is favourable, or limiting, to this parasite, is unknown; the question centres around the interaction(s) of *L. salmonae* spores with filter-feeding organisms within an IMTA site. We have chosen to gain insight into this question by examining the fate of *L. salmonae* spores within blue mussels (*Mytilus edulis*). Specifically, (1) do blue mussels extract *L. salmonae* spores from the water column, and (2) what is the fate of spores within blue mussels (do they become inactivated or do they retain infectivity?).

CIMTAN PhD candidate Sarah McConnachie (left) and technical research associate Dr. Nicole Guselle (right) sampling fish following co-habitation with blue mussels previously exposed to water with *Loma salmonae* spores (photo credit: Sean Landsman).

Past research efforts have established a reliable research study model through which the pathogenesis and pathobiology of MGDS have been elucidated. *Loma salmonae* spores delivered through various means to salmonids result in established infections that proceed with relative synchrony to the hallmark final stage – the "xenoma". Morphometry (xenoma counts per gill arch) provides a measure of infection intensity as a continuous variable. However, as with many disease models of fish and other animals, the pathogen exposure (dose rate) used experimentally is often very high such that variability is reduced; high variability in disease-trial outcomes is problematic for many reasons and a specific issue, from an animal use point of view, is the necessity of using far more target organisms (salmonids). Therefore, as an initial step in our studies, our aims were to develop a reliable "ultra-low dose infection model" with minimal variability as a more realistic model through which we can study transmission of a pathogen between trophic levels in an IMTA setting.

In a first-of-its-kind result, a co-habitation study model was developed (Harkness and Speare, 2011) such that 100% of trout exposed to only a low dose of *L. salmonae* spores within the water column became infected; characteristics of MGDS developed in these fish over a time-course

typical for other high-dose models. The work benefitted from a previous finding (Lovy *et al.*, 2008) that dexamethasone suppression of specific interleukins serves to down regulate innate immunity to intracellular organisms thereby resulting in an augmented xenoma count despite low dose exposure. From this, a standard ultralow dose spore exposure protocol was developed in which target fish are pre-treated with dexamethasone *per os* (oral administration). This has proven to be a robust method, and is not influenced or negated even in the face of immunostimulants such as beta-glucans (Speare *et al.*, 2011).

The low-dose exposure model set the stage for key *proof-of-principle* studies in which the transfer of *L. salmonae* from mussels to salmonids was formally assessed. PhD student Sarah McConnachie completed a connected series of experiments (McConnachie *et al.*, 2013) in which she found that: (1) blue mussels efficiently extract *L. salmonae* spores from the water column, (2) the spores retain viability within the viscera of blue mussels and this can be used to initiate infection in salmonids, (3) spores remain viable in blue mussels for up to 7 days, and (4) although mussels extract spores from water, the water does not become bioremediated or less infective. Several infection transfer efficiency values were developed from these results and suggest that the retained infectivity of spores captured by blue mussels is very high (the spores are not being digested or otherwise inactivated).

Sarah's work continues on this theme, to study the role of environmental variables on spore uptake and transfer, and also to develop *in vitro* methods to study spore retention to replace, and improve on, *in vivo* methods currently in use. Announcements to follow! As the objectives unfold during these studies, we feel that the results will provide a useful picture of disease transmission potentials and dynamics between trophic levels within an IMTA setting.

The references of the papers are:

Lovy J., Speare D.J., Stryhn H. and Wright G.M. 2008 - Effects of dexamethasone on host innate and adaptive immune responses and parasite development in rainbow trout *Oncorhynchus mykiss* infected with *Loma salmonae*. *Fish and Shellfish Immunology* 24: 649-658.

Harkness J. and Speare D.J. 2011 - Resistance to *Loma salmonae* in rainbow trout *Oncorhynchus mykiss* subsequent to recovery from an initial low-dose cohabitation exposure. *Fish Pathology* 46: 34-37.

Speare D.J., Guselle N. and Harkness J. 2011 - Pilot study demonstating that immunostimulation with beta-glucan fails to moderate the immunosuppressive effects of dexamethasone when co-administered to rainbow trout *Oncorhynchus mykiss* experimentally challenged with the microsporidian *Loma salmonae*. *Fish Pathology* 46: 95-97.

McConnachie S., Guselle N. and Speare D.J. 2013 - Retention of viable microsporidial (*Loma salmonae*) spores within the blue mussel (*Mytilus edulis*): use of an experimental laboratory model probing pathogen transfer within a multi-trophic aquaculture setting. *Aquaculture* 376: 1-5.

Read the papers: for copyright reasons, these papers cannot be posted here, but you can contact David Speare (speare@upei.ca) and he will send you a copy for individual use.

S… S… S… Ah ha, we got your attention! You thought it was Sea, Sex and Sun…! It's even better… Check Thierry Chopin's website at http://www2.unb.ca/chopinlab/S...S...S/index.html
Marah Hardt – researcher, writer and creative consultant from California – contacted him to talk about IMTA. He also discovered that she is involved in other activities and that led to the above entry… educate yourself and have fun!

The **4th BioMarine Business Convention** was held in Halifax, Nova Scotia, Canada, September 9-12, 2013. More than 300 participants, from 21 countries, took part in a conference with a full agenda: Discovery Tours on Day 1, followed by three days with 14 sessions, an Innovation Forum, business to business (B2B) partnering meetings, keynote speakers, 2 special sessions, a plenary discussion and a live inerview of H.S.H. Prince Albert II of Monaco. There were also ample opportunities for networking and social interactions.

The panel of the "Seaweed Biorefinery" session: Pia Winberg (Shoalhaven Marine and Freshwater Centre, Australia; moderator), Pål Bakken (Seaweed Energy Solutions AS, Norway), Thierry Chopin (CIMTAN, Canada), Peter Countway (Bigelow Molecular Microbial Ecology Laboratory, USA) and Franck Hennequart (Oilean Glas Teo, Ireland) (photo credit: Véronique Erwes).

Thierry Chopin was one of the **8 selected speakers** at the **Innovation Forum** and gave a presentation entitled **"The Turquoise Revolution: Research, Development and Commercialization of Integrated Multi-Trophic Aquaculture (IMTA) and its Diversified Products"**. Thierry Chopin was also a **panelist** in the session **"Seaweed Biorefinery"**. The presentation and panel comments triggered some interesting Tweets:

- UNBSJ's Thierry Chopin rocking it at #BioMarine2013 Innovation Forum #theturquoiserevolution
- "#Seaweed isn't just #biomass. It's also integral in providing #ecosystem services" - Thierry Chopin at #BioMarine

Pierre Rioux (New Brunswick Department of Agriculture, Aquaculture and Fisheries) and Thierry Chopin (CIMTAN) in full networking activity (photo credit: Véronique Erwes).

Read the Final Report of the conference:
http://www.biomarine.org/wp-content/uploads/2013/10/Final-report-2013.pdf
The pager on the presentation of Thierry Chopin is on p. 44.
The report on the session "Seaweed Biorefinery" in on p. 98-106.

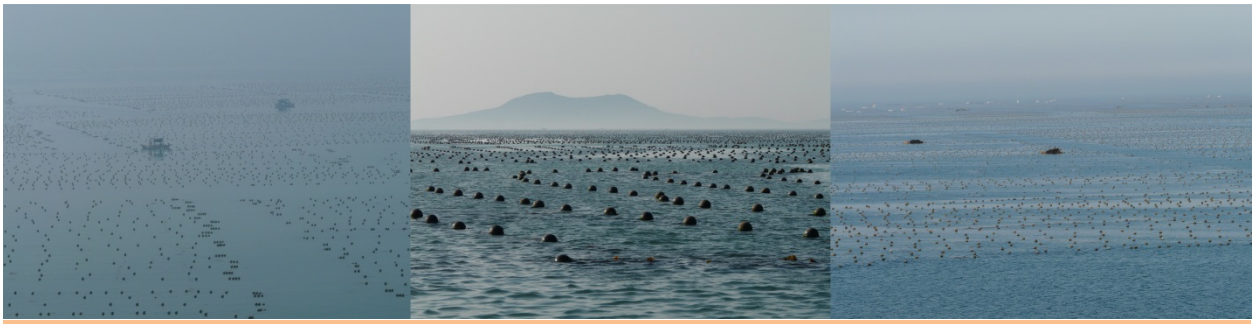
Read the Session Take Away Messages:
http://www.biomarine.org/wp-content/uploads/2013/10/BioMarine-2013-key-take-away-messages.pdf

A very interesting paper on the environmental and economic benefits of IMTA in Sanggou Bay, China, has just been published in *Aquaculture*

Shi H., Zheng W., Zhang X., Zhu M. and Ding D. 2013 - Ecological-economic assessment of monoculture and integrated multi-trophic aquaculture in Sanggou Bay of China. *Aquaculture* 410-411: 172-178.

As mentioned by the authors, many problems – including diseases, pollution and reduced biodiversity – have been encountered by the mariculture industry in China in recent decades. These problems may pose considerable challenges at the environmental, economic and societal levels. Consequently, sustainable mariculture practices providing benefits in these three areas are being considered.

As an approach designed to mitigate the ecological effects of monoculture, integrated multi-trophic aquaculture (IMTA) is attracting increased interest among researchers and commercial growers worldwide. However, sufficient quantitative assessment of IMTA is still lacking for decision makers, ecosystem managers and farmers to opt for the development of IMTA in open-water systems. In this study, the authors investigated three types of culture models in Sanggou Bay: the monoculture of kelps (*Saccharina japonica*), the monoculture of scallops (*Chlamys farreri*), and the IMTA of kelps and scallops, in order to compare the ecological and economic benefits between monocultures and IMTA.



The level of the vastly large scale of aquaculture development in Sanggou Bay is what is always striking to a visitor discovering the region for the first time (photo credits: Thierry Chopin).

Sanggou Bay, in the Yellow Sea near the city of Rongcheng, Shandong Province, is one of the most important mariculture regions in China, where Westerners interested in aquaculture development are often taken. Beyond the first bewilderment at the scale of the farming operations, one has to realize that this bay is also not without its problems. Water quality has deteriorated due to the considerable amount of mariculture, which occupies more than 50% of the area in this semi-enclosed bay. The very dense raft culture systems have led to reduced water exchange (40% reduction of the average current speed and 71% increase in the average half-life of water exchange). Fouling on the scallop, oyster, abalone, sea cucumber (and other

invertebrates) cultivation systems also reduces water exchange and oxygen availability. **Thierry Chopin** has visited Sanggou Bay twice, with Drs. **Jianguang Fang** (Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao) and **Shaojun Pang** (Institute of Oceanology, Chinese Academy of Sciences, Qingdao) and has discussed several issues with each of them. When one realizes that fed aquaculture (*i.e.* fish aquaculture) is a very small component of these large scale operations, and calculates the amount of total inorganic nitrogen excreted by the animals cultivated in Sanggou Bay, it is obvious that the large biomass of kelps does not find its nutrient requirements only from the Bay, but also from terrestrial discharges, such as agricultural, land-based aquacultural, human population waste and industrial run-offs. The decline in water quality has resulted in reduced sizes of cultivated organisms, lower production and higher mortality of animals. Moreover**,** one also has to consider the possibility that the large biomass of eroded kelp blades and *Gracilaria* filaments accumulating on the bottom in June-July each year could also generate a significant amount of organic matter deposition and decomposition in this shallow bay (the average water depth is 7-8 m). This could have negative impacts on the oxygenation levels of the sediments and the biodiversity of benthic organisms. Therefore, it is clear that more sustainable culture methods are needed and decision-makers need to be provided with data to help them select the best practices to implement. It will also be necessary to consider IMTA in a broader context of integrated coastal zone management.

Left: fish aquaculture is comparatively small in Sanggou Bay; middle: IMTA operations with seaweed harvesting in the foreground and skiffs with white sun protective cloth for invertebrate farmers in the background; right: abalone and sea cucumbers, fed with fragments of kelp blades, are cultivated in these black containers suspended between the kelp lines (photo credits: Thierry Chopin).

The authors developed an effective method to ensure the validity of the assessment of IMTA's ecological and economic benefits, the emergy analysis, which evaluates the values of nature and human economy according to the thermodynamics principles, system theory and system ecology. The nutrient removal of kelp monoculture, scallop monoculture, and kelps/scallops IMTA was 193, 49, and 202 Mt nitrogen/km^2, respectively. This indicates that IMTA was better for the environment than the other two models in terms of nutrient removal. IMTA also had the highest emdollar value, indicating that IMTA has the least negative impact on the environment among the three culture models. IMTA exhibited the highest environmental sustainability index (ESI), followed by kelp monoculture and scallop monoculture. This indicates that IMTA is more sustainable than the monocultures from both the economic and ecological aspects.

Left: harvesting of the kelp, *Saccharina japonica*, in Sanggou Bay; middle: a group of skiffs, loaded with kelps, is towed back to the processing plant; right: marine spatial planning at work with massive aquaculture fields and channels for the navigation of large commercial vessels (photo credits: Thierry Chopin).

The authors also used a cost-benefit analysis (CBA), which is a widely accepted economic tool for rational and systematic decision-making. It may also be used to assess the environmental impacts of projects from an economic perspective, particularly those with a large number of environmental externalities and effects extending over a long period of time. The prices of kelps and scallops were 4 yuan/kg and 2.8 yuan/kg in 2009, respectively. The yield of kelp monoculture was 3750 Mt/km^2, that of scallop monoculture was 4725 Mt/km^2 and that of IMTA was 7650 Mt/km^2, including 3000 Mt/km^2 of kelps and 4650 Mt/km^2 of scallops. The cost of IMTA was higher than those of the monocultures; however, the percentages of salary and wages, energy and maintenance of IMTA were all lower than those of the monocultures, indicating that IMTA is more economical than the monocultures. IMTA exhibited the highest net present value (NPV), benefit to cost ratio (BCR) and relative coefficient (RC), followed by the kelp monoculture and scallop monoculture, further demonstrating that IMTA is the optimal model with both economic and environmental benefits. RC is a new index developed by the authors to balance the benefits and the efficiency of culture models.

IMTA meal on board as the boat sails for hours in Sanggou Bay and the visitors wonder where and when the limits of the aquaculture operations will be reached. Left: kelp stew; middle: kelp snacks, abalones and sea cucumbers; right: scallops (photo credits: Thierry Chopin).

Several advantages of IMTA are potential contributors to its sustainability. First, IMTA can maximize the economic benefit, which was 25.02 million yuan/km^2, significantly higher than that of kelp monoculture (15 million yuan/km^2) or scallop monoculture (13.23 million yuan/km^2). Second, IMTA can enhance the recycling of dissolved inorganic nutrients and particulate organic matter. This is because the seaweeds in an IMTA setting can reutilize the

waste products (nitrogen and phosphorus) from the co-cultivated species (scallops) and are a renewable protein-enriched feed for them. Third, IMTA can remove more nutrients than monocultures: the nitrogen removal of IMTA was 202 Mt/km^2, higher than those of kelp monoculture (193 Mt/km^2) and scallop monoculture (49 Mt/km^2). Several studies have shown that seaweeds can act as biofilters that significantly utilize nutrients, control eutrophication and, consequently, improve the health and stability of marine ecosystems, and promote their sustainable development. Fourth, IMTA has lower economic risks than monocultures. On one hand, seaweeds have high nutrient uptake capabilities that can improve water quality and thus decrease disease/mortality in scallops. On the other hand, since IMTA is growing different species, the harvest of a diversified portfolio of species can still ensure the income and reduce market risks, even when mortality or price fluctuations are registered with one of the species. By combining environmental and economic methods (emergy and CBA analyses) in an interdisciplinary approach, this study has, therefore, clearly and elegantly demonstrated that IMTA is a sustainable mariculture practice, appropriate for a wide range of applications in China's open-water systems.

Memoirs of a GoPro

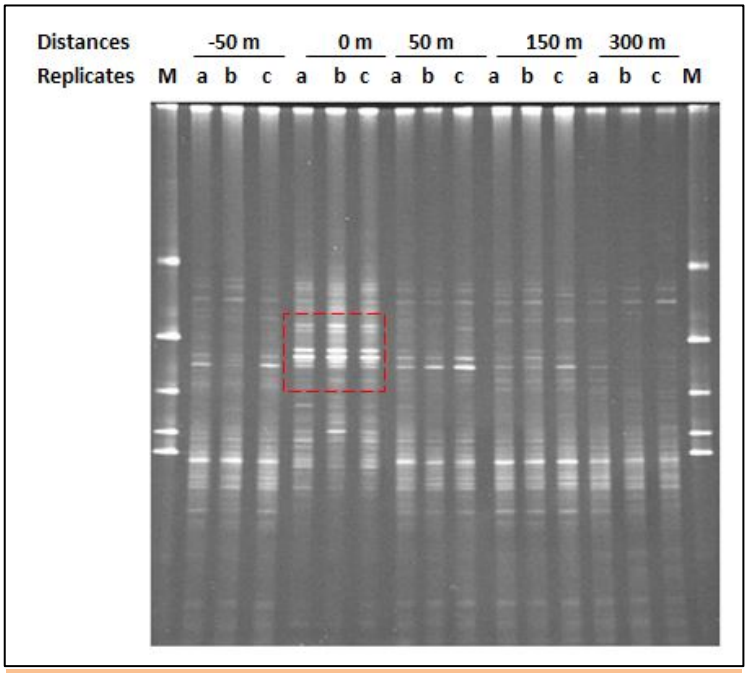
The reduced size and price and increased memory of modern video cameras, such as GoPros, have resulted in more frequent use of video documentation in research. Anyone who has used these devices to observe prolonged phenomena such as animal behaviour will be familar with the heavy-lid, tongue biting tediousness of watching the videos afterward. Often the most entertaining part of these videos is the inadvertant and unscripted documentation of bustling researchers executing the trial. The odd angles, lighting and settings generate unique and comical images.

CIMTAN researcher Gregor Reid and CIMTAN MSc graduate student Amanda Babin examine a GoPro after deployment of a sediment platform (to measure fish feces degradation) from the deck of the Canadian Coast Guard Ship (CCGS) Viola M. Davidson (photo credit: The GoPro!).

Dr. David Thumbi obtained his PhD degree in microbiology from the University of Guelph, Ontario, Canada (Department of Molecular and Cellular Biology) in 2008 where he specialized in baculovirus DNA replication. Following his graduation, David relocated to Fredericton, New Brunswick, to work for Sylvar Technologies, a company that deals with the production of baculoviruses as environmentally benign insect pest control agents. While there, David also did

David Thumbi performing sequencing analysis of bacteria isolated from aquaculture sites using a 3130xl Genetic DNA Analyzer. This technique is a key step in identification of new species (photo credit: Ben Forward).

some research with the Department of Natural Resources (NRCAN-CFS), where he contributed to genome sequencing and analysis of several baculovirus species. David joined CIMTAN last year as a postdoctoral fellow to work on the D1P3 project led by Dr. Shawn Robinson at the St. Andrews Biological Station (DFO) on the role of bacteria in nutrient recycling. David is based in the Department of Food, Fisheries and Aquaculture (FFA) at the Research and Productivity Council (RPC) in Fredericton, under the supervision of Dr. Ben Forward and the co-supervision of Dr. Thierry Chopin in the Department of Biology at the University of New Brunswick in Saint John (UNBSJ). His research focus is on bacterial profiling and species identification in samples collected from aquaculture sites using molecular tools such as Denaturing Gradient Gel Electrophoresis (DGGE) and DNA sequencing approaches. To date, David has contributed in sampling and identification of numerous bacterial populations in water column and benthic samples at sites in the Passamaquoddy Bay. The spatial-temporal profiles of benthic samples indicate variations in bacteria abundance with distance from the site and the predominant species show high sequence similarities to members in the group *Gamma (γ)*-, *Epsilon (ε)*-, and *Delta (δ)* - proteobacteria.

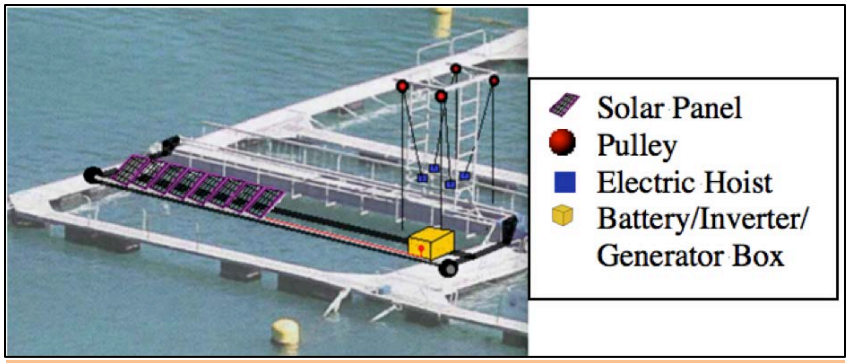


Spatial bacterial profiles, using Denaturing Gradient Gel Electrophoresis (DGGE), of benthic samples collected in August 2013 at an aquaculture site in Passamaquoddy Bay, New Brunswick, Canada. Triplicate samples (a, b and c) were collected at various distances including -50 m (upstream), 0 m, 50 m, 150 m and 300 m (downstream) from the site. Gel markers (M) are indicated in the end wells for reference. Each band represents a different bacterial species and the most abundant species are represented by brighter (dense) bands. The dominant species at the site (0 m) are depicted in a red box and their abundance decreases when moving away from the site (photo credit: David Thumbi).

First CIMTAN member quote of the month: "Working with CIMTAN has made me appreciate the value of the marine ecosystem as a natural resource for novel microbial species. The key is to know who they are and what role they are playing in the cycling of nutrients." (*David Thumbi, CIMTAN postdoctoral fellow*).

Adam Gray canoeing in the Canadian Rockies (photo credit: Crystal Cook).

Adam Gray joined CIMTAN in September 2011, and is working towards a Master of Applied Science (MASc) in Mechanical Engineering at the University of Victoria, British Columbia. Originally from Calgary, Alberta, Adam obtained his Welding Engineering Technology Diploma from the Southern Alberta Institute of Technology (SAIT Polytechnic) in 2006, and his B. Eng. in Mechanical Engineering from the University of Victoria in 2011. Studying under the supervision of Dr. Curran Crawford, his research is focused on the integration of renewable energy and demand side management. It is being applied at the IMTA Kyuquot SEAfoods Ltd. facility, where a renewable energy system has been designed and is in the procurement and installation phase. Specific challenges related to the IMTA environment, as well as rapidly varying electrical loads, required the use of a detailed MATLAB model to select the optimal system. The physical layout of the system is also unconventional in the renewable energy sense, and required the design of a SEATram Outrigger module to support the system components. Once the renewable energy system is installed and commissioned, the system performance will be measured and used to validate the MATLAB model. Due to the large intermittent electrical loads imposed on the system by large sorting winches, conventional design simulations resulted in undersized system designs by as much as 25%. This was addressed by using a MATLAB simulation with a higher resolution time-step for the optimization, resulting in a system with a 6360 Ah battery bank and a 2.75 kW solar photovoltaic array, which should satisfy the design loads year-round. The system should also eliminate approximately 12,000 kg of greenhouse gas emissions compared to an equivalent diesel powered system. When not working on his thesis, you will likely find Adam enjoying the beautiful Canadian West Coast, hiking, bicycling and surfing whenever he gets the chance.



Sketch of the SEATram Outrigger supporting renewable energy system (photo credit: Brian Vincer).

Second CIMTAN member quote of the month: "The unique IMTA environment has required us to think of unconventional solutions to deliver a robust, effective and scalable renewable energy system to aid in farm operations while reducing greenhouse gas emissions." (*Adam Gray, CIMTAN MASc candidate*).