



# Bulletin

A warm Halifax welcome to  
**Aquaculture Canada  
& Sea Farmers  
2017 Conference**

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Association Aquacole du Canada

2017-2



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

### **2017-2**

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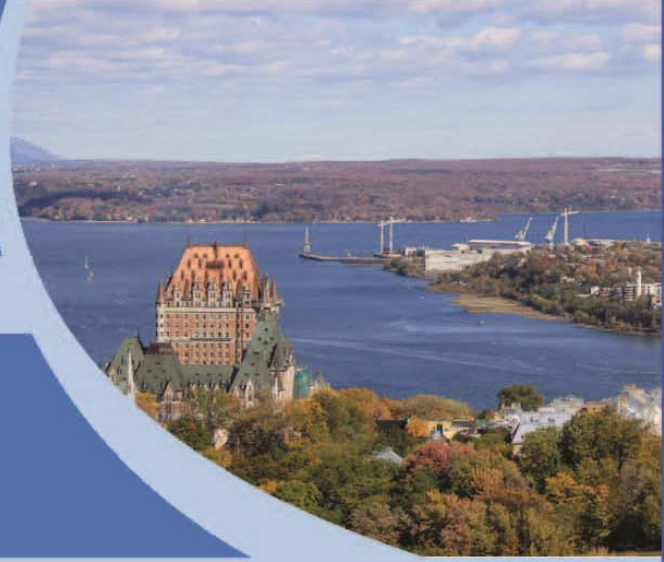
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## 2017 RESEARCH AWARD OF EXCELLENCE

Réjean Tremblay has been a professor of aquaculture at the Institut des sciences de la mer (Université du Québec à Rimouski) since 1999. He is involved in several research projects with the training of 40 MSc and PhD students working presently in the production and the management of aquatic resources. He is co-author of 118 scientific papers and 41 technical reports. By his participation in the administrative council of several organizations, his experience also includes development of fish and shellfish farming and government services. He holds a BSc in biology from UQAC, an MSc in oceanography from UQAR and a PhD in biology from Université Laval. He's a specialist in ecophysiology applied to aquaculture in shellfish and fish farming and also in lobster enhancement. His laboratory participates each year in the AAC meeting by several presentations. He is an active advocate of communication between researchers and industry to develop strong scientific quality research programs oriented to technological transfers.



Réjean Tremblay est professeur en aquaculture à l'Institut des sciences de la mer (Université du Québec à Rimouski), depuis 1999. Il a été impliqué dans plusieurs projets de recherche intégrant la formation jusqu'à maintenant de 40 étudiants MSc et PhD, qui travaillent dans la production et la gestion des ressources aquatiques. Il est coauteur de 118 articles scientifiques et 41 rapports techniques. En participant à différents conseils d'administration, il est impliqué également directement dans le développement des fermes aquacoles et le service gouvernemental. Il a un BSc en biologie de l'UQAC, une MSc en océanographie de l'UQAR et un PhD en biologie de l'Université Laval avec une spécialité en écophysiologie appliquée à l'aquaculture des bivalves et des poissons, mais également sur l'ensemencement du homard. Il croit fortement à la communication entre chercheurs et aquaculteurs afin de développer des programmes de recherche de grande qualité scientifique ayant un fort potentiel de transfert technologique.



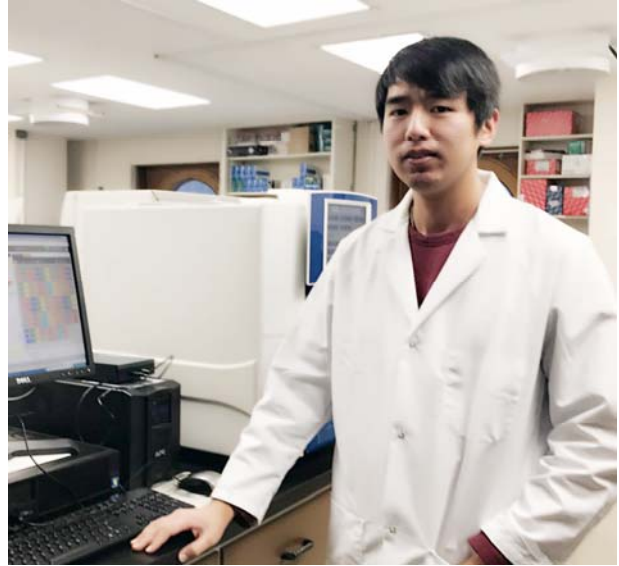
## 2017 STUDENT AWARD RECIPIENTS

### 2017 Scholarship Recipients

Garth Covernton (University of Victoria)



Xi Xue (Memorial University)



### 2017 Student Poster Presentation Award

Holly Fisher (Dalhousie University)



### 2017 Student Oral Presentation Award

Jenny Weitzman (Dalhousie University)



## **Aquaculture Canada & Sea Farmers Conference May 29 - 31, 2017**

For our annual general meeting in 2017 we partnered with the Aquaculture Association of Nova Scotia to put on the Aquaculture Canada / Sea Farmers Tradeshow and Conference at the World Trade and Convention Center in Halifax, from May 28 to 31. The Province of Nova Scotia was a presenting sponsor and co-host of the event.



Under the theme of “Cultivating Our Future”, we had keynote presentations from Henry Demone (Chair, High Liner Foods), George Chamberlain (President, Global Aquaculture Alliance), Linda Sams (Head of Sustainability, Tassal) and Jon Grant (NSERC-Cooke Industrial Research Chair in Sustainable Aquaculture, Dalhousie University), as well as 15 technical sessions and a tradeshow with 36 exhibitors. We also tasted seafood products donated and proudly displayed by Canadian farmers at multiple social venues: the President’s Reception, the Dr. Joe Brown BBQ and Silent Auction, the AANS-sponsored Nova Scotia Night, and our Gala dinner. A total of 405 people registered for the conference, and through sponsorships, auctions and the sale of raffle tickets, we raised \$11,400 for our student endowment fund to support student awards and scholarships.

Réjean Tremblay (Institut des sciences de la mer, Université du Québec à Rimouski) was presented with the AAC’s 2017 Research Award of Excellence at our closing Gala dinner. Congratulations to him, and also to Xi Xue (Memorial University of Newfoundland) and Garth Covernton (University of Victoria) for receiving AAC student scholarships, and to Jenny Weitzman and Holly Fisher (both Dalhousie University), for receiving the awards for Best Student Presentation and Best Student Poster, respectively.

Putting on a conference like this requires a big team, and there was no shortage of people who helped. I’d like to express my personal thanks in this regard to Joanne Burry (AAC conference manager), Catriona McLanaghan (AAC office manager), Joanne Liutkus (Program Committee Chair), and Tom Smith (AANS Executive Director), but in doing so also acknowledge the many people who contributed their time on organizing committees and as session chairs. I also thank the many sponsors who contributed seafood or made cash donations to offset our costs. And finally thanks to Helen Gurney-Smith (Publications Committee Chair) and Tara Daggett (Bulletin Editor) for putting this bulletin together.

Tillmann Benfey (AAC President, 2016-2017)

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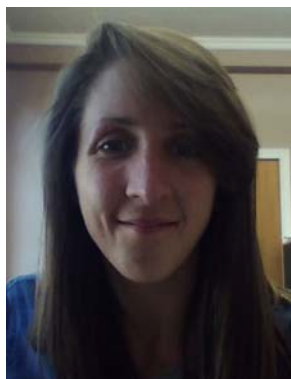
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## **PRESCRIPTIVE POLICY: A SERVICE PROVIDER'S PERSPECTIVE OF SEDIMENT COLLECTION FOR ENVIRONMENTAL MONITORING AT MARINE FINFISH FARMS**

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### **Abstract**

In Atlantic Canada, regulatory agencies have relied on the use of sulphides to monitor the environmental performance of marine finfish farms. Since the implementation of the Aquaculture Activities Regulations (AARs) by the Department of Fisheries and Oceans Canada (DFO) in 2015, harmonization efforts have been made to align provincial Environmental Monitoring Programs (EMPs) with the AARs to avoid duplication and standardize methodologies. The alignment process has resulted in two significant alterations to the provincial EMPs which include: 1) the incorporation of sample acceptability criteria to the soft-bottom protocol and 2) the implementation of a hard-bottom protocol which utilizes visual indicators (e.g., bacterial mats) to identify impact. Additionally, in 2016, provincial EMPs proposed a decision tree to select sediment sampling devices based on site-specific conditions (e.g., depth, current, and substrate type). Given the limited knowledge and practice of the various samplers listed in the decision tree in Atlantic Canada, marine farms may inadvertently be classified as hard bottom sites as research remains on-going for the establishment of equivalent thresholds of visual indicators with sediment sulphide concentrations. There is therefore a requirement to re-evaluate the inclusion of a decision tree where science advice is limited for site-specific conditions in Atlantic Canada.

### **Introduction**

The use of sediment sulphides to monitor environmental performance of marine finfish farms is accepted and employed in Atlantic Canada. The overall objective

is to retrieve the required volume of undisturbed sediment that is representative of the benthic environment at each sampling location [New Brunswick Department of Environment and Local Government (NBDELG), 2016a; Nova Scotia Department of Fisheries and Aquaculture (NSDFA), 2016a]. There is a preference to employ the soft-bottom protocol as correlation between sediment sulphide concentration and environmental impact is established (NBDELG, 2016b; NSDFA, 2016b). Marine finfish sites were traditionally situated in sheltered and shallow coves or inlets, categorized as depositional areas, where sediment samples could easily be collected via the use of divers and core tubes. However, improvements in marine net pen engineering have enabled marine finfish producers to establish sites in exposed coastal zones (e.g., erosional areas) which are generally accompanied by greater depths, coarser substrate and / or stronger currents. These marine rearing conditions have triggered many jurisdictions to limit the use of divers and adopt the use of surface-deployed equipment as an appropriate method for sediment collection (DAFF, 2008; SEPA, 2008; DFO, 2015; NBDELG, 2016a; NSDFA, 2016a).

To ensure sediment samples collected by surface-deployed equipment are representative of benthic conditions, sediment quality guidelines [Environment Canada, 1994; United States Environmental Protection Agency (U.S. EPA), 2001] were implemented by NBDELG in 2014 followed by NSDFA and the Department of Fisheries and Oceans Canada (DFO) in 2015. A decision tree was also included in the provincial Environmental Monitoring Programs (NBDELG, 2016a; NSDFA, 2016a) in 2016 to assist service providers in choosing an appropriate sampler that meets the sediment quality guidelines under site specific conditions (e.g., depth, current, and substrate type). Although the decision tree remains as guidance, provincial and federal governing agencies continue to actively discuss the implementation of the decision tree as a regulatory requirement. This is of concern due to the limited knowledge on the use, function and performance of the various samplers in Atlantic Canada. It is also important to note that many of the sampler options in the decision tree are currently being investigated as part of an Aquaculture Collaborative Research and Development Program (ACRDP) project (Page et al., 2015). In the preliminary trials, many of the samplers tested were found to not operate or collect samples successfully. The inclusion of a decision tree as a regulatory requirement not only precedes scientific advice, it also limits the tools available for service providers.

A brief literature review revealed that the same sampler may vary in size, shape, operation and performance between sources (e.g., manufacturer specifications, guidance documents). Consequently, an inappropriate sampler may inadvertently be approved for use and the site falsely categorized as hard bottom due to the limited success in retrieving acceptable sediment samples.



This is of concern as research remains on-going for the establishment of equivalent thresholds of visual indicators (e.g., bacterial mats) to sediment sulphide concentrations. The main objective of this review was to identify the differences between the manufacturer specifications, guidance documents, and the prescribed regulatory requirements of a select group of surface-deployed equipment. A secondary purpose of this review was to identify marine finfish sites incorrectly categorized for bottom type (e.g., soft bottom, mixed bottom or hard bottom) based on data from 2015 and 2016 environmental monitoring surveys.

## **Materials and Methods**

For the comparative review, the information collected was compiled based on the three (3) main sources consulted, which included the following:

- 1) Supplier specifications (supplier websites, personal communications);
- 2) Guidance documents (Environment Canada, 1994; Mudroch & MacKnight, 1994; U.S. EPA, 2001; Environment Canada, 2002);
- 3) Provincial and Federal regulatory requirements (NBDELG, 2016a; NSDFA, 2016a; DFO, 2017).

The suppliers (i.e., Hoskin, WildCo, Rickly Hydrological, KC Denmark, and OSIL) were chosen based on local or regular dealers who were used or contacted in the past (refer to Section 6.0 - References for sourcing information). Although core-type devices are known to collect relatively undisturbed and intact sediment samples, it has been documented that few can successfully operate in substrates with sand, gravel, clay or till (Environment Canada, 1994) whereas grab type devices are known to function better in the presence of larger grained sediments (U.S. EPA, 2001). Core-type devices were therefore not considered in this review. Grab type devices, such as Peterson, Smith-McIntyre, and Shipek grabs, were also excluded from this review as these devices demonstrate sampling limitations due to lack of access to the sediment sample for sub-sampling or a clamshell pivot which significantly disturbs the sediment-water interface. In the end, a total of five (5) sediment samplers were chosen and the information was compiled in a similar manner as categorized in the provincial and federal decision trees (i.e., based on depth, currents, and substrate type).

For the field review component, success rates for the collection of undisturbed sediment samples from environmental monitoring surveys were compared

between 2015 (no decision tree) and 2016 (inclusion of a decision tree) for the same eight (8) marine finfish sites.

## Results

For the comparative review, the manufacturer recommendations in relation to depth, currents, and substrate type of the five (5) chosen sediment samplers were assembled. The same type of information was then retrieved from the guidance documents and compared to the manufacturer specifications as shown in Table 1. The major difference between the manufacturer specifications and the guidance documents occurs within the substrate type category. According to the guidance documents, most of the sediment samplers in this review would only be suitable in sandy substrates or finer, except for the box corer which requires a minimum depth of one (1) metre of unconsolidated sediment for the collection of an undisturbed sediment sample.

**Table 1. Comparative review: supplier information vs. guidance documents**

	Grab Type	Petite Ponar	Standard Ponar	Van Veen	Ekman / Birge-Ekman	Box Corer
	COMPARISON	SUPPLIER INFORMATION VS. GUIDANCE DOCUMENTS				
Criteria	1. Depth	Shallow / Deep	Shallow / Deep	Shallow / Deep	Shallow / Deep	Shallow / Deep
	2. Currents	Weak	Weak / Strong	Weak / Strong	Weak	Weak
	3. Substrate Type	Soft / Hard - Loose clay, consolidated marl, sand, mixtures of sand, stone, coarse debris, gravel <b>From soft, fine-grained to firm, sandy material</b>	Soft / Hard - Loose clay, consolidated marl, sand, mixtures of sand, stone, coarse debris, gravel. <b>From soft, fine-grained to firm, sandy material</b>	Soft / <b>Hard</b> - Soft substrates to sand <b>Fine-grained to coarse which include sand, silt, and clay</b>	Soft - Finely divided muck, clay, mud, ooze, submerged marl or fine peaty materials. Not intermixed with sand and/or stones. <b>Sand or mixture of silt and sand.</b>	Soft / Hard - Finely divided muck, clay, mud, ooze, submerged marl or fine peaty materials. <b>At least 1 m depth of unconsolidated sediment</b>
	4. Disturbance	Low / Little bottom disturbance	Low bottom disturbance	Relatively undisturbed sediments	Low bottom disturbance	Minimum disturbance

**Blue:** Not previously specified by the Manufacturers **Red:** Recommended by the Guidance Documents in contravention to the Manufacturers **Grey:** Not recommended by the Guidance Documents in contravention to the Manufacturers.

It is assumed that the guidance documents were assembled based on extensive consultations with experts and regular users of surface-deployed grab samplers, and as such, the recommendations made by the guidance documents are likely valid. The recommendations provided by the guidance documents were accepted then further compared to the same type of information retrieved from the regulatory requirements (e.g., provincial and federal decision trees) as

shown in Table 2. Once more, the major difference occurred between the accepted recommendations provided by the guidance documents in comparison to the regulatory requirements within the substrate type category. In this case, the same samplers would only be suitable in fine-grained sediments which essentially consist of mud.

**Table 2. Comparative review: guidance documents vs. regulatory requirements**

	Grab Type	Petite Ponar*	Standard Ponar*	Van Veen*	Ekman / Birge-Ekman*	Box Corer
	COMPARISON	GUIDANCE DOCUMENTS VS. REGULATORY REQUIREMENTS				
Criteria	1. Depth	Shallow / Deep	Shallow / Deep	Shallow / Deep	Shallow / Deep	Shallow / Deep
	2. Currents	Weak	Weak / Strong	Weak / Strong	Weak	Weak / <b>Strong</b>
	3. Substrate Type	Soft / Hard - From soft, fine-grained to firm, sandy material	Soft / Hard - From soft, fine-grained to firm, sandy material	Soft / Hard- Fine-grained to coarse which include <b>sand*</b> , silt, and clay (e.g., mud)	Soft - Finely divided muck, clay, mud, ooze, submerged marl or fine peaty materials, and sand or mixture of silt and sand.	Soft - Finely divided muck, clay, mud, ooze, submerged marl or fine peaty materials, and min. 1 m depth of sediment <b>and sand</b>
	4. Disturbance	Low / Little bottom disturbance	Low bottom disturbance	Relatively undisturbed sediments	Low bottom disturbance	Minimum disturbance

**Blue:** Not previously specified **Red:** Recommended by the Regulatory Agencies in contravention to the Guidance Documents **Grey:** Not recommended by the Regulatory Agencies in contravention to the Guidance Documents.

For the field review, the categorization of the same eight (8) sites were compared between 2015 and 2016. In 2015, only the sediment quality guidelines were part of the regulatory requirement and as such, four (4) sites were analyzed as per the soft-bottom protocol, one (1) as mixed, and three (3) followed the hard-bottom protocol. A total of 45 acceptable sediment samples were collected. The same eight (8) sites were sampled in 2016 abiding by the sediment quality guidelines in addition to a decision tree, which resulted in all eight (8) sites being analyzed as per the hard-bottom protocol as zero (0) acceptable samples were retrieved (Table 3).

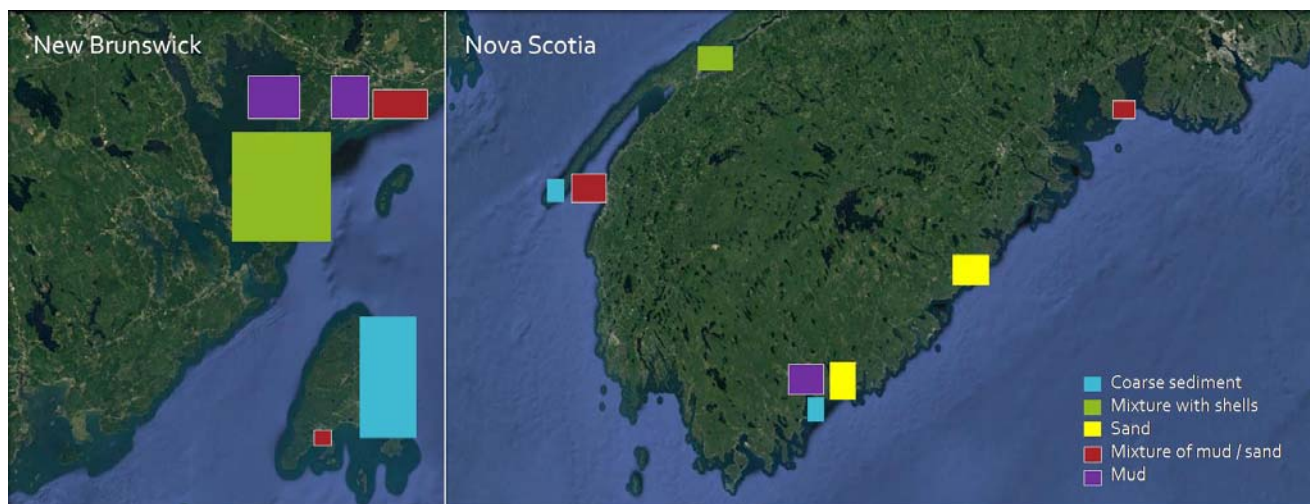
**Table 3. Benthic monitoring protocols followed during environmental monitoring surveys: 2015 vs. 2016**

Sampling Year	Protocol Followed		
	Soft	Mixed	Hard
2015	4	1	3
2016	0	0	8



## Discussion

It is evident from the comparative review that all three (3) sources of information differ in what is considered ideal conditions for these five (5) sediment samplers to collect an undisturbed sediment sample. In most cases, natural benthic conditions are composed of complex mixtures of substrate types (Fig. 1) and generally exhibit an uneven plane thus potentially altering the efficacy of the appropriate sampler. Shell debris, naturally occurring or discarded waste (e.g., scallop and mussel shells) are also generally not included in these reviews yet should be considered as a significant contributor to the success or failure of the sampler in question. In addition, the field review has shown that, in some cases, a sediment sampler chosen based on current regulatory requirements does not necessarily achieve the overall goal of retrieving the required volume of undisturbed sediment sample in comparison to previous years.



**Figure 1**  
**General classification of substrate types.**

## Conclusion

Although the sediment quality guidelines are valued as an essential tool to ensure sediment samples are collected in a consistent and representative manner of benthic conditions, the decision tree as a regulatory requirement is viewed as a limiting factor by service providers. It is generally understood that climatic, oceanographic, and benthic conditions, to name a few, differ between marine farms, yet it should also be acknowledged that these conditions significantly differ within the marine farms themselves. As a result, each marine farm should be evaluated on a case-by-case basis which increases the difficulty

in identifying an appropriate sampler based on categorized site conditions as shown in the current decision tree. Mudroch and MacKnight (1994) recognized the difficulty in choosing an appropriate sampler without any knowledge of site conditions and suggested collecting all available information, in addition to consulting with experienced personnel, for the final choice of an appropriate sampler. Service providers are, as such, experienced personnel with extensive knowledge of localized and site-specific conditions based on repeated visits. Flexibility should therefore be given within the regulation as to what service providers deem fit as an appropriate sampling device based on their knowledge of the marine farm in question.

Rather than restrict the tools available to collect an undisturbed sediment sample, a set of minimum requirements should be included as part of the regulations. Environment Canada (2017) has acknowledged new users of surface-deployed equipment, including the aquaculture industry, and have thus confirmed a review of their current guidance document is required. In the meantime, Environment Canada (1994) published a list of factors for the ideal sampler that remains applicable to this day. Replacing the prescriptive requirement of the decision tree, which is restrictive and inefficient, with Environment Canada's (1994) minimum set of requirements will allow flexibility in using various devices and in experimenting with other devices as long as the sediment-quality guidelines are achieved. Additionally, this would allow scientific and regulatory agencies (e.g., Environment Canada, Department of Fisheries and Oceans Canada) time to synthesize and evaluate applied field data specific to the aquaculture industry in Atlantic Canada for regulatory analyses.

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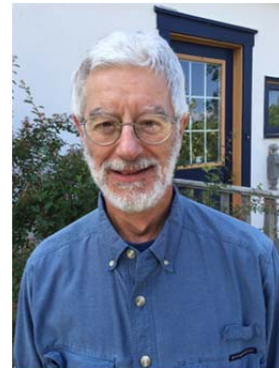
# SERIAL KNOTS IN MUSSEL CULTURE ROPES INCREASE SPAT COLLECTION AND REDUCE DUCK-RELATED MORTALITY

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## Abstract

Predation by sea ducks is a threat to mussel culture in many sites. Anti-predator refuges are required to achieve acceptable survivorship. Loosely knotted spat collectors exhibit crevices and substrate features that may act as refuges against ducks. Our experiments tested their efficiency at promoting spat survivorship. The knots used were the chain sinnet and variants thereof. Two collector setups were tried, the standard autocollector method used in Cascapédia Bay in 2015 and U-shaped collectors hanging from long lines in 2016.

In the 2015 experiment, control ropes collected 1.5 times as much as unknotted controls did. In June 2016, mussel abundance was roughly 10 - 15% of initial level on knotted ropes but was less than 5% on control ropes. Survivors and spat smaller than 0.5 cm in spring provided a residual population which grew during the summer and reached about 1500 individuals per 30.5 cm in October 2016 (versus roughly 400 on control ropes). In the 2016 experiment, the chain sinnet and its variants collected 2900 and roughly 5900 individuals per 30.5 cm, respectively, with slight differences between variants. This was a 2- to 4-fold increase as compared to controls.

Adding serial knots in spat collector ropes increases spat collection, which is useful wherever spat is in short supply. Survivorship also increased, but to an uncertain level, as this study is still under way.

## Introduction

Predation by sea ducks is a threat to mussel culture in many sites (Dionne et al., 2006). Various techniques have been developed to reduce duck-related mortality. Frightening methods such as boat chasing, sound deterrents, laser beams, etc., are of limited efficiency in the long term because of bird habituation (Dionne et al., 2006; Varennes et al., 2013). On the other hand, methods based on physical exclusion such as protective socking material (Dionne et al., 2006) and exclusion nets around mussel rafts are highly efficient (Varennes et al., 2013) but are costly.

High efficiency of deterrent methods is required because total costs of sleeving is a function of initial sleeving density (among other factors) and spat population density is adjusted to some desired (low) level to minimize mussel fall-off as growth proceeds. Any further reduction is of course detrimental to yield. In Cascapédia Bay, however, mussel growers use the autocollector technique (also described as the self-regulated collector technique; Lachance-Bernard et al., 2010). Cascapédia Bay is an open body of water in Baie des Chaleurs, Quebec. It has large fetch from all directions except from the North. Prevailing wind direction restricts the amount of time available for work at sea. Therefore, mussel growers in Cascapédia Bay have developed the autocollector method, whereby mussel spat is left on the spat collectors – autocollectors – for the entire production cycle. Because sleeving and population density adjustment are bypassed, the amount of time devoted at operations at sea is minimal. For a short summary of the structure and functioning of an autocollector longline, see Lachance-Bernard et al. (2010).

According to the autocollector method, initial spat numbers are typically well above optimal stocking density. In the absence of significant predation by ducks, population density decreases from high initial levels to lower numbers, about 15 - 20% of initial levels, because of mussel fall-off as individual growth proceeds (Lachance-Bernard et al., 2010). Thus, in the absence of predation, competition entails the loss of a large proportion of mussels anyway. It follows that with autocollectors anti-predator strategies need not be highly efficient to be satisfactory. Assuming that size-related effects are negligible, only about 20% survivorship is required. The problem arises because ducks drive down population density to levels well below the usual 15 - 20% figure.

Our approach to the problem was to take advantage of a natural feature of mussel population dynamics. In many natural situations, mussel survivorship is enhanced by crevices and substrate features (Bergeron and Bourget, 1986; Bertness et al., 2002). We mimicked the effect of crevices by using loosely



knotted spat collector ropes. Knots provide troughs, hollows and ridges analogous to roughness elements of a natural substrate. We tested the hypothesis that hollows and troughs of knots would provide shelter to spat to a point where survivorship would reach acceptable levels.

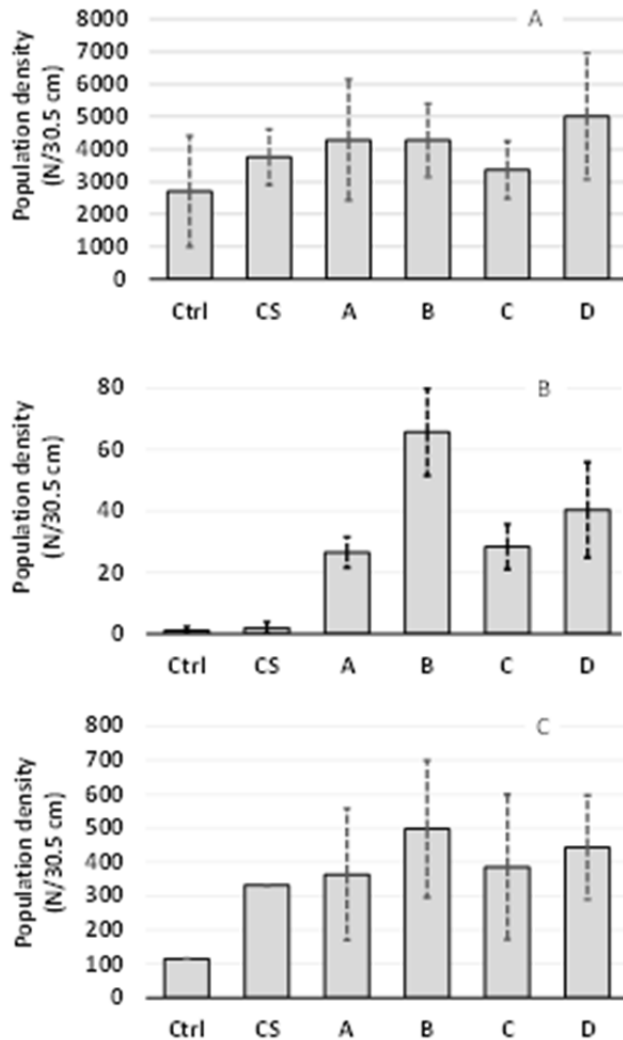
## Methods

The study site is located in Cascapédia Bay. The knots tested were the chain sinnet and variants thereof, which were compared against unknotted controls (Fig. 1). These are serial knots which can be knotted easily for spat collection and socking, and undone easily at harvest. The chain sinnet had two loops per 30.5 cm of knotted collector length. The variants were more complex configurations, based on the general principle of the chain sinnet (i.e., serial knots that can be locked for culture operations and undone when required). Two suspended culture variants were used, the standard autocollector method used in Cascapédia Bay in 2015 and U-shaped collectors hanging from long lines in 2016.

Autocollectors were immersed on two replicated longlines in early July (2015) or late June (2016) and were sampled after 3.5 months and 11 months to assess initial conditions and survivorship after duck migration, respectively. The 2015 longlines were further sampled in October 2016 and in May 2017. We sampled 35.5-cm segments of knotted collectors at high and low positions along the collectors (two replicates of each). Mussels smaller than shell length  $L = 0.5$  cm were not included in samples. Estimates of survivorship after 11 months were based on the abundance of  $L \geq 1.5$  cm mussels.



**Figure 2**  
Immersion of knotted collectors in 2015 – standard autocollector configuration.



**Figure 2**  
**2015 experiment. Effect of rope configuration on mussel abundance. Panel A, in October 2015 (shell length  $L \geq 0.5$  cm). Panel B, in June 2016 (shell length  $L \geq 1.5$  cm). Panel C, in June 2016 (shell length  $0.5 \leq L < 1.5$  cm). Treatment labels: Ctrl, controls; CS, chain sinnet; and A, B, C and D, variants of the chain sinnet.**

## Results and Discussion

In the 2015 experiment, initial population density  $N$  on control ropes averaged  $N = 2704 \pm 1707$  individuals per 30.5 cm (Fig. 2A; mean  $\pm$  SD;  $L \geq 0.5$  cm). Mean population density on the chain sinnet and its variants ranged between  $N = 3363 \pm 899$  and  $N = 5011 \pm 1935$  individuals per 30.5 cm, with little indication of variability among knotted treatments. Therefore, initial spat abundance was 1.5 fold higher on knotted rope than on controls.

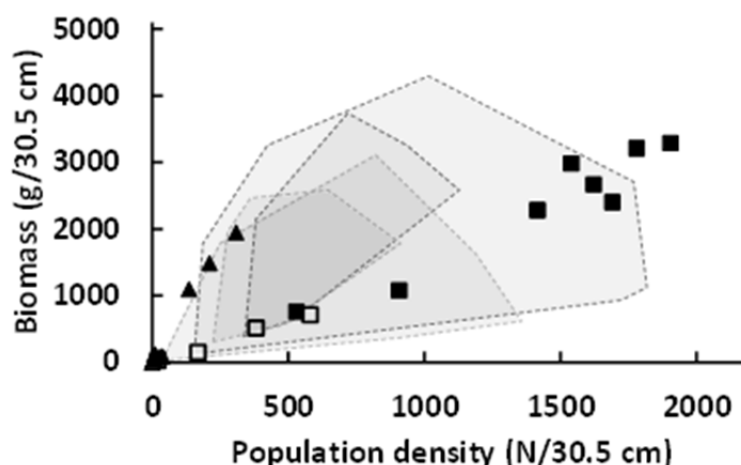
In June 2016, mean population density on control ropes was  $N = 1.1 \pm 1.3$  individuals per 30.5 cm (Fig. 2B;  $L \geq 1.5$  cm). Population density on the chain sinnet ropes also was very low and averaged  $N = 1.9 \pm 1.9$  individuals per 30.5 cm. Population density on the variants was higher and ranged between  $N = 27 \pm 5$  and  $N = 66 \pm 14$  individuals per 30.5 cm. There was indication of variability among the variants. This will be assessed statistically elsewhere. Therefore, there was evidence that survivorship increased with the presence of knots. Population density of survivors ( $L > 1.5$  cm mussels only), however, remained much lower than required for routine operations.

In June 2016, we also assessed the abundance of mussels  $0.5 \leq L < 1.5$  cm (Fig. 2C). We had only one data point for the controls ( $N = 115/30.5$  cm) and the chain sinnet ropes ( $N = 332/30.5$  cm). Mean abundance on the variants varied between  $N = 364 \pm 193/30.5$  cm and  $N = 498 \pm 202/30.5$  cm, again with little evidence of variability among the variants. Spat abundance of this size class provided additional evidence of a protective

effect of knots, especially the variants of the chain sinnet. Therefore abundance after 11 months was roughly 10 - 15% of initial abundance on knotted ropes but was less than 5% on control ropes.

To assess the situation further, we monitored autocollectors in October 2016 and May 2017 ( $L \geq 1.5$  cm shell length mussels). Results are shown in Fig. 3, where biomass ( $B$ ) is plotted as a function of population density. Such biomass-density diagrams were developed in plant ecology (Westoby, 1984) and are

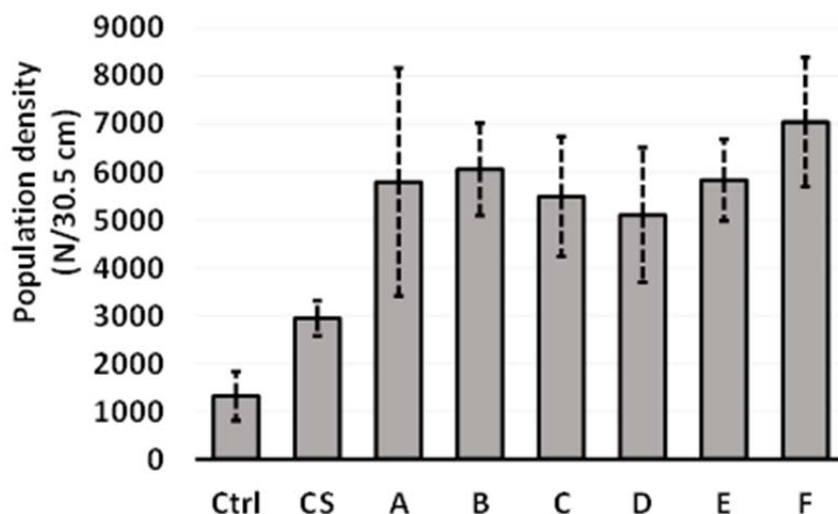
useful for the analysis of cultured bivalves as well (e.g., Fréchette et al., 2000; Fréchette et al., 2010; Fréchette et al., 2013; Lachance-Bernard et al., 2010). The four shaded areas in Fig. 3 are limited by the envelopes of individual data points obtained by Lachance-Bernard et al. (2010) for two unknotted autocollector longlines sampled at two depths for the whole production cycle in Cascapedia Bay ( $L \geq 1.5$  cm). The samples were obtained from 2004 through 2007, before duck predation became a significant issue. Therefore, these surfaces provide a template of expected B-N data points on control ropes in the absence of duck predation. They act as a background picture of predator-free autocollector yield. Thus, the performance of various rope treatments in 2016 and 2017 can be assessed from the position of data points in the B-N space shown in Fig. 3.



**Figure 3**  
**2015 experiment ( $L \geq 1.5$  cm).** Biomass-density diagram for longline 222 in October 2016 (squares) and May 2017 (triangles). Open symbols show the controls and chain sinnet, solid symbols show the variants. The grey areas depict the distribution of data points for unknotted autocollectors from 2004 through 2007, when duck predation was negligible.

The data points in Fig. 3 for the October 2016 and May 2017 samples are shown by squares and by triangles, respectively. Controls and chain sinnet are shown by open symbols and the variants are shown by solid symbols. Generally speaking, abundance on longline 223 was one order of magnitude lower than abundance on longline 222, although the ranking between the treatments did not appear to change much (detailed analyses will be provided elsewhere). Therefore, the protective effect of knots appeared weak on longline 223. The reasons for such a difference between the replicate longlines are unclear.

On longline 222, however, the situation was quite different. In October 2016 most data points for the variants were located near or beyond the outer fringe of the template areas of B-N data points. The controls and chain sinnet ropes, however, were located well inside the template areas, with maximum abundance at  $N = 581/30.5$  cm. This suggests that the mussel population on the variants had largely recovered from the predation episodes. In May 2017, mussel abundance on the controls and the chain sinnet were very low (in some cases, we had  $N = 0/30.5$  cm) and B-N data points were located near the origin. Mean abundance on the variants, however, was  $N = 186 \pm 126/30.5$  cm and



**Figure 4**  
**2016 experiment. Effect of rope configuration on initial mussel abundance.** Treatment labels: Ctrl, controls; CS, chain sinnet; and A, B, C, D, E and F, variants of the chain sinnet.

most B-N data points were located near the upper limit of the template areas. This suggests that mortality was significant during the second winter of the production cycle but that knots provided a reasonable level of protection and that mean mussel size was not negatively affected by predation.

In the 2016 experiment, control ropes collected about 1300 individual spat per 30.5 cm. The chain sinnet and its variants collected 2900 and roughly 5900 individuals per 30.5 cm, respectively, with slight differences between variants (Fig. 4). This is a 2- to 4-fold increase as compared to controls.

## Conclusion

Adding serial knots in spat collector ropes increases spat collection, which is useful wherever spat is in short supply. Survivorship also increases, thus providing a potential method for reducing duck predation. The evidence presently available suggests, however, that the level of protection provided by knots varies among longlines.

## Acknowledgments

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## THE EFFECTS OF DIETARY BUTYRIC ACID ON EARLY JUVENILE STRIPED BASS (*Morone saxatilis*) FATTY ACID PROFILES AND ESSENTIAL FATTY ACID CONTENT

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### Abstract

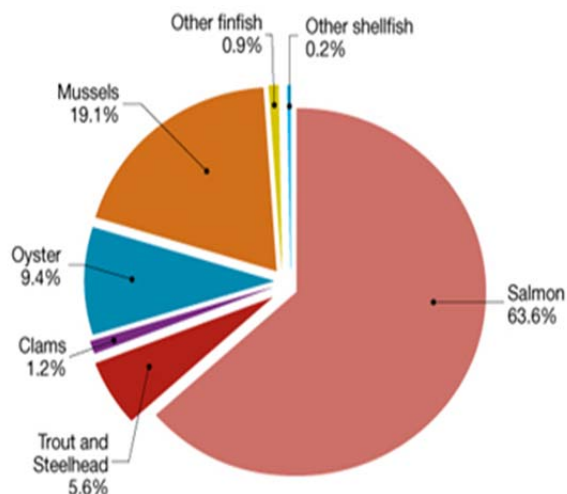
Diversification of the Canadian aquaculture industry is important to sustain production that will reflect the rising global population. First, larvae must overcome a production bottleneck that currently plagues their most-vulnerable life stage, resulting in high mortality and low yields of market-sized adults. Essential fatty acids (EFAs) are paramount to the growth and development of embryos and larvae. Deficiencies in EFAs result in developmental deformities in organs such as the brain and eyes, leading to high mortality and poor survival. BA is a short-chain fatty acid linked to significant improvements in growth factors of finfish, as well as increasing EFAs in human colon cells. In this experiment, early juvenile striped bass were weaned at 39 days post hatch onto an extruded pellet (0.6 mm) containing either 0.0, 0.5, or 1.0% butyric acid (BA), and fed for 10 days. Total fats, docosahexaenoic acid (DHA), oleic acid (OA) and linoleic acid (LA) (mg/g) in early juveniles consuming 1.0% BA were significantly greater after 10 days compared to the control (0.0%) ( $p < 0.05$ ). Eicosapentaenoic acid (EPA) content was significantly greater in the 1.0% BA treatment. Arachidonic acid (ArA) content remained constant throughout the experiment and the treatment groups. Dietary butyric acid has positive implications for the aquaculture industry, such as a complete fatty-acid profile, and an increase in certain EFAs during crucial developmental life stages of economically viable fish.

## Introduction

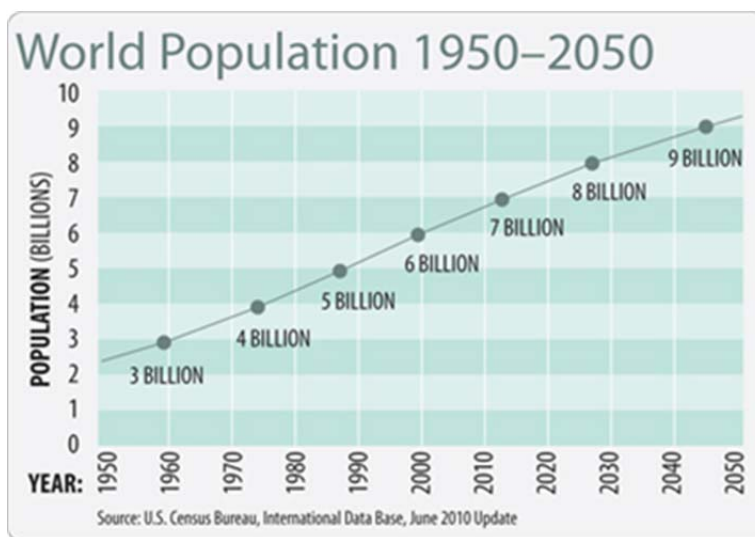
Canada has a 1 billion-dollar aquaculture industry that is dominated by Atlantic salmon, which as of 2014 accounted for over 60% of aquaculture production (Fig. 1; DFO 2014). With global populations rising well beyond our production capacity, it is crucial to diversify the Canadian aquaculture industry by introducing new, high-value, low-impact species that can be sustainably grown (Fig. 2; FAO 2014). Striped bass (*Morone saxatilis*) was the model perciform species used in this experiment, as its development and ontogeny are typical of most marine fish.

Butyric acid is a short-chain fatty acid that is the product of bacterial fermentation in the digestive tract of vertebrates (Smith et al., 1998). It is involved in promoting the digestive health of animals; for fish in particular, butyric acid has been linked to significant increases in growth, survival, and epigenetic enhancement of protein absorption (Romano et al., 2014). As with any novel dietary additive, fatty-acid analysis is vital to examine any positive or negative effect on the fatty-acid profile (Hixson and Parrish 2014).

In juvenile sea bream, the requirement for DHA and EPA is 0.5% and 1.0%, respectively; within this percentage, DHA was twice as effective as EPA on the growth of the sea-bream juveniles. However, there was no cumulative effect of the two omega-3 HUFAs on growth (Takeuchi et al., 1990). Regardless of whether the fatty acid comes in the form of an inert diet, or enriched in a live prey, increasing the amount of EFAs results in a significant improvement on the growth, swim bladder development, survival, and stress resistance of larval sea bream (Koven et al., 1990). In general, EFAs such as DHA are vital to the proper growth and development of larval fish; deficiencies in DHA can lead to gill erosion, muscular



**Figure 1**  
Breakdown of national production percentages of aquaculture species in Canada in 2014 (DFO).



**Figure 2**  
World population estimate (billions) indicating the rise from 1950 - 2050 (FAO).

dystrophy, poor brain and eye development, and impaired behaviours such as predation and schooling (Mourete, 2003; B  nitez et al., 2007).

In the past, DHA and EPA were preferentially used in fish nutrition experiments due to their prevalence in fish tissues, which led to a tendency to view them as the more important EFAs. Although both DHA and EPA are EFAs, ArA is ubiquitous and essential in its role as the primary eicosanoid precursor, regardless of the amount of EPA present in fish tissue (Bell & Sargent, 2003). ArA is an omega-6 EFA, so its presence in the diet will have different implications than DHA and EPA, the omega-3 EFAs. Nevertheless, ArA is irreplaceable in the diet of marine finfish, especially at younger, more vulnerable stages. Newly hatched larvae are delicate in many ways: in their environment, in their unique nutritional demands, and also in their small, fragile nature that leaves them exposed to stress and mortality due to handling. Larval sea bream (5 - 40 days post hatch) were given rotifers enriched with either ArA or DHA and then an Algamac diet, were transported to grow-out tanks, and then measured to see which treatment resulted in the highest mortality (Koven et al., 2001). The ArA/Algamac treatment group experienced significantly greater survival than DHA/Algamac group when fed prior to the transportation (Koven et al., 2001).

A similar study was conducted to determine if ArA has an effect on the survival of gilthead sea bream larvae, as well as the stress response due to handling and the associated salinity changes (Koven et al., 2003). The authors also included different age ranges of the larvae to determine if the previous results were only applicable to certain larval stages, or if they could also be applicable to juvenile and adult sea bream (Koven et al., 2003). The age groups included larvae, between 3 - 19 days post hatch, 20 - 30 day-old premetamorphosing larvae, and 30 - 42 day-old metamorphosing larvae (Koven et al., 2003). In sea bream and summer flounder (*Paralichthys dentatus*), ArA improved the growth, survival and resiliency to stress, and when present between 0 - 25% of total fatty acids of the diet, ArA increased the cortisol levels under osmotic stress (Koven et al., 2003; Willet et al., 2003; Van Anholt et al., 2004).

Different species have different requirements, and developmental stages will dictate these requirements. Although fatty acids in hybrid striped bass have been studied, it remains necessary to understand how the profile will change with the addition of a short-chain fatty acid such as BA at the early juvenile stage. Since it is currently unknown how varied amounts of BA in the diet of striped bass will affect the fatty-acid profile of the juveniles, the present experiment examines not only the effect of the BA treatment, but also the effect of time. The results will indicate how EFAs and ratios will change over the ten-day trial, between the three treatment groups of 0.0%, 0.5%, and 1.0% BA.



## Materials and Methods

The trial ran for ten days, with striped bass that were 39 days post hatch. There were a total of 9 tanks; 3 replicate tanks for each of the 3 diets (0.0% BA, 0.5% BA, and 1.0% BA). The tanks were cleaned and siphoned twice per day, during which time the number of mortalities were counted and recorded while being removed from the tanks.

The volume of the black tanks was 155 L, and they were 27 cm in diameter. Each tank was stocked with 200 early juveniles. The tanks were on a flow-through system and the temperature and salinity were monitored twice per day and were held constant at 20°C and 22 parts per thousand (ppt), respectively. All tanks received 24-hour light at a constant intensity of 20 lux. Vibratory feeders were programmed to dispense meals every two hours for 24 hours a day, alternating with hand feeding took place every 2 hours between 06:00 and 20:00 for the entire trial.

Sampling for fatty acids was done on day 0 of the trial, and then on the final day (day 10) of the trial. No less than 200 mg of wet weight in whole fish were sampled on these days and kept frozen at -80°C until freeze drying (lyophilization). Freeze-dried samples were stored in 50% nitric-acid-rinsed vials with Teflon caps, in freeze-dried form for fatty-acid analysis and profiling. These samples were transported to the National Centre for Mariculture in Eilat, Israel, for fatty-acid extraction and analysis.

A series of steps and protocols were then followed (Table 1) in order to properly extract the fatty acids and prepare the samples for analysis via gas chromatography and mass spectrophotometry (GC/MS). Once the samples were analyzed and the data was organized into a spreadsheet, a one-way analysis of variance was used to determine any statistical significance between the variations of fatty-acid content. A Tukey post hoc test was then conducted to analyze the significance between the treatments to determine if 0.5% was significantly different from 1.0%.

**Table 1. Protocol for fatty acid extraction and analysis on experimental striped bass samples at the start of the trial (39 dph) and after ten days of treatment (0.0%, 0.5%, or 1.0% butyric acid) (49 dph).**

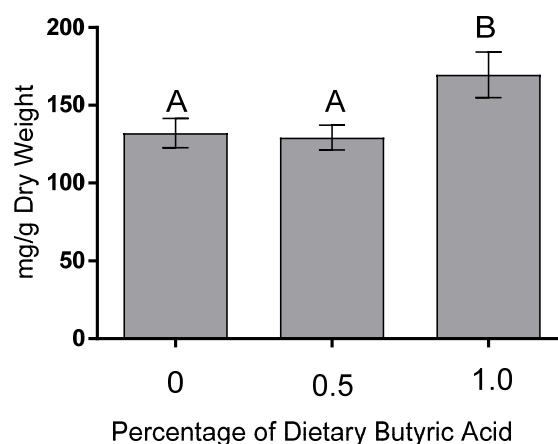
1. Freeze dry the sample overnight
2. Weigh 10 - 20 mg of dry weight (DW) of the sample into a 10-mL tube
3. Add 6 mL of extraction solution (chloroform + methanol 2:1, + 0.01% butylated hydroxytoluene (BHT)) to the tube
4. Homogenize the sample with an ultrasonic electrode on crushed ice for 2 minutes
5. Filter the homogenized sample into a pre-weighed 10-mL tube (filter No. 2 Wattman)
6. Add 2 mL of 0.08% potassium chloride (KCl) to the tube
7. Centrifuge the tube at 4°C overnight
8. Remove the upper phase and wash 3 times with the upper phase solution (chloroform + methanol + distilled water (3:48:47). Be careful not to remove the lower phase, as it contains the lipids
9. Evaporate at 50°C under nitrogen
10. Weigh the preweighed tube and calculate the percentage of total lipid content based on the difference in tube weight
11. Add 0.1 mg of standard (17:0) to the tube
12. Vortex for 10 seconds
13. Add 1.0 mL of boron trifluoride (BF<sub>3</sub>, 14%) for every 1 mg of lipid
14. Vortex for 10 seconds
15. Fill the tube with nitrogen and put the cover on
16. Place the tube in an ultrasonic bath at 50°C for 1 hour
17. Take the sample out of the bath and wait 5 - 10 minutes until the sample is at room temperature
18. Add 0.5 mL of distilled water
19. Add 1 mL of hexane for gas chromatography (GC) for every 1 mg of lipid
20. Vortex for 10 seconds
21. Centrifuge the tube for 2 minutes at 2300 revolutions per minute (rpm)
22. Remove the upper phase and place into GC bottle
23. Store at -20°C until injection of the sample into the GC
24. Inject the sample into the GC
25. Organize GC/MS output into an excel spreadsheet

## Results

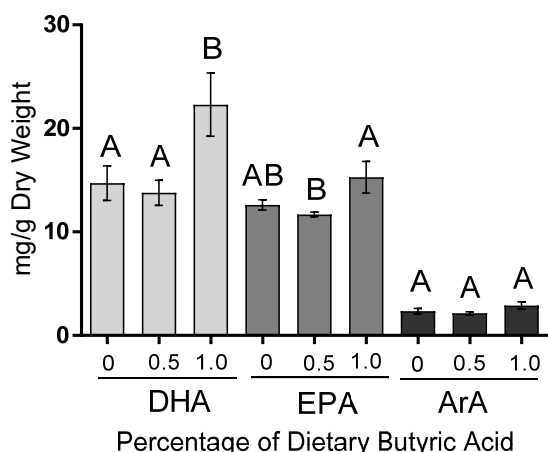
The fatty-acid profiling of the striped-bass early juveniles revealed that the mean percentage of total fatty acids (mg/g of dry weight) after the ten-day feeding trial was significantly greater in the tanks consuming 1.0% dietary BA, compared to the 0.5% treatment and the control (0.0%) ( $p = 0.038$ ) (Fig. 3). The control treatment and the 0.5% BA treatment were not significantly different from one another after ten days (Fig. 3). The 1.0% BA treatment resulted in greater mean percentage of fatty acids present after the ten-day trial than the other treatments (Fig. 3).

After 10 days of feeding altered butyric acid diets, there were statistically significant differences in lipid concentrations in fish tissue ( $p < 0.05$ ), with post-hoc Tukey analysis revealing a significant increase in total fats in fish fed 1.0% diets compared to 0.5% and control (0%) diets (Fig. 3). A diet containing 0.5% butyric acid did not increase total fats in fish tissue, with no difference observed compared to controls.

The mean percentage of docosahexaenoic acid (DHA) present was significantly greater in the 1.0% BA treatment than any other treatment ( $p = 0.028$ ) (Fig. 4). Similarly, the mean percentage of EPA was significantly greater in the 1.0% BA treatment than in the 0.5% treatment (Fig. 4). Arachidonic acid (ArA) and linoleic acid (LA) were the two omega-6 fatty acids of interest that were analyzed and profiled. The mean percentage of ArA was highest at the end of the ten-day trial for the 1.0% BA treatment, and remained consistent between the other treatments, although these differences were not found to be significant ( $p > 0.05$ ) (Fig. 4). The mean percentage of linoleic acid was also highest after ten days for the 1.0% BA treatment; however, this difference was statistically significant ( $p = 0.001$ ) (Fig. 5). The results for oleic acid (OA) were similar to LA, as the mean percentage of OA was significantly greatest when the striped bass consumed 1.0% BA for ten days compared to the control (0.0%) and 0.5% BA (Fig. 5).



**Figure 3**  
Mean concentration of total fats (mg/g dry weight of fish) of 39-day-old striped-bass early juveniles, with standard error, after ten days of receiving 0.0% BA, 0.5% BA, or 1.0% BA. Different letter groupings indicate significant differences between treatments.



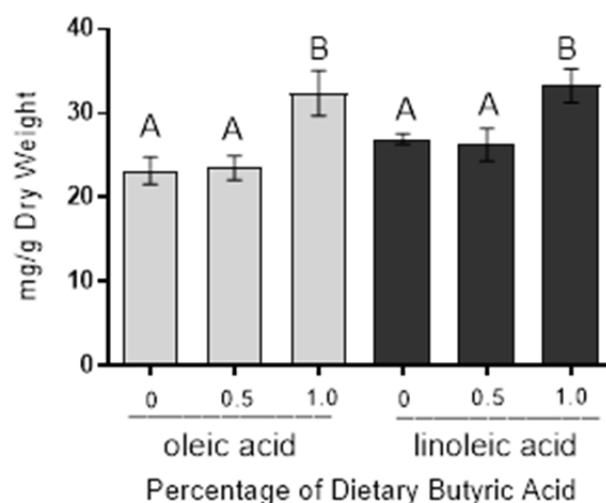
**Figure 4**  
**Mean concentration of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ArA) (mg/g dry weight of fish) of 39-day-old striped-bass early juveniles, with standard error, after ten days of receiving 0.0% BA, 0.5% BA, or 1.0% BA (left). Different letter groupings indicate significant differences between treatments.**

## Discussion

Total fatty acid content and certain essential fatty acids were profiled to determine the effects that butyric acid had on these crucial values in larval development. The results indicate that 1.0% BA significantly increased the levels of DHA and EPA, despite having no effect on ArA (Fig. 4). Although the presence of BA was unable to impact ArA, it has been shown that DHA is essential for larval growth, and that DHA is in fact superior to EPA and ArA at the larval stage in grouper (*Epinephelus malabaricus*) (Nematipour & Gatlin 1993; Wu et al., 2002). Furthermore, DHA and ArA are vital for satisfying the long-chain polyunsaturated-fatty-acid requirements in juvenile California yellowtail (*Seriola dorsalis*), highlighting the importance of DHA in larval diets compared to EPA (Rombenso et al., 2016).

The 1.0% BA treatment significantly increased the DHA content in the striped-bass juveniles and, although the levels of ArA remained the same throughout the experiment, a past experiment on human digestive tract cells revealed that BA significantly increased the DHA and ArA content in the digestive tract (Hofmanová et al., 2009). ArA could still be improving or influencing growth and survival, as it has been shown to reduce mortality due to handling-stress responses in sea bream (Koven et al., 2003). In first-feeding black sea bass (*Centropristis striata*), growth and survival was best when the diet contained 10% DHA and 6% ArA, suggesting that roughly a 2:1 DHA/ArA ratio is the most beneficial (Rezek et al., 2010). Our striped-bass results are somewhat consistent with this; comparing the DHA and ArA content after ten days on 1.0% BA, there is approximately six times as much DHA present as ArA (Fig. 4). Although a 6:1 ratio is much greater than 2:1, the ArA portion of the ratio remains the same, while DHA is triple the recommended amount based on black sea bass (Rezek et al., 2010). DHA is regarded as the most important EFA in larval rearing, as it is involved in many crucial developmental pathways. The 1.0% BA treatment tripled the DHA portion of the ratio, which indicates that the available DHA is greater in developing and newly hatched larvae. According to Rezek et al., (2010), ArA is most beneficial for first-feeding black sea bass when present with DHA as one part ArA. The striped-bass results show that ArA is still present in the ratio as one part, indicating that the striped-bass larvae contain sufficient ArA to reduce rearing stress and promote survival, as well as more than enough DHA for healthy development.

To emphasize the necessity for DHA in larvae, Senegalese sole (*Solea senegalensis*) larvae were weaned onto a first-feeding DHA-deficient diet, as it has been thought that they can biosynthesize DHA from its precursors (Pinto et al., 2016). The results indicate that with low, total, fatty-acid and DHA content in the early larval diets, the growth performance of sole significantly decreased compared to individuals with normal levels (Pinto et al., 2016). DHA can also be used preferentially over EPA during periods of compensatory growth and nutrient deficiency in the larval stage (Wu et al., 2002), so an increase in DHA is a particularly important result from this experiment. This preferential metabolism is also noted for both OA and LA in Greenland halibut (*Reinhardtius hippoglossoides*) eggs during embryogenesis (Mejri et al., 2017); therefore, a significant increase in OA and LA as seen in this striped-bass experiment has a meaningful impact on larval fatty-acid requirements in aquaculture.



**Figure 5**  
Mean concentration of oleic acid and linoleic acid (mg/g dry weight of fish) of 39-day-old striped-bass early juveniles, with standard error, after ten days of receiving 0.0% BA, 0.5% BA, or 1.0% BA. Different letter groupings indicate significant differences between treatments.

Overall, dietary butyric acid has not elicited negative effects on the fatty-acid profile of early juvenile striped bass, and for DHA, OA, and LA, it has significantly increased the whole body content. Since these EFAs are linked to larval and juvenile growth and survival via proper neural development, 1.0% dietary butyric acid is beneficial as a potential novel metabolite in the husbandry of aquaculture species. Increasing the growth, survival, total fat content, and levels of EFAs will have positive implications for the aquaculture industry and promote the diversification of species that the industry needs to expand and satisfy the growing global population.

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APPENDIX 1

Past AAC Award Winners

List of Lifetime Achievement Award Recipients

Date Bestowed	Name	Residence
May 30, 2000	Neil Bourne	Nanaimo, British Columbia
May 9, 2001	David Aiken	Saint Andrews, New Brunswick
September 20, 2002	Rene Lavoie	Dartmouth, Nova Scotia
October 31, 2003	Bill Pennell	Nanaimo, British Columbia
October 19, 2004	Ovila Daigle	Richibucto, New Brunswick
July 5, 2005	Louis Deveau	Dartmouth, Nova Scotia
November 21, 2006	Lucien Poirier	Rimouski, Quebec
May 13, 2008	Yves Bastien	Chelsea, Quebec
May 19, 2010	Al Castledine	Victoria, British Columbia
May 2012	Chris Frantsi	Saint Andrews, New Brunswick
June 3, 2014	Cyr Couturier	St. John's, Newfoundland & Labrador
June 3, 2014	Santosh Lall	Halifax, Nova Scotia
September 21, 2016	Rod Carney	Saint Andrews, New Brunswick

### List of Research Award of Excellence Recipients

<b>Date Bestowed</b>	<b>Name</b>	<b>Residence</b>
May 30, 2000	Santosh Lall	Halifax, Nova Scotia
May 9, 2001	Joseph Brown	St. John's, Newfoundland
September 20, 2002	Joel de la Noue	Laval, Québec
November 1, 2003	Tillmann Benfey	Fredericton, New Brunswick
October 20, 2004	Ed Donaldson	West Vancouver, British Columbia
July 6, 2005	John Castell	Saint Andrews, New Brunswick
November 22, 2006	David A. Higgs	West Vancouver, British Columbia
September 25, 2007	Richard Moccia	Guelph, Ontario
May 12, 2009 (joint)	Thierry Chopin	Saint John, New Brunswick
	Shawn Robinson	Saint Andrews, New Brunswick
May 11, 2011	Debbie Martin-Robichaud	Saint Andrews, New Brunswick
June 4, 2013	Marcel Fréchette	Mont Joli, Québec
June 3, 2014	Fred Page	Saint Andrews, New Brunswick
June 2, 2015	Céline Audet	Rimouski, Québec
May 31, 2017	Réjean Tremblay	Rimouski, Québec



