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Condition and biochemical profile of blue mussels (*Mytilus edulis* L.) cultured at different depths in a cold water coastal environment

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ABSTRACT

The growth and health of cultured blue mussels (*Mytilus edulis*) are affected by environmental conditions. Typically, culture sites are situated in sheltered areas near shore (i.e., < 1 km distance from land, < 20 m depth); however, land runoff, user conflicts and environmental impact in coastal areas are concerns and interest in developing deep water (> 20 m depth) mussel culture has been growing. This study evaluated the effect of culture depth on blue mussels in a cold water coastal environment (Newfoundland, Canada). Culture depth was examined over two years from September 2012 to September 2014; mussels from three shallow water (5 m) and three deep water (15 m) sites were compared for growth and biochemical composition; culture depths were compared for temperature and chlorophyll *a*. Differences between the two years examined were noted, possibly due to harsh winter conditions in the second year of the experiment. In both years shallow and deep water mussels presented similar condition; in year 2 deep water mussels had a significantly better biochemical profile. Lipid and glycogen analyses showed seasonal variations, but no significant differences between shallow and deep water were noted. Fatty acid profiles showed a significantly higher content of omega-3 s (20:5ω3; EPA) and lower content of bacterial fatty acids in deep water sites in year 2. Everything considered, deep water appeared to provide a more favorable environment for mussel growth than shallow water under harsher weather conditions.

1. Introduction

In Canadian waters, blue mussel (*Mytilus edulis*) aquaculture is undergoing a period of expansion. Typically, mussel culture sites are situated in shallow and sheltered areas nearshore (i.e., < 20 m depth, < 1 Km from coast), such as river mouths, estuaries and bays. However, mussel culture in nearshore zones is subjected to a number of issues such as land runoff, exposure to contaminants of land origin, user conflicts, carrying capacity limits and consequent food depletion (McKindsey et al., 2006; Cheney et al., 2010; Duarte et al., 2012). Aquaculture-related benthic deposition and impact, due to mussel drop-off and organic biodeposits, have become important issues of concern (Hartstein and Rowden, 2004; Fabi et al., 2009; Frechette, 2012). Moving farms to more offshore and deeper water sites (> 1 km from coast, > 20 m depth) may help to reduce exposure to contaminants of land origin, increase carrying capacity limits and avoid food depletion, diminishing environmental effects of production (McKindsey et al., 2006; Cheney et al., 2010; Duarte et al., 2012). Sub-surface chlorophyll *a* maximum layers are possible due to thermal and saline stratification; taking advantage of chlorophyll *a* maxima could avoid phytoplankton shortages due to expansion of bivalve culture (Ogilvie et al., 2004). Offshore deep water culture presents a more stable environment i.e. lower fluctuations in temperature and salinity, and has the potential to improve mussel condition, increasing growth, reducing turbidity and fouling, and improving water exchange (Cheney et al., 2010). Therefore, changes in culture technology and improvement in protocols have the potential to increase the environmental sustainability of mussel aquaculture in Canada and worldwide. Recent studies have highlighted an improved condition for a variety of shellfish species when grown in deep water, e.g. blue mussels grown off the coast of New Hampshire reached market size in 12–14 months, in contrast to the 18 months for ones located in nearby bays (Langan and Horton, 2003; Buck, 2007; Yu et al., 2010). However, there is a lack of information on how water depth and distance from the coast specifically affect and possibly benefit blue mussel (*Mytilus edulis*) condition compared to traditional coastal shallow water areas. It is important to understand these relationships and their environmental and production benefits in order to make decisions on the feasibility of deep water sites for mussel culture,

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since its development may involve considerable effort and expense (Cheney et al., 2010).

To evaluate the specific effects of water depth on *M. edulis*, blue mussels were grown in deep and shallow water sites under commercial standards and were compared for condition and biochemical parameters for two consecutive years. Condition and biochemical composition responses are useful indicators of physiological state, nutritional and commercial quality for bivalves; they follow seasonal environmental changes and vary among and within geographical locations (Thompson, 1984a; Lucas and Beninger, 1985; Orban et al., 2002; Toro et al., 2002; Hemachandra and Thippeswamy, 2008; Irisarri et al., 2015). In mussels, biochemical composition varies seasonally and in relation to water temperature, food availability, and reproductive cycle. When food is abundant reserves are accumulated in the form of lipids, glycogen, and proteins and subsequently utilized for gamete and meat production (Mathieu and Lubet, 1993; Okumus and Stirling, 1998; Kopp et al., 2005; Karayücel et al., 2013). Lipids provide the highest energy yield; they include fatty acids (FA), such as omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) which influence growth, reproduction, and immunity (Parrish, 2013). In bivalves, neutral lipids such as triacylglycerols (TAG) are used for energy storage and dominate during spring (Beninger, 1984; Freites et al., 2002; Li et al., 2007; Parrish et al., 2009; Prato et al., 2010; Martínez-Pita et al., 2012).

The main objective of this study was to compare cultured blue mussels grown at two different depths (5 m and 15 m), to highlight differences in condition and biochemical profile. Other secondary objectives were to evaluate inter-annual and seasonal variability of mussels cultured at different depths.

2. Materials and methods

2.1. Study site, experimental set-up and sampling protocol

This study was conducted between September 2012 and September 2014; mussel seed originated from Bulley's Cove and South Arm (Notre dame Bay, Newfoundland and Labrador, Canada; Fig. 1). During September 2012 mussels from the 2011 year class were collected from three culture areas in Notre Dame Bay: South Arm, Bulley's Cove and Mouse Island. Each culture area included shallow water (headlines at 5 m) and deep water (headlines at 15 m) sites: South Arm had one shallow water site and one deep water site. Bulley's Cove had two shallow water sites and one deep water site. Mouse Island included only one deep water site, due to the frequent presence of pack ice during the spring occurring in this area. South Arm and Bulley's Cove are located in the same semi-enclosed bay while Mouse Island is located in a channel, further offshore. The deep water sites were located in water deeper than 30 m (up to 60 m) while the shallow water sites were located, for the most part, in water depths < 20 m (Fig. 1). Mussels of large grade (25.4–38.1 mm) were socked (~220–250 mussels per 0.3 m of sock) two weeks prior to the start of the experiment in order to allow a period of acclimation. Mussels were collected in all six sites in September 2012 (Initial), January 2013 (4 months), May 2013 (8 months) and September 2013 (12 months) when the Year 1 experiment was concluded and mussels were harvested and processed. In September 2013 a new deployment was completed using 2012 year class seed. In Year 2, mussels were collected from the 6 sites in October 2013 (Initial), May 2014 (8 months) and September 2014 (12 months). In January 2014 (4 months) the sampling was suspended due to harsh winter conditions and the presence of ice which prevented access to the sampling sites from December 2013 until May 2014. *Mytilus edulis* were cultured using the traditional longline system and harvested using standard commercial protocols. For each collection, five socks (5 m length) were randomly sampled from the same longline (366 m length) at each study site. After transport to the commercial processing facility mussels were sampled randomly for each analysis (condition measurements, glycogen, lipid and fatty acid analyses).

2.2. Environmental data analysis

Multiparameter water quality instruments (YSI sondes 6600 V2, YSI; Yellow Springs Instrument, 2012) moored at each site (SAS, SAD, BCS 1 and 2, BCD and MID) were used to continuously monitor water temperature (°C) and chlorophyll *a* ($\mu g \cdot L^{-1}$). Data were collected monthly and processed to obtain a concatenation with consistent date/time stamp and variables output. 'Out-of-water' data (i.e. any data with depth < 1 m) and large chlorophyll *a* spikes (any data > 50 $\mu g\ L^{-1}$) were removed and a low-pass filter was applied to all the variables (i.e., Depth, Temperature, Chlorophyll) based on running-averages with a 24 h window. Monthly averages were calculated from these filtered time series data for each site which were then grouped (averaged ± SD) by depth, i.e. shallow water (5 m) and deep water (15 m).

2.3. Condition analysis

For each sampling time, 150 mussels were sampled at each site: the 5 socks collected were sectioned in three parts (top, middle and bottom) and 50 mussels from each part were sampled; they were transported on ice back to the North Atlantic Fisheries Centre (NAFC) facilities in St. John's for measurement and analysis. For each individual, the valves were separated and the interstitial water drained and the total wet weight was measured to the nearest 0.001 g, after which the meat was dissected from the shell and placed in pre-weighed aluminum trays and dried to a constant weight for 48–72 h at 80 °C (modified from Lutz et al., 1980); shells were allowed to air dry for 48–72 h at room temperature. Shell dry weight was measured to the nearest 0.001 g. Condition index was calculated as the ratio of dry tissue weight to wet tissue weight (Lucas and Beninger, 1985; Gallardi et al., 2014).

2.4. Glycogen analysis

A total of 15 mussels was sampled at each site and each time point for glycogen analysis. The mussels were dissected, placed in glass vials and quickly frozen on dry ice. Ten randomly chosen samples were then processed using the KOH method described by Gallardi et al. (2014). Glycogen content ($mg\ g^{-1}$ of wet weight) was determined by colorimetric reaction, measuring absorbance at 490 nm on a multi-detection microplate reader (Synergy HT, BIO-TEK). The concentration of glycogen in the samples was calculated based on a mussel glycogen standard (Sigma, Saint Louis, MO).

2.5. Lipid and fatty acid analyses

2.5.1. Lipid classes

A total of 15 mussels was sampled at each time point for each of the six study sites for lipid extraction, using a modified Folch method (Parrish, 1999; Gallardi et al., 2014). Meat was dissected from the shell and stored in chloroform at −80 °C until analysis. Lipids were determined in 10 individuals, randomly selected from the original 15 mussels adding methanol and homogenizing with a Polytron homogenizer (Brinkmann Instruments). Then chloroform: methanol 2:1 solution and chloroform-extracted water (2:1 ratio) were added, the samples sonicated and placed in a −20 °C freezer for 10 min. The lower, organic layer was then removed using a double pipetting technique (Parrish, 1999). Lipid classes were determined by thin layer chromatography (TLC) with flame ionization detection using an Iatroscan analyzer (MK-6 TLC-F1D, Iatron Laboraories Inc., Japan) and a three-stage development system to separate lipid classes (Parrish, 1999). The resulting chromatograms were analyzed with PeakSimple software (SRI Instruments, Torrance, CA). Total lipid content was expressed as $mg \cdot g^{-1}$ of wet weight and triacylglycerol to sterol (TAG: ST) ratios were calculated (Fraser, 1989; Freites et al., 2002).