# A comparison of the physiology of geographically distinct blue mussels (Mytilus edulis) in Prince Edward Island

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# A COMPARISON OF THE PHYSIOLOGY OF GEOGRAPHICALLY DISTINCT BLUE MUSSELS (*MYTILUS EDULIS*) IN PRINCE EDWARD ISLAND

by

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# TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF FIGURES

## ABSTRACT

Blackwood, C., Steeves, L., Davidson, J., Poirier, L.A., Comeau, L.A., Pousse, E., Filgueira, R. 2023. A comparison of the physiology of geographically distinct blue mussels (*Mytilus edulis*) in Prince Edward Island. Can. Tech. Rep. Fish. Aquat. Sci. 3551: v + 24 p.

The blue mussel (*Mytilus edulis*) is a widely distributed marine bivalve that is ecologically and commercially important. To predict mussel growth rates and optimize the production of mussels in spatially-limited coastal regions, it is important to understand how physiological traits differ between geographically distinct sources of mussels. This study was designed to compare the growth rates and physiology of four groups of mussels initially collected as spat from different mussel growing areas in Prince Edward Island and reared together at a common location. After growth in the common location, size distributions from a sample of each source were compared and small, but significant differences, in shell length were found. A common-garden experimental design was used to account for physiological plasticity, allowing for the observation of potential physiological differences due to adaptation of the four mussel sources. During three separate trials (including laboratory and dock-side trials), feeding and metabolism were compared under flow-through laboratory conditions and measurements were used to estimate scope for growth. Feeding and metabolism did not differ significantly among the sources of mussels under those conditions, however, variability in scope for growth estimates provide an indication of how differences in growth rates among the sources could arise over the long term (months to years). Inter- and intra-individual variation in *M. edulis* physiology observed in this study outline the importance of using longitudinal (repeated measurements) over cross-sectional (single point in time measurements) studies.

## RÉSUMÉ

Blackwood, C., Steeves, L., Davidson, J., Poirier, L.A., Comeau, L.A., Pousse, E., Filgueira, R. 2023. A comparison of the physiology of geographically distinct blue mussels (*Mytilus edulis*) in Prince Edward Island. Can. Tech. Rep. Fish. Aquat. Sci. 3551: v + 24 p.

La moule bleue (*Mytilus edulis*) est un bivalve marin largement distribué et présentant un intérêt écologique et commercial important. Pour modéliser la croissance et optimiser la production de moules dans des régions côtières spécifiquement identifiées, il est important de comprendre comment les traits physiologiques de moules ayant des origines géographiques variées diffèrent. Cette étude a été réalisée dans le but de comparer les taux de croissance et la physiologie de quatre groupes de moules collectées au stade de naissain dans différentes baies de l'île du Prince Edouard et élevées ensemble dans un lieu identique. Les distributions de taille initiale des moules ont été comparées selon leur origine et ont montrées une différence faible mais significative de la longueur de coquille. Dans le but d'étudier la plasticité phénotypique, un protocole expérimental de jardin commun a été mis en place dans lequel les différences physiologiques conséquentes à de potentielles adaptations en lien avec l'origine des moules ont été explorées. Durant trois expériences indépendantes (en laboratoire et sur quai), l'alimentation et les taux métaboliques mesurés en flux ouvert ont été comparés et ont permis d'estimer le Scope for Growth (énergie restante disponible pour la croissance). L'alimentation et les taux métaboliques n'ont pas présenté de différences; cependant, les variations dans les estimations du Scope for Growth pourraient fournir des indications à plus long terme (mois, années) sur d'éventuelles différences de taux de croissance selon l'origine des moules. Au regard des variabilités inter- et intra-individuelles observées au niveau de la physiologie de *M. edulis*, cette étude a permis de souligner l'importance de réaliser plusieurs mesures répétées dans le temps sur un individu.

# 1. INTRODUCTION

The blue mussel (*Mytilus edulis*) is a widely distributed filter-feeding marine bivalve that thrives in temperate subtidal and intertidal waters globally, including the waters off the Atlantic coast of Canada (Jones et al., 2010). Blue mussels are ecologically important, being recognized as both ecosystem engineers (Gutiérrez et al., 2003) and keystone species (Han et al., 2017). Mussels feed on phytoplankton and detritus by filtering water through their gills. This feeding mode and their prolific natural abundance make mussels integral to nutrient cycling and seston dynamics in coastal areas (Gallardi, 2014; Lindahl et al., 2005). In addition to their ecological significance, the cultivation of mussels is important economically (Government of Canada, 2021) and is a source of sustainable seafood (Shumway et al., 2004). For context, mussel production in PEI during 2021 was valued at over $30 000 000 CAD (Government of Canada, 2023) Within the context of a growing global human population, bivalve aquaculture presents an opportunity to supply low cost and sustainable protein (FAO, 2016). Unsurprisingly, bivalve aquaculture and the production of mussels have been increasing steadily since the 1970's (Aksnes et al., 2017). An important aspect of mussel aquaculture is the collection and transfer of wild spat from varying sources (different bays) to a final grow-out site. This practice presents challenges as mussels collected from different locations, even if geographically nearby, can perform differently in terms of growth and survival. It is important to understand what factors drive these differences and how they relate to collection location in order to optimize mussel production.

Growth rates and morphological characteristics have been shown to be highly variable in mussels (Capelle et al., 2021), with differences arising over small- and large-scale geographical distances (Kandratavicius & Alejandro, 2014; Lajus et al., 2015). Relatedly, physiological rates also exhibit variability between mussel sources over both small and large distances, which can often be attributed to phenotypic plasticity. Mussels display remarkable phenotypic plasticity in relation to their physiology, meaning they can respond to different environmental conditions by modifying physiological processes (Bayne, 2004). Under natural conditions, the plasticity of these processes allows mussels, which are largely sessile, to live in an environment subject to a high degree of both short- and long-term environmental variation (Jimenez et al., 2015). Plasticity can be observed in differences in physiology that arise over microgeographic scales (Lesser et al., 2010) and when mussels are transplanted to new locations (Steeves et al., 2020). Despite the high level of phenotypic plasticity in mussel physiology, adaption may contribute to physiological differences observed between mussel sources, even under common conditions (Jimenez et al., 2015). In the context of the present study, adaptation refers to heritable changes made to DNA that give individuals an advantage in certain local environments (Fallet et al., 2020; King et al., 2018). Epigenetic changes during larval development, can have long term effects on gene expression and thus physiology and have been found to be influenced by environmental factors such as temperature. (Fellous et al., 2015).

Variability in growth rates can be driven by differences in processes related to energy acquisition from the environment and allocation to growth and maintenance (Filgueira et al., 2011). The main physiological processes that underpin energy acquisition and allocation are feeding and metabolism (Widdows & Johnson, 1988). In general, the energy an organism has available for growth, i.e. scope for growth (SFG), is the difference in energy acquired from the environment through feeding and assimilation and the amount of energy expended through metabolism and lost through excretion and egestion (Bayne et al., 1999; Brett, 1976). Increased rates of food acquisition and increased metabolic efficiency are processes that contribute to increased growth rates in bivalves (Bayne et al., 1999; Fuentes-Santos et al., 2018; Tamayo et al., 2011; Zhang et al., 2018). However, the plasticity of mussel physiology makes it

difficult to determine if physiological differences are a result of location/origin (adaptation) or plastic processes in relation to local environment. To account for this, common-garden experiments, in which mussels are observed under the same conditions, can be used (Villemereuil et al., 2016). Common-garden experiments involving the transplant of juveniles from different origins to a common location (e.g. Babarro et al., 2000a; Mallet & Carver, 1989) can be used to disentangle the effects of plasticity and adaptation on bivalve physiology and growth rates, which are intrinsically related processes (Bayne et al., 1999). Studying differences in physiology between mussel sources, in a common-garden setting, provides an unbiased approach to determining the influence of plasticity and adaptation in key processes that relate to mussel growth (feeding and metabolism).

Two important measurements for the characterization of feeding and metabolism include pumping rate (PR) and metabolic rate (MR) (Thompson & Bayne, 1974). Pumping rate is the volume of water an individual can move over its gills per unit of time and is crucial in order to determine ingestion (Riisgård & Larsen, 2001). However, PR is not a direct proxy for feeding as mussels capture and sort particles for ingestion or rejection with different efficiencies, based on size and/or particle surface properties (Rosa et al. 2018). In the present study, capture efficiency (CE) is the proportion of particles of a given size captured by the gills compared to the proportion of particles of that size that are in the water (Rosa et al., 2018). In the absence of rejection (pseudofaeces production), the combination of PR and CE provide information on ingestion rate (Riisgård & Larsen, 2001), which in combination with diet characteristics and assimilation efficiency can determine energy acquisition. In the present study, MR refers to the consumption of oxygen (Thompson & Bayne, 1974) and can generally be used alone to estimate energy expenditure (Bayne et al., 1999; Brett, 1976) as the energy expenditure via excretion corresponds often to less than 5% of the total SFG in bivalves (Bayne and Newell, 1983). Accordingly, the combined measurements of PR, CE, and MR allow for strong characterization and comparison of physiological traits that directly affect the available energy for growth. In the absence of rejection or when pseudofaeces production is negligible, scope for growth can be estimated with these measurements, allowing for a quantifiable comparison of the energy organisms have available for growth (Widdows & Johnson, 1988). Condition index (CI) typically relates the dry weight and shell length of mussels, and is associated with differences in PR and MR (Riisgård et al., 2014). Therefore, a comparison of the above traits is crucial in predicting and comparing the performance of mussels from different sources under common environmental conditions.

It is the prevalent thought among mussel farmers in Atlantic Canada that seed collected from different locations will vary in terms of their growth rates when cultured in a common location (John Davidson, Fisheries and Oceans Canada, personal communication). The purpose of the present study was to determine if differences in physiological processes could drive variations in growth rates among four geographically distinct sources of *Mytilus edulis* in Prince Edward Island, using a common-garden experimental design. Flow-through chambers in both dock-side and laboratory experiments were used to measure feeding activity (PR and CE) and metabolic rates (MR). These measurements were compared among the four seed sources, over the course of three trials. Understanding how origin can affect physiology in mussels cultured at the same location provides insight into the degree of local adaptation and the limits of plasticity, which can be important for improving local mussel cultivation. In a broader context, the results presented here will provide insight into the potential for settling location to cause long-term individual changes in physiology.

# 2. MATERIALS AND METHODS

## 2.1. EXPERIMENTAL DESIGN AND ACCLIMATION

This study compared blue mussels from four geographically distinct locations in Prince Edward Island, Canada (i.e. Brudenell River, New London Bay, St. Peters Bay, Tracadie Bay, Fig. 1). In each location, juveniles were collected in October 2019 and brought to the common-garden location (Brudenell River) where all mussels were grown in socks (length: ~2.5 m) suspended from a single line at 1-2 m deep. The study consisted of two laboratory trials and one dockside trial, which took place in July, October, and November of 2021, respectively (Table 1). The physiology of mussels from these locations under the common-garden design was examined using flow-through chambers described in the following section. In all three trials, four individuals of approximately the same size, according to shell length, were selected from each location for a total of 16 mussels per trial (Table 1). Each trial was run for 4 days with constant water temperature, 20°C, intended to represent an average summer temperature, growing season, for this region in PEI.

Mussels were collected approximately 10 days before each trial and brought to the Aquatron laboratory facility at Dalhousie University, Halifax, Nova Scotia for the laboratory trials (1 and 2). Mussels (~ 8 per location) were maintained in two flow-through (~1.5 L $min^{-1}$) aerated holding tanks (45 L) using sand-filtered (50 µm) seawater collected at 9−12-m depth. Mussels were fed a concentrated mixed phytoplankton diet containing *Isochrysis, Pavlova, Tetraselmis, Thalassiosira weissflogii Thalassiosira pseudonana,* and *Chaetoceros* (Reed Mariculture, Shellfish Diet 1800) (Nielsen et al., 2021). The diet was kept at a constant level (~10,000 cells $mL^{-1}$) using a fluorometer (Cyclops 7-F, Turner Designs; blue excitation for *in vivo* chlorophyll *a*), which was placed in the header tank and connected to a 3-point control system (RIU3, InWater Technologies). This allowed for the feedback-initiated addition of diluted diet to the header tank. In the dockside trial (St. Peters Bay, PEI), mussels (~ 15 per location) were held in two 200-L aerated holding tanks, in static conditions with unfiltered water pumped directly from St. Peters Bay. Mussels were acclimated to experimental conditions for at least 5 days before beginning each trial. To avoid physiological stress, mussels were acclimated from the ambient temperature at which they were collected to 20°C by changing the temperature of holding tanks using in water heaters at a maximum rate of 2°C $day^{-1}$. Water temperatures were raised, using aquarium tank heaters, by 2°C in trial 1, 8°C in trial 2, and 12°C in trial 3.

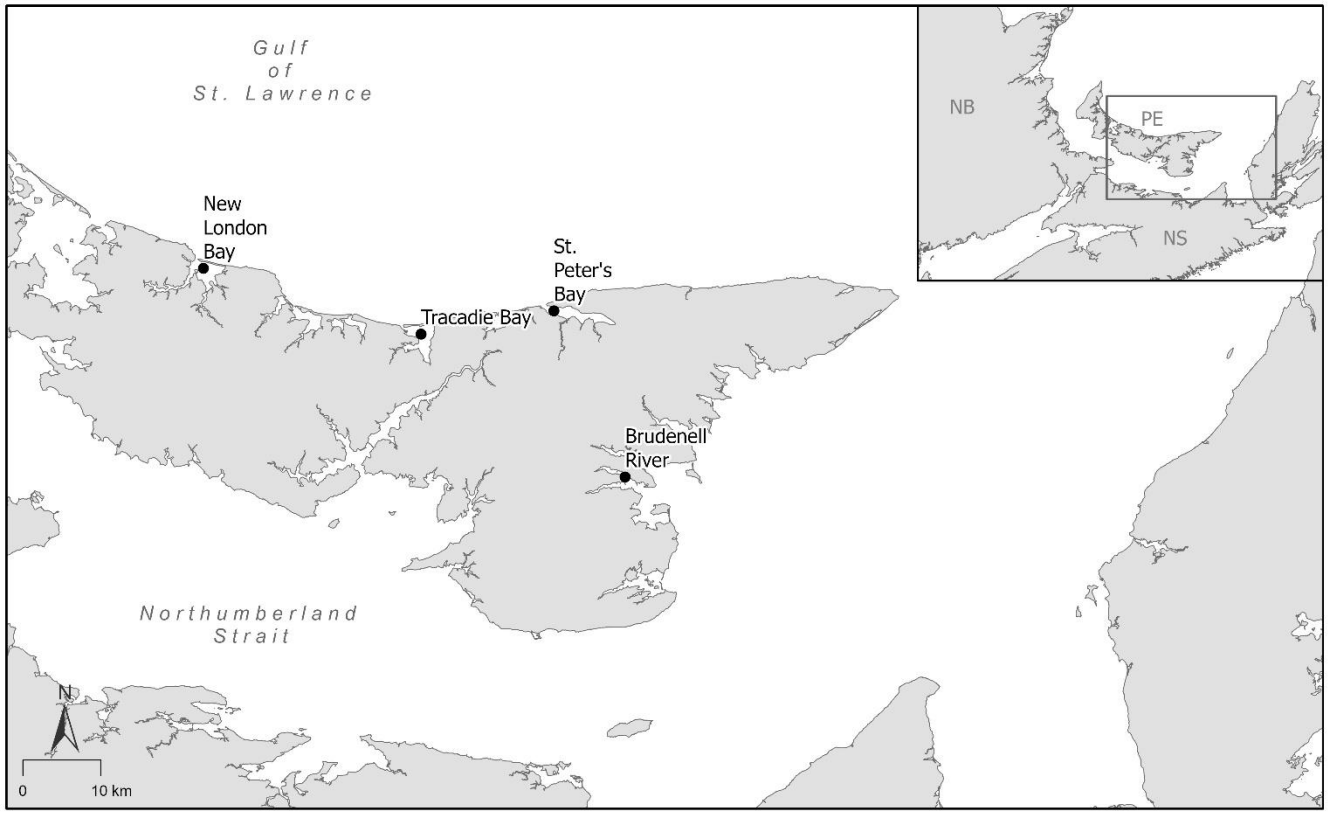


*Figure 1. Geographical distribution of the four collection sites (Brudenell River, New London Bay, St. Peter's Bay, and Tracadie Bay) located in Prince Edward Island, Canada.*

## 2.2. TRIAL CONDITIONS

The individual flow-through chambers used for the physiological studies were cylindrical and ranged in volume from 670 mL (diameter = 14.2 cm, height = 17 cm) to 805 mL (diameter = 15.5 cm, height = 17 cm). Water was pumped, using peristaltic pumps (Ismatec IP65), to each of the chambers at an average rate of 70 mL $min^{-1}$ (trials 1 and 2) and 50 mL $min^{-1}$ (trial 3). Flow was reduced during trial 3 due to reduced availability of heated 20°C water. For all trials, water was pumped to the chambers from a common header tank, which was used to keep temperature, food levels (for laboratory trials), and oxygen concentration consistent within each individual chamber. Food levels during trials 1 and 2 (Table 1) were kept stable in the header tank using the methods outlined in the previous section. To account for low initial PR, food levels were increased by approximately 50% in trial 2 after the second day (Table 1). Due to the natural variability in ambient food quantity and quality during trial 3, seston measurements were taken three times each day to provide a more thorough description of food availability. Seston concentration was calculated by filtering 0.7−1.3 L of seawater on a 1.2-µm filter (Whatman GF/C). Filters were rinsed with 25 mL of 0.5-M ammonium formate to remove any salts. To determine seston concentration (mg $L^{-1}$), filters were dried at 60°C until a stable weight was achieved. Particulate organic matter (POM), which is the fraction of organic matter of the seston, was calculated as the weight differential pre- and post-combustion of the Whatman filters for 36 hours at 450°C.

To begin each trial, 16 chambers each containing one mussel (four per source) were filled with water directly from the header tank and sealed to minimize any air exchange between the chambers and the atmosphere. During the trials, flow through the chambers was periodically stopped for 15 minutes to create static conditions within the chambers, permitting the measurement of MR and PR. In the first two trials, flow was stopped every 3 hours and in the third trial every 2 hours. Each trial ran for a minimum of three days allowing at least 24 sampling periods per trial. Mussels were suspended above the bottom of the chambers on plastic mesh to allow for placement of a magnetic stir bar to maintain mixing in the chambers. Each chamber was equipped with temperature (PreSens pt 100) and oxygen (PreSens OXY-4 SMA) probes. Further, in trials 2 and 3, 12 chambers were equipped with a fluorometer (Cyclops 7, Turner Designs; blue excitation, for *in vivo* chlorophyll *a*). At the end of the trials, mussels were removed from the individual chambers and dissected to measure shell length and tissue dry weight according to the process outlined below.

To account for background decreases in oxygen and to determine background decreases in fluorescence during the trials, control measurements were taken prior to and immediately after each trial. Control measurements followed those outlined in Vajedsamiei et al. (2021) and consisted of running the flow-through system and recording measurements in the same manner as the trials, but without mussels present. During trials 1 and 2, controls were run for 24 hours before and after the trials. Due to time constraints, controls for trial 3 were run for approximately 4 hours before and after the trial. A minimum of eight control measurements were collected for each chamber in all trials. All control measurements were taken at the same frequency as trial measurements, aside from trial 3 post controls which were compressed to 60-minute intervals. The decrease in oxygen during static periods allowed for the determination of net average background oxygen consumption within the chamber. These values were subtracted from oxygen consumption measurements with mussels present. Fluorometer readings during controls were used to determine the degree to which food levels changed in the absence of mussels.

At the beginning of trial 1, a subset of 20 individuals ranging in size (shell length: 41 – 66 mm) were collected from each source. The mussels were measured for length (L in mm), then dissected. After drying the samples at 65 °C for 48−72 hours (dried until constant weight), tissue dry weight (DW in g) was measured. Finally, condition index (CI) was calculated using the equation $CI= (DW/L^3)*1000$ (Riisgård et al., 2014). Length, DW, and CI were also measured/calculated for the mussels used in each trial.

## 2.3. PUMPING RATE, CAPTURE EFFICIENCY, AND FEEDING INDICATOR

Pumping rate (PR) and capture efficiency (CE) were determined by measuring the exponential decline in particle concentration during static conditions using an electronic particle counter (PAMAS S4031 GO, PAMAS). PAMAS measurements were taken throughout each day of the trials during static conditions. On average, 12 samples were recorded daily using 3 PAMAS. Chambers were sampled at 30 second intervals for the duration of the 15-minute static period when MR measurements were taken. Particles in the water were classified into 30 different 0.5-µm size classes ranging from 1.25 to 16.75 µm. Particle sizes were restricted to this range as the comprise the bulk of the shellfish diet, and to avoid introducing error into the calculations from estimates of low particle counts. Further, it is assumed that particles larger than 16.75 µm are fully captured by the gill of *M. edulis*. The rate of decline in particle counts due to bivalve pumping follows an exponential decline (Coughlan, 1969). For this reason, particle concentrations were converted to their natural logarithms to produce linear declines. Capture efficiency

was estimated by comparing the slopes of the linear decline (λ) in concentration of particles for the 0.5-µm size classes. CE for a given size class is represented by a value between 0 and 1, where 0 indicates particles of this size were not captured by the animal and 1 indicates particles of this size were fully captured. Capture efficiency for a given particle size follows Steeves et al. (2020) and is determined by:

$$CE = \frac{\lambda sample}{\lambda average} \quad (1)$$

where $\lambda_{sample}$ represents the slope of the linear decline (after conversion to the natural logarithm) in particle concentration of a certain size class and $\lambda_{average}$ represents the average slope of the linear decline in particle sizes that are assumed to be fully captured by *M. edulis*. In the present study, $\lambda_{average}$ represented the slope of the linear decline of particles from sizes 8.25 to 10.75 µm (Steeves et al., 2020). Although particles larger than 10.75 µm are also fully captured by M. edulis, they are excluded from the calculation as the low particle counts of large particles introduces uncertainty in the calculations (Cranford et al. 2016, Steeves et al. 2020). The slope of particles that are fully captured was used to calculate the pumping rate (PR) of individuals using the following equation (Steeves et al. 2020):

$$PR = \lambda\, average \times V \times 3600 \quad (2)$$

where $V$ represents the volume of the chamber in litres and 3,600 is a conversion factor to obtain PR in units of L $h^{-1}$. PR was then standardized ($PR_{std}$) for a standard mussel length ($L_{std}$) using the following equation (Steeves et al. 2020):

$$PR_{std} = PR \times \left(\frac{L_{std}}{L_{obs}}\right)^{b} \quad (3)$$

where $L_{std}$ is the length of a standard mussel (60 mm – which is close to the individuals used in this study and a common length used in the literature as it is close to individuals of 1 g dry weight), $L_{obs}$ is the length of the mussel being measured, and $b$ is the allometric coefficient (2.19) (Jones et al., 1992). Mussel length was used to standardize pumping rate because length scales with gill area, which is proportional to PR (Jones et al., 1992).

An indicator of feeding (IF, L $h^{-1}$) throughout the study was approximated for trials 2 and 3 by measuring the concentration of chlorophyll in the chambers using fluorometers. Given that the fluorometers can measure chlorophyll of cells that are not necessarily fully captured, these measurements do not fully incorporate the effect of CE on the estimation of PR and is thus only an indication of feeding activity. IF was not measured for trial 1 due to issues with the food pump which caused spikes in fluorescence within the header tank, making accurate calculations impossible. Fluorometers were placed in 12 (trial 2) or 11 (trial 3) of 16 chambers and recorded measurements every 10 seconds. Additionally, a fluorometer was placed in the header tank. Measurements, taken in millivolts (mV), were recorded using a datalogger (Windmill ML75x). Spikes and troughs in fluorescence were accounted for by removing the top and bottom 2.5% of measurements from each data set. Additionally, all measurements during static conditions were removed. To account for variations in the calibration of the fluorometers, control values for each chamber were scaled to header tank measurements based on the following equation:

$$V_{CC} = (V_H - V_{HC}) + V_C \quad (4)$$

where $V_{CC}$ is the voltage of the corrected control, $V_H$ is the voltage in the header tank, $V_{HC}$ is the average voltage output in the header tank during controls, and $V_C$ is the average voltage output in the chamber

during controls (all values in mV). The corrected control and chamber measurements were then averaged over 10 minutes (moving average) to minimize high-frequency noise. Using these measurements, an indication of feeding (IF) value was calculated following Vajedsamiei et al. (2021) according to the following equation:

$$IF = \left(\frac{V_{CC} - V_M}{V_M}\right) \times FR \qquad (5)$$

where $V_M$ is the voltage measurement with a mussel in the chamber and *FR* is the flow rate (L $h^{-1}$) going through the chamber (Vajedsamiei et al., 2021). IF measurements were calculated for each averaged chamber measurement and negative values were set to 0. Subsequently, a 45-minute moving average was applied to these measurements to further minimize the high-frequency noise caused by the delays between changes in the header tank and the chambers. These values were calculated for all chambers equipped with fluorometers and plotted over time.

## 2.4. METABOLIC RATE

Oximeters (PreSens OXY-4 SMA) recorded measurements of dissolved oxygen concentration (DO) at 10-second intervals throughout the course of the trials. Metabolic rate was calculated from the decline in oxygen concentration when chambers were under static conditions (periodically for 15 minutes, see above). Metabolic rate (MR) for mussels in the study was calculated following Casas et al. (2018) as:

$$MR = (m - m') \times Vol \times 60 \qquad (6)$$

where *m* refers to the slope of the linear regression for the decline in DO over time (mg $O_2$ $L^{-1}$ $h^{-1}$) with a mussel in the chamber and *m'* refers to the average decline in DO for the same chamber without a mussel present (control), *Vol* represents the volume of the chamber in litres, and 60 is a conversion factor to obtain MR in mg $O_2$ $h^{-1}$. Only *b* values from linear regressions with an $r^2$ value > 0.95 were used. Individual metabolic rates were standardized to account for potential differences in size using:

$$MR_{std} = MR\left(\frac{W_{std}}{W_{obs}}\right)^b \qquad (7)$$

where $MR_{std}$ is the standardized metabolic rate, $W_{std}$ is the dry weight of a standard mussel (1 g), $W_{obs}$ is the observed dry weight of the mussel used, and *b* represents the allometric coefficient (0.73) (Smaal et al., 1997). Dry weight was used for standardization due to the significant relationship between dry weight and MR found in mussels and the seasonal variation in the relationship between length and weight (Smaal et al., 1997).

## 2.5. SCOPE FOR GROWTH

Scope for growth (SFG, J $g^{-1}$ $h^{-1}$) was calculated for each source of mussels in trial 3 in which diet consisted of natural seston. Average particulate organic matter from trial 3, expressed as mg POM $L^{-1}$ (see section 2.2), was multiplied by standardized pumping rate (60 mm standard, which is approximately the shell length of a 1 g individual) to estimate organic ingestion rate (OIR, mg POM $h^{-1}$). Transforming these measurements to energetic units was done using the conversion factor 23 J mg $POM^{-1}$ (Widdows & Johnson, 1988). The converted values represent ingestion rate (IR). Absorption efficiency (AE) was not measured, instead pseudo-values were chosen to represent high, medium, and low AE for use in different

estimates. SFG (J $g^{-1}$ $h^{-1}$) was calculated using the following equation:

$$SFG = [(IR \times AE) - MR] \quad (8)$$

where the multiplication of *IR* and *AE* represent assimilation rate (AR) and MR represents metabolic rate (mg $O_2$ $h^{-1}$), which was converted to J $h^{-1}$ by multiplying original values by 14 (Pousse et al., 2020). SFG was calculated for each source in trial 3 using a factorial design, with each of the variables IR, AE, and MR represented by 3 levels, being 0.5, 0.7, and 0.9 for AE (Bayne et al., 1989; Widdows et al., 1984) and the 1st quartile, mean, and 3rd quartile (estimated from the measurements for each mussel source during trial 3) for IR and MR.

## 2.6. STATISTICAL ANALYSIS

A two-way analysis of variance (ANOVA) with trial and source as fixed factors was used to test for differences in MRs, PRs, and CIs, followed by a Tukey HSD post-hoc test to examine pairwise comparisons among the four sources. In all cases, the interaction between mussel source and trial was not significant ($P > 0.05$). To verify assumptions for these tests, Levene's test was used to check for homoscedasticity and a Shapiro-Wilk test was used to check for the normality of residuals. Metabolic rates passed these assumptions ($P > 0.05$), whereas PRs and CIs needed to be log transformed to satisfy both tests ($P > 0.05$). All statistical analyses were computed using R-studio version 1.2.5001. A statistical comparison of the SFG results was not included due to the use of multiple scenarios in SFG calculations. Using many SFG scenarios can inflate statistical power, leading to statistical results that may not be biologically relevant.

*Table 1. Summary of conditions during each of the three trials.*

| Trial | Location | Dates | Mussel Shell Length Range (mm) | Food Concentration in Header Tank for particles > 4.25 µm (particles $mL^{-1}$) | Mean ± SD Chamber Temperature (°C) |
|---|---|---|---|---|---|
| 1 | Laboratory, Dalhousie University, Aquatron facility | 07/16/2021 – 07/20/2021 | 55 − 57 | ~30,000 | 20 ± 0.5 |
| 2 | Laboratory, Dalhousie University, Aquatron facility | 10/07/2021 – 10/11/2021 | 59 − 61 | ~15,000 − ~22,000 | 20 ± 0.5 |
| 3 | Dockside, St. Peters Bay, PEI | 11/15/2021 – 11/19/2021 | 57 − 71 | ~3,000 − ~20,000 | 20 ± 3 |

# 3. RESULTS

## 3.1. FOOD CONCENTRATION AND CONDITION INDEX

Concentration of artificial diet was highest in trial 1 with levels in the header tank being maintained at approximately 30,000 ± 6,000 cells (> 4.25 µm) $mL^{-1}$. In trial 2, the artificial diet was maintained in the header tank at approximately 15,000 ± 300 cells (> 4.25 µm) $mL^{-1}$ for the first 48 hours and then was increased to approximately 22,000 ± 300 cells (> 4.25 µm) $mL^{-1}$. In trial 3, PAMAS estimates indicated that particle concentrations in the header tank varied over the course of the trial from 3,000 to 20,000 particles (> 4.25 µm) $mL^{-1}$.

Mussels differed significantly in mean shell length between each trial (ANOVA: df = 2, $F = 48.51$, $P < 0.0001$). Mean (± SD) shell lengths were 55.31 ± 0.60 mm (trial 1), 59.75 ± 0.68 mm (trial 2), and 63.87 ± 4.16 mm (trial 3). Mean condition index measurements did not differ significantly among sources of mussels (ANOVA: df = 3, $F = 1.66$, $P = 0.16$, Fig. 2, Table 2), nor among trials (ANOVA: df = 2, $F = 2.40$, $P = 0.11$, Fig. 2, Table 2). The highest mean condition index observed was 4.51 ± 1.15, occurring in trial 3 (New London Bay), whereas the lowest observed was 2.88 ± 0.19, occurring in trial 1 (New London Bay).

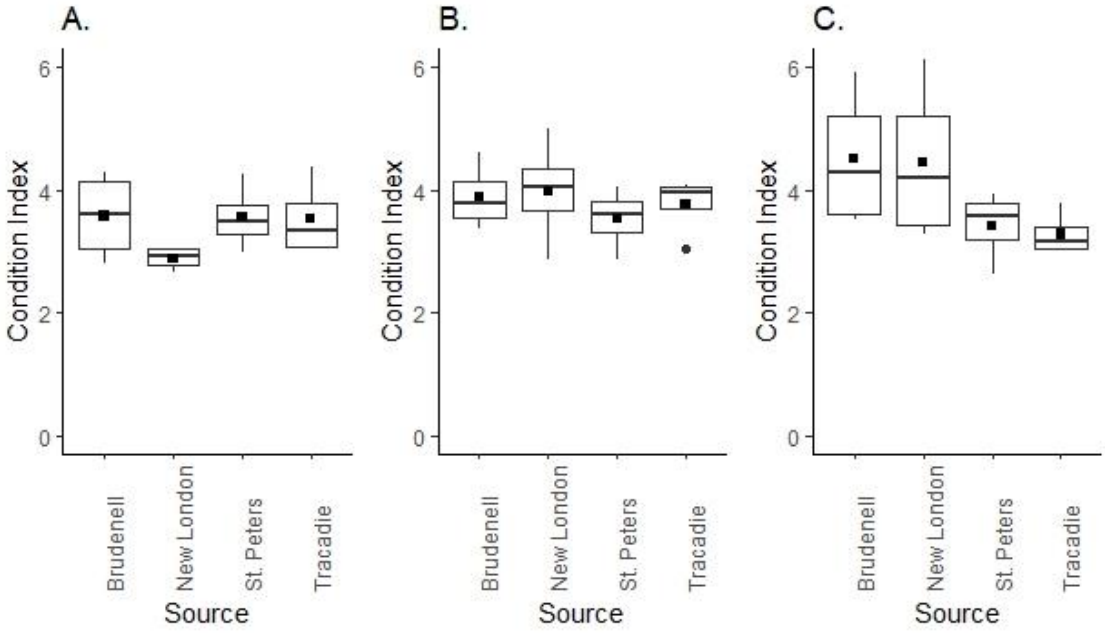


*Figure 2. Condition index measurements for each source of mussels during each trial: (A) trial 1, (B) trial 2, (C) trial 3. Mean values are represented by black squares. Boxes represent the interquartile range, with the middle bold line showing the median. Vertical lines above and below represent the largest and smallest values respectively, with circles representing outliers.*

*Table 2 . ANOVA results comparing the mean condition index among the four sources of mussels (Brudenell River, New London Bay, St. Peters Bay, Tracadie Bay) across all three trials.*

| | df | Sum Sq | Mean Sq | *F*-value | *P*-value |
|---|---|---|---|---|---|
| Source | 3 | 0.12 | 0.04 | 1.09 | 0.36 |
| Trial | 2 | 0.16 | 0.08 | 2.40 | 0.11 |
| Source * Trial | 6 | 0.33 | 0.06 | 1.66 | 0.16 |
| Residual | 36 | 1.20 | 0.03 | | |

## 3.2. FEEDING

No significant effect of mussel source (ANOVA: df = 3 $F$ = 0.19, $P$ = 0.89, Fig. 3, Table 3) nor trial (ANOVA: df = 2, $F$ = 2.70, $P$ = 0.08, Fig. 3, Table 3) were observed on mean pumping rates. The highest mean pumping rate was 1.83 ± 0.40 L $h^{-1}$, occurring in trial 1 (New London Bay), while the lowest mean pumping rate was 0.74 ± 0.85 L $h^{-1}$, occurring in trial 3 (Brudenell River). Throughout the trials, within source variation ranged from 22% (trial 1, New London Bay) to 115% (trial 3, St. Peters Bay), as calculated by the Pearson's Coefficient of Variation.

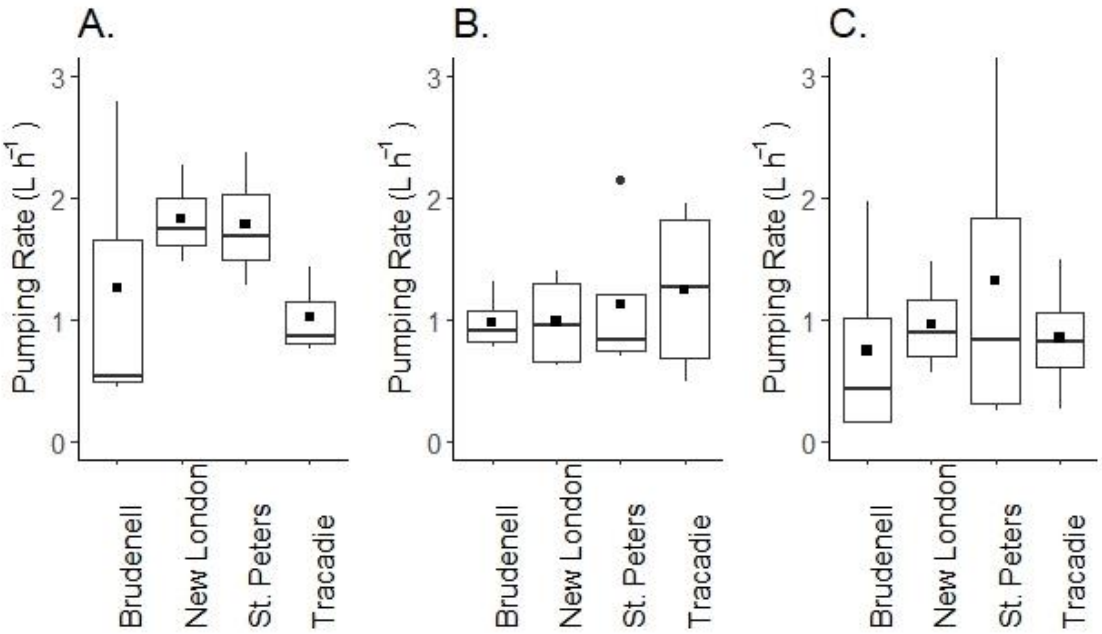


*Figure 3. Standardized pumping rates (L $h^{-1}$) for each source of mussels during each trial: (A) trial 1, (B) trial 2, (C) trial 3. Mean values are represented by black squares. Boxes represent the interquartile range, with the middle boldened line showing the median. Vertical lines above and below represent the largest and smallest values respectively, with circles representing outliers.*

*Table 3. ANOVA results comparing the mean pumping rate among the four sources of mussels (Brudenell River, New London Bay, St. Peters Bay, Tracadie Bay) across all three trials.*

| | df | Sum Sq | Mean Sq | *F*-value | *P*-value |
|---|---|---|---|---|---|
| Source | 3 | 1.43 | 0.48 | 0.92 | 0.44 |
| Trial | 2 | 2.80 | 1.40 | 2.70 | 0.08 |
| Source * Trial | 6 | 1.15 | 0.19 | 0.37 | 0.89 |
| Residual | 32 | 16.57 | 0.52 | | |

Indication of feeding (IF, L $h^{-1}$) measurements in trials 2 (Fig. 4) and 3 (Fig. 5) also exhibited a high degree of individual variation across time and inter-individual variation within sources. In trial 2, IF ranged from 0 to a maximum of 4.97 L $h^{-1}$ (Tracadie Bay). In general, feeding appeared to increase after increasing food concentrations in trial 2. In trial 3, measurements ranged from 0 to a maximum of 7.14 L $h^{-1}$ (St. Peters Bay).

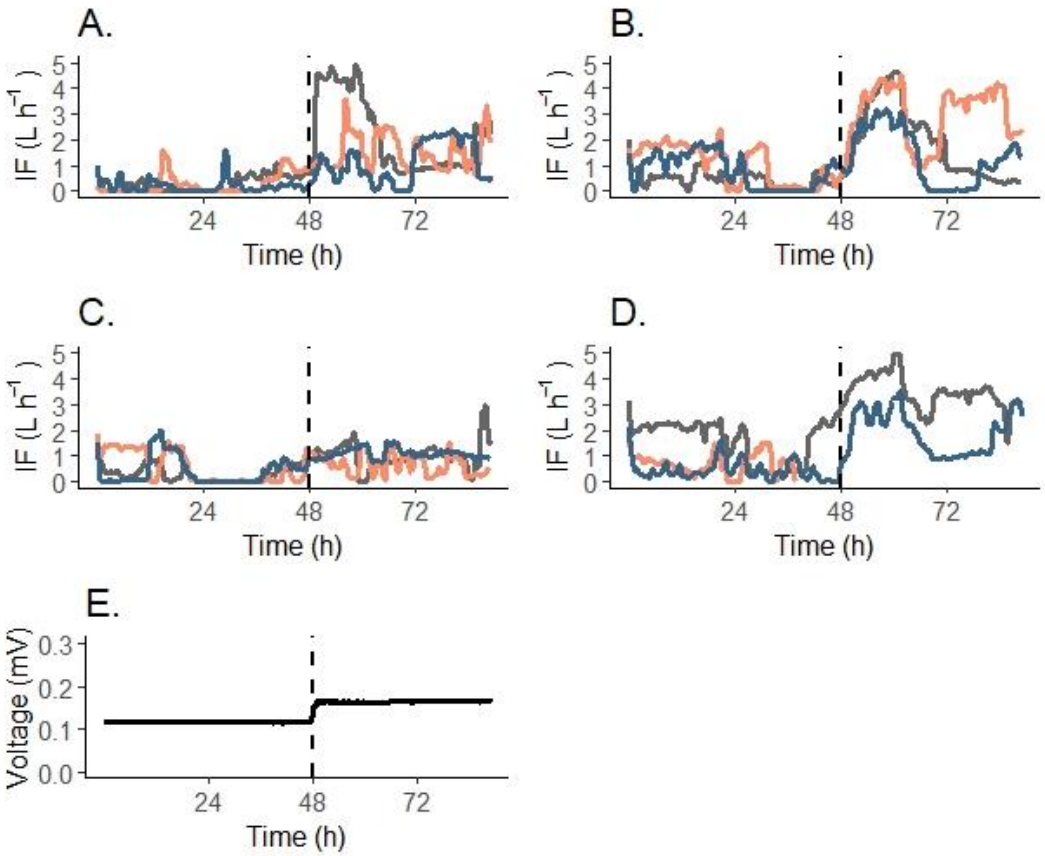


*Figure 4. Indication of feeding rates (IF) (L $h^{-1}$) for individual mussels (blue, gray, and orange lines) over time during trial 2: (A) Brudenell River, (B) New London Bay, (C) St. Peters Bay, (D) Tracadie Bay. Relative food concentrations in the header tank, as measured by fluorescence (mV), are also displayed (E). The dashed line represents an increase in food concentration.*

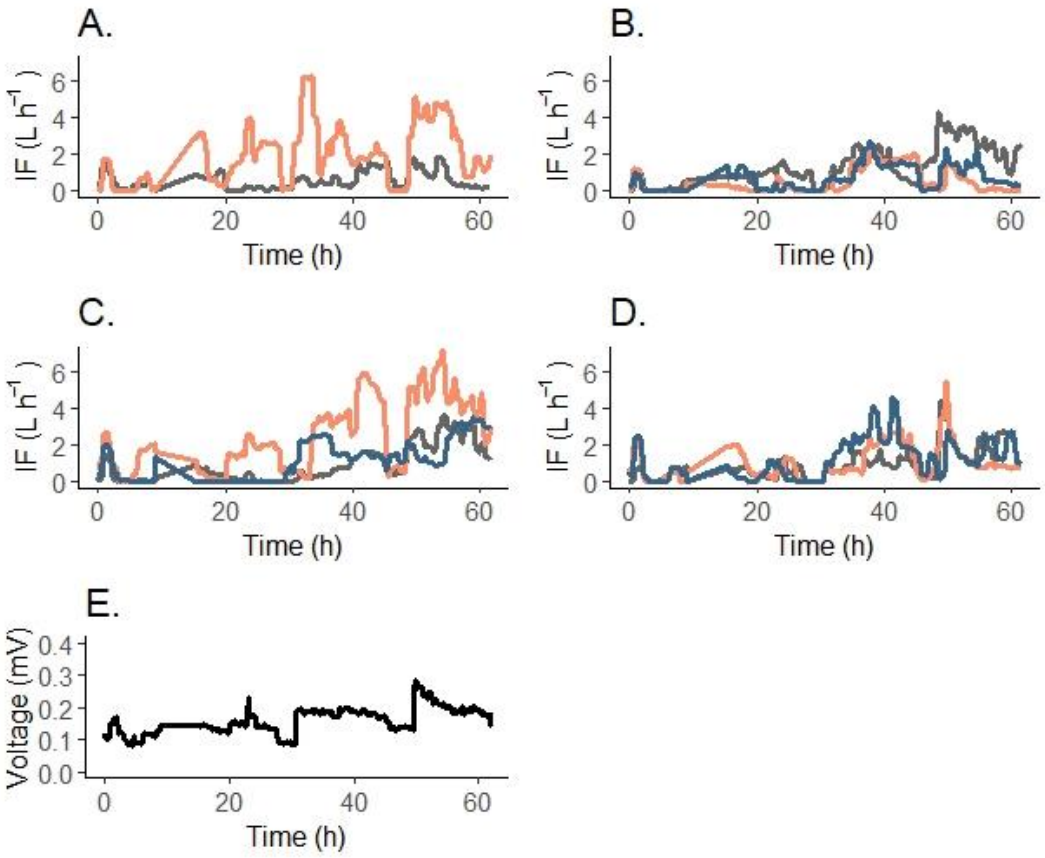


*Figure 5. Indication of feeding rates (IF) (L h$^{-1}$) for individual mussels (blue, gray, and orange lines) over time during trial 3: (A) Brudenell River, (B) New London Bay, (C) St. Peters Bay, (D) Tracadie Bay. Relative food concentrations in the header tank, as measured by fluorescence, are also displayed (E).*

### 3.3. METABOLIC RATE

There was no significant effect of mussel source on mean metabolic rate (ANOVA: df = 3, $F$ = 1.42, $P$ = 0.24, Fig. 6, Table 4), however, trial had a significant effect on mean metabolic rate (ANOVA: df = 2, $F$ = 52.16, $P$ < 0.0001, Fig. 6, Table 4). The highest mean metabolic rate was 1.25 ± 0.24 mg $O_2$ h$^{-1}$, occurring in trial 1 (St. Peters Bay), while the lowest mean metabolic rate was 0.44 ± 0.12 mg $O_2$ h$^{-1}$, occurring in trial 3 (New London Bay). Using post-hoc pairwise comparisons, mean metabolic rates were significantly higher in trial 1 compared to trial 3 for all mussel sources ($P$ < 0.05). In the sources New London Bay, St. Peters Bay, and Tracadie Bay mean metabolic rates were also significantly higher in trial 1 compared to trial 2 ($P$ < 0.05). No significant differences were observed between trials 2 and 3 ($P$ > 0.05). Throughout the trials, within source variation in metabolic rates ranged from 12% (trial 1, Brudenell River) to 26% (trial 1, Tracadie Bay), as calculated by the Pearson's Coefficient of Variation (Fig. 6).

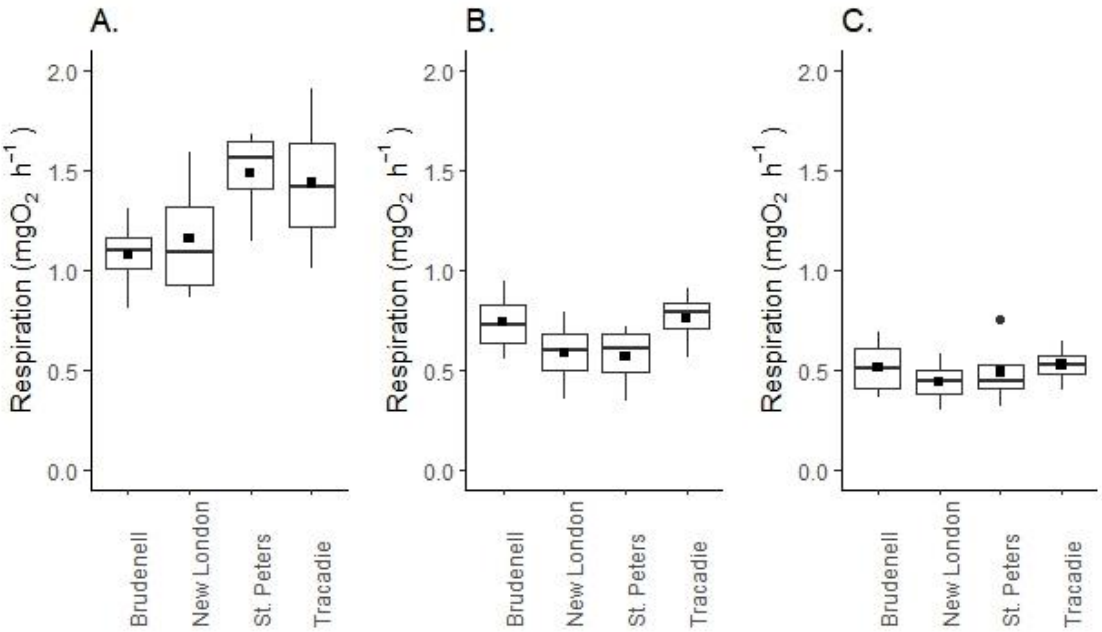


*Figure 6. Standardized metabolic rates (mg $O_2$ $h^{-1}$) for each source of mussels during each trial: (A) trial 1, (B) trail 2, (C) trial 3. Mean values are represented by black squares. Boxes represent the interquartile range, with the middle boldened line showing the median. Vertical lines above and below represent the largest and smallest values respectively, with circles representing outliers.*

*Table 4. ANOVA results comparing the mean metabolic rate among the four sources of mussels (Brudenell River, New London Bay, St. Peters Bay, Tracadie Bay) across all three trials.*

| | df | Sum Sq | Mean Sq | *F*-value | *P*-value |
|---|---|---|---|---|---|
| Source | 3 | 0.11 | 0.04 | 1.13 | 0.35 |
| Trial | 2 | 3.44 | 1.72 | 52.16 | $2.32e^{-11}$ |
| Source * Trial | 6 | 0.28 | 0.05 | 1.42 | 0.24 |
| Residual | 36 | 1.19 | 0.03 | | |

## 3.4. SCOPE FOR GROWTH

Considering all calculations of IR, AE, and MR, SFG estimates ranged from -6.66 (Brudenell River) to 39.27 J $g^{-1}$ $h^{-1}$ (St. Peters Bay) (Fig. 7). The mean (±SD) SFG estimates are as follows, Brudenell River: 4.95 ± 7.78 J $g^{-1}$ $h^{-1}$, New London Bay: 8.82 ± 4.48 J $g^{-1}$ $h^{-1}$, St. Peters Bay: 16.47 ± 13.92 J $g^{-1}$ $h^{-1}$, Tracadie Bay: 9.03 ± 5.53 J $g^{-1}$ $h^{-1}$ (Fig. 7). St. Peters Bay had the highest potential SFG, but also the widest degree of variance. New London Bay displayed the lowest maximum potential SFG, but also had the smallest variance in estimates.

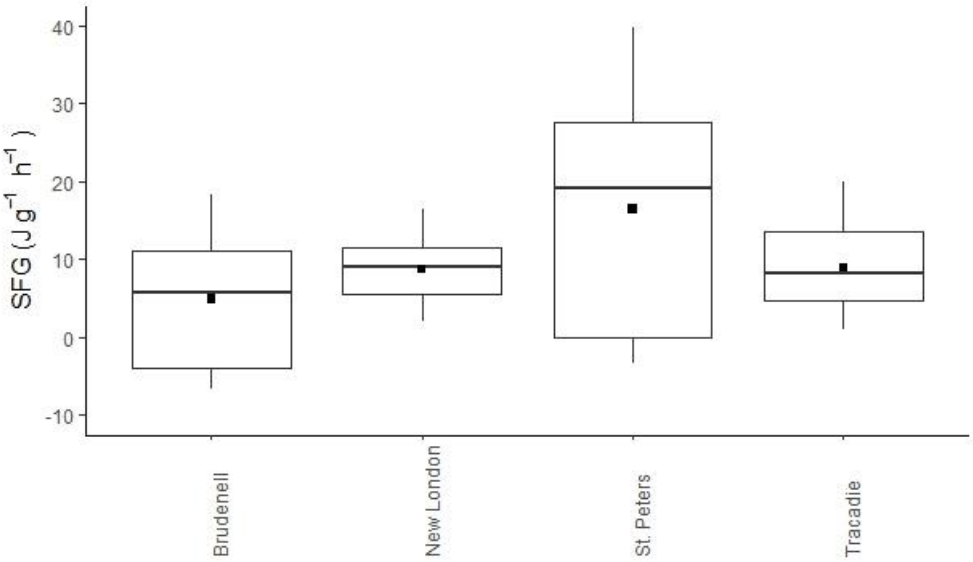


*Figure 7. Scope for growth estimations for the four sources of mussels during trial 3. Values estimated from varying ranges of MR and IR with pseudo values for AE. Mean values are represented by black squares. Boxes represent the interquartile range, with the middle boldened line showing the median. Vertical lines above and below represent the largest and smallest values respectively.*

## 4. DISCUSSION

The present work was designed to explore potential differences in important aspects of physiology among mussels from four geographically distinct sources, reared together in a common location. This study demonstrated that, at 20°C and under common conditions, pumping and metabolic rates did not significantly vary among geographically distinct sources of mussels when using both cultured and natural diets. These findings contradict our hypothesis that aspects of mussel physiology would vary among these four sources. Accordingly, we cannot directly conclude that source influences the pumping and metabolic rates of these mussels, when reared together. However, using integrative methods that combine different physiological rates, such as SFG, an explanation for differences in growth rates over a longer term may emerge.

### 4.1. FEEDING PHYSIOLOGY

The measurement of PR is an important aspect for comparing energy acquisition in mussels. Differences in pumping rates between sources of mussels would indicate that the initial step in energy acquisition varies between these sources, which may affect bioenergetics and thus growth rates (Thompson & Bayne, 1974). However, under the present trial conditions, PRs did not vary significantly among sources across all trials. Pumping rate is a highly plastic trait that can be affected by a variety of factors including environmental variables, seasonality, mussel condition, and mussel source (Riisgard & Randlov, 1981.; Sukhotin et al., 2003; Tedengren et al., 1990). Generally, mean pumping rates observed in this study, ($0.74 \pm 0.85$ to $1.83 \pm 0.40$ L $h^{-1}$) were within the range observed in the literature, ~ 0.45 to 6 L $h^{-1}$ (Cranford & Hill, 1999; Petersen et al., 2004; Smaal et al., 1997; Strohmeier et al., 2009; Tedengren et al., 1990), indicating mussels used in these trials were feeding at normal rates.

Between the trials, food concentrations and types (artificial/natural) were changed to explore feeding response under a variety of conditions. Temporal variations in concentration and quality of food are common in environments inhabited by mussels and often lead to differences in feeding behaviour over time (Smaal et al., 1997). Despite trial-by-trial differences, no significant changes in pumping rates or feeding indicators among trials were observed. This may be because changes in diet (in quality and quantity) were too minimal to observe this effect, or because individual variation in PR or feeding was too high. Notably, food concentrations were increased midway through trial 2, resulting in a general increase in feeding when using the fluorometer as an indicator of feeding activity.

In general, there was a high degree of inter-individual variation within sources of mussels when comparing pumping rates across all trials. Variability in feeding activity among individuals taken from the same location and held in a common setting has been observed in previous studies of *Mytilus* sp. and is mainly attributed to differences in physiological plasticity (Fernández-Reiriz et al., 2016; Fuentes-Santos et al., 2018). The use of the fluorometers as an indicator of feeding activity throughout the course of trials 2 and 3, showed that individual feeding can change considerably over small-time frames (minutes to hours) despite stable food conditions (trial 2), highlighting the relevance of intra-individual variability. Large intra-individual variability in feeding for mussels has been demonstrated previously over short (hours) and long (months) time frames (Fuentes-Santos et al., 2018; Strohmeier et al., 2009). This temporal variation demonstrates how discrete pumping rate measurements can misrepresent the feeding activity of individuals and sources of mussels and validates the use of methods that continuously measure feeding over time such as the one outlined in the present study (Vajedsamiei et al., 2021). More generally, given the intra-individual variability observed, it is apparent that the use of longitudinal (repeated measurements) over cross-sectional (single point in time measurements) studies is important to accurately characterize bivalve feeding physiology.

## 4.2. METABOLIC RATE

The measurement of MR is another important aspect for comparing energy availability for growth in mussels. Differences in MR may indicate the ability of some mussels to use energy more efficiently, which can contribute to increased growth rates (Thompson & Bayne, 1974). However, like the PRresults, under the tested conditions, MR did not vary significantly among the sources of mussels. A variety of intrinsic and environmental factors such as temperature, food concentration, stress exposure, and seasonality contribute to the range of MR values in the literature (Bayne & Widdows, 1978; Smaal et al., 1997; Sukhotin et al., 2003). Mean metabolic rates observed in this study ($0.44 \pm 0.12$ to $1.25 \pm 0.24$ mg $O_2$ $h^{-1}$) were within the range observed in the literature (~ 0.3 to 1.7 mg $O_2$ $h^{-1}$) for *Mytilus sp.* in equivalent environmental conditions (Schluter & Josefsen., 1993; Smaal et al., 1997; Sukhotin et al., 2003; Tedengren et al., 1990), indicating metabolic activity was normal for the mussels used in these trials.

Although lesser than PR, there was considerable variability in mean metabolic rates within sources of mussels. Intrinsic variability within sources is common in the literature and under similar conditions can be attributed to differences in plasticity (Fuentes-Santos et al., 2018; Smaal et al., 1997). Ecologically, having an important range of phenotypic plasticity within a population provides a better capacity to face changing and stressful environmental conditions. High levels of intra-individual variation in MR over short time scales (hours) were also observed in this study (not shown). Similar to PR, the intra-individual variation indicates that individual MR can change with time and illustrates the importance of multiple measurements of MR over time. As respiration is linked to the energy released from stored food, and is thus dependant on feeding, the observation of this intra-individual variation is unsurprising when considering variations in PR. When comparing trials, it is noteworthy to highlight the significantly higher

metabolic rates of all individuals in trial 1. This can be attributed to the considerably higher levels of food (Thompson & Bayne, 1974) and/or the seasonal timing of trial 1, which in turn affects the reproductive cycle of the mussels, and consequently their metabolism. Seasonal changes in energy availability and increases of energy allocation towards reproduction can result in a peak in metabolism during the spring and early summer, corresponding to the timeframe of trial 1 (June collection) (Smaal et al., 1997).

### 4.3. SCOPE FOR GROWTH

Scope for growth estimations in trial 3 represent the energy available for growth under different scenarios of assimilation efficiencies to capture the expected variability within the population. The SFG estimated here ($4.95 \pm 7.78$ to $16.47 \pm 13.92$ J $g^{-1}$ $h^{-1}$) are within the normal range for this species (-1.54 to 22 J $g^{-1}$ $h^{-1}$) (Widdows et al., 2002; Widdows & Johnson, 1988). The variability in SFG results reflects the high variability observed in physiological rates. Observed metabolic and pumping rates indicate that they do not differ among sources, suggesting a plastic response of all sources of mussels when exposed to the common garden. Despite observing similar physiological rates, adaptation cannot be discarded, as adaptive responses could emerge under different environmental conditions. Differences in physiology of bivalves are often observed after initial transfer to common locations, but plastic changes regarding the new environment can negate these differences shortly thereafter (days) (Babarro et al., 2000b; Labarta et al., 1997). Variability in physiology can indicate a broad range of plastic responses to the local environment, which must be considered when exploring adaptation.

Although both PR and MR did not differ significantly, growth rates may still vary among these sources over the long term (e.g., Albentosa et al., 2012). When interpreting the results from physiological measurements and SFG, it is important to consider that they represent instantaneous rates collected in the short term, but differences in growth rates often only become apparent in the long term, after months to years of growth (Bayne & Worrall, 1980; Mallet & Carver, 1989).. The short timeframe of these trials (days) introduces challenges in observing physiological differences that could explain differences in growth, which only manifest over the long term (years). The variability in our data and the corresponding wide range of SFG estimates observed provide a basis for how differences may appear over broader time-scales.

### 4.4. FUTURE WORK

Despite prior observations of different growth rates in *M. edulis* from geographically-distinct sources (John Davidson, Fisheries and Oceans Canada, personal communication; Mallet et al., 1987), our study did not find differences in pumping rate or metabolic rate among *M. edulis* sources reared in a common location. Regarding SFG measurements, in the future, absorption efficiency should be calculated for each source of mussels. Adding absorption efficiency measurements would allow for a stronger comparison of SFG results, by taking into account any source-by-source differences in digestive physiology that may contribute to variations in SFG calculations. The common-garden design was implemented to disentangle effects of physiological plasticity from adaption. However, due to high levels of intra- and inter-individual variability in both PR and MR, a higher sample size would be needed to observe statistically significant differences that would indicate local adaptation. Intra-individual variability was observed in small-scale temporal variations (hours) in feeding and metabolic rate. The feeding indicator (fluorometers) used in this study allowed us to explore this intra-individual variation and represents a useful tool for future comparisons. Further, the design of the present study (repeated measurements) provided an advantage over cross-sectional studies (single point in time measurements) that may misrepresent the physiology of bivalves due to short-term variations within individuals. Similarly, considerable inter-individual variability regarding physiology, which has a substantial effect in

cohort studies (Fuentes-Santos et al., 2018), was observed. Increasing replicates presents a potential solution to minimize the effects of this variation. The variation in PR and MR observed demonstrates how physiology of individual mussels can vary, but to observe differences at the source level, the effects of inter- and intra-individual variation must be accounted for. These challenges may be resolved by incorporating more replicates and measuring over longer periods of time, respectively.

Beyond the feeding and metabolic rates observed under these conditions, other external and internal factors may contribute to differential growth rates in mussels. External factors such as temperature and food concentration can influence the physiology of mussels (Riisgard & Randlov, 1981; Thompson & Bayne, 1974). As both factors can affect feeding and metabolism, it is possible that physiological variation among sources, driven by adaptation, is only apparent at varying levels of either factor. For example, physiological responses to environmental stressors (thermal stress) can vary between sources (Lesser et al., 2010). Further, it is possible that there are differences in the allocation of energy towards reproduction between these sources, which is another key driver of differential growth rates (Bayne, 2004). Despite differences in feeding and metabolism not being observed, the methods used to obtain our results represent an ideal way to compare physiological processes. Common-garden experimental designs allow for the comparison of mussels without having to consider plastic responses to different local environments. In future studies, measuring SFG and allocation of energy to reproduction in common-garden settings over a longer timeframe and with high temporal resolution, or with more individuals, may provide further insight into the physiology of mussels from each source.

### 4.5. CONCLUSIONS

Determining how physiology differs among mussel sources and how these differences affect growth rates have important implications for aquaculture. To better understand growth rates of mussels from different sources, the physiology of mussels reared in common grow-out locations needs to be better understood. Further, in a global context, studies like this are vital for determining how local adaptations can affect the long-term physiology and plasticity of organisms. In the present study, PR and MR did not vary among *M. edulis* sources, implying there is a lack of local adaptation in settling location, or that physiological plasticity was able to overcome the potential effects of adaptation. However, integrative, and long-term estimates of growth may provide better quantifications of these processes. Despite the lack of differences in feeding and metabolism observed, the methods used in the present study represent a significant improvement in techniques to examine plasticity and adaptation. The use of common-garden experiments and multi-day measurements of individuals provides a reliable characterization of mussels without the draw backs of cross-sectional studies.

## 5. ACKOWLEDGMENTS

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