Biochemical and genetic characteristics of suspension-cultured mussels (*Mytilus edulis*) in relation to byssal thread production and losses by fall-off

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**Abstract** – Mussel culture relies on the mussel self-attachment capacity through byssus production. By doing so, no cages or containment devices are needed. It has been previously suggested that thread production requires a non-negligible part of the energy expenditure in blue mussels *Mytilus edulis*. Therefore our work investigates the relationships between byssal thread production, mussels energetic reserves and phospholipids profiles in the foot. The relationship between thread production and heterozygosity was also examined. The study was realized in a small lagoon of the Îles-de-la-Madeleine, southern Gulf of St. Lawrence, Canada, on four sampling dates between June and September encompassing the pre- to post-spawning periods. Our results show a significant relationship between the thread numbers and attachment strength. However, no correlations were found between energy reserves, phospholipids composition of the foot or heterozygosity level and byssal production. Our results suggest that mussels in suspension culture in this lagoon were not energy-limited so that the energy reserves did not influence the byssal thread production.

**Key words:** Mussel culture / Byssus / Attachment / Energy reserves / Heterozygosity / Phospholipids

1 Introduction

Mussels need to be firmly attached to the substratum to survive; otherwise they could be dislodged and moved to unfavourable environments. Aquaculture takes advantage of this self-attachment capacity for suspension-culture on ropes and sleeves. However, mussels could be subject to fall-off under turbulent conditions and/or through adjustment of densities (i.e. self-thinning) with time (Fréchette et al. 1996; Myrand et al. 2009a). At sleeving, mesh tubes are filled with juveniles that must then reorganize and attach themselves to the culture substratum (rope or mesh tube) to maintain their position and access to space and food (Mallet and Myrand 1995; Sénéchal et al. 2008).

Attachment is achieved through the secretion of byssal threads (Mahé 1968) made of collagenous proteins (Bairati and Vitellaro-Zuccarello 1976; Benedict and Waite 1986). The byssal thread production, and the resulting attachment strength, varies along with abiotic (temperature, turbulence, current velocity, food availability) and biotic (reproductive condition) factors (Clarke 1999; Carrington 2002; Hunt and Sheibling 2002; Alfaro 2005; Zardi et al. 2007; Babarro and Reiriz 2010). The attachment strength is defined as the force needed to dislodge a mussel from its substrate (Lachance et al. 2008). The byssal thread production is often considered to be costly in terms of energy requirements (Pieters et al. 1980; Hawkins 1985; Thieltges and Buschbaum 2007; Zardi et al. 2007). Hawkins and Bayne (1985) reported that byssal threads can require about 8% of the mussel’s total energy costs as well as high proportions of its carbon (44%) and nitrogen (21%) budgets during summer. During this period, energy availability could be limited and mussels must trade-off the available energy between byssus production, and therefore greater attachment strength, versus growth and reproduction. Thus, it can be hypothesized that mussels having limited available energy reserves could face difficulties to produce byssal threads in sufficient numbers and/or at a lower rate.

Byssus production could also be related to the genetic characteristics of the mussel, mainly its heterozygosity (mean number of heterozygous loci per individual) which is linked to...
energy metabolism (Tremblay et al. 1998a; Myrand et al. 2002; LeBlanc et al. 2008). Indeed, a negative relationship between heterozygosity and the metabolic cost of maintenance has been observed in mussel where more heterozygous individuals need to invest less energy in their vital functions (Hawkins and Day 1996; Myrand et al. 2002). The surplus of available energy in more heterozygous mussels could, thus, be allocated to other metabolic functions such as byssal thread production.

Secretion of byssus is a dynamic process occurring in the foot after its contact with an appropriate substrate. Byssal gland produces an adhesive plate, before the foot relays and releases the new formed byssal thread via a polymer injection-molding process (Waite 2002). The foot can produce a new thread in less than 5 min (Coyne et al. 1997) and mussel attachment is usually achieved with 20 to 60 byssal threads (Bell and Gosline 1996). We hypothesize that the variation in the composition of the foot, particularly the membrane lipids, could influence the byssus production. Membrane lipids are mainly composed of phospholipids that contain fatty acids (hydrophobic part) and a polar head group (hydrophilic part). As most sites of cell function are to some degree membrane-dependent, or at least membrane-associated, using protein that are embedded in the lipid bilayer, membrane lipids play an important role in numerous cell mechanisms (Stuart et al. 1998; Calder and Yaqoob 2007). This assumption is supported by the Hulbert’s theory of membranes as pacemakers of metabolism which suggests that higher incorporation of polyunsaturated fatty acids in membrane increases the molecular activity of many membrane-bound proteins (Hulbert and Else 1999; Hulbert 2003). A growing number of studies also demonstrated that, not only the global fatty acid composition but rather the specific lipid composition of the cell membrane bilayer could have important roles on cell functions (Phillips et al. 2009). This specific membrane phospholipid composition is determined by particular phospholipid polar heads, linked to particular FA, by different linkages. Recent investigations on membrane phospholipid composition in marine bivalves have hypothesized physiological roles of peculiar phospholipids and fatty acid in these organisms (Kraffe et al. 2004; Le Grand et al. 2011) which could influence membrane functions and acts on biochemical activities such as byssal threads production.

Finally, we could expect that mussels with lower attachment strength would be more prone to fall-offs from the sleeves. Heavy losses could impact negatively the profitability of mussel culture. The aim of this study was to verify the possible relationships between the number of byssal threads produced by individual mussels relative to their attachment strength, their available energy reserves (lipids, glycogen and proteins) in different tissues, their heterozygosity level and the structure of membrane lipids of the foot. Experiments were performed at different periods between June and September to obtain mussel groups with different levels of energy reserves. These experimental periods were chosen according to spawning, a biotic factor acting directly on the attachment strength of mussels in suspension culture (Lachance et al. 2008). Finally, to establish the impact of byssal production on the productivity of mussels in suspension culture, losses by fall-off were quantified for each experimental period.

2 Materials and methods

2.1 Experimental design

The experiment took place in the House Harbour lagoon (Îles-de-la-Madeleine, southern Gulf of St. Lawrence, Canada; Fig. 1), a semi-enclosed and relatively shallow lagoon (mean depth of 6 m) where *Mytilus edulis* is cultured. Thirty mussels were randomly sampled at each of four sampling dates (22 and 29 June, 12 August and 13 September 2005) from three 60 cm long sections (10 mussels per section) of mussel sleeves suspended on a longline kept at 2 m below the surface (see Lachance et al. 2008). These mussels had been sleeved about 18 months earlier. Mussels were detached carefully, thread by thread, to avoid damaging the byssal gland before the byssus was cut off at the shell margin. Mussels were then placed in individual spherical plastic cages (diameter = 20 cm) with numerous slots allowing water circulation. These cages were made of two perfectly fitting half-spheres and weights were attached to the cages. The 30 cages were then suspended on the same longline at a 2 m depth to allow for the production of new byssal threads. After 7 days, the cages were brought back to the laboratory and kept vertical in a tank supplied with running seawater at temperature of lagoon until measurements of attachment strength and byssal thread number were carried out usually within 12 h.

For each sampling date, losses by fall-off were estimated using 15 traps of 1.5 m diameter each installed under five different longlines that held commercial sleeves with 18-month-old mussels (Mallet and Myrand 1995). Each trap was installed at ∼30 cm above the bottom of the lagoon and under three adjacent sleeves each measuring ∼2.5 m in length. The traps were cleaned twice a week by scuba divers. On each occasion, mussels were collected, their wet mass was determined and data presented per day. Each commercial sleeve produces ∼7 kg of mussels m⁻¹ in this area (Bourque and Myrand 2006). Thus, each trap was suspended under approximately 52.5 kg of mussels or 787.5 kg for all the 15 traps combined.

2.2 Laboratory analyses

First, each cage was opened and the number of byssal threads produced by the enclosed mussel was counted. To quantify the attachment strength (in Newton), a small hole was drilled through the mussel shell. A hook was inserted into the hole and connected to an AFG 250 N ± 0.05 Quantrol™ dynamometer (Dillon, Fairmont, MN, USA) coupled to the Q Graph Quantrol™ (Dillon, Fairmont, MN, USA) software (Fig. 2). The mussel was then pulled up vertically at a constant low speed until complete dislodgment (Lachance et al. 2008) while the cage was kept fixed on a table. The shell length of the dislodged mussel was measured using a caliper. Because the variation in individual shell length was low throughout the experiment (mean 67.8 mm ± 0.4 SE; N = 116), there was no need to standardize attachment strength values according to mussel size. The mussel was then dissected into three parts: the digestive gland, the mantle (which contains the gonads) and the remaining tissues. Each part was weighed and kept at −80 °C until further analyses. Additional mussels collected on
the same sleeves and at the same time as those used for the determination of byssal strength attachment were sampled to measure their gonad index calculated as the dry mantle weight (70 °C for 72 h) divided by the dry whole body weight.

2.3 Biochemical analyses

For protein and glycogen analyses, 100 mg of tissue (digestive gland and gonads) from each individual were homogenized in 0.1 M citrate buffer at pH = 5. For lipid class analyses, approximately 200 mg of tissues were used. The protein concentration was measured with spectrophotometer microplates (Molecular Devices, Sunnyvale, CA, USA) using a method based on protein–coomassie blue dye binding using bovine serum albumin (Sigma, fraction V) as the standard (Bradford 1976). Total glycogen was measured using a colorimetric method based on the enzymatic digestion of the digestive gland or gonad homogenates with amyloglucosidase (Carr and Neff 1984). Glycogen from *Mytilus edulis* (Sigma, type VII) was used as the standard, and samples were analyzed with a spectrophotometer (UV-VIS Beckman DU 640, Brea, CA, USA). For lipids analysis, after extraction (Folch et al. 1957), lipids extracts were spotted onto S-III chromarods (Iatron Laboratories Inc., Tokyo, Japan) and lipid classes (triacylglycerols [TAG], free fatty acids [FFA], acetone mobile polar lipids [AMPL], sterols [ST] and phospholipids [PL]) were separated for quantification by the Iatroscan MK-VI analyzer (Parrish 1987).

Three groups of 3–4 mussels producing an average of 46, 78 and 92 byssal threads were created to analyze the lipid
composition of the foot in relation to byssal thread production and attachment strength. Firstly, an aliquot of the lipid extract was evaporated to dryness and lipids were recovered with three washings of 500 μl of CHCl₃/MeOH (98/2, v/v) and deposited at the top of a silica gel micro-column (30 × 5 mm i.d., packed with Kieselgel 60 70–230 mesh previously heated at 450 °C and deactivated with 6 wt% H₂O) (Marty et al. 1992). Neutral lipids were eluted with 10 ml of CHCl₃/MeOH and phospholipids recovered with 20 ml of MeOH and stored at −20 °C for further phospholipids class separation by high performance liquid chromatography (HPLC) and analysis of their respective FA composition. Phospholipid classes determination used HPLC to separate plasmalogen (1-alkenyl-2-acyl-) from diacyl and the phospholipid class CAEP (ceramideaminoethylphosphonate) (Kraff et al. 2004). The “diacyls” fraction contained the diacyl forms of glycerophosphatidylethanolamine (PE), glycerophosphatidylcholine (PC), and glycerophosphatidylserine (PS) along with cardiolipin (CL) and glycerophosphatidylinositol (PI), while the plasmalogen fraction contained only the lyso-analogues derived from the plasmalogen forms of PE, PS and PC. Each fraction was collected and evaporated at 40 °C in a vacuum centrifuge, and, after transesterification (MeOH/HCl, 10 min at 100 °C), analyzed by GC for FA composition. Due to the relative difficulty to split the amide bond linking fatty acids to sphingosine-type bases, CAEP was transesterified for 5 h at 100 °C (Le Grand et al. 2011). Fatty acid methyl esters (FAME) obtained were identified and quantified using polar (CPWAX 52 CB-50 m × 0.25 mm i.d.; 0.2 μm thickness) capillary columns and C23:0 FA internal standard. FA were expressed as the molar percentage of the total FA content of each class. For plasmalogen, the total percentage was adjusted to 50% to take into account the absence of alkenyl chains of the sn-1 position hydrolyzed by the acid mobile phase. The quantities of each phospholipids class were determined from their respective FA spectrum obtained by gas chromatography (Kraff et al. 2008).

2.4 Genetic analysis

A small piece of digestive gland from each individual was used to examine enzyme polymorphism for glucose phosphate isomerase (GPI, EC 5.3.1.9), mannosephosphateisomerase (MPI, EC 5.3.1.8), phosphoglucomutase (PGM, EC 2.7.5.1), octopine dehydrogenase (OcDH, EC 1.5.1.11), leucineaminopeptidase (LAP, 3.4.11) and two esterases (EST, EC 2.2.1.12) according to Tremblay et al. (1998a). Variability at the MPI locus was used to discriminate Mytilus trossulus from M. edulis (Gosling 1992). No M. trossulus were identified among the experimental mussels. Such finding is in agreement with the low occurrence of this species at the Îles-de-la-Madeleine (Tremblay et al. 1998a; Myrand et al. 2009b).

2.5 Statistical analysis

Statistical analyses were performed using Systat 11 (Systat Software Inc, San Jose, CA, USA). Data were log-transformed when needed to satisfy test conditions. One-way ANOVAs followed by post-hoc Tukey tests were performed to compare mean thread numbers, losses by fall-off, gonad index, attachment strength, as well as mean values of glycogen, proteins, total lipids and lipid classes for the four sampling dates (22 June, 29 June, 2 August and 13 September). The relationships between thread number and attachment strength, energy components (glycogen, protein and lipids), and lipid classes (triacylglycerols, sterols and phospholipids) in the digestive gland and in the gonads were evaluated for each sampling date and also for all dates pooled together using simple Pearson correlations, or Spearman correlations when data did not satisfy the test conditions. For each phospholipid class, one-way ANOVAs followed by post-hoc Tukey tests were used to compare their content between each group of mussels differing by their number of byssal thread produced. The PRIMER software V6 was used for multivariate analysis of the composition of fatty acids of each phospholipids class of mussel’s foot (Clarke and Warwick 2001). Triangular similarity matrices, based on Bray-Curtis similarity coefficient, were created followed by non-metric multidimensional scaling (n-MDS). The number of heterozygous loci per mussel (zero to seven) was used to characterize individual multi-locus heterozygosity (MLH) and the relationships with thread numbers were estimated by simple Pearson correlations. Descriptive genetic statistics (not presented) were calculated using the Genetix version 4.05 software (Belkhir et al. 1998). The FSTAT software (Goudet 2001) was used to calculate the Fis statistic, a measure of the deviation of the observed heterozygosity compared to the expected heterozygosity (Weir and Cockerham 1984). A permutation test (1000 permutations) was used to test the null hypothesis of F is = 0.

3 Results

Throughout the experiment, the attachment strength varied between 1.6 and 29 N (Fig. 3) and varied significantly among dates (df = 3, F = 10.21, p < 0.001) with higher values in September. There was a significant correlation between attachment strength and the number of threads produced by the mussels over the whole experiment (4 dates pooled; N = 116, r = 0.70, p ≤ 0.001). The mean number of threads produced over a 7-d period varied significantly among dates (df = 3, F = 6.09, p = 0.001), with values ranging from 58.8 (2 August) to 79.3 (13 September) threads per individual (Table 1). The number of threads varied considerably among individuals for each sampling date. For example, thread numbers varied between 14 and 110 per mussel on 2 August (Table 1). Mussel biomass losses resulting from fall-offs into the traps were low: the highest value being obtained at the late June with a mean of 0.28 kg per trap per days or about 4.2 kg for the 15 traps combined (Table 1).

The gonad index, which represents the mussel’s reproductive condition, varied significantly among sampling dates (df = 3, F = 142.6, p < 0.0001), with a major decrease of 55% (from 0.42 ± 0.01 to 0.19 ± 0.01) between 22 and 29 June (Fig. 4). The gonad index remained low until 2 August and then increased slightly but significantly during fall. The energy components (glycogen, proteins, total lipids) provided...

3.1 Relationships between thread numbers and biochemical components

As the number of threads is representative of the byssal production, the mean thread number was used to describe the relationships with energy reserves and multi-locus heterozygosity. The quantity of glycogen varied between 0 and 35 mg g$^{-1}$ (Fig. 5a, b) and proteins between 60 and 170 mg g$^{-1}$ (Fig. 5c, d) in the digestive gland and the gonads. In the gonads, the glycogen and the protein values were lower in June in comparison with other dates (Table 2). In the digestive gland, the glycogen level was two to four times higher in September than at any other dates (Table 2). Total lipids varied over a wider range in the gonads (0–100 mg g$^{-1}$) than in the digestive gland (0–70 mg g$^{-1}$) (Fig. 5e, f), with a significant decrease in the gonads between 22 and 29 June (Table 2). This decrease in lipids is associated to a mass spawning as shown by the concurrent and important significant decrease in the gonad index (Fig. 4). All these energetic components showed no significant correlation with the number of threads produced for any sample date or for the four dates pooled (Table 3).

The relative quantity of triacylglycerols (%TAG) in the digestive gland varied between 0 and 80% (Fig. 6a), with a significant decrease between 22 and 29 June, in parallel with the mass spawning. While there was no significant correlation with the thread numbers for any sampling date, a significant relationship was observed when all dates were pooled (Table 4). Several mussels sampled on 22 June had higher %TAG (30–65%) in their gonads compared to the other sampling dates (Fig. 6b), but there were no significant correlations with thread numbers for any sampling date or for all dates pooled (Table 4).

3.2 Relationship between production of thread numbers and heterozygosity

Experimental mussels showed a high mean observed heterozygosity (0.58) with no significant deviation from Hardy-Weinberg equilibrium ($F$ is was not significantly different from 0). Allelic frequencies are not presented here since they are generally similar to published values (Tremblay et al. 1998a, b). There were no significant correlations between individual multi-locus heterozygosity (MLH) and thread numbers for any of the four sampling dates ($r_s = -0.003; r = 0.06; r = 0.23; r = 0.23$ from 22 June until 13 September; all $p > 0.32$), as well as for all dates pooled ($r_{obs} = -0.03$, $<r_{crit} = 0.17$, $N = 102$) (Fig. 7).
Table 2. Mean (±SE) values of the energy components (glycogen, proteins, total lipids) expressed as mg g\(^{-1}\) of wet mass (WT) and lipid classes (triacylglycerols, sterols, phospholipids) expressed as percentages for each sampling date in the digestive gland (DG) and the gonads (GO). For each energy component and lipid class, significant differences between dates revealed by post-hoc Tukey tests following one-way ANOVAs are indicated by different letters.

<table>
<thead>
<tr>
<th>Date</th>
<th>Glycogen (mg g(^{-1}) WT)</th>
<th>Proteins (mg g(^{-1}) WT)</th>
<th>Total lipids (mg g(^{-1}) WT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DG</td>
<td>GO</td>
<td>DG</td>
</tr>
<tr>
<td>22 June</td>
<td>4.2 ± 0.3 (^{a})</td>
<td>3.2 ± 0.2 (^{c})</td>
<td>88.6 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>85.5 ± 6.5 (^{c})</td>
<td>17.4 ± 2.1</td>
<td>49.6 ± 5.1 (^{a})</td>
</tr>
<tr>
<td>29 June</td>
<td>5.1 ± 0.7 (^{bc})</td>
<td>4.4 ± 1.1 (^{b})</td>
<td>85.4 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>64.4 ± 3.3 (^{c})</td>
<td>16.7 ± 2.2</td>
<td>31.2 ± 4.2 (^{b})</td>
</tr>
<tr>
<td>12 August</td>
<td>7.4 ± 0.5 (^{c})</td>
<td>19.4 ± 0.7 (^{b})</td>
<td>89.0 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>82.0 ± 3.6 (^{b})</td>
<td>19.0 ± 2.4</td>
<td>31.3 ± 3.4 (^{b})</td>
</tr>
<tr>
<td>13 Sept.</td>
<td>16.2 ± 0.9 (^{a})</td>
<td>25.3 ± 0.6 (^{a})</td>
<td>90.1 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>100.3 ± 4.4 (^{a})</td>
<td>19.6 ± 2.3</td>
<td>41.9 ± 3.7 (^{b})</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>% Triacylglycerols</th>
<th>% Phospholipids</th>
<th>% Sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DG</td>
<td>GO</td>
<td>DG</td>
</tr>
<tr>
<td>22 June</td>
<td>20.9 ± 3.9 (^{ab})</td>
<td>23.8 ± 4.8 (^{a})</td>
<td>69.5 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>69.6 ± 13.2 (^{b})</td>
<td>2.3 ± 0.2 (^{b})</td>
<td>1.1 ± 0.2 (^{c})</td>
</tr>
<tr>
<td>29 June</td>
<td>11.2 ± 1.7 (^{bc})</td>
<td>6.6 ± 1.2 (^{b})</td>
<td>76.5 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>89.5 ± 16.6 (^{c})</td>
<td>3.4 ± 0.4 (^{b})</td>
<td>1.9 ± 0.1 (^{b})</td>
</tr>
<tr>
<td>12 August</td>
<td>17.2 ± 2.7 (^{ab})</td>
<td>7.9 ± 1.0 (^{b})</td>
<td>71.7 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>87.1 ± 15.9 (^{a})</td>
<td>3.1 ± 0.3 (^{ab})</td>
<td>2.5 ± 0.1 (^{b})</td>
</tr>
<tr>
<td>13 Sept.</td>
<td>24.8 ± 2.9 (^{a})</td>
<td>11.6 ± 1.1 (^{b})</td>
<td>63.7 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>83.5 ± 15.5 (^{a})</td>
<td>2.6 ± 0.2 (^{ab})</td>
<td>2.9 ± 0.1 (^{a})</td>
</tr>
</tbody>
</table>

Table 3. Pearson or Spearman correlations between the energy components expressed as mg g\(^{-1}\) of wet mass (WT) in the digestive gland (DG) or the gonads (GO) and the thread numbers for each sampling date and for all dates pooled. (\(N = 25–29\) per sampling date; \(N = 109–116\) for pooled dates; \(r = \) correlation coefficient; \(r_{\text{observed}}\) was always \(< r_{\text{critical}}\) for the Spearman correlations or these results are indicated as NS [not significant].)

<table>
<thead>
<tr>
<th>Dates</th>
<th>Glycogen (mg g(^{-1}) WT)</th>
<th>Proteins (mg g(^{-1}) WT)</th>
<th>Total lipids (mg g(^{-1}) WT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DG</td>
<td>GO</td>
<td>DG</td>
</tr>
<tr>
<td>22 June</td>
<td>0.4 ± 0.05</td>
<td>0.23 ± 0.2</td>
<td>0.09 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>-0.13 NS</td>
<td>0.11</td>
<td>0.09 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>0.64</td>
<td>0.32</td>
</tr>
<tr>
<td>29 June</td>
<td>-0.06</td>
<td>-0.11</td>
<td>-0.08 ± 0.69 (^{a})</td>
</tr>
<tr>
<td></td>
<td>0.13 ± 0.52</td>
<td>0.52</td>
<td>0.16 NS</td>
</tr>
<tr>
<td></td>
<td>-0.29 NS</td>
<td>-0.28 NS</td>
<td>-0.29</td>
</tr>
<tr>
<td>12 August</td>
<td>-0.04</td>
<td>0.83</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>0.20 ± 0.11</td>
<td>0.11</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>-0.29 NS</td>
<td>-0.28 NS</td>
<td>-0.29</td>
</tr>
<tr>
<td>13 Sept.</td>
<td>-0.3 ± 0.12</td>
<td>0.13</td>
<td>0.19 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>0.34</td>
<td>0.98</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>0.05 NS</td>
</tr>
<tr>
<td>All dates</td>
<td>0.14 ± 0.13</td>
<td>0.14</td>
<td>0.12 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>0.09 ± 0.34</td>
<td>0.32</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td>0.27</td>
<td>0.05 ± 0.01</td>
<td>-0.12</td>
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<tr>
<td></td>
<td>0.59</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>-0.13 NS</td>
<td>-0.51 NS</td>
<td>-0.19</td>
</tr>
<tr>
<td></td>
<td>0.33 ± 0.11</td>
<td>0.01</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>0.02</td>
</tr>
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3.3 Attachment strength and phospholipids characteristic of the foot

The mean content (% of total phospholipids) of diacyls, plasmalogens and ceramide aminoethylphosphonate (CAEP) showed no significant differences among the mussel groups producing a mean of 46, 78 or 92 byssal threads (\(df = 2, F = 1.99, p = 0.22\); \(df = 2, F = 2.76, p = 0.14\) and \(df = 2, F = 3.38, p = 0.10\) respectively). Their overall means were 32.4 ± 4.8% for diacyls, 44.8 ± 1.5% for plasmalogens and 22.8 ± 3.5% for CAEP. For each subclass and class of phospholipids (diacyls, plasmalogens and CAEP) in the mussels’ foot, the n-MDS analyses clearly showed no grouping of the fatty acids chain profile according to the mussels production of byssus (46, 78 or 92 threads). These results demonstrated no significant differences among the fatty acids chain profiles of mussel groups for each phospholipids subclass and class. The mean fatty acids chain profiles of diacyls, plasmalogens and CAEP in the foot of the mussels are presented in Table 5.

4 Discussion

Hawkins and Bayne (1985) suggested that energy costs for byssal thread production were relatively high. Thus, we wanted to evaluate if mussels with higher available energy reserves could invest more energy in byssus production and thus increase their attachment strength. We had observed a significant relationship between the number of byssal threads produced over one-week periods and the mussels’ attachment strength.
Fig. 5. Energy components (glycogen, proteins and total lipids) in the digestive gland (left) and the gonads (right) in mg g\(^{-1}\) of wet mass vs. the number of threads produced over a 7-d period by individual mussels at each sampling date.

Fig. 6. Main lipid classes (triacylglycerols [TAG], sterols [ST], and phospholipids [PL]) in the digestive gland (left) and the gonads (right) vs. the number of threads produced over a 7-d period by individual mussels at each sampling date.

strength as previously reported (Lee et al. 1990; Bell and Gosline 1997; Carrington 2002). However, no significant correlations were seen between the number of threads produced over one-week periods and any of the energy component measured. These results suggest that the energy level in suspension-culture mussels in this Îles-de-la-Madeleine lagoon was not a limiting factor between June and September in terms of byssal production. To our knowledge, this is the only study, with the exception of the work of Babarro and Reiriz (2010) that looked at the influence of endogenous factors on individual byssus production capacities.

As shown in Figure 3, there was a strong temporal variation in attachment strength in the House Harbour lagoon with a two-fold increase from summer to autumn consistent with Lachance et al. (2008). Mussels with fewer threads are attached less firmly to the substrate and can be more vulnerable to dislodgement by waves, currents and turbulence (Bell and Gosline 1997; Hunt and Sheibling 2002). Nevertheless, losses by fall-off in this study were low between June and September with weekly values below 0.5% of the mass of mussels suspended over the trap (about 787.5 kg). Thus, the decrease in byssal production in early August, and the associated attachment strength, did not seem to be low enough to result in an important fall-off.

The energy assimilated by mussels from available food is allocated to different metabolic functions including growth, gamete production, and maintenance or production of inert tissue like byssal threads (Bayne and Newell 1983; Hawkins and Bayne 1991). Some metabolic functions could require large
amounts of energy and thus compete with the others (Bayne and Newell 1983; Blanco et al. 2006). For example, gamete production can mobilize up to 90% of the energy in sexually mature mussels (Rodhouse et al. 1984). Energy reserves are accumulated primarily in the digestive gland and in the gonads (Cartier et al. 2004; Blanco et al. 2006) mostly as glycogen and lipids stored as energy reserves (Freites et al. 2002; Pernet et al. 2007). In our study, we observed generally no relationship between glycogen and lipids with the number of byssal threads produced when we compared pre and post-spawned mussels in natural environment. The only exception was the significant correlation observed between the %TAG and thread numbers in the digestive gland for all dates pooled, with a correlation coefficient of 0.20.

There was a strong inter-individual variation in the number of threads produced over a 7-d period under the field conditions. Although the number of threads produced by individual mussels varied between 14 and 110 over the 7-d periods for all four experimental dates, factors related to the individuals’ energy status such as their reserve components in the digestive glands and in the gonads or multi-locus heterozygosity did not explain this inter-individual variability in byssal production. Most studies (Young 1985; Bell and Gosline 1997; Clarke 1999; Hunt and Scheibling 2001; Alfaro 2006; Lachance et al. 2008) have looked at the influence of various factors on the mean production of byssal threads or on the mean attachment strength of mussels. Only Babarro and Reiriz (2010) looked at inter-individual variation in the secretion of thread number. In their study the number of threads secreted by the mussels (Mytilus galloprovincialis) during 6 days was less variable than that observed in our study, with values around 15 to 30 threads. However, these results have been obtained in specific experimental conditions. Several studies showed that the production of byssal threads varied between mussel species and also between populations from the same species (Young 1985; Bell and Gosline 1997; Seed and Richardson 1999). All experimental mussels used in the present study belonged to Mytilus edulis, and to the same population (Tremblay et al. 1998a). Moreover, the experimental mussels measured on a given date were placed in identical environmental conditions. They were also of similar size and in the same reproductive condition, as it is usual for suspension-cultured mussels (Myrand et al. 2000). Thus, none of these factors could explain the observed variations.

Differences in the number of threads and the attachment strength were not related to phospholipid composition of mussels’ foot cellular membranes, as the content of the phospholipid classes and subclasses investigated (diacyl, plasmalogen and CAEP) as well as their specific fatty acid compositions showed no significant differences between mussels with different byssal production. CAEP is a particular class of phospholipids in mollusks and it is similar to sphingomyelin in the cell membranes of mammals (Le Grand et al. 2011). This class of phospholipids is characterizedly found in lipids rafts, which are regions of the membranes that would act as platforms to localize proteins involved in intracellular signalling pathways (Zehmer and Hazel 2005; Calder and Yaqoob 2007). The phospholipids in the rafts are mainly made of saturated fatty acids.
chains and could allow the closed packing of lipids within rafts (Calder and Yaqoob 2007). Similarly, our study (Table 5) shows that the sum of saturated fatty acids chains in CAEP is over 70%. Plasmalogens are glycerophospholipids reported to reduce transmembrane solute fluxes and minimise proton leak across mitochondrial membranes (Hazel and Williams 1990). Despite a potential role of these phospholipids classes on muscles foot functions, our results indicate that it could not be a parameter that explains the differences in the byssus production.

Mussels measured on 22 June were in a pre-spawning stage as shown by their high gonad index (0.42 ± 0.01) and the high percentage of TAG in their gonads, which reflects the egg content in females (Brazao et al. 2003; Pernet et al. 2003). A massive spawning event occurred during the following week as the gonad index declined by 55% and TAG content by 72%, indicating a massive egg release. Gametogenesis uses a large amount of glycogen to produce gametes, and mussels actively involved in gametogenesis could have lower available quantities of glycogen and lipids for byssal thread production (Carrington 2002; Zardi et al. 2007). However, the non-significant correlations between these energetic components and threads production suggest that no trade-off was needed between gametogenesis and byssus production. After spawning, energy reserves were low as revealed by the low glycogen content in the digestive gland and the gonads (below 5 mg g−1). Thus, it could be hypothesized that spawned mussels recovering from spawning and with low energy reserves could produce lower number of threads. The lack of correlations in this study does not support this hypothesis. In August and September, the glycogen increase was higher in the gonads than in the digestive gland, thus suggesting that the digestive gland is an organ for short-term storage before the assimilated energy is transferred to other tissues (Bayne and Newell 1983; Racotta et al. 2003). This glycogen increase is possibly related to an increase in food availability (Bayne and Widdows 1978; Lemaire et al. 2006) but with no apparent relationship between energy reserves and byssal production, even though the thread production was higher in September. This increase in byssal threads production could be related to environmental factors such as turbulence or temperature leading to a stronger attachment in autumn (Dolmer and Svane 1994; Hunt and Scheibling 2001; Carrington 2002; Alfaro 2005; Lachance et al. 2008).

Although more heterozygous mussels have a lower maintenance metabolism and could thus potentially have more energy available for other uses (Hawkins and Day 1996; Tremblay et al. 1998b; Myrand et al. 2002; LeBlanc et al. 2008), in the present study, this has not been translated into higher numbers of byssal threads produced. It has been shown that the heterozygosity–fitness relationship is more apparent under stressful conditions (Gentili and Beaumont 1988; Scott and Koehn 1990). Myrand et al. (2002) showed an inverse relationship between number of heterozygous loci and standard metabolism for mussels under stressful conditions. However, the experimental mussels were probably not under stressful conditions in our study. Since the mussels sampled show high number of heterozygous loci, it is possible that MLH values were not variable enough to reveal a significant correlation between thread numbers and multi-locus heterozygosity.

5 Conclusion

The lack of correlation between energy components in digestive gland and gonad and byssal thread production could be due to an overestimation of the energy required for thread production. Furthermore, mussels use their energy reserves mostly during gametogenesis and when food is scarce (Bayne and Newell 1983; Hawkins 1985). It is possible that suspension-cultured mussels in the House Harbour lagoon are not starved since seston concentration is relatively constant throughout season (Lachance et al. 2008). As a result, mussels may not be forced into energy trade-offs detrimental to byssal production. The energy invested in byssal thread production in this particular population could also differ from other populations (Rodhouse et al. 1984; Hawkins and Bayne 1991). Hawkins and Bayne (1985) quantified the energy costs for byssal production in a population of *Mytilus edulis* from the English coast, which is subjected to high wave action and strong currents. In such an environment, mussels may need to invest a higher portion of their energy into byssal production compared to mussels in suspension culture in a semi-enclosed lagoon.

We observed no correlation between all energetic characteristics and byssal production during this experiment. Hypotheses about the possible importance of heterozygosity or the role of membrane phospholipids in the foot were not supported by conclusive results neither. Further studies should be conducted to investigate other environmental parameters such as metals ions as these ions are utilized for cross-linking of the byssus proteins (Monahan and Wilker 2004; Soo Hwang et al. 2010; Holten-Andersen et al. 2011).

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