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# Comparative analysis of Byssal thread production, mechanical properties, and composition in diploid and triploid *Mytilus edulis*

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ABSTRACT

Mussel fall-off continues to pose a major challenge for suspension-culture farming, leading to substantial crop losses. Although studies on juvenile mussels (<30 mm) have indicated that triploids may exhibit enhanced byssal thread attachment and reduced fall-off, their performance in adult mussels remains insufficiently explored. In this study, we investigated the production, mechanical strength, biochemical composition, and structural features of byssal threads in diploid and triploid mussels of commercial size (>50 mm). We also calculated metabolic and filtration rates, as well as scope for growth. Our results revealed that triploid mussels produced 25 % more byssal threads with significantly enhanced mechanical properties. Triploid mussels conditioned at 20 °C exhibited a 48 % higher clearance rate, a 57 % greater scope for growth, and a 40 % stronger valve (breaking strength) compared to diploids. These findings suggest that triploid mussels over 50 mm have superior attachment strength relative to wild mussels, primarily due to increased thread production.

## 1. Introduction

Over the last 30 years, mussel (*Mytilus edulis*) aquaculture has been flourishing in Canada, representing an important economic income for coastal populations. In 2023, nearly 22,000 tons of *Mytilus edulis* were produced in suspension culture with a worth of 45 million Canadian dollars (Statistics Canada, 2024). Significant quantities of mussels are lost du to fall-off during production, especially during handling procedures (Bourque and Myrand, 2006). Comeau et al. (2017) reported that approximately 46 % of mussels detach between socking and harvest. This loss is even more pronounced in juveniles on collectors, where fall-off rates can reach up to 75 % (Comeau et al., 2015). Mussel fall-off leads to sub-optimal production and significant economic losses for mussel farmers.

Mussel detachment from collectors and socks can arise from a combination of environmental and biological stressors. Key contributing factors include wave action turbulence, predation, handling of mussel lines, self-thinning, decay of byssal threads due to elevated water temperatures, biofouling, and reproductive status (Young, 1985; Rupp and Parsons, 2004; Alfaro, 2006; Lachance et al., 2008; Fréchette, 2012; Babarro and Reiriz, 2010; Babarro and Carrington, 2013; Seguin-Heine et al., 2014; Comeau et al., 2015; South et al., 2022). Overall, mussel fall-off occurs once the attachment strength provided by byssal threads is exceeded by mechanical stress applied on mussel clusters, leading to thread breakage and detachment (Carrington et al., 2015; Gagnon, 2019). The attachment strength is itself dependent on multiple intrinsic factors including the number and quality of the threads (Smeathers and Vincent, 1979; Bell and Gosline, 1996; Bell and Gosline, 1997), their

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diameter (Hennebicq et al., 2013; Bouhlel et al., 2017), as well as amino acid and metal composition (Hedlund et al., 2009; Seguin-Heine et al., 2014). Mussel spawning can also result in lower byssogenesis and reduced byssal threads production (Babarro and Reiriz, 2010) or byssus diameter, (Hennebicq et al., 2013) decreasing the attachment strength by up to 40 % (Lachance et al., 2008; Seguin-Heine et al., 2014).

Triploidy in bivalves has been widely studied to produce stocks with faster growth due to reduced reproductive investment (Allen Jr. and Downing, 1986; Allen Jr. et al., 1986; Allen Jr. and Downing, 1990; Brake et al., 2002; Brake et al., 2004). However, to our knowledge, the impact of triploidy on the attachment strength of bivalves has not been investigated, except for our previous study on small (<30 mm) triploid *M. edulis* (Osterheld et al., 2023) in which we showed that they produced up to 65 % more byssal threads compared to diploid mussels of the same size range. Although the diameter of both diploid and triploid mussel threads was similar, those of triploids elicited 43 % higher Young modulus values compared to diploid ones, suggesting that triploid mussel threads have better resistance against deformation. Indeed, Young's modulus determines the stiffness of the threads before reaching the yield point and can suggest a better resistance against deformation for triploid mussels (Bouhlel et al., 2017; Osterheld et al., 2023).

Bivalve size and developmental stage can alter byssal production and thread properties. Due to their greater mass, larger individuals require proportionally stronger byssal attachment. For example, Babarro and Carrington (2013) revealed that mussel size correlated with the threads' thickness and byssogenesis; specifically, small mussels produce thinner and a higher number of threads compared to large mussels. Consequently, the attachment strength and thread production may vary significantly between mussels of different size ranges. Additionally, reproductive status significantly influences byssal strength. Spawning has been shown to reduce attachment strength by up to 30 % (Lachance et al., 2008), as mussels divert substantial energy toward reproduction at the expense of other metabolic functions such as growth and byssal thread production (Carrington et al., 2015). In a previous study, we found that triploid mussels exhibited low reproductive investment and reduced gamete maturity, whereas diploids were sexually mature and heavily invested in reproduction (Osterheld et al., 2024). Due to their reduced fertility, triploids may allocate more energy toward somatic growth, shell formation, and byssal development. Consequently, triploid mussels could exhibit stronger attachment during the reproductive season compared to wild diploids, potentially reducing stock loss and enhancing production yields for mussel farmers.

Furthermore, studies on triploid bivalves already demonstrated the possibility that size, age, or developmental stage could alter their metabolic needs and scope for growth. For instance, the study of Kesarcodi-Watson et al. (2001) on oysters (*Saccostrea commercialis*) reported that the clearance rate of diploid and triploid juveniles decreased with increasing concentrations of food, while in adults, the clearance rate decreased significantly only for triploid oysters, suggesting less energy spent for respiration in adult triploids compared to diploids. Moreover, their result of scope for growth (SFG) showed that adult triploid oysters had 79 % more energy available for growth and reproduction than the diploids.

This study aims to characterize byssal thread production and properties in adult triploid *Mytilus edulis*, and to assess how they may differ from both diploid mussels and juvenile stages. These differences may reflect underlying variations in metabolic demands and energy allocation strategies. We therefore tested the hypothesis that adult triploid mussels invest more energy in byssogenesis than diploids, leading to byssal threads with distinct mechanical and compositional traits. To evaluate this hypothesis, we compared respiration rates, clearance rates, absorption efficiency, and scope for growth between adult diploid and triploid mussels, and analyzed how these physiological parameters could be related with byssal thread production and characteristics (i.e. thread strength, diameter, amino acid profile, metal content, and molecular structure).

## 2. Materials and methods

### 2.1. Experimental design

At the end of May 2019 in Saint Peters Bay (46° 26′ 30.7" N, 62° 44′ 51.3" W) in Prince Edward Island, Canada, two-year old, cultured mussels *Mytilus edulis,* (shell length 50–60 mm) were harvested for spawning and production of triploid mussels as described in Osterheld et al. (2021). Briefly, fertilization was performed at an oocytes/spermatozoids ratio of 1:10. At 17 min post-fertilization, when most of the eggs had extruded their first polar body, larvae were treated for triploidy induction by bathing fertilized eggs in 400 μM 6-dimethylaminopurine in UV ultra-filtered seawater (0.2 μm) for 10 min at 18 °C. Then the eggs were washed with UV ultra-filtered seawater and reared in static tanks at 18 °C until reaching the D-shaped stage. The treated larvae were then reared in 60-L conic tanks at 18 °C in UV ultra-filtered seawater with upwelling flow-through system, and fed continuously at 30,000 cells/min with a mixture of microalgae containing *Pavlova lutheri* (CCMP 459), *Nannochloropsis oculata* (CCMP525), *Chaetoceros neogracilis* (CCMP1317), *Tisochrysis lutea* (CCMP 1324) and *Tetraselmis suecica* (CCMP 904) at a ratio of 1:1:1:1:1 equivalent biomass according to culture algal dry weight, as described by Bassim et al. (2014). At the pediveliger stage, mussels were transferred into a downweller system with similar conditions until they reached the juvenile stage of over 5-mm shell length. Every 48 h, the water temperature was lowered by 2 °C until it reached 10 °C, which was the ambient water temperature in Georgetown Harbour, PEI. At this point, mussels were transferred to a Portable Aquatic Laboratory obtaining water from Georgetown Harbour. There, the mussels were in natural water conditions thanks to the flow through water system, while avoiding weather (wave, wind) and predators (crab, ducks, starfish) risks.

Between August and September 2021, once mussels reached around 50 mm, they were transferred to the aquicole station at Pointe-au-Père near Rimouski, Québec, Canada. There, haemolymph sample from each mussel was collected and used to determine their ploidy through flow cytometry with CytoFLEX (Beckman Coulter, IN, USA) as described in Osterheld et al. (2021, 2024). Mussels that were confirmed as diploids were used as control mussels.

### 2.2. Byssal production

A total of 32 diploid and 32 triploid mussels were used for the production and harvest of byssal threads. Both the production and strength of byssal threads were compared under standardized conditions of feeding regime, temperature, light, flow speed, and salinity. The induction of the production was carried out as described in Osterheld et al. (2023) in a racetrack recirculating flume with an experimental section of 0.91 m in length and 0.45 m in width. The flume was filled with seawater filtered at 10 μm with a controlled temperature of 18.0 ± 0.7 °C and salinity of 27.0 ± 0.5 PSU. Every day, mussels were fed with one pulse-dose of 60,000 phytoplankton cells/mL with the species described above (CCMP 1324, CCMP1317, CCMP 459, CCMP 525). Mussels were fixed individually to the vertical side of an L-shaped glass, using cyanoacrylate glue (Locite Gel Control Super Glue, Henkel Consumer Adhesive, Avon, NY, USA), 5 to 10 mm above the horizontal glass with their posterior end facing upstream. They were then randomly placed, parallel to each other but separated by 5 cm, in the middle of the experimental area, facing the incoming flow. For 72 h, mussels were facing a unidirectional current that was increased with a maximal acceleration of 1 cm $s^{-2}$ and maintained at 10 cm $s^{-1}$. After the 72 h exposure, the L-shaped glasses were collected, byssal threads were counted and their distal parts were harvested (7 ± 0.2 cm). As mussels had to be kept alive for further physiological measurements, only the distal part of the byssal thread was used for mechanical properties analyses, preventing measurement of full thread length. These distal parts were stored at 4 °C in open plastic bags for a maximum of 2 months and were used for individual mechanical analyses as well as for byssal composition comparison.

### 2.3. Mechanical properties

Prior to mechanical measurements, byssal threads were rehydrated overnight in artificial seawater at 27 PSU using Instant Ocean salts (Aquarium Systems, Inc. • OH 44060 USA) at 4 °C. A total of 110 diploid and 105 triploid mussel distal threads (5 to 6 threads per mussel) were used to determine the mechanical properties.

As described by Osterheld et al. (2023), the diameter of the distal section of each individual thread was measured under an optical microscope (Olympus BX41, Japan, with a 200× magnification using the software Image Pro Plus (Media Cybernetics, Canada). Measurements were taken from four randomly selected segments of each distal thread, spaced at least 0.5 mm apart, over a minimal length of five millimeters. The means of all diploid and triploid distal threads diameter was calculated and compared between the two groups. Furthermore, the diameter of the cuticle of the distal section of each thread was measured using a Keyence VHX-2000 (Digital Microscope, Mississauga, ON, Canada).

A mechanical analyzer (Instron 5565, Norwood, MA, USA) equipped with a BioPuls bath filled with artificial seawater (pH 7.9) was used to measure the tensile characteristics of distal byssal threads, including the initial Young's modulus, the stress-at-break, breaking force, and strain-at-break, as described in Osterheld et al. (2023). Briefly, the ends of each thread were glued between small sheets of cellulose acetate (5 × 5 mm) using cyanoacrylate gel (Locite Gel Control Super Glue, Henkel Consumer Adhesive, Avon, NY, USA). The glued ends of the distal threads were placed between the grips of the mechanical analyzer and extended, until rupture, at a rate of 5 mm/min. Only threads that broke at their middle or close to the middle were considered valid, while those that broke at the jaws of the clamps or slipped were discarded. As a result, 99 diploid and 91 triploid threads were retained for analysis. The calculation of stress, strain, ultimate force, and Young's modulus were performed as described in Bouhlel et al. (2017) and Osterheld et al. (2023).

The stress ($\sigma$) represents the resistance of the thread of cross-sectional area A against an applied load (F) and is calculated as $\sigma = F/A$. The relative elongation of the thread under a load is expressed as the strain ($\varepsilon$, %) and is calculated as $\varepsilon = \left(\frac{\Delta L}{L}\right)*100$, where $\Delta L$ is the length change under load and L is the initial length of the thread. The initial stiffness of the thread, defined as the Young's modulus (E, Pa), was calculated as the slope of the stress-strain curve in the 5–10 % strain range (Bouhlel et al., 2017).

### 2.4. Amino acid composition

Byssal material from 50 diploid and 50 triploid mussels was collected and pooled separately for each treatment. Each pool was then divided into technical duplicates of 100 mg. The pools were grounded separately (Retsch Mixer Mill MM400; Retsch GmbH, Haan, Germany) for 5 min at 30 Hz until a fine powder was obtained. The byssal threads powders were then analyzed in duplicates by SPARC BioCentre (SickKids Proteomics, Analytics, Robotics & Chemical Biology Centre, Toronto, Canada) to determine the amino acid composition. Samples were dehydrated in pyrolyzed borosilicate tubes under a vacuum centrifugal concentrator. Then they were exposed to a vapour phase hydrolysis at 110 °C with 6 N HCl with 1 % phenol for 24 h under pre-purified nitrogen atmosphere. After hydrolysis and removal of excess HCl by vacuum, the hydrolyzates were washed with redrying solution. Then the samples were derivatized with phenyisothiocyanate (PITC) at room temperature to produce phenylthiocarbamyl (PTC) amino acids. The derivatized amino acids were redissolved in a phosphate buffer and loaded into an autosampler for injection as described by the protocol of SPARC BioCentre (sickkids.ca).

### 2.5. Nuclear magnetic resonance

Comparison of the amino acid structural composition of the byssal threads was performed by solid-state nuclear magnetic resonance (SS-NMR). All spectra were recorded on a Bruker Avance-III HD spectrometer (Bruker Biospin, Canada) operating at frequencies of 150.86 and 599.90 MHz for $^{13}$C and $^{1}$H respectively. The probe used was a 4 mm Magic-Angle Spinning double resonance probe and the spinning frequency was 10 kHz. The $^{13}$C spectra were initiated using either a 2 s-long low power irradiation of $^{1}$H followed by a single 90° pulse on $^{13}$C, or a 1.5 ms-long cross polarisation from $^{1}$H to $^{13}$C. The $^{13}$C signal was detected under $^{1}$H TPPM decoupling. The applied radiofrequency fields were always 75 kHz for both $^{1}$H and $^{13}$C except during the 2 s long low power irradiation of $^{1}$H (RF = 180 Hz) or cross-polarisation during which the 13C radiofrequency field was 65 kHz. The one-dimensional spectra were acquired with a total recycle delay of 5 s (3 s plus 2 s low power $^{1}$H irradiation for the single pulse spectra or 5 s for the cross-polarisation spectra), an acquisition time of 15 ms, a spectral width of ca. 45 kHz (300 ppm) while 1024 scans were accumulated. The same spectral windows were used for the two-dimensional spectra and the mixing time was 50 ms during which dipolar-assisted rotary resonance (DARR) was established by applying a 10 kHz radio-frequency field on the $^{1}$H channel. 96 transients were accumulated for each of the 256 increments acquired in the indirect dimension. To reduce the total experimental time, the two-dimensional spectra were recorded with a recycle delay of 3 s. All spectra were recorded at room temperature and processed using the MNova software (Mestrelab Research S.L., Santiago de Compostela, Spain).

### 2.6. Metal composition

The metal composition of byssal threads was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the *Centre de recherche sur les biotechnologies marines* (CRBM, Rimouski, QC, Canada) using the remaining byssal material from the same 50 diploid and 50 triploid mussels previously sampled for amino acid analysis. Byssus pools from 15 individuals per treatment were processed in triplicate. Prior to the analyses, technical triplicates of 10 mg of byssal threads by ploidy treatment (pool from different individuals) were digested in 0.5 mL of $HNO_3$ (Aristar Plus BDH 87003–261 2,5 L lot: 1116100) and 0.5 mL of $H_2O_2$ (solution Sigma Aldrich 16,911-250 mL lot BCBT9332). The samples were heated at 60 °C for 1 h, then collected, and the volume was adjusted to 10 mL by addition of Milli-Q water.

### 2.7. Physiological and metabolic analyses

Physiological and metabolic analyses were performed on the same diploid and triploid mussels used for byssogenesis conditioned at 10.0 ± 0.1 °C and 20.0 ± 0.1 °C for 1 month (16 mussels by temperature and ploidy treatment), following temperature acclimation of a rate of 1 °C by day. These temperatures were chosen as 20 °C represents the summer temperature present, while 10 °C is associated with the widely reported reproductive threshold in this area (Myrand et al., 2000; Comeau et al., 2017; Poirier et al., 2021). Determination of the scope for growth (SFG), oxygen consumption ($VO_2$), and clearance rate (CR) was performed as detailed in supplementary material. Briefly, clearance rate, respiration rate expressed as $VO_2$, and food assimilation rate were measured and used to calculate the scope for growth of diploid and triploid mussels. The scope for growth was calculated as follow:

$$SFG = A - R$$

where SFG is the scope for growth of dry mussel's mass (J $h^{-1}$ $g^{-1}$), R is the energy expended through respiration, and A is the assimilation rate. A is determined as follows:

$$A = C^{*}abs$$

where C is the estimating the organic ingestion rate (CR $\mathrm{l\ h^{-1}\ g^{-1}}$ X POM $\mathrm{mg\ l^{-1}} \times 23.5\ \mathrm{J\ mg^{-1}}$; Widdows and Johnson, 1988), and abs is the absorption efficiency. R is calculated as follows:

$$R = VO2 \times 20.33$$

$$VO2 = DOSlope^{*}(Vr - Va)^{*}3600/m$$

where $VO_2$ is the oxygen consumption (mg $O_2$ $\mathrm{g^{-1}\ h^{-1}}$), DOSlope is the rate of the decrease in dissolved oxygen (mg $O_2$ $\mathrm{L^{-1}\ s^{-1}}$), Vr is the volume of the respirometer (L), Va the volume of the animal (L), and m is the wet mass of the animal (g) according to the Q-Box AQUA protocol. As excretion represents <5 % of the energy budget in most bivalves, it was not estimated.

### 2.8. Mussel size and shell thickness

Following physiological measurements, each mussel was dissected to determine the shell and total tissue dry mass (dried at 72 °C for 72 h). The shell length, height and width were assessed, and the thickness was measured on the left valves with a micrometer (342–371-30 Mitutoyo, Takatsu Ward, Japan) at the highest part of the shell. Valve resistance was measured using a dynamometer (AFG 250 N ± 0.05 Quantrol, Dillon, Minnesota, USA). Isolated valve was positioned horizontally with the shell edge resting on a flat surface. A compressive force was applied using a 5 mm diameter steel tip placed on the curved surface at the valve's highest point. The maximum force (in Newtons) exerted before dislodgement was recorded using QGraph Quantrol software (Dillon). While this method does not yield absolute values for shell compression strength, it allows for comparative analysis of mechanical behavior across different shell types (Babarro and Abad, 2013).

### 2.9. Statistical analysis

To compare diploid and triploid mussels, the means ± standard error of means of every experiment were calculated. The normality and equality of variance were verified by Sigmaplot V14.5 using Shapiro-Wilk test. When the conditions required for Shapiro-Wilk test could not be obtained, Mann-Whitney Rank sum test was used instead. Following the verification, Student's *t*-tests were applied.

Byssal production was tested following Log conversion performed to pass Shapiro-Wilk's test conditions. Mechanical analyses of byssal threads were performed on 99 and 91 threads of diploid and triploids mussels respectively. Log conversion was performed for Young's modulus while square root correction was applied to validate local peak maximum. Both groups were tested by Student *t*-test. The comparison of amino acids composition, metal composition and structure in the byssal threads was performed on triplicates diploid and triploid mussels. Amino acids and metal compositions were compared by permutational multivariate analysis of variance (PERMANOVA) applied by using PRIMER® version 7.0.13 with PERMANOVA+1 add-on. The Euclidian distance matrix was used on normalized data with 9999 permutations (Anderson and Walsh, 2013) to test if amino acids or elemental composition of byssal threads change between diploid and triploid mussels. *P*-values were calculated with, partial sums of squares (Type III), and Monte-Carlo random draw probability (hereafter "$p_{(MC)}$") under a reduced model to avoid issues of too few available permutations with small sample sizes (Anderson and Robinson, 2003). Similarity percentage analysis (SIMPER) was used to identify which element contributed most to dissimilarities among diploid and triploid mussels byssal threads. Analyses of scope for growth, clearance rate, $VO_2$, absorption, valve breaking strength, shell thickness, and size comparison were performed on 15 and 16 diploid and triploid mussels for each temperature group, respectively by Student *t*-test.

## 3. Results

### 3.1. Byssogenesis and mechanical properties

Adult triploid mussels (shell length > 50 mm) produced 25 % more byssal threads than diploid adults, but there was no difference in the distal thread mean diameter or cuticle thickness between ploidy treatments (Table 1).

Tensile mechanical analyses demonstrated that triploids ultimate stress (MPa), Young modulus (MPa), and ultimate load (N) were higher by 32 %, 20 %, and 9.5 %, respectively (Table 2). Multiple yield points were observed in both groups on 20 and 22 % of diploid and triploid byssal threads, respectively.

### 3.2. Byssal thread composition

Amino acid composition of the distal part of byssal threads was found to be nearly identical between diploids and triploids (DF = 1 and 5, Pseudo-F = 0.11509, $p = 0.9164$; Fig. 1). With a relative abundance of ~15 %, glycine is the most abundant residue, followed by aspartic acid and asparagine (Asx) accounting for ~9 %, then glutamine and glutamic acid (Glx) with a combined value of ~8 %. The other charged amino acids arginine (Arg), lysine (Lys) and histidine (His) account for about 8 %, 6 % and 4 %, respectively. Altogether, negatively and positively charged amino acid residues have a total abundance of ~36 %. Both alanine (Ala) and proline (Pro) represent 7 % of the amino acids. Unfortunately, L-DOPA and tryptophan content could not be estimated due to a lack of byssal material.

$^{13}C$ SS-NMR analyses were also performed to compare the amino acid profile of intact mussel threads, i.e., without chemical treatment. Fig. 2 shows no difference in peak intensity and chemical shifts between byssal threads of diploid and triploid, indicating similar abundance and molecular environment.

### 3.3. Metal composition of byssal threads

The relative abundance of various elements and metals in byssal threads revealed clear compositional differences between diploid and triploid mussels with average dissimilarity estimated by SIMPER analysis of 38.8 % (DF = 1 and 5, Pseudo-F = 22.975, $p = 0.0064$; Fig. 3). SIMPER analysis showed that iron (Fe) concentrations were twice as high in byssal threads from diploid mussels, accounting for 49 % of the observed variation in elemental composition. Calcium (Ca) and magnesium (Mg) were also more abundant in diploid threads, contributing 28 % and 14 % of the respective differences. In contrast, byssal threads from triploid mussels generally exhibited higher concentrations of trace elements, although SIMPER analysis indicated that the individual contribution of each of these elements to the overall compositional differences was less than 1 %.

### 3.4. Physiological parameters

Measurement of physiological variables of diploid and triploid mussels at 10 and 20 °C are summarized in Table 3. No differences in food assimilation efficiency between both groups either at 10 °C or at

**Table 1**

Byssal thread production by diploid and triploid *M. edulis* mussels over three days under hydrodynamic flume conditions of 10 $\mathrm{cm \cdot s^{-1}}$ (mean and the standard errors). Bold indicate significant difference. *U* test = Mann-Whitney Rank sum test, *t*-test = Student's *t*-test.

| Thread characteristics | Diploid | Triploid | Statistics | p |
|---|---|---|---|---|
| Number of threads | 57.7 ± 6.3 | 72.1 ± 6.9 | t-test = 1.811 | **0.042** |
| Distal thread diameter (μm) | 61.5 ± 4.0 | 60.5 ± 5.4 | t-test = 0.098 | 0.923 |
| Cuticle thickness (μm) | 8.0 ± 0.5 | 8.0 ± 0.5 | U test = 111 | 0.972 |

**Table 2**
Tensile mechanical measurements of diploid and triploid mussels for each treatment (mean and the standard errors). Bold indicate significant difference. U test = Mann-Whitney Rank sum test, t-test = Student's *t*-test.

| Mechanical test | Diploid | Triploid | Statistics | p |
|---|---|---|---|---|
| Young's modulus (MPa) | 229 ± 19 | 275 ± 26 | U test = 7699 | **0.011** |
| Ultimate load (N) | 0.21 ± 0.01 | 0.23 ± 0.01 | U test = 7708 | **0.038** |
| Ultimate stress (MPa) | 73 ± 5 | 96 ± 8 | U test = 7786 | **0.035** |
| Ultimate strain (%) | 76 ± 3 | 72 ± 3 | t-test = 1.43 | 0.887 |

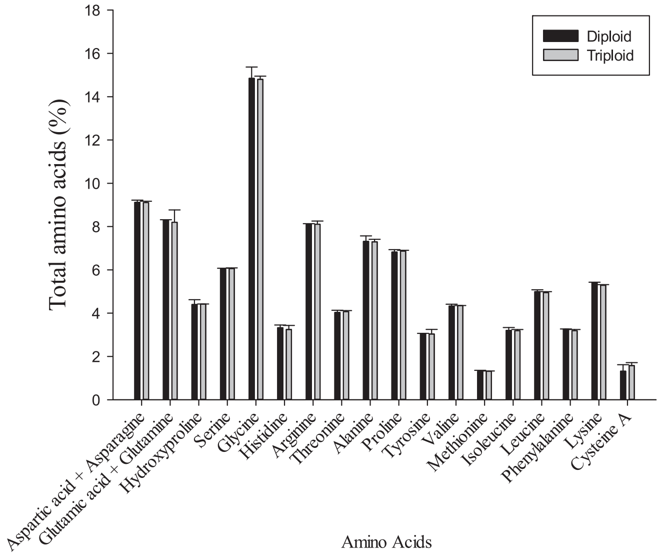

**Fig. 1.** Composition of amino acids (relative proportion) of byssal threads from diploid and triploid mussels (mean and standard error).

20 °C were observed. The $VO_2$ of triploid mussels at 10 °C was 19 % higher than diploid. However, no difference in the $VO_2$ at 20 °C was observed between both groups. The clearance rate was higher in triploid than in diploid for the two temperature conditions with 55 % and 48 % increase at 10 and 20 °C, respectively. Assimilation rate was higher in triploids compared to diploid for both treatments with an increase of 53 % at 10 °C and 49 % at 20 °C. The scope for growth was higher in triploid than in diploid for both temperature conditions (58 % greater at 10 °C; 57 % greater at 20 °C).

Interestingly, while triploids had higher clearance rate and scope for growth than diploids at 10 °C; the values of triploids' clearance rate and scope for growth at this low temperature were both similar to the values produced by diploids at 20 °C. Meanwhile, clearance rate and the scope for growth of triploids at 20 °C increased from 35 % compared to 10 °C. For both temperature conditions, triploid mussels showed thicker shell and their valves elicited stronger resistance against breaking. However, once shell breaking strength is normalized by shell thickness, no significant difference is observed at 10 °C, while it is barely significant at 20 °C (Table 3).

Shell morphology of diploid and triploid mussels showed that both length and height of triploid mussels were respectively 3 and 5 % higher in triploid mussels compared to diploids mussels. However, no difference in shell width was observed between the two groups. Triploid shells showed higher length/width, height/length, and height/width ratios than diploids (Table 4).

## 4. Discussion

This study showed that adult triploid mussels produced significantly more byssal threads than diploids. Byssal thread production in diploid mussels (57 ± 13) was consistent with previous observations from Saint Peters Bay farms (PEI, Canada), where Arens et al. (2011) recorded 58 ± 6 threads. However, adult triploid mussels produced 25 % more byssal threads, similar to our previous work on juvenile (<30 mm) triploid *Mytilus edulis* (Osterheld et al., 2023). These studies consistently showed increased thread production in triploid mussels, suggesting enhanced attachment strength under aquaculture conditions. Attachment strength depends, among other factors, on the number of threads. This is particularly relevant given the gregarious nature of mussels, which tend to form large clumps along farming lines. These clumps can be quite heavy, and their overall attachment strength relies on the collective recruitment of threads by the mussels within the group (Lachance et al., 2008). In this context, triploid mussels are expected to exhibit not only stronger individual attachment but also improved collective anchoring capacity. In addition, mechanical properties of triploid byssal threads tend to support the concept of improved attachment strength. Indeed, measures performed in commercial size mussels indicated higher Young's modulus in triploid byssal threads compared to diploids. Young's modulus calculated for diploid mussels was close to the value of 146 ± 29 MPa obtained by Bouhlel et al. (2017).

Further, higher ultimate stress and ultimate load in triploids. Such results tend to confirm the improved mechanical properties of triploid mussels and suggest better attachment properties with increased resistance against stress. This higher resistance against stress provides an advantage for triploid mussels in frequently turbulent environment subjected to important wave effects. Byssal threads possess self-healing capabilities following damage (Carrington and Gosline, 2004; Harrington and Waite, 2007; Harrington et al., 2009; Krauss et al., 2013). However, under highly turbulent conditions such as wave exposure, the rapid and repeated mechanical stress can impair their recovery (Vaccaro and Waite, 2001; Carrington and Gosline, 2004; Harrington and Waite, 2007; Reinecke et al., 2016). Owing to their enhanced mechanical properties, triploid threads may offer greater resistance to deformation and be better adapted to such dynamic environments than their diploid counterparts. These properties could help reduce mussel fall-off, particularly during handling, when individuals are subjected to substantial mechanical stress. However, further research under relevant field conditions is needed across farms to assess whether the observed increase in thread strength and number effectively reduces falloff losses and enhances crop yield.

Mechanical properties of byssal threads depend mostly depend on their diameter and composition (Hennebicq et al., 2013; Bouhlel et al., 2017; Waite et al., 2002; Harrington and Waite, 2007), but no differences were observed between threads of triploid and diploid adult or juvenile mussels (this study, Osterheld et al., 2023). These findings suggest that thread diameter does not contribute to the enhanced mechanical properties observed in triploid mussels. The threads produced by commercial-size mussels used in this study were thicker than those of juveniles, with a mean diameter of 63.2 ± 1.4 μm compared to 48 ± 2 μm, respectively (Osterheld et al., 2023). Smaller thread formation in juvenile mussels is a consistent trait observed across multiple mussel species (Babarro and Carrington, 2013; Garner and Litvaitis, 2013).

In addition to diameter of threads, their composition also affects the attachment and mechanical properties of the threads (Waite et al., 2002, Harrington and Waite, 2007). The protein gradients in byssal threads can improve the modulus and decrease the stress on byssal threads (Waite et al., 2002). Moreover, the flanking domains of preCols are also expected to be of importance for the threads' mechanical properties (Waite et al., 2002, Harrington and Waite, 2007). These authors suggested that changes in the sequence of the flanking domains could affect the stiffness of the byssal threads. The content of specific residues such as histidine and DOPA are also expected to play a major role in the mechanical behavior of the threads (Waite et al., 1998; Sun and Waite, 2005; Harrington and Waite, 2007). Indeed, these residues are involved in metal binding and are in the self-healing properties of the threads (Schmitt et al., 2015; Reinecke et al., 2016; Xu et al., 2019; Zechel et al., 2019). Thus, modification of their expression could strongly affect the

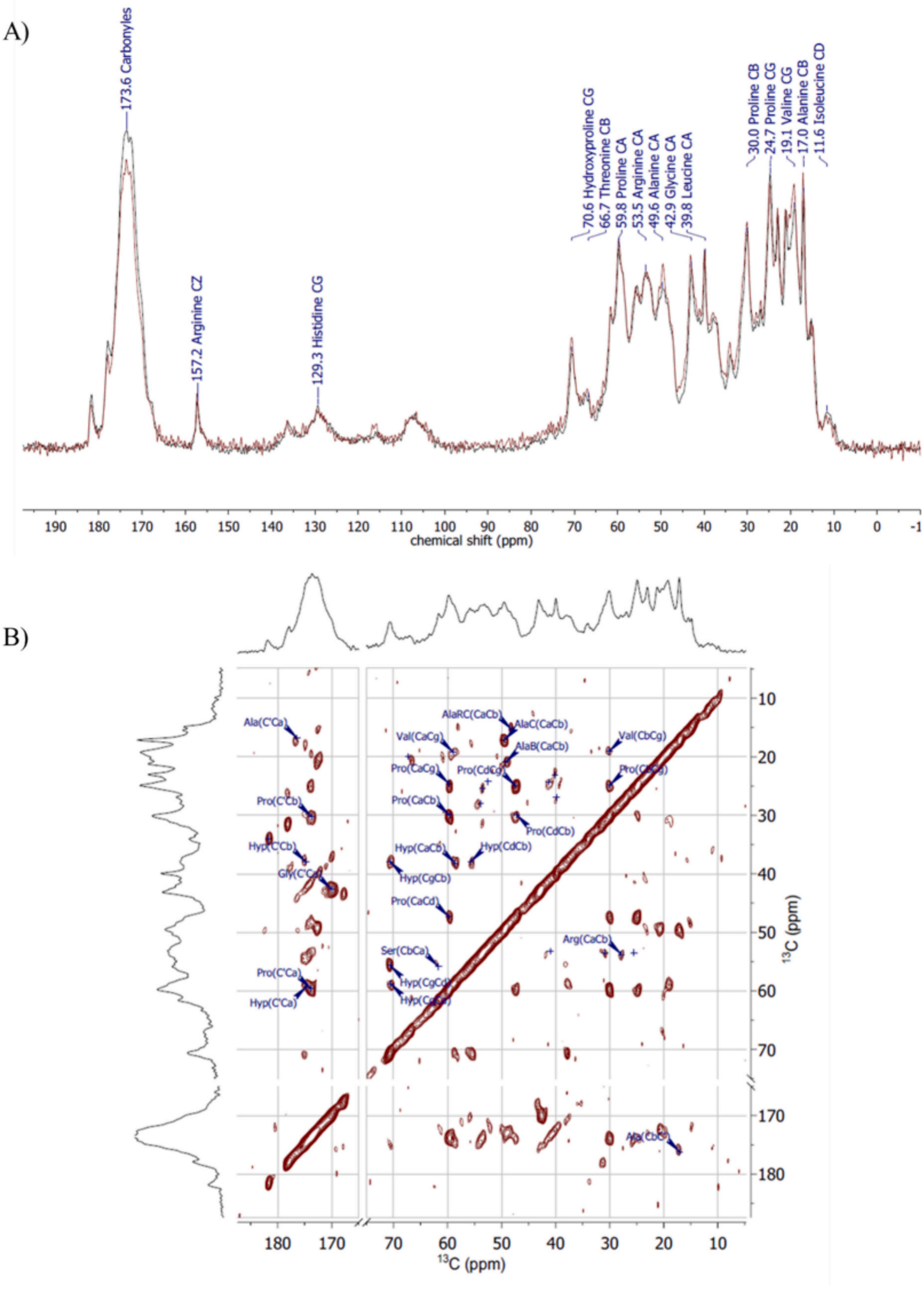

**Fig. 2.** (A) One- and (B) two-dimensional $^{13}$C solid-state NMR spectra of diploid (black) and triploid (red) byssal threads recorded at 10 kHz MAS. The amino acid peaks assignment is displayed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

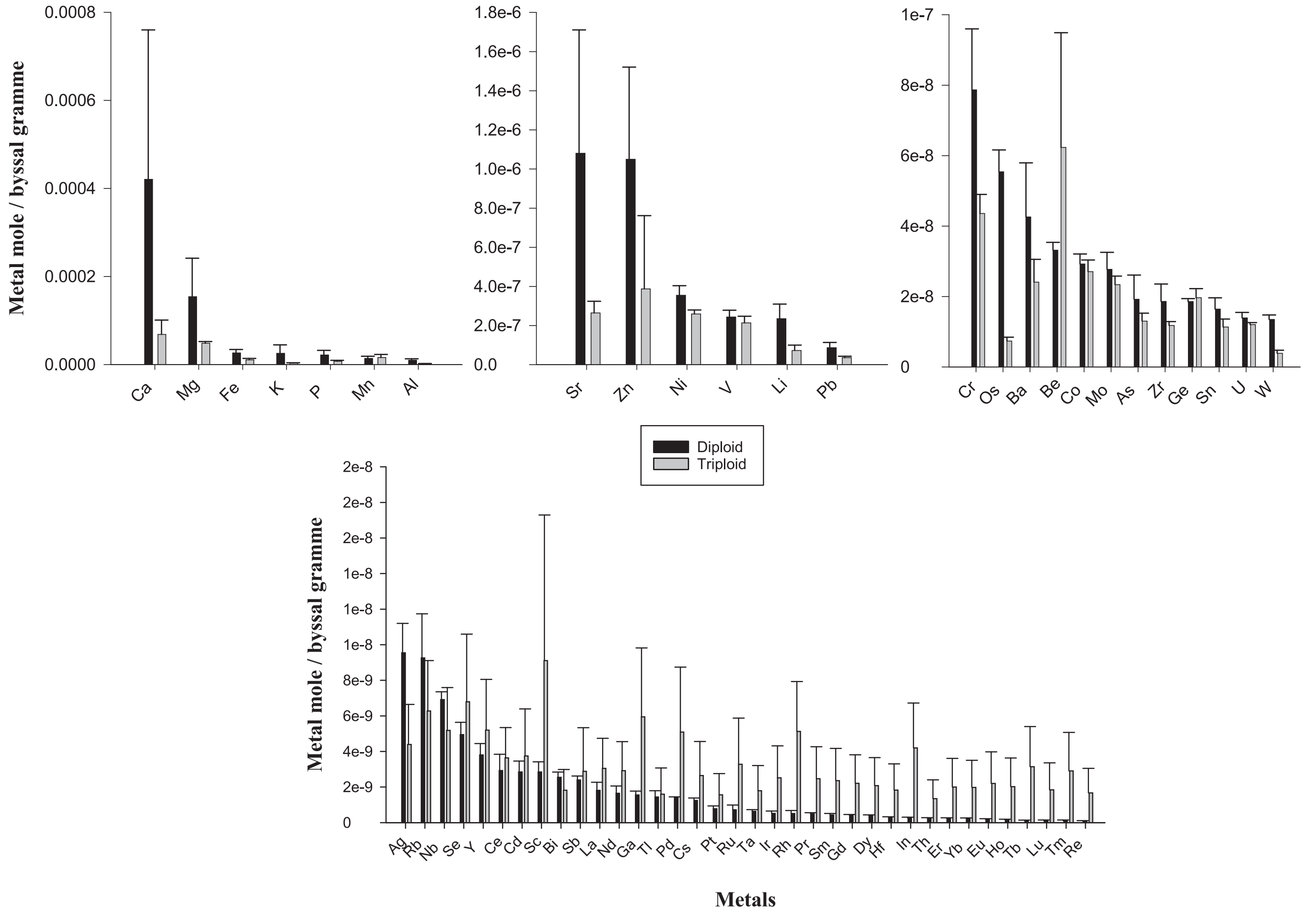

**Fig. 3.** Concentrations of each metal in diploid and triploid byssal threads (for more to less concentrated) expressed as relative percentage (mean and standard error).

**Table 3**
Metabolic analyses of diploid and triploid mussels performed at 10 and 20 °C (mean and standard error). Bold indicate significant difference. U test = Mann-Whitney Rank sum test, t-test = Student's *t*-test.

| Measurements | 10 °C | | | | 20 °C | | | |
|---|---|---|---|---|---|---|---|---|
| | Diploid | Triploid | Statistics | p | Diploid | Triploid | Statistics | p |
| Scope for Growth (J $h^{-1}$ $g^{-1}$) | 184 ± 19 | 291 ± 15 | t-test = 4.37 | **0.00016** | 285 ± 14 | 447 ± 29 | U test = 17,000 | **< 0.001** |
| $VO_2$ ($mgO_2$ $h^{-1}$ $g^{-1}$) | 1.16 ± 0.06 | 1.38 ± 0.07 | t-test = 2.93 | **0.024** | 2.06 ± 0.05 | 2.04 ± 0.07 | t-test = 0.22 | 0.827 |
| Clearance Rate (l $h^{-1}$ $g^{-1}$) | 1.45 ± 0.13 | 2.25 ± 0.11 | t-test = 4.73 | **0.00006** | 2.28 ± 0.11 | 3.38 ± 0.23 | U test = 24,000 | **< 0.001** |
| C | 255 ± 23 | 395 ± 19 | t-test = 4.73 | **0.00006** | 399 ± 14 | 593 ± 40 | U test = 24,000 | **< 0.001** |
| A | 208 ± 19 | 319 ± 15 | t-test = 4.72 | **0.00006** | 327 ± 15 | 488 ± 30 | U test = 18,000 | **< 0.001** |
| Absorption efficiency (%) | 82 ± 1 | 81 ± 1 | t-test = 0.49 | 0.63 | 82 ± 1 | 83 ± 1 | t-test = 0.53 | 0.603 |
| Shell Thickness (mm) | 0.86 ± 0.02 | 0.96 ± 0.02 | t-test = 3.53 | **0.0014** | 0.85 ± 0.05 | 0.93 ± 0.03 | U test = 69,500 | **0.048** |
| Valve breaking strength (N) | 130 ± 20 | 185 ± 20 | t-test = 2.35 | **0.026** | 122 ± 16 | 171 ± 14 | U test = 67,000 | **0.038** |
| Valve breaking strength normalized by shell thickness (N $mm^{-1}$) | 150 ± 23 | 194 ± 21 | t-test = 2.35 | 0.084 | 138 ± 13 | 182 ± 11 | U test = 70,000 | **0.05** |

properties of byssal threads. The results of the amino acids content of diploid and triploid mussels showed no difference between the two groups, suggesting no impacts of their composition on the attachment strength and tensile properties of the byssal threads.

Absence of difference in molecular composition between threads from diploid and triploid mussels was also confirmed by absence of profile differentiation with NMR analyses. NMR data were similar to NMR results from Arnold et al. (2013) performed on distal byssal threads of *Mytilus edulis* diploids, highlighting the same peaks as in our study. Previous studies also showed that byssal threads with the same content

**Table 4**
Shell size characteristics for each ploidy treatment (mean and the standard errors). Bold indicate significant difference. U test = Mann-Whitney Rank sum test, t-test = Student's *t*-test.

| Shell Properties | Diploid | Triploid | Statistics | p |
|---|---|---|---|---|
| Length (mm) | 59.25 ± 0.44 | 62.77 ± 0.46 | t-test = 2.64 | **0.018** |
| Height (mm) | 22.41 ± 0.17 | 24.58 ± 0.20 | U test = 230,000 | **0.001** |
| Width (mm) | 31.57 ± 0.23 | 31.73 ± 0.25 | U test = 404,000 | 0.642 |
| Length/Width | 1.88 ± 0.01 | 1.92 ± 0.008 | t-test = 2.81 | **0.004** |
| Height/Length | 0.38 ± 0.004 | 0.39 ± 0.002 | U test = 330,000 | **0.018** |
| Height/Width | 0.71 ± 0.007 | 0.74 ± 0.004 | t-test = 2.94 | **0.001** |

in amino acid residues could have different attachment strength (Hennebicq et al., 2013). For instance, Hennebicq et al. (2013) found no significant variation in amino acid residues, except for glutamine, between threads of *Mytilus edulis* with different mechanical properties. In their study, the reduction of the attachment strength was mostly due to the reduction of the diameter of the byssal threads. In our study, glycine, Asx, Glx, arginine, alanine, and proline counted for more than 54 % of the total of the amino acids. These observations were consistent with other studies showing that the major amino acids present in the distal part of byssal threads were first glycine, followed by alanine, asparagine, glutamine, arginine, and proline (Lucas et al., 2002; Hennebicq et al., 2013; Bouhlel et al., 2017). As for Hennebicq et al. (2013), we observed that the dominant amino acid was glycine with level of 18 %. The higher levels of glycine are supposedly due to the presence of repetitive sequences containing glycine (Gly-Xaa-Yaa) in the preCols (Waite et al., 1998; Lucas et al., 2002; Silverman and Roberto, 2010; Hagenau et al., 2011). These last domains are flanked by silk fibroin like domains and are believed to mediate the function between the distal and proximal regions (Silverman and Roberto, 2010). Moreover, glycine-rich sequences with histidine such as Gly-His-Gly are present in the N-terminus of preCol-NG and C-termini of preCol-P and -D and are involved in binding with metals such as Cu(II) (Waite et al., 1998; Waite et al., 2002). Furthermore, Glycine forms triplets with proline and hydroxyproline (Gly-Pro-Hyp) which helps the stabilization of the triple-helix conformation (Engel et al., 1977; Hennebicq et al., 2013).

The comparison of metal content in diploid and triploid byssal threads with other studies showed that the metal proportions measured in this study are different from what was previously observed. Indeed, we measured that Ca, Mg, and Fe were respectively the most abundant elements in byssal threads, while Bouhlel et al. (2017) showed that Mn was the most abundant element in *M. edulis* byssal threads followed by Ca, and Mg. In their study, Hennebicq et al. (2013) observed Al, Fe, I, and Mg were, in this order, the most abundant elements. Such differences in the proportion of elements of byssal threads may be due to the feeding of the mussels as well as their location. For instance, Szefer et al. (2002) showed highly variable metals concentrations in byssal threads depending on the mussels harvesting site where Fe concentrations ranged from 243 to 10,308 μg/g dry weight. Similarly, Seguin-Heine et al. (2014) demonstrated differences in metal composition between the Open Sea and Lagoon as well as metal variability with seasons. Nevertheless, while differences in proportions were observed between these studies and ours, the major metals remained the same. Thus, as proposed by Bouhlel et al. (2017) metals were divided into 3 different groups. Group I with B, Fe, Al, Mg, Ca, Mn, and K; group II containing Ti, V, Cr, Ni, Cu, Zn, Sn, Sr, Mo, and, Ba; and group III would represent trace metals. Our results also suggest that Os and W could both be included in group II metals. The elemental composition of byssal threads differed between triploid and diploid mussels, with over 91 % of the variation attributed to higher concentrations of Fe, Ca, and Mg in diploids. These metals, particularly Fe, are known to enhance the mechanical properties of byssal threads and their cuticle by forming sacrificial bonds through interactions with histidine or DOPA residues (Holten-Andersen et al., 2009; Waite and Harrington, 2022). Although concentration differences were observed between diploid and triploid threads, they appear insufficient to fully explain the superior mechanical performance of triploid mussel threads. Further research is needed to determine whether this trend is consistent.

Triploid mussels elicited significantly higher valve breaking strength compared to diploid mussels at both 10 and 20 °C. This higher resistance of triploid shell could therefore provide them with a better protection against high energy environments, as well as predators such as shell-crushing fishes, birds, crustacean, gastropods, and starfishes (D'Amours et al., 2008; Wilding and Nickell, 2013; Sardenne et al., 2019; Johnson, 2020). The increased shell resistance of triploids seems to be due, at least partially, to their thicker shell as thinner shells are easier for predators to penetrate (Grey et al., 2007; Johnson, 2020). However, normalization of the valve breaking strength by shell thickness demonstrates that shell thickness alone cannot explain the superior shell resistance in triploids and other factors such as shell composition or shell shape could be involved. Indeed, results of shell shape demonstrated that triploid shells had higher length and height for similar width. Therefore, more resistant shells of triploids had slightly different shapes than diploids, as demonstrated by the altered length/width, height/length, and height/width ratios. Johnson (2020) determined that bivalve shell shape plays an important role in its resistance against pressure and thus potential predators. Based on their observation, we may hypothesize that the better resistance of triploid shells may be partially due to their slightly different shape compared to diploids, that could possibly have a better repartition of the stress applied on them.

Differential byssogenesis and shell resistance of triploid mussels could be partially explained by their physiological rates resulting in different energetic status at 10 and 20 °C. As expected, temperature significantly influenced mussel metabolism, with both diploid and triploid individuals exhibiting higher scope for growth, oxygen consumption ($VO_2$), and clearance rates at 20 °C compared to 10 °C. Moreover, across both temperature treatments, triploids consistently showed greater scope for growth, assimilation rates, and clearance rates than their diploid counterparts. Outside from potential genetic factors, the higher clearance and assimilation rates could be affected by the increased shell volume associated with higher shell height and length. This might lead to increased gill surfaces which might explain the higher filtration abilities of triploids as proposed by Prieto et al. (2020) when comparing fast- and slow-growing mussels. No differences were observed for other physiological rates measured, except for $VO_2$ of triploid mussels at 10 °C, indicating higher metabolic rates, which may be linked with the higher clearance rate requiring more energy compared to diploids (Norkko et al., 2005). Although not directly tested in this study, the lack of difference in $VO_2$ rates at 20 °C between triploid and diploid mussels may be linked to the higher reproductive investment observed in diploids. Mussels typically allocate more energy to reproduction at 20 °C than at 10 °C, resulting in increased oxygen consumption (De Vooys, 1976; Iglesias and Navarro, 1991). In diploids, $VO_2$ would thus reflect the combined effects of elevated temperature and reproductive effort. In contrast, triploid mussels, known for their reduced reproductive investment (Kiyomoto et al., 1996; Brake et al., 2004; Qin et al., 2020; Osterheld et al., 2024), are expected to show a lower reproductive contribution to $VO_2$, making temperature the primary driver. However, further analyses are required to validate this hypothesis. At 10 °C, triploid mussels exhibited significantly higher $VO_2$, indicating that they maintain relatively elevated metabolic activity at lower temperatures compared to diploids. This trait may confer advantages in growth, survival, and resilience to stress or pathogens under cooler aquaculture conditions. However, further research is needed to determine whether this increased metabolic activity translates into enhanced performance and productivity in real-world farming environments.

Thus, the higher energy availability in triploid mussels measured at 10 and 20 °C were mostly related to their higher clearance rate. With similar food assimilation level (absorption efficiency), the higher ingestion of food by triploid mussels would induce higher energy available for different functions, like byssus and shell production. Furthermore, the elevated clearance rates observed in triploid mussels suggest that their farming may help to mitigate eutrophication at aquaculture sites. As extractive species, mussels play a key role in reducing nutrient enrichment, and mussel farming is widely recognized as an efficient and environmentally sustainable strategy for nutrient removal (Lindahl et al., 2005; Petersen et al., 2016; Smaal et al., 2019). By filtering and consuming algae, mussels remove anthropogenic nutrients either by storing them in their tissues, recoverable upon harvest, or by producing feces and pseudo-feces that promote sedimentation (Songsangjinda et al., 2000; Petersen et al., 2016, 2019; Smaal et al., 2019). Triploid mussels, with their higher filtration capacity, may enhance these processes by consuming more algae and thereby increasing nutrient retention or sedimentation. While triploids are unlikely to replace natural populations or produce large-scale effects on eutrophication, they could offer localized benefits when farmed near nutrient-rich areas. Nonetheless, further research and modeling are needed to assess the magnitude of these effects under practical aquaculture conditions.

## 5. Conclusion

In this study, we supported our previous work on triploid spat of *Mytilus edulis* showing higher energy available to produce more byssal threads with enhanced mechanical properties. Such improved triploid byssal properties were also observed in adult mussels, suggesting lower losses due to fall-off for mussel farmers. However, we cannot explain difference in mechanical properties by their differential biochemical and molecular composition as amino acids and NMR analyses showed similar results between diploid and triploid mussels. Although elemental composition did not account for the differences in mechanical properties between diploid and triploid byssal threads, the observed compositional variations suggest a potential relationship that warrants further investigation. The higher energy availability to produce more byssal threads and more resistant shells of triploid mussels was mostly related to their higher clearance rates. The higher clearance rate suggested that triploid farming could be of interest as an ecological species to reduce localized eutrophication. Future research integrating predictive modeling with field experiments will be crucial for assessing the ecological value of triploid mussels in commercial farming conditions.

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## CRediT authorship contribution statement

**Kevin Osterheld:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **John Davidson:** Writing – review & editing, Project administration, Methodology, Conceptualization. **Luc A. Comeau:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Tiago Hori:** Writing – review & editing, Project administration, Funding acquisition. **José M. F. Babarro:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Isabelle Marcotte:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Alexandre A. Arnold:** Writing – review & editing, Validation, Methodology, Formal analysis. **Christian Pellerin:** Writing – review & editing, Validation, Supervision, Methodology. **Richard Saint-Louis:** Writing – review & editing, Validation, Supervision, Methodology. **Réjean Tremblay:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2025.743469.

## Data availability

Data will be made available on request.

## References

Alfaro, A.C., 2006. Byssal attachment of juvenile mussels, *Perna canaliculus*, affected by water motion and air bubbles. Aquaculture 255 (1–4), 357–361. https://doi.org/10.1016/j.aquaculture.2005.11.059.

Allen Jr., S.K., Downing, S.L., 1986. Performance of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). I. Survival, growth, glycogen content, and sexual maturation in yearlings. *Journal of Experimental Marine Biology*. Ecology 102 (2–3), 197–208. https://doi.org/10.1016/0022-0981(86)90176-0.

Allen Jr., S.K., Downing, S.L., 1990. Performance of triploid Pacific oysters, *Crassostrea gigas*: gametogenesis. Can. J. Fish. Aquat. Sci. 47 (6), 1213–1222. https://doi.org/10.1139/f90-14.

Allen Jr., S.K., Hidu, H., Stanley, J.G., 1986. Abnormal gametogenesis and sex ratio in triploid soft-shell clams (*Mya arenaria*). Biol. Bull. 170 (2), 198–210. https://doi.org/10.2307/1541803.

Anderson, M.J., Robinson, J., 2003. Generalized discriminant analysis based on distances. Austral. New Zeal. J. Stat. 45 (3), 301–318. https://doi.org/10.1111/1467-842X.00285.

Anderson, M.J., Walsh, D.C., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecol. Monogr. 83 (4), 557–574. https://doi.org/10.1890/12-2010.1.

Arens, C.J., Paetzold, S.C., Davidson, J., 2011. The effect of high-pressure spraying for tunicate control on byssal thread characteristics in the cultured blue mussel (*Mytilus edulis* Linnaeus, 1758). Aquat. Invasions 6 (4), 507–510. https://doi.org/10.3391/ai.2011.6.4.15.

Arnold, A.A., Byette, F., Séguin-Heine, M.-O., LeBlanc, A., Sleno, L., Tremblay, R., Pellerin, C., Marcotte, I., 2013. Solid-State NMR structure determination of whole anchoring threads from the blue mussel *Mytilus edulis*. Biomacromolecules 14 (1), 132–141. https://doi.org/10.1021/bm301493u.

Babarro, J.M.F., Abad, M.J., 2013. Co-existence of two mytilid species in a heterogeneous environment: mortality, growth and strength of shell and byssus attachment. Mar. Ecol. Prog. Ser. 476, 115–128. https://doi.org/10.3354/meps10122.

Babarro, J.M., Carrington, E., 2013. Attachment strength of the mussel *Mytilus galloprovincialis*: effect of habitat and body size. J. Exp. Mar. Biol. Ecol. 443, 188–196. https://doi.org/10.1016/j.jembe.2013.02.035.

Babarro, J.M., Reiriz, M.J.F., 2010. Secretion of byssal threads in *Mytilus galloprovincialis*: quantitative and qualitative values after spawning stress. J. Comp. Physiol. B. 180 (1), 95–104. https://doi.org/10.1007/s00360-009-0392-y.

Bassim, S., Tanguy, A., Genard, B., Moraga, D., Tremblay, R., 2014. Identification of *Mytilus edulis* genetic regulators during early development. Gene 551 (1), 65–78. https://doi.org/10.1016/j.gene.2014.08.042.

Bell, E., Gosline, J., 1996. Mechanical design of mussel byssus: material yield enhances attachment strength. J. Exp. Biol. 199 (4), 1005–1017. https://doi.org/10.1242/jeb.199.4.1005.

Bell, E.C., Gosline, J.M., 1997. Strategies for life in flow: tenacity, morphometry, and probability of dislodgment of two *Mytilus* species. Mar. Ecol. Prog. Ser. 159, 197–208. https://doi.org/10.3354/meps159197.

Bouhlel, Z., Genard, B., Ibrahim, N., Carrington, E., Babarro, J.M., Lok, A., Flores, A.A., Pellerin, C., Tremblay, R., Marcotte, I., 2017. Interspecies comparison of the mechanical properties and biochemical composition of byssal threads. J. Exp. Biol. 220 (6), 984–994. https://doi.org/10.1242/jeb.141440.

Bourque, F., Myrand, B., 2006. Étude descriptive du dégrappage en milieu lagunaire aux Îles-de-la-Madeleine. MAPAQ, DIT, Rapport de R-D no 152, 9.

Brake, J., Davidson, J., Davis, J., 2002. Triploid induction of *Mytilus edulis* using 6-dimethylaminopurine. Shellfish Res. 21 (2), 503–508.

Brake, J., Davidson, J., Davis, J., 2004. Field observations on growth, gametogenesis, and sex ratio of triploid and diploid *Mytilus edulis*. Aquaculture 236 (1–4), 179–191. https://doi.org/10.1016/j.aquaculture.2003.09.016.

Carrington, E., Gosline, J.M., 2004. Mechanical design of mussel byssus: load cycle and strain rate dependence. Am. Malacol. Bull. 18 (1/2), 135–142.

Carrington, E., Waite, J.H., Sara, G., Sebens, K.P., 2015. Mussels as a model system for integrative ecomechanics. Annu. Rev. Mar. Sci. 7 (1), 443–469. https://doi.org/10.1146/annurev-marine-010213-135049.

Comeau, L.A., Filgueira, R., Guyondet, T., Sonier, R., 2015. The impact of invasive tunicates on the demand for phytoplankton in longline mussel farms. Aquaculture 441, 95–105. https://doi.org/10.1016/j.aquaculture.2015.02.018.

Comeau, L.A., Filgueira, R., Davidson, J.D.P., Nadeau, A., Sonier, R., Guyondet, T., Ramsay, A., Davidson, J., 2017. Population structure and grazing capacity of cultivated mussels in Prince Edward Island, Canada. In: Canadian Technical Report of Fisheries Aquatic Science, 3228. N.B. Fisheries and Oceans Canada, Moncton, p. 23.

D'Amours, O., Archambault, P., McKindsey, C.W., Johnson, L.E., 2008. Local enhancement of epibenthic macrofauna by aquaculture activities. Mar. Ecol. Prog. Ser. 371, 73–84. https://doi.org/10.3354/meps07672.

De Vooys, C.G.N., 1976. The influence of temperature and time of year on the oxygen uptake of the sea mussel *Mytilus edulis*. Mar. Biol. 36 (1), 25–30. https://doi.org/10.1007/BF00388425.

Engel, J., Chen, H.T., Prockop, D.J., Klump, H., 1977. The triple helix⇌ coil conversion of collagen-like polytripeptides in aqueous and nonaqueous solvents. Comparison of the thermodynamic parameters and the binding of water to (L-Pro-L-Pro-Gly) n and (L-Pro-L-Hyp-Gly) n. Biopolym. Origin. Res. Biomol. 16 (3), 601–622. https://doi.org/10.1002/bip.1977.360160310.

Fréchette, M., 2012. Self-thinning, biodeposit production, and organic matter input to the bottom in mussel suspension culture. J. Sea Res. 67 (1), 10–20. https://doi.org/10.1016/j.seares.2011.08.006.

Gagnon, M., 2019. Self-organization and mechanical properties of mussel culture suspensions: a critical review. Aquac. Eng. 87, 102024. https://doi.org/10.1016/j.aquaeng.2019.102024.

Garner, Y.L., Litvaitis, M.K., 2013. Effects of injured conspecifics and predators on byssogenesis, attachment strength and movement in the blue mussel, *Mytilus edulis*. J. Exp. Mar. Biol. Ecol. 448, 136–140. https://doi.org/10.1016/j.jembe.2013.07.004.

Grey, M., Lelievre, P.G., Boulding, E.G., 2007. Selection for prey shell thickness by the naticid gastropod *Euspira lewisii* (Naticidae) on the bivalve *Protothaca staminea* (Veneridae). Veliger 48 (4), 317–322.

Hagenau, A., Papadopoulos, P., Kremer, F., Scheibel, T., 2011. Mussel collagen molecules with silk-like domains as load-bearing elements in distal byssal threads. J. Struct. Biol. 175 (3), 339–347. https://doi.org/10.1016/j.jsb.2011.05.016.

Harrington, M.J., Waite, J.H., 2007. Holdfast heroics: comparing the molecular and mechanical properties of *Mytilus californianus* byssal threads. J. Exp. Biol. 210 (24), 4307–4318. https://doi.org/10.1242/jeb.009753.

Harrington, M.J., Gupta, H.S., Fratzl, P., Waite, J.H., 2009. Collagen insulated from tensile damage by domains that unfold reversibly: In situ X-ray investigation of mechanical yield and damage repair in the mussel byssus. J. Struct. Biol. 167 (1), 47–54. https://doi.org/10.1016/j.jsb.2009.03.001.

Hedlund, J., Andersson, M., Fant, C., Bitton, R., Bianco-Peled, H., Elwing, H., Berglin, M., 2009. Change of colloidal and surface properties of *Mytilus edulis* foot protein 1 in the presence of an oxidation (NaIO4) or a complex-binding (Cu2+) agent. Biomacromolecules 10 (4), 845–849. https://doi.org/10.1021/bm801325j.

Hennebicq, R., Fabra, G., Pellerin, C., Marcotte, I., Myrand, B., Tremblay, R., 2013. The effect of spawning of cultured mussels (*Mytilus edulis*) on mechanical properties, chemical and biochemical composition of byssal threads. Aquaculture 410, 11–17. https://doi.org/10.1016/j.aquaculture.2013.06.011.

Holten-Andersen, N., Mates, T.E., Toprak, M.S., Stucky, G.D., Zok, F.W., Waite, J.H., 2009. Metals and the integrity of a biological coating: the cuticle of mussel byssus. Langmuir 25 (6), 3323–3326. https://doi.org/10.1021/la8027012.

Iglesias, J.I.P., Navarro, E., 1991. Energetics of growth and reproduction in cockles (*Cerastoderma edule*): seasonal and age-dependent variations. Mar. Biol. 111 (3), 359–368. https://doi.org/10.1007/BF01319407.

Johnson, E.H., 2020. Experimental tests of bivalve shell shape reveal potential tradeoffs between mechanical and behavioral defenses. Sci. Rep. 10 (1), 1–12. https://doi.org/10.1038/s41598-020-76,358-x.

Kesarcodi-Watson, A., Klumpp, D.W., Lucas, J.S., 2001. Comparative feeding and physiological energetics in diploid and triploid Sydney rock oysters (*Saccostrea commercialis*): II. Influences of food concentration and tissue energy distribution. Aquaculture 203 (1–2), 195–216. https://doi.org/10.1016/S0044-8486(01)00623-8.

Kiyomoto, M., Komaru, A., Scarpa, J., Wada, K.T., Danton, E., Awaji, M., 1996. Abnormal gametogenesis, male dominant sex ratio, and Sertoli cell morphology in induced triploid mussels, *Mytilus galloprowincialis*. Zool. Sci. 13 (3), 393–402. https://doi.org/10.2108/zsj.13.393.

Krauss, S., Metzger, T.H., Fratzl, P., Harrington, M.J., 2013. Self-repair of a biological fiber guided by an ordered elastic framework. Biomacromolecules 14 (5), 1520–1528. https://doi.org/10.1021/bm4001712.

Lachance, A.A., Myrand, B., Tremblay, R., Koutitonsky, V., Carrington, E., 2008. Biotic and abiotic factors influencing attachment strength of blue mussels *Mytilus edulis* in suspended culture. Aquat. Biol. 2 (2), 119–129. https://doi.org/10.3354/ab00041.

Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.O., Olrog, L., Rehnstam-Holm, A.-N., Svensson, J., Svensson, S., Syversen, U., 2005. Improving marine water quality by mussel farming: a profitable solution for Swedish society. AMBIO J. Human Environ. 34 (2), 131–138. https://doi.org/10.1579/0044-7447-34.2.131.

Lucas, J.M., Vaccaro, E., Waite, J.H., 2002. A molecular, morphometric and mechanical comparison of the structural elements of byssus from *Mytilus edulis* and *Mytilus galloprovincialis*. J. Exp. Biol. 205 (12), 1807–1817. https://doi.org/10.1242/jeb.205.12.1807.

Myrand, B., Guderley, H., Himmelman, J.H., 2000. Reproduction and summer mortality of blue mussels *Mytilus edulis* in the Magdalen Islands, southern Gulf of St. Lawrence. Mar. Ecol. Prog. Ser. 197, 193–207. https://doi.org/10.3354/meps197193.

Norkko, J., Pilditch, C.A., Thrush, S.F., Wells, R.M.G., 2005. Effects of food availability and hypoxia on bivalves: the value of using multiple parameters to measure bivalve condition in environmental studies. Mar. Ecol. Prog. Ser. 298, 205–218. https://doi.org/10.3354/meps298205.

Osterheld, K., Davidson, J., Comeau, L.A., Hori, T., Belzile, C., Tremblay, R., 2021. Efficiency of various strategies to induce triploidy in *Mytilus edulis* in relation to their first-stage performance. J. Appl. Aquac. 1–16. https://doi.org/10.1080/10454438.2021.1985032.

Osterheld, K., Davidson, J., Comeau, L.A., Hori, T., Babarro, J.M., Marcotte, I., Pellerin, C., Tremblay, R., 2023. Triploidy in *Mytilus edulis* impacts the mechanical properties of byssal threads. Aquaculture 566, 739191. https://doi.org/10.1080/10454438.2021.1985032.

Osterheld, K., Davidson, J., Comeau, L., Audet, C., Hori, T., Tremblay, R., 2024. Reproductive investment and gonad development in triploid mussels, *Mytilus edulis*. Aquaculture 593, 741315. https://doi.org/10.1016/j.aquaculture.2024.741315.

Petersen, J.K., Saurel, C., Nielsen, P., Timmermann, K., 2016. The use of shellfish for eutrophication control. Aquac. Int. 24 (3), 857–878. https://doi.org/10.1007/s10499-015-9953-0.

Petersen, J.K., Holmer, M., Termansen, M., Hasler, B., 2019. Nutrient extraction through bivalves. In: Goods and Services of Marine Bivalves. Springer Nature, pp. 179–208. https://doi.org/10.1007/978-3-319-96,776-9.

Poirier, L., Ramsay, A., Coffin, M., Davidson, J., Comeau, L., 2021. Price Edward Island's mussel monitoring program and associated environmental data. In: Canadian Manuscript Report of Fisheries and Aquatic Science 3226. Fisheries and Oceans Canada, Moncton, New Brunswick. E1C 9B6, Canada.

Prieto, D., Tamayo, D., Urrutxurtu, I., Navarro, E., Ibarrola, I., Urrutia, M.B., 2020. Nature more than nurture affects the growth rate of mussels. Sci. Rep. 10 (1), 3539. https://doi.org/10.1038/s41598-020-60,312-y.

Qin, Y., Li, X., Noor, Z., Li, J., Zhou, Z., Ma, H., Xiao, S., Mo, R., Zhang, Y., Yu, Z., Yu, Z., 2020. A comparative analysis of the growth, survival and reproduction of *Crassostrea hongkongensis*, *Crassostrea ariakensis*, and their diploid and triploid hybrids. Aquaculture 520, 734946. https://doi.org/10.1016/j.aquaculture.2020.734946.

Reinecke, A., Bertinetti, L., Fratzl, P., Harrington, M.J., 2016. Cooperative behavior of a sacrificial bond network and elastic framework in providing self-healing capacity in mussel byssal threads. J. Struct. Biol. 196 (3), 329–339. https://doi.org/10.1016/j.jsb.2016.07.020.

Rupp, G.S., Parsons, G.J., 2004. Effects of salinity and temperature on the survival and byssal attachment of the lion's paw scallop *Nodipecten nodosus* at its southern distribution limit. J. Exp. Mar. Biol. Ecol. 309 (2), 173–198. https://doi.org/10.1016/j.jembe.2004.03.018.

Sardenne, F., Forget, N., McKindsey, C.W., 2019. Contribution of mussel fall-off from aquaculture to wild lobster *Homarus americanus* diets. Mar. Environ. Res. 149, 126–136. https://doi.org/10.1016/j.marenvres.2019.06.003.

Schmitt, C.N., Winter, A., Bertinetti, L., Masic, A., Strauch, P., Harrington, M.J., 2015. Mechanical homeostasis of a DOPA-enriched biological coating from mussels in response to metal variation. J. R. Soc. Interface 12 (110), 20150466. https://doi.org/10.1098/rsif.2015.0466.

Seguin-Heine, M.O., Lachance, A.A., Genard, B., Myrand, B., Pellerin, C., Marcotte, I., Tremblay, R., 2014. Impact of open sea habitat on byssus attachment of suspension-cultured blue mussels (*Mytilus edulis*). Aquaculture 426, 189–196. https://doi.org/10.1016/j.aquaculture.2014.02.006.

Silverman, H.G., Roberto, F.F., 2010. Byssus formation in *Mytilus*. In: Biological adhesive systems. Springer, Vienna, pp. 273–283. https://doi.org/10.1007/978-3-7091-0286-2_18.

Smaal, A.C., Ferreira, J.G., Grant, J., Petersen, J.K., Strand, Ø., 2019. Goods and Services of Marine Bivalves. Springer Nature. https://doi.org/10.1007/978-3-319-96,776-9.

Smeathers, J., Vincent, J., 1979. Mechanical properties of mussel byssus threads. J. Molluscan Stud. 45 (2), 219–230. https://doi.org/10.1093/oxfordjournals.mollus.a065497.

Songsangjinda, P., Matsuda, O., Yamamoto, T., Rajendran, N., Maeda, H., 2000. The role of suspended oyster culture on nitrogen cycle in Hiroshima Bay. J. Oceanogr. 56 (2), 223–231. https://doi.org/10.1023/A:1011143414897.

South, P.M., Delorme, N.J., Skelton, B.M., Floerl, O., Jeffs, A.G., 2022. The loss of seed mussels in longline aquaculture. Rev. Aquac. 14 (1), 440–455. https://doi.org/10.1111/raq.12608.

Statistics Canada, 2024. Table 32-10-0107-01 Aquaculture, Production and Value. https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210010701.

Sun, C., Waite, J.H., 2005. Mapping chemical gradients within and along a fibrous structural tissue, mussel byssal threads. J. Biol. Chem. 280 (47), 39332–39336.

Szefer, P., Frelek, K., Szefer, K., Lee, C.B., Kim, B.S., Warzocha, J., Zdrojewska, I., Ciesielski, T., 2002. Distribution and relationships of trace metals in soft tissue, byssus and shells of *Mytilus edulis trossulus* from the southern Baltic. Environ. Pollut. 120 (2), 423–444.

Vaccaro, E., Waite, J.H., 2001. Yield and post-yield behavior of mussel byssal thread: a self-healing biomolecular material. Biomacromolecules 2 (3), 906–911. https://doi.org/10.1021/bm0100514.

Waite, J.H., Harrington, M.J., 2022. Following the thread: Mytilus mussel byssus as an inspired multi-functional biomaterial. Can. J. Chem. 100 (3), 197–211. https://doi.org/10.1139/cjc-2021-0191.

Waite, J.H., Qin, X.-X., Coyne, K.J., 1998. The peculiar collagens of mussel byssus. Matrix Biol. 17 (2), 93–106. https://doi.org/10.1016/S0945-053X(98)90023-3.

Waite, J.H., Vaccaro, E., Sun, C., Lucas, J.M., 2002. Elastomeric gradients: a hedge against stress concentration in marine holdfasts? Philos. Trans. R. Soc. Lond. B Biol. Sci. 357 (1418), 143–153. https://doi.org/10.1098/rstb.2001.1025.

Widdows, J., Johnson, D., 1988. Physiological energetics of *Mytilus edulis*: scope for growth. Mar. Ecol. Prog. Ser. 46, 113–121. https://doi.org/10.3354/meps046113.

Wilding, T.A., Nickell, T.D., 2013. Changes in benthos associated with mussel (*Mytilus edulis* L.) farms on the west-coast of Scotland. PLoS One 8 (7), e68313. https://doi.org/10.1371/journal.pone.0068313.

Xu, Q., Xu, M., Lin, C.Y., Zhao, Q., Zhang, R., Dong, X., Tian, U., Zhang, Y., Tian, S., Xia, Z., 2019. Metal coordination-mediated functional grading and self-healing in mussel byssus cuticle. Adv. Sci. 6 (23), 1902043. https://doi.org/10.1002/advs.201902043.

Young, G., 1985. Byssus-thread formation by the mussel *Mytilus edulis*: effects of environmental factors. Mar. Ecol. Prog. Ser. 24 (3), 261–271. https://doi.org/10.3354/meps024261.

Zechel, S., Hager, M.D., Priemel, T., Harrington, M.J., 2019. Healing through histidine: bioinspired pathways to self-healing polymers via imidazole–metal coordination. Biomimetics 4 (1), 20. https://doi.org/10.3390/biomimetics4010020.