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Effect of emersion on soft-shell clam, *Mya arenaria* and the mussel, *Mytilus edulis* seeds in relation to development of vitality indices

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Abstract – Blue mussels (*Mytilus edulis*) and soft-shell clams (*Mya arenaria*) are both aquaculture species in east coast of Canada and US shellfish farmers take advantage of the byssal threads production of mussels for suspension culture and the burrowing behaviour of soft-shell clams for enhancement practices. It is important that these animals attach and burrow efficiently to minimize losses during rearing. The aim of this work was to study two potential vitality indices on mussels (23.6 ± 0.1 mm) and clams (22.6 ± 0.1 mm) seeds following various periods of emersion: attachment strength of *Mytilus edulis* and burrowing ability of *Mya arenaria.* The effect of emersion on energy content (proteins, lipids, glycogen) was also examined. We observed no significant decrease in the attachment strength of mussels after air exposure for 78 h or in the burrowing efficiency of soft-shell clams after 54 h. Air exposure had no effect on different lipid classes, proteins, or glycogen content in either mussel or clam tissues. The stressful emersion event induced in our study may not have been high enough to induce detectable behavioural responses. This can be explained by the bivalves' ability to adapt their metabolism to minimize activity during air exposure. In doing so, they do not consume their energy reserves, which are then still available when specimens are reimmersed. Thus mussels are able to efficiently produce byssal threads and clams to burrow into sediments as soon as they are back in the water.

Keywords: Bivalve aquaculture / Stress indicator / Energy storage / Emersion effect

1 Introduction

Blue mussel (*Mytilus edulis*) and soft-shell clam (*Mya arenaria*) are two bivalve species used in aquaculture activities. Mussel farming began in Canada in the late seventies and is based on submersible longlines to avoid damage from winter ice cover (Mallet and Myrand 1995). Mussels are the most important cultivated bivalve on the east coast of Canada (Statistic Canada 2010). There is also a strong market potential for soft-shell clams, thus a growing interest in its cultivation (Pariseau et al. 2007). Mussel and clam growers take advantage of the behavioural characteristics of these organisms to develop farming techniques. Mussels secrete byssal threads to attach themselves to substrate. Soon after sleeving (the operation by which seed mussels are loaded into the mesh sleeves; Mallet and Myrand 1995), spat attach to the rope and mesh sleeves on which they will grow until harvest. In contrast, soft-shell clams are endobenthic: after seeding, clam seed must burrow into the substrate where they will grow until harvest (Pariseau et al. 2007).

Attachment and burrowing are two energy-consuming activities that are ecologically relevant and important for successful culture production. The byssal thread production is often considered to be costly in terms of energy requirements (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. Moeser et al. (2006) suggested that the energetic shifts to reproduction could explain the changes observed in the mechanical properties of the byssal threads. This hypothesis have been confirmed by others studies showing that spawning influences the attachment strength of mussels (Lachance et al. 2008; Seguin-Heine et al. 2014), because such stressful event for the mussels could weaken the fibers by the production of thinner threads (Hennebicq et al. 2013) or a decrease of the byssogenesis (Babarro and Reiriz 2010). Babarro and Carrington showed also that mussels (*Mytilus galloprovincialis*) changed their energy allocation in relation to their habitat, as mussels at wave exposed sites allocated resources to reduce risk of dislodgment (smaller and thicker

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Table 1. Length and mass of experimental seed used (mean ± SE).

Species	Total length (mm)	Wet mass		
		Total (g)	Digestive gland (g)	All other tissues (g)
Mytilus edulis (n = 525)	23.6 ± 0.1	1.45 ± 0.02	0.077 ± 0.003	0.436 ± 0.007
Mya arenaria (n = 450)	22.6 ± 0.1	1.43 ± 0.02	0.043 ± 0.001	0.447 ± 0.008

shell, stronger byssal threads) instead of growth and reproduction. However, in Magdalen Islands lagoon, we noted that the energy level for adult suspension-culture mussels was not a limiting factor in terms of byssal production (Lachance et al. 2011). Burial involves muscular movements requiring energy (Pariseau et al. 2007). Indeed, mussels have to attach to sleeves to avoid slippage (fall-off) to the bottom while clams have to burrow rapidly to avoid benthic predators as well as dislodgement by waves and currents (Hunt and Mullineaux 2002). In their natural intertidal habitat, mussels and clams undergo periodic air exposure and are well adapted to such environmental conditions. Mussels have the ability to hermetically close their valves to avoid desiccation. soft-shell clams are not as threatened as mussels by desiccation because the substrate in which they burrow never completely dries, thus providing them a permanently wet habitat. Their valves do not close hermetically, but they will withdraw their siphons into the shell. During aquaculture operation, this emersion periods could be more important than the periodic air exposure undergo in natural environment and combined with manipulation, these stressful emersion event could have a significant energetic impact on clams and mussels (Tremblay and Pellerin-Massicotte 1997; Leblanc et al. 2005; Babarro et al. 2007; Brenner et al. 2012) and eventually impact the mussels' byssogenesis and clams' burrowing behaviour.

The aim of this work was to test the effects of extended air exposure with 100% humidity on the attachment capacity of young blue mussels and on the burrowing ability of young soft-shell clams. We hypothesized that attachment and burial could be reduced due to the higher energy investment needed to maintain basal metabolism when faced with the stressful emersion event. Even though bivalves can switch from aerobic to anaerobic metabolism to limit energy demands during emersion (Shick and Widdows 1981; Newell 1991), the by-products of anaerobic metabolism accumulated during emersion are expulsed by overshooting filtering (Newell 1991) when animals are returned to the water. Due to these energetic costs, mussels might produce weaker threads while soft-shell clams might take longer to bury completely. The effect of reimmersion after emersion on energy storage was also studied in the digestive gland and in all other tissues combined.

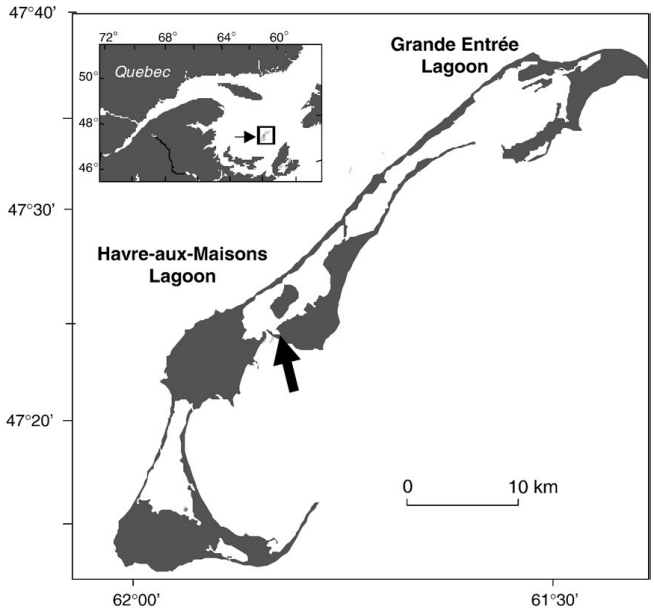

Fig. 1. Sampling sites for *Mytilus edulis* (Havre-aux-Maisons) and *Mya arenaria* (Dune du Nord) seed in the Magdalen Islands, Québec, Canada.

2 Materials and methods

2.1 Collection and emersion of mussels and clams

In late May 2007 (seawater temperature of 10 °C), seeds of blue mussels were sampled from longlines and soft-shell clams were manually collected at low tide from a natural population both in Havre-aux-Maisons Lagoon from the Magdalen Islands (Québec, Canada) (Table 1, Fig. 1). These young mussels and clams are generally immature in May in this area (Rodhouse et al. 1986; Brulotte and Giguère 2007), so there was no energy invested in gonadal development. All specimens were placed in flow-through tanks for three days to recover from harvesting operations; tanks were supplied with sand-filtered water pumped from the nearby Havre-aux-Maisons Lagoon. After this period, 600 specimens of each species were brought into a temperature-controlled room (10 °C) and placed between two layers of brown paper saturated with seawater to avoid tissue desiccation. Air exposure treatments lasted 6, 24, 30, 48, 54, and 78 h for mussels and 6, 24, 30, 48, and 54 h for soft-shell clams. Every morning, the paper was sprayed with seawater to maintain humidity at 100%. Control mussels and clams representing air exposure of 0 h, were left in the acclimation tank until the behavioural experiments.

2.2 Tank conditions

We used 3 tanks (1.9 m long × 1.1 m wide × 0.6 m high), each supplied with seawater at a flow of 10 L min^{-1}. The water level was maintained at 30 cm above the bottom, and lights above the tanks were on during all experiments. Water salinity and temperature were measured in experimental tanks at the beginning, middle, and end of each

experiment with a YSI-85 thermosalinometer (Yellow Springs Instruments, OH, USA). Triplicate samples were taken in each of the 3 experimental tanks at the beginning and at end of each experiment for total particulate organic matter (TPM) analysis.

2.3 Behavioural analysis

Mussels (n = 75 per treatment) were randomly chosen for the seven emersion periods (0, 6, 24, 30, 48, 54, and 78 h) and two hours prior the reimmersion, each mussel was glued (Bostik cyanoacrylate adhesive glue) onto an individual plastic plate by its right valve (ventral edge facing down). Each individual plastic plate with its glued mussel was then perpendicularly inserted into one of the five grooves of a larger main plastic plate. Each groove could hold five individual plastic plates. This system allowed the mussels to attach their newly secreted byssal threads to the main plate. Each plate with its mussel was subsequently pulled away to measure the mussel's attachment strength to the main plate without interfering with the attachment of its neighbours (see below). Each main plate measured 60 cm long × 60 cm wide × 1 cm thick and held 25 individual plates arranged vertically. The individual plates were 10 cm long × 5 cm wide × 1 cm thick. The plate systems were placed at the bottom of each of the three tanks for reimmersion for 22 h. Long pieces of stainless steel were put on each row of individual plastic plates to keep the plate systems at the bottom. Water was distributed along each row of the main plate via a perforated pipe. After 22 h of reimmersion, the attachment strength of individual mussels was determined. A small hook joined by string to an endless screw was attached to an individual mussel plate and connected to a digital force gauge. Mussel plates were drawn up mechanically at constant low speed by a small motor until the plate dislodged, as described by Lachance et al. (2008). The minimum force required to dislodge a plate with its glued mussel was recorded with a 10 N digital force gauge (Q-graph Quantrol™ software; Dillon, Fairmont, MN, USA). The force needed to dislodge each plate with no mussel had been recorded beforehand and was subtracted from the force measured during the experiment to eliminate the individual plate weight. The resulting value is the net attachment strength. After attachment strength measurements, shell length was determined with an electronic caliper (Fowler Sylvac) and total wet weight was measured. Each mussel was then dissected and the tissues separated into digestive gland and remaining tissues. Tissues were weighed separately and kept at −80 °C for subsequent biochemical analyses.

Before reimmersion of soft-shell clams, three trays of approximately 70 cm long × 40 cm wide × 20 cm high were filled with pre-sifted (800 μm) medium sand and placed in the middle of each of the three experimental tanks. Twenty-five clams (n = 75 per treatment) were placed in five rows of five clams in each tray (triplicates) for each air exposure trial (0, 6, 24, 30, 48, 54 h). At this point, the degree of burial (not buried at all, 50% buried, or completely buried) was recorded every 15 min for four hours. The time required for clams to start burrowing and the percentage of clams completely buried were recorded after four hours of reimmersion. After burial measurements, shell length and total weight were measured. As was done with mussels, each clam was dissected and the digestive gland and remaining tissues were separated. Wet tissue was weighed and kept at −80 °C until biochemical analyses.

2.4 Biochemical analysis

For each species, the digestive gland and remaining tissues of 12 specimens by treatment were randomly sampled and analyzed individually to compare the two treatments: control (no emersion) vs. 78 h of emersion following by 22 reimmersion for mussels and control vs. 54 h of emersion for soft-shell clams. Proteins were determined by colorimetry with a spectrophotometer (Molecular Devices Vmax Kinetic Microplate Reader, USA) according to Bradford (1976). Lipid classes (triacylglycerols [TAG], sterols [ST], and phospholipids [PL]) were determined by chromarod thin-layer chromatography coupled with an Iatroscan flame ionization detection system (Iatroscan MK-6) according to Parrish (1987). Because of the microquantities of tissues, it was impossible to use the same specimen's tissues for all biochemical analyses. Thus, glycogen analyses were made on 5 different specimens randomly chosen per tank and per emersion period and determined by colorimetry and spectrophotometry (Spectrophotometer Beckman DU 640) according to Carr and Neff (1984).

2.5 Statistical analysis

Mussel behaviour experiments were run in triplicate (three different tanks), with 25 mussels per emersion period. The percentage of totally buried clams was calculated for each tank, thus providing triplicate values for each emersion period. 20 completely buried clams were randomly chosen to quantify the burrowing activity. For biochemical analysis, 5 mussels and clams per tank (replicate) per emersion period were analyzed. For behavioural analysis, one-way ANOVAs were used to compare the effect of the emersion period on the attachment strength of mussels and on the percentage of clams completely buried and the burrowing initiation time. Factorial ANOVAs (main factors: emersion duration and tissue) were used for biochemical analyses. Tukey tests were applied after Bonferroni corrections if assumption of ANOVA were met. When needed, data were transformed (log10 or square root) to respect test conditions. Residual distribution normality was analyzed with a Kolmogorov-Smirnov non-parametric test and homogeneities of variance were determined with Levene's test. All statistical analyses were performed using Systat 11 (Systat Software Inc., San Jose CA, USA).

3 Results

3.1 Water analysis

Temperature and salinity were similar for all treatments for both species. Mean water temperature in the 3 tanks during the experiment with mussels was 10.3 ± 0.1 °C while salinity was 31.17 ± 0.03. Concentrations of total particulate matter (TPM) and particulate organic matter (POM) during

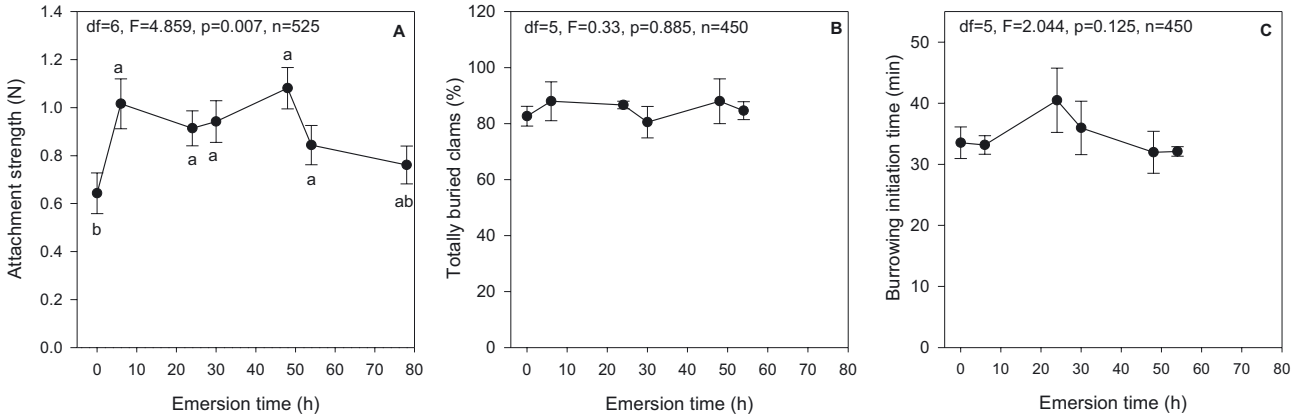

Fig. 2. Attachment strength of mussels (A), percentage of totally burrowed clams (B), and time required to initiate burrowing in clams (C), for different lengths of time (mean ± SE) exposed to air (10 °C and 100% humidity), 0 represent the control always submerged in seawater.

Table 2. Biochemical variables analyzed in *Mytilus edulis* and *Mya arenaria* digestive gland and all the other tissues (mean ± SE). Concentrations were pooled when no significant difference between tissues was found. TAG: triacyglycerols, ST: sterols, PL: phospholipids.

	Variable	Digestive gland	All other tissues
Mytilus edulis	% TAG	55.7 ± 7.2	24.1 ± 2.6
	% ST	3.0 ± 0.6	6.7 ± 0.4
	% PL	22.0 ± 3.9	46.9 ± 2.0
	TAG/PL	43.4 ± 11.0	2.3 ± 0.2
	TAG/ST	5.1 ± 1.9	0.7 ± 0.1
	Glycogen (μg g^{-1}*)	33.7 ± 4.1	
	Proteins (mg g^{-1}*)	6.6 ± 0.3	8.6 ± 0.6
Mya arenaria	% TAG	17.4 ± 1.8	8.4 ± 0.9
	% ST	5.4 ± 0.4	9.9 ± 0.4
	% PL	42.3 ± 2.0	65.3 ± 3.2
	TAG/PL	8.4 ± 0.7	
	TAG/ST	3.4 ± 0.5	0.9 ± 0.1
	Glycogen (μg g^{-1}*)	10.6 ± 1.4	
	Proteins (mg g^{-1}*)	4.4 ± 0.3	

* Per gram of wet mass.

this experiment were respectively 0.60 ± 0.14 mg L^{-1} and 0.51 ± 0.01 mg L^{-1}. During the experiment with clams, mean water temperature was 12.8 ± 0.2 °C, salinity 31.04 ± 0.03, TPM 0.84 ± 0.10 mg L^{-1} and POM 0.38 ± 0.06 mg L^{-1}.

3.2 Behavioural analysis

Behavioural analyses have been realized on seed around 23 mm for both species (Table 1). For *Mytilus edulis*, air exposure had a significant effect on their attachment strength comparatively to control (mussels without emersion, Fig. 2A). Mussels emerged from 6 to 54 h and reimmerged for 22 h were attached significantly more strongly (nearly twice) to the plate compared to control mussels (no emersion at all, Tukey $p < 0.05$). The mean strength measured in control animals was 0.64 N while mussels showing maximal value (48 h emersion) showed mean attachment strength of 1.08 N.

For *Mya arenaria*, the percentage of individuals per tank that were completely buried after 4 h of reimmersion was not significantly different after emersion of 6 to 54% with an overall mean of 86 ± 2% (Fig. 2B). The time required for clams to initiate burial was not related to the duration of air exposure and started after 30 min in all emersion treatments (Fig. 2C). Less than 5% of clams do not burrow during all the experiment.

3.3 Biochemical analysis

There was no significant effect of emersion or emersion duration on the various biochemical parameters for either species (Tables 2 and 3). However, we did find differences in the concentrations of the most biochemical constituents in the two tissue groups examined (Tables 2 and 3).

In mussels, biochemical contents in the digestive gland were significantly different from those found in the remaining tissues except for the glycogen (Table 3). TAG percentage and the TAG/PL and TAG/ST ratios were higher in the digestive gland while % ST, % PL, and protein content were higher in the remaining tissues (Table 2).

Table 3. Results of factorial ANOVA examining the effects of emersion time and tissues on the biochemical constituents (TAG: triacylglycerols, ST: sterols, PL: phospholipids, ratio between TAG and ST, ratio between TAG and PL, proteins and glycogen) of mussels (*Mytilus edulis*) and soft-shell clams (*Mya arenaria*). Bold indicated significant effect of factors. TAG, ST and PL were tested on % of total lipids and proteins and glycogen on concentrations in mg g^{-1} and ug g^{-1} respectively (n = 12).

Variables	Factors	*Mya arenaria*			*Mytilus edulis*		
		df	*F*	*p*	*df*	*F*	*p*
% TAG	Emersion	1	0.031	0.865	1	0.011	0.919
	Tissues	1	14.107	**0.006**	1	16.451	**0.004**
	Emersion*Tissues	1	0.215	0.655	1	0.067	0.803
% ST	Emersion	1	0.000	0.993	1	2.146	0.181
	Tissues	1	22.144	**0.002**	1	55.595	**0.001**
	Emersion*Tissues	1	0.711	0.424	1	0.001	0.980
% PL	Emersion	1	0.055	0.820	1	0.482	0.507
	Tissues	1	26.271	**0.001**	1	33.738	**0.001**
	Emersion*Tissues	1	0.084	0.779	1	0.774	0.405
TAG/ST	Emersion	1	0.376	0.557	1	0.173	0.689
	Tissues	1	8.750	**0.018**	1	22.827	**0.001**
	Emersion*Tissues	1	0.069	0.799	1	0.008	0.929
TAG/PL	Emersion	1	0.025	0.877	1	1.866	0.209
	Tissues	1	14.197	**0.005**	1	2.228	0.174
	Emersion*Tissues	1	0.032	0.862	1	0.024	0.881
Proteins	Emersion	1	0.673	0.436	1	4.448	0.068
	Tissues	1	8.556	**0.019**	1	0.068	0.800
	Emersion*Tissues	1	0.508	0.496	1	0.579	0.468
Glycogen	Emersion	1	0.131	0.726	1	1.313	0.285
	Tissues	1	3.223	0.110	1	5.264	0.051
	Emersion*Tissues	1	0.091	0.770	1	0.112	0.747

Except for the TAG/PL ratio, protein content, and glycogen, the energy components of clams were different between the two groups of tissue (Table 3). TAG percentage and the TAG/ST ratio were higher in the digestive gland while ST and PL percentages were higher in the remaining tissues (Table 2).

4 Discussion

In this study, the duration of air exposure (⩽78 h for mussels and ⩽54 h for clams) had no negative effect on attachment of mussels or on burial of clams after reimmersion. Thus, our hypothesis that attachment and burial efficiency are reduced by the higher energy investment needed to maintain basal metabolism, when faced with the stressful emersion event, was not confirmed. Significant higher attachment strength were observed from mussels emerged for 6 h and have been maintained until 78 h emersion. Our results suggest that emersion periods of 54 h for clams and 78 h for mussels at 10 °C and 100% humidity are not stressful enough to alter these behaviours. In a previous study on *Mya arenaria*, Pariseau et al. (2007) found a similar result with individuals held out of the water for only 4 h in the sun without modification of burial behaviour compared to controls. A possible explanation could be that the behavioural indices examined here (attachment strength and burrowing) are not good indicators of vitality for mussels and clams or that the stress used (emersion for the facility to control the intensity) was not appropriate to test the seed vitality. It was also surprising to observe that seed clams need more that 30 min to initiate burrowing. Generally, healthy seed clams (around 15 mm in shell length) burrow within 10 min (Emerson et al. 1990; Beal and Vencile 2001; Appelbaum et al. 2011). These results could suggest that vitality of clams' seed in end of May in this area could be too low to observed significant changes. This longer emersion time could be also related to higher seed size used in our study (around 22 mm), as Pariseau et al. (2007) observed an inverse relationship between size and burial level. We observed an increase of attachment strength after 6 h emersion in *M. edulis* seed. Results on *Perna canaliculus*, where 4 h of emersion followed by 20 h of reimmersion reduced significantly the activity of seed mussels, the vital staining (heat movements, gill cilia, foot, shell valves, mantle and siphon) and rate of retention estimated by byssal thread attachment (Webb and Heasman 2005; Carton et al. 2007). These results could suggest that *M. edulis* seem largely more resistant to emersion than *P. canaliculus*.

4.1 Emersion effect

The capacity of many bivalve species to reduce their energy demands during emersion might explain the fact that no

behavioural differences were observed in mussels and clams following different emersion periods. In addition to disrupting their respiratory activities, air exposure prevents bivalves from feeding and might add a desiccation stress. During this period, mussels and clams must rely on anaerobic metabolism (de Zwaan and Wijsman 1976; Schick et al. 1986; Sobral and Widdows 1997; Babarro et al. 2007), which should lead to a marked metabolic depression (Shick and Widdows 1981; Le Moullac et al. 2007). The energy requirements met by anaerobiosis are only 4% of normal aerobic metabolism in the blue mussel (Widdows 1989). During emersion, metabolic activities are thus reduced to a minimum to diminish the effects to this stress until reimmersion. Furthermore, when mussels are exposed to air, they show a gaping behaviour (periodic opening and closing of the shells), which provides them with a low oxygen renewal in the tissues (Widdows et al. 1979; Guderley et al. 1994). Significant reductions of burial behavioral following exposition to high hypoxia level have been already demonstrated for other clams' species (Long et al. 2008; Lee et al. 2012). During this hypoxia, the metabolism was supported by anaerobic activity related to a decline of glycogen and protein content (Lee et al. 2012). In our results, the absence of modification of glycogen and protein use in clams and mussels tissues suggests that the emersion conditions seem not impact energetic reserves. We suggest that emersion in 100% humidity and 10 °C and the potential air gaping and metabolic despression, seem sufficient to maintain seed vitality during 54 h for clams and 78 h for mussels.

When reimmersed, mussels increase their respiration rate to compensate for the oxygen debt accrued during anaerobic metabolism and start filtering and feeding to rebuilding their energy stores (Zandee et al. 1986). During the 22 h of reimmersion, energy transfer between mussel digestive gland and other tissues probably did not have time to occur: Thompson et al. (1974) found that energy transfer between the digestive gland and other tissues takes about 7–10 days in summer and fall but up to 35 days in winter, when food is scarce. Thus, the energy component measured in tissues other than the digestive gland after reimmersion would reveal the energetic cost of emersion, attachment, and the increased respiration rate required to evacuate the by-products of anaerobic metabolism. Our results show that there was no impact of these three potentially energy-consuming activities on energy components in non-digestive gland mussel tissues, meaning that animals did not have to consume their energy resources, or at least not to a detectable degree.

This stability of the behavioural response and in the different energy components analyzed is in agreement with Harding et al. (2004), who found that dry storage (emersion) of mussels at high humidity and 2–4 °C for a period up to 12 days followed by a 24 h reimmersion can reduce the stress response and provide a better quality product with longer shelf life. In contrast, Almeida et al. (2005) found that emersion of *Perna perna* for 24 h caused a clear increase in the levels of lipid peroxidation and oxidative DNA damage. Nevertheless, when these mussels were reimmersed in seawater for three hours, the levels of damage to lipids and DNA went back to control values. Hole et al. (1995) also found that 2- to 4-year-old blue mussels exposed to a 24 h hypoxia and hyperthermia (28 °C) event showed lysosomal destabilization, but their metabolic rate returned to control levels 12 h after reimmersion. Similar results were observed in intertidal conditions for *M. edulis* and *M. arenaria* (Tremblay and Pellerin-Massicotte 1997).

As was the case with the behavioural responses, the biochemical results suggest that our experimental emersion treatments (time, humidity, and temperature) did not stress blue mussels or soft-shell clams. However, mussels and clams may have had time to recover when reimmersed for 22 h. It is possible that mussels and clams were stressed by emersion but that their subsequent reimmersion allowed them to return to control levels. In this context, the intensity and duration of the stress events could have been insufficient to be measured by the chosen indicators (energy reserves and behaviour) at the end of the reimmersion.

4.2 Tissue effect

Differences were observed in most biochemical concentrations between the two tissue groups depending on the component. TAG percentage was higher in the digestive gland than in the other tissues for both species. TAG is an important energy source for bivalves (de Zwaan and Mathieu 1992), and the digestive gland is where digestion, energy storage, and the distribution of metabolic reserves occur. Our results suggest that mussels and clams had enough energy available to resist physiological stress. The high TAG concentration can probably be explained by an accumulation of plankton rich in TAG that were ingested previous to emersion and during the reimmersion period (Caers et al. 2000). In contrast to TAG, the percentages of structural lipids (ST and PL) were higher in the remaining tissues as already observed in literature (Moreno et al. 1976; Pernet et al. 2007). These tissues use energy from these lipids for growth, which could explain why structural lipids are more abundant in these tissues than in the digestive gland, which is an energy storage organ.

While glycogen concentration was the same for both groups of tissues in the blue mussel, its level in *M. arenaria* digestive gland was about half that found in the remaining tissue. This might be explained by the fact that glycogen content increases in *M. arenaria* mantle tissue when food is abundant (Gabbott and Bayne 1973), suggesting that clams ingested and stored glycogen before emersion. Even though clams were not supposed to be sexually mature, some small individuals, like our individuals around 23 mm, can have limited fertility and reproduce (Brousseau 1978; Roseberry et al. 1991). It is possible that glycogen was invested in gonadal development in such individuals.

5 Conclusion

Relatively simple behavioural tests could be very useful to farmers, providing them with a rapid and inexpensive way to determine the vitality of their seed stock. The aquaculture industry needs to develop easy and rapid vitality indicators to limit fall-off and losses. Our results suggest that an emersion duration of 2–3 days at 10 °C and 100% humidity is probably not stressful enough to be measured with the behavioural

and biochemical indicators chosen. A longer duration, higher temperature, or drier environment during emersion, or another kind of stressful event, may be needed to produce an effect on these indicators. Thus, the use of these behavioural indicators for aquaculture purposes needs further investigation. Nevertheless, behavioural indicators are valuable because of their low cost and ease of use. The behavioural indicators we chose are known to be influenced by temperature, salinity, food availability, size, reproductive cycle and sediment carbonate geochemistry (Matthiessen 1960; Lardies et al. 2001; Pariseau et al. 2007; Lachance et al. 2008; Clements and Hunt 2014). Our results show that emersion at low temperature (10 °C) and high humidity for as long as 78 h for mussels and 54 h for clams has no effect on the energy reserves and capacity of mussels to produce byssus or of clams to burrow. Thus, in shellfish aquaculture practices, if low temperature and high humidity conditions are provided during emersion periods associated with the transfer of spat to culture sites, shellfish vitality and production profitability will not be affected.

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