Modeling the depuration potential of blue mussels (*Mytilus* spp.) in response to thermal shock

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Abstract

When contaminated, most molluscs destined for human consumption require a depuration regime lasting 48 h at a minimum temperature of 5 °C to ensure elimination of coliforms. However, this regime is unsatisfactory in northern temperate regions, where temperatures are frequently below 5 °C. A series of tests were undertaken to determine the filtration activity of mussels (*Mytilus* spp.) under cold temperatures. We used physiological measures rather than the more traditional method of bacterial analysis to evaluate mussel acclimation to cold temperatures. Mussels were acclimated for 4 weeks at three different temperatures (8, 4, and −1 °C) and their scope for growth was evaluated each week to determine the level of acclimation. Mussels were then exposed to a thermal shock and clearance rates were measured after 2 h and 72 h. We observed a clearance rate of 2.45 l h^{-1} g^{-1} (g DW) for the 8 °C control group. Thus, within a 48-h depuration period, *Mytilus* spp. could filter a standard volume of 117.47 l. We used a von Bertalanffy exponential model to estimate the time required for an individual from each thermal shock treatment to filter that standard volume. We found that thermal shock had an important effect on the volume filtered by a mussel in 48 h. For example, mussels acclimated at 8 °C were able to filter the standard volume of 117.47 l in an average of 75 h at 4 °C, whereas those acclimated at 4 °C and transferred to 8 °C required only 23 h on average.
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1. Introduction

In the process of food ingestion, bivalves can accumulate bacterial contamination, such as faecal coliforms (Trollope and Webber, 1977; Prieur et al., 1990). Blue mussels retain 100% of the particulate

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matter larger than 4 μm and up to 20–30% of the particulate matter that is smaller than 1 μm (see the review of Hawkins and Bayne, 1992). Furthermore, coliform aggregation contributes to the retention and assimilation of coliforms by mussels (Bernard, 1989). Thus, in bad environmental conditions, the coliform level is sometimes higher than the standard level acceptable for human consumption and may prevent the commercial exploitation of bivalves (Desbiens et al., 2000). A solution to this problem is depuration, which uses the large filtration capacity required in molluscs for feeding and respiration (Furfari, 1966; Perkins et al., 1980). The depuration process is carried out by holding molluscs in tanks with non-contaminated water (Furfari, 1966; Bernard, 1989). Generally, the time required to eliminate nearly 100% of the coliform *Escherichia coli* is less than 48 h (Furfari, 1966; Trollope and Webber, 1977; Bernard, 1989). As depuration efficiency is directly related to the filtration capacity of the bivalve, it is necessary for depuration to occur in conditions that preserve the physiological state of the bivalves. Thus, bivalves should be submitted to minimal stress levels to allow for optimal filtration activity (Perkins et al., 1980).

Bivalves are ectothermic organisms, thus temperature is a major determinant of their physiological status (Bayne, 1976). Depuration can occur over a large range of temperatures, but 5 °C is considered as the minimum threshold below which physiological activity is strongly inhibited (Furfari, 1966; Perkins et al., 1980). Nevertheless, the local environmental conditions have an important and long-term effect on the physiological phenotype of bivalves (Hatcher et al., 1997; Sukotin et al., 2003). Thus, bivalves from northern temperate regions are adapted to low temperatures and have an ability to feed and filter at temperature below 5 °C because of compensations in the enzyme systems related to carbon and ATP demands. In this paper, we consider thermal acclimation as a physiological response observed in the laboratory and acclimatization to be the physiological responses observed in the field (following Blackstock, 1984; Lesser and Kruse, 2004). From monthly physiological measurements, Smaal et al. (1997) observed that mussels generally acclimate to temperature variations between 0 and 20 °C. Results obtained in eastern Canada demonstrated that mussel metabolism is significantly lower in winter (Hatcher et al., 1997), but

these authors suggested that this metabolic depression could be related to food limitations during ice cover. Finally, Thompson and Newell (1985) demonstrated that the thermal sensitivity of mussel metabolic rates differs among populations from different latitudes. Mussels from Newfoundland (Canada), where the maximum water temperature is 17 °C, catabolized more protein and showed severe depressions in the clearance rate at 25 °C compared to mussels from Stony Brook (NY, USA), which are frequently exposed to temperatures of 25 °C (Thompson and Newell, 1985). All these results suggest that mussels from northern regions probably maintain their physiological capacity to filter at temperatures below 5 °C. Another important temperature effect is the thermal shock suffered by bivalves. Metabolic and filtration rates react rapidly in response to short-term thermal fluctuations within the range of tolerated temperatures. Thus, physiological rates increase with a short-term increase in temperature and conversely decrease with a short-term decrease in temperature (Bayne, 1976; Tremblay et al., 1998a).

Our objective was to estimate the theoretical depuration potential of mussels in relation to thermal shock and acclimation to cold temperatures. Scientific studies on the depuration process have mainly been made on the basis of bacterial reduction using coliforms as indicators. The choice of this purification criterion gives only a positive or negative result, indicating that bivalves are depurated or not (Boulter and Wilson, 1998). It is not possible to determine the level of acclimation in relation to environmental changes with this type of analysis. Furthermore, bacteriological analyses need high initial bacterial counts in bivalves to observe the depuration mechanism (Boulter and Wilson, 1998), and this characteristic is difficult to obtain on the east coast of Québec, Canada (Desbiens et al., 2000). Since the depuration efficiency of mussels is directly related to their physiological capacity, the theoretical depuration potentials in this study were estimated under laboratory by measuring (1) the physiological acclimation capacity of mussels in low temperatures, and (2) the filtration capacity following a thermal shock. This second evaluation was achieved using a simple mathematical model that generated the theoretical filtration time required for complete depuration. We hypothesized that the depuration capacity of mussels is proportional to the

clearance rate as measured by a volume of water cleared of suspended particles per unit of time.

2. Methods

Mussels were sampled in early May 2000 from an aquaculture farm in the baie de Gaspé (Québec, eastern Canada, 48°50′N; 64°27′W) during gametogenesis (the spawning period in this location begins in late June; Thomas, 1996). A subsample of 60 mussels (53.4 to 69.9 mm) was frozen at −80 °C for species identification by Polymerase Chain Reaction (PCR) using the diagnostic genetic marker *Glu*-5 (Rawson et al., 1996). This method allows the differentiation of the two species present on the east coast of Canada (*Mytilus edulis* and *Mytilus trossulus*) as well as hybrid individuals. For mussel DNA isolation, we used the QIAGEN® DNeasy™ Tissue Kit, and 50 ng of DNA served as a PCR template.

In the laboratory, 90 mussels (15.2 to 72.7 mm) were kept for allometric determination and another 216 (51.2 to 70.9 mm) for acclimation (72) and thermal shock (144) experiments. All these mussels were numbered with a bee tag, kept in seawater at 12 °C (temperature of the sea at harvest time; >90% oxygen saturation), and continuously fed a mixture of *Isochrysis galbana* and *Chaetoceros gracilis* (10^6 cells ml^{-1}; a ration of 6–7% of their body mass daily).

2.1. Allometric experiment

Using six metabolic chambers simultaneously, the clearance rate then the oxygen consumption rate were measured from five individual mussels and one blank. Between the clearance rate and oxygen consumption measurements, the filtered seawater in the chambers was changed. After these measurements, the animals were returned to the aquarium and placed into a small container in order to collect faeces for the determination of the absorption efficiency. At the end of all physiological measures, individual shell length and body mass (dry weight, DW) were determined for each mussel.

Clearance rates were measured using a static (not flow-through) system in which the rate of particle density decrease (algal depletion) in the metabolic chamber was monitored (Petersen et al., 2004). These particle density measurements were carried out using a Beckman Z1 particle Coulter-counter fitted with a 120-μm orifice tube. The experimental water within the chambers was mixed with gentle aeration. The clearance rate was evaluated at 10-min intervals over 1 h, and the degree to which the valves were open was monitored continuously. Following Gilek et al. (1992), the greatest difference between two consecutive measurements during this period was assumed to be the clearance rate. This method avoided the underestimation of the actual clearance rate, which is related to the degree to which the exhalant siphon is open and to valve gape (Newell et al., 2001; Riisgård et al., 2003). The clearance rate ($1 h^{-1}$) was then used to estimate the amount of ingested or consumed energy. This calculation was obtained by assuming that the diet had an energy content of 23 J mg^{-1} (Widdows and Johnson, 1988). Oxygen consumption rates for individual mussels were determined by sealing the metabolic chambers and measuring the decrease in dissolved oxygen with a YSI (5331) polarographic analyzer and electrode. The water was mixed with a magnetic stirrer. Because mussels had an abundant food supply, we assumed that the measured rates of oxygen uptake represented the routine metabolism as described by Bayne (1976). The output signal was monitored continuously on a chart recorder until a minimum decrease of 20% O_2 was observed. Oxygen concentration was not allowed to fall below 75% saturation. The rate of oxygen consumption was calculated in ml O_2 h^{-1} and converted to energy equivalents using the conversion factor of 1 ml O_2 = 20.33 Joules as described by Widdows and Johnson (1988). Assimilation represents the product of ingested energy and absorption efficiency (Widdows and Johnson, 1988). We took samples of the diet mixture as well as faeces from individual mussels, making sure that pseudofaeces were excluded (Honkoop et al., 2003). Samples were filtered through pre-combusted, pre-weighed 47 mm GFC glass fibre filters, rinsed with isotonic ammonium formate (3.2%), dried at 80 °C for 48 h, cooled to room temperature in a desiccator, weighed, combusted at 450 °C overnight, cooled to room temperature in a desiccator, and finally weighed to estimate the organic and inorganic fractions contained in the food and faeces. Absorption efficiency was measured using Conover's (1966) ratio of food dry weight to ash-free dry weight in relation to faeces dry weight to ash-free dry weight.

2.2. Acclimation experiment

Physiological characteristics (scope for growth) were determined on mussels 48 h (t_0) after the sampling, after which all mussels were transferred to three different temperatures (-1, 4, and 8 °C) regimes for 21 days. To obtain -1 °C, we used water baths with glycerol in the cooling system. For the acclimation experiment, we used nine 5-l aquaria (three aquaria for each temperature treatment) and 18 aquaria for the thermal shock experiments (two aquaria for each thermal shock treatment). Each aquarium contained eight mussels and was supplied with air. Mussels were randomly distributed among aquaria and aquaria were randomly distributed among treatments to ensure sample independence. To evaluate the progress of the thermal acclimation, physiological measurements on the same mussels were made for each temperature treatments on days 1, 14, and 21. The effect of temperature on mussel acclimation capacity was quantified by determining the scope for growth of 24 mussels (obtained from three different aquaria) by acclimation temperature. The scope for growth is an integration of all the basic physiological processes (food ingestion, food assimilation, respiration, and excretion) and is a good index of the energetic state of an organism under different environmental conditions (Widdows and Johnson, 1988). Scope for growth (P) was determined by the equation:

$$P = A - (R + U) \tag{1}$$

where A is food assimilation and R and U are the energy losses by respiration and excretion, respectively. However, excretion represents less than 5% of total energy budget in mussels (Tremblay et al., 1998b) and has thus been ignored in this study, as suggested by Bayne et al. (1999) and Honkoop et al. (2003). The body mass (g DW) of each mussel was determined using an allometric equation according to the formula given by Bayne (1976) and using the slope determined for the mussels of this study, to determine the physiological rates for an individual with a standard mass of 1 g.

2.3. Thermal shock experiments

At the end of the acclimation period (21 days), mussels from each temperature (-1, 4, and 8 °C)

were reciprocally transferred to all thermal conditions. Clearance rates (filtration) were then measured after 2 and 72 h to evaluate the thermal shock. Experimental controls consisted of mussels that were transferred to the same temperature (i.e., 8 to 8 °C; 4 to 4 °C; -1 to -1 °C). For each transfer treatment, clearance rates were measured as previously described on 16 mussels at 2 and 72 h after the thermal shock. The clearance rate was used to estimate the volume of water filtered by a mussel per unit of time and for the evaluation of the theoretical depuration capacity in experimental conditions of long- and short-term thermal variation.

2.4. Rationale for the model

In this study, the time required to complete a theoretical depuration was estimated by a mathematical model. This time period was calculated for a target volume needed to be filtered by the mussels to achieve complete depuration. We postulated that the clearance rate of a control mussel at 8 °C, which has experienced the same handling procedure as the other thermal shock combinations, would provide the best clearance target volume, representing a complete purification after 48 h (as suggested by Furfari, 1966). This target volume was estimated and used to determine how long the mussels from each treatment would take to filter an equivalent volume of water. We used a simple model to evaluate the time required for a mussel to filter the target volume while undergoing the various thermal shock treatments. By knowing the individual clearance rates at 2 and 72 h after the thermal shock as well as the control clearance rate at the transferred temperature, it was possible to estimate the clearance rate at any other time using a von Bertalanffy exponential model. More specifically, the instantaneous clearance rate at time t, denoted by $Y(t)$, was predicted as follows:

$$Y(t) = a\left(1 - be^{-kt}\right) \tag{2}$$

where

$$b = \frac{1 - C_2/a}{e^{-2k}} \quad \text{and} \quad k = \frac{1}{70}*\log\left(\frac{a - C_2}{a - C_{72}}\right)$$

In this expression, C_2 and C_{72} stand for the clearance rates recorded 2 and 72 h, respectively, after a thermal shock and a stands for the clearance rate observed for the control at the transferred temperature.

The volume of water filtered until time t, denoted by $V(t)$, is thus easily obtained from the area under the $Y(t)$ curve:

$$V(t) = \int_0^t Y(u)du = \frac{a}{k}\left(kt + be^{-kt} - b\right) \qquad (3)$$

The time needed to filter the target volume is the time t such that $V(t)$=target, which was solved numerically.

We discarded observations if they showed (i) a deceleration in the clearance rates in response to a transfer to a higher temperature or (ii) an acceleration after transfer to a lower temperature, since these observations go against the assumptions of our theoretical model and are critical for the application of our model. Therefore, for a transfer to a higher temperature, the clearance rate needed to be greater 2 h after transfer than the clearance rate at 72 h, and the rate of clearance at 72 h needed to be greater than or equal to the clearance rate of the control temperature to which the mussels had been transferred. Conversely, for a transfer to a lower temperature, the value of the clearance rate had to have been less at 2 h after transfer than at 72 h, and the 72-h value must have been less than or equal to the clearance rate of the control. We discarded mussels if they were not filtering at both measurement times (i.e., at 2 and 72 h after the thermal shock). For controls, the determination of the time required to process the target volume was computed using a linear model with the mean clearance rate between the measurements at 2 and 72 h. A SAS® program (SAS, 1999) was used and is available on request from the authors.

2.5. Data analysis

Simple regression analyses were used to examine the relationships between dry weights and other biological variables (i.e., oxygen uptake, clearance rate, length of shell, and absorption efficiency). For the first two variables, a square-root transformation was necessary and the length of shell was log transformed to achieve data normality and homogeneity of variances.

ANOVAs were used to examine if the average weight and length variables were different among the acclimation temperatures. Logarithmic transformations and inverse transformations (X^{-1}) were ap-

plied to the weight and length variables, respectively. Repeated-measures ANOVAs were used to test the effects of acclimation temperature and acclimation date on absorption efficiency rate, oxygen uptake, clearance rate, and scope for growth. A squared (X^2) transformation for the absorption efficiency rate and square-root transformations for the three other variables [$(X+19)^{0.5}$ for scope for growth] were applied to these variables. The appropriate error term for each source of variation was used.

The basic assumptions (normality, homogeneity of variances) were respected after the specified transformations as verified by a Box–Cox test for the majority of the variables measured. However, the variances were not perfectly homogeneous in certain cases. Nevertheless, ANOVA is relatively robust to heteroscedasticity, particularly when there are several replicates and when the model is balanced (Milliken and Johnson, 1992). The normality of residuals was examined with a Shapiro–Wilk test (Zar, 1984) and homogeneity of variance was visually examined for the residuals (Montgomery, 1991). A significance level of 0.05 was adopted for all statistical tests. When a source of variation was significant, a posteriori multiple comparisons (LS means; SAS, 1999) were carried out with the Bonferonni-adjusted level to identify differences.

3. Results

3.1. Species identification

M. trossulus was the predominant species in our sample from the baie de Gaspé, making up 89% of our sample compared to 8% *M. edulis* and 3% hybrids. This agrees with another study in this location (Thomas and Tremblay, 1999).

3.2. Allometric relations

The results showed significant positive relationships between body mass (g dry weight, DW) and oxygen consumption ($n=89$; $F=44.25$; $p<0.0001$; $R^2=0.34$), body mass and clearance rate ($n=85$; $F=91.53$; $p<0.0001$; $R^2=0.52$), and body mass and mussel length ($n=90$; $F=1228.14$; $p<0.0001$; $R^2=0.93$). There was no significant relationship be-

tween % absorption efficiency and body mass ($n=88$; $F=0.26$; $p=0.6119$). For the oxygen consumption, we used a slope of 0.497 (based on individual measurements of consumption against body mass) for the allometric correction. For the clearance assessment, we used a slope of 0.792 as determined by individual measurements.

3.3. Acclimation

For each temperature treatment, mean body mass (g DW) and mean length were similar (ANOVA; $F_{2,266}=0.98$; $p=0.3768$; $F_{2,258}=0.81$; $p=0.4446$, respectively). The acclimation conditions (-1, 4, and 8 °C) significantly influenced values of the % absorption efficiency, the clearance rate, and the scope for growth (Fig. 1; Table 1). Indeed, for these physiological variables, we observed low values at -1 °C, intermediate values at 4 °C, and high values at 8 °C (Fig. 1b,d,f). In most cases, there was a significant decrease in all physiological measures 1 day after the beginning of the acclimation (cf. Fig. 1a,c,e; Table 1). This was followed by a recovery of the absorption efficiency (Fig. 1a), oxygen consumption (Fig. 2), and the scope for growth after 14 days (Fig. 1e), and a recovery of the clearance rate after 21 days.

For the oxygen consumption, there was a significant interaction between the acclimation temperature and the time required for recovery following the transfer (Fig. 2; Table 1). Oxygen consumption generally decreased 1 day after the beginning of the acclimation. Recovery was longer (21 days) for the mussels transferred to 4 °C whereas 14 days were enough for mussels exposed to the other temperatures. The mussels in each treatment were significantly different in mean body mass (-1 °C: 0.49; 4 °C: 0.56; and 8 °C: 0.53 g dry weight; ANOVA; $F_{2,281}=2.84$; $p=0.0025$) and mean shell length (-1 °C: 59.1 mm; 4 °C: 60.8 mm; 8 °C: 59.6 mm; ANOVA; $F_{2,281}=10.19$; $p=0.0001$). Despite these differences in weight and length, ranges were small and should not have had a great influence on specific clearance rates after allometric corrections.

3.4. Thermal shock

A positive thermal shock (transfer to a higher temperature) caused an increase in the clearance rate
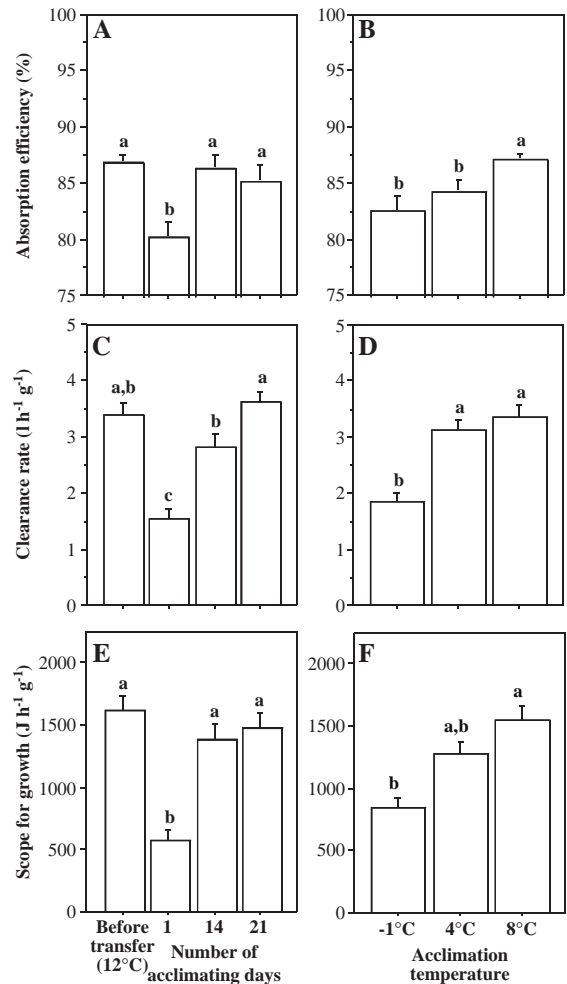

Fig. 1. Absorption efficiency (%), clearance rate (l h^{-1} g^{-1} dry weight: DW), and scope for growth (J h^{-1} g^{-1} DW) with time following the acclimation (before and after 1, 14, and 21 days) (a, c, e) and acclimation temperature (-1, 4, and 8 °C) (b, d, e). Different letters indicate statistically different values. Errors bars are S.E.

followed by a decrease (Fig. 3). A negative thermal shock (transfer to a lower temperature) caused a decrease in the clearance rate followed by an increase (Fig. 3). There was no difference in clearance rates between measurements at 2 and 72 h for the -1, 4, and 8 °C controls (LS mean comparisons; respectively $p=0.6529$, $p=0.5483$, and $p=0.9861$). However, mussels in the -1 °C control showed a significantly lower clearance rate than at the other two temperatures (LS means; $p<0.0001$). For all other treatments, the transfer to different temperatures involved a signifi-

Table 1
Summary of ANOVAs showing the effect of acclimation temperature (Temp), acclimation date (Date), and crossed factors on (a) absorption efficiency, (b) oxygen consumption (c) clearance rate, and (d) scope for growth

Source of variation	df	MS	F-value	p
(a) Absorption efficiency				
Temp	2	8.5×10^6	6.18	0.0068
Date	3	13.3×10^6	9.68	0.0002
Temp * Date	6	2.5×10^6	1.85	0.1322
Error	204	1.3×10^6		
Corrected total	239			
(b) Oxygen consumption				
Temp	2	0.068	2.99	0.0691
Date	3	0.268	11.74	<0.0001
Temp * Date	6	0.083	3.63	0.0105
Error	211	0.019		
Corrected total	246			
(c) Clearance rate				
Temp	2	4.283	17.25	<0.0001
Date	3	5.990	24.12	<0.0001
Temp * Date	6	0.562	2.26	0.0715
Error	214	0.180		
Corrected total	249			
(d) Scope for growth				
Temp	2	1191.6	6.91	0.0043
Date	3	2712.1	15.73	<0.0001
Temp * Date	6	380.9	2.21	0.0774
Error	199	184.5		
Corrected total	234			

A square (X^2) transformation for the absorption efficiency and square-root transformations for the three other variables [$(X+19)^{0.5}$ for scope for growth] were applied to normalize the data.
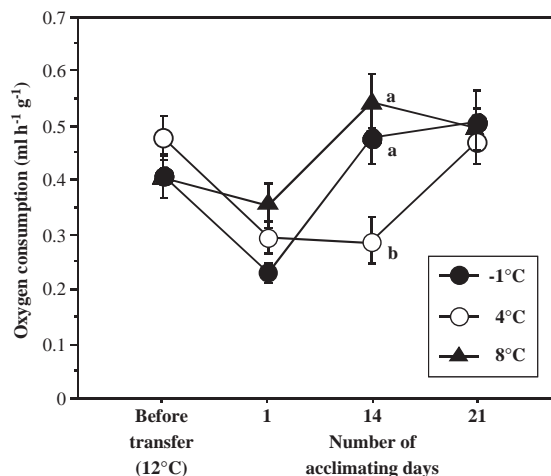

Fig. 2. Oxygen consumption (ml h^{-1} g^{-1} dry weight) according to the acclimation time (before and after 1, 14, and 21 days) and acclimation temperature (−1, 4, and 8 °C). Different letters indicate statistically different values. Errors bars are S.E.

(3). Simulated filtration times of the target volume are presented in Fig. 4. We observed that control mussels (transfer to the same temperature) at −1 °C needed significantly more time (90.7 ± 9.7 h) to filter the standard volume of 117.47 l than controls at 4 and 8 °C (LS means; $p < 0.0001$). Nevertheless, there was no difference in the filtration times between the 4 and 8 °C controls (LS means; $p = 0.0947$; 57.0 ± 1.6 h and 48.7 ± 1.6 h, respectively). Thermal shocks (transfer to a different temperature) had an important effect on the time a mussel required to filter the standard vol-
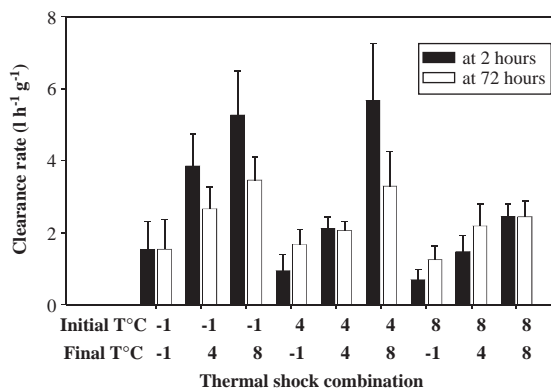
cant change in the clearance rates after 2 h and a trend to re-establish the initial clearance rate 72 h after the transfer (rate at initial temperature; Fig. 3).

3.5. Simulation

The volume of water filtered was estimated using the assumption that the clearance rate of the 8 to 8 °C control transfer (2.4474 l h^{-1} g^{-1}) could process 117.47 l in a 48-h period. This volume was used as a target value for all the other mussels in order to estimate a time of complete purification. Clearance rates of 1.5706, 2.0806, and 2.2274 (l h^{-1} g^{-1}) were observed for the −1, 4, and 8 °C controls, respectively, and were used as the *a* parameters in Eqs. (2) and

Fig. 3. Clearance rates (l h^{-1} g^{-1}) at 2 and 72 h following thermal shock in relation to temperature of acclimation and transfer temperature. Errors bars are S.E.
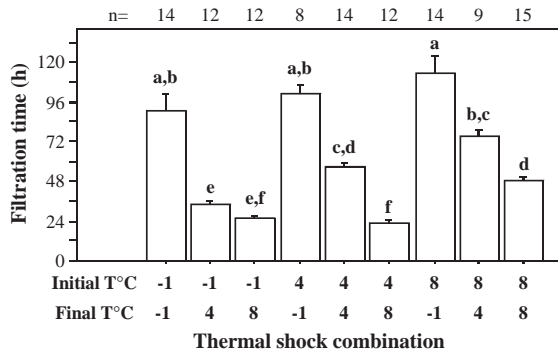
Fig. 4. Simulation of required time (in hours) to filter the target volume (117.47 l) for each treatment combination of acclimated (initial) and transferred (final) temperature. Bars having different letters above them differ significantly ($p < 0.05$). Number of data included in each simulation is shown above bars. Errors bars are S.E.

ume. For example, mussels acclimated at 8 °C filtered the standard volume of 117.47 l in 75.4 ± 4.2 h at 4 °C and those acclimated at 4 °C and transferred at 8 °C took only 23.1 ± 1.8 h. The mussels acclimated to 8 °C and transferred to −1 °C required the longest time process the standard volume (113 ± 9.9 h).

In some cases, 36% of a batch was discarded after thermal shock. We discarded mussels if they were not filtering when the two measurements were taken (i.e., 2 and 72 h after the thermal shock). We discarded mussels that spawned in the metabolic chambers or those that did not meet the assumption of our theoretical model of (i) a filtration slowdown after transfer to a lower temperature and then acclimating or (ii) an acceleration of filtration after transfer to a higher temperature and then acclimating.

4. Discussion

4.1. Physiological responses to temperature

We observed that the mussels cultivated in suspension from the Gulf of St. Lawrence (specifically from the baie de Gaspé) that we used in these experiments tolerate temperatures down to −1 °C and still maintain a positive scope for growth in the presence of food. Using the scope for growth measurements, it would be possible to evaluate the global acclimation of the mussels to temperature variations after several weeks. Scope for growth evaluates the energetic bal-

ance of the mussels, integrating all processes related to energy gain and loss (Widdows and Johnson, 1988). In general, this index is considered to be highly sensitive to environmental changes and has a high degree of precision (Grant and Cranford, 1991; Tremblay et al., 1998b; Widdows et al., 2002). Our results are in accordance with several studies using temperatures near or under 0 °C, where no limiting effect on growth rate has been found at low temperatures during the spring phytoplankton bloom (Kautsky, 1982; Thompson, 1984; Smaal et al., 1997).

However, physiological measurements taken at −1 °C indicated that acclimation was not fully complete after 21 days in contrast to the general pattern of 14 days reported in the literature (Bayne, 1976). Scope for growth was significantly lower in these mussels than in those acclimated to 8 °C for the sampling periods; this effect appeared to be related to clearance rates. Clearance rates were significantly lower in mussels maintained at −1 °C compared to the other acclimation temperatures. In field studies on the east coast of Canada, Hatcher et al. (1997) observed that the metabolic rate of *M. edulis* during winter (characterized by ice cover) at temperatures less than 0 °C was generally lower than the metabolic rate in spring and autumn. In a study on the northeast coast of the USA, Lesser and Kruse (2004) observed a significant decrease in overall ATP demand (defined as a decreased whole-animal rate of respiration) in winter compared to summer in the mussel *Modiolus modiolus*. However, this decrease was less than 20%. In our laboratory experiments, we observed full acclimation of the routine metabolic rate after 21 days at −1 °C. The differences observed in the scope for growth were mainly related to differences in the clearance rate, which were only partially acclimated at −1 °C. Thus, the lower scope for growth observed at −1 °C is probably not related to metabolic depression associated with starvation in the field, as suggested by Hatcher et al. (1997), since mussels in our experimental conditions were continuously fed. Even when the scope for growth showed a significant decrease at −1 °C, the values remained largely positive, indicating that mussels had energy to invest in growth and reproduction, as has already been observed in mussels at this temperature by Kautsky (1982) in the Baltic Sea and by Thompson (1984) in Canada. Jorgensen et al. (1990) noted a lack of temperature acclimation for

filtration rates and, as suggested by Riisgård (2001), the inconsistent results between no acclimation and full acclimation in the *M. edulis* filtration rate remain to be explained. In our results, we observed a trend towards acclimation of filtration rate when water temperatures decrease to −1 °C, but acclimation was not complete even after 21 days.

Our results indicate that when temperature decreases towards −1 °C, mussel clearance rates decrease significantly and more time is required for a mussel to filter a standard volume of water. Our model estimated this time to be 90.7 h, which is much greater than the standard 48 h normally used in North American and UK depuration facilities (Furfari, 1966; Boulter and Wilson, 1998). These results may be explained by two hypotheses. Firstly, mussels did not undergo complete temperature acclimation at −1 °C. Secondly, mussels need more than 21 days to fully acclimate their clearance rate at this temperature. Jorgensen et al. (1990) and Jorgensen and Ockelmann (1991) have observed a lack of complete clearance rate acclimation in relation to temperature and related these observations to a change in the beat frequency of the water-pumping lateral cilia or to a change in water viscosity (viscosity affects the resistance of cilia to water flow). Other experiments in long-term acclimation are necessary to respond to this question, ideally studies that include field validation of the results.

Because the estimated clearance rate varies directly with the short-term variations in temperature (Jorgensen et al., 1990; Tremblay et al., 1998a), thermal shock has a significant impact on depuration potential. Boulter and Wilson (1998) demonstrated that physiological activity (oxygen consumption, ammonia excretion, and estimation of filtration by dye removal in seawater) increases with seawater temperature in the range of 5 to 20 °C. They concluded that the rate of the mollusc activity at 15 °C is at least double that at the minimum temperature of 5 °C currently specified in the UK for depuration system operation. In our laboratory study, we observed that the time required for a mussel to filter the standard volume was inversely related to temperature. Thus, when mussels are subjected to an increase in temperature, the amount of time to filter the standard volume is less than the 48 h. For example, in mussels transferred from 4 to 8 °C, the filtration time required to process the standard

volume is only 40.4% of the required time for the control mussels at 4 °C. The same relationship was observed with a decrease in temperature: a transfer to low temperatures increased the time required for mussels to filter the standard volume. Indeed, a transfer from 4 °C to −1 °C caused a 177% increase in the filtration time compared to the control at 4 °C for the theoretical complete depuration as determined by the mathematical model.

4.2. Use of an exponential model

It is accepted that metabolic recovery after a stress follows an asymptotic curve rather than a simple linear response (Hochachka and Guppy, 1987). From a biological perspective, the use of an exponential model, like the von Bertalanffy function, appears to be a more realistic approach to describe both the acceleration and deceleration in the clearance rate after a thermal shock. Indeed, the von Bertalanffy function has often been used to describe biological models and is one of the most frequently used relationships to describe growth functions (Allen, 1966; Ricker, 1975). This function has also been used to describe different metabolic responses, such as the photosynthetic recovery of lichens after wetting (Groulx and Lechowicz, 1987) as well as bioaccumulation experiments in mussels (Güngör et al., 2001).

Assumptions linked to the use of the theoretical models of metabolic acceleration–deceleration impose hypothetical constraints to give useful estimates. Thus, we discarded mussels that spawned (12% of the discarded mussels). No spawning events were observed in aquaria, but some mussels spawned in the metabolic chambers and thus altered the measurements of particle counts. The main reason for eliminating data in our model (over 20%) was a very weak or absent clearance rate at one of the observation times (2 or 72 h), which prevented us from estimating the effects of thermal shocks on clearance rates for these individuals. We observed that these cases were associated with mussels having slightly opened valves or a limited opening of the exhalant siphon aperture and slow beating of the lateral cilia. There is a positive relationship between mussel filtration rates, valve gape, and exhalant siphon area (Newell et al., 2001; Riisgård et al., 2003).

4.3. Recommendation for management

Our results demonstrated that, in the commercial operation of depuration facilities, it is necessary to consider the thermal variations undergone by mussels, particularly between sampling and depuration. Moreover, as acclimation to temperatures less than 0 °C appears to be difficult for mussels, the time required to depurate at low temperatures should be significantly greater. Since the process of commercial mollusc depuration greatly increases the cost of production (from 16% to 20%, estimated by Cerebral Marine Research, 1990), the choice of management strategy in relation to temperature variation is important. The results of this study indicate that the difference in temperature between the harvest and depuration sites can be used to predict the amount of time required for the depuration process. Bacterial analysis of mussels to validate the depuration process seems particularly important if mussels are submitted to temperatures near 0 °C or to thermal shock.

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