Modeling of blue mussel (*Mytilus* spp.) depuration potential in reaction to thermal shock

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ABSTRACT

In most habitats contaminated by coliform, a depuration regime lasting 48 h at a minimum temperature of 5°C is generally satisfactory for ensuring purification of most molluscs for commercial use. This regime, however, would be unsatisfactory in temperate-northern regions where temperature are frequently less than 5°C, although bivalves such as *Mytilus* spp. are able to maintain a relatively high rate of filtration at these low temperatures. To estimate depuration potential at low temperatures, we measured clearance rates and modelled the impact of thermal shock on *Mytilus* spp. in a laboratory analysis. Mussels were acclimated for four weeks at three different temperatures (8, 4 and -1°C). They were then subjected to thermal shocks for all thermal combinations including controls (when the temperature to which they were transferred was equal to that for which they had been acclimated). Clearance rates were measured on the same mussel after 2 h and 72 h of thermal shock. We observed a clearance rate of 2.45 l h^{-1} g^{-1} (gDW) for the 8°C control batch. Within a 48 h depuration period, *Mytilus* spp. could filter a standard volume of 117.47 litres within this period. We used both a piecewise regression model and 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 acclimatised at 8°C filtered the standard volume of 117.47 litres for an average of 75 h at 4°C, whereas those acclimatised at 4°C and transferred to 8°C required only 23 h on average.

Key words: Clearance rate, thermal cold acclimation, depuration, mussels, mytiliculture, *Mytilus* spp.

INTRODUCTION

In the process of food ingestion, bivalves accumulate pollutants such as fecal coliform (Trollope and Webber 1977; Plusquellec et al., 1990; Prieur et al., 1990). Blue mussels retain 100 % of particulate matter larger than 4 µm and up to 20 to 30 % of particulate matter smaller than 1 µm (see review of Hawkins and Bayne, 1992). Furthermore, the aggregation process of coliform, both with itself and with other particles, contributes towards the retention and assimilation of coliform by mussels (Bernard, 1989). Thus, pollutant concentrations in bivalves depend on the pollutant levels of their habitat. Despite attempts to decrease pollutant uptake in littoral inhabitants, the level of coliform concentration in seawater 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 for this problem is *depuration* which uses the large filtration capacity of molluscs, which are required for feeding and respiration (Furfari, 1966, Haven et al., 1978; Perkins et al., 1980). The depuration process is characterized by the holding of molluscs in tanks with non-polluted water (without bacteria) for a minimum period of time responding to satisfactory human health standard (Furfari, 1966; Bernard, 1989; Power and Collins, 1989). Generally, the time required to eliminate near 100% of coliform, *Escherichia coli*, is 48 h (Furfari, 1966; Trollope and Webber, 1977). As depuration efficiency is directly related to the filtration capacity of bivalves, it is necessary for depuration to occur in conditions that preserve the physiological state of bivalves to obtain good results. Thus, stress level suffered by bivalves should be minimal to allow optimal filtration activity. Power and Collins (1989) have observed that excretion and elimination of coliform *E. coli* in depuration conditions is dramatically decreased in period of stress.

Bivalves are ectotherm organisms and, thus, temperature is a major determinant of physiological status (Bayne et al., 1976). Depuration can occur throughout a large range of temperatures, but generally a temperature of 5°C is considered as a minimum threshold, below which physiological activity is strongly decreased (Furfari, 1966; Haven, 1978). Nevertheless, the local environment has an important and long-term effect on the physiological phenotype of bivalves (Widdows, 1978, Hatcher et al., 1997; Sukotin et al., 2003). Thus, bivalves from temperate-northern regions are adapted to low temperatures and have an ability to feed and filter at temperatures less than 5°C. Thermal acclimation and acclimatization of metabolism for maintenance, growth and

gametogenesis require temperature compensation in the enzymatic machinery to supply the carbon and ATP demand and have been well-described (Hochachka and Somero, 1984; Clarke, 1993). We considered thermal acclimation as physiological response observed in laboratory and acclimatization as physiological responses observed in field (following Blackstock, 1984; Lesser and Kruse, 2004). Smaal et al. (1997) have observed from monthly physiological measurements that mussels were generally acclimated to temperature variations between 0 and 20°C. Results obtained in Eastern Canada demonstrated that the metabolism of mussels is significantly lower in winter than in autumn and spring (Hatcher et al., 1997), but the authors suggested that this metabolism depression could be especially related to the nutritional stress observed in winter during ice cover. Finally, Thompson and Newell (1985) have demonstrated that the thermal sensitivity of metabolic rates of mussels differed between populations from different latitudes. Mussels from Newfoundland (Canada), where the maximum temperature is 17°C, catabolized more protein and showed severe depressions of the clearance rate at 25°C in comparison to mussels from Stony Brook (NY, USA) which are frequently exposed to this temperature. The thermal sensitivity of the metabolic rate of mussels from Magdalen Island differs somewhat from the pattern established for mussels from Newfoundland (similar latitude) and showed a higher thermal tolerance (Tremblay et al., 1998a). This response should be related to the adaptation of mussels from Magdalen Island to life in the semi-closed lagoons, where they are frequently exposed to temperatures greater than 20°C. All these results suggest that mussels from Nordic regions probably maintain their metabolic capacity to filter at temperature less than 5°C. Another important relationship with temperature is the thermal shock suffered by the bivalves between the harvest and the depuration area. In response to short–term thermal fluctuations within the range of tolerated temperatures, metabolic rates react rapidly. Thus metabolic rate increases with a short-term increase in temperature and conversely decreases with a short-term decrease of temperature (Widdows and Bayne, 1971; Tremblay et al., 1998a).

Our objective is to estimate the theoretical depuration potential of mussels in cold waters, in the context of different depuration and harvest sites location (possibility of thermal shock) by measuring, under laboratory conditions, (1) physiological acclimated capacity of mussels in low temperatures [with measure of absorption efficiency, O_2 consumption, clearance rate and scope for growth (Boulter and Wilson, 1998)], and (2) filtration capacity, estimated by clearance rate, following a thermal shock.. This evaluation was achieved, using a simple mathematical model, by

an evaluation of the theoretical filtration time required for a complete mussel's depuration. We hypothesized that the mussel's depuration capacity is proportional to the clearance rate measured by a volume of water cleared of suspended particles per unit of time.

Methods

Mussel acclimatization

The effect of temperature on mussel acclimation capacity was quantified by the scope of growth. The scope of growth is an integration of all 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 (cf. Bayne, 1976; Widdows and Jonhson, 1988) so may be used to evaluate mussel acclimation. Scope of growth (P) was determined by:

$$P = A - (R + U) \quad (1)$$

Where *A* is food assimilation, and *R* and *U* are respectively the energy loses by respiration and excretion. 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).

Mussels were sampled in May 2000 from a cultivated farm in Gaspé Bay (Québec, Eastern Canada) and were kept in seawater at 12°C (environmental temperature; > 90% oxygen saturation) before their transfer to 3 different temperatures (-1, 4 and 8°C) for 21 days of acclimation period in laboratory conditions. To obtain -1°C, we used bains-marie with glycerol in the cooling system. For each acclimated temperature treatment, we used 18 bottles of 5 litres in size, supplied with air in a double boiler, with each bottle containing 8 mussels. Mussels were randomly distributed between bottles and bottles were randomly distributed between treatments to assure the sample independence.

Mussels were fed continuously by 10 kcells ml^{-1} (a ration of 6-7% of their body mass daily) with a mixture of *Isochrysis galbana* and *Chaetoceros gracilis*. Because mussels had an abundant food supply, we assumed that the measured rates of oxygen uptake represented the routine metabolism (Thompson and Bayne, 1972). Physiological measurements were taken before

transfer (at 12°C, temperature of the sea at harvest time) and 1, 14 and 21 days after transfer to evaluate the progression of the thermal acclimation. For each date and temperature, the physiological features of 24 individuals were measured, using 3 randomly chosen bottles from the entire 18 bottles that were available. Mussels were left in the metabolic chambers of 500 ml for 1 hour before each physiological measure and empty shells were used as a blank. Using six metabolic chambers simultaneously, oxygen consumption and clearance rate were measured from five individual mussels and one blank. Animals that remained closed or spawned within the metabolic chamber were excluded from further analysis. After these measurements, the animals were returned to the aquarium tank 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 length and body mass (dry weight, DW) were determined.

Rates of oxygen consumption for individual mussels were determined by sealing the metabolic chamber and measuring the decrease in oxygen with an YSI (5331) polarographic analyser and electrode. The water in the metabolic chamber was mixed with a magnetic stirrer and the output signal monitored continuously on a chart recorder until a minimum decrease of O_2 of 20%. Oxygen concentration was not allowed to fall below 75% of saturation. The rate of oxygen consumption in ml O_2 h^{-1} was calculated as described by Widdows and Johnson (1988) and transformed to energy equivalents using the conversion factor 1 ml O_2 = 20.33 Joules. Clearance rates were measured using a static system, in which the rate of decrease in particle density (algal depletion) in the metabolic chamber was monitored (Jorgensen et al., 1990; Riisgård, 1991; Navarro et al., 2000). These measurements were carried out using a particle Beckman coulter-counter Z1, fitted with a 120-μm orifice tube. The experimental medium was maintained homogeneous by gentle aeration and the clearance rate was evaluated at 10 minute intervals for one hour. Following Gilek et al. (1992), the greatest difference between two consecutive measurements during this period was assumed to be the clearance rate. The clearance rate (l h^{-1}) was then used to estimate the amount of ingested or consumed energy with the multiplication of the amount of organic fraction of the diet assumed to have an energy content of 23 J mg^{-1}.

Assimilation represents the product of ingested energy and absorption efficiency (Widdows and Johnson, 1988). Absorption efficiency was measured by the Conover (1966) ratio that consists of the ratio of food dry weight and ash-free dry weight by faeces dry weight and ash-free dry

weight. Samples of diet mixture were collected during the experiments, as well as faeces in each container. Faeces from individual mussels were collected, making sure that no pseudofaeces were produced and mixed with faeces (Iglessias et al., 1998; Honkoop et al., 2003). Samples were filtered through pre-ashed, pre-weighed 47-mm glass fiber filters GFC, rinsed with isotonic ammonium formate (3.2%), dried at 80ºC for 48 h, cooled to room temperature in a desiccator, weighed, combusted at 450ºC for overnight, cooled to room temperature in a desiccator and finally weighed to estimate the organic and inorganic fraction contained in the food and faeces. The scope for growth integrates all physiological variables as previously indicated.

The body mass (gDW) of each mussel was determined to balance the physiological rates for an individual standard mass of 1 g using an allometric equation according to the formula given by Bayne (1976). In order to determine this equation for the mussel stock used, physiological measurements were taken, at the same temperature as the sampled seawater (12°C), on 90 mussels between 15.2 and 72.7 mm in length one week after they were harvested.

At the end of the acclimation period (21 days), mussels from each temperature (-1, 4 and 8°C) were reciprocally transferred to all the thermal conditions. Experimental controls represented mussels that were transferred to the same temperature (i.e. 8 to 8ºC; 4 to 4ºC and -1 to -1ºC). From each transfer treatment, clearance rates (n = 16) were measured, as previously described, on the same mussel at 2 and 72 hours after the thermal shock. The clearance rate was used for estimation of the volume of water filter by a mussel per unit of time, and thus for the evaluation of the theoretical potential of depuration capacity of mussels in experimental condition of long and short term thermal variation.

Rationale for the model

The time required to complete a theoretical depuration was estimated by a mathematical model. This required time was calculated with a target volume, which was assumed as the required volume to a completed purification. We postulated that the clearance rate of a control mussel, acclimated and transferred at high temperature (8°C), which has experienced the same handling procedure as the other thermal shock combinations, provided a best clearance target volume representing a complete purification after 48 hours (as suggested by Furfari 1966). This target volume was estimated and used, as a basis to determine how long the mussels from all treatments will 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 rate at 2 and 72 hours after a 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:

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

where

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

In this expression, C_2 and C_{72} stand for the clearance rates recorded respectively after 2 and 72 hours following 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 filtered the target volume is thus the time *t* such that *V(t)* = target, which was solved numerically.

According to our model, assumptions to use data have been stated in relation to the type of transfer. We discarded observations if they did not agree to the assumptions of our theoretical model of (i) a deceleration in clearance rates in response to a transfer to a higher temperature or (ii) acceleration after transfer to a lower temperature. These assumptions were critical for the application of our model. Therefore, for a transfer to a higher temperature, the clearance rate

must be greater 2 h after transfer than 72 h, and the rate at 72 h must be greater 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 must be less at 2 h after transfer than at 72 h, and the 72 h value must be less or equal to the clearance rate of the control temperature to which the mussels had been transferred. We discarded mussels if they were not filtering at both measured 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. Program in SAS® software language (SAS 1999) was used and is available on request from the authors.

Data analysis

Simple regression analyses were used to examine the relations between dry weights and oxygen uptake, clearance rate, length ($\log_{10}$), and absorption efficiency. For the first two variables, a square root transformation was necessary in order to answer the basic postulates of data normality and variances homogeneity.

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

Repeated measures ANOVAs were used to test the effect of the factors of acclimation temperature and acclimation date on absorption efficiency rate, oxygen uptake, clearance rate and scope for growth. A square (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, variances homogeneity) were respected, after suggested transformations by a Box-Cox test, in the majority of the analyses. However, the variances were not perfectly homogeneous in certain cases. In addition, ANOVA is relatively robust to the 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 variance homogeneity was examined by visual examination of the residuals (Montgomery, 1991). A significance threshold of 0.05 was adopted for all statistical tests. When

a source of variation was significant, *a posteriori* multiple comparisons (LSmeans, SAS 1999) were carried out with the Bonferonni-adjusted threshold to identify the differences.

Results

Allometric relations

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

Acclimation

The results were based on a total of 269 mussels with length from 51.2 to 70.9 mm. For each temperature treatment, means body mass (gDW) and lengths were similar (ANOVAs; $F_{2, 266} = 0.98$; $p = 0.3768$; $F_{2, 258} = 0.81$; $p = 0.4446$ respectively). The acclimation conditions (-1, 4 and 8°C) influenced significantly values of the absorption efficiency rate, 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 (see Fig. 1b, d, f). In most cases, there was a significant decrease of all physiological measures one day after the beginning of the acclimation (cf. Fig 1a, c, e; Table 1). This was followed by a recovery of the absorption efficiency, oxygen consumption and the scope for growth potential after 14 days, and a recovery of the clearance rate after 21 days.

There was a significant interaction between the temperature of acclimation and time following the transfer for the oxygen consumption (cf. Fig. 2; Table 1). Oxygen consumption was generally low 1 day after the beginning of the acclimation and the recovery took more time (21 days) for the mussels transferred to 4°C, whereas 14 days are enough for other temperatures. For each treatment of initial temperature, the mussels had values slightly different in mean body mass (-1°C: 0.49; 4°C: 0.56, and 8°C g dry weight: 0.53; ANOVA; $F_{2, 281} = 2.84$; $p = 0.0025$) and mean

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 such difference in weight and length, ranges were small and should not have a great influence on specific clearance rate.

Thermal shock

A positive thermal shock (transfer to a higher temperature) caused an increase in the clearance rate followed by a deceleration, and a negative thermal shock (transfer to a lower temperature) caused a decrease in the clearance rate followed by an acceleration (Fig. 3). There was no difference in clearance rate between measurements at 2 and 72 h for -1, 4 and 8°C controls (LSmeans comparisons; respectively $p = 0{,}6529$; $p = 0{,}5483$ and $p = 0{,}9861$). However, mussels of the control at -1°C showed a significant lower clearance rate than at the two other temperatures (LSmeans; $p < 0{,}0001$). For all other treatments, the transfer to different temperatures involved a significant change of the clearance rates after 2 hours and a trend to re-establishment of the initial rate after 72 h of transfer (rate at initial temperature; cf. Fig. 3).

Simulation

The amount of filtered and cleared water volume was estimated with the assumption that the clearance rate at the 8 to 8°C control transfer (2.4474 l h^{-1} g^{-1}) could process 117.47 litres 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 and is used in the model in order to consider the time necessary for a complete purification. Clearance rates of 1.5706, 2.0806, and 2.2274 (l h^{-1} g^{-1}) were observed respectively for controls -1, 4 and 8°C and were used as the parameter *a* in the equation 2 and 3.

Simulated filtration times of the target volume are presented in Figure 4. We observed that control mussels (transfer to the same temperature) at -1°C needed significant more time (90.7 ±9.7 h) to filter the standard volume of 117.47 litres than controls at 4 and 8°C (LSmeans; $p < 0.0001$). Nevertheless, there was no difference in the filtration time between controls at 4 and 8°C (LSmeans; $p = 0.0947$ with respectively 57.0 ±1.6 h and 48.7 ±1.6 h). Thermal shocks (transfer to a different temperature) had an important effect on the time a mussel required to filter the standard volume. For example, mussels acclimatised at 8°C filtered the standard volume of 117.47 l in 75.4 ±4.2 h at 4°C and those acclimatised at 4°C and transferred at 8°C took only 23.1 ±1.8 h. The longest filtration time to process the standard volume (over 113 ±9.9 h) was

represented by the mussels acclimatized to 8°C and transferred to -1°C.

Discussions

Physiological responses to temperature

With the use of scope for growth measures it would be possible to evaluate global acclimation of the mussels to temperature variations after several weeks. Scope for growth evaluates the energetic balance of the mussels represented by the integration of all processes related to gain and loss of energy (Widdows and Bayne, 1971). Thus, it would be possible to determinate the quantity of energy available for the growth and reproduction of the individual (Widdows and Johnson, 1988). Generally, this index is considered highly sensitive to environmental changes and has a high degree of precision (Grant and Cranford, 1991, Tremblay et al., 1998b; Widdows et al., 2002). We observed that the mussels cultivated in suspension from Saint-Lawrence Gulf (specifically from Gaspé Bay, 48:50N; 64:27W) used in these experiments, support temperatures encountered in their environment until -1^{o}C and maintain positive scope for growth in the presence of nutritive input. These results are in accordance with several studies characterized by winter temperature near or under 0^{o}C (Kautsky, 1982; Jorgensen et al. 1990, Small et al. 1997, Lesser and Kruse, 2004). As demonstrated for mussels from other areas, a period of 14 to 21 days is generally sufficient for mussels to fully acclimate to temperature changes (Bayne, 1976). Thus, our results suggest that for mussels maintained at temperatures between 4 and 8°C the time required to filter the standard volume of water, calculated to theoretically eliminate the presence of coliform in depuration conditions, should be the same.

However, physiological measures occurring at winter temperature of -1°C indicated that acclimation was not fully complete after 21 days. Scope for growth was significantly lower than acclimation to 8°C for all periods of time and was particularly related to clearance rate. Clearance rates were significantly less than all other acclimation temperatures and for all periods. 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 less than the metabolic rate in spring and autumn. 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

mussels, *Modiolus modiolus.* However this decrease was less than 20%. In our results, we observed a full acclimation of the metabolic routine rate for temperature of -1°C compared to 4 and 8°C after 21 days. The differences observed in the scope for growth were mainly related to differences in the clearance rate, partially acclimated to a temperature of -1°C. Furthermore, a lower scope for growth observed at -1°C would not be related to metabolic depression associated with starvation in the field as suggested by Hatcher et al. (1997), as mussels in our experimental conditions were continuously fed. Even when 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 already observed by Loo (1992) on mussels at this temperature. Jorgensen (1990) has noted a lack of temperature acclimation on filtration rates and as suggested by Riisgård (2001) the inconsistent results between no and full temperature acclimation in the filtration rate in *M. edulis* remain to be explained.

Thus, when temperature decreased towards -1°C in winter conditions, the clearance rates of mussels decreased significantly, and significantly more time was required for a mussel to filter the standard volume of water and eliminate the presence of coliform by the natural processes of ventilation, defecation and extracellular digestion (Bernard, 1989; Power and Collins, 1989). Our model estimated this time at 90.7 hours. This is much greater than the standard of 48 hours normally used in North American depuration facilities (Furfari, 1966; Haven, 1978). However, as we stopped the experiment after 21 days, acclimation to temperature under 0°C should be established after more time.

Because the estimated clearance rate varied directly with short-term variation of temperature (Widdows and Bayne, 1971; Bayne, 1976; Jorgensen et al. 1990; Tremblay et al., 1998a), thermal shock had a significant impact on depuration potential (Boulter and Wilson, 1998). We observed that the time a mussel required 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 hours that is usually required. For example, the filtration time required to process the standard volume from mussels transferred from 4 to 8°C represents only 40.4% of the required time for the control mussels at 4°C. The same relation was also observed with a decrease in temperature. Thus, a transfer to low temperatures increased the time required for mussels to filter the standard volume. Indeed, a transfer of mussel from 4°C to -1°C caused an increase of 177% in the filtration time compared to control at 4°C for a theoretical complete

depuration evaluated by the mathematical model.

Use of an exponential model

It has been accepted that metabolic recovery after a stress follows an asymptotic curve rather than a simple linear response (Hochachka and Guppy, 1987). The use of an exponential model, like the von Bertalanffy function, appears to be more realistic approach, from a biological perspective, to describe both 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 been also used to describe different metabolic responses such as a photosynthetic recovery of lichen 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. Indeed, in some cases after thermal shock, 36% of a batch was discarded due to a non-response or the non-correspondence to the theoretical scheme after a stress. We discarded mussels if they were not filtering at the times of the two measurements (i.e. 2 and 72 hours) after the thermal shock, and discarded those that did not agree with our theoretical model of (i) filtration slowdown after transfer to a lower temperature and then acclimating or (ii) acceleration of filtration after transfer to a higher temperature and then acclimating. This strategy of discarding mussels appeared to be justified because nearly identical results of simulated clearance time were observed if all mussels had been kept. However, we propose that the calculation of an individual clearance time ensured the sample independence and allowed better comparison among treatments. We observed that the non-correspondences to our criteria were associated with irregular comportment of mussels such as having slightly open valves, or a limited opening of the exhalant siphon aperture and beating of the lateral cilia, or a displacement of mussel by foot movement and/or production of byssal threat. There is a positive relationship between filtration rates of mussels, valve gape and the exhalant siphon area (Famme et al., 1986; Jorgensen et al., 1988; Jorgensen, 1990; Newell et al., 2001; Rissgård et al. 2003).

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 acclimatization to temperatures less than 0°C appears to be difficult for mussels, the time required to depurate mussels at this temperature would be significantly greater. As the process of commercial mollusc depuration greatly increases the cost of production (until 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 paper show that the difference in temperature between the harvest and depuration sites may be used in the prediction of the amount of time required by the depuration process.

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FIGURE LEGENDS

Fig. 1. Values of 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, 1, 14 and 21 days) (a, c, e) and acclimation temperature (-1, 4 and 8°C) (b, d, e). Distinct letters indicate statistically different values. Errors bars are SE.

Fig. 2. Values of oxygen consumption (ml h^{-1} g^{-1}Dry weight) according to the interaction acclimation time (before, 1, 14 and 21 days) and acclimation temperature (-1, 4 and 8°C). Distinct letters indicate statistically different values. Errors bars are SE.

Fig. 3. Clearance rates after 2 and 72 h following thermal shock in relation to temperature of acclimation and transfer temperature. Errors bars are SE.

Fig. 4. Simulation of required time (in hours) to filter the target volume (117.47 litres) for each treatment combination of acclimated (initial) and transferred (final) temperature. Bars having different letters above them differ significantly. Number of data included in each simulation is shown above bars. Errors bars are SE.

Figure 1

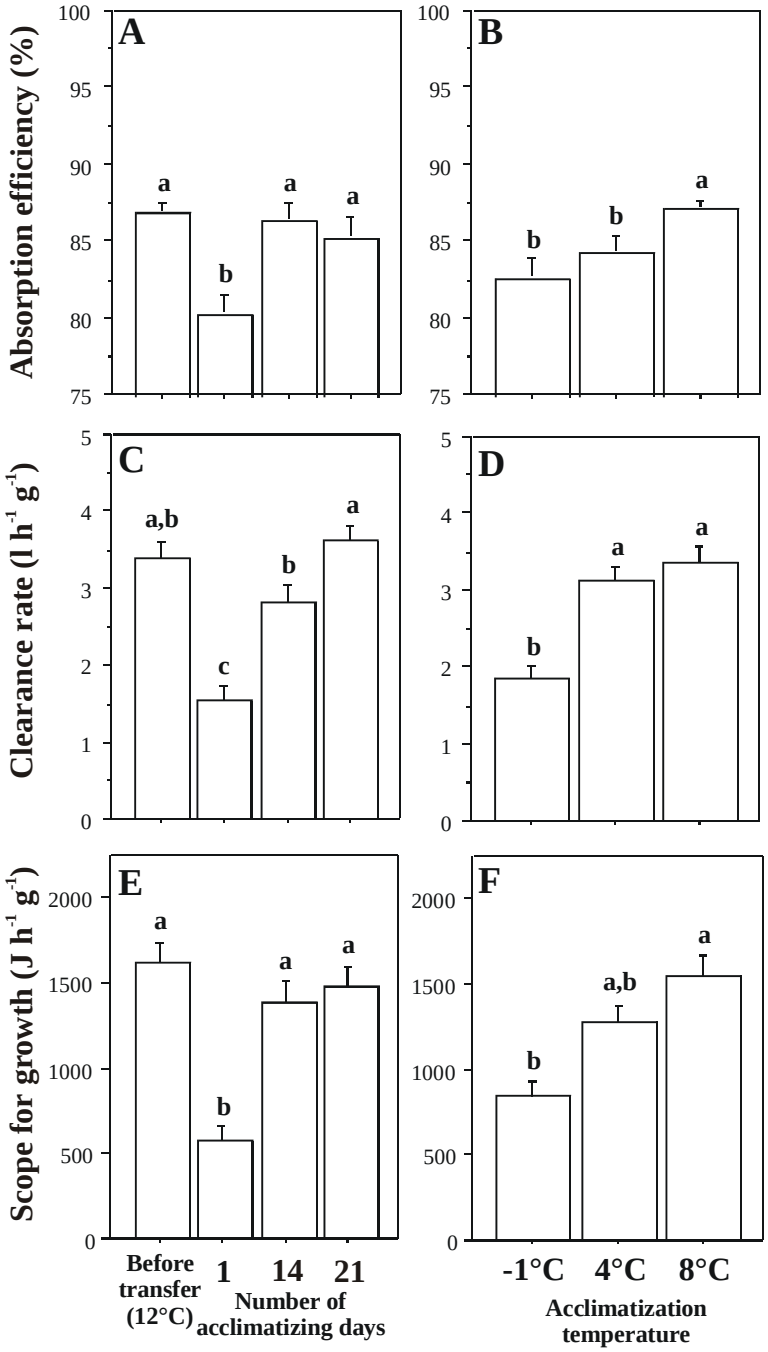
A
B
C
D
E
F
Absorption efficiency (%)
Clearance rate (l h-1 g-1)
Scope for growth (J h-1 g-1)
100
95
90
85
80
75
5
4
3
2
1
0
2000
1500
1000
500
a
b
a
a
a
b
b
a
a,b
c
b
a
b
a
a
b
a
a
b
a,b
a
Before transfer (12°C)
1
14
21
Number of acclimatizing days
-1°C
4°C
8°C
Acclimatization temperature

Figure 2

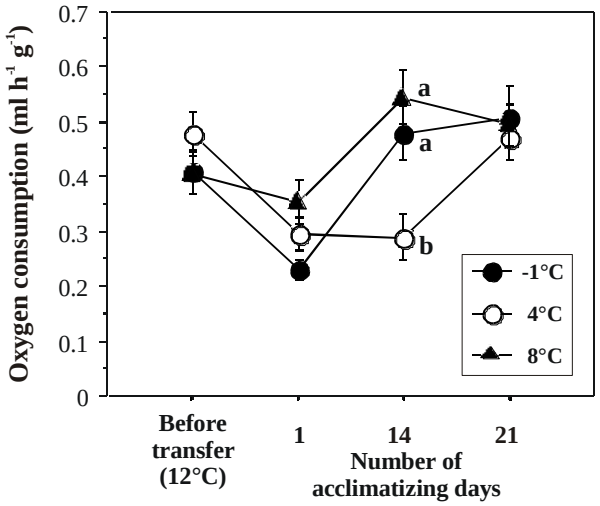

Figure 3

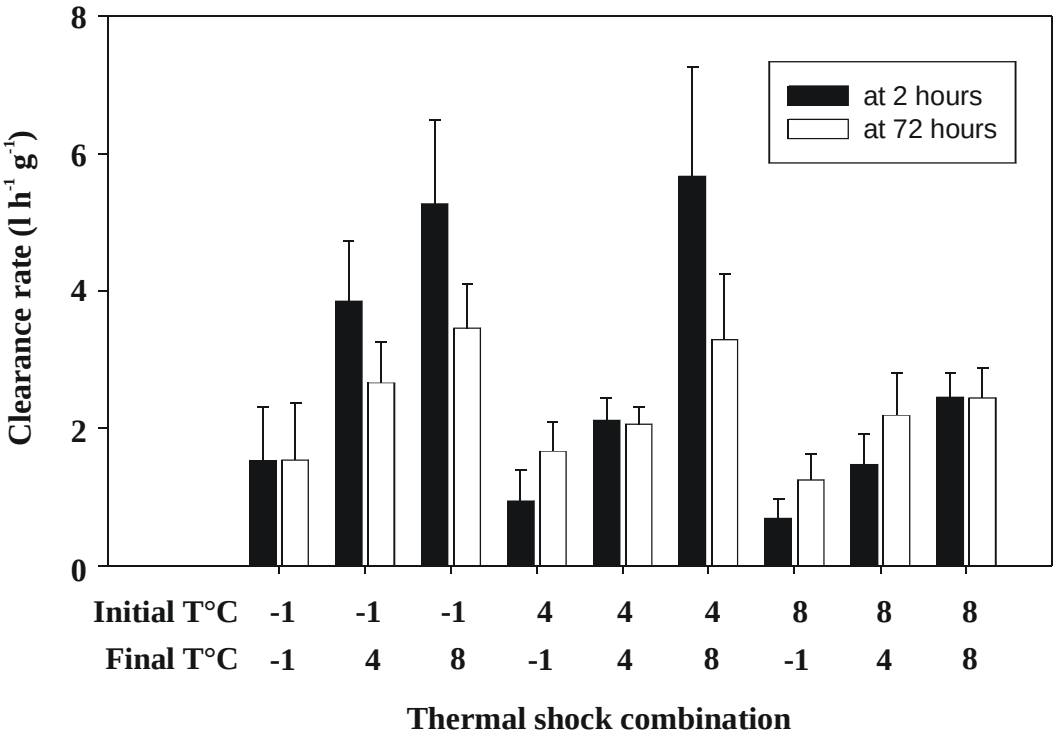

Figure 4

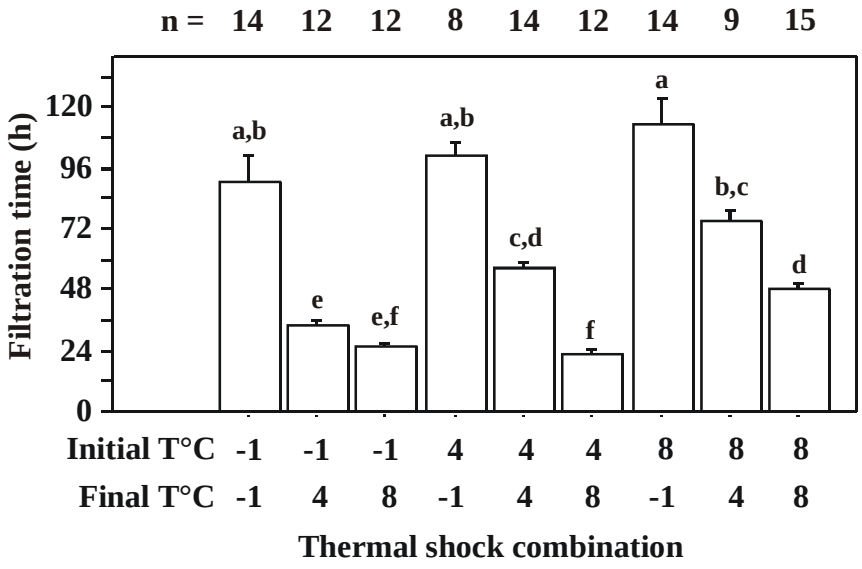
n = 14 12 12 8 14 12 14 9 15
Filtration time (h)
120
96
72
48
24
0
a,b
e
e,f
a,b
c,d
f
a
b,c
d
Initial T°C -1 -1 -1 4 4 4 8 8 8
Final T°C -1 4 8 -1 4 8 -1 4 8
Thermal shock combination

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. 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.

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			