Effect of temperature on filtration rate in the mussel *Mytilus edulis*: no evidence for temperature compensation

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ABSTRACT: The filtration rate of *Mytilus edulis* as a function of acute change in temperature was measured by means of the clearance method in a group of mussels seasonally acclimated to 18°C. This was done by stepwise changes in temperature in order to both determine the temperature-tolerance interval within which the mussels were fully open, and to ensure that the acute effects were reversible. The filtration rate (F, ml min^{-1} ind.$^{-1}$) as a function of temperature (T, °C) could be expressed by a regression line with the equation: $F = 3.27\,T + 38.2$ in the temperature-tolerance interval between 8.3 and 20°C. A reduction in temperature to below 8.3°C initiated valve closure, and at 6.1°C all mussels were completely closed. The same group of mussels was then acclimated to 11°C over a period of 5 d before the measurements were repeated, and the filtration rate as a function of temperature was subsequently found to be: $F = 3.27\,T + 38.1$ in the temperature-tolerance interval which had extended down to 4.1°C. Next, a group of mussels seasonally acclimated to about 15°C was split up into 3 subgroups which were exposed to 10.2, 15.6 and 20.3°C over the following 23 d. During the acclimation period, the filtration rate of fully open mussels was measured every 3 to 4 d in the 3 groups. Because none of the slopes of the 3 regression lines appreciably differed from 0, it is concluded that the acute effect of a change in temperature is not modified in *M. edulis* over a 3 wk acclimation period; i.e., there is no evidence for temperature compensation.

KEY WORDS: Clearance rate · Temperature tolerance · Acclimation · Adaptation · Feeding behaviour

INTRODUCTION

There is extensive literature dealing with the effect of temperature on filtration rate in suspension-feeding bivalves (e.g. Widdows & Bayne 1971, Widdows 1976, 1978, Winter 1978, Brock & Kofoed 1987, Jørgensen et al. 1990, Bougrier et al. 1995, Lei et al. 1996, Haure et al. 1998, Petersen et al. 2003, Sylvester et al. 2005), but the subject is controversial due to often inconsistent results (Jørgensen 1990, Bayne 2001, Riisgård 2001a,b, Riisgård & Seerup 2003). However, when dealing with effects of temperature on the filtration rate of suspension-feeding bivalves, it is necessary to distinguish between the effect of temperature on the maximum filtration rate measured on wide open bivalves under

optimal conditions, and the effect of high/low temperature on the valve-opening state (tolerance) (Riisgård et al. 2003). When exposed to changes in temperature, mussels may attempt to maintain the filtration rate to minimize the impact of the temperature change. The process of becoming adjusted to or tolerant of a new temperature is called acclimatization. Further, when mussels acclimate to changes in temperatures under laboratory conditions and if complete acclimation occurs, this is called thermal compensation which implies that the filtration rate is maintained as the temperature changes.

Jørgensen et al. (1990) studied the acute effect of temperature on filtration rate capacity in *Mytilus edulis* within the temperature-tolerance interval, and the

results indicated a lack of temperature acclimation. In a recent study conducted by Riisgård & Seerup (2003) on the soft clam *Mya arenaria*, it was found that only minor, or possibly no, temperature acclimation takes place in soft clams. Likewise, the results of Petersen et al. (2003) indicate a lack of temperature compensation in filtration rate in Arctic and temperate specimens of the clam *Hiatella arctica*. However, the above findings are inconsistent with Hawkins & Bayne (1992) who suggested that *M. edulis* achieves at least partial temperature independence by effecting complete or near complete acclimation of filtration rate over a 2 to 3 wk period following transfer to constant temperatures between 5 and 20°C. Further, Hawkins & Bayne (1992) suggested that the biochemical basis of temperature compensation in mussels involves acclimation of the rate with which vesicles containing serotonin are transported along axons to the nerve terminal. Bayne (1993) stated that the model for feeding behaviour proposed by Jørgensen et al. (1990) fails to explain 'many recorded features of feeding behaviour, such as temperature acclimation of filtration rate', and further, Bayne (1998) argued that the '*a priori* expectation' is that 'flexibility' will lead to physiological compensation in suspension-feeding bivalves. However, most of the experimental data that support temperature compensation in bivalves have only been published in a few rather old papers. Thus, Widdows & Bayne (1971) reported that complete acclimation of the filtration rate occurs within 2 wk in *M. edulis.* Widdows (1976) suggested that *M. edulis* has compensatory mechanisms that allow the mussels to acclimate the filtration rate to both cyclic and constant temperatures, and further, Widdows (1978) suggested that the feeding activity of *M. edulis* is maintained at a relatively constant level throughout the year as a result of the acclimation of filtration rate to temperature.

The inconsistent results between no and full temperature acclimation in filtration rate call for a more rigorous experimental design and precise filtration rate measurements. The aim of the present work was to further study the effects of temperature on filtration rate in *Mytilus edulis*, with special emphasis on possible temperature compensation.

MATERIALS AND METHODS

Specimen collection. Blue mussels *Mytilus edulis* were collected in the southeastern part of Kerteminde Fjord (Funen, Denmark) in August 2002 and June 2003 (ca. 20‰) and brought to the

nearby Marine Research Centre where all the experiments were conducted.

Filtration rate measurements. The filtration rate was measured as the volume of water cleared of suspended algal cells *Rhodomonas* sp. (almost spherical, about 6.3 μm in diameter) per unit time using the clearance method (Riisgård 2001b). Algal cells were added to a well-mixed, strongly aerated aquarium with a known volume of Whatman GF/C-filtered (1.2 μm) seawater (V) and a group of mussels. The reduction in the number of algal cells was followed as a function of time by taking samples (15 ml) every 10 min and measuring the algal concentration with an electronic particle counter (Elzone 5380). After measuring, the remaining water (ca. 13 ml) was immediately returned to the experimental aquarium to ensure a negligible reduction in the total water volume. The individual clearance rate (Cl) was determined from the exponential decrease in algal concentration (verified as a straight line in a semi-log plot) as a function of time using the formula (Riisgård & Seerup 2003): $Cl = aV/n$, where V = volume of water, n = number of filtering mussels, and a = slope of the regression line in a semi-ln plot of the reduction in algal concentration with time in the aquarium with mussels. Repeated measurements of clearance rate over time were performed by adding algal suspension to re-establish the initial algal concentration in the aquarium; see example of experimental procedure in Fig. 1 and Table 1. Control experiments without mussels showed that no significant sedimentation of algal cells took place. Since the suspended algal cells are 100% efficiently retained by the gills of *Mytilus edulis* (Møhlenberg & Riisgård 1978), this implies that Cl = filtration rate (F) (filtration rate = pumping rate). All experiments were conducted at naturally low algal concentrations (i.e. 1000 to 4000 *Rhodomonas* sp. cells ml^{-1}) equivalent to about 1 to 5 μg chl a l^{-1} (Clausen & Riisgård 1996).
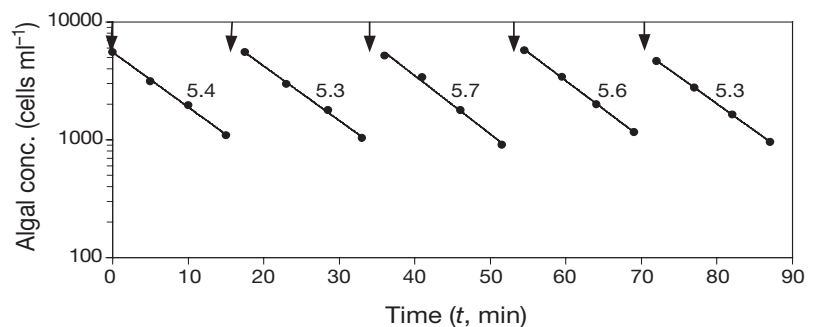

Fig. 1. *Mytilus edulis.* Exponential decrease in algal cell concentration as a function of time (t, min) due to fully open and actively filtering mussels (Group 7, Table 2) during a clearance experiment on Day 1 at 20.3°C in an aquarium with well-mixed seawater. Regression lines and estimated filtration rates (l h^{-1} ind.$^{-1}$) are shown. Arrows indicate addition of algal cells. The mean individual filtration rate ± SD is shown in Table 1 and Fig. 3A

Table 1. *Mytilus edulis*. Example of data obtained in a filtration rate experiment performed on Day 1 at 20.3°C (see Fig. 1). Volume of water in experimental aquarium = 5.0 l; no. of actively filtering mussels = 6; no. of samples taken during each clearance experiment = 4; C_0 and C_t: algal (*Rhodomonas* sp.) concentration at time 0 and *t*, respectively; *a*: slope of regression line for reduction of algal concentration with time in semi-ln plot; r^2: determination coefficient; *F*: estimated filtration rate. The mean ± SD value of *F* is shown in Fig. 3A

t (min)	C_0 (cells ml^{-1})	C_t (cells ml^{-1})	*a* (min^{-1})	r^2	*F* (l h^{-1} ind.$^{-1}$)
15.0	5572	1092	−0.1072	0.9984	5.36
15.5	5552	1036	−0.1066	0.9979	5.33
15.5	5192	908	−0.1142	0.9933	5.71
14.5	5764	1160	−0.1114	0.9993	5.57
15.0	4672	960	−0.1055	1	5.28
					Mean: 5.45 ± 0.18

Acute (hours) effects of temperature. *Mytilus edulis* (length = 5.054 ± 0.055 cm, n = 5) collected in the field (18°C) were placed in a strongly aerated aquarium with temperature-controlled (18°C; Hetofrig cooler/ heater) seawater and subsequently fed algal culture (1000 to 5000 *Rhodomonas* cells ml^{-1}) for 5 h. The next day, the mussels were stimulated to maximal valve opening by adding algal suspension to the aquarium. The water temperature was reduced in a stepwise manner from 18 to 6.1°C with intervals of 2°C. At each temperature step, the filtration rate was measured and the valve gape registered. Subsequently, the temperature was gradually elevated with intervals of 2 to 20°C and measurements of the filtration rate were performed at each temperature. During the period of temperature adjustment (ca. 1 h), the mussels were fed with algal culture to keep the mussels maximally open. Using the same mussels, a second set of experiments was performed, where the mussels were acclimated to 11°C for 5 d and fed algal cells 5 h d^{-1}. The water temperature was elevated in a stepwise manner from 11 to 20.4°C with intervals of 2°C, then likewise reduced to 4.1°C and finally, the temperature was gradually elevated to 11°C with 2°C steps. At each temperature, the filtration rate was measured and the valve gape registered.

Short-term (days) effects of temperature. Three groups of *Mytilus edulis* of the same mean size (Groups 5, 6, 7; Table 2) that had been naturally acclimated to 15°C were placed in 3 aerated aquaria with 5 l of filtered seawater (Whatman GF/C-filter, 1.2 µm). The temperature of the water in the 3 aquaria was adjusted and con-

trolled (using a Hetofrig heater/cooler systems) over time to 10.3, 15.6 and 20.3°C, respectively. The mussels were fed for 8 h with algal culture (1000 to 5000 *Rhodomonas* sp. cells ml^{-1}). The next day (Day 1), the mussels were stimulated to maximally open their valves by addition of algal cells so that the filtration rate capacity could be determined. Over the following 22 d, the mussels were kept at constant temperature and fed for 8 h d^{-1} (about 3000 *Rhodomonas* cells ml^{-1}), and approximately every third day the filtration rate was measured before the water was changed to maintain high water quality.

Shell length and flesh dry weight (24 h at 110°C) were determined for all mussels used in the filtration experiments.

RESULTS

Acute effects of temperature

Mussels acclimated to 18°C were completely open and thus filtered with a maximum rate in the temperature interval between 8.3 and 20°C (Fig. 2A), hereafter referred to as the temperature-tolerance interval. Lower temperatures initiated closure of valves and the mussels were completely closed below 6°C. Within the temperature-tolerance interval, the mean individual filtration rate (*F*, ml min^{-1} ind.$^{-1}$) could be expressed as: $F = 3.27T + 38.2$. After 5 d of acclimation to 11°C, the mean individual filtration rate as a function of temperature could be expressed by the equation: $F = 3.27T + 38.1$ (Fig. 2B). In the lower part of the temperature interval, the cold-adapted (11°C) mussels tolerated temperatures down to at least 4.1°C without closing their valves. This indicates that the temperature-tolerance interval, within which the valves remain fully open and the maximum filtration rate of *Mytilus edulis* is realized, can be displaced depending on the length of the acclimation period to low temperatures. The temperature-dependent filtration rates of mussels acclimated to 18 and 11°C were nearly identical in the temperature-tolerance interval, and the acute temper-

Table 2. *Mytilus edulis*. Data for groups of mussels used in filtration-rate acclimation experiments (Fig. 3). T_E: experimental temperature; L_S and L_E: mean ± SD shell length at start and end of experiments, respectively; W_w and W_d: mean ± SD wet- and dry weight, respectively at the end of the experimental period

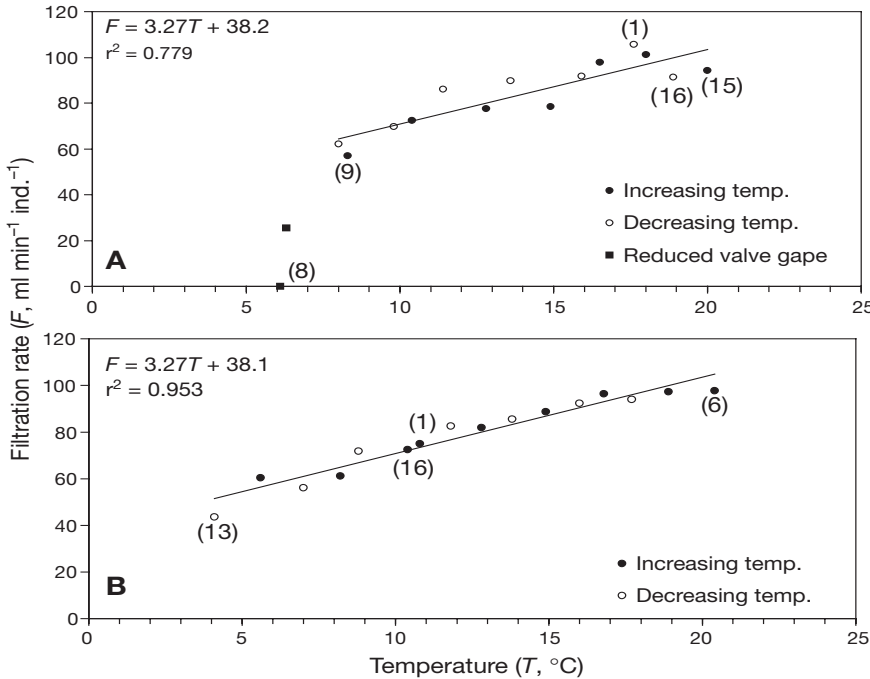| Group	n	T_E (°C)	L_S (cm)	L_E (cm)	W_w (g)	W_d (g)
5	6	10.3	4.398 ± 0.048	4.408 ± 0.101	4.019 ± 0.519	0.426 ± 0.142
6	6	15.6	4.398 ± 0.052	4.435 ± 0.080	4.209 ± 0.639	0.439 ± 0.150
7	6	20.3	4.389 ± 0.048	4.390 ± 0.035	4.106 ± 0.702	0.424 ± 0.134

Fig. 2. *Mytilus edulis*. Mean individual filtration rate of mussels (length = 5.054 ± 0.055 cm, n = 5) as a function of acute stepwise increasing and decreasing temperature as indicated by the inserted numbers starting at (1). (A) Mussels seasonally acclimated to 18°C. (B) The same group of mussels after 1 wk of acclimation to 11°C. The regression lines for filtration rate as a function of temperature in the temperature-tolerance interval and their equations are shown
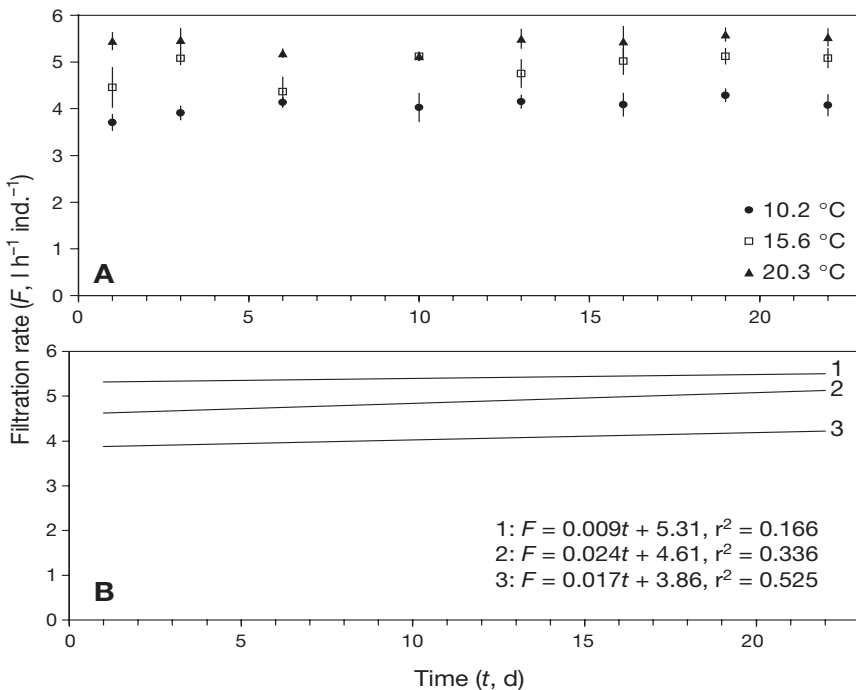

Fig. 3. *Mytilus edulis*. (A) Mean individual filtration rate of 3 groups of mussels (Groups 5, 6, 7; Table 2) exposed to 10.3, 15.6 and 20.3°C, respectively, over a period of 23 d. Each data point represents the mean ± SD of 5 measurements. (B) Mean individual filtration rate (F, l h^{-1} ind.$^{-1}$) as a function of time (t, d) shown by means of regression lines based on the data shown in (A)

ature effects were reversible in the tolerance interval since the filtration rates measured during stepwise temperature reduction equalled those of stepwise temperature elevation.

Short-term effects of temperature

Fig. 3 shows the mean individual filtration rates of 3 groups (Groups 5, 6, 7) of *Mytilus edulis* kept at 3 different temperatures (10.3, 15.6 and 20.3°C) as a function of time (days of acclimation). No significant differences in shell length were observed between the groups at the start or the end of the experiment, nor were there any changes in shell length during the acclimation period (2-way ANOVA, p > 0.45; Table 2). No differences in either wet weight (1-way ANOVA, p > 0.87) or dry weight (1-way ANOVA, p > 0.98) were found among the groups at the end of the experiment (Table 2). Over the 23 d temperature acclimation period, the filtration rate of each group was nearly constant. According to the temperature compensation theory, regression lines 1 and 3 in Fig. 3B should have decreased and increased, respectively, while regression line 2 should have remained constant (slope = 0). In practice, none of the slopes of the 3 regression lines appreciably differed from 0. This indicates that the acute effect of a change in temperature was not modified in *M. edulis* during the 3 wk acclimation period; i.e. there was no evidence for temperature compensation.

DISCUSSION

When evaluating the effects of temperature on the filtration rate, bivalves in similar opening states should be compared. This requirement was fulfilled by Jørgensen et al. (1990) and by Riisgård & Seerup (2003), who studied the effects of temperature on filtration rate of mussels *Mytilus edulis* and soft clams *Mya arenaria*. In the temperature-tolerance interval, within which

the bivalves remain fully open, the relationship between temperature and maximum filtration rate was found to be linear, as also observed in the present work (Fig. 2). Further, the above studies, along with the present work (Fig. 3), indicate a general lack of temperature acclimation in bivalves.

In a recent study, Petersen et al. (2003) measured the filtration rate capacity in Arctic clams *Hiatella arctica* and *Mya* sp. In both species, filtration rates were found to increase linearly with temperature in the temperature-tolerance interval between –1.3 and 4°C. Filtration rates were also determined in temperate (Swedish) specimens of *H. arctica* and these specimens had a temperature-tolerance interval between 0 and 20°C. The results also suggest a lack of temperature compensation in bivalves because the filtration rates measured at 1°C were not significantly different in clams from the 2 collection sites.

The lack of temperature acclimation in bivalves reported here and previously suggested by Jørgensen et al. (1990), Riisgård & Seerup and Petersen et al. (2003) is in conflict with Widdows & Bayne (1971) and Widdows (1976, 1978). Widdows & Bayne (1971) suggested that complete acclimation of the filtration rate occurs within 14 d in *Mytilus edulis*. Further, Widdows (1976) reported that *M. edulis* possesses compensatory mechanisms that enable the mussels to acclimate their filtration rates to cyclic as well as to constant temperatures. According to Widdows, the filtration rate completely acclimates and becomes totally independent of temperature change, and 'this process of thermal compensation ... takes place over a period of approximately 14 days'. In a later work, Widdows (1978) suggested that the feeding activity of *M. edulis* can be maintained at a relatively constant level throughout the year as a result of acclimation of the filtration rate to temperatures between 5 and 20°C. However, in the last 3 cited papers, the mussels were filtering at low rates, varying between only 10 and 30% of the rates of fully open mussels; the low rates can be explained by incorrect use of the flow-through chamber method (Riisgård 2001b,c,d, Petersen 2004, Petersen et al. 2004). The same objection applies to data on the effects of temperature on filtration rates in oysters *Crassostrea gigas* (Bougrier et al. 1995) and *Ostrea edulis* (Haure et al. 1998).

The insufficient documentation of acute (hours) or short-term (weeks) temperature acclimation in bivalves does not imply that bivalves are unable to adapt to high and cold temperatures. Thus, filter-feeding bivalves adapted to high temperatures typical of the season of growth (27°C in Georgia) appear to maintain about the same filtration rates as bivalves adapted to relatively low temperatures (13°C in Denmark) (Riisgård 1988). As pointed out by Jørgensen (1990), tem-

perature adaptation of filtration rates (feeding rates) are presumably evolutionary 'fixed adaptations' that are integrated with other bioenergetic rate functions, such as respiration rate, thus maintaining a potential for growth that is relatively independent of latitude, but more likely adjusted to prevailing concentrations of food.

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