Viscosity of seawater controls beat frequency of water-pumping cilia and filtration rate of mussels *Mytilus edulis*

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ABSTRACT: The small-scale movements of cilia make the temperature-dependent viscosity of water a key physical/mechanical factor. In mussels and other suspension-feeding bivalves, bands of water-pumping lateral cilia produce the feeding current through the interfilamentary canals of the enlarged gill (which mainly serves as a water-pumping and food-particle collecting organ). Because the viscosity of seawater is inversely related to temperature, the linear increase of filtration rate with temperature in mussels cannot be explained solely by increased biological activity. To resolve the question of whether change in filtration rate with temperature in mussels *Mytilus edulis* is under mainly biological/physiological or physical/mechanical control, we conducted laboratory experiments on mussel-gill preparations stimulated with serotonin, and on intact mussels. In gill-preparations, the beat frequency of the water-pumping lateral cilia was measured both as a function of temperature and as a function of kinematic viscosity in dextran- and PVP-manipulated seawater at constant temperature. Data on kinematic viscosity of seawater and viscosity-manipulated seawater were converted to 'temperature equivalents' of seawater by measurement of the viscosities, and this showed that the effect of temperature on lateral cilia activity in gill-preparations is purely mechanical, controlled by the viscosity of the ambient seawater. Further, the ciliary beat frequency was found to depend on kinematic viscosity to the power $-3/2$ ($f \approx v^{-3/2}$), a relation we used to develop a mussel-pump model for the filtration rate of intact mussels vs. viscosity. The model is in agreement with filtration rates measured in intact mussels, suggesting that viscosity of seawater is the controlling factor for the beat frequency of lateral pump-cilia and filtration rate in mussels. According to the model, the mussel pump yields a constant power within the temperature-tolerance interval.

KEY WORDS: Beat frequency · Lateral cilia · Kinematic viscosity · Dextran · PVP · Filtration rate · Mussel-pump model

INTRODUCTION

In the temperature-tolerance interval, within which ciliary suspension-feeding bivalves keep their valves fully open, the relationship between temperature and filtration rate is approximately linear (Jørgensen et al. 1990, Riisgård & Seerup 2003, Kittner & Riisgård 2005). Further, there is a general lack of temperature compensation in bivalves examined experimentally (Jørgensen et al. 1990, Petersen et al. 2003, Riisgård & Seerup 2003, Kittner & Riisgård 2005). Lack of physiological compensation implies that bivalves do not achieve temperature independence through complete physiological acclimation of the filtration rate following transfer to a higher or lower constant temperature, although this acclimation mechanism was suggested by Widdows & Bayne (1971), Widdows (1976, 1978), and Bayne (1998).

It is obvious that the filtration rates of mussels must be correlated to the beating activity of the water pumping lateral cilia on the gill filaments. The inner-

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vations of the 15 µm long lateral cilia are both cilio-excitatory serotonergic and cilio-inhibitory dopaminergic, and the nervous regulation of the activity of the lateral cilia is complex (Aiello 1960, Jørgensen 1975, Jørgensen & Ockelmann 1991). Whether mussels fine-tune the beat frequency of the lateral cilia as a regulatory response to ambient temperature remains unknown (Riisgård 2001a).

The metabolism of poikilothermic animals generally increases with increasing temperature, but the linear increase of filtration rate with temperature in the blue mussel *Mytilus edulis* and other ciliary suspension-feeding bivalves (Kittner & Riisgård 2005) cannot be explained solely by increased biological activity of the lateral cilia, because the viscosity of seawater is inversely related to temperature. The lateral cilia provide the power that drives the water flow through a mussel, but for cilia operating at very low Reynolds numbers, and for flows through narrow conduits, the viscosity of the ambient water exerts a crucial mechanical effect (Jørgensen et al. 1990). The hydrodynamic scale is characterized by the dimensionless Reynolds number (Re) = object length (l) × velocity (u)/kinematic viscosity of seawater (ν) ($\sim 10^{-6}$ m^2 s^{-1}), which indicates the relative importance of inertial and viscous forces (Purcell 1977, Jørgensen 1983, Sleigh 1989). In mussels, the mean velocity (u) of the cilia driven through current is about 1 mm s^{-1}, and the length (l) of a solid perpendicular to the flow is given by the diameter of the lateral cilia (0.2 µm), and thus Re = lu/ν = 0.0002. Other length scales, such as the width of interfilament canals (40 µm), also lead to Re <<1, hence viscous forces dominate. Cilia driven flows, forces acting on beating cilia, and major contributions to the flow resistance through the mussel all increase with increasing kinematic viscosity. Therefore, the effect of temperature-dependent viscosity is highly relevant to a better understanding of the physical basis of ciliary suspension feeding in aquatic organisms.

A certain increase in viscosity by either temperature-change or by addition of a high molecular weight polymer (dextran) at constant temperature causes a strong reduction in the ingestion rate of particles in ciliary suspension-feeding larvae of the sand dollar *Dendraster excentricus*, but only about 50% of the reduction is thought to be attributable to viscosity change (Podolsky 1994). Similar observations were made on trochophore larvae of the serpulid polychaete *Galeolaria caespitose* (Bolton & Havenhand 1998). Clearly, the ingestion rate of particles depends on both the pumped volume flow (filtration rate) and the particle capture process. To separate the two, and to resolve underlying basic mechanisms, the present study set out to measure beat frequency of the lateral cilia in mussel-gill preparations (using video-microscope recordings) and to measure filtration rate of intact blue mussels in response to the effects of temperature, and manipulated viscosities at constant temperature. Such results should also aid the development of an improved ciliary-pump model for an intact mussel.

MATERIALS AND METHODS

Video-observations. All video-microscope observations were made at the Marine Biological Research Centre, Kerteminde, Denmark on locally collected blue mussels *Mytilus edulis.* The mussels were kept in aerated seawater (20°C, 20 psu) until video-microscope recordings could be made. Paths of algal cells were studied using the observation vessel submerged-microscope objective technique of Nielsen et al. (1993), Riisgård et al. (1996), and Riisgård & Larsen (2005). Gill-filament preparations were isolated in an approximately 10×10 cm temperature-controlled microscope observation vessel filled with seawater to a depth of 2 cm. The gill preparations were fixed 1 cm above the bottom by means of clips attached to 2 movable (rotational and translational) rods, leaving approximately 1 cm of seawater above the free surface of the gill filaments. The vessel was placed on a microscope stage (Nikon with 60 µm depth of focus at 40× magnification) with the cross-table removed so that the microscope objective could be immersed in the water above the gill preparation, which was illuminated through a thin glass window in the bottom of the chamber. The objective was sufficiently far (6 to 7 mm) from the gill filaments not to affect the rate and direction of flows.

Ciliary beat frequency and effect of 5-HT. The metachronal waves of lateral cilia, moving in opposite directions on 2 adjacent filaments were observed and recorded using a video camera (Kappa CF 11/1) attached to the microscope, and a 50 half-frames-per-second video recorder (Panasonic NV-FS200 HQ). The wavelength (λ, µm) was determined as the distance between 2 wave crests; the wave speed (c, µm s^{-1}) was estimated on the basis of the distance a wave crest had moved during 0.2 s (10 half-frames). We subsequently estimated the lateral cilia beat frequency as: $f = c/\lambda$, using mean values of 10 determinations of both wavelength and wave speed. In isolated *Mytilus edulis* gill filaments, the frequency of beating of the lateral cilia decreased soon after preparation. The cilio-excitatory effect of 5-HT (5-hydroxytryptamine; serotonin) on isolated gill filaments is depicted in Fig. 1. The beat frequency of the lateral cilia increased from 14.0 ± 0.5 (SD) Hz at a concentration of 10^{-7} M 5-HT in the surrounding water (18°C) to 16.6 ± 0.3, 20.0 ± 0.1, 24.6 ± 0.4, and 26.6 ± 2.7 Hz at final 5-HT concentrations of 5×10^{-7}, 10^{-6}, 5×10^{-6}, and 10^{-5} M in the
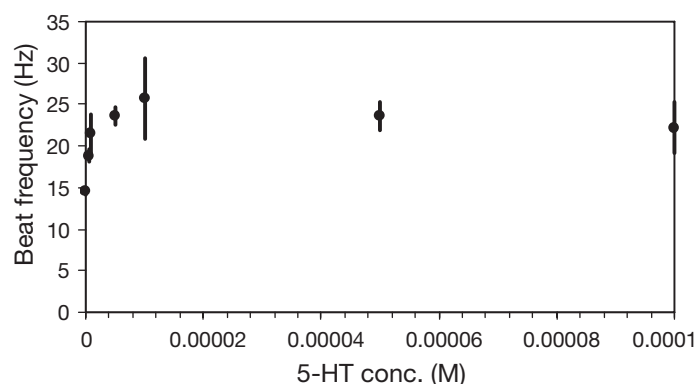
Fig. 1. *Mytilus edulis*. Mean (±2 SD, n = 8) beat frequency of lateral cilia in gill preparation (18°C) stimulated with different concentrations of 5-HT in seawater (20 psu)
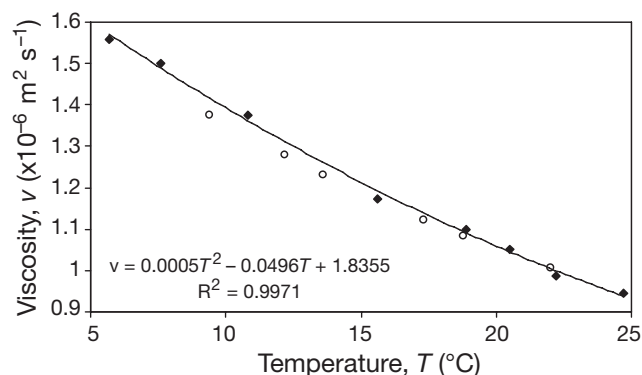

Fig. 2. Measured kinematic viscosity (v) of 20 psu seawater as a function of temperature T. (◆) Table-values for kinematic viscosity of 35 psu seawater at different temperatures (O) (Rawson & Tupper 1968)
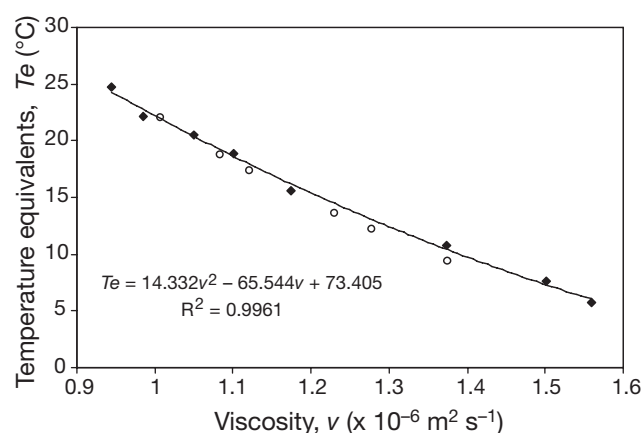

Fig. 3. Temperature data from Fig. 2 as a function of measured kinematic viscosity. Te: temperature equivalents. Symbols as in Fig. 2

microscope observation vessel, respectively. At higher 5-HT concentrations there was no further stimulation, but a slight tendency for decreased beat rate with increasing concentration. It appears that 5-HT is highly potent in stimulating the activity of the lateral cilia on the gill filaments of *Mytilus edulis* when added to the surrounding water (Jørgensen 1975). Maximal stimulation of the lateral cilia was obtained at about 10^{-5} M 5-HT. At this concentration, the laterofrontal cirri were also overstimulated and thus removed from the interfilament canal (Jørgensen 1975, Jørgensen et al. 1988, Nielsen et al. 1993, Riisgård et al. 1996). Therefore, 10^{-5} M 5-HT was adopted as a standard treatment in subsequent video-microscope experiments with gill-preparations in which the beat frequency of lateral cilia was studied as a function of seawater (20 psu) temperature in the observation vessel (regulated step-wise up and down using a Lauda RE 04 heater/cooler system).

Viscosity measurements. *Seawater:* The kinematic viscosity of 20 psu seawater was measured as a function of temperature using a calibrated viscometer (Ubbelohde Viscometer fitted with a tempering jacket; Schott). Measured kinematic viscosity as a function of temperature is shown in Fig. 2 along with table-values for 35 psu seawater obtained from Rawson & Tupper (1968). The temperature data in Fig. 2 (denoted 'temperature equivalents', Te) plotted as a function of kinematic viscosity (v) can be described by the power trend line shown in Fig. 3, and the fitted equation has been used subsequently in the present work.

Dextran: The viscosity of dextran-manipulated seawater (v_d) was measured at constant temperature (22°C) using an Engler viscosimeter and applying the equation: $v_d = v_w(\Delta t_d / \Delta t_w)$, where v_w = viscosity of seawater, and Δt_d and Δt_w are the flow-through time for seawater with added dextran (D4772 Blue Dextran, mol wt. ~ 2 000 000; Sigma-Aldrich) and clean seawater (20 psu), respectively. Concentrations of 0.75, 0.68,

0.61, 0.46, 0.23, and 0 g dextran l^{-1} were used to manipulate the viscosity of seawater. According to Table 1, $v_w = 1.00691 \times 10^{-6}$ m^2 s^{-1} (20 psu, 22°C).

PVP: The viscosity of seawater in the observation vessel was manipulated by adding polyvinyl pyrrolidone (PVP360 Polyvinylpyrrolidone = Polyvidone = PVP, average mol wt. 360 000; Sigma-Aldrich). The actual viscosity was determined using a calibrated viscometer (Ubbelohde Viscometer fitted with a tempering jacket; Schott). The effect of added PVP on the kinematic viscosity of seawater is shown in Table 1, which also shows for comparison temperature equivalents of 35 psu seawater, according to Rawson & Tupper (1968). The effect of salinity was negligible (20 vs. 35 psu). Concentrations of 1.2, 2.2, 3.6, 0.5 and 7.2 g PVP l^{-1} were needed to manipulate the viscosity of 22°C seawater (20 psu) to those viscosities of seawater at temperatures of 19.2, 17.9, 14.5, 13.1 and 10.4°C, respectively.

Table 1. Effect of added dextran and polyvinyl pyrrolidone (PVP) on measured mean (±SD, n in parentheses) kinematic viscosity of seawater (20 psu, 22°C). Temperature equivalents (*Te*) estimated according to equation shown in Fig. 2, or obtained from table-values of viscosity of seawater (35 psu) vs. temperature given by Rawson & Tupper (1968)

Conc. (g l^{-1})	Kinematic viscosity (10^{-6} m^2 s^{-1})	Estimated *Te* (20 psu) (°C)	Table-values for *Te* (35 psu)(°C)
0	1.0069 ± 0.0029 (4)	21.9	22.0
Dextran			
0.23	1.1117 ± 0.0325 (6)	18.3	17.8
0.46	1.1746 ± 0.0325 (6)	16.2	15.5
0.61	1.2690 ± 0.0619 (6)	13.3	12.4
0.68	1.4368 ± 0.0257 (6)	8.8	7.9
0.75	1.6466 ± 0.0257 (6)	4.3	3.3
PVP			
0.12	1.0833 ± 0.0047 (4)	19.2	18.8
0.22	1.1222 ± 0.0029 (4)	17.9	17.3
0.36	1.2302 ± 0.0074 (4)	14.5	13.6
0.50	1.2777 ± 0.0029 (4)	13.1	12.2
0.72	1.3757 ± 0.0055 (4)	10.4	9.4

Effect of viscosity on filtration rate of intact mussels.
Blue mussels *Mytilus edulis* were collected in the southeastern part of Kerteminde Fjord (Fyn, Denmark) in September 2006 and brought to the nearby Marine Biological Research Centre where the experiments were conducted. The filtration rate was measured as the volume of seawater (20 psu) 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 seawater (V = 1.5 l) and a group (n = 6) of mussels. The reduction in the number of algal cells was followed as a function of time by taking samples (15 ml) every 5 min and measuring the algal concentration with an electronic particle counter (Elzone 5380). After measurement, the remaining water (ca. 13 ml) was returned to the experimental aquarium to ensure a negligible reduction in the total water volume. The individual filtration rate (F) was determined from the exponential decrease in algal concentration as a function of time using the formula: $F = bV/n$ where V = volume of water, n = number of filtering mussels, and b = 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 filtration rate over time were performed by adding algal suspension to re-establish the initial algal concentration in the aquarium, and the effect of manipulated water viscosity was studied by adding a certain volume of dissolved PVP (10.5 g in 100 ml seawater) to the aquarium in which the water temperature was kept constant at 22°C using a cooler/heater system (Lauda). In a control without mussels, there was

no significant sedimentation of algal cells. Since suspended algal cells are retained with an efficiency of 100% by the gills of *M. edulis* (Møhlenberg & Riisgård 1978), this implies that the measured clearance rate, or filtration rate (F), is equal to the pumping rate. The clearance measurements were conducted at naturally low algal concentrations (i.e. ca. 500 to 4000 *Rhodomonas* sp. cells ml^{-1}), equivalent to about 0.5 to 7.5 µg chl *a* l^{-1} (Clausen & Riisgård 1996). After each clearance measurement, the viscosity was measured (Ubbelohde Viscometer) at 22°C and expressed as a temperature equivalent (cf. Fig. 3). The mean shell length of the group of mussels used was 32 ± 2.1 (SD) mm (n = 6).

RESULTS

We determined (for mussel-gill preparations stimulated with 10^{-5} M 5-HT) the beat frequencies of lateral cilia at different dextran-manipulated viscosities of seawater (22°C, 20 psu) expressed as temperature equivalents (*Te*) (Fig. 4, □). The viscosity had a pronounced effect on the ciliary beat frequency, which increased in an approximately linear relationship with *Te*. Also shown in Fig. 4 are similar results for PVP-manipulated viscosities of seawater (◉), and for changing temperature of seawater (◇). The data sets were modelled by linear regression models (Fig. 4). The model slopes for PVP-manipulated viscosities and for temperature-manipulated viscosities are very similar (see equations in Fig. 4), indicating that the effect of temperature on lateral cilia activity in gill-preparations was purely mechanical, controlled by the viscosity of the ambient seawater.

At constant temperature (22°C), the beat frequency (f) decreased when exposed to increasing dextran- or PVP-manipulated viscosities of the seawater (Fig. 5). It is remarkable that beat frequency was not simply inversely proportional to kinematic viscosity, v, but behaved as $f \approx v^{-3/2}$, a relationship that we used in the subsequent development of a pump model for the intact mussel.

Fig. 6 shows the exponential decrease in algal cell concentrations as a function of time due to grazing by a group of mussels in an aquarium with well-mixed seawater (22°C), without and with different concentrations of PVP added in order to manipulate the viscosity. The corresponding filtration rates are shown in Fig. 7. The mussels were fully open in clean seawater and at
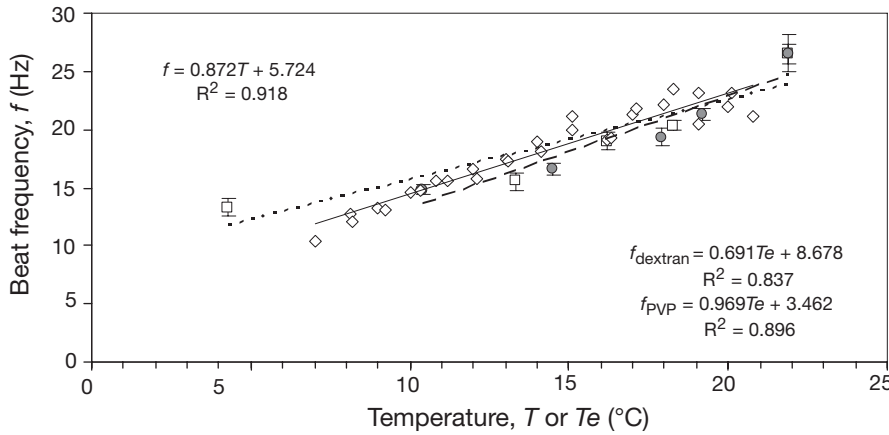
Fig. 4. *Mytilus edulis*. Beat frequency of lateral cilia in mussel-gill preparations stimulated with 10^{-5} M 5-HT versus temperature (T) or temperature equivalents (Te); ($\diamond$) exposed to different temperatures of the seawater; ($\square$) exposed to different dextran-manipulated viscosities of seawater (22°C, 20 psu); ($\odot$) exposed to different PVP-manipulated viscosities of seawater (22°C, 20 psu). Mean (± 2 SD, n = 8) indicated for PVP and dextran

the 2 lowest PVP-concentrations; but, at the 2 highest PVP-concentrations there was a noticeable effect on the exhalant opening (some constriction), and the mantle edges were pulled inwards. This may have lowered the filtration rates.

The ciliary response (as beat frequency of lateral cilia versus changes in kinematic viscosity determined for mussel-gill preparations) may also be expected to apply to intact mussels, and accordingly we use this assumption in construction of the following mussel-pump model.

MUSSEL-PUMP MODEL

To study the effect of viscosity on pumping rate of intact mussels, consider the flow model of Jørgensen et al. (1986), comprising a leaky, viscous pump with an operating pump pressure ΔH_P (1.38 mm H_2O), viscous resistances to flow in the interfilament canals (ΔH_{ifc}, 0.44) and in the exhalant siphon (ΔH_{es}, 0.14), a kinetic energy loss at the exit (ΔH_{ex}, 0.38), and resistance due to the

laterofrontal cirri (ΔH_{lf}, 0.4). According to Jørgensen et al. (1986, Table 3 therein) the percentage contributions of the 4 main components of system resistance (ΔH_S) are approximately: $\Delta H_{ifc} = 31\%$, $\Delta H_{es} = 10\%$, $\Delta H_{ex} = 28\%$, and $\Delta H_{lf} = 29\%$ for a '35 mm standard' mussel at the reference temperature of 17°C. However, for the 10^{-5} M 5-HT stimulated gill-preparation studied in the present work, the frictional loss in the interfilament canal is the only contribution to the system resistance.

According to the usual filter-pump analysis (Riisgård & Larsen 1995, 2000), the flow adjusts itself so that the pump pressure exactly balances the total resistance of the pump system, that is: $\Delta H_p = \Delta H_S$, where according to Jørgensen et al. (1990, Eqs. 3 to 6 therein):

$$\Delta H_S = \Delta H_{ifc} + \Delta H_{es} + \Delta H_{ex} + \Delta H_{lf} = \\ C_1 \nu V + C_2 \nu^{1/2} V^{3/2} + C_3 V^2 + \Delta H_{lf} \quad (1)$$

Here, ν (= μ/ρ) is the kinematic viscosity, μ is dynamic viscosity, ρ is the density of seawater (assumed to be constant), and V denotes the pumping rate (the filtration rate, or clearance rate of particles retained by the gills with 100% efficiency). Constants C_1, C_2 and C_3 include fixed geometric parameters of the interfilament canal and siphon.

The leaky, viscous gill pump represented by bands of beating lateral cilia is modeled phenomenologically by (Jørgensen et al. 1990, Eq. 7 therein):

$$V = V_d - V_l \quad (2)$$

where the net volume flow (V = pumping rate) equals the difference between the volume flow driven by the cilia (V_d), and the return flow (V_l) leaking back through the pump. This return flow is proportional to the pressure rise over the pump, $V_l \approx \Delta H_P$, so Eq. (2) may be written in the usual form of a pump characteristic:
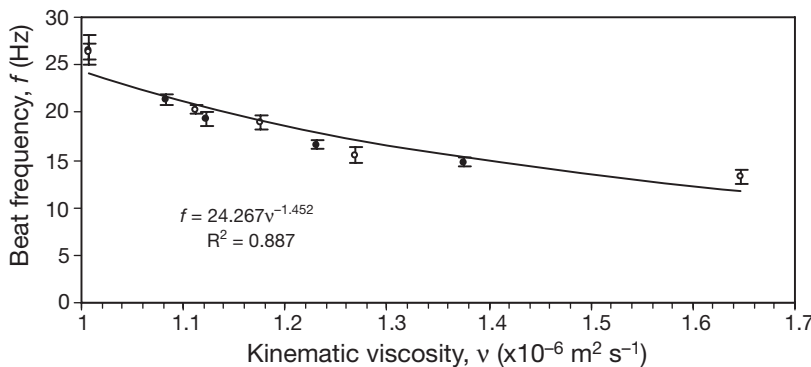

Fig. 5. *Mytilus edulis*. Mean (± 2 SD, n = 8) beat frequency of lateral cilia in gill preparations stimulated with 10^{-5} M 5-HT at constant temperature (22°C) and exposed to different dextran-manipulated (o) or PVP-manipulated ($\bullet$) viscosities of the seawater (20 psu). The common power-function line and its equation are shown
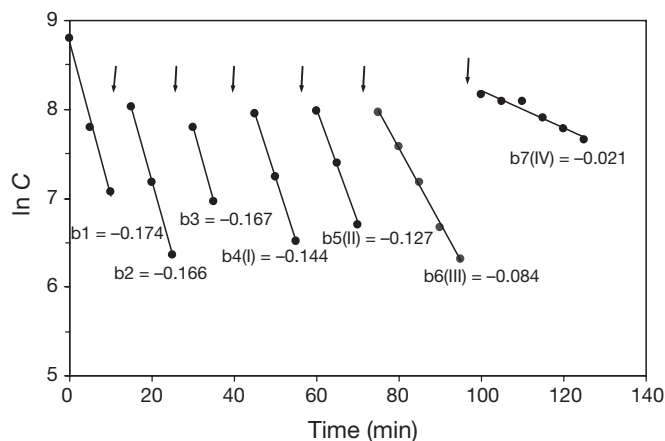
Fig. 6. *Mytilus edulis*. Exponential decrease in algal cell concentration (C, cells ml^{-1}) as a function of time due to grazing by a group (n = 6) of mussels at 22°C in an aquarium with well-mixed seawater, without (b1, b2, b3) and with different concentrations of PVP (b4, b5, b6, b7) added in increasing concentrations (I, II, III, IV). Regression lines and estimated slopes (b) are shown. Arrows indicate addition of algal cells. Estimated filtration rates are shown in Fig. 7

$$\Delta H_P = \Delta H_P^0 \, (1 - V/V_d) \; ; \; \Delta H_P^0 \equiv C\nu^m V_d \qquad (3)$$

where C is a constant, and the expression for the maximum pressure at zero pumping, ΔH_P^0, is based on experimental observations and on the assumptions that the driving velocity is proportional to the beat frequency of water pumping lateral cilia, $V_d \approx f$, and that f depends only on viscosity of the water, and is not influenced by the added 10^{-5} M 5-HT necessary for ensuring a steady lateral ciliary beat frequency in gill-preparations. Thus, as shown experimentally by Jørgensen et al. (1986, Table 2 therein; 1988, Table 2 therein; 1990, Table 3 therein), in fully open mussels, for the 3 cases, ΔH_P^0 is essentially constant (about 3.8 ±

Fig. 7. *Mytilus edulis*. Relationship between filtration rate of intact mussels at constant temperature (22°C), exposed (□) and not exposed (◆) to different PVP-manipulated viscosities of seawater (20 psu). Regression line and its equation for all data points are shown

1.0, 3.5 ± 0.4 [3.7 ± 0.4; 5-HT stimulated], and 3.19 ± 0.32 mm H$_2$O, respectively) and independent of temperature. The present data on beat frequency vs. kinematic viscosity in gill-preparations (Fig. 5) suggest $f \approx \nu^{-m}$, $m \approx 3/2$.

Equating Eqs. (1) and (3) gives:

$$C\nu^m V_d \, (1 - V/V_d) = C_1 \nu V + C_2 \, \nu^{1/2} V^{3/2} + C_3 V^2 + \Delta H_{lf} \qquad (4)$$

which, after dividing by the reference value $\Delta H_{P,ref}$ (= 1.38 mm H$_2$O at 17°C), using $\nu^m V_d \approx \nu_{ref}^{\,m} V_{d,ref}$, and introducing $\tilde{\nu} = \nu/\nu_{ref}$ and $\tilde{V} = V/V_{ref}$, may be rearranged as

$$(1 - C_0 \tilde{\nu}^m \tilde{V})/(1 - C_0) = \\ 0.31 \, \tilde{\nu}\tilde{V} + 0.10 \, \tilde{\nu}^{1/2}\tilde{V}^{3/2} + 0.28 \tilde{V}^2 + 0.29 \qquad (5)$$

where $C_0 = V_{ref}/V_{d,ref}$ and the coefficients to terms on the right hand side of Eq. (5) are recognized as the fractions of total head loss given above for the 4 main contributions at the reference state. Clearly, Eq. (5) is identically satisfied at the reference state ($\tilde{\nu} = 1$, $\tilde{V} = 1$), and it shows how the various contributions to system resistance change as $\tilde{\nu}$ and $\tilde{V}$ change from their values at the reference state. The constant C_0 is determined by use of the reference values $\Delta H_{P,ref} = 1.38$ mm H$_2$O and $\Delta H_P^0 \approx 3.8$ mm H$_2$O from Jørgensen et al. (1986), since Eq. (3) evaluated at the reference state gives $V_{ref}/V_{d,ref} = 1 - \Delta H_{P,ref}/\Delta H_P^0 = 1 - 1.38/3.8 = 0.637$.

The result Eq. (5) is an equation for $\tilde{V}$ as function of $\tilde{\nu}$, i.e. a quantity proportional to filtration rate, F, of an intact mussel vs. kinematic viscosity. Solutions to Eq. (5) at $m = 3/2$ and scaled to the 3 reference states of $F_{ref} = 34$ (present Fig. 7), 62 (Jørgensen et al. 1990, Fig. 3 therein), and 93.6 (Kittner & Riisgård 2005, Fig. 2B therein) ml min^{-1} ind.$^{-1}$, respectively, all at $\nu_{ref} = 1.15 \times 10^{-6}$ m^2 s^{-1} (17°C), i.e. setting $F = F_{ref} \, \tilde{V}/\tilde{V}_{\nu=1}$ at each $\tilde{V}$ and $\nu = \nu_{ref} \tilde{\nu}$ at each corresponding $\tilde{\nu}$, are shown in Fig. 8 along with linear regression lines in Table 2.

The model results in Fig. 8 for the 3 reference states show general agreement with corresponding measurements on intact mussels given for a 35 mm mussel (this study) (slope: −40.7 ± 4.2 [SE]), for a 39 mm mussel (data from Jørgensen et al. 1990, Fig. 3 therein) (slope: −51.5 ± 5.3) and for a 50 mm mussel (data from Kittner & Riisgård 2005, Fig. 2B therein) (slope: −89.3 ± 5.1). According to the present model, slopes of regression lines increase linearly with the value of F_{ref} to which results are scaled, which tends to reflect the trend of the data for the 3 cases, with slopes of −31.0 ± 1.2, −56.4 ± 2.1 and −85.2 ± 3.2, respectively. It should also be stressed that use of the reference state taken from a given set of data makes the model prediction exactly
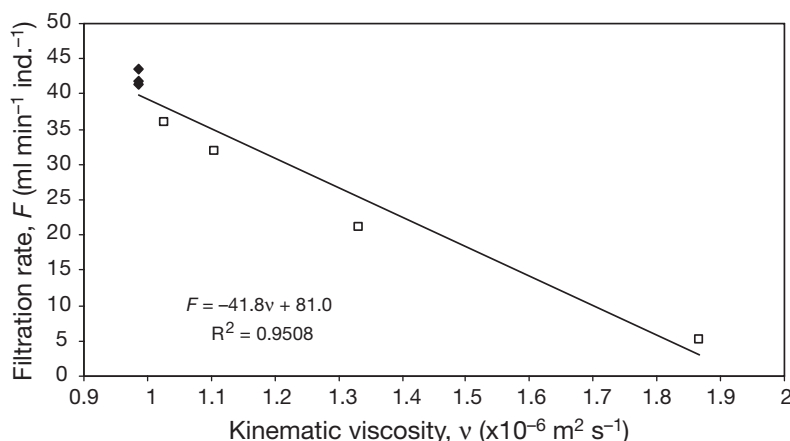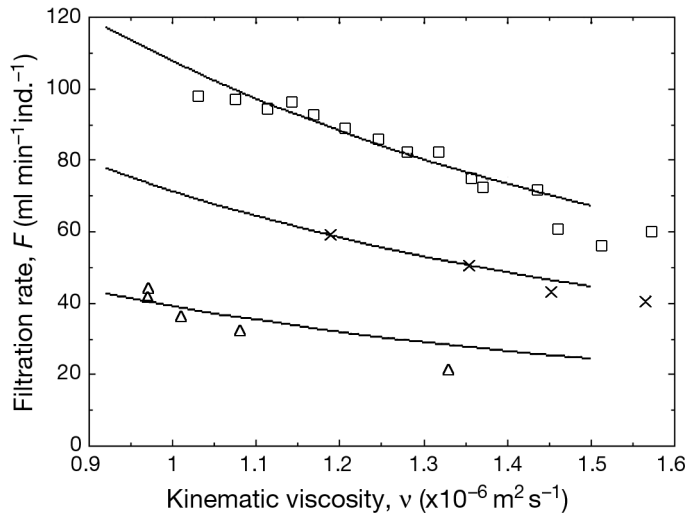
Fig. 8. *Mytilus edulis*. Experimental data (△, ✕, □) compared to model predictions (curves) of filtration rate vs. kinematic viscosity for intact mussels corresponding to the 3 reference states of F_{ref} = 34, 62 and 93.6 ml min^{-1} ind.$^{-1}$, respectively, all at ν_{ref} = 1.15 ×10^{-6} m^2 s^{-1}, 17°C. Table 2 gives data sources and regression lines fitted to data and model predictions

match the data at this state. However, the main objective of the model was to determine the slope of the F,ν-curve, which is in no respect an input taken from the data. Also, the 5 constants introduced in Eq. (5) (the ratio $\Delta H_{P,ref}/\Delta H_P^0$, and the 4 fractions of total head loss from the 4 main contributions) may vary among specimens and by size, but have been kept unchanged for the 3 cases considered, because of lack of information. Within the assumptions mentioned we conclude that the model is able to predict the response of filtration rate of intact mussels to change in viscosity, be it due to temperature change or viscosity-manipulation at constant temperature.

Solutions to Eq. (5) for values other than m = 3/2 show less agreement with available data on filtration rate vs. kinematic viscosity. Specifically, when m = 1, which would correspond to a linear, viscous pump model, as used in Jørgensen et al. (1990, Eqs. 8 & 9 therein), the slope values of F,ν-curves are too small.

In the present pump model, the driving flow, V_d, is assumed to be proportional to the beat frequency of lateral cilia, and the average force exerted on the cilia

is assumed to increase with increasing viscosity as ν^m so that the power delivered by the pump is of the form, $P_P \approx \nu^m V_d$. This expression has the same form as ΔH_P^0 in Eq. (3), which according to observations by Jørgensen et al. (1986, Table 2 therein; 1988, Table 2 therein; 1990, Table 3 therein) appears to be approximately constant, hence we conclude that the ciliary mussel pump yields a constant power. Note that this pump power is proportional to the metabolic power expended by the model of the mussel and not the useful power received by the water.

DISCUSSION

The present study has demonstrated a pronounced effect of viscosity on ciliary beat frequency in mussel-gill preparations (Fig. 4), suggesting the relation $f \approx \nu^{-3/2}$ (Fig. 5). This observation was used to develop a pump model of an intact mussel, based on the further assumptions that (1) the lateral cilia activity is the same in a gill-preparation as in an intact mussel, and (2) that the ciliary driving velocity is proportional to beat frequency, f. Possible evidence for the latter assumption comes from Petersen et al. (1999), who measured at different temperatures the beat frequency of the water-pumping lateral stigmatal cilia in an intact ascidian. The beat frequency increased linearly with temperature, and it was closely correlated with the increase in filtration rate. An argument for the former assumption is that the power delivered by the ciliary mussel pump appears to be constant. Petersen et al. (1999) suggested that physiological rather than physico-mechanical mechanisms controlled beat frequency and hence pumping rate, although no observations of response to a viscosity change at constant temperature were made to support this statement.

The present mussel-pump model predicts for 3 sets of experimental data the dependence of filtration rate on kinematic viscosity (Fig. 8). Based on this result, and the closely matching slopes of regression lines in Fig. 4 for the response of ciliary beat frequency in gill preparations to changes in temperature and to changes in viscosity by additives to seawater at constant temperature, we conclude that physical/mechanical factors, rather than biological/physiological mechanisms control the

Table 2. *Mytilus edulis*. Linear regression of filtration rate (F, ml min^{-1} ind.$^{-1}$) on kinematic viscosity (ν, 10^{-6} m^2 s^{-1}), $F = a + b\nu$, for experimental data and model predictions shown in Fig. 8

Shell length (mm)	F_{ref} (ml min^{-1} ind.$^{-1}$) at ν_{ref} = 1.15 ×10^{-6} m^2 s^{-1}	—— Experimental data —— a ± SE	b ± SE	——— Model ——— a ± SE	b ± SE	Source
35	34	79.1 ± 5.3	−40.7 ± 4.2	69.9 ± 1.4	−31.0 ± 1.2	Present study, Fig. 7
39	62	119.9 ± 7.4	−51.5 ± 5.3	127.5 ± 2.6	−56.4 ± 2.1	Jørgensen et al. (1990)
50	93.6	195.8 ± 6.7	−89.3 ± 5.1	192.5 ± 3.9	−85.2 ± 3.2	Kittner & Riisgård (2005)

filtration rate of mussels and possibly other ciliary suspension-feeding organisms. In mussels and other bivalves with compound eulaterofrontal cirri, the so-called 'cirral particle trapping mechanism' operates (Riisgård et al. 1996, Riisgård & Larsen 2005), and mucus is not involved in either the particle capture process or the subsequent transport of captured particles towards the mouth. But at high silt concentrations (>1 mg silt l^{-1}), *Mytilus edulis*, for example, produces mucus for cleaning the overloaded gill. Here, a mixture of food and silt particles enrolled in mucus is carried to the labial palps where the food particles are sorted from the silt particles, which are subsequently rejected along with the mucus as pseudofaeces (Jørgensen 1990). In the present work, the addition of PVP did not cause production of pseudofaeces, and since the measurement of filtration rate of mussels is based on the clearance method, we also conclude that the particle-capture process itself (in this case involving only one particle size) is apparently not affected adversely by temperature or viscosity, as found by Podolsky (1994) and Bolton & Havenhand (1998), who considered a range of particle sizes and species. Further, it may be concluded that the ciliary mussel pump not only yields a constant power within the temperature-tolerance interval, but apparently, to judge from the lack of temperature compensation in mussels (Kittner & Riisgård 2005), the performance of the mussel pump also remains constant following transfer to a higher or lower constant temperature.

The general lack of temperature compensation in ciliary suspension-feeding bivalves (Kittner & Riisgård 2005) shows that the beat frequency of the lateral cilia is not likely to be adjusted as a regulatory response to ambient temperature. In aquatic invertebrates, the oxygen consumption rate (respiration rate) behaves like that of an *in vitro* biochemical system, and typically doubles with every 10°C increase in temperature (van't Hoff's rule), which implies that the change of respiration rate (R) with increasing temperature is exponential, a relationship which is usually described mathematically by the function $Q_{10} = (R_2/R_1)^{10/(t_2-t_1)}$, where R_2 and R_1 are the respiration rates at 2 temperatures, t_2 and t_1 (Schmidt-Nielsen 1970). The filtration rate in ciliary suspension-feeding invertebrates is another important physiological function, but although a 'filtration rate Q_{10}' is frequently reported in the literature (e.g. Widdows & Bayne 1971, McLusky 1973, Bayne et al. 1976, Lei et al. 1996, Yukihira et al. 2000, Petersen et al. 2003), a number of recent studies show that the concept is inappropriately applied to these animals, because it is the temperature-dependent viscosity of the ambient water that mechanically controls the beat frequency of the water pumping cilia and thus the filtration rate. This statement is supported by a

number of studies showing that the relationship between filtration rate and temperature is linear (not exponential) in ciliary suspension-feeding invertebrates, e.g. in the sponge *Halichondria panicea* (Riisgård et al. 1993), the polychaete *Sabella penicillus* (Riisgård & Ivarsson 1990), the bryozoans *Electra pilosa*, *Conopeum reticulatum* (Menon 1974), *Celleporella hyalina* (Riisgård & Manríques 1997), the mussel *Mytilus edulis* (Jørgensen et al. 1990, Kittner & Riisgård 2005), the soft clam *Mya arenaria* (Riisgård & Seerup 2003), and the ascidian *Ciona intestinalis* (Petersen & Riisgård 1992).

It seems unlikely that the filtration rate of bivalves may be physiologically regulated by adjustment of the lateral ciliary beat frequency. Thus, higher or lower filtration rates may be gained only through an evolutionary change in cilia length, or increase/decrease of the total length of the lateral ciliary band. The latter hypothesis is illustrated by the following example.

In the scallop *Aequipecten opercularis*, the total length of the lateral ciliary band is 446 m g^{-1}, and the filtration rate is 10.7 l h^{-1} g^{-1}, giving a length-specific filtration rate for the lateral ciliary band of 0.24 ml h^{-1} cm^{-1} (Riisgård & Larsen 2005). These values may be compared to similar data obtained for *Mytilus edulis* (Table 3). The weight-specific filtration rate is nearly twice as high in *A. opercularis* and the total length of the lateral ciliary band is 2.8 times longer, whereas the length specific filtration rate is somewhat lower. Because the length of the lateral cilia is 15 µm in both species, this supports the hypothesis that *A. opercularis* may have evolved to subsist at lower algal concentrations (in deeper water) than *M. edulis* by a conspicuous increase of the total length of the water pumping lateral ciliary band. Table 4 shows the increase in lateral ciliary beat frequency with temperature (i.e. decreasing viscosity) in different marine bivalves and the ascidian *Ciona intestinalis*. Most of the data indicate an increase in beat frequency with temperature on the order of 0.7 to 0.9 Hz/°C, but larger values appear in some species. Such differences may

Table 3. Weight specific filtration rate (F), total length of lateral ciliary band per g dry body weight (T_{lc}), and length-specific filtration rate of lateral ciliary band (F_{ls}). [a]Data from Riisgård & Larsen (2005); [b]authors' unpublished data on gills combined with 5°C temperature-corrected (Kittner & Riisgård 2005) filtration rate obtained from Møhlenberg & Riisgård (1979)

Species	F ($l\ h^{-1}\ g^{-1}$)	T_{lc} ($cm\ g^{-1}$)	F_{ls} ($ml\ h^{-1}\ cm^{-1}$)
Aequipecten opercularis[a]	10.7	44 583	0.24
Mytilus edulis[b]	5.53	15 851	0.35

Table 4. Increase in lateral ciliary beat frequency with temperature in different marine bivalves and the ascidian *Ciona intestinalis*

Species	Comments	Slope of regression line[a] or increase in beat frequency with temperature (or temp. equivalent[b]) (Hz/°C)	Source
Mytilus edulis	Gill preparation	$(10.4-6.8)/(10-2) = 0.45$ $(22-10.4)/(23-10) = 0.89$	Aiello (1960)
Modiolus modiolus	Gill preparation + 10^{-5} M 5HT	$(31-15.7)/(21-11) = 1.53$	Jørgensen et al. (1990)
Mytilus edulis	Intact, young transparent mussel	$(14-9)/(20.5-14) = 0.77$	Jørgensen & Ockelmann (1990)
Abra nitida	Intact, young transparent mussel	$(15-10)/(20.5-14) = 0.77$	Jørgensen & Ockelmann (1990)
Abra alba	Intact, young transparent mussel	$(19-10.5)/(20.5-14) = 1.30$ $(14-10.5)/(20.5-14) = 0.54$	Jørgensen & Ockelmann (1990)
Spisula subtruncata	Intact, young transparent mussel	$(20.5-12.8)/(20.5-14) = 1.19$	Jørgensen & Ockelmann (1990)
Corbula gibba	Intact, young transparent mussel	$(19.5-10)/(20.5-14) = 1.46$	Jørgensen & Ockelmann (1990)
Cultellus pellucidus	Intact, young transparent mussel	$(19.8-13.5)/(20.5-14) = 0.97$ $(15.1-10.2)/(20.5-14) = 0.75$	Jørgensen & Ockelmann (1990)
Mytilus edulis	Gill preparation + 10^{-5} M 5HT	0.87[a]	Present study; Fig. 4
Mytilus edulis	Gill preparation + 10^{-5} M 5HT + dextran (22°C)	0.66[b]	Present study; Fig. 4
Mytilus edulis	Gill preparation + 10^{-5} M 5HT + PVP (22°C)	0.97[b]	Present study; Fig. 4
Ciona intestinalis	Intact ascidian	0.70[a]	Petersen et al. (1999)

be a result of differences in the way viscosity affects the pumping performance due to differences in pump morphology.

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