Marine Biology (1999) 133: 185–192

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Beat frequency of cilia in the branchial basket of the ascidian *Ciona intestinalis* in relation to temperature and algal cell concentration

Received: 6 October 1997 / Accepted: 8 October 1998

Abstract To elucidate the effects of temperature and algal cell concentration on pumping of water in the ascidian *Ciona intestinalis* a number of different experiments were performed. Beat frequency of the lateral cilia in the openings of the branchial sac was measured in intact specimens using a microprojection objective and a monochrome CCD video camera. At constant low algal cell concentration, beat frequencies increased linearly with temperature from 4.0 Hz (±0.5) at 7.4 °C to 13.6 Hz (±1.6) at 20.1 °C. At a constant temperature of 15 °C, beat frequency decreased with increasing algal cell concentration from approximately 3000 to >10 000 *Rhodomonas* sp. cells ml^{-1}. The decrease was observed both in experiments where the ascidians had been acclimated to a fixed algal cell concentration and in experiments with changing concentrations. Effect of algal cell concentration on squirting/siphon closure and flow velocity in the exhalent siphon was measured using a thermistor. At low algal cell concentrations, flow velocity in the exhalent siphon was stable, apart from a few short squirts. At very high algal cell concentrations, the flow velocity was reduced and much less stable, with prolonged squirting. The effect of gut content on filtration was studied in experiments with specimens acclimated to high algal cell concentrations. Results showed a close relation between gut clearance and filtration rate. From the experimental results and a qualitative analysis of the *Ciona*-pump it was concluded that the ciliary beat frequency is proportional to the water flow through the sea squirt and that changes in pumping caused by temperature or algal cell concentration are under nervous control or governed by enzyme kinetics, rather than being a result of physico-mechanical properties, i.e. pump efficiency versus flow resistance, of the ascidian pump.

Communicated by L. Hagerman, Helsingør

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Introduction

Most suspension-feeding macroinvertebrates live in the coastal zone where environmental factors vary to a great extent. Changes in environmental conditions can be expected to influence vital functions such as filtration, and it has often been shown that filtration rate in different suspension-feeding macroinvertebrates is dependent on various environmental factors. To what extent different environmental factors influence filtration and whether variations in filtration rate due to changing environmental parameters are regulated physiologically, including nervous control and/or enzyme kinetics, or purely by the physico-mechanical properties of the suspension-feeder pump are points still under debate (see, e.g. Jørgensen 1990).

In the temperate zone, temperature varies on an annual basis from around 0 to >20 °C in many coastal areas. A positive relationship in various ciliary suspension-feeders between filtration and temperature, up to a certain level, has previously been recorded (Winter 1978; Brock and Kofoed 1987; Jørgensen et al. 1990; Riisgård and Ivarsson 1990; Petersen and Riisgård 1992; Riisgård et al. 1993). It has been discussed whether the observed changes in filtration rate with temperature purely reflect changes in the viscosity of the water (Jørgensen et al. 1990; Riisgård and Ivarsson 1990) or if changes in ciliary activity, and thus some kind of physiological control,

may be involved (Petersen and Riisgård 1992; Riisgård et al. 1993; Riisgård and Larsen 1995). Others have reported, primarily on mussels, no apparent effect of temperature on filtration rate (Widdows and Bayne 1971; Bayne 1973; Smaal et al. 1997). The contrasting results between none and full temperature acclimation remain to be explained, but it is noteworthy that in most of the experiments showing no effect of temperature on filtration rate, the mussels were filtering at very low rates corresponding to 10–30% of maximum reported rates.

Concentration of suspended material in the water is also highly variable in the coastal zone and has, in all investigations, been found to affect filtration rate in suspension-feeding macroinvertebrates (Navarro and Winter 1982; Robbins 1983; Riisgård 1991; Petersen and Riisgård 1992). With increasing particle load (functional response) or at very low particle concentrations, filtration rates have been observed to decrease. The functional response has been interpreted as a regulatory mechanism controlling filtration rate in order to keep ingestion constant (Navarro and Winter 1982), but it has also been viewed as a response to sub-optimal conditions (Jørgensen 1990) or a protective reaction against overloading of the digestive system (Riisgård 1991; Willows 1992). The latter was supported by Petersen and Riisgård (1992) who found a close relation between filtration rate in *Ciona intestinalis* and calculated gut capacity. However, no conclusive evidence has been provided.

Ascidians feed by pumping the surrounding water through a continuously produced mucus net, which lines the branchial basket and traps suspended particles. The water current through the ascidian is created by beating of the lateral cilia in the stigmata openings of the branchial basket (MacGinitie 1939). In juvenile specimens of *Ciona intestinalis* the tunica surrounding the animal is transparent, and it is possible to observe the stigmata openings and beating of the lateral cilia in intact specimens. Upon disturbance, ascidians close the siphons for a shorter or longer period of time or squirt, whereby contraction of the mantle wall causes expelling of the water in the branchial basket through the inhalent siphon (Day 1919; MacGinitie 1939; Werner and Werner 1954). Siphon closure or squirting will cause a cessation of filtration and is, besides changes in ciliary activity, the only means by which ascidians can regulate filtration/ingestion over time, since no particle-sorting mechanism similar to that found in mussels has been observed.

In order to elucidate the mechanisms behind variations in filtration rate in suspension-feeding macroinvertebrates under changing environmental conditions, it is our purpose to study the effects of (1) temperature and (2) particle concentration on the pump of the ascidian *Ciona intestinalis*. Further (3) we want to demonstrate how the functional response is triggered. Since the ascidian net does not become leaky in response to changes in temperature or particle concentration (Randløv and Riisgård 1979; Robbins 1984), it is our main hypothesis that previously observed fluctuations in filtration rate (Petersen and Riisgård 1992) in *C. intestinalis* with variations in temperature and particle concentration are primarily caused by changes in ciliary activity. Further, we hypothesize that changes in ciliary activity are governed by physiological processes, i.e. processes inherent to the life functions of the ascidian, like enzyme kinetics or nervous control, rather than by the physico-mechanical properties of the ascidian pump, i.e. pump efficiency versus flow resistance.

Materials and methods

Beat frequency of lateral cilia

The experimental set-up was in principle the same as used by Mayer (1994) and consisted of an aerated 40-litre temperature-controlled aquarium and a 15-litre test aquarium connected to the constant-temperature aquarium.

In the test aquarium, individual specimens of *Ciona intestinalis* were fastened to an acrylic plastic holder fixed to a three-axis traversing rig, so that the ascidian could be positioned without disturbance of feeding. The test animal was placed in front of a window made of optical flat glass with a red LED (Light Emitting Diode, wavelength 660 nm) behind the specimen to light up the branchial basket. The microscope, which was placed behind the window, consisted of a microprojection objective and a monochrome CCD video camera connected to a 50 half-frame s^{-1} S-VHS video recorder.

The lateral cilia of the ascidian stigmata normally produce well-defined metachronal waves (Takahashi et al. 1973). For each video recording per treatment and individual, the number of frames needed for ten waves to pass a given location in the stigmata were counted three times, in five different stigmata in both the temperature and constant algal cell concentration experiments, and in three or four stigmata in both the experiments with varying algal cell concentration. From measurements of the average number of frames per wave and number of frames per second, average beat frequency (B) per specimen and treatment could be calculated as:

$$B = 50/F_w \ ,$$

where F_w is the number of frames per passing wave. Beat frequencies during different treatments were compared using a one-way analysis of variance followed by a Scheffé F-test (1% significance level).

Experiments with changing temperature were performed with ascidians that had been kept at 10 °C and acclimated in the experimental set-up to experimental temperature and an algal cell concentration of approximately 3000 *Rhodomonas* sp. cells ml^{-1}. Six to eight *Ciona intestinalis* (body dry wt: 6.7 to 34.0 mg) were tested at 7.4 °C (±0.2), 10.2 °C (±0.1), 15.1 °C (±0.1) and 20.1 °C (±0.1 °C) and an algal cell concentration of 1000 to 3000 cells ml^{-1}. When pumping, stigmatal openings of the test animal were recorded for 7 to 15 min in different parts of the branchial basket.

Effect of algal concentration was tested in two ways. In experiments where ascidians were exposed to constant algal concentration, specimens were acclimated in the experimental set-up to 15.2 °C (±0.1 °C) in sea water that had been filtered through a Sartorius Sartobran 0.45 μm filter. A suspension of dense algal culture was added to the experimental set-up to the required concentration at least 14 h prior to video recording of stigmatal openings, and algal cell concentration was adjusted just prior to recordings. Stigmatal openings in pumping ascidians were recorded in six individuals (body dry wt: 4.0 to 17.9 mg) for 7 to 15 min in different parts of the branchial basket at estimated algal concentrations of 0, 3000 and 15 000 cells ml^{-1} of the flagellate *Rhodomonas* sp.

In experiments with changing algal cell concentrations, specimens were acclimated to 15.0 °C (±0.1 °C) in filtered sea water. The water was replaced with newly filtered sea water 14 to 18 h prior to the start of the experiment. Two individuals were mounted on the traversing rig and video recorded alternately for approximately 10 min. Algal cells were added to an estimated concentration of 2000 to 4000 cells ml^{-1}, and the test animals were alternately recorded for 3- to 5 min at intervals over 1 to 2 h, allowing the ascidians time to recover after the rig had been gently moved to bring an individual into focus. Further algal cells were added to a concentration of 10 000 to 19 000 cells ml^{-1}, and the ascidians were recorded for 3- to 5-min periods during the following 4 to 5 h. The water in the experimental set-up was then gradually changed to bring algal concentrations down to 2000–3000 cells ml^{-1}, and stigmatal openings of the test animals were recorded for 3- to 5-min periods during the following 1 to 3 h and again between 12 and 16 h after reduction. Water samples were taken during the experiments and subsequently analysed for particle concentration in the size range 4 to 10 μm on a Coulter Counter. A total of six *Ciona intestinalis* (body dry wt: 2.3 to 13.4 mg) were studied.

Squirting

The experimental set-up consisted of a 10-litre test aquarium submersed in a temperature-constant bath. Flow speed out of the exhalent siphon of the ascidian was measured using a thermistor (LaBarbera and Vogel 1976) mounted in a micromanipulator. The thermistor was calibrated in a rotation tank and connected to a computer (PC-Labdas Data Acquisition Software) with a data sampling frequency of 1 Hz. During measurements of flow speed, the exhalent siphon was simultaneously recorded on a video camera so that variations in flow speed could be related to squirting/closure of the siphon. Diameter of the exhalent siphon was measured using the video camera.

Specimens ($n = 6$, body dry wt: 20 to 120 mg) were placed in the experimental set-up at least 14 h before experimental start for acclimation to experimental conditions (T: 15.2 ± 0.1 °C) and *Rhodomonas* sp. cell concentrations of respectively 1100 cells ml^{-1} (range: 800 to 1300 cells ml^{-1}) and 22 000 cells ml^{-1} (range: 20 000 to 27 000 cells ml^{-1}). For each test animal, flow speed was measured for 20 min, three times at three different occasions for each algal cell concentration.

Gut content

In order to evaluate relationships between gut content and filtration rate, experiments with *Ciona intestinalis* acclimated to high algal cell concentrations were performed.

Two groups of ascidians, large (L: 60 to 70 mm) and small (S: 40 to 50 mm), were transferred to an experimental set-up (T: 15 °C, salinity: 20‰) as used by Petersen et al. (1995) and acclimated for 1 to 2 d to *Rhodomonas* sp. cell concentrations of respectively 20 000 to 30 000 cells ml^{-1} (S) and 40 000 to 50 000 cells ml^{-1} (L). Filtration rate, defined as clearance of 100% efficiently retained particles and in ascidians equal to pumping rate, in the experimental set-up was calculated from the difference between algal concentration in inflowing and outflowing water as:

$$F = (C_i V - C_o V)/C_o \ ,$$

where C_i and C_o are mean cell concentration (cells ml^{-1}) in inflowing and outflowing water, and V is flow rate through the experimental aquarium. For each size group of animals, three to five specimens were transferred at the start of the experiment (t_0) to beakers kept at constant temperature (15.1 °C), and filtration rates were determined at algal cell concentrations of 1000 to 3000 cells ml^{-1} (Petersen and Riisgård 1992) at intervals over 24 h. The rest were transferred to running filtered sea water with a low concentration of suspended baker's yeast. Eight ascidians were sampled at t_0 and thereafter at intervals during 24 h for analysis of plant pigments. The tunics were removed and body parts homogenised and put into 10 ml of 96% ethanol for extraction and subsequent analysis according to Jespersen and Christoffersen (1987).

Results

The movements of the lateral cilia of *Ciona intestinalis* were co-ordinated, producing a well-defined metachronal wave travelling counterclockwise when viewed from the outside, thus being a dexioplectic metachronal wave (Knight-Jones 1954). Measured from the video recordings, the wavelength was 10 μm and the cilia were 16 to 18 μm long. The size and shape of the stigmatal openings showed considerable difference, but they were normally 35 to 45 μm wide or approximately double the length of the cilia.

When the ascidian was pumping undisturbed almost all stigmatal openings had beating cilia. Upon disturbance the cilia stopped and lay flat against the stigmatal walls. Following such a ciliary arrest or stoppage the cilia gradually rose to an upright position, thus closing the stigmatal openings before they shortly afterwards resumed beating. The inactive state could last for a different amount of time in different stigmata.

Effect of changes in temperature

Beat frequencies of lateral stigmatal cilia were significantly ($p < 0.01$) different at the different tested temperatures and increased linearly with temperature from 4.0 Hz (±0.5) at 7.4 °C to 13.6 Hz (±1.6) at 20.1 °C. The relation between temperature and beat frequency is shown in Fig. 1 together with calculated filtration rates (Petersen and Riisgård 1992) for the tested *Ciona intestinalis*. From the linear relations, mean increase in the interval 5 to 20 °C was 4.6 times for filtration rate and 4.5 times for beat frequency.

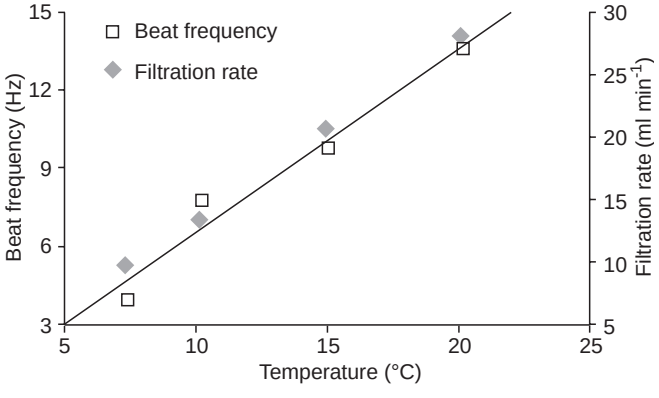

Fig. 1 *Ciona intestinalis*. Beat frequency of lateral cilia in stigmatal openings and filtration rate, calculated according to Petersen and Riisgård (1992), as a function of temperature. Line describes the linear relation between temperature (T, in °C) and beat frequency (B, in Hz): $B = 0.7T - 0.47$, $r^2 = 0.96$

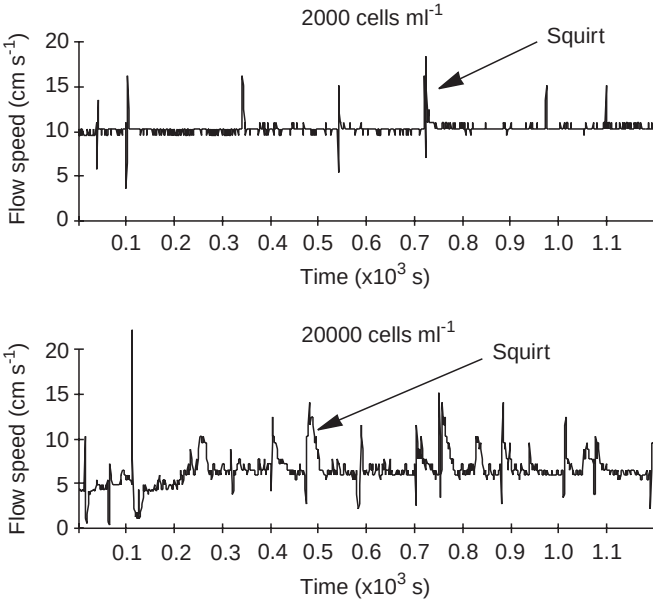

Fig. 2 *Ciona intestinalis*. Flow speed measured in the exhalent siphon of Sea Squirt A at 10 °C and approximately 2000 and 20 000 *Rhodomonas* cells ml^{-1}

Effect of changing algal concentration

At low algal cell concentrations, flow velocity out of the exhalent siphon of the test animals was stable, apart from a few short squirts, and there was a continuous stable flow for 98.5% (±1.1) of the time used for measuring flow velocity. At very high algal concentrations (>20 000 cells ml^{-1}), the flow pattern was much less stable with prolonged squirting, and the tested sea squirts were open and pumping for 88.8% (±4.1) of the time (Fig. 2). Flow speed varied from 9.9–20.7 cm s^{-1} at low algal cell concentrations to 4.7–13.7 cm s^{-1} at high algal cell concentrations, depending on the size of the specimen (Table 1).

In ascidians that had been acclimated overnight to expected fixed concentrations of 0, 3000 or 15 000 cells ml^{-1}, beating of the lateral cilia in the stigmata was uniform in different parts of the branchial sac and had a frequency of 8.6 (±0.5), 9.8 (±0.6) and 4.9 Hz (±0.3), respectively. Figure 3 (summarised in Table 2) shows beat frequencies of *Ciona intestinalis* that were exposed to changing algal cell concentrations. At the beginning of the experiments particle concentrations were 200 to 600 cells ml^{-1} in sea water with no addition of algal cells and beat frequencies were between approximately 7 to 10 Hz (Section A in Fig. 3). After addition of algae to concentrations of 2500 to 6000 cells ml^{-1} beat frequencies increased to approximately 9 to 12.5 Hz (Section B in Fig. 3). Further addition of algae to 10 000–19 000 cells ml^{-1} reduced beat frequencies in all specimens to ca. 4 to 7 Hz depending on actual algal cell concentration (Section C in Fig. 3). Subsequent reduction of algal cell concentration level to 1500–4000 cells ml^{-1} did not result in an immediate return to high beat frequencies (Section D in Fig. 3). At high algal cell concentrations in the test aquarium, the dorsal lamina appeared red from the high numbers of *Rhodomonas*-cells. Upon reduction in algal cell concentration, the red colour rapidly disappeared again. The following day, beat frequencies had resumed normal level (Table 2).

Figure 4 shows beat frequencies as a function of particle concentrations from experiments where particle concentration was measured. Where algae were added, an inverse relation between particle concentration and beat frequency is seen. Including all data collected at 15 °C, there are significant differences ($p < 0.01$) between all combinations of no addition of algae (8.6 ± 1.0 Hz), low algal level (9.9 ± 1.1 Hz) and high algal level (5.1 ± 0.6 Hz).

Gut content

Gut content of plant pigments (chlorophyll *a* and phaeopigment) decreased for 9 to 11 h after transfer to filtered sea water in both small and large specimens. During the same period, filtration rate increased from 5–10% to 75–95% of filtration rate at the end of the experiment (F_{max}). Gut content and filtration rate as a function of time are shown in Fig. 5, expressed as percentages respectively of average content at t_0 and F_{max}. The F_{max} value was 27.8 ml min^{-1} for large (body dry wt: 60 to 80 mg) and 12.0 ml min^{-1} for small sea squirts (body dry wt: 18 to 22 mg).

Table 1 *Ciona intestinalis*. Flow velocity (V, cm s^{-1}), diameter of the exhalent siphon (D, mm) and calculated filtration rate (F, ml min^{-1}) in exhalent siphon at low (<2000 cells ml^{-1}) and high (>20 000 cells ml^{-1}) concentrations of *Rhodomonas* cells. Filtration rate was calculated as F = (siphon area × V)/2. No estimate of siphon diameter could be obtained for Specimen C

Specimen (body dry wt)	Low algal level			High algal level		
	V (cm s^{-1})	D (mm)	F (ml min^{-1})	V (cm s^{-1})	D (mm)	F (ml min^{-1})
A (38 mg)	10.5 ± 0.3	3.2	25.3	5.4 ± 1.5	3.0	11.5
B (62 mg)	17.9 ± 3.3	2.7	30.7	9.5 ± 3.4	2.5	13.9
C (54 mg)	13.2 ± 1.3	–	–	6.5 ± 2.0	–	–
D (71 mg)	20.7 ± 6.4	2.5	30.5	13.7 ± 1.6	2.2	15.6
F (20 mg)	9.9 ± 1.9	2.2	11.3	6.3 ± 1.0	2.0	5.9
H (26 mg)	12.5 ± 1.8	1.9	17.0	4.7 ± 1.3	1.9	4.0

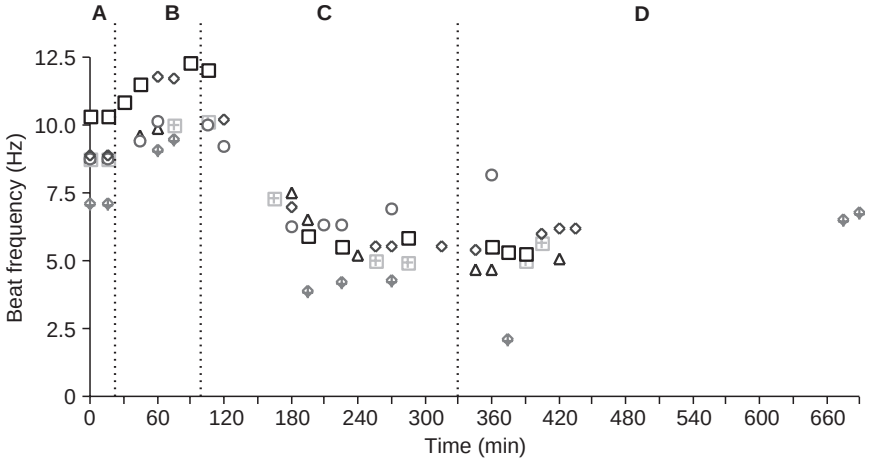

Fig. 3 *Ciona intestinalis*. Beat frequency of lateral cilia in the stigmatal openings, measured at 15 °C and changing concentrations of *Rhodomonas* cells. **A** 200 to 600 cells ml^{-1}; **B** 2500 to 6000 cells ml^{-1}; **C** 10 000 to 19 000 cells ml^{-1} **D** 1500 to 4000 cells ml^{-1}. Each symbol denotes a different specimen

Table 2 *Ciona intestinalis*. Beat frequency (mean ± SD) of lateral stigmatal cilia in experiment with changing algal cell concentration. Time elapsed is counted from the start of the experiment (see Fig. 3)

Cell conc. (cell ml^{-1})	Time elapsed (min)	Beat frequency (Hz)	No. of specimens
200–600	0–30	9.3 (±0.8)	4
2500–6000	60–90	10.7 (±1.1)	6
10 000–19000	220–310	5.3 (±0.8)	6
1500–4000	360–690	5.6 (±1.4)	6
2000–4500	1200–1400	10.0 (±1.4)	3

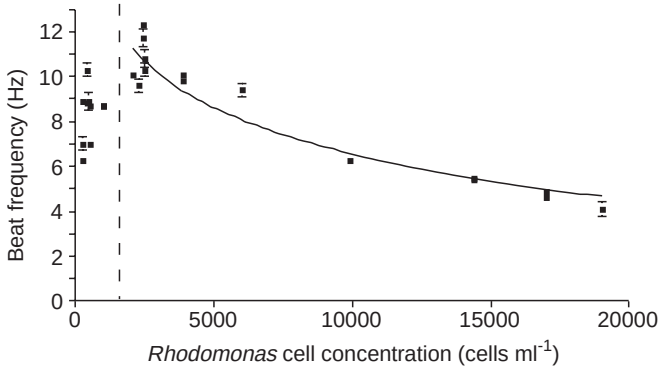

Fig. 4 *Ciona intestinalis*. Beat frequency (mean ± 5D) of lateral cilia in the stigmatal openings, as a function of cell concentration at 15 °C. Line [$B = 33.8–6.82 \log(C)$, $r^2 = 0.92$] describes the relation between *Rhodomonas* algae concentration (C, in cells ml^{-1}) and beat frequency (B, in Hz), where algal cells were added to the experimental set-up (on the right side of the dotted line)

Discussion

Our observations on geometry and beating of the lateral stigmatal cilia of *Ciona intestinalis* are in accordance with previous observations in solitary ascidians. In isolated pieces of the branchial basket, Takahashi et al. (1973) found that the lateral cilia were 20 μm long and produced a dexioplectic metachronal wave with a wavelength of about 10 μm and a beat frequency of 6 to 13 Hz at 24 to 26 °C. The low beat frequency observed by Takahashi et al. (1973) may be due to rapid decline in excised material that is not stimulated with serotonin, or adversely high temperatures. In intact specimens of the transparent *Corella wilmeriana* the lateral cilia were 20 μm long and their beating also produced a dexioplectic metachronal wave with a beat frequency of 10 to 13.5 Hz at 18 °C (Mackie et al. 1974). In both species, it was found that periods with actively beating cilia alternated with periods of ciliary arrest, which could be separated into two phases: an inactive state where the cilia lay flat lining the stigmata and a relaxed state where the cilia were kept motionless in an upright position just before resuming beating activity (Takahashi et al. 1973; Mackie et al. 1974). Lower estimates of lateral cilia length in the present study are probably not in conflict with previous studies but are presumably due to difficulties measuring the length of the cilium in an upright, maximally extended position with the present technique.

A close correlation between filtration rates and beat frequencies in the temperature range 7 to 20 °C was demonstrated. The similar increase in rates in the studied temperature interval for both filtration and beating of the lateral cilia indicates that changes in filtration rate in response to temperature reflect changes in activity of the lateral cilia. Though linear, the increase in beat frequency with increasing temperature corresponds to a Q_{10} of 2.3, which is in accordance with previous observations. In excised gills of *Mytilus edulis*, the beat frequency varied with temperature with a Q_{10} of 1.8 (Aiello 1960). In *M. edulis* followed at environmental temperatures rising from 14 to 22 °C, the beat frequency of lateral cilia in excised gill material increased from about 10 to about 18 Hz, corresponding to a Q_{10} of 2.1 (Stefano et al. 1977). In various mussels, Jørgensen and Ockelmann (1991) found that newly settled intact juveniles had an increase in beat frequency of lateral cilia in

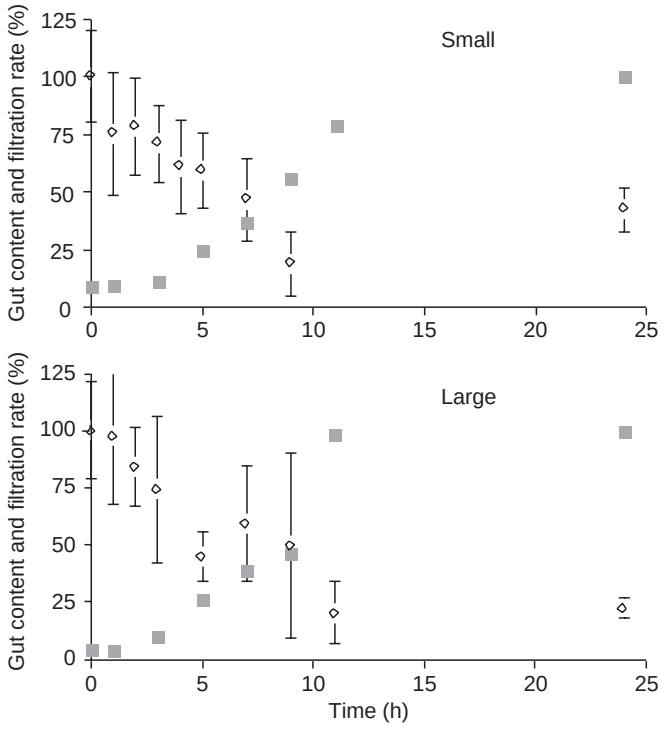

Fig. 5 *Ciona intestinalis*. Mean gut content (±SD, *open symbols*) and filtration rate (*filled symbols*), as a function of time in a group of small (body dry wt: 18 to 22 mg) and a group of large (body dry wt: 60 to 80 mg) *C. intestinalis* that had been transferred from high (>20 000 cells ml^{-1}) concentrations of *Rhodomonas* cells to filtered sea water. Gut content was measured as plant pigments (chlorophyll *a* plus phaeopigment) and expressed as percentage of gut content at the beginning of the experiment. Filtration rate is in percentage of F_{max} that was calculated according to (Petersen and Riisgård 1992) using dry weight of body parts

the temperature range 14 to 21 °C, corresponding to a Q_{10} of, on average, 2.1. In groups of adult blue mussels, *M. edulis*, adapted to either summer or winter conditions, filtration rate was, however, found to increase in the temperature range 10 to 20 °C, corresponding to a Q_{10} of 1.33, which is proportional to the decrease in the viscosity of sea water (Jørgensen et al. 1990). This led to the conclusion that the dimensioning and geometry constitute important properties of the mussel pump, affecting resistance to water flow in the canal system with changes in viscosity and leaving little room for factors other than viscosity in influencing the rate of water pumping (Jørgensen et al. 1990). Sleigh and Aiello (1972) found, however, a close relation between filtration rate and beating of the lateral cilia, and showed that measured flow velocities caused by the lateral cilia could produce the filtration rates observed in mussels. Most experimental evidence thus indicates a close correlation between beat frequency and filtration rate. A qualitative analysis of the ascidian pump may further support these results.

The resistance in the flow system of *Ciona intestinalis* may be expressed as a head loss ΔP_r, generally depending on the volume flux F through the individual as

$$\Delta P_r = \mu AF + BF^2 \ , \quad (1)$$

A and B being factors dependent on system geometry, see also e.g. Riisgård and Larsen (1995) for discussion of the characteristics of ciliary pump systems. The first term on the right-hand side of the equation is due to the viscous resistance, with μ denoting the dynamic viscosity of the water. The second term denotes the loss of kinetic energy in the exhalent siphon or in connection with turbulence generation. In order to maintain a steady flow through the animal, the ciliary pump must produce a pump pressure head ΔP_p, which balances the system resistance

$$\Delta P_p = \Delta P_r \ . \quad (2)$$

The dependency of ΔP_p on F is strongly contingent upon the pump type. For example, the flow rate driven by a piston-type displacement pump is only dependent on the displacement rate. Given the piston kinematics, F will only depend on the piston frequency f, as $F = kf$, where k is a geometrical factor. However, the ciliary pump in *C. intestinalis* and other ciliary filter feeders may be described as a viscous pump in which moving structures (cilia) drive the fluid due to the viscous friction in the water. Hence, without viscosity, the motion of cilia would not cause any pump work. Since open spacing exists between individual cilia and in particular between ciliary tips on either side of the stigmata, an opposing pressure may drive a leak flux opposite to the main flow direction. Due to the small Reynolds numbers characterising the internal flow in *C. intestinalis*, it may be idealised as creeping flow. The volume flux produced by the ciliary pump may then be expressed as

$$F = kf - \Delta P_p/(C\mu) \ , \quad (3)$$

where f denotes the ciliary beat frequency and C is a geometrical factor, see e.g. Riisgård and Larsen (1995) for more details. Solving Eqs. 1, 2 and 3 for ΔP_p yields

$$\Delta P_p = \mu C(kf - F) \ , \quad (4)$$

and inserting Eqs. 1 and 4 into Eq. 2 gives

$$kf/F = 1 + A/C + BF/(\mu C) \ , \quad (5)$$

which may then be used for solving for F. Assuming the ciliary beat frequency f to be constant, an increase in dynamic viscosity μ implies an increase in both ΔP_p and ΔP_r. However, according to Eq. 5, F is unaltered for varying viscosity, if convective losses are neglected ($B = 0$). Further, including convective losses in the analysis ($B > 0$), the flux F is seen to even increase with increasing viscosity. Consequently, since the head loss in the flow system of *C. intestinalis* is assumed to be mainly due to viscous losses, neglecting terms in Eq. 1 of the form BF^2, we believe that changes in the ciliary beat frequency reflect quite closely the relative changes of the volume flux through the animal.

An increase in viscosity alters the rate of mechanical work W, exerted by the cilia, such that

$$W = F\Delta P_r = \mu A F^2 \quad , \tag{6}$$

for $B = 0$, since viscous resistance is increased in the flow system. Additionally, and probably even more important, the viscous resistance related to the local flow field around individual cilia is increased. The influence on the flow rate of changes in viscosity is thus mainly a question of the response of ciliary motion to changes in work load. Studying the abfrontal gill cilia of *Mytilus edulis*, Yoneda (1962) found that increasing the relative viscosity 2.4 times the viscosity of sea water had no effect on the relative angular velocity of the effective stroke of the cilium. Similarly, it has been shown that beat frequency of lateral cilia of *M. edulis* (Aiello 1960) and the peristomial cilia of *Stentor polymorphus* (Sleigh 1956) decreased by a maximum of 10% when 0.3% methylcellulose was added, corresponding to an increase in viscosity of 2.2 times that of sea water. Hence, taking into account the small change in viscosity (approx. 50%) in the temperature range 4 to 24 °C, it is not likely that the viscous resistance is altered to a degree that impairs the cilia significantly. In summary, temperature-dependent changes in filtration rate are a result of changes in beat frequency of the lateral cilia.

A close relation between beating of the lateral cilia and filtration rate was further demonstrated from the experiments with different algal cell concentrations. At high algal cell concentrations of $>10\,000$ *Rhodomonas* cells ml^{-1} beat frequency was reduced significantly. Reduction in filtration rate to about 50% of F_{max} at algal concentrations of 8000 to 20 000 cells ml^{-1} has been found at both acute (Petersen and Riisgård 1992) and long-term exposure (Petersen et al. 1995) to high algal cell concentrations. Increased squirting or closure of the siphon openings at high cell concentrations leading to longer periods with no active filtration can account for only a minor part of the reported reduction in filtration rate. In fact, the reduced flow out of the exhalent siphon at high algal cell concentrations is the result of reduced beating of the lateral cilia. In experiments with both acute and long-term exposure to high algal cell concentrations, the ascidians responded by reducing beating frequency of the lateral cilia by approximately 50%, depending on actual concentration and time of exposure to high algal concentrations. It is not likely that the reduction in beat frequency at high algal cell concentration was the result of increased resistance to water flow through the branchial sac caused by algal cells blocking the mucus net, since experiments with changing algal concentrations showed that beat frequencies remained reduced after algal concentration had been adjusted to low levels and the dorsal lamina no longer was coloured by captured particles. Reduced filtration rate at high particulate concentrations in ascidians is thus primarily a result of reduced water processing by the stigmatal cilia and only to a minor degree of disturbance of the pump by, e.g., increased squirting or clogging of the mucous filter. The steady, reduced beat frequencies at high algal concentrations indicate that the functional response is physiological in nature, in the sense that it must reflect some kind of nervous control of the beating of the stigmatal cilia.

The close relation between gut fullness and filtration rate, corresponding to observations by Petersen and Riisgård (1992), indicates that reduced beat frequencies at high algal cell concentrations most likely are triggered by the degree of gut filling. The functional response can thus be viewed as a protective reaction against overloading of the digestive system (Petersen and Riisgård 1992) or that filtration rate is constrained by the digestibility of the food and limitations on the rate of absorption of energy within the gut (Willows 1992).

Finally, reduction in beat frequency at low algal cell concentrations was significant but not of the same magnitude as the reduction in filtration rate reported by Petersen and Riisgård (1992). This difference may be caused either by the fact that it was impossible to keep the water in the experimental set-up absolutely particle free (min. conc.: 200 to 600 cells ml^{-1}) or because some of the reduction in filtration rate observed by Petersen and Riisgård (1992) was caused by cessation of production of mucus net in water deprived of particles. The reduction of beat frequency at low algal concentrations observed in the present study is of the same magnitude as the reduction of filtration rate at low algal cell concentrations observed in experiments with long-term exposure to constant concentration (Petersen et al. 1995).

In summary, filtration in *Ciona intestinalis* is primarily a result of beating of the lateral cilia in the stigmatal openings. With changes in environmental parameters like temperature and particle concentration, beat frequency and thus filtration rate changes. These changes are thus physiological in nature rather than a result of physico-mechanical properties of the ascidian pump. Changes in beat frequency due to changes in temperature may be a result of biochemical regulation (enzyme kinetics), as indicated by the observed Q_{10}, while the changes caused by differences in particle load must involve nervous control of the cilia, triggered by the fullness of the gut. Nervous control of beat pattern and cilia arrest have been demonstrated in *C. intestinalis* (Takahashi et al. 1973; Bergles and Tamm 1992) and *Corella willmeriana* (Mackie et al. 1974). Responses to changes in environmental parameters on filtration may thus involve different kinds of regulatory mechanisms.

Acknowledgements We would like to thank Prof. P.S. Larsen for lending facilities at the Dept. Fluid Mechanics and critical reading of the manuscript, and Prof. Strømberg for kindly providing facilities at Kristineberg Marine Research Station. The experiments performed in this study comply with the current law in Denmark.

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