Vol. 215: 133–141, 2001 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published May 31

Feeding activity, retention efficiency, and effects of temperature and particle concentration on clearance rate in the marine bryozoan *Electra crustulenta*

Dennis Lisbjerg, Jens Kjerulf Petersen*

National Environmental Research Institute, PO Box 358, Frederiksborgvej 399, 4000 Roskilde, Denmark

ABSTRACT: Various factors influencing clearance rate were elucidated on the bryozoan *Electra crustulenta* (Pallas). Measurements of clearance rates were performed using the algae *Rhodomonas* sp. (6 µm in diameter). Clearance rates were related to the area of the active zooids within the colonies in order to obtain area-specific clearance rates. Specific area was 42% of the total colony area. Several replicates were performed with each colony to obtain maximum clearance rate (F_{max}). F_{max} increased with temperature from 90 ml h^{-1} cm^{-2} at 6°C to 229 ml h^{-1} cm^{-2} at 22°C. Clearance rate decreased at increasing algal cell concentration from 1600 to 19 000 cells ml^{-1}. The decrease in clearance corresponded to a maximum ingestion rate at particle concentrations >8500 *Rhodomonas* sp. cells ml^{-1}. *E. crustulenta* zooids are capable of retaining and ingesting particles in the range from ca 5 to ca 30 µm in diameter. Smaller particles are less efficiently retained due to the structure of the feeding apparatus, the lophophore and larger particles due to the size of the mouth (30 µm in diameter). Feeding activity was observed on single zooids and it was found that zooids have periodical retraction of the lophophore. At low particle concentrations (ca 1500 cells of *Rhodomonas* sp. ml^{-1}) the lophophore is retracted $5 \times h^{-1}$ for periods of 38 s. Zooidal activity measured as the time of protruded lophophore thus leads to an activity of 95% of the total time. At high algae concentrations, zooidal feeding activity decreased to 70% as the lophophore was retracted more frequently ($10 \times h^{-1}$) and for longer periods of time (107 s). Despite the decreased activity at high algae concentration, this could only account for 50% of the decrease in clearance rate. Thus, regulatory mechanisms of the clearance rate other than retraction of the lophophore must be considered in bryozoans.

KEY WORDS: Clearance rate · Feeding activity · Filtration · Functional response · Ingestion rate · Particle spectrum · Regulation of filtration rate · Retention efficiency

INTRODUCTION

In the last few years, different aspects of filtration biology have been studied in bryozoans. Pumping rate (Riisgård & Manríquez 1997), particle capture (Nielsen & Riisgård 1998) and clearance rate (e.g. Riisgård & Goldson 1997, Lisbjerg & Petersen 2000) have been studied applying techniques used in the studies of other benthic suspension-feeders (e.g. Riisgård & Ivarsson 1990, Petersen & Riisgård 1992, Riisgård et al. 1993).

Bryozoans are abundant in coastal zones and, as for other suspension-feeders in this very variable environment, their clearance is subject to variation due to differences in external factors. In general, temperature and particle concentration are 2 major components contributing to changing clearance rates for benthic suspension-feeders (e.g. Petersen & Riisgård 1992, Clausen & Riisgård 1996, Petersen et al. 1999). Effects of temperature on bryozoan clearance rate have been studied by Menon (1974), Riisgård & Manríquez (1997), Lisbjerg & Petersen (2000) and particle concentration by Bullivant (1968), Best & Thorpe (1983, 1986), and Riisgård & Goldson (1997). Other factors may also in-

*Corresponding author. E-mail: jkp@dmu.dk

fluence clearance rate, e.g. the size of particles. Due to the open structure of the bryozoan filter, small particles are not efficiently retained. Distance between laterofrontal cilia varies from 3 to 4 µm in *Crisia* (Nielsen & Riisgård 1998) and experiments show that particles smaller than ca 5 µm in diameter are retained less efficiently than larger particles in the filtering apparatus of *Electra crustulenta* (Riisgård & Goldson 1997) and *Celleporella hyalina* (Riisgård & Manríquez 1997).

In an encrusting bryozoan colony, there is a difference in the activity level of the zooids. Just behind the growing edge of the colony, zooids seem constantly active on a large time scale, looking at activity in weeks or months. As the growing edge of the colony keeps expanding, the position of the zooids within the colony changes and as the colony grows, new zooids occupy the zone just behind the growing edge. When observing the activity of a single zooid, after 2 to 72 d of activity a resting phase follows, during which the polypide degenerates into a brown body (e.g. Barnes & Clarke 1994, 1998, Bayer et al. 1994). A new polypide is formed around the brown body within 2 to 17 d and gives rise to a new active period (Ryland 1976).

Short-scale variation (hours, minutes) in zooid activity has yet to be studied. Shutting down events where the polypides retract into the zooecium have been reported (e.g. Menon 1974, Hunter & Hughes 1993, Riisgård & Goldson 1997). Hunter & Hughes (1993) noted a decrease in the time spent feeding at increasing algae concentrations. The duration of the non-feeding (shut down) episode, and whether the behaviour applies for zooids which have been exposed to high particle concentrations for prolonged time, is uncertain.

It is currently being debated whether suspension-feeders in general regulate their clearance rate. Petersen et al. (1999) reported a decrease in the beat frequency of the lateral cilia in ascidians in order to regulate clearance rate at increasing particle load, while others report to have found no evidence for physiological regulation in bivalves and bryozoans (e.g. Clausen & Riisgård 1996, Riisgård & Goldson 1997). The present study of bryozoans contributes to the discussion by examining the influence on clearance rate by various external factors. The study encompasses the major components expected to influence clearance rate: temperature and particle concentration as previously studied. Also, this study presents new data on retention efficiency of the lophophore filter and short-scale zooidal activity. All experiments were performed on the same bryozoan species, which makes analysis comparing data from the different experiments possible.

Throughout the text we will use the word 'regulation' in the context of any change in behaviour or other animal activity due to changes in the environmental parameters.

MATERIALS AND METHODS

The bryozoan *Electra crustulenta* (Pallas) usually has encrusting colonies but can produce erect forms. It is found in brackish water on all available substrata on the lower shore and in shallow water (Hayward & Ryland 1990). In this study, colonies of *E. crustulenta* were collected in the inner part of Roskilde Fjord, Denmark. In Roskilde Fjord salinity varies from 7 to 19‰, and yearly temperatures range from 0 to 22°C (Flindt et al. 1997). Only colonies of *E. crustulenta* settled on shells of *Mytilus edulis* were collected. The soft body parts of *M. edulis* were removed and the halved shells, with the *E. crustulenta* colonies, were placed in a 20 l container with a continuous flow-through of water (18‰ S, 17°C) and algae suspension (*Rhodomonas* sp.). Periodically, the colonies were cleaned of debris using a soft brush. Numerous glass slides were suspended into the container hanging from a float or placed on the bottom. After some time, larvae were released and settled on the slides. These young colonies were used in all clearance experiments.

Clearance rate experiments were performed and calculated according to Lisbjerg & Petersen (2000). Clearance rate (F) is based on the clearance of *Rhodomonas* sp. in a 200 ml beaker and calculated as

$$F = V \times \ln(C_0/C_t)/(A \times t) \quad (1)$$

where A is the area of the colony, t is time, V is the volume of the beaker and C the algal cell concentration. An exponential line was fitted to the decline in algal cell concentration, and only experiments with an r^2 value > 0.95 were considered. Several replicate experiments were performed and the 2 to 3 replicates where the colony achieved the highest clearance rates were regarded as the colony potential (maximum clearance rate, F_{max}). Concentrations of particles were measured on an Elzone® 180 XY particle counter mounted with a 95 µm orifice tube. The decline of particles was followed in a 10 ml subsample every 8 to 15 min. Immediately after counting the subsample, the remainder of the sample was returned to the experimental beaker. This is a different procedure than used in Lisbjerg & Petersen (2000), who found a change in zooid behaviour when returning the subsample to the experimental beaker, and instead added an equal volume containing approximately the same algae concentration. Bullivant (1968) and Markham (in Best & Thorpe 1994) also noted this change in zooidal behaviour. However, in preliminary experiments in this study no behavioural effect was detected. Riisgård & Goldson (1997) and Riisgård & Manríquez (1997) have had similar results. Common to all of the experiments showing no effect was the use of an Elzone®, whereas a Coulter® particle counter has been used when an effect has been observed.

After each sequence of clearance experiments, a video recording of the colony was made with a Sony digital DCR-VX1000E video camera. Using a computer, colony area could be estimated from the video recordings. As not all of the zooids within a colony are actively feeding due to the brown body formation, both the total area and the area of the active zooids (specific area) were measured. Total area was estimated by marking the circumference of the colony on the digital pictures using a computer. Specific area was the area of zooids which had fed on the red-coloured *Rhodomonas* sp., which could easily be seen in their gut through the transparent frontal membrane.

To study effects of particle concentration, clearance rates were estimated for 3 colonies at 18°C using *Rhodomonas* sp. at various initial cell concentrations, ranging between 1600 and 19 000 cells ml^{-1}. Ingestion rate (I) was calculated based on the decline in concentrations of *Rhodomonas* sp.

$$I = (C_0 - C_t) \times V/(A \times t) \quad (2)$$

Effects of temperature on clearance rate were studied by measuring clearance rate at 6, 10, 14, 18, 22 and 24°C on colonies of different size. At each temperature, results of maximum clearance rate were plotted as a function of colony size. Between experiments, temperature was increased 2°C every second day. Initial algal cell concentrations were kept at ca 2500 *Rhodomonas* sp. cells ml^{-1} in all experiments.

Particle retention efficiency of different sized particles was quantified from experiments in which 2 different algae species were fed to a colony simultaneously. As the clearance rates of the 2 algae are assumed to be equal, any difference in clearance is considered a difference in retention efficiency due to the difference in particle size. In each setup, *Rhodomonas* sp. was used as the reference particle and the retention of the other species was estimated relative to this. *Rhodomonas* sp. has an estimated spherical diameter (ESD) of 6 µm and clearance rates were compared with 2 unidentified small flagellates of 3.6 and 4.3 µm ESD, *Heterocapsa triqueter* (15 µm ESD), and *Prorocentrum micans* (23 µm ESD). Prior to these experiments, zooidal behaviour was studied when feeding on the mentioned particles using a stereo microscope. Observations also included *Scripsiella* sp. (19 µm ESD), *Fragilidium subglobosum* (48 µm ESD), eggs of the copepod *Acartia tonsa* (75 µm), cellulose beads (100 to 250 µm), and biosilon beads (125 to 250 µm).

Diurnal activity was investigated by video recording zooids. As video recording requires ample light, influence of light was studied in other experiments measuring the clearance of *Rhodomonas* sp. in light and dark conditions. To perform the video recording, the video camera was placed above the colony and set for interval recordings of 2 s with 28 s breaks. Zooids were monitored for 22 h. After continuous recording for the first 3 h, the recording was paused every second hour for the next 15 h, whereupon recording was continuous for 4 h. In all, 14 h of recording was obtained within the 22 h period. Recordings were performed at *Rhodomonas* sp. concentrations of ca 1500 cells ml^{-1}. The experimental beaker was linked to a 2 l container acting as a buffer to maintain constant algae concentration. A peristaltic pump on the hoses connecting the 2 containers provided water circulation. Particle concentrations were followed by sampling every hour in the adjoining 2 l container to keep animals undisturbed. Feeding activity at different algae concentrations was studied by video recording in the same setup.

To establish a relation between area and weight, exact area measurements were performed on colonies settled on slides. After measuring colony area, colonies were dried for >48 h in 60 to 70°C and weighed. Later, they were ashed at 475°C for a minimum of 4 h, to obtain ash-free dry weight.

RESULTS

Though nearly 100 % of the zooids behind the growing edge of the colony were active, overall zooidal activity was less. Fig. 1 shows the area of active zooids (specific area) in relation to the total colony area. By fitting a linear regression, it was estimated that the specific area was 42 ± 2.7 % (95 % confidence interval, CI) of the total colony area.

The ingestion rate increased with increasing initial cell concentration at concentrations below 8500 cells ml^{-1} (Fig. 2). Between 8500 and 19 000 cells ml^{-1}, results showed some sort of saturation as the ingestion rate reached a maximum. Iteratively fitting data to an Ivlev equation gave a maximum ingestion rate of

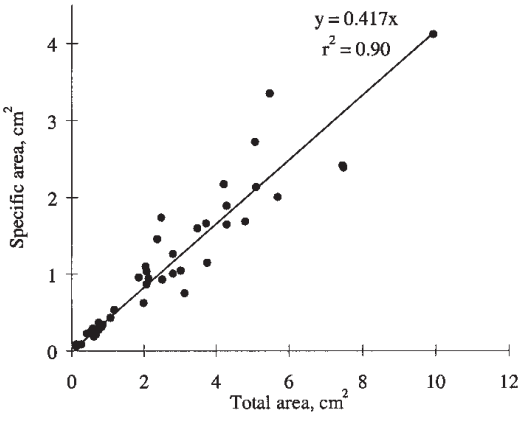

Fig. 1. *Electra crustulenta.* Area of active zooids (specific area) as a function of total colony area. n = 44

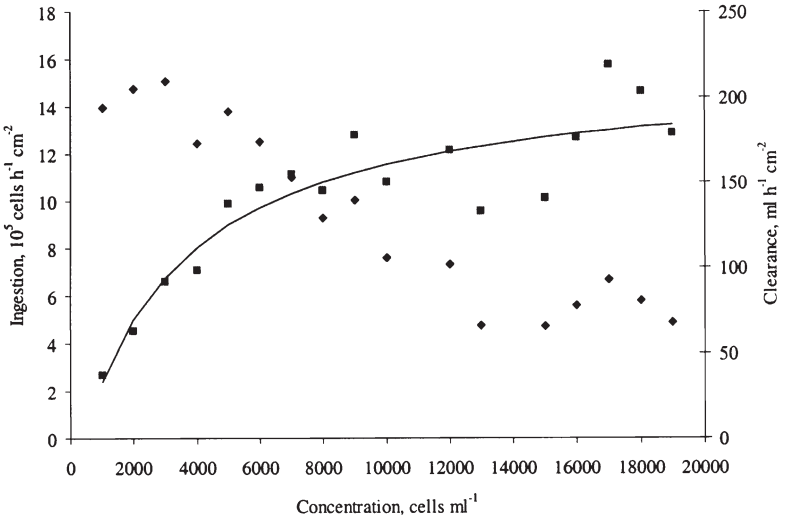

Fig. 2. *Electra crustulenta.* (■) Ingestion and (◆) clearance of *Rhodomonas* sp. as a function of cell concentration. Based on 3 colonies at 18°C. The curve represents a fit to an Ivlev equation: Ingestion, $I = 1.2 \times 10^6 [1 - \exp(-2.3 \times 10^{-4} X)]$

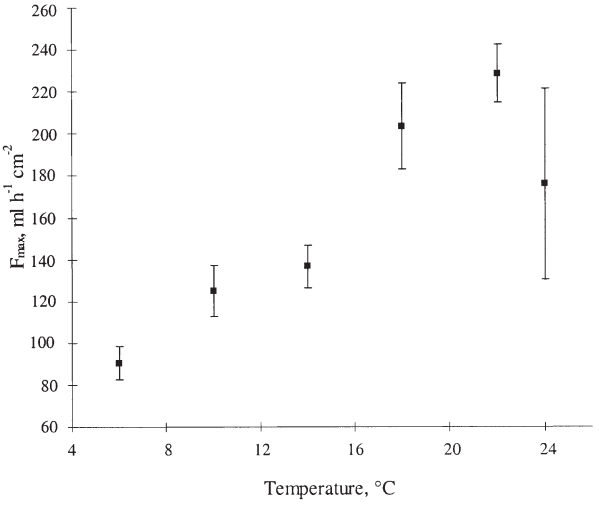

Fig. 3. *Electra crustulenta.* Maximum clearance (F_{max}) as a function of temperature. Bars represent 95% CI

Table 1. *Electra crustulenta.* Clearance at different temperatures, found as the regression of maximum clearance (F_{max}) as a function of specific area. Mean ratio is ratio of specific to total area at each temperature (±95% CI)

Temp. (°C)	Clearance (ml h^{-1} cm^{-2})	p	r^2	No. of colonies	Mean ratio (%)
6	90	<0.001	0.95	9	45 ± 9
10	125	<0.001	0.93	11	39 ± 8
14	137	<0.001	0.95	12	48 ± 4
18	204	<0.001	0.85	12	37 ± 7
22	229	<0.001	0.95	12	46 ± 6
24	176	<0.001	0.41	6	48 ± 14

1.30×10^6 cells h^{-1} cm^{-2}. Mean ingestion rate was calculated at concentrations >8500 cells ml^{-1}. $I = 1.24 \pm 0.16$ (95% CI) 10^6 cells h^{-1} cm^{-2}. Clearance rate decreased from 203 ml h^{-1} cm^{-2} at low cell concentrations to 89 ± 27 (95% CI) ml h^{-1} cm^{-2} at concentrations >8500 cells ml^{-1}.

Effects of temperature were studied on colonies of different sizes at algal cell concentrations well below concentrations leading to saturation (between 2000 and 2800 *Rhodomonas* sp. cells ml^{-1}). Maximum clearance rate for each colony was plotted as a function of specific area at each temperature (as in Fig. 2). The slope of the regressions (Table 1) gives the area-specific clearance rates. Maximum clearance rate increased from 90 ml h^{-1} cm^{-2} at 6°C to 229 ml h^{-1} cm^{-2} at 22°C (Fig. 3). Results at 24°C show a sudden decrease in clearance (176 ml h^{-1} cm^{-2}), and the clearance rates were much more variable between colonies resulting in a wide 95% CI, indicating some sort of stress. $Q_{10} = 1.8$ in the interval 6 to 22°C. At 22°C the area-specific F_{max} was estimated to be 229 ml h^{-1} cm^{-2}. This corresponds to 0.23 ml h^{-1} $zooid^{-1}$, as there were approximately 990 zooids cm^{-2} colony area.

Particle capture was observed for different sized particles (Table 2). In experiments using small particles, only the activity of the zooids was recognized. At larger particle sizes, the particles were distinct dots that could be easily followed under the microscope. Particles smaller than *Fragilidium subglobosum* (48 µm ESD) were captured by the lophophore and disappeared into the mouth. *F. subglobosum* was also retained by the lophophore and led to the mouth, but never ingested. The particles were at the opening of

Table 2. *Electra crustulenta.* Visual observation of ingestion of different particles. Yes = successfully ingested, No = not ingested, Try/no = attempted but not ingested

Particle	Estimated spherical diameter (ESD) (µm)	Ingestion
Small flagellate 1	3.6	Yes
Small flagellate 2	4.3	Yes
Rhodomonas sp.	6.2	Yes
Heterocapsa triqueter	15	Yes
Scripsiella sp.	19	Yes
Prorocentrum micans	23	Yes
Fragilidium subglobosum	48	Try/no
Eggs of *Acartia tonsa*	75	No
Cellulose beads	100–250	No
Biosilon beads	125–250	No

the mouth for <95 s before rejection. Occasionally, a particle was released into the lophophore, and sucked back into the mouth, before final rejection. Particles larger than *F. subglobosum* were immediately rejected, sometimes by an outward bending of a tentacle as soon as the particle reached the lophophore.

In order to expose colonies to approximately the same level of biovolume in all retention efficiency experiments, the initial concentration of algae was reduced with increasing particle size (Fig. 4). However, when counting the particle concentration on the particle counter, the low concentrations were encumbered with greater errors.

The 2 small flagellates were cleared less efficiently than *Rhodomonas* sp. whereas the 2 larger particles, *Heterocapsa triqueter* and *Prorocentrum micans*, were retained as efficiently as *Rhodomonas* sp. (Fig. 5).

In 6 colonies, activity measured as clearance rate was not affected by changes from light to dark (Fig. 6) (2-way ANOVA, between: light/dark $p = 0.28$, colonies $p < 0.001$). Three replicates were performed in light within 2 h, whereupon colonies were kept in darkness for a 4 h period, during which 6 replicates were performed.

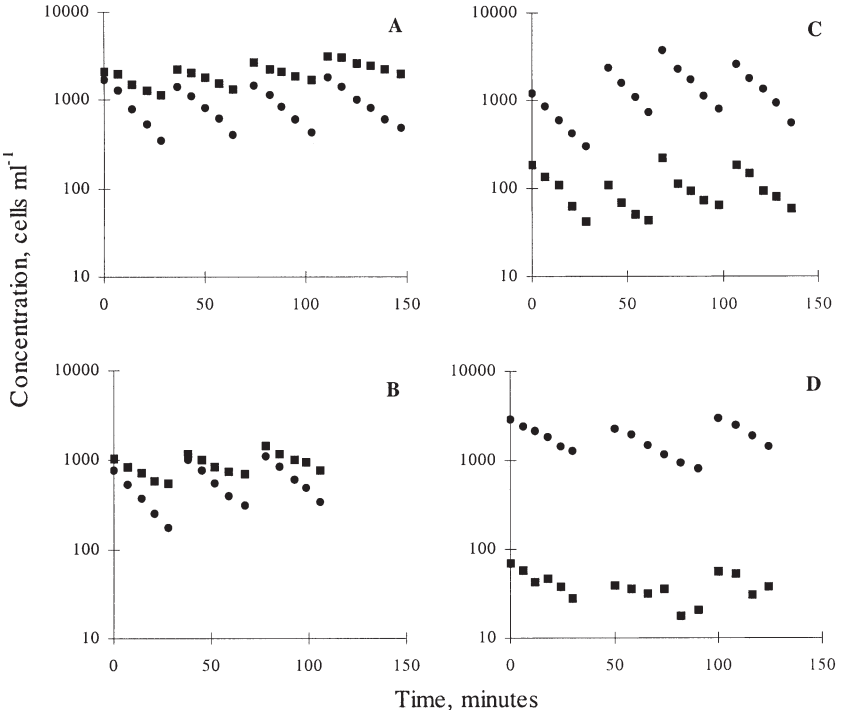

Fig. 4. *Electra crustulenta.* (■) Simultaneous clearance of *Rhodomonas* sp. and (●) algae of other sizes. (A) Small flagellate 1 (3.6 µm ESD), (B) small flagellate 2 (4.3 µm ESD), (C) *Heterocapsa triqueter*, (D) *Prorocentrum micans*

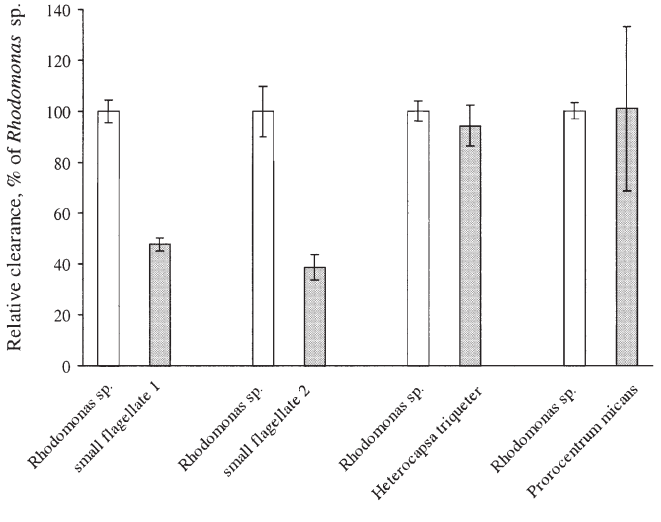

Fig. 5. *Electra crustulenta.* Relative clearance (±1 SE) of *Rhodomonas* sp. and algae of other sizes. Small flagellate 1 (3.6 µm ESD), n = 7. Small flagellate 2 (4.3 µm ESD), n = 4. *Heterocapsa triqueter* (15 µm ESD), n = 19. *Prorocentrum micans* (23 µm ESD), n = 12

In continuous light conditions, 1 colony was followed on a video recording for a 22 h period at a concentration of ca 1500 (1580 ± 75, 1 SE) *Rhodomonas* sp. cells ml^{-1}. Recording was initiated immediately after moving the colony to the experimental beaker. Thus, in calculation of activity/inactivity of zooids, the first 2.5 h of recording were omitted, as behaviour appeared disturbed. The zooids were continuously active (having protruded lophophore) throughout the day, only interrupted by short intervals of retraction into the zooecium. After 10 h, all zooids in the frame were retracted for ca 1 h. This break in activity was considered to be due to some external disturbance of the colony and was also excluded in the calculations. The zooids had protruded lophophores 95% of the total time recorded (Table 3). Activity at other food concentrations were studied on zooids feeding at ca 3000 (2895 ± 271, 1 SE) cells ml^{-1} for 3.5 h and ca 10 000 (10855 ± 238, 1 SE) cells ml^{-1} for 10.5 h. The difference in activity at 1500 and 3000 cells ml^{-1} was not as pronounced when comparing median values rather than means (Table 3). Zooid activity level at 10 000 *Rhodomonas* sp. cells ml^{-1} decreased in comparison to activity at lower food concentrations (Fig. 7). The time of active periods decreased (*F*-test for variance, *t*-test assuming unequal

Table 3. *Electra crustulenta.* Activity of zooids at 3 different *Rhodomonas* sp. concentrations (I, II, III) from video recordings. Activity is time of protruded lophophore and inactivity is time of retracted lophophore

Activity		*Rhodomonas* sp. cells ml^{-1}								
		ca 1500 Zooid			ca 3000 Zooid			ca 10 000 Zooid		
		I	II	III	I	II	III	I	II	III
Active periods (s)	Median	480	450	503	375	345	420	240	180	210
	Mean	836	675	594	388	354	432	340	206	227
95% CI		294	215	136	63	42	76	74	28	27
Inactive periods (s)	Median	30	30	30	30	30	30	60	60	90
	Mean	54	34	29	46	30	31	94	108	116
95% CI		32	8	4	10	3	4	19	20	20
Active (% of total time)		94	95	95	89	92	93	78	66	66
Frequency of retraction/protrusion events (h^{-1})		9	11	12	16	19	15	17	23	21

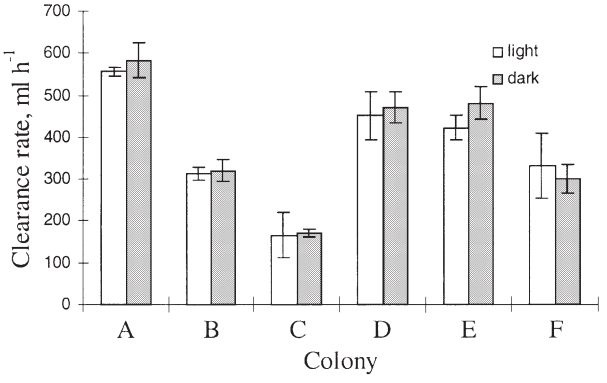

Fig. 6. *Electra crustulenta.* Clearance rate (±95% CI) in 6 colonies during daylight (light) and dark periods. Daylight, 3 replicates; dark, 6 replicates

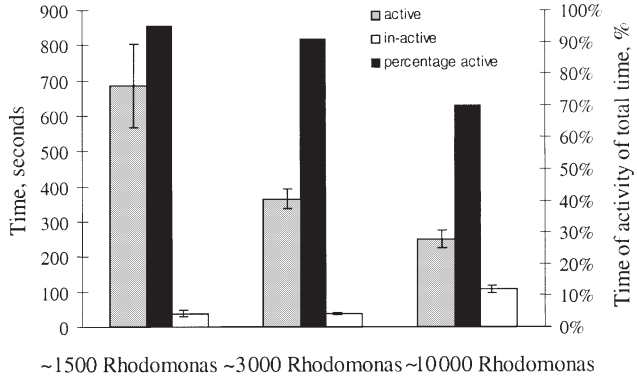

Fig. 7. *Electra crustulenta.* Activity of single zooids of 3 different colonies at 3 *Rhodomonas* sp. concentrations, measured as mean time of protruded lophophore and inactivity as mean time of retracted lophophore. Overall activity level is calculated as % of total time. At 1500 cells ml^{-1}, n = 3; 3000 cells ml^{-1}, n = 4; 10 000 cells ml^{-1}, n = 3. Bars represent 95% CI

variance, $p < 0.001$ at 1500 and 10 000 cells ml^{-1}) and the time of inactivity increased (*t*-test assuming unequal variance, $p < 0.05$ at 1500 and 10 000 cells ml^{-1}), though not in the same order of magnitude, which led to a higher frequency of retraction/protrusion events.

Wet weight (WW), dry weight (DW) and ash-free dry weight (AFDW) of colonies were related to the colony area. The uncertainty in acquiring WW is greater than for DW and this is reflected in a lower correlation between WW and area (Table 4). Correlations of DW were higher for the total colony area than specific area. Specific area excludes the zone of growth and area of inactive zooids, areas with no functional polypide but which hold ample calcium and add to the weight. On the contrary, AFDW correlates with specific area. Zooids in the specific area contained a lot of degradable material.

DISCUSSION

In this study, specific area of *Electra crustulenta* colonies as a function of total area showed a high correlation (Fig. 1). All the colonies used in these experi-

Table 4. *Electra crustulenta.* Area-specific weight found as the slope of the regression between weight and area of colonies. (±95% CI)

		Weight (mg cm^{-2})	r^2	No. of animals
Wet weight	Total area	13.1 ± 1.3	0.91	25
	Specific area	33.2 ± 6.0	0.85	17
Dry weight	Total area	3.9 ± 0.3	0.95	25
	Specific area	12.6 ± 1.4	0.93	17
Ash-free dry weight	Total area	0.46 ± 0.06	0.79	22
	Specific area	2.0 ± 0.1	0.97	17

ments were reared in the laboratory. They grew at constant conditions, which probably induced uniform growth patterns and polypide activity cycling, leading to a high correlation between total and specific area. A variable environment has an influence on brown body formation. Thus, correlation of clearance rate to total area could be less clear in colonies grown in nature, and would vary with season. Barnes & Clarke (1998) found great seasonal variation amongst 3 different species of bryozoans, ranging from peaks of around 75% active zooids during summer months, to periods with <10% during winter.

Results of clearance rate as a function of specific area in the present study showed an increase with temperature. A Q_{10} of 1.8 for clearance rate is in agreement with other bryozoans, which range from 1.4 to 1.7 (Menon 1974, Riisgård & Manríquez 1997, Lisbjerg & Petersen 2000). Clearance rates in bivalves and ascidians (Petersen et al. 1999) also have a Q_{10} of around 2. It has been debated whether the increase in clearance rate is due to physical effects or increased biological activity (e.g. Jørgensen et al. 1990, Riisgård & Larsen 1995, Petersen et al. 1999).

Q_{10} values for metabolic rates are usually between 2 and 3 (Randall et al. 1997). The frequency of the lateral cilia generating the water flow through the lophophore could be limited by enzymatic kinetics in the cells. At increasing temperatures, enzymatic reactions would increase, leading to higher beat frequency of the cilia and thus to increasing clearance rate. Q_{10} values of the clearance rates in *Electra crustulenta* correspond well to Q_{10} values of the beat frequency by the lateral cilia in ascidians and bivalves (Jørgensen et al. 1990, Petersen et al. 1999).

The decline in clearance at 24°C for *Electra crustulenta* (Fig. 2) indicates that the colonies have reached their heat tolerance level. Looking at mortality at increasing temperature, Menon (1972) found the temperature LD_{50} (where half of the zooids were dead within 24 h) for *Membranipora membranacea*, *Electra pilosa*, and *Conopeum reticulum*, to be 25, 28.6, and 29°C, respectively. Menon also noted that colonies of *E. pilosa* and *C. reticulum* grown at 22°C never achieved sexual maturity.

In *Electra crustulenta*, the zooids of the specific area show no difference in diurnal activity. Barnes & Clarke (1994) conducted a 24 h study of 2 Antarctic species by looking at numbers of protruded lophophores from photos, which showed no diurnal variation in activity. Snapshots of numerous zooids showed an activity level of ca 96%. Barnes & Clarke (1994) estimated activity in the most active region, just behind the outer growing zone. In this region polypide degeneration rarely occurs and all zooids could be considered as having the ability to be active. The estimated activity level can therefore be interpreted as 1 zooid on average being active 96% of the time. This is in agreement with single zooid activity at low food concentrations in this study (Fig. 7), in which activity was only interrupted by short periods of retraction. These results indicate that undisturbed zooids are almost continuously filtering. However, frequency and duration of shutting down increased at higher food concentrations. A similar behaviour pattern has been observed in ascidians, where frequency and duration of squirting increases (Petersen et al. 1999). In this study, the phenomenon was observed at food concentrations of about 10 000 cells ml^{-1}, which is well above saturation concentration (Fig. 2).

Ingestion rate shows a typical functional response. Functional response has also been found in numerous studies of other suspension-feeders (e.g. Petersen & Riisgård 1992, Petersen et al. 1995). Ingestion rate reaching a maximum indicates some sort of regulatory mechanism. Whether suspension-feeders are capable of regulating ingestion is the basis for much discussion. However, in bryozoans several mechanisms could be of relevance. In laboratory experiments at very high algae concentrations, bryozoans have shown expulsions of boli formed in the pharynx (Hunter & Hughes 1993, Best & Thorpe 1994). This behaviour would lead to decreased ingestion. In the present study, direct observations showed no expulsion of boli, and this behaviour is probably induced only at extremely high algae concentrations. Furthermore, boli formation would lead to an overestimate of clearance rates using methods measuring decline in algae concentration, as in this study. However, the levelling off of ingestion rate was followed by a corresponding decrease in clearance rate. The decrease in clearance rate at food concentrations from 1500 to 10 000 cells ml^{-1} was ca 50%. The results of zooidal activity showed a decline in feeding periods, which could serve as an explanation for the reduced ingestion. Total time of active feeding periods decreased ca 26%, but the longer shutdown periods at high cell concentrations only account for about half of the decreased clearance rate. This implies that other regulatory mechanisms also may be of relevance. As observed, zooids, when feeding on larger particles, make an outward bending of the tentacle. This behaviour, which would make the lophophore filter more leaky and thus lead to a lower retention and a decrease in clearance rate, was not evident from the video recordings in this study. Finally, a shift in beat frequency of the lateral cilia on the tentacles would alter clearance rate and thus could act as a regulation mechanism. This has not been investigated for bryozoans, but Petersen et al. (1999) found a decrease in beat frequency of the cilia at increasing algae concentrations in an ascidian. The regulating mechanisms leading to the constant particle ingestion rate might be a reaction

to an overloading of the digestive system as in ascidians (Petersen & Riisgård 1992, Petersen et al. 1999). As a consequence, large particles, rather than small particles, would lead to gut fullness and regulation of the clearance rate at lower concentrations.

In this study the functional response was found using *Rhodomonas* sp., which is one of the smallest particles retained fully by the filter for *Electra crustulenta* (Fig. 5). The minimum size range of particles is limited due to the structure of the filter, i.e. the distance between laterofrontale cilia (Nielsen & Riisgård 1998). *E. crustulenta* is able to retain and feed on particle sizes from 5 µm to at least 23 µm (Table 2). Particles larger than 23 µm are retained by the filter, though they might not be ingested. For example, *Fragilidium subglobosum* cells were retained by the lophophore but were not ingested by the zooids. The size of the mouth gives a physical limit to the collected particles that can be ingested. *E. crustulenta* zooids have a mouth of about 30 µm in diameter. In a study of 53 species, it was shown that size of the mouth ranged from 15 to 91 µm. Species with mouths smaller than 30 µm in diameter have round mouths, whereas in larger species they have elongated shapes (Winston 1978). Thus, restrictions in mouth size and form indicate that bryozoans would favour small particles without appendages as a food source. This might be single-celled algae or small chain-forming species with few spines.

Within the size range that bryozoans are able to ingest, results show a maximal retention for *Rhodomonas* sp. and larger particles, i.e. a retention efficiency of 100% of the laterofrontal cilia (RE_{lfc}). Considering this as the maximum for the bryozoan filter, Riisgård & Manríquez (1997) found a ratio between clearance rate (F) and pumping rate (Q) of 0.26 for single zooids of *Electra pilosa*. This indicates that, due to the leaky structure of the lophophore filter, only about 25% of the water pumped through the lophophore is filtered, thus the retention efficiency of the tentacle crown (RE_t) is 25%. In this comparison of F and Q, the level of F is estimated using the general formula,

$$F = V \times \ln(C_0/C_t)/(n \times t) \quad (3)$$

where n is the number of zooids, t is time, V is the volume of the beaker and C the algal cell concentration. However, in this formula, it is assumed that the zooids are constantly active during the experimental period, which is not necessarily the case (Table 3). Even at low food concentrations, zooids have periods of inactivity. Clearance is therefore performed by less zooids than expected from pictures of the colony area, as a fraction of the feeding zooids are inactive at all times. Calculating clearance per zooid thus underestimates clearance of the zooid in its active periods. When comparing zooidal clearance with pumping rate, activity accounts for some of the difference and not all of the difference, between F and Q is due to the open structure of the lophophore. However, at low algae concentrations the zooidal activity is 95% and the error in assuming 100% activity is small. RE_t is 30% for zooids of *Electra crustulenta*, based on the pumping rate found by Riisgård & Manríquez (1997). The corrected efficiency, based on an activity level of 95%, is 32%.

The periodical shutting down at low algal cell concentrations has not previously been considered in calculations. It has been argued that the shutting down is an unnatural phenomenon caused by the stress of high particle load in the laboratory which in turn would affect colony growth rate (Riisgård & Goldson 1997). Several studies of bryozoan growth rates show no decrease in growth rate at *Rhodomonas* sp. cell concentrations from 4000 to 200 000 cells ml^{-1} (Hunter & Hughes 1993, Bayer et al. 1994, Riisgård & Goldson 1997). Only at 300 000 cells ml^{-1} (ca 375 µg chl *a* l^{-1}) did cell concentration cause a decreased growth rate (Hunter & Hughes 1993). This implies that despite the increased time of shutting down at high algae concentrations, the animals are in good condition, and shutting down could be a natural regulating mechanism.

Previous studies have concluded that bryozoans have no physiological regulation of the filter-pump in nature. Bryozoans utilize their clearance capacity because the lowest algal concentration that causes maximum growth rate corresponds to the mean summer phytoplankton levels in fjords and coastal waters (Riisgård & Goldson 1997). However, the bryozoans do not experience mean concentrations. They are exposed to actual fluctuations in the benthic boundary layer, that are depending on factors like season, currents, sunlight and wind. For example, mean concentration of 8 µg chl *a* l^{-1} in the Port Erin Bay area (Isle of Man, Northern Irish Sea) is based on concentrations ranging from 3 to 16 µg chl *a* l^{-1} due to tidal cycling (Sanderson et al. 1996). A concentration of 16 µg chl *a* l^{-1} corresponds to ca 13 000 cells ml^{-1} of *Rhodomonas* sp. (Riisgård & Goldson 1997), which is well above saturation concentration for *Electra crustulenta*. Variation has also been found in microtidal areas (Petersen et al. 1997). They measured daily particle concentrations for 16 d in a cove belonging to the same fjord system as Roskilde fjord, and found concentrations between 1 and 18 µg chl *a* l^{-1} at depths 15 to 100 cm from the bottom. *E. crustulenta* colonies on the leaves of *Zostera marina* would be exposed to these concentrations and would probably react to the high chlorophyll levels by increasing time periods of shutting down, and use of the other regulation mechanisms previously discussed.

Bryozoans may not actually be exposed to the chl *a* concentrations mentioned, as a boundary layer would

affect particle availability, but this aspect was not investigated in this study. Also, the discussion of particle load is limited to the chl *a* fraction and does not consider the non-fluorescent particle fraction, which would contribute to the load that bryozoans are exposed to.

In conclusion, bryozoans are indeed influenced by external factors like temperature and particle concentration. Shutting down is a natural phenomenon and contributes to the regulation of clearance rate in bryozoans at high particle concentrations. This behaviour is not a symptom of stress, as other studies show that bryozoans maintain maximum growth rates at high particle concentrations.

Acknowledgements. We thank Dr Bent Vismann at the Marine Biological Laboratory of the University of Copenhagen for fruitful discussions. We are most grateful to Jacob Carstensen for statistical advice, and Sebastian Knudtzon for valuable suggestions on improving the manuscript.

LITERATURE CITED

Barnes DKA, Clarke A (1994) Seasonal variation in the feeding activity of four species of Antarctic bryozoan in relation to environmental factors. J Exp Mar Biol Ecol 181: 117–133

Barnes DKA, Clarke A (1998) Seasonality of polypide recycling and sexual reproduction in some erect Antarctic bryozoans. Mar Biol 131:647–658

Bayer MM, Cormack RM, Todd CD (1994) Influence of food concentration on polypide regression in the marine bryozoan *Electra pilosa* (L.) (Bryozoa: Cheilostomata). J Exp Mar Biol Ecol 178:35–50

Best MA, Thorpe JP (1983) Effects of particle concentration on clearance rate and feeding current velocity in the marine bryozoan *Flustrellidra hispida*. Mar Biol 77:85–92

Best MA, Thorpe JP (1986) Effects of food particle concentration on feeding current velocity in six species of marine Bryozoa. Mar Biol 93(2):255–263

Best MA, Thorpe JP (1994) Particle size, clearance rate and feeding efficiency in marine Bryozoa. In: Hayward PJ, Ryland JS, Taylor P (eds) Biology and palaeobiology of bryozoans. Olsen and Olsen, Fredensborg, p 9–14

Bullivant JS (1968) The rate of feeding of the Bryozoan, *Zoobotryon verticillatum*. NZ J Mar Freshw Res 2:111–134

Clausen I, Riisgård HU (1996) Growth, filtration and respiration in the mussel *Mytilus edulis*: no evidence for physiological regulation of the filter-pump to nutritional needs. Mar Ecol Prog Ser 141:37–45

Flindt MR, Kamp-Nielsen L, Marques JC, Pardal MA, Bocci M, Bendoricchio G, Salomonsen J, Nielsen SN, Jørgensen SE (1997) Description of the three shallow estuaries: Mondego River (Portugal), Roskilde Fjord (Denmark) and the Lagoon of Venice (Italy). Ecol Model 102:17–31

Hayward PJ, Ryland JS (1990) The marine fauna of the British Isles and northwest Europe, Vol 2. Molluscs to chordates. Oxford University Press, New York

Hunter E, Hughes RN (1993) The effect of cell concentration on colony growth and feeding in the bryozoan *Celleporella hyalina*. J Mar Biol Assoc UK 73:321–331

Jørgensen CB, Larsen PS, Riisgård HU (1990) Effects of the temperature on the mussel pump. Mar Ecol Prog Ser 45: 205–216

Lisbjerg D, Petersen JK (2000) Clearance capacity of *Electra bellula* (Bryozoa) in seagrass meadows of Western Australia. J Exp Mar Biol Ecol 244:285–296

Menon NR (1972) Heat tolerance, growth and regeneration in three North Sea bryozoans exposed to different constant temperatures. Mar Biol 15:1–11

Menon NR (1974) Clearance rates of food suspension and food passage rates as a function of temperature in two North Sea bryozoans. Mar Biol 24:65–67

Nielsen C, Riisgård HU (1998) Tentacle structure and filter-feeding in *Crisia eburnea* and other cyclostomatous bryozoans, with a review of upstream-collecting mechanisms. Mar Ecol Prog Ser 168:163–186

Petersen JK, Riisgård HU (1992) Filtration capacity of the ascidian *Ciona intestinalis* and its grazing impact in a shallow fjord. Mar Ecol Prog Ser 88:9–17

Petersen JK, Schou O, Thor P (1995) Growth and energetics in the ascidian *Ciona intestinalis*. Mar Ecol Prog Ser 120: 175–184

Petersen JK, Schou O, Thor P (1997) In situ growth of the ascidian *Ciona intestinalis* (L.) and the blue mussel *Mytilus edulis* in an eelgrass meadow. J Exp Mar Biol Ecol 218:1–11

Petersen JK, Mayer S, Knudsen MÅ (1999) Beat frequency of cilia in the branchial basket in the ascidian, *Ciona intestinalis*, in relation to temperature and algal cell concentration. Mar Biol 133:185–192

Randall D, Burggren W, French K, Fernald R (1997) Eckert animal physiology: mechanisms and adaptations. WH Freeman and Company, New York

Riisgård HU, Goldson A (1997) Minimal scaling of the lophophore filter-pump in ectoprocts (Bryozoa) excludes physiological regulation of filtration rate of nutritional needs. Test of hypothesis. Mar Ecol Prog Ser 156:109–120

Riisgård HU, Ivarsson NM (1990) The crown-filament pump of the suspension-feeding polychaete *Sabella penicilus*: filtration, effects of temperature, energy cost, and modelling. Mar Ecol Prog Ser 62:249–257

Riisgård HU, Larsen PS (1995) Filter-feeding in marine macro-invertebrates: pump characteristics, modelling and energy cost. Biol Rev 70:67–106

Riisgård HU, Manríquez P (1997) Filter-feeding in fifteen marine ectoprocts (Bryozoa): particle capture and water pumping. Mar Ecol Prog Ser 154:223–239

Riisgård HU, Thomassen S, Jakobsen H, Weeks JM, Larsen PS (1993) Suspension feeding in marine sponges *Halichondria panicea* and *Haliclona urceolus*: effects of temperature on filtration rate and energy cost of pumping. Mar Ecol Prog Ser 96:177–188

Ryland JS (1976) Physiology and ecology of marine bryozoans. Adv Mar Biol 14:285–443

Sanderson WG, Harding SP, Thorpe JP (1996) An investigation of the effects of tidal fluctuations on the availability of seston as food for intertidal Bryozoa. In: Gordon DP, Smith AM, Grant-Mackie JA (eds) Bryozoans in space and time. National Institut of Water and Atmospheric Research, Wellington, p 259–269

Winston JE (1978) Polypide morphology and feeding behavior in marine ectoprocts. Bull Mar Sci 28(1):1–31

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: February 22, 2000; Accepted: September 6, 2000
Proofs received from author(s): April 30, 2001