

# Flow and conduit formation in the external fluid-transport system of a suspension feeder

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## Summary

To what extent is the development of a fluid-transport system related to flow within the system? Colonies of the bryozoan *Membranipora membranacea* have a simple external fluid-transport system with three components: the canopy of lophophores (crowns of ciliated tentacles), the edge of the canopy, and chimneys (raised openings in the canopy). The lophophores pump seawater into the colony and capture food particles from the seawater. The chimneys and canopy edge let the water back out of the colony. New chimneys form at the canopy edge as the colony grows. I tested whether there was a correlation between chimney formation and excurrent flow speed at

the canopy edge by measuring excurrent flow speeds prior to chimney formation. Excurrent flow speeds were higher in regions that produced chimneys than in regions that did not form chimneys. Observations of changes in chimney shape after anesthetization with  $MgCl_2$  suggest that both growth and behavior determine chimney shape. Together, the results suggest that there is a strong correlation between growth and flow in this external fluid-transport system, with new chimneys forming at sites of high flow.

Key words: fluid-transport system, Bryozoa, suspension feeding, biomechanics, development.

## Introduction

Systems that transport fluids are important for many different functions in different organisms (LaBarbera, 1990; LaBarbera and Vogel, 1982). For example, the vertebrate circulatory system and the gastro-vascular system of hydroids are internal fluid-transport systems that carry oxygen, nutrients or wastes through pipe-like conduits inside the organism (LaBarbera, 1990; LaBarbera and Vogel, 1982). Many other fluid-transport systems are used for suspension feeding or gas exchange and have conduits that form simple openings onto the ambient fluid, such as the oscula and ostia of sponges (LaBarbera, 1990; LaBarbera and Vogel, 1982). These openings allow the organism to pump the ambient fluid through itself to capture food particles or oxygen.

Fluid flow affects the development of pipe-like, internal conduits in a number of internal fluid-transport systems. In vertebrates, increasing the flow rate through blood vessels causes them to increase in diameter, and reducing the flow rate causes them to decrease in diameter (Kamiya and Togawa, 1980; Langille, 1995). Fluid flow appears to have similar effects on the dimensions of conduits in the gastro-vascular system of hydroids (Buss, 2001; Dudgeon and Buss, 1996) and, possibly, the veins of plasmodial slime molds (Nakagaki et al., 2000). Fluid flow also appears to affect the rate of formation of new conduits in the vertebrate circulatory system and, possibly, the hydroid gastrovascular system, with higher flow rates inducing more conduit formation (Brown and Hudlicka, 2003; Buss, 2001; Dudgeon and Buss, 1996; Prior et al., 2004).

In systems involved in suspension feeding and gas exchange, does fluid flow affect the formation of conduits that form openings to the ambient fluid? The bryozoan *Membranipora membranacea* Linnaeus 1767 is a good system in which to investigate this question because colonies of *M. membranacea* possess a simple, external fluid-transport system that is amenable to flow visualization. Colonies of *M. membranacea* consist of an array of physiologically connected individuals (zooids) bearing crowns of ciliated tentacles (lophophores; Fig. 1). The lophophores form a canopy over most of the colony, broken by excurrent openings called chimneys (Fig. 1; Banta et al., 1974). The chimneys are formed by groups of lophophores that lean away from each other and are held higher than their neighbors (Fig. 1B,C; Banta et al., 1974; Dick, 1987; Lidgard, 1981). The lophophores capture suspended food particles from seawater that they pump from above the colony, between their tentacles and then under the canopy to exit the colony either at the canopy edge or at one of the chimneys (Fig. 1B–D). Dick (1987) suggested that the difference in height between chimney and non-chimney lophophores results from differences in growth between zooids, based on the apparent absence of muscles that could account for the differences in lophophore shape.

Previous authors have suggested that fluid flow determines where new chimneys form in *M. membranacea* (Dick, 1987; Grünbaum, 1997; Okamura and Partridge, 1999). The spacing of the chimneys depends on the ambient flow speed in which

the colonies are grown (Okamura and Partridge, 1999) and on the presence or absence of spines (Grünbaum, 1997), an inducible defense produced by the bryozoans against predatory nudibranchs (Harvell, 1984). The spines stick up between the lophophores, so they may affect chimney spacing by interfering with the flow under the canopy (Grünbaum, 1997). The shape of the chimneys is also affected by the shape of the substrate (which determines the shape of the colony), possibly because the pattern of flow around and through the colony depends on the substrate shape (Grünbaum, 1997). However, the position of existing chimneys does not appear to be affected by either changes in the ambient flow speed or by injury to the colony (von Dassow, 2005). This suggests that fluid flow may affect where new chimneys form but may not affect their position after they have formed (von Dassow, 2005).

What local hydrodynamic factors control chimney formation? Previous authors suggested that high pressure under the canopy might induce chimney formation (Dick, 1987; Larsen and Riisgard, 2001). However, chimneys form at the canopy edge, which is a region of excurrent flow (Fig. 1A; von Dassow, 2005). Hydrodynamic models suggest that the canopy edge should be a site of low pressure (Grünbaum, 1995; Larsen and Riisgard, 2001), so that hypothesis is not supported (von Dassow, 2005). An alternative hypothesis is that high excurrent flow speed at the canopy edge induces chimney formation (von Dassow, 2005). This hypothesis predicts that there should be a positive correlation between high excurrent flow speed and chimney formation at the canopy edge.

In this study, I tested whether chimney formation correlates with differences in local excurrent flow speed at the canopy edge and whether chimney morphology results from differences in growth between zooids. To test whether chimneys form at sites of high excurrent flow speed at the canopy edge, I measured the excurrent flow speeds at sites along the canopy edge prior to chimney formation. To determine whether the difference in height between chimney and non-chimney lophophores depends on growth or behavior, I anesthetized colonies with  $\text{MgCl}_2$  and observed the changes in height of the chimney lophophores relative to the canopy lophophores.

## Materials and methods

### *Measurements of flow at the canopy edge*

I measured excurrent flow speeds at the canopy edge by tracking particle trajectories in videos of the canopy edge in plan-view (Fig. 1D). Colonies were illuminated with a sheet of red (635  $\mu\text{m}$ ) laser light using a World Star Tech (Toronto, ON, Canada) diode laser. The sheet of laser light was <1 mm thick and oriented parallel to the colony surface. The laser light was focused at the level of the stalks supporting the lophophores (Fig. 1B,D) to maximally illuminate the excurrent flow.

For flow visualization, I used particles of carmine alum lake (Allied Chemical and Dye Corp., New York, NY, USA) at a concentration of  $6.7 \times 10^{-6} \text{ g ml}^{-1}$ . A stock suspension of carmine in seawater ( $2.0 \times 10^{-3} \text{ g ml}^{-1}$ ) was prepared fresh

every day and filtered through 40  $\mu\text{m}$  Nitex mesh. I made a new stock suspension every day because I noticed that the concentration of particles seemed to change over the course of several days. Aliquots of this stock suspension were mixed thoroughly into the seawater in the video tank.

Most particles appeared to be between 30 and 50  $\mu\text{m}$  in diameter in the videos, but there may have been smaller particles below the limit of resolution. The carmine did not appear to clump, but there were a few particles up to 100  $\mu\text{m}$  in diameter. These larger particles may have been naturally occurring debris or plankton in the seawater.

The colonies were videotaped in plan-view every other day until 2 days after a chimney first appeared. The colonies were videotaped using a Watec 902 low-light, analog video camera and a macro lens (Watec Co. Ltd, Kawasaki City, Japan). In the video taken 2 days prior to the appearance of the chimney, 10 s of video (recorded approximately 1 min after the addition of the carmine) was captured using a Scion LG-3 frame-grabber (Scion Corp., Frederick, MD, USA) and a Hotronic AR31 TBC/Frame Synchronizer (Hotronic Inc., Campbell, CA, USA) using Scion Image 1.62A software (Scion Corp.). Videos were processed and analyzed in NIH Image 1.62. The odd and even video fields were separated to give a framing rate of 60 video fields per second.

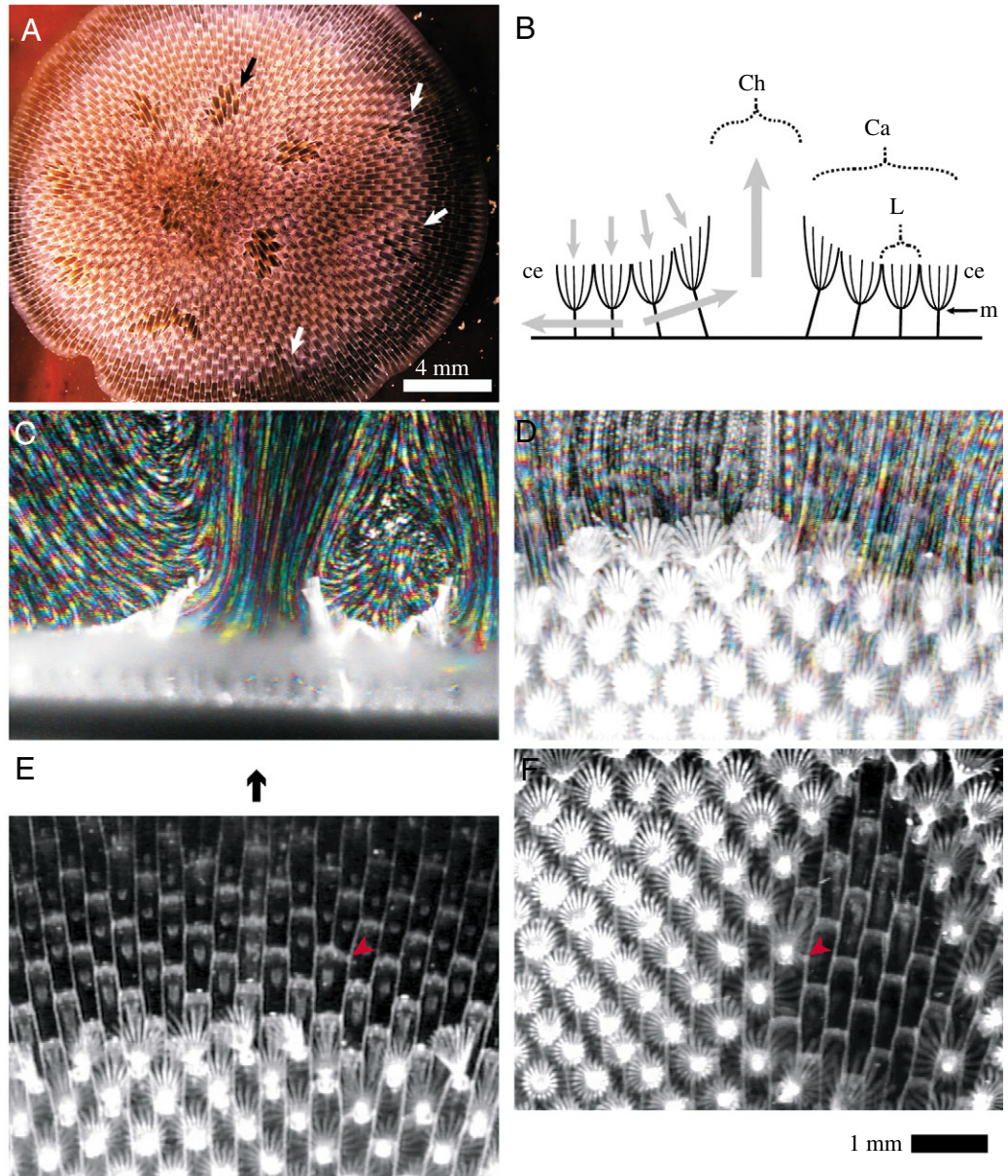
In each video field, particles appeared as short streaks. A series of 10–22 fields were superimposed, skipping every other field. Using this method, the trajectory of each particle appears as a series of streaks, spaced apart much as in a strobe photograph. To calculate particle speeds near the canopy edge, I measured the distance from the end of one streak (within 0.35 mm from the canopy edge) to the end of the next streak in the series of streaks produced by the particle. I tested this method for measuring particle speeds by filming carmine dust moving at known, calibrated speeds of  $10 \text{ mm s}^{-1}$  and  $5 \text{ mm s}^{-1}$ . The measured speeds of individual particles were within 7% of the calibrated speeds (this error is small relative to the variability in particle speeds at any given location on the canopy edge; Fig. 2A).

Each zooid in an *M. membranacea* colony produces one, or occasionally two, daughter zooids so that the zooids appear in regular columns that occasionally branch (Fig. 1E,F). I sorted the zooid columns into two groups: (1) ‘chimney zooid columns’ were the zooid columns that formed the new chimney and (2) ‘non-chimney zooid columns’ were those that did not. I took the mean particle speed (measured 2 days prior to chimney formation) at the canopy edge at each zooid column to calculate the excurrent flow speed at each zooid column. Only zooid columns with at least three measurements of particle speed were included. To compare the chimney-forming regions with the non-chimney-forming regions, I calculated the median excurrent speed of the chimney zooid columns and of the non-chimney zooid columns for each colony.

### *Colony collection and culturing*

For this experiment, colonies of *M. membranacea* growing on various species of foliose red algae were collected from the

Fig. 1. (A) A colony in plan-view: a fully formed chimney (black arrow) and partially formed chimneys at the canopy edge (white arrows). (B) A diagram of a colony viewed from the side: Ch, chimney; Ca, canopy; L, lophophore; ce, canopy edge; m, mouth position at lophophore base; gray arrows, direction of flow. (C) Flow into a colony and out of a chimney (scale bar for C–F is in F). Successive frames from a black-and-white video were colored and superimposed so that moving particles appear as rainbow streaks. The streak length is proportional to the particle speed. Lophophores appear white since they did not move. The colony was viewed from the side and illuminated with a light sheet perpendicular to the colony surface. (D) An image of the flow out of the canopy edge 2 days before chimney appearance, produced as in C but with the colony in plan-view, illuminated with a light sheet at the level of the mouths. (E) A video frame showing the canopy edge of the colony in D, in plan-view, 2 days before chimney appearance. Note the columns of zooids (rectangular boxes) oriented in the direction of growth (black arrow). Red arrowheads indicate the same point on the colony in E and F (on the left edge of the chimney in F). (F) A video frame of the same colony 2 days after chimney appearance.



dock at the Friday Harbor Laboratories, Friday Harbor, WA, USA. Pieces of colonies were gently peeled off the algal blade and glued, using Duro™ ‘Quick Gel™’ superglue (Loctite Corp., Cleveland, OH, USA), to pieces of black acrylic that were 1.2–1.4 cm wide, 5 cm long and 0.3 cm thick. Colonies were transferred to pieces of acrylic because the algae often decays within several days of collection. The acrylic pieces were wide enough for two chimneys to form along the width of the piece. The colonies were allowed to grow onto the acrylic for several days in sea tables with running seawater until they were used for an experiment.

During the experiment, I grew all the colonies in still water to insure that measurements of excurrent flow were done under the same ambient flow conditions as those in which the chimneys formed. I fed the colonies daily with *Rhodomonas* at a concentration of  $1.2 \times 10^6$  to  $2.1 \times 10^6$  cells  $\text{cm}^{-2}$  of colony (resulting in approximately  $2.3 \times 10^3$  to  $5.6 \times 10^3$  cells  $\text{ml}^{-1}$  in

the aquarium). I changed the seawater in the aquarium daily. In addition, I used a pump on a timer to stir the seawater in the aquarium for 15 min every 2 h. The aquarium was cooled by keeping it in a sea table with running seawater. The temperature in the aquarium ranged from 12 to 16°C (median, 13°C). Under these conditions, the colony edge grew at  $\sim 0.3$ – $1.8$  mm  $\text{day}^{-1}$  (median, 0.8 mm per  $\text{day}^{-1}$ ).

The videos were made in still water in a small video tank. The video tank was cooled by running seawater past one wall. The difference in temperature between the video tank and the aquarium was  $-0.5^\circ\text{C}$  to  $1.5^\circ\text{C}$  (median,  $0.5^\circ\text{C}$ ).

After I videotaped the colonies for the last time, I used these colonies in the anesthetization experiment described below.

#### *Components of velocity perpendicular to the colony surface*

In the experiment described above, only the components of velocity parallel to the colony surface were measured. To



determine how much the component of velocity perpendicular to the colony surface contributes to the excurrent flow speed, I made videos with the sheet of laser light oriented in a plane perpendicular to both the colony surface and the canopy edge. I calculated the components of excurrent velocity and the resultant excurrent flow speed at the canopy edge, based on particle trajectories in the same manner as described above. Colonies were grown and videotaped following the protocols described above.

#### *Testing for an effect of carmine on excurrent flow speed and growth*

I tested for an effect of carmine on excurrent flow speed by measuring the change in excurrent flow speed after adding either carmine suspension or plain seawater (as a control). Carmine suspension was prepared as described above. For this experiment, the colonies were collected growing on pieces of laminarian kelp. Pieces of algae with small colonies (0.4–1.5 cm diameter) were collected and glued to pieces of acrylic using Duro Quick Gel superglue. The colonies were left for 1 day in the sea table prior to use. Filming was done as described above with the laser sheet parallel to the colony surface.

To test for an effect of carmine on flow speed, I measured the ratio of the flow speed after adding either carmine or seawater (as a control) to the flow speed before adding the carmine or seawater. Particles occurring naturally in the seawater were used to measure flow speeds before adding carmine and in the seawater controls. To measure particle speeds, I traced particle positions by hand on a transparency taped to a video monitor. I calculated the ratio of the flow speed after adding carmine (or seawater) to the initial flow speed by taking the average ratio of speeds for 5–6 pairs of particles distributed along the canopy edge. The speed of the first particle in each pair was measured prior to adding the carmine or the seawater control. I chose the second particle in each pair by finding the particle – appearing 50–70 s after adding the carmine or seawater control – that came the closest to the initial position of the first particle in the pair.

I tested for an effect of carmine on colony growth by exposing colonies to carmine twice daily for 5 days. The colonies were grown in the sea table and placed in dishes with  $6.7 \times 10^{-6}$  g ml<sup>-1</sup> carmine in seawater for 10 min twice each day for 5 days to simulate carmine exposure during experiments on chimney formation. The diameter in two orthogonal directions was measured with an ocular micrometer before and after the start of the experiment. Growth was measured as the average of the ratio of the diameter of the colony after the experiment to its diameter before the experiment for the two directions.

#### *Anesthetization experiments*

I observed chimneys in colonies anesthetized with 0.35 mol l<sup>-1</sup> MgCl<sub>2</sub> to test whether the difference in height between chimney and non-chimney lophophores depends on muscle activity. MgCl<sub>2</sub> is commonly used as an anesthetic for

marine invertebrates (Kaplan, 1969). I measured the ‘chimney height’ – the maximum height difference between the tentacles on the chimney lophophores and the tentacles on canopy lophophores – before and after anesthetization. Seawater was replaced by draining the seawater completely and then pouring in either 0.35 mol l<sup>-1</sup> MgCl<sub>2</sub> or seawater. The lophophores re-extended quickly after replacing the seawater with either MgCl<sub>2</sub> or seawater in this sudden manner but tended to retract and remain retracted if I tried to increase the MgCl<sub>2</sub> concentration gradually. Measurements were made 15–22 s prior to replacing the seawater with 0.35 mol l<sup>-1</sup> MgCl<sub>2</sub> and 1.6–2.0 min after replacing the seawater. Colonies were illuminated with a sheet of laser light perpendicular to the colony surface and videotaped from the side. The colonies were held so that the lophophores faced downward. Colonies were grown as described for the experiment on differences in excurrent flow between sites that formed chimneys and sites that did not form chimneys.

To test whether or not the colonies were actually anesthetized, I touched the colonies with a wooden probe (~1.5 mm diameter) and observed whether or not the lophophores retracted. Lophophores in *M. membranacea* colonies normally retract extremely quickly in response to disturbance (Thorpe et al., 1975). The measurements of chimney height were made between 9 and 22 s before touching the colonies to see if the colonies were anesthetized.

#### *Statistics*

Statistical tests were done in Statview 5.0 for Macintosh (SAS Institute, Inc., Cary, NC, USA). Calculations of confidence intervals for the experiments testing for effects of carmine on growth and excurrent flow speed were implemented in Mathematica 3.0 for Macintosh (Wolfram Research, Inc., Champaign, IL, USA). I used non-parametric tests because they require fewer assumptions than parametric tests. Box plots show median, 1st and 3rd quartile, 1st and 9th decile, and minimum and maximum values.

## **Results**

### *Excurrent flow speed at the canopy edge*

There was high variability in flow speed at the canopy edge at spatial scales smaller than 1 mm (smaller than the size of chimneys; Fig. 2A; Lidgard, 1981). There was also a broad range in the speeds of individual particles measured at any single position along the canopy edge (Fig. 2A). The particle speed data was smoothed by calculating the mean speed of all particles appearing in each zooid column (Fig. 2B). Only zooid columns in which  $\geq 3$  particles were visible were used.

To test for a difference in the excurrent flow speed between regions that formed chimneys and adjacent regions that did not form chimneys, in each of seven colonies, the median speed was calculated 2 days prior to chimney formation, both for zooid columns that formed chimneys and for those that did not (Fig. 2C). The excurrent flow speed was significantly greater in regions that subsequently formed chimneys than in regions

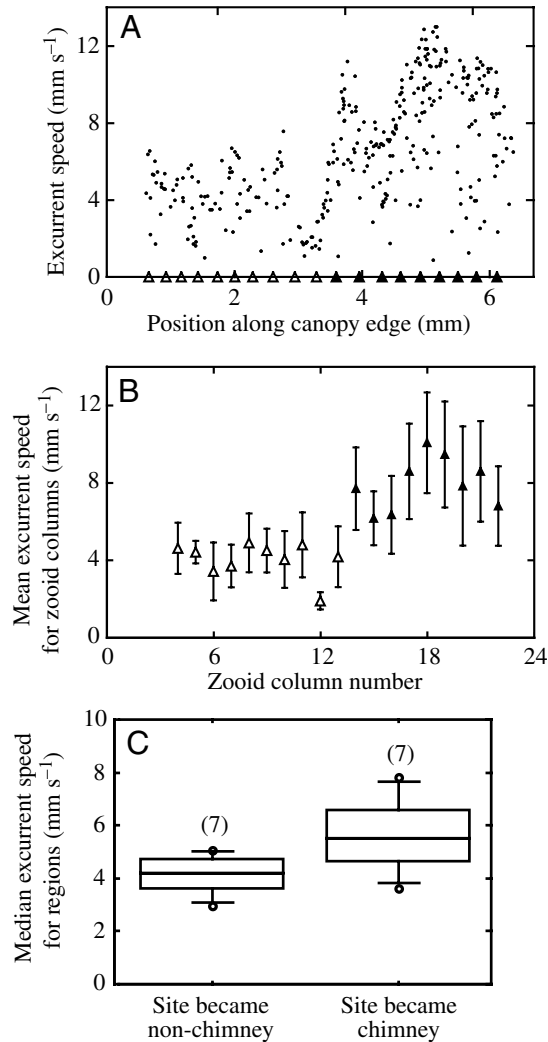


Fig. 2. (A) A plot of particle speeds at the canopy edge in the colony shown in Fig. 1D–F. Triangles indicate center positions of zooids at the canopy edge: zooid columns that formed part of the chimney after 4 days (filled triangles) and zooid columns that did not form a chimney (open triangles). (B) Mean particle speed ( $\pm$  S.D.) in each zooid column of the colony in A: chimney zooid columns (filled triangles) and non-chimney zooid columns (open triangles). (C) Box-plot of median excurrent flow speed in regions that subsequently gave rise to chimneys *versus* regions that did not: one chimney formed in each colony ( $N=7$  colonies). Plot shows median, 1st and 3rd quartile, 1st and 9th decile, and minimum and maximum values.

that did not form chimneys ( $P=0.028$ ,  $N=7$  colonies; Wilcoxon signed ranks test).

#### Components of velocity away from the colony

The measurements of excurrent flow speed described above included only the components of velocity parallel to the colony surface and not the component of velocity perpendicular to the colony surface. To determine how much difference this made to the measurements of flow speed, I measured the components of velocity at the canopy edge in colonies viewed from the side so that I could measure the component of velocity

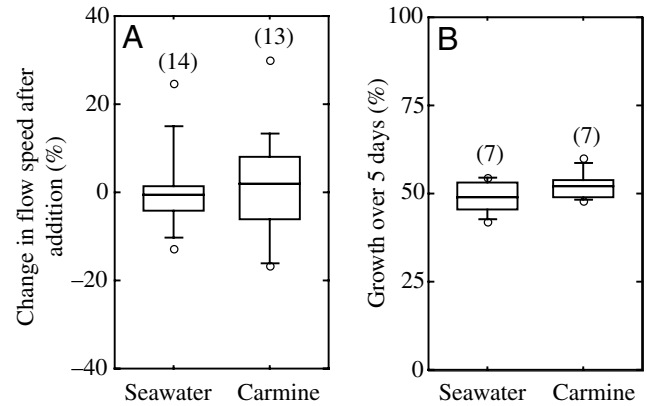


Fig. 3. (A) Box-plot showing changes in excurrent flow speed after addition of either seawater or a suspension of carmine in seawater. (B) Box-plot showing growth over 5 days in colonies exposed to either seawater or carmine suspension. Numbers of colonies are in parentheses. Box-plots show median, 1st and 3rd quartile, 1st and 9th decile, and minimum and maximum values.

perpendicular to the colony surface. The ratio of the resultant flow speed (calculated from both the perpendicular and parallel components of velocity) to the component of velocity parallel to the colony surface was 1.04 (range 1.02–1.10,  $N=9$  colonies). There was no correlation between the ratio of the flow speed to the component of velocity parallel to the colony surface and the excurrent flow speed ( $P=1$ ; Kendall rank correlation). These results suggest that missing the component of velocity perpendicular to the colony surface had little effect on measurements of flow speed.

#### Does carmine affect flow speed or growth?

To test for an effect of carmine on excurrent flow speed, I measured the change in flow speed at the canopy edge after adding either carmine or seawater (Fig. 3A). Carmine did not have a statistically significant effect on the change in excurrent flow speed at the canopy edge ( $P=0.63$ ; Mann–Whitney  $U$  test; Fig. 3A). The 95% confidence interval (of the Mann–Whitney  $U$  test) for the difference between the carmine-treated colonies and the seawater controls was  $-6\%$  to  $+9\%$ .

To test for an effect of carmine on colony growth, colonies were exposed to either carmine suspension or seawater control for 10 min twice a day for 5 days (Fig. 3B). Carmine did not have a statistically significant effect on colony growth ( $P=0.28$ ; Mann–Whitney  $U$  test). The 95% confidence interval (for the Mann–Whitney  $U$  test) for the difference between the carmine-treated colonies and the seawater controls was  $-2\%$  to  $+9\%$ .

#### Does growth or behavior determine chimney shape?

If chimney height (Fig. 4A,B) is determined solely by the behavior of zooids, chimney height should go to zero after anesthetization. However, if chimney height is determined solely by differences in growth between zooids, then chimney height should not change. Chimney height did not decrease to zero after anesthetization with  $MgCl_2$  ( $N=6$ ; Fig. 4B,C).

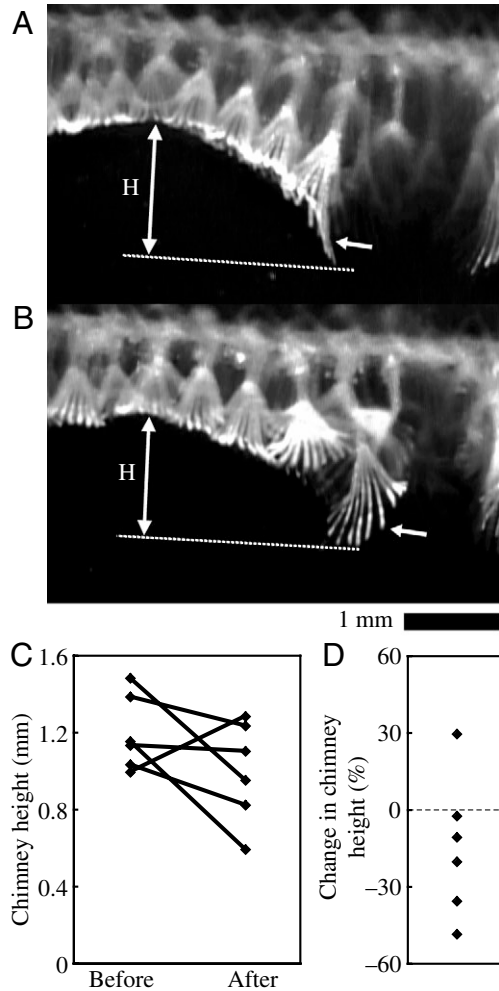


Fig. 4. (A) An image of a colony before anesthetization. (B) The same colony after anesthetization with  $\text{MgCl}_2$ . Single arrows indicate a lophophore that has turned around after anesthetization; chimney height (H) is the difference in height between the tallest part of the chimney and the shortest part of the canopy (double arrows). These images were taken in side view with the light sheet perpendicular to the colony. (C) Scatter plot of chimney height before and after anesthetization ( $N=6$ ). (D) Scatter plot of the change in chimney height after replacing seawater with anesthetic ( $0.35 \text{ mol l}^{-1} \text{ MgCl}_2$ ).

However, chimney height decreased by as much as 49% and increased by as much as 29% in some colonies after anesthetization (Fig. 4D). Also, the orientation of lophophores occasionally changed dramatically after anesthetizing the colony (Fig. 4A,B).

The lophophores of colonies placed in  $0.35 \text{ mol l}^{-1} \text{ MgCl}_2$  never retracted when the colony was touched with a wooden probe ( $N=6$ ) but lophophores of control colonies in seawater always retracted when the colony was poked ( $N=5$ ), indicating that colonies placed in  $0.35 \text{ mol l}^{-1} \text{ MgCl}_2$  were anesthetized. This difference in retraction between  $\text{MgCl}_2$ -treated colonies and control colonies was statistically significant ( $P=0.0009$ ;  $\chi^2$ -test).

## Discussion

### *Relationships between flow and chimney formation*

In this study, chimneys formed at regions with high excurrent flow speeds, as predicted by the hypothesis that high flow speed induces conduit formation in this system. The relationship between flow and conduit formation has been addressed in several internal fluid-transport systems, in which fluid moves through pipe-like conduits to transport material within the organism. Increased flow is correlated with conduit formation or increased conduit size in the vertebrate circulatory system (Brown and Hudlicka, 2003; Langille, 1995; Prior et al., 2004), the gastrovascular canals of hydroid colonies (Buss, 2001; Dudgeon and Buss, 1996) and the veins of plasmodial slime molds (Nakagaki et al., 2000). This study suggests that similar relationships between flow and conduit formation also exist in external fluid-transport systems. Unlike systems studied previously, this system is involved in suspension feeding rather than internal transport, and its conduits (the chimneys) are simple openings rather than pipes.

It is possible that high excurrent flow speed might induce chimney formation indirectly by affecting patterns of incurrent flow or feeding. For example, excurrent flow speed may affect the extent of recirculation of filtered seawater since the filtered seawater may be ejected further from the colony at sites with higher excurrent flow speeds. This might result in higher feeding rates and faster growth of lophophores at sites on the canopy edge with high excurrent flow speeds. Reduction in re-filtration may be an advantage of arranging lophophores in a tight canopy broken by chimneys (Eckman and Okamura, 1998; Pratt, 2004).

Alternatively, it is possible that high excurrent flow speed could induce chimney formation directly *via* shear-stress-induced changes in the growth of the lophophores and the stalks supporting the lophophores. One would expect higher shear stress on the lophophores or the stalks of the lophophores at the sites with higher flow speeds where chimneys formed in this study. Shear stress is known to affect the development of some internal fluid-transport systems. In vertebrates, increasing shear stress causes blood vessel size to increase and may induce blood vessel formation (Brown and Hudlicka, 2003; Kamiya and Togawa, 1980; Langille, 1995; Prior et al., 2004). In hydroid colonies, shear stress in the gastrovascular canals may influence where new polyps and stolons form (Buss, 2001; Dudgeon and Buss, 1996).

However, the observation that chimneys tend to form at sites of high excurrent flow speed could also be explained if new chimneys tend to form far from existing chimneys, even if chimney formation is not affected by flow speed. Both existing chimneys and the canopy edge provide outlets for filtered seawater. Therefore, one might expect low excurrent flow speeds at parts of the canopy edge near old chimneys and high excurrent flow speeds at parts of the canopy edge far from old chimneys. Therefore, if new chimneys tend to form far from old chimneys, one might expect them to form at sites of high excurrent flow speed.

The hypothesis that high excurrent flow speed induces chimney formation directly might explain the effects of ambient flow (Okamura and Partridge, 1999) and the presence of spines (Grünbaum, 1997) on chimney spacing, and the responses of lophophores to injury of adjacent zooids (Dick, 1987; von Dassow, 2005). Both high ambient flow speed (Okamura and Partridge, 1999) and the presence of defensive spines (Grünbaum, 1997) result in reduced chimney spacing. High ambient flow speed also resulted in smaller lophophores in field studies of *M. membranacea* (Okamura and Partridge, 1999). Both small lophophore size and the presence of spines might result in high shear stress if the volumetric flow rate remains constant, since the flow will be forced through narrower channels under the canopy. Therefore, one might expect chimneys to form sooner – and closer to each other – in colonies with smaller lophophores or colonies bearing spines than in colonies with large zooids or without spines. Note that increased resistance to flow through the system due to the presence of spines (Grünbaum, 1997) or small lophophore size might counteract this effect.

Ambient flow speed could also affect the rate of flow through the colony independently of effects on zooid size. Ambient flow speed is known to enhance flow through many suspension feeders (Knott et al., 2004; Vogel, 1977; Young and Braithwaite, 1980), but I found no such effect in *M. membranacea* chimneys (von Dassow, 2005; but see Stewart, 2000).

Dick (1987) found that chimneys sometimes form at sites of injury to the colony. By contrast, I found that injuring parts of the colony within the canopy of lophophores did not induce chimney formation (von Dassow, 2005). Instead, the lophophores surrounding the injury closed or partially closed the gap in the canopy formed by the injury. However, I found that only lophophores bordering injuries large enough to produce lasting openings in the canopy (where one would expect excurrent flow) became asymmetrical, like chimney lophophores (Figs 1B,C, 4A). Lophophores bordering smaller injuries that left no lasting opening in the canopy did not become asymmetrical. This is consistent with Dick's hypothesis that excurrent flow past lophophores induces them to become asymmetrical (Dick, 1987).

In this study, colonies were grown in still water and the excurrent flow speeds were measured in still water, whereas in nature, colonies grow in sites with ambient currents. This difference between the experimental and natural conditions does not affect interpretation of the results since the hypothesis that high excurrent flow speed induces chimney formation predicts that excurrent flow speeds should be higher at sites that subsequently form chimneys regardless of ambient flow conditions.

#### *Possible implications for performance*

Because chimneys function to let filtered water out of the colony (Banta et al., 1974; Lidgard, 1981) and to reduce recirculation of the filtered water (Eckman and Okamura, 1998), the correlation between flow and chimney formation

may be advantageous for these colonies. Forming chimneys at sites of high excurrent flow at the canopy edge might insure that the excurrent stream out of the new chimney will be strong enough to push filtered water far from the colony, thereby reducing recirculation. Also, if sites of high excurrent flow at the canopy edge are sites that are far from existing chimneys, one would expect that they would be sites where chimneys would help the most to reduce the pressure under the canopy. Hydrodynamic models suggest that high pressure under the canopy should reduce feeding rates (Grünbaum, 1995; Larsen and Riisgard, 2001; but see Pratt, 2004). Experiments to manipulate flow through the colony will be necessary to test these hypotheses and the hypotheses described above relating to specific mechanisms of chimney induction.

#### *Both growth and behavior contribute to chimney morphology*

In bryozoan species in which chimneys are not associated with skeletal structures, do the differences between chimney and non-chimney zooids result from differences in growth between zooids or differences in behavior?

Many species of bryozoans produce chimneys, but the morphological and behavioral mechanisms producing these chimneys vary considerably. In some species of bryozoans, chimneys are associated with bumps on the colony surface produced by growth of the zoecium (the box around the zooid) or by budding of zooids perpendicular to the colony surface (Banta et al., 1974; Cook, 1977; Ryland, 2001; Shunatova and Ostrovsky, 2002; Winston, 1978, 1979). Sometimes the chimneys are even associated with the position of male zooids (Ryland, 2001). However, there are also species in which the position of chimneys or lophophore clusters is due to behavior alone: the position of the chimneys changes every time the lophophores re-extend (Shunatova and Ostrovsky, 2002; Winston, 1978). In others, such as *Membranipora membranacea*, the chimneys do not change position even over the course of several days (von Dassow, 2005) and are associated with degenerate zooids (Lidgard, 1981), but are not associated with obvious skeletal features. Note that there is one report suggesting that chimney zooids may be larger than non-chimney zooids in *M. membranacea* (Cook and Chimonides, 1980), but the authors did not report how they sampled the zooids or whether this relationship was statistically significant.

The present study suggests that chimney morphology depends both on differences in growth between zooids and on behavior in *M. membranacea*. Chimney height never decreased to zero after anesthetization, indicating that the chimney height cannot be solely determined by behavior of zooids. This suggests that there must be differences in growth or morphogenesis between chimney and non-chimney zooids. However, chimney height changed by almost 50% in some colonies, and chimney lophophores occasionally changed their orientation after anesthetization, indicating that behavior also contributes to chimney shape.

#### *Summary*

The present study suggests that new chimneys form at sites



of high excurrent flow speed and that chimney morphology results from a combination of behavior and differences in growth between zooids in *M. membranacea*. These results are consistent with the hypothesis that high flow rates can induce the formation of new conduits in this external fluid-transport system. The results show that the tight correlation between flow and development observed in internal fluid-transport systems may also occur in external fluid-transport systems.

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