WATER PROPULSION SPEEDS AND POWER OUTPUT BY COMB PLATES OF THE CTENOPHORE *PLEUROBRACHIA PILEUS* UNDER DIFFERENT CONDITIONS

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Summary

Parameters of ciliary beating and water propulsion can be studied in a unique fashion in ctenophores because the beat frequency can be controlled. *Pleurobrachia pileus* comb plates were driven at frequencies between 2 and 25Hz and at temperatures between 10 and 25˚C. As frequency is increased from 5 to 25Hz, the rest period between beats is first shortened and then disappears: the duration of the effective stroke is reduced because the angular velocity (which is directly proportional to the sliding velocity of the microtubules) and the tip speed of each plate increase whilst the amplitude of the beat remains unchanged. The recovery stroke is shortened because the recovery bend is propagated more quickly to the tip of the plate. The phase difference between adjacent plates in the metachronal wave (expressed as a percentage of the cycle) is increased in spite of a sharp decrease in the time delay between adjacent plates, a reduction in the number of plates per wave and an increase in the metachronal wave velocity. The water flow speed becomes more continuous and increases in approximate proportion to the tip speed whilst the estimated power output of a metachronal wave increases exponentially, from 10^{-10} W at a tip speed of about $20 \, \mathrm{mm \, s^{-1}}$ to 10^{-8} W at a tip speed of about $75 \, \mathrm{mm \, s^{-1}}$. When comb plates are driven to beat at 10Hz and the temperature is raised from 10˚C towards 20˚C, the duration of the effective stroke is reduced and the comb plates have a somewhat higher angular velocity and tip speed; the duration of the recovery stroke is reduced with a faster propagation of the recovery bend; a rest phase first appears between successive beats and then becomes longer. The phase difference between adjacent plates in a metachronal wave (expressed as a percentage of the cycle time) is decreased, as is the time delay between successive plates in a metachronal wave, so that the number of plates per wave and the wave velocity are increased. The water flow speed and power output are increased by a modest amount (a rise in temperature from 10 to 20˚C produces changes equivalent to those produced by a 5Hz increase in frequency at 20˚C). The cooperation between adjacent plates in the antiplectic metachronal wave makes a major contribution to the dramatic increase in power output of each metachronal wave that is seen as the beat frequency is increased.

Introduction

Ctenophore comb plates are unusual among cilia in that they normally remain quiescent unless stimulated and are individually triggered to beat in cydippids, if not in all

ctenophores, by a visco-mechanical signal (Sleigh, 1972; Tamm, 1982). The repertoire of forward movements of cydippid ctenophores like *Pleurobrachia* ranges from a slow drifting motion with a low frequency of metachronal waves to rapid swimming with a high frequency of waves, according to their 'mood' and orientation (Horridge, 1965; Tamm, 1982). The beat frequency is controlled over a wide range by coordinating signals that normally originate at the aboral apical balance organ (Chun, 1880; Horridge, 1965; Tamm, 1982). During feeding, the animal rotates to bring towards its mouth food that has been caught on a tentacle, and in this movement the comb plates of some comb rows beat forwards while others beat in reverse (Tamm, 1982); mechanical stimuli may also cause reversal of beating (Sleigh and Barlow, 1980, 1982).

It is possible to drive the beating activity of ctenophore comb plates at predetermined frequencies (Sleigh and Jarman, 1973). This makes it possible systematically to examine ciliary beating, metachronal coordination and propulsive effects under a range of prescribed conditions and to seek answers to questions about how the motion of individual cilia responds to changes in beat frequency, how the metachronal wave changes, how the speed of water propulsion is affected and the extent to which the power output of the system changes.

The mechanical response of a forward-beating comb plate has been shown to vary with the frequency of beating, with both effective and recovery strokes decreasing in duration and the rest phase being reduced in length and then lost, as the frequency of beat is increased (Sleigh and Jarman, 1973). By comparing the beat pattern of comb plates at frequencies between 4 and 17.5Hz, these authors found that the decrease in duration of the effective stroke was due to an increase in the angular velocity of the effective stroke, without any decrease in the amplitude of beat. It might therefore be assumed that the rate of water propulsion by a comb plate would increase at higher frequencies not only because the plate beats more often but also because its tip speed is increased. Two important features influence the speed of water propulsion produced by a single beat of a comb plate; one is the tip speed, the other is the effect of interaction with other plates on either side of it in the metachronal sequence that cooperate in the water-pumping cycle and particularly in the shedding of volumes of fast-flowing water from the tips of the plates near the end of the effective stroke (Barlow *et al.* 1993). If changes in phase difference between adjacent plates in the row occur as frequency changes, these could therefore also alter the rate of water propulsion, so the properties of the phase linkage between adjacent comb plates may be very important. It would therefore be interesting to find out whether the power output of a beating comb plate increases at a faster or slower rate than the tip speed, when the frequency is increased.

The propulsion of water by comb plates at two different frequencies was briefly compared by Sleigh and Aiello (1972). They found that not only was the maximum flow rate more rapid at 12Hz than at 5Hz, but also that the distance the water was propelled in a single beat was higher at 12Hz; the forward flow above the plates was maintained at a higher and more steady rate throughout the beat cycle at 12Hz than at 5Hz, but the backflow between the comb plates during the recovery stroke was more obvious at the higher frequency than at 5Hz.

In this paper, we report how both tip speed and phase difference changed with

experimentally induced changes in the frequency of beat, and we use measurements of water flow to estimate the power output of a section of the comb row at different beat frequencies. We also report on changes in ciliary beat parameters with temperature change, for in preliminary experiments at various temperatures we found it was possible to extend the range at which the cilia could be driven by mechanical stimuli, but we also found that at extreme temperatures there were changes in the activity of the comb plates.

Materials and methods

Pleurobrachia pileus Mueller were collected from Southampton Water, stored in a cold room at 4˚C and prepared for filming as described by Barlow *et al.* (1993). The beat frequency of the comb plates was controlled using the method of Sleigh and Jarman (1973). High-speed ciné films of the beating comb plates were taken in the presence of 'Saran' microspheres (Dow Corning), and computer-derived contour maps of the flow speed and direction around the comb rows were prepared according to the method used by Barlow *et al.* (1993).

When required for temperature experiments, individual ctenophores were transferred from the cold room to the experimental temperature (up to 20˚C) in a 250ml beaker of 4 ˚C sea water and allowed to warm up slowly to the selected temperature. Once warmed, the animals were allowed to adjust to the experimental temperature for 1h before filming commenced. For temperatures over 20˚C, the animals were warmed to 20˚C and kept at this temperature before being transferred to the experimental temperature shortly before filming. This was because exposure to high temperature caused rapid deterioration of the animals. After 1h at 25˚C, experimental animals were observed to be in very poor condition; therefore, films were taken within 15min of exposure to these higher temperatures.

Estimates of the swimming power output were made by measurement of the power in the jet of water produced by tethered ctenophores. Since the animal could not move, the total power production should be expressed in the water flow behind the animal. The power (P) in a water jet (momentum jet) can be estimated from the equation:

$$P = \tfrac{1}{2}\rho u^3 A \,,$$

where ρ is the density of water, u is the velocity of water and A is the cross-sectional area of the jet; an explanation of momentum jet theory has been given by Ellington (1978). To apply the equation it is necessary to know the cross-sectional area of the jet and the velocity of flow in it. Within a water jet the velocity varies across the cross section and so the component cross-sectional area of each velocity (from the mapped flow contours) must be integrated to calculate the total power output:

$$P = \tfrac{1}{2}\rho \int u^3 \mathrm{d}A \,.$$

Because the jet rapidly dissipates in low Reynolds number flow and diffuses the momentum through a wide area, the greatest accuracy in estimating the power output is likely to be obtained in the fastest region of the flow close to its source. The power in the jet is seen from the momentum jet equation to be proportional to the third power of the

velocity, and so small regions of high-speed flow at the centre of the jet contain a high proportion of the output energy. Although the flow changes rapidly in the region close to the comb plates, and accurate assessments of velocity profiles are not easy to obtain, the contour maps referred to above (e.g. Fig. 1) provide information on velocity profiles at different stages of the beat cycle which can be used to estimate the power output in the jet at these different stages. The velocity profile of a jet is reconstructed by drawing a line perpendicular to the flow (see example in Fig. 1), i.e. where it may be regarded as the radius of a cross section through a cylindrical jet. When the profile is measured at the peak flow speed in the cycle, the peak power of the wave of activity can be calculated.

The accuracy of this method of estimating the power output depends on three assumptions: (1) that the shape of the output flow is approximately cylindrical (we are satisfied from polar views of the animal that this is the case); (2) that the velocity contours give a reasonable measure of particle flow speeds (the number of data points included gives us confidence of this); and (3) that the amount of the jet that could be integrated was a reasonable proportion of the whole. In fact, it was usually only possible to integrate as far out as the 20% contour, i.e. to a velocity level that was 20% of the maximum speed detected. Water moving at a lower velocity is beyond the edge of the field of view at the magnification required to see details of high-velocity regions around the comb plates. This limitation involves some underestimates because the energy contained in the jet outside this contour is ignored, but, because the power in a region of the jet is proportional to the cube of the velocity, we estimate that more than 90% of the power will be included
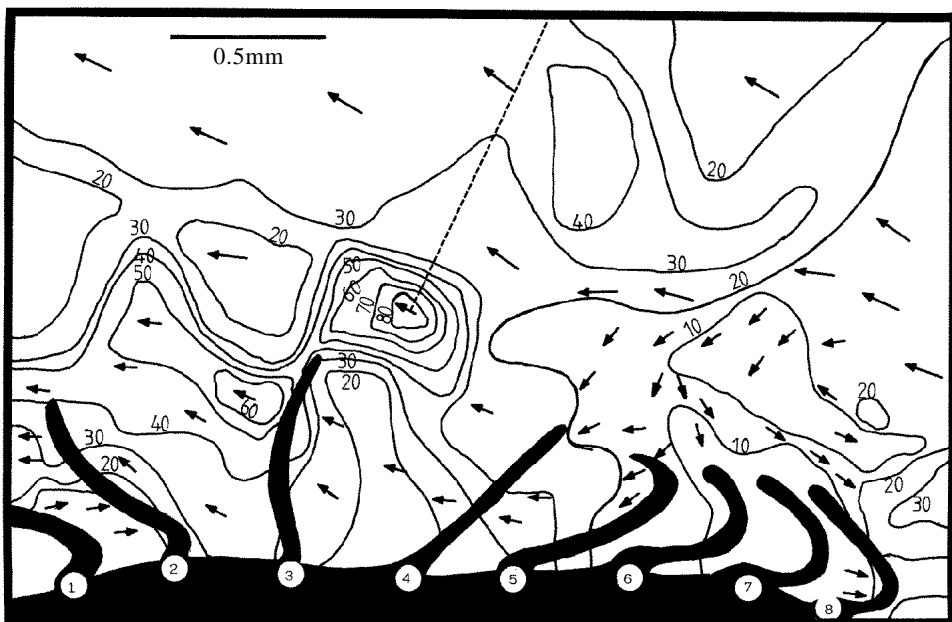

Fig. 1. Water flows around a comb row beating at 20Hz (20°C), showing water speed contours (in mm s^{-1}) and flow directions derived by the method described by Barlow *et al.* (1993). The dashed line indicates the radius of the output jet used in the construction of a velocity profile for calculation of the power output. 1–8 refer to eight adjacent comb plates.

by integrating over the fastest 80% of the jet. It is especially important that the contour maps are based on an adequate number and on an even distribution of data points and that there are data points defining the upper and lower limits of the jet. Power estimates were calculated from the average of at least five profile radii for each specimen.

Results

Changes in parameters of beating and coordination with change in beat frequency

The observations of Sleigh and Jarman (1973) that an increase in beat frequency reduced the duration of all parts of the cycle were confirmed and extended to cover more parameters and a wider frequency range. Over the frequency range from 5 to 25Hz (at 20˚C), the angular velocity of the effective stroke more than doubled (Fig. 2A) and the tip speed increased by a similar proportion (Table 1). Over this range of frequencies, the number of plates per wave dropped from 23±3 to 7±2 (N=5, mean ± s.d.) (Fig. 2B) and the velocity of propagation of metachronal waves increased from $56\pm14\,\mathrm{mm\,s^{-1}}$ to $95\pm23\,\mathrm{mm\,s^{-1}}$ (Student's *t*-test; t=2.7, P<0.05).

The phase difference between adjacent comb plates in the wave, measured as the percentage of the cycle (including any rest phase) between the start of the effective stroke of one plate and the start of the same stroke of the next plate in the metachronal sequence, progressively increased with frequency (Fig. 3D). In one example, the phase difference increased from about 4% at 5Hz to 11% at 25Hz; these phase differences represent a time lag between successive plates of 8ms and 4.4ms respectively. There were about nine plates within one wave at 25Hz and 25 plates per wave at 5Hz in this example; all of the plates were active at 25Hz, there being no rest period, whilst at 5Hz about 59% of the cycle was taken up by the rest period and less than half of the plates were active.

As frequency increases, the proportion of the cycle occupied by the effective stroke and
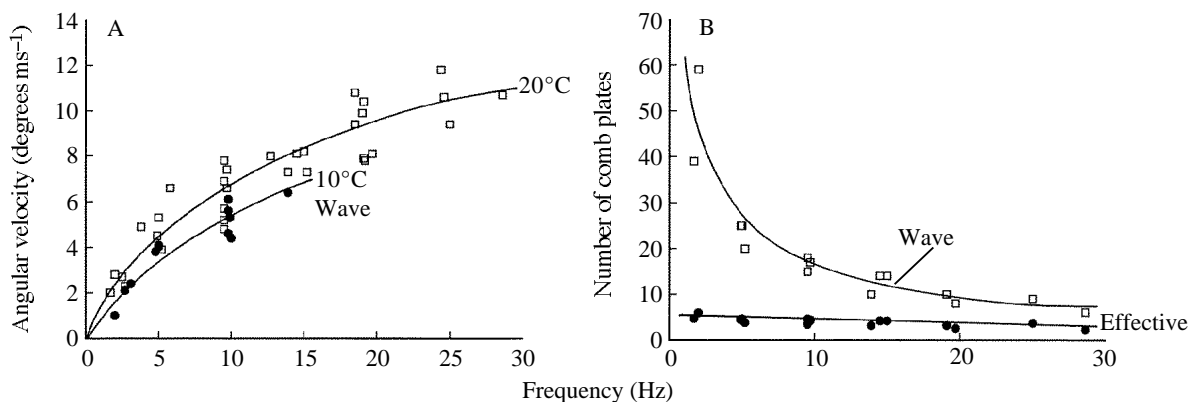

Fig. 2. (A) The change in angular velocity of comb plates beating at frequencies of 2–28Hz at 20˚C and of 2–14Hz at 10˚C. (B) The change in the number of comb plates in a wavelength (Wave) and in the effective stroke (Effective) at various frequencies. There was a significant difference between the number of effective stroke plates per wave at 5Hz and at 20Hz (t=3.31, P<0.01).

the recovery stroke both get larger as the rest period shortens (Fig. 3A–C), the latter being eliminated at a frequency between 15 and 20Hz (at 20°C). At higher frequencies where the plates beat continuously, the cycle time is divided between the effective and recovery strokes. Over the frequency range from 5 to 25Hz, the ratio of the duration of the effective stroke to the duration of the recovery stroke remained at 1:1.8±0.3 (N=39), although below 5Hz there was a tendency for the two strokes to become more equal. Above 25Hz, there was a tendency for the percentage of the cycle occupied by the effective stroke to increase at the expense of the recovery stroke, largely because the next effective stroke tended to commence before the recovery bend of the previous stroke has reached the tip of the plate.

Table 1. *Summary of beat parameters of comb plates and the fastest water flows measured in the region of the ciliary tip*

Frequency (Hz)	Total cycle time (ms)	Temperature (°C)	Maximum tip velocity		Water flow* (mm s⁻¹)	Ratio of flow/ plate tip speed	10^{10}×power (W)
			Effective (mm s⁻¹)	Recovery (mm s⁻¹)			
24.3	41	24	60±4	41±4	46	0.76	49.0
25.0	40	20	109±4	47±2	82	0.75	630.0
19.4	52	24	63±2	36±1	60	0.95	110.0
19.6	51	24	63±6	45±1	62	0.98	150.0
19.6	51	20	72±12	34±1	70	0.97	66.0
19.7	51	20	102±3	40±1	86	0.84	420.0
14.3	70	24	60±4	32±1	50	0.83	45.0
13.4	75	24	63±9	43±5	50	0.79	27.0
14.1	71	20	72±2	34±1	70	0.97	110.0
11.1	90	24	51±5	38±5	45	0.88	12.0
9.6	104	24	54±5	27±1	35	0.65	15.0
9.7	103	20	73±2	43±2	64	0.88	85.0
9.8	102	20	68±3	30±1	61	0.90	53.0
9.6	104	10	61±1	24±1	45	0.73	26.0
9.7	103	10	66±6	29±3	64	0.97	85.0
5.4	185	24	21±2	17±1	15	0.71	0.6
4.3	233	24	34±2	20±3	25	0.73	4.7
4.3	233	20	57±4	27±3	44	0.77	9.8
5.2	192	10	45±1	19±1	30	0.67	11.0
4.9	202	10	49±1	24±1	35	0.71	14.0
2.0	500	20	32±3	32±4	33	1.03	1.9
1.8	552	20	22±1	18±1	18	0.82	2.4
2.0	500	10	14±1	7±1	9	0.64	–

*Fastest flow detected.

Values for velocity are mean ± S.D., N=5.

Within any wave, 3–4 plates could usually be seen at stages of the effective stroke. There was a small reduction in the number of effective stroke plates in each wave as frequency increased (Fig. 2B), and there was a significant difference between the number of effective stroke plates per wave at 5Hz and 20Hz (t=3.31, P<0.01). Since the amplitude of the effective stroke remains much the same over the main frequency range, the reduction in the number of effective stroke plates per wave suggests that there must be an increase in the angle between adjacent plates and, therefore, in the inter-plate volume, at higher frequencies. Measurements of the maximum inter-plate angle showed an increase from about 40° at 5Hz to about 50° at 25Hz. In animals with an average length of 8.0±1.4mm (N=25), the comb plate bases were separated by 0.46±0.06mm (N=40) and the comb plates had a length of 0.79±0.13mm (N=40), so that an increase in inter-plate angle of 10° increased the inter-plate volume by about 15%. Therefore, an increase in beat frequency from 5 to 25Hz not only increased the tip speed of the plates by more than 100%, it also resulted in the ejection of 15% more water from each inter-plate space in each cycle and in the performance of these actions five times as frequently.

Effects of temperature on parameters of beating and coordination

When comb plates were driven by a mechanical stimulator, the range of frequencies over which the beating was propagated along the comb rows varied with temperature: the maximum frequency was reduced at low temperature and the minimum frequency was
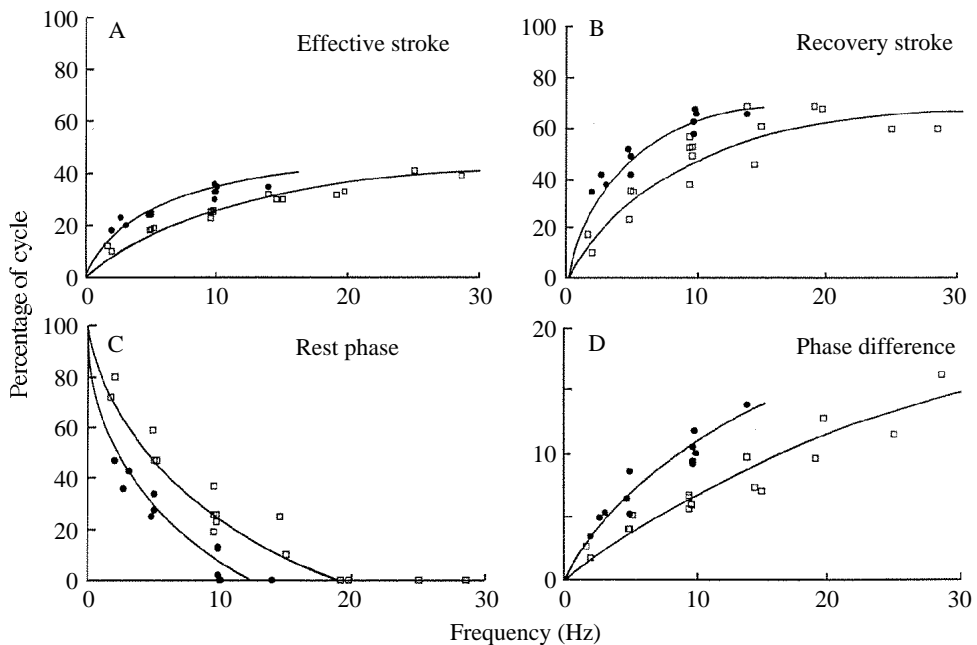

Fig. 3. The effect of frequency on the proportions of phases of the beat cycle. The proportions are expressed as percentages of the total cycle time. (A) The duration of the effective stroke; (B) the duration of the recovery stroke; (C) the duration of the rest phase; (D) the phase difference between adjacent comb plates. (●) 10°C, (□) 20°C.
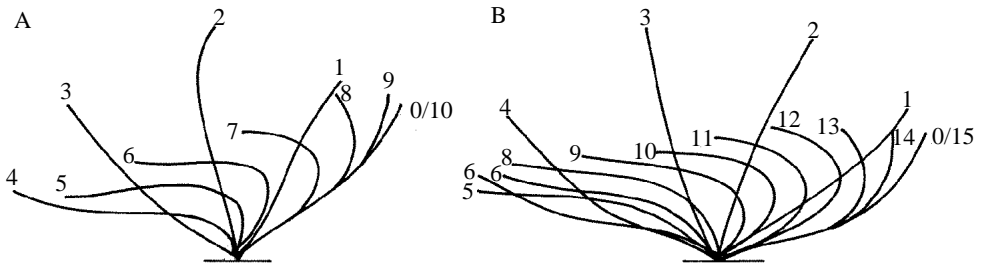
Fig. 4. Profiles at 6.3ms intervals of a comb plate beating at 10Hz, (A) at 24°C and (B) at 10°C. The duration of the rest phase between active cycles was 37ms in A and 5ms in B.

raised at high temperature. The average maximum frequency at which plates could be regularly driven increased from 14.7Hz at 10°C to 31.1Hz at 25°C. At the higher temperatures the comb plates began to fray at the ends, and the distal ends became floppy so that they no longer remained fully extended throughout the effective stroke, even at 24°C (Fig. 4A). This effect occurred quickly above 25°C and prolonged exposure to these temperatures resulted in the beat losing its polarisation. Under these conditions, the effective and recovery strokes became similar and the beat appeared flagellar with symmetrical bends propagated from base to tip; the plates projected perpendicularly from the surface and all the plates along the row beat in synchrony, propelling water perpendicularly outwards from the body surface. Eventually, after longer exposure, the tips of the comb plates began to be shed, with consequent shortening. Over the temperature range from 10 to 20°C, the comb plates remained intact and showed a wide stable frequency range, so that studies of the effects of temperature on beat parameters were largely concentrated within this range; 10Hz was chosen as the standard frequency at which comparisons were made because plates could be driven at this frequency across the whole chosen temperature range.

A comparison between the beat profiles of a comb plate beating at 10Hz at 10°C and at 24°C (Fig. 4) shows that the effective strokes and recovery strokes are both completed more quickly at the higher temperature. Plates beating at 10Hz normally showed little or no rest phase at 10°C since the effective and recovery strokes occupied the whole cycle, but with the shortened duration of the effective and recovery strokes at 20°C, a prominent

Table 2. *A comparison of parameters measured at 10°C and at 20°C on sets of comb plates beating at 10 Hz*

Parameter	10°C	20°C
Angular velocity (degreesms^{-1})	5.2±0.7	6.9±0.8
Average length (mm)	0.85±0.14	0.87±0.09
Average tip speed (mm s^{-1})	77±9	104±16
Number of comb plates per wave	10.2±0.8	16.4±1.3
Wave velocity (mm s^{-1})	47±5	80±12
Number of cilia in effective stroke	4.1±0.4	3.3±0.3

Each value is a mean of 25 measurements, derived from five beat cycles of each of five animals.

rest phase was present at the same frequency (Figs 3C, 5C). The shortening of the effective stroke with increase in temperature evident at 10Hz occurred because the angular velocity increased (Fig. 4B); a comparison of two sets of measurements on comb plates beating at 10Hz (Table 2) showed a significant increase in angular velocity between 10°C and 20°C (t=3.46, P<0.01). The average length of the comb plates of these two groups was not significantly different so the average tip speed at 20°C increased by some 35% (Table 2).

The reduction in the proportion of the cycle occupied by the effective and recovery strokes over the whole temperature range studied is shown in Fig. 5A,B, and the increasing proportion of the cycle occupied by the rest phase is shown in Fig. 5C. The combination of a higher angular velocity and a smaller proportion of the cycle occupied by the effective stroke resulted in a pronounced decrease in the phase difference between adjacent comb plates, expressed as a percentage of the cycle time, as the temperature increased (Fig. 5D). Thus, each wave of comb rows beating at 10Hz contained fewer comb plates at 10°C than at 20°C (Table 2), the rest phase increasing from a very short pause to a prolonged rest of around 25% of the cycle, so that the number of active comb plates per wave increased from about 10 to about 12. The change in wavelength resulted in a 70% increase in wave velocity between 10 and 20°C (Table 2), whereas the number of cilia in the effective stroke at any instant decreased somewhat.

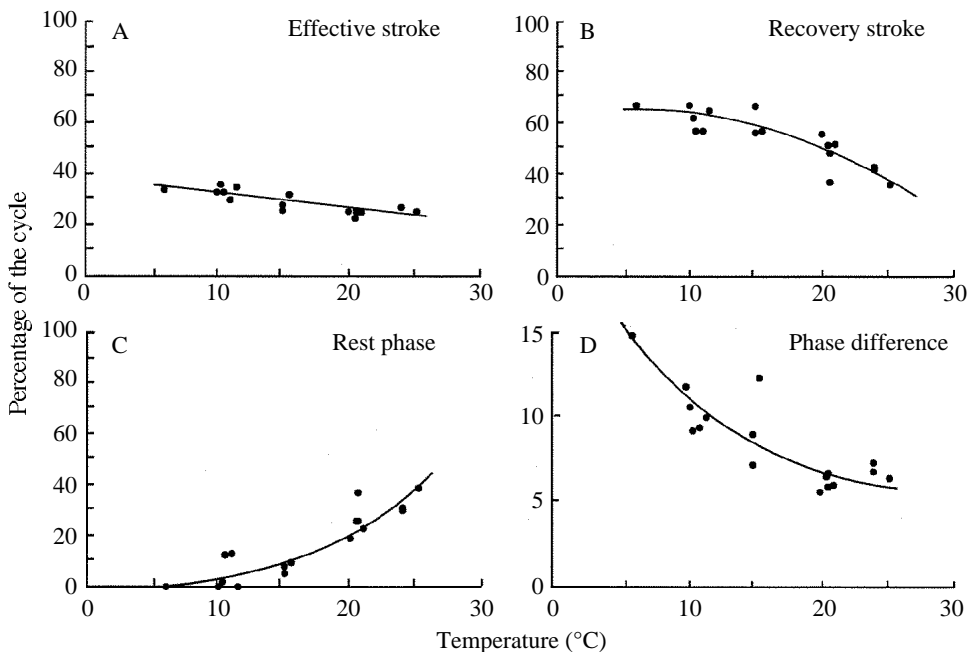The appearance of the flow patterns around the comb plates did not show any

Fig. 5. The effect of temperature on the proportions of the whole beat cycle of comb plates beating at 10Hz. Proportions are expressed as a percentage of total cycle time. (A) Duration of the effective stroke; (B) duration of the recovery stroke; (C) duration of the rest phase; (D) the phase difference between adjacent comb plates.

differences attributable to temperature changes. An increase in temperature from 10°C to 20°C at a constant frequency of 10Hz caused modest increases in the tip speed, the angular velocity and the inter-plate volume. Increases in beating parameters for a 10°C rise in temperature were equivalent to those caused by an increase in beat frequency of about 5Hz at 20°C (e.g. Fig. 2A).

Water flow and estimates of power output from comb plates

An analysis of the flow patterns around the comb plates of six animals over a range of frequencies from 1.8 to 15Hz and over a temperature range from 10 to 24°C was performed by the preparation of contour maps with the aid of two computer programs (Barlow *et al.* 1993). The peak tip velocities of a comb plate during effective and recovery strokes were measured directly from projected frames of the high-speed ciné film, and the water flow speeds generated by ciliary beating were read from contour maps of the type shown in Fig. 1. The values of these parameters are shown in Table 1, where it can be seen that the ratio of maximum water flow speed to comb plate tip speed remained within fairly narrow limits over the full frequency range.

At very low beat frequencies, the phase difference between the effective strokes of adjacent comb plates in a row was reduced and the plates beat more synchronously; at the same time, the excursion angle of the effective stroke decreased and the number of plates involved in each wave increased. The maximum velocity of the tips of the comb plates was about $15–30\,\mathrm{mm\,s^{-1}}$; water could be seen moving at $15–18\,\mathrm{mm\,s^{-1}}$ within the inter-
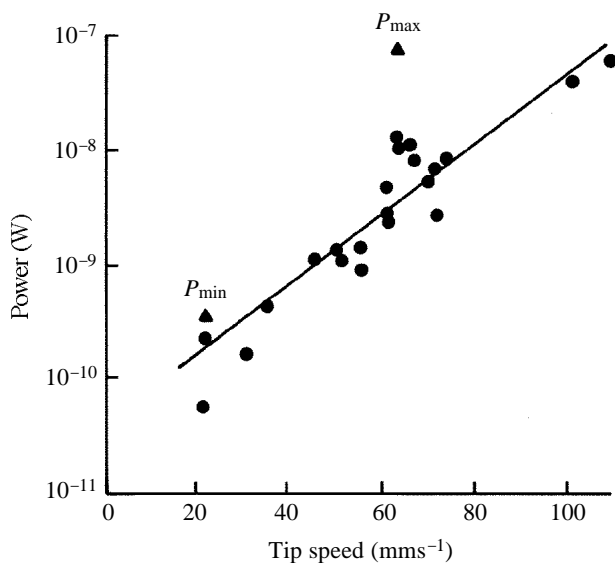

Fig. 6. The power output of comb rows in relation to peak tip speed. The power value plotted is the integrated output of the jet for the passage of one wave. Values marked (▲) show the expected total output for the whole organism estimated from the values for single waves. P_{max} is the estimated total power output for the organism with all comb rows beating at 20Hz, while P_{min} is the estimated minimum power output for low-frequency (5Hz) orientation movements affecting only one quadrant (two comb rows).

plate space during the second half of the effective stroke, but the water speed dropped rapidly beyond the tips of the comb plates. Pulses of water could be seen being squeezed out from between the comb plates. In their recovery stroke, the comb plates dragged water upstream and this helped to decelerate the pulses of water emerging from between the plates. Once the wave of activity had passed and the resting phase had begun, the water flow slowed to less than $3\,\mathrm{mm\,s^{-1}}$ (about 10% of the maximum), though some residual upstream flow could be seen near the plate, associated apparently with the end of the recovery stroke.

The water flow became more continuous at higher beat frequencies, as shown in Fig. 1. At 20Hz, when tip speeds of 60–$100\,\mathrm{mm\,s^{-1}}$ were reached in the effective strokes, the water flow reached maximum speeds of 60–$90\,\mathrm{mm\,s^{-1}}$, and even between effective strokes the water continued to flow at 30–40% of the maximum tip speed.

The estimated power output in the exit jet of water close to the comb plate tips, calculated using the momentum jet equation with data at different frequencies and different temperatures from six specimens, is shown in Table 1. The peak power output of one metachronal wave clearly rose sharply with the frequency, but showed a closer correlation with peak tip velocity, which we saw earlier is related to the peak speed of water flow, irrespective of the temperature. Comparison of power output with tip speed also eliminates variation in plate length of different individuals at the same temperature and frequency. The peak power output values are plotted against the maximum tip speed of the comb plates in Fig. 6, where it is seen that the power increases exponentially with the tip speed.

Discussion

Cilia normally beat autonomously at a frequency that depends on physico-chemical conditions, including ionic and hormonal concentrations. In ctenophores, the beat frequency of the comb plates is precisely controlled. The comb plates have a low intrinsic activity and all of the plates of a row can be driven to beat at a selected frequency by stimulating a plate at the head of the row; in life this is achieved by a wave of activity propagated along the ciliated groove from the apical organ, or in experiments by mechanical stimulation. This ability to control the beat frequency externally enables us to study the effects on ciliary beat parameters and the resultant water flows of varying beat frequency at a constant temperature or of varying temperature at a constant frequency of beat in a way that has not proved possible with any other cilia.

The angular velocity of the effective stroke is increased by raising the frequency of beat at a constant temperature and is also increased by raising the temperature at a constant frequency. The angular velocity is proportional to the rate of sliding between microtubules in the effective stroke. At 20°C, the rate of sliding between microtubules in *Pleurobrachia pileus* cilia is about $100\,\mathrm{rad\,s^{-1}}$ at 7.5Hz and rises to $200\,\mathrm{rad\,s^{-1}}$ at 30Hz; this compares with a rise from about $100\,\mathrm{rad\,s^{-1}}$ at 30Hz to $200\,\mathrm{rad\,s^{-1}}$ at 45–100Hz in the distal region of mechanically vibrated sperm tails of the sea urchin *Hemicentrotus pulcherrimus* (Shingyoji *et al.* 1991). The finding that the rate of sliding of axonemal microtubules increases as the interval since the previous beat decreases was compared by

Sleigh and Jarman (1973) with a comparable effect on contractility in heart muscle (Bowditch, 1871; Koch-Weser and Blinks, 1963).

In spite of the substantial increase in energy supply required to move the comb plates (and the surrounding water) more quickly after a reduced interval for recovery at a higher beat frequency, the cilia continued to perform a normal beat, suggesting that neither ATP synthesis nor its diffusion along the length of the ciliary axonemes limits the activities of dynein in generating active sliding. It is only at very high frequencies that the ciliary tip begins to lose its stiffness, which may indicate that an inadequate supply of ATP is available in that region to activate the dynein cross-bridges that are assumed to provide a major part of ciliary stiffness.

The rate of sliding in the recovery stroke presumably controls the rate of propagation of the ciliary bend along the axoneme in this phase of the beat. The rate at which the effective and recovery strokes speed up is less than the rate of increase of the frequency, so that effective and recovery strokes take up a progressively greater proportion of the cycle time at higher frequencies, with first a reduction and then a loss of the rest phase between beats. Sanderson *et al.* (1992) have found that, when the beat frequency of cilia of mammalian respiratory epithelia increases from about 2Hz to above 16Hz, the duration of the effective stroke shortens a little, but most of the increase in frequency results from a marked shortening of both the recovery and rest phases; in this case, the rest phase persists. Although the rest phase in *Pleurobachia pileus* may be eliminated at high frequencies, the ratio of the duration of the effective stroke to the duration of the recovery stroke remains practically unchanged over a wide frequency range, so that the shape of the active part of the metachronal wave remains very much the same, with only a very small decrease in the number of plates active in the effective stroke at any instant as frequency rises. The stability of the wave shape over a wide frequency range presumably relates to the tight coupling between adjacent plates during metachronal triggering and is vital for the maintenance of the inter-plate pump that contributes so much to propulsion (Barlow *et al.* 1993). The phase difference between adjacent comb plates must be kept within close limits for this pump to work properly; if the plates become too closely synchronised and the angle between adjacent plates is reduced, the capacity of the pump falls, and if the time interval between adjacent plates becomes too long, then the cooperative effect in shedding the fast-flowing water from the plate tips is reduced.

In metachronal coordination of cydippid ctenophores, the motion of one plate individually triggers the motion of the next by viscous-mechanical forces (Sleigh, 1972; Tamm, 1973). At the start of the beat adjacent plates lie close together. The rapid acceleration of a leading plate produces a strong negative pressure in the space between this leading plate and the next one because of a high resistance to flow into the narrow space that separates them, and this suction raises the lower plate, stimulating it to begin its own effective stroke (although the precise mechanism of activation of contraction is unknown) (Sleigh, 1972). When the angular velocity of the leading plate is increased by a rise in the frequency of beat, the following plate should be triggered more quickly because faster flows will occur around the leading plate. The angular velocity is more than doubled by increasing the frequency of beat from 5 to 25Hz, but the time interval

between adjacent plates at 25Hz is a little more than half that at 5Hz. There is therefore a slight increase in the angle between adjacent plates during their effective strokes as frequency is increased as well as a more rapid rate of increase in volume; these increase suction and bring about a stronger stimulation. The shortening of the time interval between the commencement of the effective stroke by successive comb plates along the row results in a faster propagation of the metachronal wave, while the wavelength decreases, since a fivefold increase in the frequency only produces a 70% increase in wave velocity. Inter-plate coupling is reduced at higher frequencies if a new beat begins before the previous recovery stroke has been completed; as a result, only alternate waves may be propagated if stimulation above about 25Hz is attempted; Sleigh and Jarman (1973) explained this by observing that the time taken for the 'unrolling' of the plates in their recovery strokes meant that there was no longer much spatial overlap between adjacent plates at high frequencies, so that inter-plate volume was reduced and insufficient suction was generated to raise the lower plate and trigger its contraction.

The tip speed of a comb plate increases in proportion to the angular velocity, but the water flow generated by the comb plate depends substantially upon the coordinated action of adjacent plates (Barlow *et al.* 1993). Analysis of flow patterns shows that the region of fastest flow moves further beyond the tip of the comb plate as frequency is increased. When this happens, the acceleration of water by the plate slackens and the comb plate overtakes the water flow. The existence of a low-pressure region behind the accelerating comb plate causes water leaving its tip to run over to the trailing face of the plate, but acceleration continues. This backflow at the tip of the comb plate indicates an influence of inertia. However, the following plate acts to minimise the drag that results from this backflow and, hence, the metachronal wave conserves energy. Water shed from the comb plate tip during acceleration is retained within the acceleration field of the adjacent plates, whereas if the plate were to beat in isolation the energy in this water would be lost as drag. At lower frequencies, the water appears to be more closely bound to the tips of the plates and less is shed from the tips.

The power output increases exponentially with frequency; more water is accelerated to tip speed at higher frequencies, but apparently it is not accelerated to speeds above the tip speed. The increase in momentum of the water results not only from an increase in the tip speed of the comb plates, but also from an increase in the volume of water expelled from the inter-plate space at the end of the beat and from the increase in velocity of this expelled water because the plates close together more quickly. A fivefold increase in frequency from 5 to 25Hz can increase the power output in water propulsion 100 times, while the tip speed of the comb plates only increases by three or four times. This indicates the major contribution to water propulsion made by the cooperation between plates within a metachronal wave. It also implies an enormous increase in ATP utilization and therefore in supply; it would be interesting to see how long a frequency of 25Hz could be maintained experimentally, recognising that such a high rate of beating is probably only produced in very short bursts, if at all, in nature.

The estimated output by each metachronal wave of *Pleurobrachia* corresponds to an energy expenditure of 1.7fJ per ciliary axoneme per effective stroke at 20Hz (assuming the comb plate contains about 100000 cilia, Afzelius, 1961). This value for the 800 μm

long cilia of *Pleurobrachia pileus* comb plates compares with calculations of an energy expenditure per beat of 0.5fJ per ciliary axoneme in the 30 μm long compound cilia of *Sabellaria* (Sleigh and Holwill, 1969) and 0.11fJ in the 10 μm long single cilium of *Paramecium* (Silvester and Sleigh, 1984). The energy expenditure clearly increases with axoneme length, but the effect of compounding cilia together cannot be deduced from these data. The work done against elastic forces may be 5 or 10 times that done against viscous forces (Sleigh and Holwill, 1969; Hiramoto, 1974).

At 5Hz the velocity of wave propagation is 56mm s^{-1}. For a comb row around 10mm in length it will take approximately 0.2s for a wave to travel its whole length, so there is always just one wave present within the length of the row at any time. Therefore, the power output for the passage of one wave also represents the continuous power output of the comb row. Below 5Hz, there are intervals between waves when none of the comb plates is active, and the continuous output per row is rather less than Fig. 6 indicates. The small rate of increase in wave propagation velocity with frequency means that by 20Hz or above there are likely to be two complete waves present on the comb row at any time, which would make the continuous power dissipated by the comb row twice that indicated by the values plotted in Fig. 6. The estimates for P_{max} and P_{min} attempt to make allowances for both the number of waves present on the comb row and the number of rows active and so to give an estimate for the range of continuous mechanical power output of the whole animal during forward locomotion. This indicates the limits of versatility for controlled swimming, which varies from a slow turning motion, with only one pair of comb rows operating at low frequency, to rapid forward swimming, with all eight rows beating at nearly full speed. The power output can be varied over a range of well over two orders of magnitude under the control of the organism. At very high frequencies (25Hz or greater), there may be three complete waves of activity on the row at any time. However, although some specimens achieved this, it is a rather artificial situation, since 20Hz is the highest frequency reported for spontaneously active animals (Tamm and Moss, 1985).

The power output estimated in this study is that displayed in the fastest jet seen near the tips of the comb plates during a beat cycle. The velocity of this jet is greater than that of the steady stream of water propelled off the rear end of the comb row, where each metachronal wave produces a high-speed flow that is drawn into an eddy by the movement of the body. Within this eddy, residual packets of high-speed flow due to the actions of individual comb plates may persist for a short while. These effects are clearly visible in dye trails left behind swimming ctenophores in underwater photographs and video films taken by Matsumoto (1991). Rapidly swimming unrestrained ctenophores display smooth forward locomotion, driven by the series of jets of water produced by successive metachronal waves, in exactly the manner predicted from studies on restrained ctenophores in our laboratory experiments.

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