The hydrodynamics of filter feeding in choanoflagellates

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Choanoflagellates are filter feeding heterotrophic nanoflagellates. Fluid flow generated by the motion of the flagellum delivers suspended prey particles to the surface of a crown-like filtering apparatus. Hydrodynamic characteristics are consequently of great importance to understanding prey capture in these organisms.

This paper reports on the use of video-microscopy to investigate the hydrodynamics of filter feeding in choanoflagellates. The cell and collar morphology, and the flagellar and fluid motion of three species of choanoflagellates, one from each family of the phylum, are described. Inter-species differences in these parameters are compared to one another and to optima theoretically determined by Higdon (1979b). The motion of a flagellum close to an infinite boundary is modelled using a line of stokeslets. This model predicts the occurrence of viscous eddies in the fluid streamlines. Viscous eddies are observed in the far field fluid flow of two species of sessile choanoflagellates.

Balancing forces over a control volume and using slender body theory allows estimates of pressure drop across the collar of the three species of choanoflagellate to be made. The magnitude of pressure drop (Δp) was found to be consistent with previous publications, suggesting that Δp may be a constraining parameter for filter feeders.

Key words: Choanoflagellates; Fluid Flow; Pressure Drop; Slender Body Theory; Viscous Eddies; Food particle filtration.

Introduction

The Choanoflagellida, or choanoflagellates, are single celled heterotrophic nanoflagellates of the phylum Choanozoa (Cavalier-Smith 1993). These organisms are widely distributed geographically and representatives of the group can be found in most aquatic habitats. Some species may be entirely pelagic but most are particularly associated with suspended debris or solid surfaces (Carrias et al. 1998). Recognised as primarily filter feeding bacterivores, the choanoflagellates occupy a vital niche in the microbial loop. Under some circumstances they may be responsible for the majority of the grazing pressure exerted on the bacterial standing stock (Fenchel 1982).

Mechanism of filter feeding

The basic mechanism of filter feeding, common to all choanoflagellates, is illustrated in Figure 1. The round or ovoid protoplast (cell), typically 10–20 µm in length, is modified at the distal end into a crown, or collar like structure. The radially symmetrical collar is formed from a palisade of identical microvilli, the number and length of
which vary in a species-specific manner. The length of microvilli can also vary between individuals of the same species, being influenced by both the size and the physiological state of the cell. Functionally, the collar acts as a filter, trapping bacteria on its outward-facing surface, before transporting them towards the cell where they are phagocytosed. Bacteria encounter the filter by being entrained in the fluid flow generated by a single smooth flagellum that extends from the cell apex at the centre of the collar. The flagellum propagates an approximately planar wave, which travels from the base to the tip, drawing fluid through the collar and driving it distally away from the protoplast.

The phylum comprises three families, distinguished mainly by differences in their extracellular structures (Thomsen and Buck 1991). In this paper the effect these extracellular structures may have on fluid flow is assessed by describing the flow in one species from each family. All species were selected on the basis of cell size and reliability of flagellar motion during microscopical examination.

i. The Codosugidae are naked apart from a very fine investment. Many species are sessile, being attached to solid surfaces via a fine, flexible stalk, termed a pedicel. The length of the pedicel is highly variable, and is dependent on species and life-cycle stage. In this paper Codosiga gracilis (Fig. 2a), a marine species, is used to assess how varying the length of the pedicel affects fluid flow. Although one pedicel may support between five and six cells in this species, this study limits itself to consideration of the fluid flow around a single stalked individual.

ii. The Salpingoecidae are identified by the possession of a firm ‘cup-like’ theca. The theca, which surrounds the protoplast, attaches cells directly to solid surfaces. Salpingoeca amphorida (Fig. 2b), a relatively large freshwater species, was selected for this study.

iii. The Acanthoecidae are exclusively marine and often pelagic. They are surrounded by a ‘basket-like’ lorica composed of siliceous costal strips. The morphology of the lorica is highly variable between species. The lorica of Stephanoeca diplocosta (Fig. 2c), comprises between 150 and 185 costal strips in total, and is divided by a waist into anterior and posterior chambers (Leadbeater 1979). Although a siliceous pedicel, by which the cell may attach to surfaces, does occur in some individuals of this species, all Stephanoeca diplocosta cells described here were pelagic.

Hydrodynamic environment

The hydrodynamic environment of size and flow speed within which choanoflagellates propel and filter water is dominated by viscous forces. Such environments are described by their low Reynolds number. The Reynolds number is the ratio of inertial to viscous forces; the two forces concerned in the motion of a fluid. Inertial force is associated with the motion of a mass of fluid. Viscous force is associated with the sliding of layers of fluid over one another (Vogel 1994). Reynolds number (Re) is defined as:

\[ \text{Re} = \frac{\rho v l}{\mu} \]
$\rho$ and $\mu$ are the density and viscosity of the fluid respectively, $l$ is a characteristic length of a solid surface around which the fluid is moving, and $v$ is a characteristic velocity of the fluid (Vogel 1994). The micrometre scale of choanoflagellates and their prey determine that fluid flow is dominated by viscous forces. Inertial forces will have no appreciable effect on flow. This circumstance is described by a Reynolds number of much less than unity. Typical Re values for flagellate cells range from $10^{-2}$–$10^{-4}$ (Higdon 1979a; Sleigh 1991). In such hydrodynamic environments flow is always strictly laminar, and small particles entrained in the fluid flow have negligible momentum. In flow environments dominated by viscous forces, it is possible to develop mathematical models of the hydrodynamics that can lead to an enhanced understanding of the observed phenomenon.

**Mathematical modelling of the flow**

Many papers (Lighthill 1976; Higdon 1979a, b, c; Liron and Blake 1981), utilise the concept pioneered by Hancock (1953) of using slender body theory to replace the flagellum by a distribution of ‘stokeslets’ and ‘potential dipoles’ along the centre line.

Higdon (1979b) analysed the generation of feeding currents by a sessile choanoflagellate from the Codosigidae (*Codosiga*) in this way. Higdon describes the choanoflagellate by a limited number of parameters. The spherical cell body radius ($A$), and the radius ($a$) and length ($L$) of the smooth cylindrical flagellum are used to geometrically model the flagellate. The cell body is situated at a height ($H$) above the surface to which the cell is attached. The flagellar motion is defined as a sinusoidal wave in terms of the amplitude ($b$), the wavelength ($\lambda$) and the total number of waves ($N$) occurring over the length of the flagellum (see Fig. 1). Using these parameters, Higdon determined the velocity field and power consumption for a cell with varying $L/A$, $a/A$ and $H/A$ ratios. Varying these ratios allowed Higdon to determine the theoretical optimal configuration of cellular dimensions and flagellar waveform. The present paper attempts to verify, or refute, the optima identified by Higdon (1979b), by comparing them to measurements on the cellular parameters and flagellar waveforms of three species of choanoflagellates.

Liron and Blake (1981) also employed ‘stokeslets’ to determine the occurrence, number and shape of viscous eddies generated by point forces.

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**Fig. 2.** Light micrographs of a) *Codosiga gracilis*, b) *Salpingoeca amphoridium* and c) *Stephanoeca diplocostata*. Scale bars indicate 5 µm.
near solid boundaries. The theoretical presence of these eddies is pertinent to choanoflagellates as many species attach directly, or via pedicels to solid surfaces. The present paper expands on the approach of Liron and Blake (1981) by using a line of stokeslets normal to a planar boundary to approximate the flow fields generated by the flagellum. The length of the line of stokeslets, and the height at which they are situated above the surface, is varied to simulate the cellular characteristics of the two sessile species of choanoflagellate used in this study, *Salpingoeca amphoridium* and *Codosiga gracilis*. This paper compares the occurrence and shape of the streamlines predicted by our stream function formulation to the pattern of fluid flow generated by these two species of choanoflagellate.

**Influence of the collar**

The presence of the collar as a structure through which fluid must pass has been largely discounted when modelling fluid flow around flagellates. Higdon (1979b) assumes that the collar tentacles are too fine to affect the dynamics of flow. We believe this assumption to be unfounded, and that the structure of the collar may significantly affect the hydrodynamics of filter feeding in choanoflagellates. The present paper aims to build upon Higdon's work by describing the effect of the collar on fluid flow in terms of the relationship between fluid velocity, pressure drop, and the variable collar geometry of the three species of choanoflagellates.

Pressure drop can be ascribed to the resistance fluid encounters as it passes through a filter, in this instance the palisade of microvilli that comprise the collar. The pressure drop across a filter is determined by the head-on velocity of the fluid, fluid viscosity, and the relative spacing and radius of the collar tentacles (the filter elements). The proportion of filter elements to filter pores (\( \gamma \)) will be a component in determining the resistance to flow. If fluid is forced through this filter at a given velocity, pressure drop will increase with increasing values of \( \gamma \). If, however, pressure drop is taken as a constant, head-on fluid velocity will fall as \( \gamma \) increases. In practice in choanoflagellates the flagellar beating drives water away from the cell, reducing the pressure within the collar. This creates a pressure drop across the collar drawing water between the tentacles. An organism can change the flow behaviour by altering the flagellar beat (frequency, wavelength, amplitude) or by changing the collar characteristics (number, length and spacing of the tentacles and their angle to the cell body).

Fenchel (1986) estimated the pressure drop across the filters of a number of protozoan filter feeders, using the model of Tamada and Fujikawa (1957). He concluded that pressure drop across a filter was relatively constant between species, and, therefore, may represent a physical constraint on filter feeders. The direct inference of this conclusion is that filter feeders with a high proportion of fibres to pores will necessarily generate lower velocities than filter feeders where values of \( \gamma \) are lower. The model of Tamada and Fujikawa (1957), used by Fenchel (1986), represents a filter as a single layer of parallel, identical filter elements. The gap between filter elements, \( h \), is therefore constant. This is not an accurate approximation for the filtering apparatus of choanoflagellates, where \( h \) increases as you move distally along the collar. The present study employs slender body theory and a control volume approach to estimate pressure drop across the collar. This model accommodates the increasing values of \( b \), and the decreasing values of \( \gamma \), encountered with distal movement along the collar. Details of the model are presented in Appendix 1.

**Materials and methods**

Late exponential stage cultures of the three species of choanoflagellates were prepared for video recording work. Cultures of *Salpingoeca amphoridium* were grown at 25 °C in Pratt’s medium (Karpov and Leadbeater 1997) with the addition of the bacterium *Klebsiella pneumoniae* as a nutritive source, and 1 cm lengths of human hair as an attachment substrate. Cultures of *Codosiga gracilis* and *Stephanoea diplocostata* were grown at 14 °C in seawater/distilled water medium. This medium was prepared as a 99:1 ratio of 0.2 µm-filtered seawater and distilled water. ‘C2’, a co-isolated bacterium, was added as a nutritive source to cultures of *Codosiga gracilis*, along with 1 cm lengths of human hair as an attachment substrate. Cultures of *Codosiga gracilis* and *Stephanoea diplocostata* were grown at 14 °C in seawater/distilled water medium. This medium was prepared as a 99:1 ratio of 0.2 µm-filtered seawater and distilled water. ‘C2’, a co-isolated bacterium, was added as a nutritive source to cultures of *Codosiga gracilis*, along with 1 cm lengths of human hair as an attachment substrate. Three ml of a nitrate/phosphate additive (5 g NaNO₃ and 0.75 g Na₂HPO₄ dissolved in 100 ml distilled water) was added to 1 L of seawater/distilled water medium for culturing of *Stephanoea diplocostata*. This additive stimulated the growth of co-cultured bacteria. The attachment substrate was omitted from cultures of *Stephanoea diplocostata*.

Sixteen hours prior to use the hair substrate with its attached cells was removed from cultures of *Codosiga gracilis* and *Salpingoeca amphoridium* and transferred to fresh medium. This ‘starvation’ step reduced ambient bacterial concentration, and increased the percentage of
the cell population showing flagellar activity and ingesting latex microspheres during observation.

Fluid motion was observed using a Leitz ortholux II microscope, fitted with phase contrast objectives. Recordings were made using a Sony hyper-HAD CCD camera and a Panasonic S-VHS recorder. The recorder was fitted with a Jog/Shuttle control to allow frame by frame replay. Playback was displayed on a 14" Toshiba monitor giving a final magnification of x3,660 for x40 objective and x4,800 for x100 oil immersion objective.

Microscope slides were specially adapted for recordings of fluid flow by creating a well 20 mm square and 0.5 mm deep. Such precautions are necessary to reduce the hydrodynamic distortions that close slide-to-cover slip spacing may impose on the flow field generated by a cell.

Latex microspheres 0.5 μm in diameter (Polysciences) were used throughout for tracking flows and estimating flow speeds. Microspheres were suspended in Pratt’s medium to a concentration of 5 × 10^7 microspheres ml⁻¹ for use with Salpingoeca amphoridium. Adsorption of bovine serum albumin (BSA), to the surface of the microspheres considerably reduced the aggregation of the microspheres when suspended in seawater (Polysciences, 1991). BSA-coated microspheres were suspended to a concentration of 5 × 10^7 microspheres ml⁻¹ in seawater/distilled water medium for use with Codosiga gracilis and Stephanoeca diplocostata.

One hundred microlitres of the appropriate microsphere suspension was added to the well of an adapted microscope slide, along with a 1 cm length of hair bearing the choanoflagellate cells. Cells were initially examined at x40 for feeding behaviour. Once a possible subject had been identified, recordings of the motion of the microspheres around the cell were made, either at x40 or x100 oil immersion. All recordings were made at room temperature, between 19 °C and 22 °C.

Video recordings were made onto Fuji Pro S-VHS tape at a frequency of 50 Hz, giving a temporal separation between adjacent frames of 0.02 s. Frame by frame playback allowed the position of individual microspheres entrained in the fluid flow around a cell, to be recorded directly from the screen onto a transparent overlay sheet. This was done either every 0.02 s or every 0.2 s depending on the velocity of the microsphere. Flagellar waveforms were recorded in a similar manner; the outline of a flagellum was traced directly onto a transparent overlay sheet. A separate tracing was made for each successive frame of the recording.

Collar parameters, tentacle number and tentacle radius, were determined for Salpingoeca amphoridium using transmission electron microscopy (TEM). Late exponential phase cultures were pelleted by centrifugation, dehydrated through an ethanol series and fixed and embedded according to Karpov and Leadbeater (1998). Dehydration of samples for TEM can be associated with a level of shrinkage in the cell tissue. The measured values of tentacle radius presented here should therefore be considered as a minimum. The same collar parameters for Codosiga gracilis were taken from Leadbeater and Morton (1974), and for Stephanoeca diplocostata from Leadbeater (1994).

Results

Cell characteristics and flagellar motion

Table 1 displays average cell characteristics for the three species of choanoflagellate studied. Height above the substratum (H) refers to the height of the mid point of the cell body above the attachment surface, consistent with Higdon (1979b). No values of H are given for Stephanoeca diplocostata, as all cells examined were freely suspended. Values of H for Codosiga gracilis are expressed as the observed range, because cells were selected to reflect the range of pedicel lengths observed in this species. The calculated values L/A and H/A respectively non-dimensionalise the flagellar length (L) and the height of the midpoint above the substratum (H), by dividing by a measure of the cell size, the cell radius (A).

It can be seen in Table 1 that cells of Codosiga gracilis and Stephanoeca diplocostata have similar dimensions. Salpingoeca amphoridium has a slightly larger cell radius and much longer flagellum resulting in a larger value for L/A, the non-dimensionalised flagellar length.

Table 2 displays measurements that describe the flagellar waveform and motion for the three species of choanoflagellate studied. All measurements were determined using traces of flagella taken direct from the video recording. The flagellar waveform in both Codosiga gracilis and Stephanoeca diplocostata appears to contain less than one full wavelength. Wavelength in these species was determined by extrapolating the closest observed approximations to a sinusoidal waveform. This must be regarded as an approximation as both species appear to have a large three dimensional component to their waveform. The value N is the average number of linear wavelengths on a complete flagellum.

Wavespeed (Vw), was calculated from the distance travelled by the maximum amplitude (β) of the waveform in consecutive frames. ĉk is a measure used in Higdon (1979b), as an indicator of the forward thrust generated by the flagellum. It is calculated as the maximum slope of the flagellum perpendicular to the cell body.
Table 1. Cell characteristics for *Salpingoeca amphoridium*, *Codosiga gracilis* and *Stephanoeca diplocostata*. Mean values, ± the standard deviation, are shown for all species and parameters with the exception of the height of *Codosiga gracilis* above the substratum, which is expressed as a range. *n* is the number of cells in the data set. Flagellar Length (*L*) was measured in non-feeding cells as the flagellum is characteristically held rigid and straight when not in motion.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Flagellar Length (<em>L</em>) (µm)</th>
<th>Cell Radius (<em>A</em>) (µm)</th>
<th>Height Above Substratum (<em>H</em>) (µm)</th>
<th><em>L</em>/<em>A</em></th>
<th><em>H</em>/<em>A</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salpingoeca amphoridium</em></td>
<td>10</td>
<td>20.65 ± 2.92</td>
<td>2.31 ± 0.68</td>
<td>6.20 ± 0.48</td>
<td>9.03 ± 0.31</td>
<td>2.75 ± 0.29</td>
</tr>
<tr>
<td><em>Codosiga gracilis</em></td>
<td>9</td>
<td>8.30 ± 1.83</td>
<td>1.84 ± 0.16</td>
<td>12.9–23.5</td>
<td>4.68 ± 0.12</td>
<td>5.96–13.07</td>
</tr>
<tr>
<td><em>Stephanoeca diplocostata</em></td>
<td>4</td>
<td>8.58 ± 1.95</td>
<td>1.81 ± 0.63</td>
<td>N/A</td>
<td>4.81 ± 0.32</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 2. Flagellar motion and waveform measurements for *Salpingoeca amphoridium*, *Codosiga gracilis* and *Stephanoeca diplocostata*. *n* is the number of cells in the data set. *N* is the number of complete waves in a flagellum. *αk* is the maximum slope of the flagellum perpendicular to the cell body, a measure of forward thrust as defined in Higdon (1979b).

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Minimum Beat Frequency (Hz)</th>
<th>Wavelength (<em>λ</em>) (µm)</th>
<th><em>N</em></th>
<th>Wavespeed (<em>Vw</em>). (µm sec⁻¹)</th>
<th><em>αk</em> (arb)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salpingoeca amphoridium</em></td>
<td>10</td>
<td>17 ± 0.31</td>
<td>17.92 ± 1.15</td>
<td>1.2</td>
<td>304.64 ± 1.58</td>
<td>1.7 ± 0.012</td>
</tr>
<tr>
<td><em>Codosiga gracilis</em></td>
<td>9</td>
<td>10 ± 0.33</td>
<td>10.27 ± 0.51</td>
<td>0.81</td>
<td>45.6 ± 9.38</td>
<td>2.0 ± 0.034</td>
</tr>
<tr>
<td><em>Stephanoeca diplocostata</em></td>
<td>4</td>
<td>10 ± 0</td>
<td>8.63 ± 0.47</td>
<td>0.96</td>
<td>41.3 ± 8.17</td>
<td>2.0 ± 0.021</td>
</tr>
</tbody>
</table>

Table 3. Average collar characteristics and velocity measurements for *Salpingoeca amphoridium*, *Codosiga gracilis* and *Stephanoeca diplocostata*, expressed as the mean and the standard deviation for the data set. *n* is the number of cells in the data set. *CN* is the number of tentacles comprising the collar, *CD* the tentacle diameter. Collar Angle describes the angle at which collar tentacles protrude from the protoplast, subtended to the vertical. Collar length is measured from the surface of the protoplast. *h*ₘᵢₙ and *h*ₙₐₓ are calculated as the narrowest and widest gaps between adjacent collar tentacles. *VHO* is the head-on fluid velocity; the distance a particle travels in the 0.1 s before intersecting with the collar. *V*ₘₐₓ is the maximum particle velocity observed.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>CN (°)</th>
<th>CD (µm)</th>
<th>Collar Angle (°)</th>
<th>Collar Length (µm)</th>
<th><em>h</em>ₘᵢₙ (µm)</th>
<th><em>h</em>ₙₐₓ (µm)</th>
<th><em>VHO</em> (µm sec⁻¹)</th>
<th><em>V</em>ₘₐₓ (µm sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salpingoeca amphoridium</em></td>
<td>10</td>
<td>27</td>
<td>0.1</td>
<td>19.9</td>
<td>8.05</td>
<td>0.1</td>
<td>0.70</td>
<td>26.0</td>
<td>204.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.64</td>
<td>± 0.01</td>
<td>± 5.12</td>
<td>± 2.50</td>
<td>± 0.012</td>
<td>± 0.081</td>
<td>± 2.36</td>
<td>± 13.21</td>
</tr>
<tr>
<td><em>Codosiga gracilis</em></td>
<td>9</td>
<td>30</td>
<td>0.15</td>
<td>17.2</td>
<td>4.5</td>
<td>0.11</td>
<td>0.39</td>
<td>20.5</td>
<td>109.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 1.67</td>
<td>± 1.49</td>
<td>± 4.5</td>
<td>± 0.009</td>
<td>± 0.078</td>
<td>± 2.11</td>
<td>± 19.72</td>
<td>± 19.72</td>
</tr>
<tr>
<td><em>Stephanoeca diplocostata</em></td>
<td>4</td>
<td>41</td>
<td>0.15</td>
<td>15.5</td>
<td>4.9</td>
<td>0.14</td>
<td>0.32</td>
<td>14.0</td>
<td>90.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 1.26</td>
<td>± 1.12</td>
<td>± 0.011</td>
<td>± 0.098</td>
<td>± 1.28</td>
<td>± 11.86</td>
<td>± 11.86</td>
<td>± 11.86</td>
</tr>
</tbody>
</table>
This study did not use stroboscopic illumination to determine the frequency of the flagellar beat in these choanoflagellates. We are therefore, unable to state categorically that the flagellar beat frequencies are, as given in Table 2, 17 and 10 Hz respectively; they may be a multiple of these numbers, but it is unlikely that any small multiple could give the series of flagellar profiles shown in Fig. 3.

Given that, in general terms, the motion of a flagellum can be described as:

\[
\text{Frequency} \times \text{Wavelength} = \text{Wavespeed},
\]

consideration of Table 2 shows that the observed wavespeeds for *Codosiga gracilis* and for *Stephanoea diplocostata* are approximately half that which would be predicted from the above equation. The likely cause for this disparity lies in the possible three-dimensional nature of the waveforms in these two species. Observations on the flagellar movement in *Codosiga gracilis* and *Stephanoea diplocostata* suggest that the waveforms may have a helical component, a motion that would result in significant movement of the flagellum in the ‘z’ plane of focus. This unrecorded motion may account for the deviation of the observed wavespeeds from those predicted. From the above equation the predicted wavespeeds for *Codosiga gracilis* and *Stephanoea diplocostata* are 102.7 µm sec\(^{-1}\) and 86.3 µm sec\(^{-1}\) respectively. These are comparable with the observed maximum flow rates in these species \((V_{\text{max}}\) in Table 3). This consistency lends credence to the hypothesis that there is a component to the flagellar waveform in *Codosiga gracilis* and *Stephanoea diplocostata* that could not be recorded using this method.

It can be seen in Table 2 that *Codosiga gracilis* and *Stephanoea diplocostata* have similar flagellar waveform and motion. The flagellar motion of *Salpingoeca amphoridium* is distinct from the other species, having a higher beat frequency and thus generating a higher wavespeed. None of the

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**Fig. 3.** Traces of the flagellar waveform in consecutive still frames, with a temporal separation of 0.02 sec. A single flagellar beat cycle for, *Codosiga gracilis* 1A–F, *Stephanoea diplocostata* 2A–F, and *Salpingoeca amphoridium* 3A–D.
species considered exhibited a strictly sinusoidal waveform. The waveform in all species showed increasing amplitude as the wave moved distally from the cell. This is illustrated in Figure 3, which shows one cycle of the flagellar beat, for each of the three species considered.

**Fluid motion**

Figures 4a–f, illustrate typical patterns of fluid flow generated by the two sessile species of choanoflagellate studied; *Salpingoeca amphoridium* and *Codosiga gracilis*. Two examples of fluid flow in *Codosiga gracilis* are presented, showing flow around a cell with a long (Fig. 4b and 4e) or a short (Fig. 4c and 4f) pedicel, respectively. Figures 4a–4c show the direction of particle movement in the fluid flow induced by the flagellar beating. Figures 4d–4f show details of some particle movements in close proximity to the collar. In Figures 4d–4f solid circles represent the position of a particle at 0.02s intervals. The dashed lines between the solid circles represent the path the particle has taken in the intervening period, and is a direct interpretation of the ‘smearing’ of particles seen on the video recording.

Figures 4g–4i show the streamlines generated by a line of stokeslets which, situated above an infinite plane, act together to create a force akin to that created by a single flagellum. The length of the line of stokeslets, and the distance above the infinite plane at which it is positioned, is varied in the formulation to approximate the parameters of flagellar length (*L*), and cell height (*H*), representative of *Salpingoeca amphoridium* and *Codosiga gracilis*. The stream function formulation is detailed in Appendix 1. Figure 4g shows the streamlines generated when values of *H* and *L* similar to those for *Salpingoeca amphoridium* are used, and is therefore directly comparable to Figure 4a. Similarly Figure 4h and 4i show streamlines generated when values of *H* and *L* similar to *Codosiga gracilis* with a long and short pedicel respectively, are used. These predicted streamlines can be compared to the actual flow lines generated by *Codosiga gracilis* with varying pedicel lengths (Fig. 4b and 4c respectively).

**Far field fluid flow**

Figures 4a–4c confirm the occurrence of re-circulating viscous eddies in the outer flow fields of *Salpingoeca amphoridium* and *Codosiga gracilis*. The far field fluid flow generated by *Salpingoeca amphoridium* (Fig. 4a) contains a toroidal-shaped viscous eddy, the cross-sectional ‘centre’ of which is approximately the same distance from the attachment substrate as the end of the flagellum. The ‘centre’ of the eddy in the fluid flow generated by *Codosiga gracilis* (Fig. 4b) is also approximately level with the end of the flagellum, but the optical cross-section represented by the recorded streamlines differs in shape from the eddy cross-section seen in *Salpingoeca amphoridium*. The cross-sectional shape of the eddy observed around *Salpingoeca amphoridium* is roughly circular with its major axis at 65° angle to the attachment substrate (see Fig. 4a). In *Codosiga gracilis*, the optical cross-section of the eddy is more elliptical in shape and is orientated so that the major axis is approximately parallel with the attachment surface.

The cross-sectional shape of the viscous eddy in *Codosiga gracilis* (Fig. 4b–4c) appears to be influenced by the parameter *H*, the height of the mid point of the cell above the attachment substrate. The positioning of the ‘centre’ of the eddy, relative to the end of the flagellum and the attachment surface remains the same. However, the shape of the eddy cross-section is not a symmetrical circle or ellipse, it is flattened on the side adjacent to the attachment surface giving the cross-section a more semicircular appearance.

The pattern of fluid flow generated by the stream function formulation (Fig. 4g–4i) also predicts the occurrence of an eddy. The shape, and the position of that eddy above the infinite plane alters as the input values of *L*, the length of the line of stokelets, and *H*, the height of that line above the infinite plane, are varied. The velocity field in the far field decays as the square of distance.
Near field fluid flow

Figures 4a–4c illustrate that there is a strong directional streaming of fluid along the flagellar axis. This streaming is due to the net force that the flagellum is generating in that direction. Examination of the flow in close proximity to the flagellum, Figures 4d–4f, shows that there is an increase in the velocity of flow close to the flagellum; the entrained microsphere travels greater distances between successive video frames. The velocity field in this region decays logarithmically with distance, a slow decay rate away from the flagellum.

In addition to an increase in the velocity of flow close to the flagellum, there is an alteration in the nature of flow. Far field fluid flow is characterised by the relatively smooth linear progression of the microsphere. In close proximity to the flagellum however, lateral movements of the particle become pronounced; flow becomes more oscillatory in nature (Fig. 4d–4f). The increase in lateral movement of the suspended microspheres may indicate that fluid motion within the collar is closely coupled to the lateral movements of the flagellum.

Figure 5 illustrates the details of fluid flow around the lorica of the pelagic *Stephanoeca diplocostata*.

Examination of Figure 5 reveals that fluid flow around *Stephanoeca diplocostata* displays similarities to and differences from the flow around the two sessile species of choanoflagellate studied. Recirculation of fluid in *Stephanoeca diplocostata* was not observed. The viscous eddy associated with flow in *Salpingoeca amphoridium* and *Codosiga gracilis* was not seen in this species. However, the strong directional streaming of fluid flow along the axis of the flagellum, a feature present in both *Salpingoeca amphoridium* and *Codosiga gracilis*, also occurs in *Stephanoeca diplocostata*. There is no perceptible increase in the velocity of flow as it passes through the lorica; the distance a suspended particle travels between successive video frames does not increase until it passes through the collar.

Pressure drop and the influence of the collar

Table 3 summarises the collar characteristics of the three species of choanoflagellate studied, along with average and maximal flow velocities generated by the flagellum. The collar angle describes the angle at which collar tentacles protrude from the protoplast, subtended to the vertical, when the vertical plane is parallel to the axis of the flagellum. Since the collar tentacles, the filter elements in these organisms, emerge from the protoplast at an angle, they do not lie parallel to one another. Consequently, the gap between tentacles, \( h \), increases with distance along the collar. Two values of \( h \) are given for each species. The minimum value of \( h \), \( h_{\text{min}} \), occurs when the filter elements are most tightly packed, i.e. as the collar emerges from the protoplast. The maximum value of \( h \), \( h_{\text{max}} \), occurs at the most distal end of the collar. \( h \) is calculated as:

\[
2\pi(A + R)\sin\theta - (CN^\circ \times CD)
\]

\[
\frac{2\pi(A + R)\sin\theta - (CN^\circ \times CD)}{CN^\circ}
\]

where \( A \) is the radius of the cell body, \( R \) is the distance along the collar, taking the surface of the cell body as zero, \( \theta \) is the collar angle, \( CD \) is the collar tentacle diameter and \( CN^\circ \) is the number of collar tentacles that comprise the collar, as illustrated in Figure 6.

The value \( V_{HO} \) is the head-on fluid velocity, and is calculated from the distance a particle moves in the 0.1 s prior to its intersection with the collar. As \( V_{HO} \) varies along the length of the collar, an average value is derived from the intersection of \( (n \times 10) \) particles with the collar halfway along its length. This was the best estimate of average \( V_{HO} \) possible. Determining the full range of \( V_{HO} \) from the proximal to the distal extremes of the collar, in order to calculate the average, was not feasible. At the distal end of the collar it was difficult to judge whether a
particle is in close proximity to the flagellum (see Fig. 4d–4g). This approach to studying fluid flow in radially symmetrical organisms produces a two-dimensional representation of what is a three-dimensional process. Consequently, it is impossible to record particle movement in the third dimension (depth), and thus the velocities stated here are ‘best estimates’

Table 3 documents a number of inter–species differences in collar morphology.

The collar of Salpingoea amphoridium has the smallest number of tentacles, which protrude from the protoplast at the largest collar angle. These two features combine to give the collar of Salpingoea amphoridium the highest values of \( h_{\text{max}} \); it is a relatively porous structure. The collars of Codosiga gracilis and Stephanoeca diplocostata are less porous than that of Salpingoea amphoridium. Although the three species have similar values of \( h_{\text{min}} \), the increased number of collar tentacles in the collar of Codosiga gracilis, and more particularly Stephanoeca diplocostata, combine with a smaller collar angle in both species, to reduce the magnitude of \( h_{\text{max}} \).

Measures of velocity, \( V_{\text{HO}} \) and \( V_{\text{max}} \), are greater in...
Salpingoeca amphoridium than in either Codosiga gracilis or Stephanoeca diplocostata. This may result from the longer flagellum in Salpingoeca amphoridium which may beat at a higher frequency.

The information summarised in Table 3 was used in conjunction with slender body theory in a control volume to estimate and provide insight on the pressure drop ($\Delta p$) across the collars of the three species studied. In our calculations, fluid velocity and viscosity were regarded as being constant along the collar, and the pressure drop ($\Delta p$) was allowed to co-vary with increasing values of $b$ (It would have been equally valid to maintain $\Delta p$ as the fixed variable, and allow velocity to vary as a function of $b$). The relationship between the pressure drop ($\Delta p$) and head-on velocity is linear, so a proportionate decrease in $\Delta p$ would occur if $V_{HO}$ changed. Appendix 2 summarises the calculations, and results are presented in Figure 7.

Figure 7 shows that for all three species of choanoflagellate studied the pressure drop decreases towards the distal end of the collar, as values of $b$ increase.

The magnitude of the pressure drop over the collar is not the same in the three species studied. The collar of Stephanoeca diplocostata experiences the largest difference in pressure drop between the base and the distal end. The pressure drop is relatively high at the base of the collar, decreasing to $0.4 \times 10^{-6}$ atm at the distal end. The averaged pressure drop over the entire collar length is $1.6 \times 10^{-6}$ atm. The collar of Salpingoeca amphoridium has the lowest pressure drop of the three collars modelled. Pressure drop in this species varies from $6 \times 10^{-7}$ atm close to the cell protoplast, to $5 \times 10^{-8}$ atm at the far distal end of the structure (not shown), with an average pressure drop over the whole length of $1.3 \times 10^{-7}$ atm. The collar of Codosiga gracilis experiences an intermediate pressure drop, ranging from $2.5 \times 10^{-6}$ atm at the base, to $2 \times 10^{-7}$ atm at the distal end of the collar, with an average over the collar length of $5.0 \times 10^{-7}$ atm.

**Discussion**

**Comparing cell and flagellar morphologies**

Higdon (1979b), derived optimal values for a number of cell and flagellar parameters that maximised flow rate ($U/V$), and/or minimised power requirement ($\eta^{-1}$). $U$ is the flow rate through a circular disk of radius $2A$ averaged over one flagellar beat cycle and $V$ is the average linear wave speed. Higdon used this as an estimate of the potential flow through the collar. The minimum power requirement is estimated as:

$$\eta^{-1} = \frac{P}{6\mu A U^2}$$

where $P$ is the power consumption of the flagellum, $\mu$ is the viscosity of the surrounding medium, $A$ the cell radius and $U$ the flow rate defined above.

The optima for some of these parameters, such as $L/A$, the non-dimensionalised flagellar length, and $H/A$, the non-dimensionalised height, are interdependent. Under such circumstances, Higdon (1979b) calculated the optima for the dependent variable for one or more values of the fixed variable. In the evaluation of this data, we derive the optima for the independent variable from the closest approximation available of the fixed variable. Thus, in assessing the optimal value for $H/A$, we would use the line of $L/A = 5$ for Codosiga gracilis and Stephanoeca diplocostata, but the line of $L/A = 10$ for predicting the optimal height for Salpingoeca amphoridium (see Table 1). Higdon’s study was based on planar sinusoidal waves whereas there is some evidence of three dimensional flagellar motion in our observations. This will introduce a more three dimensional component to our flow patterns and will result in particles moving in and out of the focal plane. In a more elaborate study of the hydrodynamics generated by a helical beat, Orme, Blake and Otto (2002) predict a more helicoidal averaged particle motion which retains the small scale oscillations seen in Figures 4d–f. Fig-

Figs 8–11. Redrawn with permission from Higdon (1979b). Figure 8, the relationship of $U/V$ (a) and $\eta^{-1}$ (b), to $L/A$. Figure 9, the relationship of $U/V$ (a) and $\eta^{-1}$ (b), to $H/A$. Figure 10, the relationship of $U/V$ (a) and $\eta^{-1}$ (b), to $N$, the number of wavelengths in a flagellum. Figure 11, the relationship of $U/V$ (a) and $\eta^{-1}$ (b), to $\alpha k$, the maximum slope of the flagellum.
ures 8–11 are adapted for the purpose of this study from Higdon (1979b) and are reproduced with permission.

Cell characteristics

The non-dimensionalised average flow rate, \( U/V \), shows a hyperbolic relationship with \( L/A \), the non-dimensionalised flagellar length (Fig. 8a). The average \( L/A \) ratio of 9.03 for *Salpingoea amorphidium* lies near the top of the linear part of the graph, suggesting that the length of the flagella in *Salpingoea amorphidium* may be optimised to maximise flow rate. The \( L/A \) ratio in this species is slightly larger than the optimum for minimising power consumption, \( \eta^{-1} \) (Fig. 8b). The relationship described in Figure 8b shows a defined minimum at \( L/A = 7 \), with \( \eta^{-1} \) increasing linearly with \( L \) thereafter. *Codosiga gracilis* and *Stephanoeca diplocostata* are cells of similar dimensions and produced \( L/A \) ratios of 4.68 and 4.81 respectively. These are smaller than would be predicted to minimise \( \eta^{-1} \), and produce values of \( U/V \) of just over half that of *Salpingoea amorphidium*.

The sensitivity of \( U/V \) to \( H/A \), the non-dimensionalised height, is dependent on the value of \( L/A \), the non-dimensionalised flagellar length (Fig. 8a). When values of \( L/A \) are smaller, \( L/A = 5 \) for example, and all portions of the flagella lie near to the cell body, the value of \( H/A \) has relatively little effect on flow rate. This results from short flagella being relatively ineffectual at generating flow close to a solid surface. As the flagellum lengthens and becomes more effective, and \( L/A \) increases, the effect of \( H/A \) on flow rate becomes more pronounced. Despite this, the values of \( U/V \) and \( \eta^{-1} \) are relatively insensitive to \( H/A \) as long as the height of the cell body exceeds the length of the flagellum (\( H > L \)). Therefore, Higdon concluded that the height of the midpoint of the cell being equal to the length of the flagellum (\( H = L \)) may be the lower limit for an efficient organism. *Salpingoea amorphidium* does not comply with this prediction. The average flagellar length (\( L \)) of *Salpingoea amorphidium* is far in excess of the height of the mid-point of the cell above the substrate (\( H \)). Consequently, flagellar motion in *Salpingoea amorphidium* is likely to be relatively inefficient in terms of power consumption. The graph in Figure 9b predicts that \( \eta^{-1} \) will be over 2000 for the case \( L/A = 10 \) (\( H/A \) for this species is 2.75). This compares to values of \( \eta^{-1} \) of 1200–1400 (\( L/A = 5 \)) for *Codosiga gracilis*. Power requirement is low in *Codosiga gracilis* as values of \( H/A \) vary between 5.96–13.07. In all but one of the *Codosiga gracilis* cells from which these calculations are derived, the height of the midpoint of the cell above the attachment substrate exceeded the flagellar length, \( (H > L) \).

Flagellar characteristics

The number of wavelengths in a flagellum (\( N \)) has a defined optimum dependent on flagellar length. For \( L/A = 10 \), the optimum \( N \) for both \( U/V \) and \( \eta^{-1} \) is between 1.1 and 1.3, Figures 10a and 10b respectively. *Salpingoea amorphidium* conforms to this prediction with a value of \( N = 1.2 \). When \( L/A = 5 \), the optimal number of wavelengths drops below 1, for both \( U/V \) and \( \eta^{-1} \). Table 2 reveals that *Codosiga gracilis* and *Stephanoeca diplocostata* both have values of \( N \) of less than 1.

The parameter \( \alpha k \), which is a measure of the maximum slope of a flagellum, gives an indication of the forward thrust the flagellum will generate. For all values of \( L/A \), \( U/V \) increases approximately linearly with increasing values of \( \alpha k \) (Fig. 11a). Consequently, no optimal value can be determined. The relationship of \( \eta^{-1} \) to \( \alpha k \), does yield \( L/A \) dependent optima (Fig. 11b). At \( L/A = 10 \), the optimum is around 2.5, dropping to 2 when \( L/A = 5 \). For all values of \( L/A \), as \( \alpha k \) falls below 1.5 the value of \( \eta^{-1} \) rises dramatically. Examination of Table 2 shows that both *Codosiga gracilis* and *Stephanoeca diplocostata* have values of \( \alpha k \) close to the predicted optimum of 2 (\( L/A = 5 \)). In *Salpingoea amorphidium* the value of \( \alpha k \) is 1.7, below the optimal value of 2.5 (\( L/A = 10 \)). The shape of the relationship of \( \eta^{-1} \) to \( \alpha k \) between the values 1.7 and 2.5, determines that power consumption is relatively insensitive to changes in \( \alpha k \) over this range.

In conclusion we can state that the flagellar waveforms of the three species of choanoflagellate examined conform to the optima predicted by Higdon (1979b) in terms of \( N \), and for *Codosiga gracilis* and *Stephanoeca diplocostata* also in terms of \( \alpha k \). The value of \( \alpha k \) for *Salpingoea amorphidium* is less than the predicted optimum, but the consequential increase in \( \eta^{-1} \) will be small with little reduction in the efficiency of the organism.

Cell characteristics show less agreement with the predicted optima. The flagellar length in both *Codosiga gracilis* and *Stephanoeca diplocostata* is shorter than would yield an optimal value of \( L/A = 7 \). *Codosiga gracilis* complies with Higdon’s assertion that \( H = L \) may be the minimum ratio of cell height to flagellar length for an efficient organism. *Salpingoe-
ca amphoridium does not, and consequently may have a higher power requirement to drive a flagellar motion adapted to maximising flow rate.

Occurrence and description of viscous eddies

Validity of viscous eddies

The occurrence of far field viscous eddies has been described previously, in the flow field generated by other protozoan species (Sleigh and Barlow 1976). Descriptions of the position and shape of these eddies has been criticised as being an artefact. It has been suggested (Liron and Blake, 1981; Fenchel, 1986), that the occurrence of these viscous eddies is due mainly to the influence of the microscope slide and coverslip on fluid flow. Two of the three species of choanoflagellate examined in this study generated a flow field that contained an eddy structure. We do not believe these eddies to be due to the external constraints of the apparatus for the following reason. The maximum dimension of any cell observed was slightly in excess of 25 µm, the minimum depth of the well in which the cells were contained was 500 µm. Therefore, in comparison with previous studies, which have used larger protozoa and unmodified microscope slides and coverslips, the choanoflagellate cells were further from the influence of these solid boundaries.

Similarities between theoretical and experimental fluid flows

It can be seen by comparing Figures 4a and 4g that the eddy in the far field fluid flow of Salpingoeca amphoridium resembles the eddy predicted by the stream function formula, for the same values of $H$ and $L$, in a number of respects. The major axes of the cross-sections of both actual and predicted eddies are orientated at an angle to the attachment substrate/infinite boundary. Observations suggest that this angle is greater for the eddy produced by the flagellar motion of Salpingoeca amphoridium, than for the eddy predicted by the model. The model predicts that the eddy will be fairly ‘tight’, that is, that there will be rapid recirculation of fluid. The fast velocities of fluid flow generated by the flagellar motion in Salpingoeca amphoridium (Table 3), suggest that this rapid recirculation may also occur. The position of the eddy relative to the Salpingoeca amphoridium cell, agrees with the predictions of Liron and Blake (1981), and Higdon (1979b), that the centre of the eddy will be parallel with the end of the flagellum.

The cross-sectional shape of the eddy predicted by the stream function formula when values of $H$ are large, and values of $L$ are small, is a good match for the shape of the fluid flow generated by Codosiga gracilis with a long pedicel. In both actual and predicted fluid flows, the major axis of the eddy cross-section is orientated parallel to the attachment substrate. The centre of the eddy generated by Codosiga gracilis occurs at a greater distance from the cell than the eddy generated by Salpingoeca amphoridium. The eddy is also more diffuse in the former species, suggesting slower recirculation of fluid. This prediction is in concordance with the documented velocity of fluid flow in Codosiga gracilis (Table 3).

Dissimilarities between theoretical and experimental fluid flows

The streamlines we use to model the flow field are represented by constant forces. In Stokes flow, this creates constant streamlines, ‘tori’, along which particles will travel. A particle entrained in a circular torus will follow the same smooth path and cycle indefinitely.

Experimental results do not support the existence of smooth, constant streamlines. Examination of Figures 4a–4f and Figure 5, reveals that the flow paths followed by particles are far from smooth. In far field flow (Fig. 4a–4c, Fig. 5), particles exhibit minor lateral movements, rather than a continuous smooth forward motion. In near field flow (Fig. 4d–4f), microspheres have a large oscillatory component to their motion. We suggest that this discrepancy between theoretical and experimental descriptions of flow results, in part, from an incomplete modelling of the reality of flagellar motion by the stream function formulation we have used.

Such discrepancies may result from taking the force created by the flagellum as being evenly distributed, in one net direction (away from the boundary/substrate interface). This is not an accurate description of the motion the flagellum actually undertakes. In practice, force is applied to the fluid by the whole surface of the flagellum, the orientation of which is changing constantly over the course of the flagellar beat cycle.

As the direction and magnitude of the force exerted by the flagellum on the surrounding fluid is
constantly changing, so are the flow streamlines. This constant re-orientation will be most pronounced in the fluid immediately adjacent to the flagellum, and may help to explain the oscillatory component of microsphere movement in the near field flow. In addition, the constant re-orientation of the force applied by the flagellum may alter the trajectory of microspheres, allowing them to cross from one streamline to another. This leads to a much more complicated pattern of particle flow, reminiscent of the chaotic flow pattern described by Orme, Otto and Blake (2001a, b).

Brownian motion may also have a rôle in moving small particles from one streamline to another. Over one period of a flagellar beat it can be estimated that the root mean displacement for a 0.5 µm microsphere attributable to Brownian motion will be around 0.25 µm to 0.5 µm (i.e. 0.5–1 particle radius). The influence of Brownian motion will be more observable in the far field, because of the weaker influence of the deterministic flow field associated with the flagellar beat pattern, but it can lead to streamline hopping by microspheres throughout the flow field, resulting in a more random behaviour.

The presence of the collar may also destabilise the streamlines. Fluid driven by flagellar motion will diverge to pass around the collar tentacles. Although the collar is referred to as a static object, most collars exhibit 1–2 µm of lateral movement, relative to the point of anchorage, in response to the lateral forces exerted by the flagellum. Consequently, streamlines are diverging around a non-stationary object. This may impose an additional level of randomisation on the particle flow pattern.

The influence of the boundary

The shape of the eddy in the flow field of Codosiga gracilis alters in response to varying values of $H$ (Fig. 4b–4c). In Figure 4b, where the pedicel length is 23.5 µm, the cross-sectional shape of the eddy is approximately symmetrical about a major axis in the horizontal plane. This axis runs through the centre of the cross-section. In Figure 4c, where the pedicel length is 13.1 µm, this is not the case, and a flattening of the underside of the eddy is apparent. It may be hypothesised that this is the influence of the boundary on flow close to its surface. Irrespective of the cause, it is worth noting that none of the particles from which traces were taken in Figure 4c, actually intersected with the collar. This change in eddy shape is not accurately predicted by changing the value of the height above the infinite plane, $H$, in the stream function formula. Reducing $H$, rather than producing the semi-circular eddy cross section observed in cells of Codosiga gracilis with a short pedicel, produces an eddy similar in cross-section to that produced by Salpingoeca amphoridium. The predicted eddy is orientated with the major axis at an angle to the attachment substrate. A reduction in the value of $H$, also predicts a smaller eddy, closer to the application of the point force. This is not observed in cells of Codosiga gracilis with short pedicles.

It is noteworthy that although the height of the mid-point of the cell ($H$) in Salpingoeca amphoridium is considerably less than for Codosiga gracilis (Table 1) the influence of the boundary is not detected as a change in the cross-sectional shape of the eddy in the former species. This is consistent with the observation that the mid-point of the cross-section of the eddy is the same distance from the attachment substrate as the tip of the flagellum. The greater flagellar length ($L$) and, to a lesser extent, cell radius ($A$) in Salpingoeca amphoridium combine to ensure that the positioning of the eddy is outside the influence of the attachment substrate.

Effect of the lorica

Examination of Figure 5, and an inter species comparison of the values of $V_{H0}$ (Table 3), offers no evidence to support the notion that the silica lorica of Stephanoeca diplocostata acts as a ‘flume’ increasing the velocity of fluid flow around the collar, as proposed by Andersen (1989). Codosiga gracilis and Stephanoeca diplocostata are species with similar flagellar waveform and dynamics, and similar collar characteristics. We may therefore, postulate that values of $V_{H0}$ in these species may be similar. However, a Students ‘T’ test shows that the head-on fluid velocity ($V_{H0}$), is significantly ($p < 0.05$) slower in Stephanoeca diplocostata than in Codosiga gracilis. Although this is an interesting qualitative comparison, a quantitative analysis into the effect of the lorica on the velocity of fluid flow would require the use of a loricate species with and without its lorica. In practical terms, this is almost impossible to achieve. Culturing loricate species in low silica media will reduce the breadth of the costal strips, but not remove them entirely (Leadbeater 1994). It may also interfere with other aspects of their physiology.
$\Delta P$ as a constraining feature on collar morphology and flagellar dynamics

Figure 7 illustrates pressure drop across the collar as a function of the distance from the protoplast and hence, increasing space between the tentacles ($b$). All the species of choanoflagellate studied show a similar response; pressure drop across the collar decreases as the collar itself becomes more porous and presents less resistance to flow. An analogous argument can be used to explain interspecies differences. The collar of *Stephanoeca diplocostata* has the highest ratio of filter elements to filter pores ($\gamma$). This results from a higher number of filter elements, 41, and a smaller collar angle subtended to the vertical, 15.5°. The collar of *Stephanoeca diplocostata* consequently presents a higher resistance to flow. This circumstance will result in either a larger pressure drop, if velocity is the fixed parameter, or a slower flow if pressure drop is the reference constant. By contrast, the collar of *Salpingoeca amphoridium* has relatively few filter elements, just 27, and a larger angle subtended to the vertical, 19.9°. Consequently, less resistance is offered to flow and pressure drop is smaller. For a given pressure drop over the collars of *Stephanoeca diplocostata* and *Salpingoeca amphoridium*, we would expect to observe a faster fluid flow for the latter, than for the former. Inspection of Table 3, reveals that that is the case.

Fenchel (1986), determined the pressure drop across the filter feeding apparatus of a range of flagellates and ciliates, using the model of Tamada and Fujikawa (1957). He concluded that pressure drop is a conserved feature between species and may therefore describe a physical limitation on filter feeding. The results presented in this paper give tacit support to this assertion.

Fenchel (1986) expressed pressure drop in the CGS unit of dyne cm$^{-2}$, and reported an estimated range of pressure drops from 9 to 16 dyne cm$^{-2}$, with an average of around 10 dyne cm$^{-2}$. One atmosphere is equivalent to $1.01325 \times 10^5$ dyne cm$^{-2}$, thus Fenchel (1986) predicted pressure drop to occur within the range of $8.8 \times 10^{-6}$–$1.5 \times 10^{-5}$ atm. Two choanoflagellates are reviewed by Fenchel, *Diaphanoeca* sp. and *Monosiga* sp. These species were calculated as having a pressure drop of $1.2 \times 10^{-2}$ atm and $1.4 \times 10^{-5}$ atm, respectively. Inspection of Figure 6 reveals that the estimates of pressure drop presented here are lower than those of Fenchel (1986). Although the lowest pressure drop calculated, that associated with the distal collar of *Salpingoeca amphoridium*, was around two orders of magnitude less than the estimates of Fenchel (1986), there was some overlap in the two sets of predictions. Averaging pressure drop over the length of the collar, results in estimates of pressure drop closer to those proposed by Fenchel (1986). Average pressure drops range from $1.3 \times 10^{-2}$ atm for the porous collar of *Salpingoeca amphoridium*, to $1.6 \times 10^{-5}$ atm for the dense collar of *Stephanoeca diplocostata*. Considering the differences in the models used to determine pressure drop in these two studies, the consistency of the data does indicate that pressure drop is relatively constant in a range of filter feeding organisms.

A number of physical constraints could contribute to the narrow range of pressure drops observed in filter feeders. Filter feeders must generate sufficient flow to deliver prey to the filter surface, and have filters with adequate surface area and configuration to intercept those prey particles. At its most basic, this configuration of flow and filter architecture will determine the lower limit to pressure drop. The upper limit of pressure drop may also be determined by considerations governing the delivery of prey to the filter surface. The majority of biological filters, so far described, are unbounded; that is the fluid is free to diverge and pass around, as well as through the filter. Increases in pressure drop, whether it be effected by an increase in flow rate or by an increase in the proportion of filter elements ($\gamma$), will encourage fluid to flow around rather than through the filter. This would result in a decrease in the rate of prey delivery to the filter and may influence the upper limit of tenable pressure drop.

In conclusion this study has revealed that filter feeding in choanoflagellates is not a standard process. There is wide variation in the feeding strategies adopted by different species. *Salpingoeca amphoridium* maximises the rate of flow through and around its porous collar by rapid beating of its long flagellum. Maximising the rate of flow rather than the efficiency of its cellular morphology and flagellar motion may ultimately be the strategy that optimises phagocytosis and energy utilisation for this species. In spite of the larger gaps between adjacent collar tentacles, $b_{\text{max}}$, *Salpingoeca amphoridium* is able to maintain the pressure drop across the collar by producing fast fluid flows. Maximising flow rate is energetically costly. This may indicate that *Salpingoeca amphoridium* is adapted to moderate or high prey environments.
Maximising flow rate has a number of interrelated consequences for the cell and its prey capture. The forces generated by the beating flagellum have to be balanced in a sessile organism by tensile or compressive forces at the point of anchorage (Sleigh, 1991). These forces will be substantial in *Salpingoeca amphoridium*, and may provide an explanation of why cells of this species attach directly to the substrate, rather than producing a pedicel. The compressive forces can be calculated as being of the magnitude \( \mu \beta^2 L/\lambda \) where \( \mu \) is the viscosity of the surrounding fluid, \( \beta \) is the radian frequency, \( \beta \) the amplitude of the flagellar beat, \( L \) is the flagellum length whilst \( \lambda \) is the corresponding wavelength.

Applying this estimation to *Salpingoeca amphoridium* suggests that the average compressive forces generated by the flagellar motion in this species will be 10–100 nN. A fine pedicel may not be able to withstand such large compressive forces. As a consequence of this, cells of *Salpingoeca amphoridium* will inhabit a region where the substrate to which they are attached may exert an influence on the prevailing flow regime. In these regions, ambient flow may be reduced, and the high velocities generated by the cell may be vital to maintaining bacterial delivery to the collar surface.

Choanoflagellates may take several minutes to phagocytose the bacteria entrapped on the outside of the collar. Retention of bacteria on the collar whilst awaiting phagocytosis is therefore an important aspect of feeding in these protozoa. As the velocity of fluid passing through and around the collar increases, so does the drag on the entrapped bacteria. Consequently, for a given prey type, as the head-on fluid velocity \( (V_{Ho}) \) increases, the retention of bacteria drops (Shimeta and Jumars 1991; Shimeta 1993). *Salpingoeca amphoridium* has very low levels of particle retention on its porous collar (Pettitt 2001). Under these circumstances the occurrence of an eddy in the flow field will be an advantage to the feeding cell, as it allows recapture of bacteria that have been lost from the collar. This is in contrast to Fenchel’s (1986), conclusions on the effect of eddies. He states that the occurrence of eddies would have a deleterious effect on sessile filter feeders, as it would result in constant re-formation of water already cleared of prey. Publications by Fenchel however, have concentrated on larger filter feeders, which may have higher retention efficiencies than choanoflagellates. Under these circumstances, an eddy would be disadvantageous, as there would be less recirculating prey, and less time for new prey to be recruited from the bulk flow into the eddy structure.

*Codosiga gracilis* and *Stephanoea diplocostata* adopt an alternative strategy to *Salpingoeca amphoridium*. They optimise their cellular morphology and flagellar motion for energy efficiency. In these two species this may be the strategy that optimises phagocytosis and energy utilisation, and may indicate that both species are adapted to prey poor or prey limited environments. The smaller gaps between adjacent collar tentacles, \( h_{max} \) in these two species impose a slower rate of flow in order to maintain pressure drop. The compressive forces, generated by the slower flagellar motion in these two species, will be lower than those experienced by *Salpingoeca amphoridium*. As a consequence, *Codosiga gracilis* is able to overcome the restrictions in fluid flow associated with its attachment substrate, by producing a pedicel.

References


Appendix 1: Estimating pressure drop

Slender Body Theory (SBT) is one technique that can be used to estimate the pressure drop across the collar of the three species of choanoflagellate studied. Other theories that could be used to estimate pressure drop include lubrication theory, which relies on the gap between the filter elements, \( h \), being of much smaller width than the radius of the filter element, \( \chi \). In the case of choanoflagellates, variation exists in the width of the gap between the collar tentacles, \( b \). The gap is small close to the cell protoplast, and large at the distal end of the collar. By varying the value of \( h \) in the formulation for pressure drop, it will be possible to demonstrate where pressure drop is greatest, or velocity smallest, as they are linearly related.

The estimate of pressure drop is derived by examining force balances between pressure drop and the force needed to move fluid through the collar. The pressure drop over the gap between the tentacles is specifically compared to the force per length needed to move fluid through the collar. All values used in the calculations are in dimensional form, and are expressed in units of seconds, \( \mu m \) and kg.

The force balance per unit length between two tentacles leads to an expression for the pressure change (Batchelor 1971). The force per unit length on each tentacle can be calculated approximately as:

\[
\log \left( \frac{b}{\chi} \right) = \frac{4\pi \mu V_{HO}}{\chi} \tag{1.2}
\]

where \( \mu \) is the dynamic viscosity of the fluid; derived from the kinematic viscosity and density of the fluid and equals \( 10^{-3} \) kg \( \mu m s^{-1} \). \( V_{HO} \) is the velocity of fluid flow in the direction perpendicular to the collar in \( \mu m s^{-1} \). \( b \) is the gap between the centre of the tentacles (\( \mu m \)) as shown in Figure 12, and \( \chi \) is the tentacle radius (\( \mu m \)).

Fig. 12. A horizontal section through two adjacent tentacles, showing the location of the parameters used in SBT to determine pressure drop (\( \Delta p \)).
Since the force per unit length must be equal to the pressure drop multiplied by the gap between the tentacles, \( \Delta p \times b \), where \( \Delta p \) denotes pressure drop, we obtain,

\[
\Delta p \times b = \frac{4 \pi \mu V_{HO}}{\log (b/\chi)} \tag{1.3}
\]

Therefore, on rearranging we obtain the relationship:

\[
\Delta p = \frac{4 \pi \mu V_{HO}}{b \log (b/\chi)} \tag{1.4}
\]

The variation in pressure drop can therefore be computed over the whole collar length as a function of the gap between adjacent tentacles. To enable us to compute the variation we require that: the radius of the collar tentacles is a fixed parameter, and that the velocity of flow approaching perpendicular to the collar is constant at all distances along the collar.

An equally valid approach that could be adopted, would be to designate pressure drop as the constant value, and determine the velocity necessary to force fluid through the varying gap, \( h \), between the filter elements. Average pressure drop over the whole collar is calculated using the approximate discretization formula as:

\[
\overline{\Delta p} = \frac{1}{n} \sum_{i=1}^{n} \Delta p_i \tag{1.5}
\]

Where \( n \) is the total number of intervals at which pressure drop is determined, and \( \Delta p_i \) is the pressure drop at each interval.

**Appendix 2: Stream function formulation**

In Liron and Blake (1981) the streamlines generated by a single point force above an infinite boundary were calculated. This paper builds on that work by more accurately representing the flagellum as a line of point forces. The length of this line, and the height at which it is positioned above the infinite boundary, are altered to approximate to the various flagellar lengths and cell heights from the substrate seen in the two sessile species of choanoflagellate studied.

The force generation by the line of point forces that model the flagellum acts, on average, normal to the boundary above which it is situated. An axisymmetric flow field will therefore develop. This allows us to introduce a Stokes stream function to represent the distribution of forces along the flagellum.

If we represent the radial and vertical directions by the polar co-ordinate system \((r, z)\) then the stream function \((\Psi)\) is defined in Aderogba and Blake (1978) by:

\[
\Psi = \frac{F}{8 \pi \mu} \left[ \frac{r^2}{(r^2 + (z + h)^2)^{1/2}} - \frac{r^2}{(r^2 + (z + h)^2)^{1/2}} + \frac{b \pi z}{(r^2 + (z + h)^2)^{3/2}} \right] \tag{1.6}
\]

where the parameters are defined as follows:

- \( \mu \) represents the viscosity of the fluid that surrounds the cell. \( b \) gives the distance above the boundary that the force is situated. When the force is spread over the length of the flagellum, \( b \) varies for each point in that spread.
- \( F \) is the strength of the force acting. The force may be acting at one point, or be distributed along a ‘stretched straight’ flagellum. We non-dimensionalise the flagellar length by dividing through by the height above the boundary. Thus the parameters of \( H \) and \( L \) used in the stream function formulation to model *Salpingoeca amphoridium* will be \( H = 1, L = 3.33 \) (see Table 1). The force that represents the action of the flagellum is then subdivided into a number of points spread at even intervals along the flagellar length. The longer the flagellum, the greater the number of points, and the greater the spread of the force. Thus the force per interval (\( F_{\text{INT}} \)) would be:

\[
F_{\text{INT}} = F/10
\]

when 10 intervals were used.

When representing the streamlines generated in two dimensions, the co-ordinates \((r, z)\) are defined as the horizontal and vertical axes respectively.