Busker & Gemmell: Ciliate PIV data Methodology

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(a) Ciliate cultures

Ciliates were isolated from a whole water sample (S approx. 32 psu) collected at Port Aransas, TX (27 35'59.11" N, 97 13'47.97" W). Cultures were enriched with phytoplankton (*Isochrysis galbana*, *Rhodomonas* sp. and *Heterocapsa* sp.) and placed into 50 ml tissue culture flasks with a few drops of each food. Flasks were then covered and placed in an incubator at 20C on a 12 L : 12 D cycle. Cultures were transferred weekly and fed every 3-4 days.

(b) Experimental set-up

Videos were recorded in a darkroom at room temperature using a Photron SA6 high-speed camera at 1125 frames per second and 1920 x 1440 pixel resolution. Recordings were performed in an optical glass filming vessel (10 x 20 x 40 mm) and illumination was provided from a 150W fibre optic illuminator with an attached collimator. Magnification was provided by 10x or 40x Nikon Extra Long Working Distance objective lenses depending on the species and desired field of view. Long working distance objectives allow the user to focus further away from the front of the lens and into the middle of the volume of interest. Thus, cells that are swimming in focus are free from any 'wall effects' that may impact locomotion [17]. The hydrodynamic stimulus to elicit an escape response of ciliates was produced by the vertical movement of a small sphere (4 mm in diameter) connected by a stainless steel rod to a piezoelectric pusher, which was lowered into the chamber and situated in the middle of the water column. A signal generator provided the stimulus pulse and was synchronized to both the high-speed camera and piezoelectric pusher. Once triggered, the camera saves half the video frames from before and half from after the stimulus. The stimulus was manually activated when a ciliate was in focus and close to the sphere. Approximately 20 escapes were recorded for each experimental condition.

(c) Data analysis

High-speed videos were imported into ImageJ (v. 1.48) software that was used to obtain kinematic information. Escape sequences were manually checked for any escape tracks that travelled out of the field and were not included in our analysis to avoid underestimating swimming kinematics. Additionally, to avoid including rapid jumps that were not a direct result of the prescribed stimulus, if a ciliate began an escape jump more than 15 ms before or after the stimulus, it was not included in our analysis. In total, 27 swimming sequences were analysed for *Pseudotontonia* sp., 16 for *Tontonia* sp. and 13 for *Strobilidium* sp. Escape response latencies were determined for *Pseudotontonia* sp. and *Tontonia* sp. by identifying the initial contraction movements of the tail after stimulus onset, and for *Strobilidium* sp. by identifying distinct changes in ciliary movement/structure associated with rapid swimming. Statistical comparisons between species were performed using SIGMAPLOT (v. 13.0) software. We compared the

difference in escape performance parameters using a one-way ANOVA or Student's t-test (when only two groups were compared). All data were log-transformed and checked for normality using a Shapiro-Wilk test. In a few cases when normality was not achieved through transformation, the non-parametric Mann-Whitney test was used to compare means between two treatment groups.

(d) Micro-particle image velocimetry

The larger (i.e. *Pseudotontonia* sp.) of the tailed ciliate species investigated in this study was chosen for this analysis because it provided a better ratio between organism and tracer particle size. To quantify fluid motion around *Pseudotontonia* sp. during rapid escape behaviour, we used high-speed micro-particle image velocimetry (μPIV) described in [14]. Briefly, this method uses long working distance objective lenses (40x) to create a thin 'optical sheet' for resolving tracer particles in the fluid surrounding the organism. Seeding particles consisted of unicellular microalgae, *Nannochloropsis* oculata, which are approximately 2 μm in diameter. Fluid velocity vectors from motion of particles within the focal plane were determined from sequential images analysed using a cross-correlation algorithm (LaVision software v. 8.2). Image pairs were analysed with shifting, overlapping interrogation windows of a decreasing size of 64 x 64 to 32 x 32 pixels. Subsequently, the instantaneous vorticity field, $\omega(x,z,t)$, was calculated from the instantaneous velocity vector field, where x and z are, respectively, the horizontal and vertical coordinate of the focal plane, and t is time.

(e) Flow data analysis

The flow imposed by the ciliate *Pseudotontonia* sp. jumping by tail contraction consists of two opposite signed viscous vortex rings: a tail-bound vortex that is around the contracting tail and a bodybound vortex that is around the jumping ciliate body (see Results). The time series of the circulation, $\Gamma(t)$, of each vortex was obtained by area-integrating the vorticity in the vortex at every time step:

$$\Gamma(t) = \iint \omega(x,z,t)dxdz$$

Subsequently, the temporal decay phase of $\Gamma(t)$ was fitted, with a virtual time origin $(t0)$, to the theoretical viscous decay of circulation based on the impulsive Stokeslet model [18,19]:

$$G_{fit}(t)=I_{fit}/4\pi\nu(t-t0)'$$

where I_{fit} is the fitted hydrodynamic impulse of the vortex, and ν is the kinematic viscosity. Spatial extension of the flow imposed by the ciliate jumping may be used to quantify the risk of the ciliate being detected by mechanoreceptive predators. To quantify the spatial extent of the imposed flow, we computed the area (S) within which the flow velocity exceeds a threshold magnitude (U^*) for the time instant when the flow reaches its maximum spatial extent. We then calculated the radius of an equivalent circular area, defined as $r = (S/\pi)^{1/2}$. By choosing a series of magnitudes for U^*, we plotted the resulting r as a function of U^*, to determine the spatial decay rate of the imposed flow. Note that the theoretical impulsive Stokeslet model has a spatial decay of r-3.

References:

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