Boundary effects on currents around ciliated larvae

To the Editor — Gilpin *et al.* described cilia-driven vortices around starfish larvae and discussed their relevance for swimming and feeding[1]. For free-swimming organisms, such as starfish larvae, these vortices are beguiling artefacts. This effect has been studied directly by others: in short, eddies often noted around ciliated organisms are usually due to the hydrodynamical influence of nearby boundaries; vortices arise within the confines of small chambers, but enlarge and disappear as boundaries recede[2–5].

To illustrate this, we recorded starfish and other larvae, either trapped between a slide and coverslip or tethered ~2 mm from the nearest boundaries, with sufficient prey density (*Isochrysis galbana*; ~5 μm) to visualize the flow field. (The data from which images in this paper and other findings of this study were derived are available from the corresponding author on request.) Tight eddies develop immediately near coverslipped, but not tethered, seastar larvae because flow that would normally exit dorsally or frontally is forced to recirculate when blocked by coverslip or slide. Gilpin and colleagues' 'defects' arise in large individuals when nearby sections of the sinuous ciliary band beat with oppositely directed components relative to the boundary plane; these divide recirculation into local cells (Fig. 1a, left). But these defects do not exist around tethered larvae (Fig. 1a, right; Fig. 1b), because streams driven by the same pair of regions converge downstream of the ciliary band (side view, Fig. 1b). Hence, no vortices develop near larvae far from surfaces. Other larval types with different ciliary bands behave similarly: tight eddies appear around bounded, but not tethered, larvae (Fig. 1b). Even the tethered flow is artefactual: first, streamlines are compressed in the direction of flow compared to freely swimming organisms[6]; second, large-scale eddies develop at millimetre distances, because even small larvae swim powerfully enough to stir small vessels. In natural conditions, flow fields around freely swimming larvae would be even less deformed than shown here for tethered larvae, with none of the features highlighted in ref. 1.

This demonstration sufficiently disproves Gilpin and colleagues' conclusions, but there are also clear theoretical reasons to dispute their analysis. In the viscosity-dominated Stokes flows surrounding small organisms, fluid forces and velocities decrease with distance from immersed objects[7–9]. Fluid particles much closer to a slide or coverslip than to a larva are necessarily affected more strongly by that boundary than the larva. The authors' choice of a two-dimensional model appropriately reflects that these boundary effects result in flows that are constrained, except very near the larva, to be primarily parallel to the slide or coverslip. However, both the classic squirmer model and their application thereof assume an organism embedded in an infinite, unbounded fluid domain. This modelling neglects viscous drag and flow constraints imposed by nearby boundaries, which were dominant influences in their experiments. Hence, neither the observations nor the model

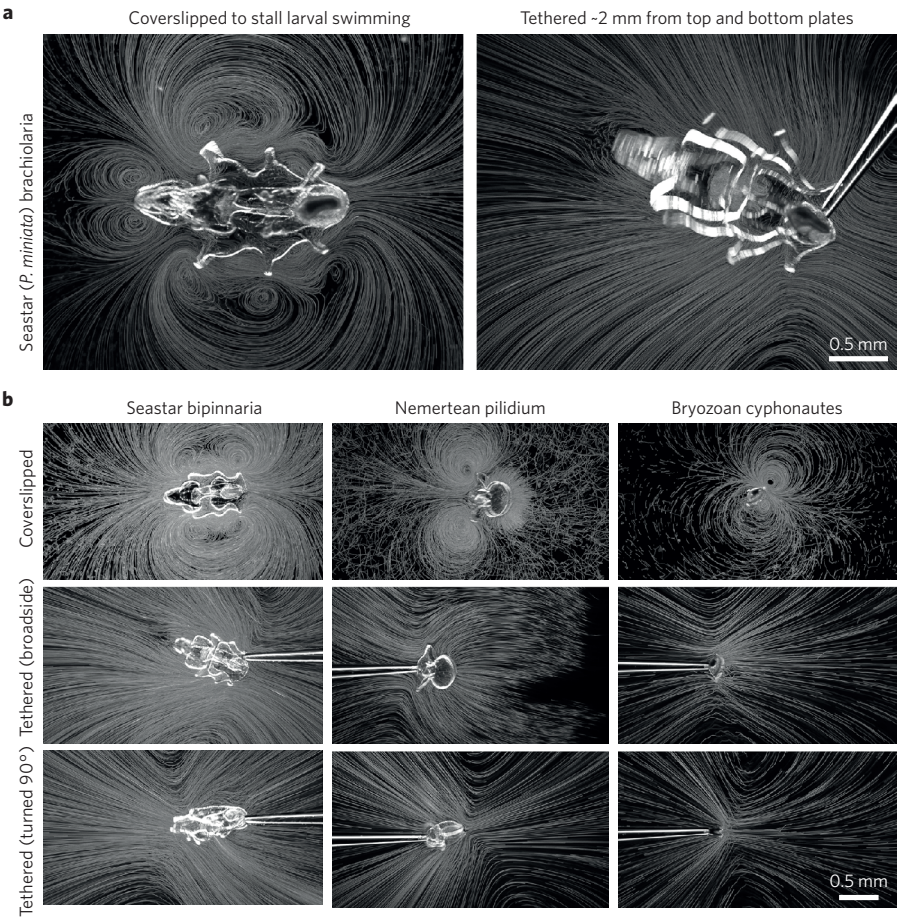

Figure 1 | Vortices around ciliated larvae are a function of nearby boundaries. **a**, Brachiolaria of *Patiria miniata* (same stage and species as studied by Gilpin *et al.*) either coverslipped or tethered by suction through fire-polished capillary. **b**, Smaller larvae: bipinnaria of *P. miniata* (~400 μm thick), a pilidium (~200 μm) and cyphonautes (~150 μm). Near-field eddies so obvious around any coverslipped larvae are absent around the same animals tethered >1 mm from any boundary. Note that the relative positions of far-field eddies depend on orientation of larval body with respect to chamber. All images are maximum intensity projections of 1,500–4,000 frames collected at 125 fps. Flow is from left to right (vortices aside).

support the authors' assertions about larval feeding and swimming, because the modelled trade-off — vortices retard swimming but increase particle capture — depends on traits and constraints not present in naturalistic settings for these organisms.

The biological relevance of boundary-induced eddies is nevertheless profound. Sessile or confined organisms that suspension feed at low Reynolds number must contend with these eddies, which might reduce food supply by recirculating the same water, or, equally, mix nearby water to overcome diffusive limits to transport. Some such organisms exhibit traits that probably constitute adaptations to nearby boundaries[5,10–12]. The functional design of internal ciliated spaces might similarly exploit circulation flow in fully internal ducts[3] or external clefts such as the bipinnaria's circumoral field. But starfish larvae swim and feed amid the boundless sea; since they are likely to approach firm surfaces only in laboratories or on terminal encounters, peripheral vortices do not factor into their normal function. ❐

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Reply to 'Boundary effects on currents around ciliated larvae'

Gilpin *et al.* reply — Whether confined with a slide or immobilized by a tether, microscopic swimmers produce currents that qualitatively differ from their freely swimming counterparts. Fortunately, this effect has been widely studied and modelled in previous literature[1–4], and is explicitly considered in our recent Letter about swimming and feeding currents produced by larval starfish[5]. The slide-confined visualizations shown in Figs 1a and 4a of our Letter produce local particle recirculation, as von Dassow *et al.* describe. However, they fail to note that our data analysis and theoretical model explicitly include this effect. Moreover, their comparison of our confined organisms to their tethered animals remains incomplete because tethering introduces a new set of confounding far-field effects that von Dassow *et al.* fail to consider when discussing our observation of topological defects in the ciliary band. Thus, von Dassow and colleagues' broad statements regarding the relevance of their observations to our conclusions regarding ciliary bands and the feeding versus swimming trade-off are inconsistent with the content of our Letter.

First, we note that, contrary to von Dassow and colleagues' statements, our original Letter shows that freely swimming animals create distorted, open particle paths rather than 'eddies' (closed loops) — this effect is clearly visible in Supplementary Movies 3 and 4 of our Letter (which show freely swimming animals), as well as in the theoretical model shown in Figs 3f and 3g (which depict unconfined feeding simulations). However, readers comparing the freely swimming and confined experiments should take note of an important distinction between what we describe as 'vortices', and what von Dassow *et al.* repeatedly refer to as 'eddies'. A vortex is typically defined as a contiguous rotational region in a flow with non-zero curl associated with the local velocity field; an eddy is a vortex region that contains closed streamlines[6,7]. Eddies (visualized by circulating tracer particles) imply the presence of vorticity, but vorticity does not guarantee the existence of eddies. This is because vorticity is independent of the reference frame, while eddies are affected by immobilization, bulk flows and confinement[3,4]. Importantly, immobilization of a swimmer causes the co-moving frame and laboratory frame to coincide, resulting in vortices appearing as closed streamlines, which we exploit only to visualize qualitatively[8] the regions associated with high vorticity in Fig. 1a and Supplementary Movie 1 of our Letter. Supplementary Figure S1 shows that instantaneous streamlines from the velocity field of a freely swimming animal can transiently form vortical flows even in the absence of closed eddies.

Correct interpretation of our Letter requires appreciating this subtle distinction between vorticity and eddies, because our observations and their associated interpretation as a particle capture mechanism do not require eddies, only regions of vorticity change near the swimmer surface. The two largest recirculation regions visible in our data arise from the lack of drag due to immobilization, and so they are also present in the far-field of von Dassow and colleagues' tethered experiments, outside of their limited field of view. However, the near-field eddies result from the combined effects of immobilization and confinement on high-vorticity regions created on the swimmer surface. But the vorticity itself is produced by sign changes in the velocity boundary conditions on the surface of the larva, which are determined by the larval cilia and not the experimental conditions. The cilia themselves are unaffected by confinement due to their small hydrodynamic cross-section (~30 μm) relative to the wall spacing (~500 μm); moreover, the ciliary reversal regions have been widely reported in the literature on invertebrate larvae[9,10]. Their direct relevance to hydrodynamics is further established by our observation of topological defects using high-magnification imaging of the ciliary band (Fig. 2 of our Letter), as well as by numerical extraction of the boundary conditions from our experimental velocity fields (Fig. 4b). The ciliary reversals and associated topological defects are directly responsible for our observed behavioural transitions, which are also visible as transient pausing in freely swimming larvae (Supplementary Movies 2 and 3).

Importantly, our theoretical 'squirmer' model and subsequent findings regarding the feeding versus swimming trade-off solely require the presence of ciliary reversal regions on the surface of a microswimmer in an open flow. These regions may give rise to eddies under a coverslip, angled streamlines near a tether, and deflected particle paths near freely swimming animals — but our model includes only their properties in biologically relevant open flows. However, we also show that the leading-order effect of confinement can be appended to the squirmer model by subtracting a Stokeslet from the stream function[1], in which case

the squirmer model creates bounded, recirculating streamlines that qualitatively match the confined experimental flow fields (Fig. 4e and Supplementary Section 3C of our Letter).

Finally, we note that the tethering experiments shown by von Dassow *et al.* introduce a new set of confounding effects, in which pathlines appear to terminate on the surface of the larvae — an effect seemingly in violation of the no-flux boundary conditions. This is because tethering induces artefacts due to poor z-plane isolation, in which particles following three-dimensional trajectories pass out of focus upon approach to the swimming body — hence obscuring essential details of the near-field boundary conditions and topological defects. Because confined flows decay more slowly ($\sim r^{-2}$) relative to open flows ($\sim r^{-3}$), tethering makes it difficult to evaluate the effective two-dimensional contribution of the ciliary band outlining the circumoral field. Thus while our choice of the two-dimensional squirmer represents a 'reduced order' model comprising the effective two-dimensional component of the flow field — which is more easily visualized in a confined setting — it is also biologically appropriate due to the one-dimensional form of the ciliary band and point-like topological defects.

Invertebrate larval forms have transfixed biologists for hundreds of years[11], and we believe our Letter demonstrates that these elegant structures produce unexpected and fascinating physics that may shed light on the subtle role that physical constraints played in the earliest stages of animal evolution. The correspondence by von Dassow *et al.* highlights the current limitations of laboratory methods for studying microscale biological flows, which provide only two-dimensional, static snapshots of rapidly changing, three-dimensional phenomena. Thus, developing truly quantitative models of behaviour will require biologists and physicists alike to look beyond slides and tethers, towards emerging imaging techniques such as light-sheet illumination[12] and light-field capture[13]. ❐

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