Shear vibes: Delineating movement and cilia stroke patterns in the planula of the hydrozoan *Clytia gregaria*

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ABSTRACT

Many factors contribute to a larva's ability to navigate its three-dimensional domain, including abiotic influences such as currents and flow as well as organismal traits including larval morphology and behaviour. Using the hydromedusa *Clytia gregaria* (L. Agassiz, 1862) (Cnidaria: Hydrozoa), we investigated the behaviour of planulae in different hydrodynamic conditions and anaesthetised and de-ciliated larvae to observe mechanisms behind movement. In this study, we provide evidence that cnidarian planula are capable of both actively righting themselves and passively moving upwards via positive buoyancy. Planulae maintain their orientation even in high shear, suggesting their ability to sense shear and control their movements in response. We also provide a first detailed description of ciliary strokes and movement patterns for a hydrozoan planula, highlighting the potential control of active and inactive cilia. Our results suggest planulae may move in a yet unknown combination of ciliary movement and muscular activity. We provide important insights into an often overlooked yet crucial cnidarian life history stage as well as a comparative reference point for further studies of larvae in this highly diverse phylum.

INTRODUCTION

Marine invertebrate larvae provide a capacity for dispersal for benthic and sessile adults. This dynamic life stage is associated with high mortality rates and dictates species recruitment (Thorson, 1950; McEdward, 2020). After a variable time in the pelagic environment, a suitable habitat must be found, which is of crucial importance, especially for sessile organisms. Success in the planktonic larval stage is necessary and plays a large role in an organism's fitness and ability for species dispersal. It is also one of the most sensitive periods, particularly in the context of changing spatiotemporal patterns and increasing environmental stressors as a result of anthropogenic climate change.

An important contributor to being a "successful" larva that grows to competency and eventually into a functioning adult, is its ability to navigate its three-dimensional domain, that is the water column. This includes factors such as environmental flow, biomechanical constraints associated with organismal morphologies, and swimming behaviour (Butman et al., 1988; Clay & Grünbaum, 2010, 2011; Fagerström, 2022; Fuchs et al., 2015). Clay & Grünbaum (2010) demonstrated that complex larval morphologies allow for distinct interactions within shear environments, and apparently simple (i.e., ellipsoid or spherical) body plans may jeopardise larval positioning in the water column due to an increased propensity to tilt into downwelling water. Cnidarian planula larvae are characterised by such an apparent simple morphology, having oblong, torpedo-like shapes, densely covered by one type of cilia. They lack apparent stabilisation mechanisms, that are present in other marine larvae, such as the pleuteal arms in certain echinoderms (e.g., Grünbaum & Strathman, 2003; Fagerström et al., 2022; Jones et al., 2015; Krasovec et al., 2021). Previously, it has been shown in planktotrophic planulae of *Desmophylum pertusum* that these larvae usually move directed at the aboral pole with an upwards swimming behaviour (even after turbulence) until the larva reaches competency (Fagerström, 2022; Freeman, 2005). Movement is thought to be mainly facilitated

by simple cilia, in contrast to compound cilia (e.g., Weis, Keen, & Buss, 1985) (Fig. 1). Although several studies investigated planulae, it is still unexplored how planulae and other simple larval types behave and interact with varying degrees of shear, and how it compares to more complex types, as discussed in Clay & Grünbaum (2010). Furthermore, cilial contribution to directional movement patterns has not been explicitly described to our knowledge, but it is assumed that coordinated ciliary beating is major contributor to planulae movement (see Leclere & Röttinger, 2017)

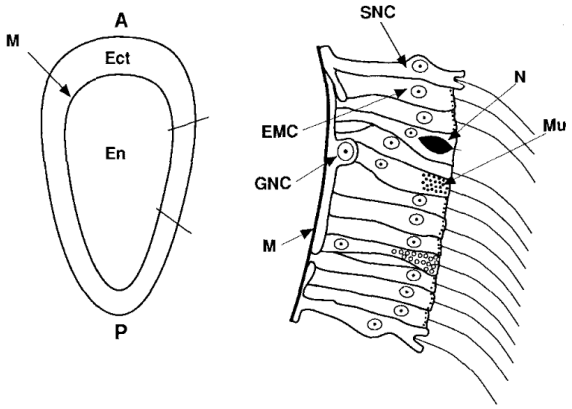

Figure 1. Illustration of a hydrozoan planula larva taken from Freeman & Ridgeway 1990 (their Fig. 1, page 64). Left: Planula composed of 2 cell layers including ectoderm (Ect) and endoderm (En), separated by mesoglea (M) with anterior/aboral (A) and posterior/oral (p) end. Right: Detail from the ectodermal wall of the planula showing Epitheliomuscular cells (EMC) which bear single cilia. The EMCs contain scattered mucus cells (Mu) and nematocysts (N). Neurosensory cells (SNC) are present throughout the ectoderm and ganglion cells (GNC) lie on the mesoglea.

A good study system to investigate these questions are hydrozoans, which are commonly found in the plankton, are easy to collect and cultured to obtain planula larvae in a short period of time. Hydrozoans boast some of the most diverse life cycles in the animal

kingdom (Bouillon et al., 2006; Collins et al., 2002) but textbook examples include the direct development of a planula or actinula larvae into a polyp stage, which can form asexually produced zooid colonies, that then produce mobile and sexually reproductive medusae (see Bouillon et al., 2006).

Several hydrozoan species are utilised as model organisms, such as *Hydra* sp., *Hydractinia echinata (symbiolongicarpus)*, or *Clytia hemisphaerica* due to their remarkable ability for regeneration and immuno-recognition (e.g., Frank et al., 2005; Lechable et al., 2020). Despite the importance of some hydromedusae as model organisms, little attention has been placed on fundamental characteristics of hydrozoan planulae, such as body movement, ability to change shape from oblong to round body plans, cilia characterisation, or larval positioning in different flows. Best studied are planulae of tropical scleractinian corals, such as *Acropora* or *Pocillopora* (e.g., Richmond, 1987), and it is assumed that the characteristics of these taxa are common to the entire diverse group. To our knowledge, no study has described the ciliary beating in hydrozoan planulae and there are few general descriptions of planulae movement in varying hydrodynamic conditions.

We used *Clytia gregaria* (L. Agassiz, 1862; Fig. 2), a common hydromedusa in Friday Harbor, San Juan Islands, Washington to investigate the behaviour of planulae in still water and different degrees of shear. Our main aims were to: (1) characterise planula movement patterns in water with and without shear, and (2) infer how ciliary movement is utilised to maintain an upward-pointing orientation/active righting. We hypothesised that planulae can maintain directionality and actively swim against or with shear forces. We also give a first description of the cilia and their movement patterns in *C. gregaria* planulae with remarks on the importance of ciliary movement as the primary method of movement and orientation in this species' larval stage.

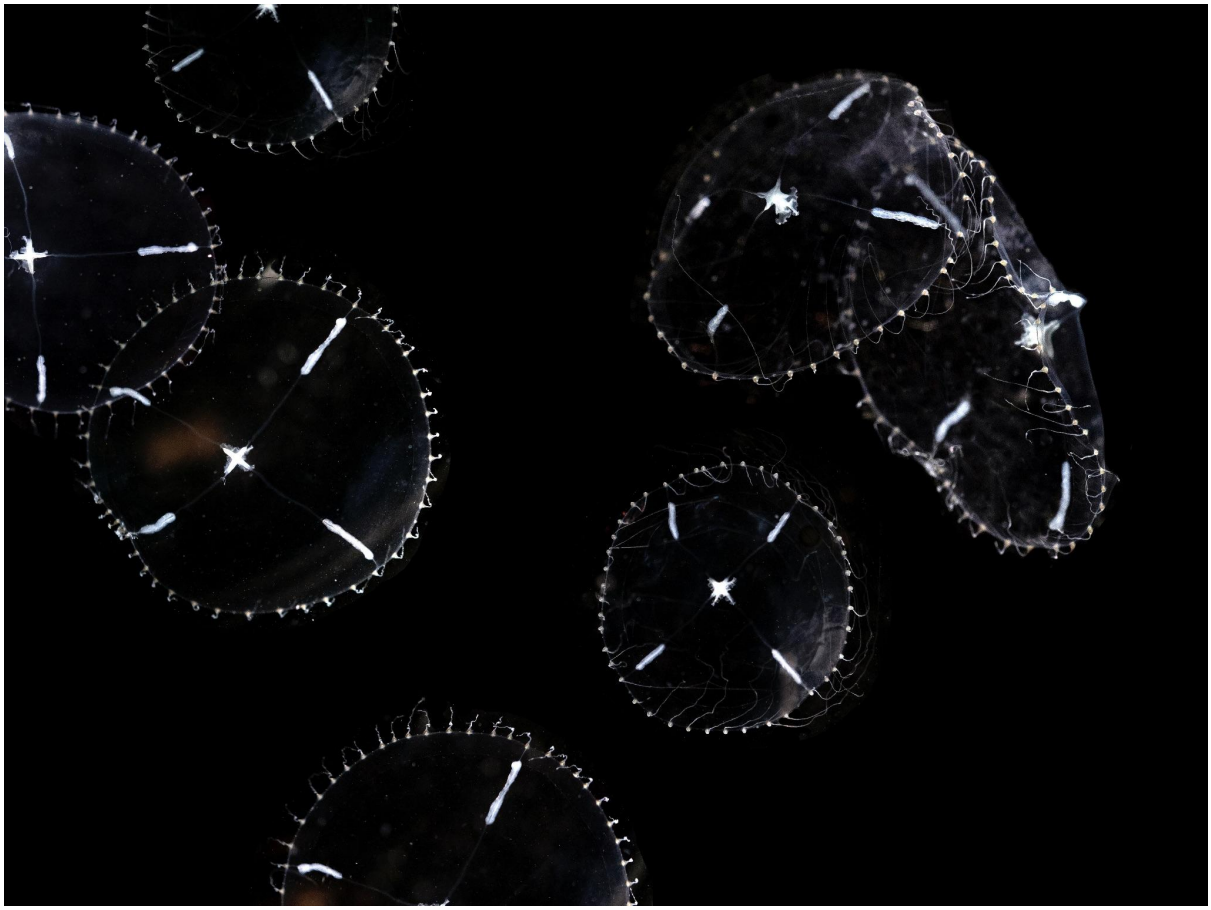

Figure 2. Individuals of *Clytia gregaria* collected at the docks of FHL. Image courtesy of Yu Kai Tan.

METHODS

Collection of organisms

We collected individuals of the hydromedusa *Clytia gregaria* at the floating docks at Friday Harbor Laboratories, San Juan Islands, WA (48.545141°N 123.012059°W, 0.5 m depth) in late June and early July 2023. Organisms were kept individually in small dishes with 0.45-µm filtered seawater (FSW) in temperatures of 13–15°C for up to 4 d. We changed the water daily.

Fertilisation and culture of larvae

We obtained gametes of *C. gregaria* by light-dark intervals and warming stress following a method adapted after Miller, 1980: small dishes were covered with aluminium foil overnight and put in direct sunlight in the morning. Female *C. gregaria* medusae usually release eggs 2–4 h after being exposed to light. Sperm was obtained similarly, though males seem to release sperm constantly in varying concentrations.

Eggs were individually pipetted into a new dish with FSW. Sperm released into the dishes was taken by pipetting 1–2 ml of seawater from male dishes into the dish with eggs. Fertilisation occurred in 13–15°C cooled by flowing water in open aquaria. 2-cell embryos were usually observed after 1–3 h. Once gastrulae developed (after approximately 10 h), they were transferred to small 12-well plates, approximately 5–10 gastrulae in each well and kept in the well plates until they developed into planulae, *circa* 10 h later. Developmental stages were assessed using a dissecting microscope and taking pictures with an iPhone 12Mini camera through the microscope ocular.

Shear trials

We constructed a tank (Fig. 3) consisting of two 50 ml cell culturing flasks that formed two enclosed water circulation systems and simultaneously served as the sidewalls of the observation chamber. The inner gap of the two flasks tanks was separated by a narrow gap of approximately 9 × 45 × 75 mm (length × width × height) and two microscope slides were hot melt glued between the flasks to act as the front and back of the observation tank. An additional black piece of acrylic was placed behind the back end for contrast. The so-created chamber could hold in total around 50 ml of liquid. Temperature could be controlled by pumping reverse osmosis (RO) water from two temperature-controlled chillers into the two cell culturing flasks. Thus, by adjusting the inflow temperature in each flask a controlled level of shear could be

obtained in the chamber between the flasks. We used temperature differences between the flasks and thus obtained three different levels of shear, from now on called trials: (1) no shear (13°C in both flasks), (2) low shear (5°C difference with left flask 18°C, right flask 8°C), and (3) high shear (10°C difference, left 23°C, right 3°C). Shear was based on the ambient temperature of saltwater, 13°C, such that the "no shear" treatment was held at 13°C.

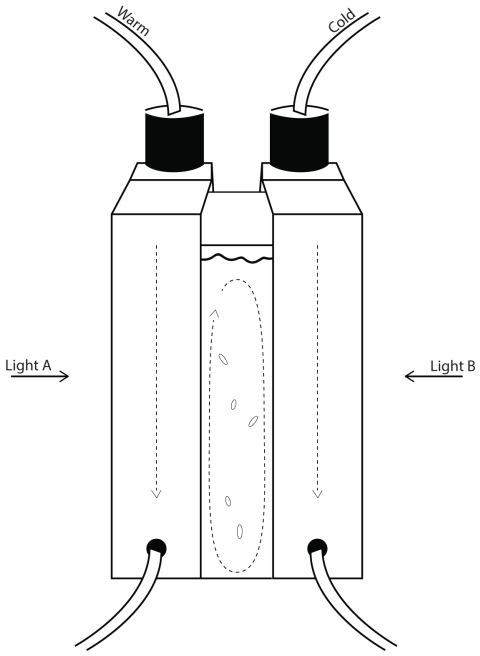

Figure 3. Shear tank set-up.

Approximately 15–30 larvae (40–50 h post-fertilisation) were placed in these three different shear trials. After the larvae were acclimated to the shear for at least two minutes, they were filmed using a video camera (Sony™ Handycam HDR-CX550, 30 fps) for approximately 5 min in each shear trial. The order of trials was randomised across two replicate rounds with different larvae. Individual videos were trimmed into three clips to reduce the number of frames to analyse. The three clips were chosen to maximise larval visibility and were between 25–35 s (900–1100 frames) each.

In addition to experiments in filtered seawater (FSW, referred to as responsive planula from now on), we also added another treatment to assess the role of active swimming. For this, we anaesthetised around 30 planulae by arresting epitheliomuscular cells (Fig. 1) using magnesium chloride (MgCl). MgCl blocks muscular activity through sodium channel inhibition and does not act on ciliary microtubules, which require energy from ATP hydrolysis (Satir et al., 2014). Planulae were relatively resistant to the MgCl anaesthesia in seawater; therefore, to maintain an immobile state, we ran subsequent flow experiments in a 7.5% MgCl solution, diluted to a 2:1 ratio of MgCl:FSW. Preliminary testing confirmed that this dilution was sufficient to arrest planular movement while under microscopic observation and was assumed to also stop ciliary movement via arresting epitheliomuscular cells. We acclimated the larvae for 3 min to become immobile in the MgCl solution before shear treatments began.

During trial analysis, we noticed that there appeared to be a leak within our shear tank. Despite several attempts to fix it, we were unsuccessful and forced to proceed with a leaky set-up. All trials and treatments were run in the same tank and were thus exposed to the leak.

To observe the behaviour and movement of planulae that lack any kind of cilia, we removed cilia by quickly dipping each larva into 2X saltwater (~10 s) at approximately 60 ppt (ambient FSW has 30 ppt).

Tracking in Fiji

To track individual planulae, we used the plugin TrackMate (Ershov et al., 2022) in Fiji (Version 2.9.0; Schindelin et al., 2012). We then imported the trimmed videos (with 900–1100 frames) via the FFMPEG option in Fiji without virtual stack. The frames were scaled with 1-cm marks on the shear tank and the frames were cropped to only include the inside of the shear tank (Fig. 4). We then converted the video to grayscale 8-bit via the split channels option (red

channel). To simplify the tracking, we subtracted the background with the "subtract background" option and a threshold of 5–50 pixels, depending on the video quality.

TrackMate was opened through the Fiji plugins, and all frames were selected for analysis. We used the LoG detector with an object detection size of 0.05 cm and no prior quality threshold. No initial thresholding but a subsequent quality filter was applied to the detected spots. We used the overlap tracker with a precise Interaction-over-Section (IuO) calculation, which uses the object's contour to compute union and intersection to ensure tracking even by overlapping of objects (also see https://github.com/trackmate-sc). We then applied a subsequent filter to select only tracks of planulae and exclude any random tracks or smaller particles in the water. The tracking data were saved as readable csv and xml files. AVI videos and tracking data were visually assessed to confirm that the resulting tracks correspond to planulae and not random particles in the water. If an individual planula was divided into separate tracks, we either added or averaged out the data to get one single track for each planula. We did not track de-ciliated planulae due to time constraints and technical issues, but we performed a qualitative analysis of movement, shape changes and sizes.

The following data points were collected for all tracked planulae for both FSW and MgCl treatments: position in tank (X and Y), water movement at larval position (up, down), larval direction (up, down, horizontal), orientation change (yes or no), and mean speed (cm frame^{-1}). We adjusted the TrackMate output of speed from cm frames^{-1} to mm s^{-1} by multiplying the mean speed by 300 (30 fps × 10 mm cm^{-1}).

Flow subtraction

To calculate the speed of shear water flow, we placed approximately 200 µl of *I. galbana* algae culture (Tahiti strain) in the shear tank to act as non-moving, neutrally buoyant particles. These particles were filmed both in FSW and MgCl treatments, under low shear and

high shear. To calculate the average speed of each particle, we digitally tracked each particle using Fiji's TrackMate plugin (see above for details, with adapted LoG detector to object detection size of 0.02 cm). The average particle speed was used as a measure of the water flow speed. Since larvae experienced different water flow depending on their position in the water column (either middle, right side, left side, bottom, or top), we categorised larval position by assigning each observed larva a position represented by the categories in Figure 4. We then only compared larval speed to water flow in similar locations in the water column. Given Fiji's TrackMate only output speed in terms of average absolute speed, we assigned categorical variables (upwards, downwards) to the water flow and larval direction as a measure of "velocity," however, we note that this method is not ideal and assumes that the flow gradient is constant instead of a gradient. To calculate the net, flow-corrected larval speed, we used the following logic: if the vertical direction of the water flow was in the same direction as the larva's movement, then water speed was subtracted from the larva's measured speed; if the direction of the water flow was in the opposite direction of the larva's movement, then water speed was added to the larva's measured speed. We performed flow subtraction on all tracked larvae (including the no shear trial) to mitigate effects of the leak present in our study system.

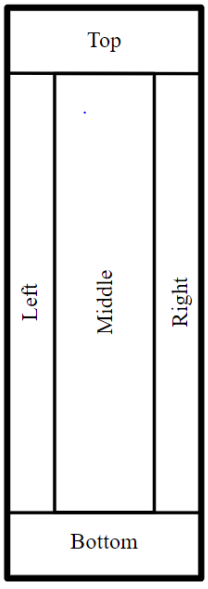

Figure 4. Divisions of the shear tank to account for different flows within the tank. Video of the shear tank was cropped to create a clean frame for analysis (outer thick black line). In Fiji, larvae were tracked and assigned relative positions based off of the rough categories depicted above (±0.5cm).

Cilia and bodily movement

To measure *C. gregaria* planulae cilia and their bodily movement, we filmed individual planulae underneath a high-speed camera (Photron MC1 HSV camera; 125 frames s^{-1}) mounted to a compound microscope (Olympus BH2 series microscope; 125X total magnification). First, individual planulae (responsive, de-ciliated and anaesthetised) in a drop of FSW on a microscope slide and under a raised cover slip, were filmed at high-speed for a qualitative analysis of their micro-movements regarding shape, direction and rotation in water.

Secondly, we isolated individual cilia in high speed videos and tracked over one beat cycle. For each cycle, we recorded the beginning and end times of both the power and recovery stroke. The power stroke is the active portion of a ciliary beat cycle, while the recovery stroke primarily functions to reset for the subsequent power stroke We repeated this process for five cilia in each of five replicate planulae.

We determined cilia length by processing still frames of high speed videos and measured using Fiji (Version 2.9.0; Schindelin et al., 2012). This process was repeated for five cilia in each of five replicate planulae. An ANOVA was performed to assess: 1) differences in cilia length across replicate planulae and 2) the duration of the power and 3) the recovery stroke. For 1) planulae were treated as a fixed factor and 2) and 3) as blocking factors and the type of stroke as a fixed effect. We used a natural log transform on the stroke duration data to satisfy assumptions of normality.

Statistics

All statistical analyses were carried out in RStudio (v. 2023.06.0). Plots and graphs were created using the *ggplot2* package (Wickham, 2016) and pictures were assembled in Adobe Illustrator 2023 (v. 27.6.1). The R packages *tidyverse* (Wickham et al., 2019) and *dplyr* (Wickham et al., 2023) were used to calculate standard statistical measures, including mean, minimum, and maximum values. The function *shapiro.test()*, a Shapiro test for multiple factors, was used to check for normality of the data and a Levene test was used to check for variance homogeneity using *LeveneTest()*. A Breusch-Pagan test was also used to check for variance homogeneity using *bp()* in the R package *lmtest* (Achim & Hothorn, 2002). For analysis of more than two independent variables and interaction between, a two-way ANOVA was performed using the R function *aov()*. A post-hoc test was performed using a *Tukey HSD()* function to determine significance levels between and across shear trials and MgCl v. FSW treatments.

RESULTS

Development of C. gregaria

2 h after fertilisation, the first signs of embryogenesis were visible. The 4-cell embryos developed within 3–5 h post-fertilisation, and blastulae developed before 24 h (Fig. 5). Fully developed, swimming planula larvae were obtained 24–30 h after fertilisation. Planulae had a length of around 260 μm and width of 120 μm while moving forward, with a general torpedo-like shape (rounded in cross section), but these dimensions changed depending on their movement.

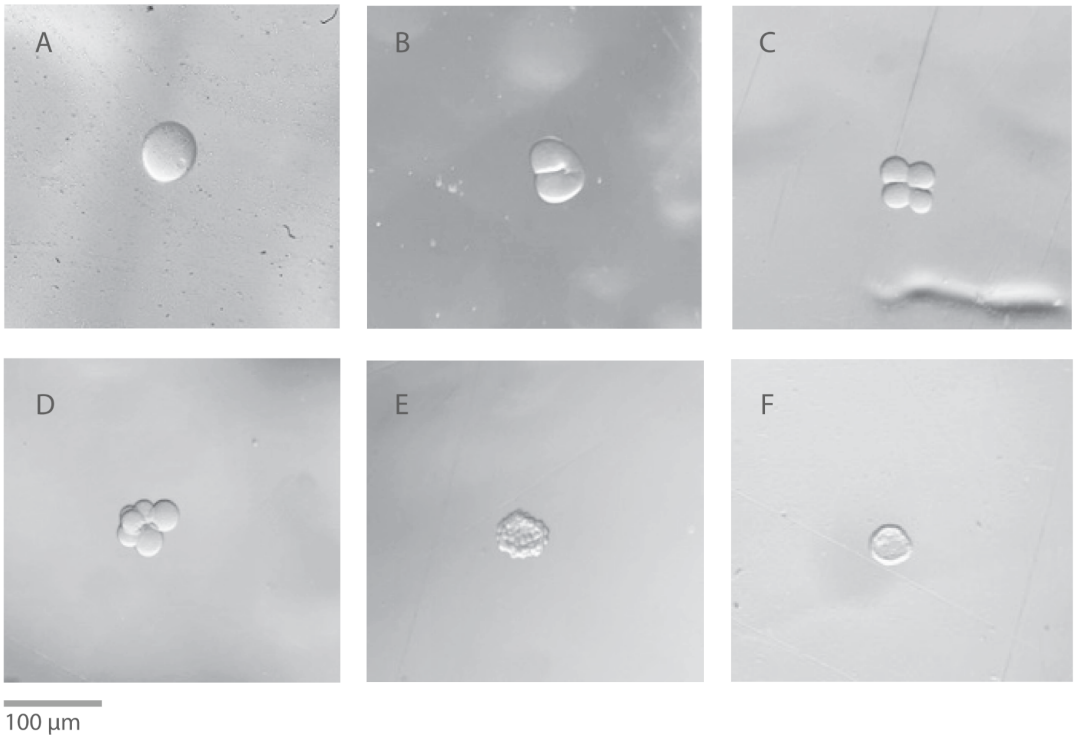

Figure 5. Developmental stages of *C. gregaria*. (A) Egg after spawning, (B) 2-cell embryo, (C) 4-cell embryo, (D) 8-cell embryo, (E) 128-cell embryo, and (F) late blastula.

High-speed observations of planula behaviour

Responsive planulae moved with their aboral side first, maintaining an oblong, torpedo-like shape with a rotating movement along their aboral-oral axis (Fig. 6). Due to the restriction of the cover slip, only horizontal movement in one plane was observed, which was constant during the high-speed recording and observations under the stereomicroscope. When planulae encountered obstacles, their body shape and swimming direction changed away from the obstacle.

In de-ciliated planulae, body shape was smaller and rounder and not as oblong as in the responsive and anaesthetised individuals. They were furthermore unable to move in a directed trajectory, even when re-exposed to FSW. However, their body appeared to stretch and elongate more, which was not observed in other treatments.

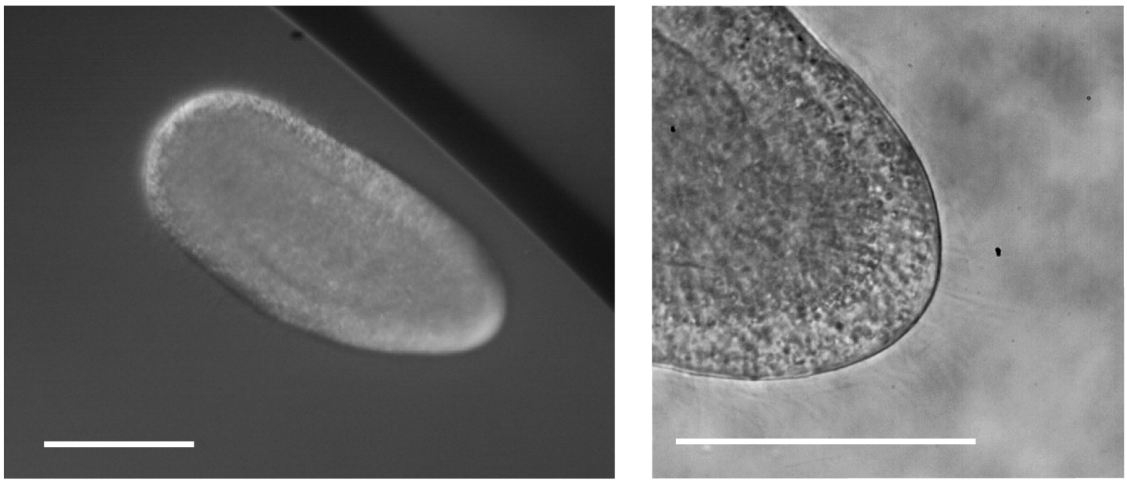

Figure 6. Left: Planula in FSW treatment with an oblong, forward moving shape (aboral side is facing the left upper corner). Right: Detail of the same planula showing the cilia at the aboral side. Scale bar is 100 μm.

Planula in shear: Orientation and directionality

In total, 83 planulae were tracked: 67 responsive larvae in FSW were observed in no shear (24), low shear (25), and high shear (18). 16 planulae were anaesthetised in MgCl and observed in no shear (8), low shear (8), and high shear (10). There was no significant difference between the two FSW replicates ($p = 0.065$). We therefore combined the two FSW replicates for comparison with the MgCl treatments. A third replicate was conducted, but was not analysed as footage analysis was impossible due to technical issues.

94% of responsive planulae maintained their orientation (showed no signs of tumbling) in the water column across all shears ($n = 59$ vs. 63). In the MgCl treatment, 20% of the tracked planulae maintained their orientation ($n = 6$ vs. 26) across all shears.

During shear trials with responsive planulae in FSW, 82% of planula showed an upward swimming behaviour under control conditions (in low shear 92%, in high shear 87%) and 62% of planulae swam in the opposite direction of water flow in still (8% in low shear, 10% in high

shear). In MgCl the anaesthetised planulae in almost all trials moved in the direction of the flow (100% in still and high shear, 90% in low shear) and in still the tracked movement was downwards in 100%, in low shear 75% and in high shear 30%.

Irrespective of direction, planulae appeared to swim in an oscillating trajectory expressing a sinusoidal path in 2D. The swimming patterns were consistent among all responsive planulae and across all shear levels and replicates. In high shear, we observed a qualitatively stronger oscillating trajectory with an increased amplitude, while planulae in low or no shear swam in more linear trajectories. Although anaesthetised planulae looked similar to responsive planulae in shape, they performed tumbling patterns, with no prominent orientation across all shear levels, including still water. 13 out of 16 anaesthetised larvae exhibited downwards movement and in the direction of waterflow. In contrast, de-ciliated planulae changed their body shape from oblong in FSW to a round shape, but tumbled in the water, like the anaesthetised planulae. We did not observe orienting behaviour in anaesthetised planulae.

Planula in shear: Movement and speed

Across both anaesthetised and responsive larvae, raw speed (i.e., not flow-corrected) increased with increasing shear (Fig. 7). Between MgCl and FSW treatments for each respective shear trial, raw speed did not significantly differ (Table 2). In FSW treatments only, raw speed was significantly different between no shear and low shear and no shear and high shear trials ($p = 0.034$ and 0.020, respectively; Table 2). In MgCl treatments only, there was no significant difference in raw speed between any shear trials (Table 2).

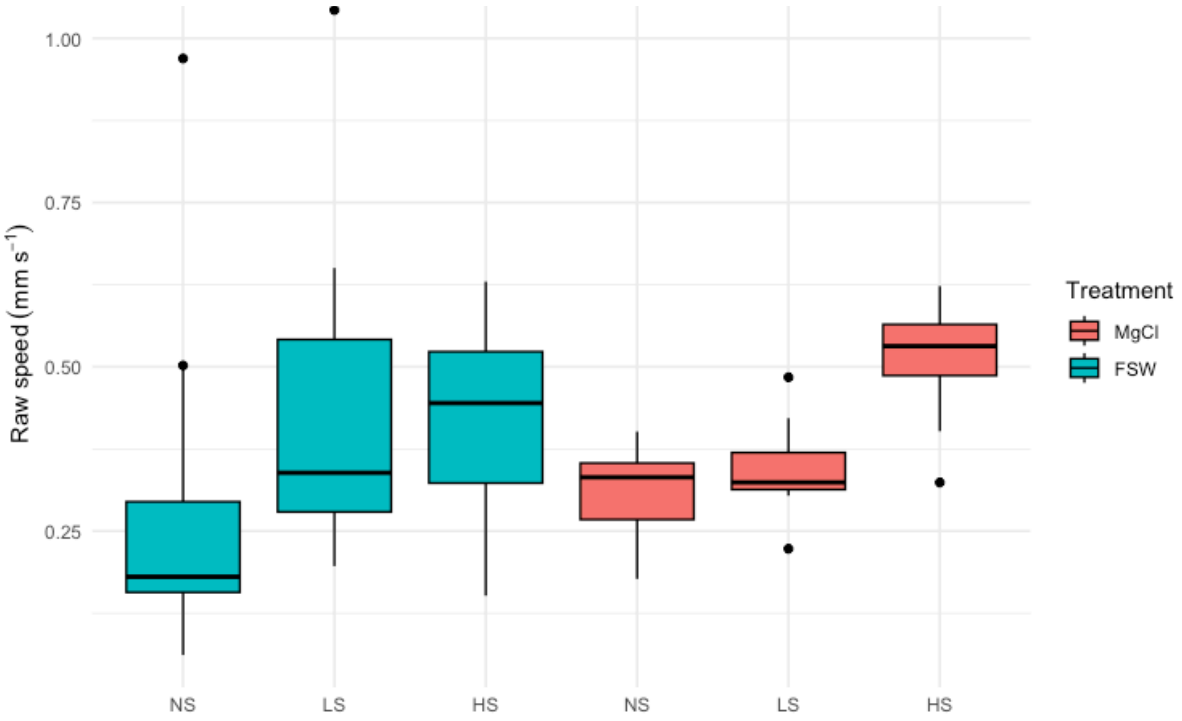

Figure 7. Raw planula speed for both responsive (FSW, cyan) and anaesthetised planulae (MgCl, in red). NS = no shear, LS = low shear, HS = high shear.

In contrast to raw speed, flow-corrected speed decreased with increasing shear for both anaesthetised and responsive larvae (Fig. 8). The majority of responsive planulae had positive flow-corrected speeds that decreased with increasing shear, whereas anaesthetised larvae primarily had negative flow-corrected speeds that increased towards zero with increasing shear (i.e., still decreasing speed; Fig. 8). These negative flow-corrected speeds indicate that anaesthetised larvae were moving slower than the surrounding water. Depending on the larva's position in the shear tank, these results can either be interpreted as sinking relative to the surrounding water when in the upwards-flowing side of the tank or positive buoyancy in the downwards-flowing side of the tank.

The interactions of both treatments (FSW v. MgCl) and shear levels were significant ($p = 0.003$; Table 1). Flow-corrected speed significantly differed between MgCl and FSW treatments for the respective no shear and low shear trials ($p < 0.001$) but not the high shear

trials (p = 0.426; Table 3, Fig. 8). In FSW treatments only, flow-corrected speed was significantly different between the no shear and high shear trials (p = 0.020) but not between no shear and low shear or between low and high shear trials (Table 3). Within only the MgCl treatments, flow-corrected speed was not significantly different between any of the shear trials (Table 3).

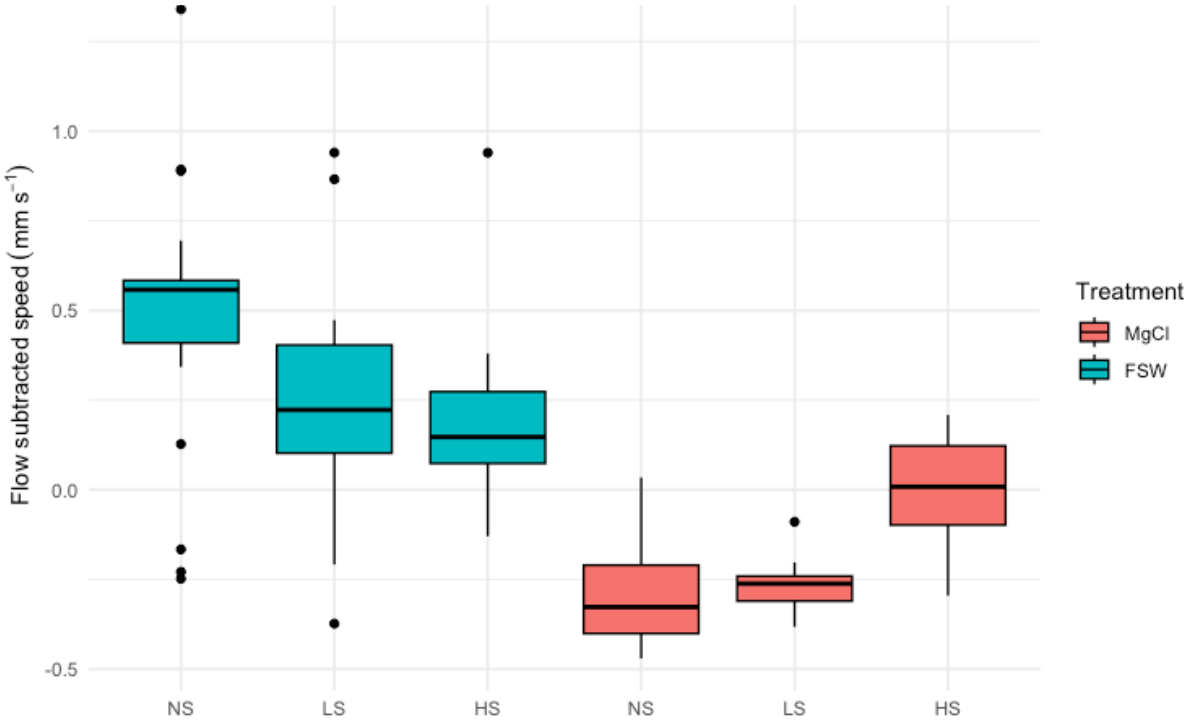

Figure 8. Flow-corrected planula speed, for both responsive (FSW, cyan) and anaesthetised planulae (in MgCl, in red). NS = no shear, LS = low shear, HS = high shear.

Table 1. ANOVA table (flow-corrected speed ~ treatment * shear) comparing all treatments of responsive planulae (in FSW) to anaesthetised (in MgCl).

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Treatment (FSW v. MgCl)	1	4.385	4.385	58.684	**3.07e-11 *****

Shear	2	0.416	0.208	2.782	0.068
Treatment:Shear	2	0.953	0.477	6.377	**0.003****
Residual	83	6.202	0.075		

Table 2. Tukey-Post hoc test results for ANOVA (raw speed ~ trial). Trial refers to both MgCl and FSW treatments in all different shears. Significant values in bold. Dark grey shading indicates comparisons within only FSW shear trials. Light grey shading indicates comparisons within only MgCl shear trials. Blue shading indicates comparisons between FSW and MgCl respective shear trials.

Interactions	**Lower 95% confidence interval**	**Upper 95% confidence interval**	***p*-value**
FSW no shear v. FSW low shear	-0.0009453882	-2.30E-05	**0.0338948**
FSW no shear v. FSW high shear	-0.00106792	-5.88E-05	**0.019595**
FSW low shear v. FSW high shear	-0.0005744645	4.16E-04	0.9971583
MgCl no shear v. MgCl low shear	-0.0008943803	6.68E-04	0.9982238
MgCl no shear v. MgCl high shear	-0.0014032067	7.91E-05	0.1072417
MgCl low shear v. MgCl high shear	-0.001290058	1.92E-04	0.2673524
FSW no shear v. MgCl no shear	-0.0008267359	4.63E-04	0.9627552
FSW low shear v. MgCl low shear	-0.0008272759	4.48E-04	0.9534368
FSW high shear v. MgCl high shear	-0.0003423303	9.03E-04	0.7768059

Table 3. Tukey-Post hoc test results for ANOVA (flow-corrected speed ~ trials). Significant values in bold. Dark grey shading indicates comparisons within only FSW shear trials. Light grey shading indicates comparisons within only MgCl shear trials. Blue shading indicates comparisons between FSW and MgCl respective shear trials.

Interactions	Lower 95% confidence interval	Upper 95% confidence interval	*p*-value
FSW no shear v. FSW low shear	-2.66E-05	0.0015426166	0.0644033
FSW no shear v. FSW high shear	9.89E-05	0.0018156938	**0.0198295**
FSW low shear v. FSW high shear	-6.43E-04	0.0010419712	0.9826091
MgCl no shear v. MgCl low shear	-1.39E-03	0.0012680257	0.9999937
MgCl no shear v. MgCl high shear	-2.16E-03	0.0003582723	0.3034265
MgCl low shear v. MgCl high shear	-2.10E-03	0.000419392	0.3815596
FSW no shear v. MgCl no shear	1.44E-03	0.0036376446	**0.0000000**
FSW low shear v. MgCl low shear	-2.81E-03	-0.0006357891	**0.0001923**
FSW high shear v. MgCl high shear	-1.74E-03	0.0003792022	0.425829

Cilia movement

We tracked cilia near the oral side of the planulae and along both flanks. Cilia appeared to display different levels of motility, with both mobile and immobile cilia observed within the row. Individual cilia were also observed to begin and arrest their movement. Cilia in *C. gregaria* displayed a power and recovery stroke beating pattern (Fig. 11). Stroke duration varied significantly between individual planulae ($p = 0.018$; Table 4) as well as between power

stroke and recovery stroke (p = 0.038; Table 4); on average, power strokes took 50.56 ms while recovery strokes took 60.48 ms (Fig. 9).

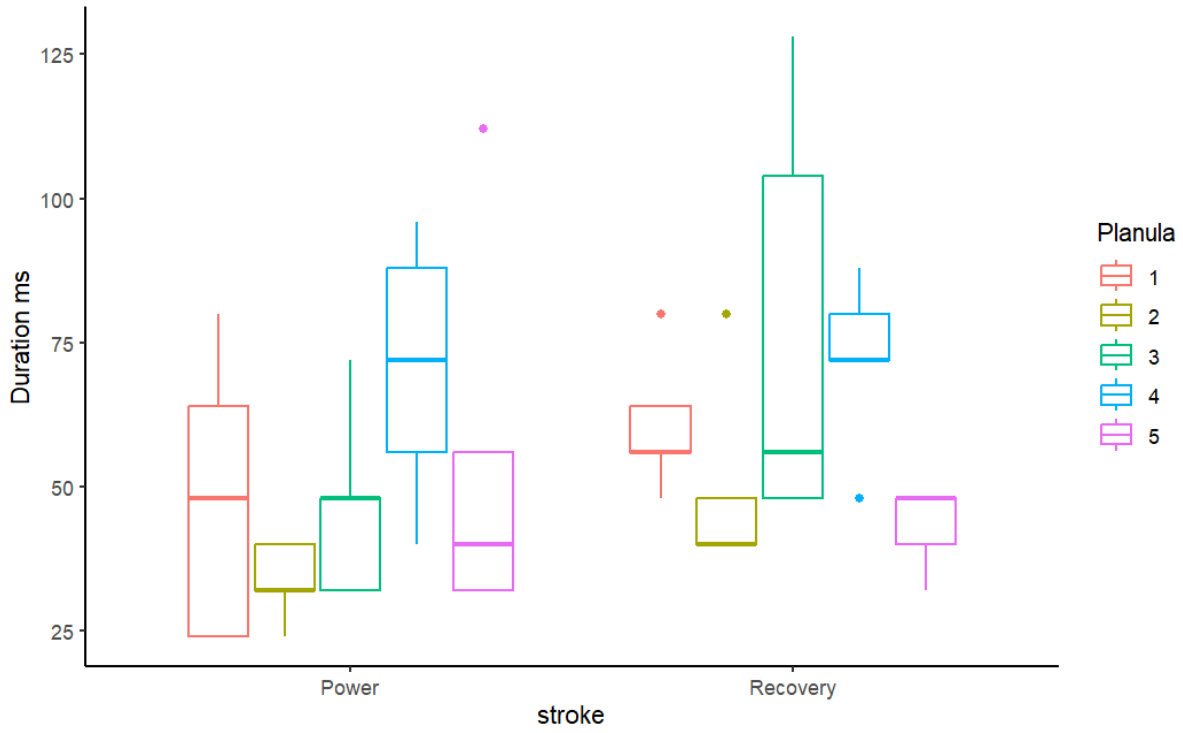

Figure 9. A boxplot of each planulae and their power and recovery stroke durations.

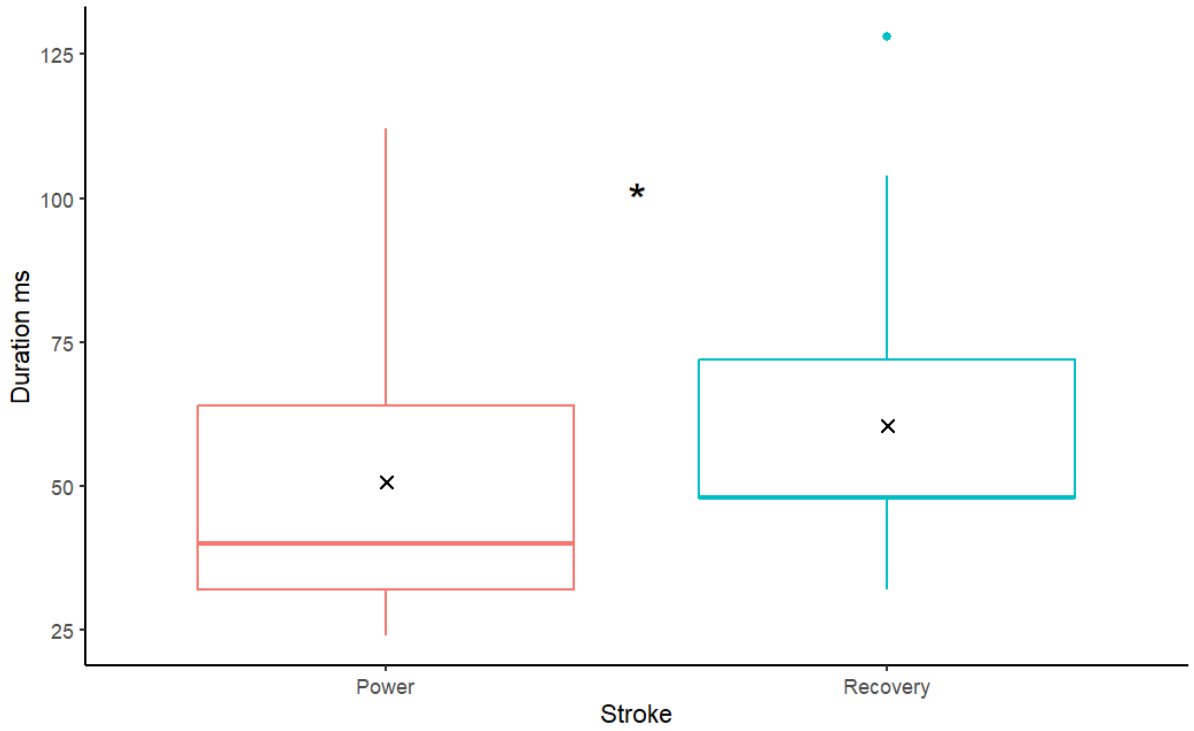

Figure 10. Power and recovery stroke duration (in ms) across all planula replicates (n = 5). The cross represents the average duration of each stroke. The asterisk indicates the significance between power and recovery stroke ($p < 0.05$).

Table 4. ANOVA results regarding the effects of planulae and stroke on the log-transformation of stroke duration. **Bold** indicates a significant difference ($p < 0.05$).

Source	*Df*	*Sum Sq*	*F value*	*Pr(>F)*
Planulae	4	1.751	3.330	**0.018**
Stroke	1	0.604	4.596	**0.038**
Residuals	44	5.784		

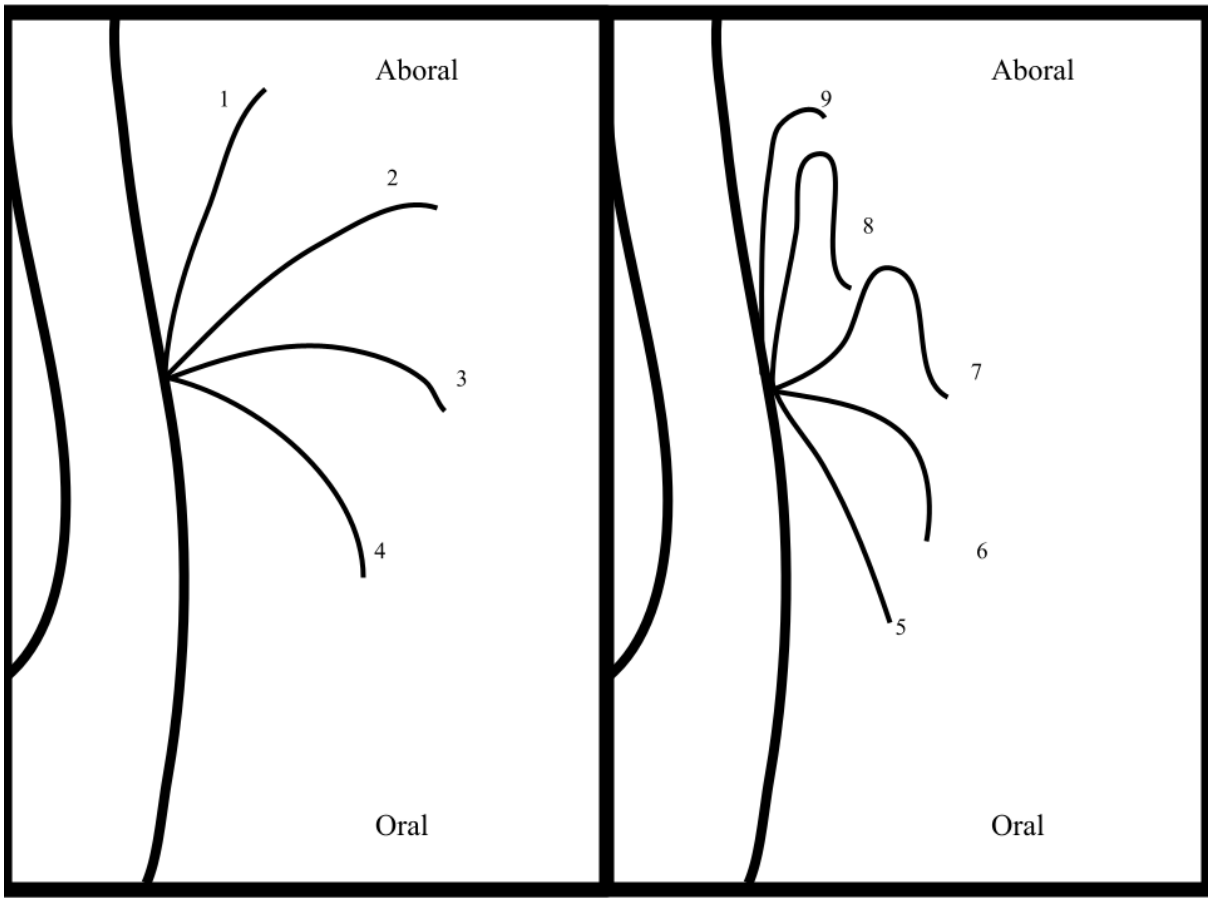

Figure 11. Schematic depiction of the power (left) and recovery (right) stroke observed in *C. gregaria* planulae.

Cilia length

There was no significant difference in cilia length among planulae ($p = 0.810$; Table 5). Cilia length was normally distributed across planulae (Shapiro-Wilk, $W = 0.981$, $p = 0.910$). Cilia averaged 19.36 µm, and ranged from 14.95 to 24.35 µm (Fig. 12).

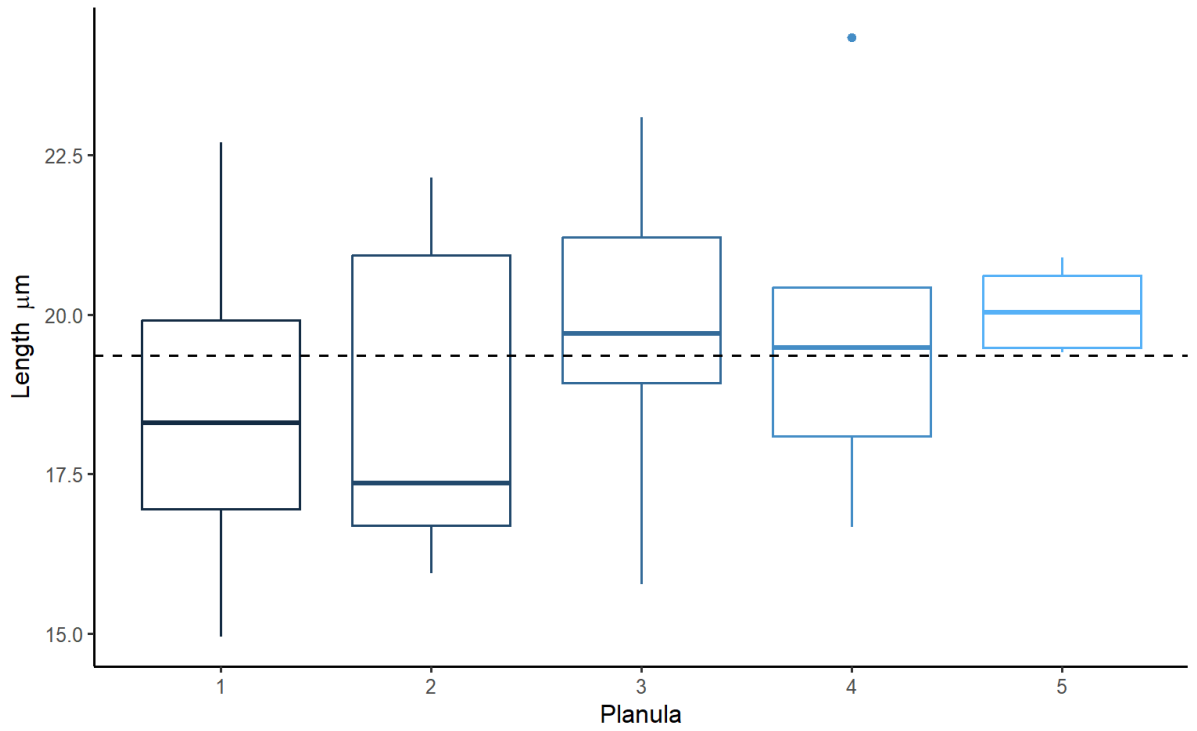

Figure 12. Cilia length in each planula replicate with a dotted line showing the overall mean length across all planulae.

Table 5. ANOVA results of the effects of planulae on cilia length. Bold indicates a significant difference ($p < 0.05$).

Source	*Df*	*Sum Sq*	*F value*	*Pr(>F)*
Planulae	4	10.28	3.330	0.810
Residuals	20	130.45		

DISCUSSION

In this study, we aimed to investigate the movement patterns in the planula of hydromedusa *Clytia gregaria* and examine how different shears affect their orientation, speed, and directionality. By exploring the ability to maintain orientation in varying hydrodynamic scenarios as well as their ciliary and muscular activity, we sought to understand the mechanisms behind their movement and shed light on the potential coordination and navigation abilities of this seemingly simple larval type.

Ciliary activity and movement

Our observations of cilia in *C. gregaria* provide a first detailed description of their morphology and strokes, which align with previous studies on coral planulae (Poon et al., 2022). The faster power strokes compared to recovery strokes are a common trend across taxa and facilitate forward trajectories in marine invertebrate larvae (Knight-Jones, 1954; Poon et al., 2022; Sleigh, 1989). Stroke duration has a significant effect on the force on extracellular fluid around the planulaes body, thereby affecting the individual's speed. The variation in stroke duration that we observed may be attributed to behavioural differences or intraspecific variation, and further investigation into the phenotypic plasticity of cilia stroke duration could provide insights into larval performance and survival.

Having only uniform motile cilia only is common in densely ciliated microswimmers and suggests that organisms navigate their environment effectively despite lacking specialised projections (Sleigh, 1989). This morphology seems to be an effective way for small larvae and other small pelagic organisms to move in the water column at low Reynold numbers. Because hydrozoan planulae are commonly lecithotrophic, it can be assumed that the ciliary activity is primarily utilised for their movement and not for feeding purposes.

Further observations also revealed that the cilia were capable of switching between movement and arresting movement. We did not find any evidence in the literature about our finding that planulae being able to change are capable of changing their ciliary activity from active to inactive states while maintaining a consistent stroke direction. This suggests that there is a yet unknown control over ciliary activity in a way that could potentially account for active regulation of orientation and trajectory of movement. Furthermore, our observations in de-ciliated larvae showed that planulae without cilia were unable to move in any direction and only expressed stationary bending and flexing of their bodies. Accordingly, we hypothesise that ciliary activity enables the majority of forward propulsion in this planula and further explore the importance of body shape and muscular activity in planulae.

Muscular and ciliary activity are important for active planular movement

The importance of bodily movement in planulae has not been extensively studied, but authors such as Leclere & Röttinger (2017) state that movement in planulae is mostly facilitated by coordinated ciliary beating. However, in the crawling planula larvae of *Clava multicornis*, it has been shown that active lateral muscular bending in combination with coordinated ciliary movement is used in a positive phototactic behaviour (Piraino et al., 2011). We tested the effect of muscular activity on movement in planulae through the use of MgCl, which is the first time this type of assay has been used on cnidarian planula to our knowledge. We hypothesised that these planulae were unable to move their bodies due to the MgCl inhibiting muscle movements, while cilia are likely still functional. Given that the anaesthetised planulae did not exhibit a normal uprighting behaviour as with the responsive planulae, we conclude that muscular activity is partially responsible for active orientation in shear, likely in combination with ciliary activity. In addition, our observations in de-ciliated larvae suggest that muscular activity alone does not appear to facilitate directed movement either, as de-ciliated planulae were inefficient

at moving both horizontally and vertically under the compound microscope. Therefore, planula capability to adapt their shape and actively use their muscles is potentially important in conjunction with cilia movement in determining planula movement.

Alternatively, it is possible that our behavioural observations were representative of unknown physiological effects of our assays. We don't know exactly how MgCl affects the planulae on a cellular level, nor how a hypersaline exposure might impact planula physiology (apart from expected osmolarity impacts). To resolve uncertainties around anaesthetising and de-ciliation, we recommend doing more specific trials investigating the effects of MgCl and hypersaline exposure on planular tissues on an ultrastructural level (e.g., comparative SEM/TEM or more detailed behavioural studies).

Planula can control their orientation in shear

In shear, planulae actively correct their trajectories to maintain an upward orientation, suggesting that they have an ability to sense shear environments and control their movements accordingly. The mechanism behind active stabilisation or potential directionality in this larval type is yet unknown, but possible avenues to investigate this further are presented by our findings in the planula's ciliary motion. How is their movement affected by the active and inactive cilia and are those states actively controlled to facilitate directional change?

In addition to active control of swimming orientation, we also found evidence for passive buoyant forces in anaesthetised larvae. The vast majority of anaesthetised larvae were recorded in downward flowing water and showed a negative flow-corrected velocity, which indicates that they were moving upwards relative to the water flow around them. A slower larval velocity relative to faster downwards-flowing water indicates that an upwards force is acting on the anaesthetised planulae, such as buoyancy. Positive buoyancy is not unexpected for a lecithotrophic larva, which contains positively buoyant lipid reserves). For a future

experiment, it would be interesting to study older larvae to compare if they are less bouyant due to lipid depletions over time.

We further observed an oscillatory movement pattern in all upwards-travelling individuals. This pattern is consistent with the movement of other cnidarian planula, such as *Acropora tenuis*, which travels in helical trajectories (Takeda et al., 2022). We qualitatively observed that oscillation path trajectory displayed larger amplitudes in high shear than in low or no shear trials. This increase in horizontal movement indicates that high shear is capable of tilting the planulae off-axis but the planulae then compensate by correcting their orientation, thus exhibiting a tendency to return to an upward-righting orientation. However, without being able to delineate the vertical velocity component from the oscillatory behaviour, we are unable to separate vertical velocity from oscillating trajectories with our dataset. In fact, the observed lower average flow-corrected speed in the high shear trials could be explained by a stronger oscillating movement, thus longer trajectory path, and slower average speed.

This also has implications for the performance of *C. gregaria* planula in natural scenarios of high shear, which as we show can influence the directionality of larval displacement in the water column. However, results from this study show that planulae are capable of retaining upward movement, despite high levels of shear. The general trend to maintain an upward movement as well as moving with the aboral front first is consistent with observations for other cnidarian species (Fagerström et al., 2022). Our findings that they can even maintain this orientation in shear, suggests that planulae may be able to navigate shear environments, and that the possession of an apparently simple body plan does not preclude them from retaining upward movement in the water column. Although planulae are unable to match the speeds of currents and wave motions, seawater movement and mass is spatially and temporally patchy and can allow for conditions with no or minimal mixing. Under these conditions, planulae would still appear capable of maintaining upward swimming trajectories.

This would allow the larvae to manoeuvre above obstacles on the benthos or into overlaying water masses that then transport larvae towards suitable habitat. This upward orientation is suggested to increase larval chances of dispersal to suitable habitats (Takeda et al., 2022).

Most larvae were tracked in upward flow, which could be a result of a biassed video analysis (tank edges were less visible and planulae only tracked in a specific area of the tank). However, we tracked all larvae consistently, making a bias in only the shear trials unlikely, though it cannot be ruled out. To address this potential bias, it would be interesting to explore how planulae behave in downwards moving flow compared to our observations. In addition, it is also possible that our binary categorization of water flow,either upwards or downwards instead of a true velocity gradient, impacted our ability to interpret the net flow-corrected speed and parse out the effect of water flow. Alternatively, the skewed presence of upward swimming larvae in our data may indicate that planulae actively seek out upwards flow; potentially as a method for achieving a desirable position within the water column for pre-competent planulae.

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