

### **Referee's General Opinion:**

This paper discusses the statistical properties of settling microswimmers in turbulence. In the first part the mathematical model for settling swimmers is derived and in the second part the results of numerical simulations in a turbulent flow are discussed.

I am not convinced that the present version of the manuscript deserves publication.

### **Author's Reply:**

We appreciate the careful reading of our manuscript and your constructive suggestions for improving the quality of the paper. In response to your major comments, we provide clarifications below and additional text is clearly marked in document. We hope that the revised version will be suitable for publication.

### **Referee's Comment #1**

The derivation of the model (section 2.1) is interesting and its study probably deserves publication. The main objection I have is that in the limit discussed in this paper (eqs. 5-8), the model is identical to the Kessler model for gyrotaxis which has been already studied in the same flow and with the same statistical approach (clustering in terms of Voronoi distribution and preferential concentration). Therefore, it is not clear what are the new results of this manuscript.

Moreover, this is not clearly discussed in the paper. After eqs. (5-8), it is written that "the second term on the rhs of (8) is analogous to the gyrotactic effect", while the model (6,8) is identical to the standard gyrotactic model.

### **Author's Reply:**

One of your criticisms is that, in the limit of our paper, our model is identical to the Kessler model for gyrotaxis. However, despite the similarities in mathematical forms, the mechanism responsible for the orientation effect is totally different from that in Kessler's model. In the Kessler model, the reorientation effect is caused by the offset between the mass center and the shape center of a plankton cell. The magnitude of the gravitational reorientation torque is determined by the distance of the offset as well as some other physical properties such as fluid viscosity and density ratio, etc. As a result, the Kessler reorientation torque is a physical mechanism regardless of whether a plankton is swimming or settling or not. In our model, however, the reorientation effect is caused by the fluid inertial torque when a plankton swim and settling relative to the ambient fluid, and thus depends on the swimming and settling speeds. The magnitude of the reorientation angular velocity is no longer an independent parameter, which is drastically different from the case of Kessler model.

We believe this difference is important and deserves investigation. First, it allows a plankton to modify its swimming speed and then control its reorientation behavior. Second, our results suggest that the settling speed also matters even if a plankton is motile, allowing fluid inertial torque to generate a reorientation effect. To address both points, we studied how clustering and preferential sampling are altered when microswimmers have different swimming and settling speeds ( $\Phi_s$  and  $\Phi_g$ ). To emphasize the dependency of the reorientation effect on the swimming and settling speeds, we discuss the clustering and preferential sampling in a  $\Phi_s$  vs  $\Phi_g$  parameter diagram in the paper. For instance, we show that clustering intensity is the largest when  $\Phi_s = 10$  and  $\Phi_g =$

0.5. This was not observed in earlier studies where fluid inertial torque and the settling speed are not considered as a mechanism of reorientation effect.

To address the difference between our model and the Kessler model, we have added a discussion in section 2.1:

Around line 90:

*“The last term of Eq.(8) indicates that fluid inertial torque drives a squirmer swimmer to swim against gravity. Here, we use a dimensionless timescale  $\Psi_1$  to quantify the effect of fluid inertial torque.  $\Psi_1$  can be understood as the dimensionless time that a swimmer in still fluid restores upward orientation from an inclined orientation under a reorientation torque. This is analogous to the gyrotactic effect induced by bottom-heaviness, which is typically expressed as  $2\Psi^{-1}(\mathbf{e}_g \times \mathbf{n})$  (Kessler, 1986). We note that, however, they are two different mechanisms. The torque generated by bottom-heaviness depends on the distance of the offset between the center of gravity and hydrodynamic forces on a cell, which is usually determined by morphology. On the contrary, fluid inertial torque depends on the swimming and settling speeds and, determined by motility”*

## **Referee's Comment #2**

In conclusion, I think that it would be interesting to investigate the model general model (3,4) and compare it with the known gyrotactic limit. This would add something new to our understanding to swimming microorganisms in turbulence. Moreover, my impression is that, with the typical values discussed after (9), the range of the Stokes numbers is comparable with the other dimensionless parameters and therefore the limit  $St \rightarrow 0$  is not justified.

## **Author's Reply:**

We appreciate your suggestion that we can study the original model (3) and (4) to compare with the Kessler model. It would be interesting to see whether new physical phenomena emerge when the Stokes number  $St$  is not negligibly small. However, we want to focus the scope of the current paper on plankton or other small microorganisms in water. Based on their typical physical properties, the Stokes number approaches to zero. For instance, we assume a plankton cell has typical size  $a = 0.1 \text{ mm}$  and it is 5% heavier than the water. For the flow environment, we use the parameter range in the main text after Eq. (9). The range of dissipation rate cited in our text gives the range of Kolmogorov time scale for ocean turbulence,  $\tau_f = 31.6$  to  $1.0 \text{ s}$ . At last, using the definition of  $St = 2 * 1.05a^2 / (9\gamma\tau_f)$ ,  $St$  number ranges from 0.0001 to 0.0023. This is much smaller than other dimensionless parameters such as  $\Phi_s$  and  $\Phi_g$  in our model. A similar but more detailed estimate of dimensionless parameters can be referred to Table II in our recent paper (Qiu et al., Physical Review Research 4, 023094 (2022)), which is attached below. As a result, it is more appropriate to focus on the limit of zero Stokes number for the present study.

To address this issue, we have added discussions in section 2.1 in the main text to demonstrate the range of Stokes number to validate our simplification.

Around Line 80:

“Typically,  $St$  of planktonic microswimmers are usually negligibly small as summarized in Qiu et al. (2022a). For instance, using  $a = 0.1\text{mm}$ ,  $\rho_p/\rho_f = 1.05$ , and using typical turbulence Kolmogorov timescale  $\tau_f = 31.6$  to  $1.0\text{ s}$ , one obtains  $St = 1.0 \times 10^{-4}$  to  $2.3 \times 10^{-3}$ .”

TABLE II. Dimensionless numbers of typical plankton species shown in Table I. The Kolmogorov scales of ocean turbulence is calculated with  $\gamma = 1.058 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ , and the energy dissipation rate  $\epsilon$  ranges from  $1 \times 10^{-9}$  to  $1 \times 10^{-6} \text{ m}^2 \text{ s}^{-3}$  [42].  $Re_p$  is calculated with  $Re_p = v_{\text{swim}}L/\gamma$  because  $v_{\text{swim}} > v_{\text{settle}}$  for many species in Table I. Superscript<sup>1</sup>: The values are calculated with  $\lambda = 2.3$  similar to *Centropages typicus*. Superscript<sup>2</sup>: The values are calculated with  $\lambda = 2.0$ .

Species		$\Phi_{\text{swim}}$	$\Phi_{\text{settle}}$	$M$	$Re_p$	$St (\times 10^{-5})$	$\Psi_I$
<i>Cochlodinium polykrikoides</i> [39]	Single cell	2.17~0.39	0.14~0.03	-0.078	0.015	0.17~5.52	20.7~655.2
	2-cells	3.32~0.59	0.1~0.03	-0.101	0.029	0.22~6.95	9.1~287.2
	4-cells	4.44~0.79	0.23~0.04	-0.136	0.077	0.45~14.11	3.6~112.5
	8-cells	4.75~0.84	0.36~0.06	-0.137	0.147	0.90~28.51	2.1~67.4
<i>Centropages typicus</i> [28,41]	early nauplius	1.83~0.33	0.28~0.05	-0.114	0.041	1.29~40.78	8.7~274.2
	late nauplius	3.99~0.71	0.78~0.14	-0.114	0.153	3.75~118.48	1.4~44.9
<i>Euterpina acutifrons</i> [28]	late nauplius <sup>1</sup>	5.99~1.06	1.44~0.26	-0.114	0.204	2.96~93.61	0.5~16.1
<i>Eurytemora affinis</i> [28]	late nauplius <sup>1</sup>	9.09~1.62	1.01~0.18	-0.114	0.313	3.02~95.49	0.5~15.2
<i>Temora longicornis</i> [28,40]	late nauplius <sup>1</sup>	3.16~0.56	1.33~0.24	-0.114	0.166	7.02~222.01	1.0~33.1
	copepod <sup>1</sup>	4.55~0.81	0.94~0.17	-0.114	0.231	6.57~207.83	1.0~32.5
<i>Ceratium tripos</i> <sup>2</sup> [27]		0.93~0.16	0.91~0.16	-0.101	0.012	0.46~14.60	5.9~186.1
<i>Ceratium furca</i> <sup>2</sup> [27]		4.32~0.77	0.34~0.06	-0.101	0.033	0.17~5.50	3.3~105.9
<i>Akashiwo sanguinea</i> <sup>2</sup> [27]		1.66~0.30	0.30~0.05	-0.101	0.012	0.15~4.81	9.9~314.3
<i>Dinophysis acuminata</i> <sup>2</sup> [27]		1.84~0.33	0.18~0.03	-0.101	0.010	0.09~2.84	15.2~481.8
<i>Alexandrium minutum</i> <sup>2</sup> [27]		1.54~0.27	0.05~0.01	-0.101	0.005	0.03~0.89	58.3~1843.6
<i>Prorocentrum minimum</i> <sup>2</sup> [27]		1.14~0.20	0.03~0.00	-0.101	0.002	0.01~0.44	159.8~5053.4

Table II in Qiu et al., Physical Review Research 4, 023094 (2022). In this table,  $\Phi_{\text{swim}}$  and  $\Phi_{\text{settle}}$  means  $\Phi_s$  and  $\Phi_g$  in the present paper. We also note that  $\Psi_I$  in this table does not refers to the reorientation time scale defined in the present paper.

### Referee's Comment #3

Minor point: the presentation of the model and the results is not always clear. For example, the settling speed  $v_g$  is not defined.

### Author's Reply:

Thank you. We remove the symbol  $v_g$  and give the definition of  $\Phi_g$  directly,  $\Phi_g = 2(\rho_p/\rho_f - 1)a^2g/(9\gamma\mu_f)$ . It can be found right below Eq. (4).