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Analysis and modelling of swimming behaviour in *Oxyrrhis marina*

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1 Boakes *et al.* Swimming behaviour of *Oxyrrhis marina*

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3 **Analysis and modelling of swimming behaviour in *Oxyrrhis marina***

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2
3 **ABSTRACT**

4
5 *Oxyrrhis marina* is a heterotrophic dinoflagellate that swims in helical paths in a three-dimensional
6 (3D) environment. It is also known to react to various environmental stimuli and resultant changes
7 in its swimming patterns have been widely observed. Understanding this swimming behaviour and
8 providing tools for quantifying swimming-related parameters will aid in interpreting its response to
9 environmental stimuli, including the presence of food items and chemical cues. We review the
10 literature on the analysis of swimming in *O. marina*. We also outline the related fields of random
11 walks, path analysis, and simulation of individual based movement where relevant to *O. marina*.
12 We then discuss some of the problems associated with trying to understand 3D helical movement
13 through analysis of two-dimensional (2D) video microscopy data. We emphasize that the current
14 models in the literature cannot accurately simulate helical walks and conclude that *O. marina* would
15 be a suitable candidate for establishing a standard 3D framework that can be used as a tool to
16 characterise observed movement patterns in the future.
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INTRODUCTION

Swimming in protists is one of the more conspicuous attributes that stimulated early studies on their ecology and taxonomy. The pioneering work by Jennings (1906) focussed on the movements in ciliates and selected flagellates and demonstrated the importance of swimming when protists explore the environment and react to external stimuli. Some of the first descriptions of the swimming behaviour in the heterotrophic dinoflagellate *Oxyrrhis marina* date back to the publications by Kent (1880) and Senn (1911). These studies identified the presence of two morphologically and functionally differentiated flagella that generate the required propulsion for swimming.

One of the reasons for the early interest in *O. marina* stems from the relative ease with which this species can be grown in the laboratory and its regular appearance in early studies of marine aquaria (Lowe *et al.*, this issue-a; Watts *et al.*, this issue). It feeds on a variety of phytoplankton and bacteria species, can survive shortages of food by cannibalistic feeding, and has high tolerance to a range of environmental conditions (Droop, 1959; Lowe *et al.*, this issue-a). These attributes make *O. marina* an attractive planktonic predatory protist for laboratory studies. Hence, it has been frequently used as a 'model' for the diverse functional group of marine microzooplankton (e.g. Hansen *et al.*, 1996; Wolfe and Steinke, 1996; Strom *et al.*, 2003; Roberts *et al.*, this issue). Clearly, this approach has its limitations since a single model organism will never display all the traits necessary to perfectly represent a diverse group. However, in practice there are many benefits to using a model (Montagnes *et al.*, this issue), and initial studies on *O. marina* often stimulated further research on other phagotrophic protists or mixed natural populations that have widened our understanding of protist biology in general (e.g. Breckels *et al.*, this issue).

Swimming has been explored in several protists (e.g. Buskey *et al.*, 1993; Crawford and Lindholm, 1997; Fenchel and Blackburn, 1999; Fenchel, 2001; Jakobsen *et al.*, 2006; Visser and Kiørboe, 2006), but relatively little is known about the fine details of swimming and its ecological relevance. *Oxyrrhis marina* is known to show a phototactic response (Jakobsen and Strom, 2004),

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2
3 much like other protists (e.g, Drescher, *et al.*, 2010), but quantitative data on its swimming
4
5 behaviour is lacking. Other protists react to hydromechanical signals (Jakobsen, 2002, Jakobsen *et*
6
7 *al.*, 2006), exhibit a gravitaxis response (Hemmersbach and Häder, 1999), gyrotaxis response
8
9 (Thorn and Bearon, 2010) and undergo diel migration (Pizay *et al.*, 2009). However, none of these
10
11 particular behaviours have been explicitly investigated by analysing *O. marina* paths and could be
12
13 possible avenues for future investigations in this species. The helical swimming path in *O. marina*
14
15 is a good example, since its significance for sampling local environments, foraging for individual
16
17 food particles and reacting to external stimuli is poorly understood.

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22 Swimming in helical paths is a result of a rotation along the length axis that has been
23
24 suggested to enable unsymmetrical organisms to follow a straight course (Jennings, 1906).
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26 Changes in the rotational component can be part of an avoidance reaction that leads to bending of
27
28 the swimming track to produce helical klinotaxis, a type of chemosensory motile behaviour
29
30 (Fenchel, 2001), or a complete reversal of the swimming direction (“U-turn response”; Fenchel and
31
32 Blackburn, 1999). The helical swimming path in *O. marina* is likely affected by external stimuli
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34 since it can vary greatly in speed, rotational movement, helical extent and directional persistence
35
36 (e.g. Bartumeus *et al.*, 2003). Additionally, directed swimming is important to exploit patchily
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38 distributed resources (Grünbaum, 2002), and changes in individual swimming behaviour will
39
40 subsequently affect spatial distribution at the population level.

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45 There are many unanswered questions about swimming in microzooplankton in general and
46
47 quantifying different swimming behaviours of *O. marina* may give insight into general processes of
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49 similar planktonic protists. Does swimming in helices give these organisms advantages over
50
51 organisms that show non-helical swimming behaviour? Is helical swimming beneficial for
52
53 foraging? How do environmental conditions and stimuli affect *O. marina* individual swimming
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55 paths and their overall dispersal at the population level? Are common analytical methods sufficient
56
57 to quantify subtle behavioural changes?
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Below we first summarise some of the older literature and discuss the recent findings on

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swimming in *O. marina*. We address the limitations of the experimental designs used and the relevant tools for the analysis and the modelling of *O. marina* swimming behaviour. We then indicate that many of the current experimental tracking systems are limited because three-dimensional (3D) helical paths are typically only captured in two dimensions. Similarly, we also discuss how standard path analysis techniques and associated movement models fail to adequately deal with helical walks. We conclude that ignoring the helical nature of the swimming path in *O. marina* results in loss of important fine detail and recommend that future studies must use a suitable 3D framework to further explore the swimming behaviour in *O. marina*.

ANALYSIS OF THE SWIMMING BEHAVIOUR IN *OXYRRHIS MARINA*

Two flagella are used to produce complex swimming behaviours

Despite the initial confusion about the number of flagella present (the original drawings by Dujardin (1841) indicate at least four flagella), further microscopic observations resolved that *O. marina* uses one longitudinal flagellum directed posteriorly that pushes the cell forward and one transversal flagellum that partially wraps around the cell from its origin in a ventral furrow (Senn, 1911). Together, the beating of these flagella causes these organisms to swim in a helical path (Cosson *et al.*, 1988; Fenchel, 2001). Studies on the ultrastructure of the flagella were conducted by Cachon *et al.* (1988) and their movement was described by Cosson *et al.* (1988). The findings of both studies are reviewed in Lowe *et al.* (this issue-b). Roberts *et al.* (this issue) provide details on the role of the flagella in feeding. Here we focus on describing the swimming behaviour and detail the technology that is used in its analysis.

Senn (1911) described three types of swimming behaviour when observing individual cells that included: 1) regular rotation when the longitudinal flagellum is trailing and the transverse flagellum is beating actively; 2) jerky movements when the longitudinal flagellum is actively beating and the transverse flagellum is inactive; and 3) wobbling movements whereby the longitudinal flagellum is inactive. Although these observations are probably affected by

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3 methodological limitations that resulted in an emphasis on slow swimming or immotile cells, this
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5 early report indicates the extensive behavioural repertoire present in *O. marina*.
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10 **Video microscopy and the study of *O. marina* swimming behaviour**

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12 The first popular study of *O. marina* swimming behaviour was conducted over 20 years ago
13 by Cosson *et al.* (1988), who analysed the movement of flagella and the swimming behaviour. The
14 organisms were observed under a microscope, and images were captured on video tape for later
15 analysis. This experimental method is often referred to as ‘two-dimensional (2D) video
16 microscopy’ and is commonly used to investigate the behaviour of microorganisms over small time-
17 scales. This study identified that the helical swimming paths exhibited by *O. marina* were mainly
18 linear, with a 44-80 μm helical width, completing a full helical rotation in 130-170 μm with a mean
19 forward displacement of 400-700 $\mu\text{m s}^{-1}$. The study further showed that *O. marina* exhibits
20 different types of rotational and translational behaviour and can produce spontaneous changes in
21 direction by folding the longitudinal flagellum. This type of action suggests that *O. marina* might
22 exhibit ‘run-and-tumble’ swimming, much like bacteria, where the organism changes direction after
23 a run time, actively reorienting itself (Berg, 1983). Many small organisms (i.e. most bacteria) are
24 confined to this type of reorientation in chemical gradients and larger organisms, including motile
25 protists such as *O. marina*, exhibit helical klinotaxis as the main component of sensory motile
26 behaviour (Fenchel, 2001). While the mechanisms for swimming can drastically vary between
27 pelagic protozoa (Dusenbery, 2009), their resultant swimming behaviour only shows three generic
28 components of locomotion: (i) Swimming punctuated by turns (i.e. run-and-tumble and correlated
29 movement), (ii) helical trajectories while swimming and (iii) rapid jumping, often referred to as
30 ‘breaks’. Thus the study by Cosson *et al.* (1988) suggests that *O. marina* exhibits the first two out
31 of the three behaviours and, therefore, could be a useful model to study them.
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59 Tarran (1991) next used video microscopy to record and qualitatively analyse swimming
60 behaviour of *O. marina* when offered various food concentrations. *Oxyrrhis marina* mainly

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3 travelled in straight lines with few turns in the absence of food. Once food was introduced, more
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5 frequent turning and shorter runs were observed. Swimming speeds of 90-179 $\mu\text{m s}^{-1}$ were
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7 recorded, ~2 to 8 times lower than what was observed by Cosson *et al.* (1988). Tracks also showed
8
9 combinations of swimming behaviour, with both intensive and extensive phases. The intensive
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11 phases were considered to be a kinetic response to colliding with food particles, increasing the
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13 encounter rate within areas of high food concentration. In this sense, *O. marina* also acts like many
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15 other protists (e.g. Fenchel and Jonsson 1988) and may be used in this sense as a model to predict
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17 the behaviour of other protists.
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22 The technology used when the studies by Cosson *et al.* (1988) and Tarran (1991) were
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24 conducted included freeze-framing an analogue video tape and using tracing paper to grid the
25
26 various positions of the organisms. This method was both lengthy and tedious, and human error
27
28 limited the precision of the collected data. Furthermore, within a single microscopic field-of-view,
29
30 it was impossible to obtain swimming paths of anything more than 2 s length due to the organism
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32 moving out of the focal plane (Tarran, 1991). Consequently, manual tracking was occasionally used
33
34 to gather longer path information. These early reports concluded that *O. marina* forages randomly
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36 and does not have the capacity to sense food particles. In contrast, more recent work using
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38 microcapillary assays laced with amino acids or the algal secondary metabolite
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40 dimethylsulphoniopropionate (DMSP) demonstrated that *O. marina* has the ability to sense and
41
42 react to chemical gradients and is likely using chemical information when foraging for prey (Martel,
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44 2006; Breckels *et al.*, this issue). Recently, the attraction of *O. marina* and other protists to DMSP
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46 and other low molecular weight compounds was confirmed using microfluidics and image analysis
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48 of their swimming behaviour (Seymour *et al.*, 2010). Such experiments reveal that *O. marina* is
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50 capable of directed swimming, but they do not reveal the details of that swimming behaviour. In
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52 the next section we explore how modern methods may provide quantitative swimming information.
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Automatic tracking facilitates the analysis of swimming behaviour

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3 Recent methodological developments using digital video technology make it easier to analyse
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5 swimming paths of organisms with imaging software that can freeze-frame a digital recording at a
6
7 precise rate. This has allowed for the development of tracking software, which comes in two main
8
9 forms. The first originates with tracing, where manual input of positional data can be recorded
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11 digitally making data collection more accurate than the manually tracing of paths into grids. More
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13 advanced software can automatically track single or multiple organisms in space and time (e.g.
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15 LabTrack at www.bioras.com), further decreasing analysis time. This technology was successfully
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17 used to demonstrate helical klinotaxis in the chemosensory behaviour of *O. marina* towards mussel
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19 tissues (Fenchel, 2000) and to analyse the effect of 20 μ M additions of amino acids on the
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21 swimming behaviour of *O. marina* and the ciliate *Favella* sp. (Strom *et al.*, 2007). The latter
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23 studies suggested that amino acids that inhibit feeding also result in changes in swimming
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25 behaviour with a decrease of helical swimming and the occurrence of 'start-stop' movements that
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27 resulted in zig-zag patterns. Thus, modern methods have been, and should continue to be applied to
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29 the analysis of *O. marina* swimming behaviour.
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39 **Problems associated with the reduction of 3D swimming paths to 2D data**

40 While automatic tracking is a significant step forward in generating swimming data for
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42 microorganisms, the method itself still has some fundamental disadvantages. We will explore these
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44 weaknesses in general before returning to specific studies that used *O. marina* to address and
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46 overcome such problems.
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50 The most obvious problem is that all swimming microorganisms move in a three-
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52 dimensional (3D) domain, but conventional video microscopy only allows for observations in two
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54 dimensions. This loss of data in the third dimension is a critical issue for the analysis of helical
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56 swimming paths, as the path differs in appearance depending on the 2D perception (Fig. 1).
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59 Another problem is that the focal length of a conventional microscope results in a low depth-of-
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field: beyond a few body lengths of travelling in the third (i.e. vertical) dimension, a cell will move

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3 out of the focal range so that automatic tracking software often loses the object in motion. Analysis
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5 is usually conducted on tracks of a minimum length, so that the shorter tracks of an organism
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7 moving through the focal length are ignored, and, hence, a comprehensive analysis of vertical
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9 motion is impossible.
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12 Another common issue causing analytical problems when recording positional data of
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14 swimming protists is error due to poor spatial resolution, whether this is because of manual grid
15
16 positioning or pixel limitations of the computer hardware. This results in a loss of minimal scale
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18 information. An organism may appear to remain stationary for a short time when it has only moved
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20 within a pixel or grid slot, leading to noisy data (note, this is homologous to the “human error”
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22 associated with the early studies, mentioned above). The capture rate (sampling rate) can also affect
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24 the apparent properties of the observed movement path, a problem that has been investigated both
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26 theoretically (e.g. Bovet and Benhamou, 1988; Codling and Hill, 2005; Plank and Codling, 2009)
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28 and experimentally (e.g. Hill and Häder, 1997). Furthermore, discretisation of paths affects the
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30 path appearance and the qualitative information that can be gained from movement data. For
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32 example, the helical details can be lost after discretisation as shown in Fig. 1c. These issues slow
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34 our progress to accurately analyse the swimming behaviour of protists.
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43 **3D analysis of swimming in *O. marina***

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45 2D video microscopy, while standard, is not the only method of recording swimming paths of
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47 microorganisms. Techniques for the 3D capture of these organisms, while harder to accomplish, do
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49 exist and have been used for many years. Berg (1978) developed a tracking microscope to
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51 accurately follow a single microorganism in three dimensions. Such techniques have only been
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53 used for very specific investigations and have not made their way into common practice. Thar *et al.*
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55 (2000) applied two cameras to allow the first 3D tracking of swimming *O. marina* and two other
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57 protists (*Euglena gracilis* and *Strombidium sulcatum*). Such systems can be used in future studies
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59 on *O. marina* to describe its swimming behaviour and elucidate its sensory abilities (e.g. Breckels *et*
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3 *al.*, this issue, Drescher, *et al.*, 2010). Additionally, the possibility to track several microorganisms
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5 simultaneously with this system allows studies on the interactions between *O. marina* and its prey.
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8 A more recent example of 3D analysis is the study by Menden-Deuer and Grünbaum (2006):
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10 using two infrared-sensitive cameras, experiments were conducted on the vertical migration of
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12 *O. marina* in response to the prey flagellate *Isochrysis galbana* and their exudates, which were
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14 distributed in thin layers. The study examined how efficiently *O. marina* locate prey patches by
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16 analysing changes in individual swimming behaviour and population dispersal. Turning rate was
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18 sampled every 0.25 s, and the rate of change of direction (RCD) increased at the boundaries of the
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20 layers from 56 degrees s⁻¹ in the absence of food cells to 70 degrees s⁻¹ in the presence of food cells.
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22 Food particles resulted in twice as much change of RCD than dissolved exudates. While vertical
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24 velocity in the presence of both food particle and filtrate were seen to decrease, speed increased
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26 from 307 μm s⁻¹ in the absence of food cells to 339 μm s⁻¹ in the presence of food cells, yet there
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28 were no significant changes in speed with the addition of dissolved exudates. Although speed and
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30 turning angles were higher, there was a net decrease in diffusivity when *O. marina* was in the
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32 presence of food, demonstrating that changes in swimming behaviour resulted in accumulation of
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34 *O. marina* in the prey layer. Menden-Deuer and Grünbaum (2006) also demonstrated that *O.*
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36 *marina* uses rapid vertical and horizontal migration when foraging for food. This is an important
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38 finding and suggests that the methods of 2D video microscopy are insufficient to completely
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40 quantify swimming behaviour in relationship to prey patches.
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48 A similar dual-view imaging system with high resolution was used by Drescher *et al.* (2009)
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50 that is optimised for monitoring the swimming of organisms of different sizes (10-1000 μm
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52 diameter). This system was successfully applied to observe phototactic and gravitactic responses in
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54 three protist species (*Chlamydomonas reinhardtii*, *Volvox barberi* and *Volvox carteri*) and enabled
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56 the recognition of 'eukaryotic run-and-tumble' behaviour in *C. reinhardtii* (Polin *et al.*, 2009). In
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58 addition to the quantification of movement paths, the high-resolution images enabled an analysis of
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60 the beating of the flagella over long periods of time. This facilitated an analysis of the timing and

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3 sequence of events that result in a tumble reorientation.
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5 Technological advances have lead to new analytical systems, such as digital holographic
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7 microscopy that can be used to reconstruct 3D paths (Sheng *et al.*, 2007). This technique was used
8
9 to investigate the swimming behaviour of dinoflagellates (*Karlodinium veneficum* and *Pfiesteria*
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11 *piscicida*) with high detail and demonstrated species-specific, complex and highly variable
12
13 swimming behaviours as quantified by their velocity, radii of helical trajectories, and direction of
14
15 helical rotation. Furthermore, it provided the opportunity to assess swimming and toxin production
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17 in the feeding ecology of dinoflagellates, and showed that toxins can immobilize prey to facilitate
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19 prey capture (Sheng *et al.*, 2010).
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24 We lack detailed understanding of the diversity of swimming behaviours in other protists
25
26 including *O. marina*, and a more widespread application of digital holographic microscopy and 3D
27
28 high resolution imaging could overcome this shortcoming in the future.
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33 **PATH ANALYSIS OF MOVEMENT DATA**

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35 Many *O. marina* movement paths appear to have periods of long-straight movements interspersed
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37 with periods of rapid-turning known as run-and-tumble (Berg, 1983). Where run-and-tumble
38
39 patterns are being investigated it is common to analyse 'flight times' or distances (step lengths) and
40
41 tumbling (turning) angles. Flight times are recorded as the time (or distance) moved before a
42
43 change in direction occurs, whilst the tumbling angles are the resultant displacement angle after a
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45 tumble. Distributions of flight times can give insight into swimming behaviour, especially when
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47 different phases of behaviour are observed in different environmental conditions due to possible
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49 foraging responses (e.g. Uttieri *et al.*, 2007).
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54 Bartumeus *et al.* (2003) investigated the swimming paths of *O. marina* in response to
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56 various food densities. After removing the fine helical information from the path by using a finite
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58 helical fit method (Crenshaw *et al.*, 2000), they discovered significant difference in flight times and
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60 tumbling angles between high and medium food densities. Although there were no significant speed

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3 changes between swimming with different concentrations of prey, they suggested that *O. marina*
4
5 exhibited a helical Lévy walk. In a Lévy walk the distribution of flight times is heavy tailed while
6
7 the turning angles are random and uncorrelated, which results in an observed path that appears to
8
9 have periods of intensive movement with high turning interspersed with long straight movements
10
11 where turning is minimal. Lévy walks have been shown to be the optimal searching strategy for
12
13 various foraging scenarios including a sparse and patchy prey distribution. Under these conditions,
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15 *O. marina* is assumed to be unable to sense target locations outside a small capture radius and has to
16
17 rely on a ‘purely random’ search (see Bartumeus *et al.*, 2003, and references therein).
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22 Given that *O. marina* shows chemosensory responses to dissolved chemicals (Breckels *et al.*
23
24 *et al.*, this issue), it seems unlikely that foraging would be a purely random search but instead we
25
26 might expect *O. marina* to adapt its behaviour to the local environment. More recent studies have
27
28 suggested that early data analyses that found evidence of Lévy movement behaviour may have been
29
30 flawed (e.g. Edwards *et al.*, 2008; Plank and Codling, 2009), and considering that the original
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32 study by Bartumeus *et al.* (2003) used only a few data points to fit an inverse power law (Fig. 4 in
33
34 Bartumeus *et al.*, 2003) it may be worth revisiting these results with further experimental data on *O.*
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36 *marina* and more detailed analysis. For example, Reynolds (2008) re-examined *O. marina*
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38 swimming behaviour using numerical simulations of foragers within patchy and non-patchy
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40 distributions of food particles and showed that movement paths generated using a chemosensory
41
42 response mechanism can appear similar to Lévy random walks.
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48 Using cinematographic techniques, Vandromme *et al.* (2010) examined the swimming
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50 response of the ciliate *Strobilidium sp.* to various food concentrations. They demonstrated “helix”,
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52 “non-helix”, and “break” swimming characteristics, a similar classification to the typical swimming
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54 in copepods (Schmitt and Seuront, 2001) and concluded that the analytical methods developed for
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56 copepods can also be used to characterize and simulate helical and non-helical swimming for
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58 ciliates. This too would be an avenue to explore when attempting to analyse and interpret the
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60 swimming of *O. marina* as it exhibits similar swimming characteristics.

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A NEW DIRECTION FOR RESEARCH ON *O. MARINA*: MODELLING SWIMMING THROUGH HELICAL RANDOM WALKS

While studies on *O. marina* have aided our understanding of behavioural responses to specific conditions, and this information has been used to derive or apply *O. marina*-based simulation models as a tool to interpret protozoan population dynamics (Davidson *et al.*, this issue), it is still not known what advantages helical movements convey at the individual level. Swimming in a helix will likely increase foraging efficiency, as the searching radius of the organism is increased by sampling a wider volume of water across the net direction of movement (Crenshaw 1996). A helical walk may also be more effective for detecting chemical gradients, as concentration gradients could be more easily recognised by helical sampling, regardless of the swimming direction through the gradient (Crenshaw, 1996; Fenchel, 2001). While observing fine scale swimming is possible, testing these hypotheses is experimentally challenging as it is difficult to observe *O. marina* foraging on individual food particles (Roberts *et al.*, this issue) and to quantify its reaction to chemical gradients (Breckels *et al.*, this issue). Modelling these helical paths would be a beneficial tool that could aid in determining the advantages of helical swimming in *O. marina*.

Although there is a well developed framework to study ‘classical’ random walk models (Codling *et al.*, 2008), there is a lack of models and consistently practiced data analysis protocols that directly consider the fine helical motion observed in *O. marina*. Continued study of the swimming in *O. marina* could rectify this shortcoming so that helical paths could be included in models of protist swimming in the future. A model for helical motion by creating a spiral around an axis of trajectory relative to the organism’s position was developed by Crenshaw and Edelstein-Keshet (1993) to show that changes in the direction of rotational velocity change the net direction of movement. Crenshaw (1993) used this model to demonstrate that organisms swimming in helical paths can align themselves along a gradient using taxis where there is no randomness in the movement mechanisms. Jékely *et al.* (2008) used the planktonic larvae of annelids (*Platynereis*

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3 *dumerilii*) to show that selective illumination of one eyespot changes the beating of adjacent cilia
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5 and results in phototaxis. Their computer simulation further demonstrates that such local effects are
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7 sufficient to direct the helical swimming trajectories towards the light and that helical swimming
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9 increases the precision of navigation.
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12 So far, studies of *O. marina* have investigated changes in behaviour in response to different
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14 stimuli including uniform layers of phytoplankton or dissolved exudates (see Breckels *et al.*, this
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16 issue). Individual level orientation of *O. marina* to such stimuli has yet to be shown. Likely
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18 progress will stem from extending the early model of Crenshaw (1993) and the more recent
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20 simulations of Jékely *et al.* (2008) to include some of the standard random walk techniques, before
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22 comparing the simulations with real 3D swimming data from *O. marina*. This approach will reveal
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24 possible advantages of helical walks, and can be used to investigate how small-scale changes to this
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26 walk affect the characteristics of swimming paths.
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33 CONCLUSIONS

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36 Quantifying the swimming behaviour in *O. marina* will aid in understanding the role of swimming
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38 in the structure and ecology of microbial ecosystems. Thus, it is important that we understand how
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40 the 3D helical movement path and run-and-tumble swimming used by *O. marina* affect its ability to
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42 sense chemical information in the natural environment. Although the specific swimming responses
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44 may not be representative of all other protists, this species provides a suitable model to analyse and
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46 quantify run-and-tumble behaviour and swimming in helical paths. Such information can then be
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48 compared with data from other flagellates and ciliates that show similar or contrasting swimming
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50 behaviours. However, there is generally a lack of studies that properly incorporate both the 3D
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52 nature of the path and the fine scale helical properties. This makes understanding population
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54 behaviour particularly difficult where there is high diversity in behaviour between individuals.
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59 We also advise that a standard 3D framework and explorational data analysis should be
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established for modelling and analysing protist swimming paths in order to qualitatively and

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3 quantitatively determine, and understand their swimming behaviour. Because of its exceptional
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5 suitability for laboratory-based research in combination with the pioneering studies on swimming,
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8 *O. marina* would be an appropriate candidate for the development of this framework. Once in
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10 place, it will assist with further experimental studies to determine, for example, advantages and
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12 disadvantages of helical over non-helical swimming under various environmental conditions.
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1 Boakes *et al.* Swimming behaviour of *Oxyrrhis marina*

2
3 **REFERENCES**

- 4
5 Bartumeus, F., Peters, F., Pueyo, S. *et al.* (2003) Helical Lévy walks: Adjusting searching statistics
6 to resource availability in microzooplankton. *Proc. Natl. Acad. Sci.*, **100**, 12771–12775.
7
8 Berg, H. C. (1978) The tracking microscope. *Adv. Opt. Electron. Microsc.*, **5**, 1–15.
9
10 Berg, H. C. (1983) *Random Walks in Biology*. Princeton University Press, Princeton, NJ.
11
12 Bovet, P. and Benhamou, S. (1988) Spatial analysis of animals movement using correlated random
13 walks. *J. Theor. Biol.*, **131**, 419–433.
14
15 Breckels, M.N., Archer, S. D., Malin, G. *et al.* (this issue) The role of dissolved infochemicals in the
16 ecology of the heterotrophic dinoflagellate *Oxyrrhis marina*. *J. Plankton Res.*, **xx**, xx–xx.
17
18 Buskey, E. J., Coulter, C. and Strom, S. (1993) Locomotory patterns of microzooplankton: Potential
19 effects on food selectivity of larval fish. *Bull. Mar. Sci.*, **53**, 29–43.
20
21 Cachon, M., Cosson, J., Cosson, M. *et al.* (1988) Ultrastructure of the flagellar apparatus of
22 *Oxyrrhis marina*. *Biol. Cell.*, **63**, 159–168.
23
24 Codling, E. A. and Hill, N. A. (2005) Sampling rate effects on measurements of correlated and
25 biased random walks. *J. Theor. Biol.*, **233**, 573–588.
26
27 Codling, E. A., Plank, M. J. and Benhamou, S. (2008) Random walk models in biology. *J. R. Soc.*
28 *Interface*, **5**, 813–834.
29
30 Cosson, J., Cachon, M., Cachon, J. *et al.* (1988) Swimming behaviour of the unicellular biflagellate
31 *Oxyrrhis marina*: *in vivo* and *in vitro* movement of the two flagella. *Biol. Cell.*, **63**, 117–128.
32
33 Crawford, D. W. and Lindholm, T. (1997) Some observations on vertical distribution and migration
34 of the phototrophic ciliate *Mesodinium rubrum* (= *Myrionecta rubra*) in a stratified brackish
35 inlet. *Aquat. Microb. Ecol.*, **13**, 267–274.
36
37 Crenshaw, H. C. (1993) Orientation by helical motion. III. Microorganisms can orient to stimuli by
38 changing the direction of their rotational velocity. *J. Math. Biol.*, **55**, 231–255.
39
40 Crenshaw, H. C. (1996) A new look at locomotion in microorganisms: Rotating and translating.
41 *Amer. Zool.*, **36**, 608–618.
42
43
44
45
46
47
48
49
50
51
52
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55
56
57
58
59
60

1 Boakes *et al.* Swimming behaviour of *Oxyrrhis marina*

2
3 Crenshaw, H. C. and Edelstein-Keshet, L. (1993) Orientation by helical motion. II. Changing the
4
5 direction of the axis of motion. *J. Math. Biol.*, **55**, 213–230.

6
7
8 Crenshaw, H.C., Ciampaglio, C. N. and McHenry, M. (2000) Analysis of the three-dimensional
9
10 trajectories of organisms: Estimates of velocity, curvature and torsion from positional
11
12 information. *J. Exp. Biol.*, **203**, 961–982.

13
14
15 Davidson, K., Fotoon, S. and Montagnes, D. J. S. (this issue) *Oxyrrhis marina* based models as a
16
17 tool to interpret protozoan population dynamics. *J. Plankton Res.*, **xx**, xx–xx.

18
19
20 Drescher, K., Goldstein, R. E. and Tuval, I. (2010) Fidelity of adaptive phototaxis. *Proc. Nat. Acad.*
21
22 *Sci.*, **107**, 11171–11176.

23
24
25 Drescher, K., Leptos, K. and Goldstein, R. E. (2009) How to track protists in three dimensions. *Rev.*
26
27 *Sci. Instrum.* **80**, 014301.

28
29
30 Droop, M. R. (1959) A note on some physical conditions for culturing *Oxyrrhis marina*. *J. Mar.*
31
32 *Biol. Ass. U.K.*, **38**, 599–604.

33
34
35 Dujardin, F. (1841) *Histoire naturelle des zoophytes. Infusoires, comprenant la physiologie et la*
36
37 *classification de ces animaux, et la manière de les étudier à l'aide du microscope*. Roret,
38
39 Paris.

40
41
42 Dusenbery, D. B. (2009) *Living at Micro Scale: The Unexpected Physics of Being Small*. Harvard
43
44 University Press, Cambridge.

45
46
47 Edwards, A. M., Phillips, R. A., Watkins, N. W. *et al.* (2007) Revisiting Lévy flight search patterns
48
49 of wandering albatrosses, bumblebees and deer. *Nature*, **449**, 1044–1048.

50
51 Fenchel, T. (2001) How dinoflagellates swim. *Protist*, **152**, 329–338.

52
53
54 Fenchel, T. and Blackburn, N. (1999) Motile chemosensory behaviour of phagotrophic protists:
55
56 mechanisms for and efficiency in congregating at food patches. *Protist*, **150**, 325–336.

57
58
59 Fenchel, T. and Jonsson, N. (1988) The functional biology of *Strombidium sulcatum*, a marine
60
oligotrich ciliate (*Ciliophora Oligotrichina*). *Mar. Ecol. Prog. Ser.*, **48**, 1–15.

Grünbaum, D. (2002) Predicting availability to consumers of spatially and temporally limited

1 Boakes *et al.* Swimming behaviour of *Oxyrrhis marina*

2
3 resources. *Hydrobiologia*, **480**, 175–191.

4
5 Hansen, F. C., Witte, H. J. and Passarge, J. (1996) Grazing in the heterotrophic dinoflagellate

6
7 *Oxyrrhis marina*: size selectivity and preference for calcified *Emiliana huxleyi* cells. *Aquat.*

8
9 *Microb. Ecol.*, **10**, 307–313.

10
11 Hemmersbach, R. and Häder, D. P. (1999) Graviresponses of certain ciliates and flagellates. *FASEB*

12
13 *J.*, **13**, S69-S75.

14
15 Hill, N. A. and Häder, D. P. (1997) A biased random walk model for the trajectories of swimming

16
17 micro-organisms. *J. Theor. Biol.*, **186**, 503–526.

18
19 Jakobsen, H. H. (2002) Escape of protists in predator-generated feeding currents. *Aquat. Microb.*

20
21 *Ecol.*, **26**, 271–281.

22
23 Jakobsen, H. H., Everett, L. M. and Strom, S. L. (2006) Hydromechanical signalling between the

24
25 ciliate *Mesodinium pulex* and motile protist prey. *Aquat. Microb. Ecol.*, **44**, 197–206.

26
27 Jakobsen, H. H. and Strom, S. L. (2004) Circadian cycles in growth and feeding rates of

28
29 heterotrophic protist plankton. *Limnol. Oceanogr.*, **49**, 1915–1922.

30
31 Jékely, G. Colombelli, J. Hausen, H. *et al.* (2008) Mechanism of phototaxis in marine zooplankton.

32
33 *Nature*, **456**, 395–399.

34
35 Jennings, H. S. (1906) *Behavior of the Lower Organisms*. Columbia University Press, New York.

36
37 Kent, W. S. (1880) *A Manual of the Infusoria*. Vol. 1. David Bogue, London.

38
39 Lowe, C. D., Martin, L. E., Watts, P. C. *et al.* (this issue-a) Isolation and culturing strategies for the

40
41 maintenance of *Oxyrrhis marina*. *J. Plankton Res.*, **xx**, xx–xx.

42
43 Lowe, C. D., Keeling, P. J., Martin, L. E. *et al.* (this issue -b) Who is *Oxyrrhis marina*?

44
45 Morphological and phylogenetic studies on an unusual dinoflagellate. *J. Plankton Res.*, **xx**, xx–

46
47 xx.

48
49 Martel, C. M. (2006) Prey location, recognition and ingestion by the phagotrophic marine

50
51 dinoflagellate *Oxyrrhis marina*. *J. Exp. Mar. Biol. Ecol.*, **335**, 210–220.

1 Boakes *et al.* Swimming behaviour of *Oxyrrhis marina*

2
3 Menden-Deuer, S. and Grünbaum, D. (2006) Individual foraging behaviour and population
4
5 distributions of a planktonic predator aggregating to phytoplankton thin layers. *Limnol.*
6
7
8 *Oceanogr.*, **51**, 108–116.

9
10 Montagnes, D. J. S., Lowe, D. D., Roberts, E. C. *et al.* (this issue) An introduction to the special
11
12 issue: *Oxyrrhis marina*, a model organism? *J. Plankton Res.*, **xx**, xx–xx.

13
14 Pizay, M. D., Lemeé, R., Simon, N. *et al.* (2009). Night and day morphologies in a planktonic
15
16 dinoflagellate. *Protist*, **160**, 565–575.

17
18 Plank, M. J. and Codling, E. A. (2009) Sampling rate and misidentification of Lévy and non-Lévy
19
20 movement paths. *Ecology*, **90**, 3546–3553.

21
22 Polin, M., Tuval, I., Drescher, K., *et al.* (2009) *Chlamydomonas* swims with two 'gears' in a
23
24 eukaryotic version of run-and-tumble locomotion. *Science*, **325**, 487–490.

25
26 Reynolds, A. M. (2008) Deterministic walks with inverse-square power-law scaling are an emergent
27
28 property of predators that use chemotaxis to locate randomly distributed prey. *Phys. Rev. E.*, **78**,
29
30 011906.

31
32 Roberts, E. C., Wootton, E. C., Davidson, K. *et al.* (this issue) Feeding in the dinoflagellate *Oxyrrhis*
33
34 *marina*: Linking behaviour with mechanics. *J. Plankton Res.*, **xx**, xx–xx.

35
36 Schmitt, F. C. and Seuront. L. (2001) Multifractal random walk in copepod behavior. *Physica A.*,
37
38
39 **301**, 375–396.

40
41 Senn, G. (1911) *Oxyrrhis*, *Nephroselmis* und einige Euflagellaten, nebst Bemerkungen über deren
42
43 System. *Z. Wiss. Zool.*, **97**, 605–672.

44
45 Seymour, J. R., Simó, R., Ahmed, T., Stocker, R. (2010) Chemoattraction to
46
47 dimethylsulfoniopropionate throughout the marine microbial food web. *Science*, **329**, 342–345.

48
49 Sheng, J., Malkiel, E., Katz, J. *et al.* (2007) Digital holographic microscopy reveals prey-induced
50
51 changes in swimming behaviour of predatory dinoflagellates. *Proc. Natl. Acad. Sci.*, **104**,
52
53 17512–17517.

54
55 Sheng, J., Malkiel, E., Katz J. *et al.* (2010). A dinoflagellate exploits toxins to immobilize prey prior
56
57
58
59
60

1 Boakes *et al.* Swimming behaviour of *Oxyrrhis marina*

2
3 to ingestion. *Proc. Natl. Acad. Sci.*, **107**, 2082–2087.

4
5 Strom, S., Wolfe, G., Holmes, J. *et al.* (2003) Chemical defense in the microplankton I: Feeding and
6
7 growth rates of heterotrophic protists on the DMS-producing phytoplankter *Emiliana huxleyi*.

8
9
10 *Limnol. Oceanogr.*, **48**, 217–229.

11
12 Strom, S. L., Wolfe, G. V. and Bright, K. J. (2007). Responses of marine planktonic protists to
13
14 amino acids: feeding inhibition and swimming behavior in the ciliate *Favella* sp. *Aquat.*

15
16
17 *Microb. Ecol.*, **47**, 107–121.

18
19 Tarran, G. A. (1991) *Aspects of the grazing behaviour of the marine dinoflagellate Oxyrrhis marina*,
20
21 *Dujardin*. Doctoral Dissertation. University of Southampton. Department of Biology.

22
23
24 Southampton, UK. pp. 182.

25
26 Thar, R., Blackburn, N. and K uhl, M. (2000) A new system for three-dimensional tracking of motile
27
28 microorganisms. *Appl. Environ. Microbiol.*, **66**, 2238–2242.

29
30 Thorn, G. J. and Bearon, R. N. (2010) Transport of spherical gyrotactic organisms in general three-
31
32 dimensional flow field. *Phys. Fluids*, **22**, 041902.

33
34 Uttieri, M., Cianelli, D., Strickler, J. R. *et al.* (2007) On the relationship between fractal dimension
35
36 and encounters in three-dimensional trajectories. *J. Theor. Biol.*, **247**, 480–491.

37
38
39 Watts, P. C., Martin, L. E., Kimmance, S. E. *et al.* (this issue) The distribution of *Oxyrrhis marina* -
40
41 a global wanderer or poorly characterised endemic? *J. Plankton Res.*, **xx**, xx–xx.

42
43
44 Wolfe, G. V. and Steinke, M. (1996) Grazing-activated production of dimethyl sulfide (DMS) by
45
46 two clones of *Emiliana huxleyi*. *Limnol. Oceanogr.*, **41**, 1151–1160.

47
48
49 Vandromme, P., Schmitt, F. G., Souissi, S. *et al.* (2010) Symbolic analysis of plankton swimming
50
51 trajectories: Case study of *Strobilidium* sp. (Protista) helical walking under various food
52
53 conditions, *Zool. Stud.*, **49**, 289–303.

54
55
56
57 Visser, A. and Ki rboe, T. (2006) Plankton motility patterns and encounter rates. *Oecologia.*, **148**,
58
59 538–546.
60

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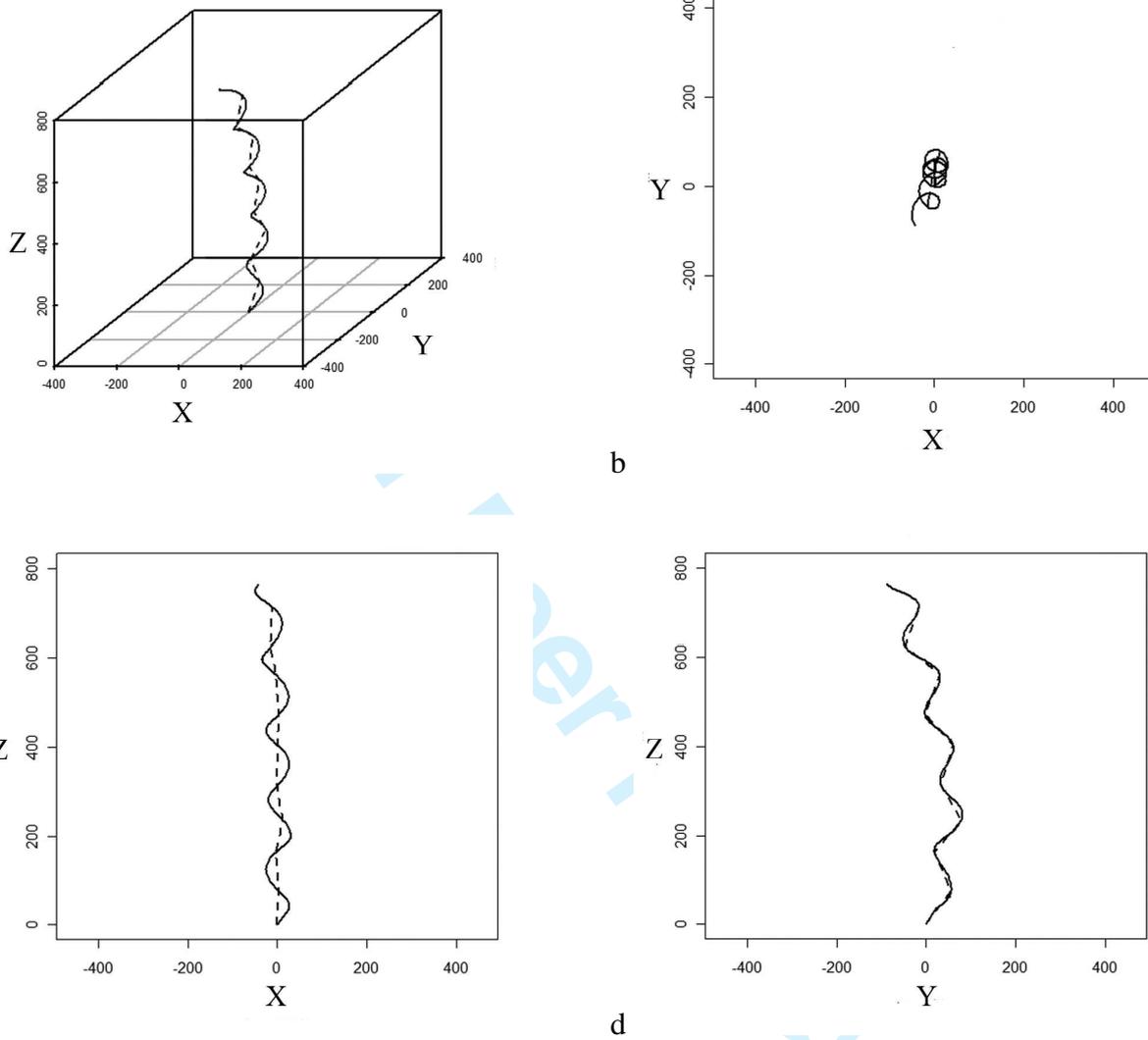
FIGURE LEGEND

Fig. 1. Example of a simulated three-dimensional (3D) helical random walk and the subsequent effect of projecting onto 2D planes. (a) Three-dimensional representation of a correlated helical trajectory, beginning at the origin, (0,0,0), with 200 steps (solid line) and a rediscretisation featuring only 8 steps where every 25th point in the original walk is included (dashed line). In this example the distance units are dimensionless and have no biological meaning. This helical path is then represented in two dimensions by projecting into (b) the *xy*-plane, (c) the *xz*-plane, and (d) the *yz*-plane. The net displacement of the movement path calculated for each of these projections is (a) 772.8 units, (b) 100.7 units, (c) 767.3 units, and (d) 771.7 units, respectively; illustrating that projecting the 3D path into a 2D plane can cause simple path analysis to be misleading. Rediscretisation of the paths can cause a loss of helical details.

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3 **Figure**

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6 **Fig. 1.**



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