Zooplankton induced currents and fluxes in stratified waters
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ABSTRACT
This paper presents experimental results for the fluid transport induced by tethered as well as freely swimming zooplankton organisms (Daphnia) in a density-stratified water column. Particle image velocimetry and planar laser induced fluorescence were used to measure the instantaneous velocity and density distributions and to estimate kinetic energy, dissipation rates, vertical mass fluxes, and apparent diffusion coefficients around individual organisms. For freely swimming organisms we found dissipation rates of kinetic energy of $10^{-6}$ W kg$^{-1}$ and an apparent diffusion coefficient of $10^{-5}$ m$^2$ s$^{-1}$. Both values are valid for a volume of influence of approximately 1 cm$^3$, which is about two orders of magnitude larger than the size of the organism. In the case of tethered Daphnia all measures are significantly larger but – associated with the artificial situation – of less environmental significance. The results indicate that swimming-induced fluid and mass transport in the vicinity of zooplankton organisms is characterized by apparent diffusion coefficients comparable in magnitude to turbulent diffusivities typically found in stratified aquatic environments. Upscaling of these results using abundances of Daphnia in lakes further suggests that biologically-induced fluid transport by zooplankton might potentially be important for vertical mixing in stratified waters if background mixing is weak.

Key words | Daphnia, diffusion, dissipation, fluid transport, kinetic energy

INTRODUCTION

Hydrodynamic interactions of swimming zooplankton with its aquatic environment (see Guasto et al. (2012) for a comprehensive review) are known to have important implications for sensing, foraging, predation, and reproduction (Kiørboe 2008). On the scale of an individual, the flow field generated by the organism determines the efficiency and energetic costs of propulsion and feeding (Morris et al. 1985; van Duren et al. 2003; Jiang & Strickler 2007). Swimming in aquatic environments also affects the persistence of ‘footprints’, which can guide potential predators (Visser 2001; Videler et al. 2002; Kiørboe et al. 2010), and limits the perception of chemical cues, which can induce mating (van Duren et al. 1998). On the other hand, swimming might help to avoid predators (Cohen & Forward 2009) or toxicants (Dodson et al. 1995). Through cumulative effects of individual performance, hydrodynamic phenomena at organism scale can influence the structural and functional properties of the plankton community and ultimately the fate of biogenic carbon in aquatic ecosystems (Alcaraz 1997).

Ongoing research effort also investigates the role of animal swimming for large-scale vertical transport and mixing in aquatic systems. Energetic arguments suggest that the biosphere contributes mechanical energy for ocean mixing in the same order of magnitude as major winds and tides (Huntley & Zhou 2004; Dewar et al. 2006). Although first indications of biological induced turbulence and mixing were observed for dense swarms of krill (Kunze et al. 2006) and schools of small fishes (Gregg & Horne 2009; Lorke & Probst 2010), experimental evidence is still limited to larger organisms. Following Visser (2007), the size for most of the small and very
abundant aquatic animals like zooplankton limits the kinetic energy imparted to the flow and associated mixing is insignificant. Numerical simulations (Dabiri 2010), however, showed that the successive interaction of zooplankton organisms with parcels of fluid lead, under certain conditions, to vertical displacement of the fluid parcels over distances much larger than the individual body size.

Although fluid transport and mixing around swimming zooplankton is a key process for the interaction of zooplankton with its physical and chemical environment, direct and quantitative observations are very limited. Existing studies are based on numerical simulations or experimental observations of flow fields. Laboratory measurements are typically performed using particle image velocimetry around tethered or freely swimming zooplankton organisms (Stamhuis & Videler 1995). Such measurements allow for quantitative flow field visualizations and estimates of dissipation rates of mechanical energy, but do not allow for direct quantification of solute transport associated with zooplankton swimming. The transport of dissolved substances and heat is, in most aquatic systems, affected by vertical density stratification. Scaling arguments and analytical flow field simulations suggest that even the flow fields around small $O\ (100\ \mu\text{m}–1\ mm)$ zooplankton organisms are affected by typical salt- or temperature-stratification (Ardekani & Stocker 2010). Doostmohammadi et al. (2012) suggested, that density stratification reduces the flow signatures that predators use to detect prey and that strong density gradients can provide a hide-out for motile zooplankton organisms. The majority of experimental observations, however, are performed without density stratification.

In this study we investigate the small-scale flow field around tethered and freely swimming millimeter-sized zooplankton ($Daphnia magna$) in laboratory experiments. Under the effect of stable density stratification, we estimate fluid and mass transport within the affected volume. We combined particle image velocimetry (Stamhuis 2006) with laser-induced fluorescence (Crimaldi 2008) to characterize the velocity field, kinetic energy, rates of viscous energy dissipation, as well as the vertical fluxes and apparent diffusion coefficients associated with the movement of individual organisms.

**METHODS**

**Experimental setup**

Experiments were conducted in a linear vertical density gradient with a buoyancy frequency $N$, of $0.07\ \text{s}^{-1}$,

$$N = (-g/\rho \cdot \partial \varphi/\partial z)^{1/2}$$

where $\rho$ is the density, $z$ is depth, $g$ is gravitational acceleration. The gradient was established in a 17.3 cm tall glass tank ($9\ \text{dm}^3$ volume) with a maximum salt (NaCl) concentration $C_{\text{NaCl}} = 0.17\%$ at the bottom and $0.0\%$ at the top using the Oster-method (Zurigat et al. 1990). The tank was filled from the bottom to the top at a constant rate and with linearly increasing salt concentration in the inflow. The resulting density gradient corresponds to a temperature gradient of approximately $3\ ^\circ\text{C}\ \text{m}^{-1}$ at $24\ ^\circ\text{C}$, which is comparable to gradients observed in the seasonal thermocline.

The gradient is stabilized against temperature fluctuations within the laboratory, which ultimately led to the generation of convectively-driven currents and mixing, by keeping the tank together with the supply tanks inside of a larger tank with water of controlled temperature.

The fluorescent tracer Rhodamin 6G was added during the filling process at a rate proportional to that of salt, resulting in a concentration of $C_{\text{Rh}} = 0.02\ \text{mg}\ \text{l}^{-1}$ at the bottom and $0.0\ \text{mg}\ \text{l}^{-1}$ at the surface. The intensity of fluorescence measured at a wavelength of 555 nm after excitation with laser light (532 nm) was used as a proxy to quantify the density distribution and to observe small-scale density disturbances within a $2\ \text{mm}$ thick light sheet. The light sheet was arranged vertically and fluorescence intensity was recorded using a 4 megapixel charge coupled device (CCD) camera synchronized with laser-pulses at a rate of $7\ \text{Hz}$ (double-frame images per second) within a $17 \times 17\ \text{cm}$ field of view near the centre of the tank. Raw images of the fluorescence distribution were corrected for inhomogeneous illumination due to light absorption and reflections and for fluctuations in laser power following Shan et al. (2004). The flow field was visualized using $20\ \mu\text{m}$ sized seeding particles, evenly distributed throughout the tank, and observed by a second CCD camera. Two-dimensional particle velocities within the laser light sheet were estimated by correlating two
subsequently captured image frames, each synchronized with a separate laser pulse and with a time lag of 35 ms between both exposures.

The analysis of the flow and the dispersion around zooplankton organisms was performed under two different conditions (Figure 1). First, a single *Daphnia* was tethered with glue on the tip of a fine nylon filament and fixed within the laser light sheet. The organism was aligned directly in the laser sheet at the centre of the tank, in a way that its second antennae movement accelerated the fluid mainly in parallel to the light sheet (Figures 1(a) and 1(b)). In the second setup, more realistic measurements have been conducted with a number of freely swimming *Daphnia* using an identical experimental setup. *Daphnia* passed through the light sheet and the field of view of the cameras randomly and for short time periods (max. 5.8 s) only (Figure 1(c) and http://www.uni-koblenz-landau.de/landau/fb7/umweltwissenschaften/uphys-de/Research/Biomix/Freely_Moving_Daphnia.gif provided in the supplementary material).

**Flow field analysis**

Instantaneous velocities were analyzed by adaptive correlation and mean velocities by the average correlation procedure of the DynamicStudio software (Dantec Dynamics) in $2.65 \times 2.65$ mm subareas, i.e. within $64 \times 64$ pixel interrogation areas with 50% overlap. Within the entire tank no advective (background) currents were present during the measurements and any current and density displacement in the aquarium could entirely be related to the flow generated by the *Daphnia*. The regions covered by the tethered and around freely swimming *Daphnia* were discarded and remaining outliers, exceeding a velocity threshold of $v_i = 1 \text{ cm s}^{-1}$, were replaced by mean values of the surrounding instantaneous velocities. The index $i$ denotes the component in horizontal $i = x$ or vertical $i = z$ direction of the velocity vector $\vec{v}$. Velocities above $1 \text{ cm s}^{-1}$ appeared only in overexposed interrogation areas, which were partly or entirely covered by the *Daphnia*. The kinetic energy $E = 0.5 \cdot \vec{v}^2$ and the viscous energy dissipation (Catton et al. 2007),

$$
\varepsilon = \mu \left[ 2 \left( \frac{\partial u_x}{\partial x} \right)^2 + 2 \left( \frac{\partial u_z}{\partial x} \right)^2 + 2 \left( \frac{\partial u_x}{\partial z} + \frac{\partial u_z}{\partial x} \right)^2 + 3 \left( \frac{\partial u_x}{\partial z} + \frac{\partial u_z}{\partial x} \right)^2 \right]
$$

with kinematic viscosity $\mu$, were estimated from observed velocities and velocity gradients. The total dissipated power $P_{\text{diss}} = \rho \cdot \int \varepsilon \, dV$ was estimated in the fluid volume $V$ affected by the *Daphnia*. $V$ was assumed to be equal to a cylindrical volume with a diameter of $dx$ over which $\varepsilon > 10^{-7.5} \text{ W kg}^{-1}$. Although $\varepsilon = 10^{-7.5} \text{ W kg}^{-1}$ appears to be a high threshold, observed spatial distributions of $\varepsilon$ showed strong spatial gradients in the range between $\varepsilon = 10^{-8.5}$ and $\varepsilon = 10^{-7} \text{ W kg}^{-1}$. Hence, the particular choice of this threshold did not significantly decrease the volume affected by the *Daphnia*.

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**Figure 1** Raw images of (a) tethered *Daphnia* for particle image velocimetry, (b) tethered *Daphnia* for laser-induced fluorescence, and (c) freely swimming *Daphnia* (full video of the freely swimming *Daphnia* is available in the supplementary material). Explanations are provided in the images, the grayscale in (b) and (c) visualizes the density distribution, which initially had a linear vertical gradient.
Analysis of scalar transport

The fluid mixing associated with the *Daphnia*-induced flow was quantified by estimating an apparent diffusion coefficient based on fluid displacement. 50 virtual particles were placed along a straight line perpendicular to the jet directly behind the *Daphnia* and their pathways have been followed numerically based on the observed mean velocity field in $10^{-5}$ s time steps. The corresponding diffusion coefficient $k_{\text{Daph}}$ was estimated from the mean two-dimensional displacement $r = \sqrt{\Delta v^2 + \Delta z^2}$ of the particles within time $\Delta t$ after release as

$$k_{\text{Daph}} = 0.25 \langle v^2 \rangle / \Delta t$$

(Berg 1993). In an independent approach, the small-scale mixing induced by the tethered *Daphnia* was estimated from the observed density fluctuations by evaluating the vertical mass flux through a horizontal plane, perpendicular to the laser light sheet. In analogy to turbulent transport, the flux $J$ can be estimated as $J = \overline{v'C}$, with $v'$ denoting the fluctuation of the vertical velocity, $c'$ the fluctuation of concentration observed by laser-induced fluorescence, and the overbar denotes temporal averaging. An alternative estimate of the diffusion coefficient can be obtained from Fick’s law as

$$k_{\text{Daph}} = -J / \langle \partial c / \partial z \rangle$$

where $\langle \partial p / \partial z \rangle \approx \langle \partial c / \partial z \rangle = 0.72 \text{ kg m}^{-1}$ is the spatially averaged ($\langle \rangle$ denotes spatial averaging) density gradient corresponding to the concentration gradient of salt and Rhodamine 6G.

RESULTS

Flow fields and kinetic energy

In case of the tethered organism, the tetherline fixing did not affect the movement of the second antennae and most of the mechanical energy introduced by their steady motion resulted in displacement of surrounding fluid, instead of propulsion of the *Daphnia*. An individual of about 4.2 mm length caused a persistent and nearly steady fluid jet. This quasi-steady flow field allows estimation of temporal averaged velocities and hydraulic parameters. The core of the jet had a longitudinal extension of approximately 60 mm, where the first part had a strong downward velocity component for about 45 mm before it reached a minimum level turned into a slight upward motion and faded away in the horizontal direction (Figure 2(a)). The upward and horizontal motion as well as its kinetic energy (Figure 2(b)) and energy dissipation rate (Figure 2(c)) is weak in comparison to the downward component of the jet. The transversal extension of the fully developed jet was approximately 14 mm corresponding to a lower threshold of $\overline{v'} \approx 1.0 \text{ mm s}^{-1}$. Assuming spherical symmetry, the jet encompassed a volume of approximately 7 cm$^3$, which is about 180 times the volume of the tethered organism (approximated as a sphere). The spatially averaged velocity in the jet was $\overline{v} = 2.8 \text{ mm s}^{-1}$ and the maximum velocity was $v_{\text{max}} = 14 \text{ mm s}^{-1}$. The kinetic energy reached a maximum value of $10^{-4}$ J kg$^{-1}$ in the centre of the jet (Figure 2(b)). The rate of viscous energy dissipation reached a maximum value of $2.2 \times 10^{-5}$ W kg$^{-1}$ (Figure 2(c)), while most of the dissipation rates range within the order of magnitude of $10^{-6}$ W kg$^{-1}$ (mean value: $\varepsilon = 8.2 \times 10^{-7}$ W kg$^{-1}$). Based on the observed magnitudes, the longitudinal extension of the jet $r_{\text{max}}$ can be estimated by balancing the observed decay of kinetic energy with its rate of viscous dissipation along the jet by $r_{\text{max}} = E / \varepsilon \approx 43 \text{ mm}$, which is in close agreement with the downward directed part of the observed jet of 45 mm. Although the jet turned to a slight upward motion, these estimates indicate that most of the kinetic energy is dissipated in vicinity to the nearly vertically directed jet and that the recirculating flow occurred at smaller velocities distributed over a much larger area in the far field of the jet, i.e. that the tethered *Daphnia* did not generate a circulating eddy. The volume of the jet, i.e. the volume of influence of the tethered *Daphnia*, increases to $V = 15.8 \text{ cm}^3$, if the proposed threshold in dissipation rate instead of velocity is used to define this volume. This larger estimate can be attributed to the increased diameter of the cylinder covered by dissipation rates above the proposed threshold. The total dissipated power $P_{\text{diss}}$ in this volume was $P_{\text{diss}} = 1.3 \times 10^{-8}$ W.

Although fixation within the light sheet and the nearly stationary flow field generated by the tethered *Daphnia*
provide ideal experimental conditions for the development and testing of observation and analysis procedures, the resulting dissipated power is certainly overestimated because the mechanical energy introduced by the organism goes entirely into acceleration of surrounding water, instead of propulsion. Because of the highly unsteady character of the flow associated with the passage of the freely swimming zooplankton organism, all parameters have been evaluated for each individual image, except for the dissipated power and the diffusion coefficient, which were averaged over the recorded sequence. Figures 2(d)–2(f) exemplify the results showing the velocity field, kinetic energy and dissipation rate around a freely swimming Daphnia. The average speed of the Daphnia was 14 mm s⁻¹ corresponding to the observed $v_{\text{max}}$ in the jet of the tethered organism. Movie files of the entire movement and its hydrodynamic characterization are provided in the supplementary material (http://www.uni-koblenz-landau.de/landau/fb7/umweltwissenschaften/uphys-de/Research/Biomix/Velocity_Free_Daphnia.avi for velocity, http://www.uni-koblenz-landau.de/landau/fb7/umweltwissenschaften/uphys-de/Research/Biomix/Kinetic_Energy_Free_Daphnia.avi for kinetic energy).
energy, and http://www.uni-koblenz-landau.de/landau/fb7/umweltwissenschaften/umphys-de/Research/Biomix/Dissipation_Rate_Free_Daphnia.avi for dissipation rate). Although the highest rate of viscous energy dissipation \( \varepsilon = 2.8 \times 10^{-4} \) W kg\(^{-1} \) induced by the freely swimming Daphnia is an order of magnitude higher in comparison to the maximal dissipation rate caused by the tethered Daphnia, the total amount of dissipated energy (dissipated power) was an order of magnitude lower, i.e. \( P_{\text{diss}} = 1.2 \times 10^{-9} \) W (Table 1). This difference can be related to a smaller volume of influence, which had a mean size of \( V = 0.63 \) cm\(^3\) based on a threshold in dissipation rate. The mean rate of energy dissipation within this volume was \( \varepsilon = 2 \times 10^{-6} \) W kg\(^{-1} \). In case of the freely moving Daphnia, the mean volume of the altered flow field \( \langle \vec{v} \rangle > 1.0 \) mm s\(^{-1}\) was only 0.08 cm\(^3\) and much smaller in comparison to the mean volume affected by high dissipation rates. The mean volume affected by freely swimming organisms was estimated as the mean area affected by the Daphnia after it has passed through the light sheet times the mean displacement of the Daphnia from image to image; i.e. it was assumed that the Daphnia affects a cylindrical volume of fixed length along its wake.

### Scalar transport

Virtual particle displacements within the observed flow field of the tethered Daphnia revealed apparent diffusivities \( k_{\text{Daph}} \), which increase with increasing time span \( \Delta t \) over which particle displacement was observed. Maximum diffusivities of \( 5 \times 10^{-5} \) m\(^2\) s\(^{-1}\) were obtained before the particles left the high velocity region of the jet, which approximately corresponds to the first 32 mm of displacement (Figure 5(a)). For further increasing \( \Delta t \), \( k_{\text{Daph}} \) decreased slightly until the particles reached the end of the jet. Based on the observed nearly stationary flow field and by neglecting vertical transport outside of the cross-sectional area of the jet, the total flux induced by the jet can be estimated as the mean vertical velocity \( \langle v_z \rangle \) \( \approx 1.4 \) mm s\(^{-1}\) multiplied by the mean salt concentration \( \langle c \rangle \) \( \approx 0.05 \) kg m\(^{-3}\) within the jet, resulting in a value of \( f = 7 \times 10^{-5} \) kg m\(^{-2}\) s\(^{-1}\) and a corresponding diffusivity of \( k_{\text{Daph}} = 9.7 \times 10^{-5} \) m\(^2\) s\(^{-1}\).

Virtual particle displacements within the observed dynamic flow passed by the freely swimming Daphnia revealed apparent diffusivities of \( k_{\text{Daph}} = 1.5 \times 10^{-5} \) m\(^2\) s\(^{-1}\) within the wake of the organism. Figure 5(b) shows that

### Table 1

Summary of hydrodynamic parameters (mean and maximum dissipation rates of kinetic energy \( \varepsilon \) and mean diffusion coefficients \( k_{\text{Daph}} \), estimated using virtual particle tracking and from flux observations) for tethered and freely swimming Daphnia, respectively.

<table>
<thead>
<tr>
<th>Case</th>
<th>( \varepsilon (v_{\text{max}}) ) [W kg(^{-1})]</th>
<th>( V ) [cm(^3)]</th>
<th>( P_{\text{max}} ) [W]</th>
<th>( k_{\text{Daph}} ) (virt. particle) [m(^2) s(^{-1})]</th>
<th>( k_{\text{Daph}} ) (flux) [m(^2) s(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tethered</td>
<td>( 8.2 \times 10^{-7} ) (2.2 \times 10^{-5})</td>
<td>15.8</td>
<td>( 1.3 \times 10^{-8} )</td>
<td>( 5 \times 10^{-3} )</td>
<td>( 9.7 \times 10^{-5} )</td>
</tr>
<tr>
<td>Freely swimming</td>
<td>( 2 \times 10^{-6} ) (2.8 \times 10^{-4})</td>
<td>0.63</td>
<td>( 1.2 \times 10^{-9} )</td>
<td>( 1.5 \times 10^{-3} )</td>
<td>( 0.8 \times 10^{-5} )</td>
</tr>
</tbody>
</table>

**Figure 3** | Example for diffusion coefficient \( k_{\text{Daph}} \) computed from the displacement of the particles with time \( t \), i.e. mean diffusion coefficient of all particle-pathways: (a) for the tethered Daphnia, (b) for the freely swimming Daphnia.
the diffusion coefficient remains approximately constant between a short period of increasing ($\Delta t < 1.8$ s) and decreasing diffusion ($\Delta t > 4$ s).

In contrast to the experiment with the tethered *Daphnia*, it is not possible to assume quasi-stationary conditions and therefore it is not possible to use mean values for evaluating the vertical mass flux from concentration fluctuations. However, it is still reasonable to consider the entire disturbance imposed by a swimming *Daphnia* as the only source of mass transport and therefore it is not possible to use mean values for the vertical salt disturbance imposed by a swimming *Daphnia* (Figure 4). The mean diffusion coefficient remains approximately constant between a short period of increasing ($\Delta t < 1.8$ s) and decreasing diffusion ($\Delta t > 4$ s).

Although the agreement between the diffusivity estimates from particle displacement and from the salt flux (Table 1) is reasonable, it has to be noted that both results depend strongly on the cross-sectional width, over which virtual particles have been released and over which the velocity and concentration fluctuations have been evaluated. Differences between both estimates can result from the unsteady motion of the *Daphnia*, which was integrated by the displacement method along the pathways (space) and by the flux method over time. Therefore both methods were repeated, i.e. the estimation of the diffusion via displacements starting from three additional lines of particle release and the estimation of the flux for four additional cross-sections at different heights $z$, each of width $D$, crossed by the swimming *Daphnia*. We achieved nearly identical results with $1.15 \times 10^{-5}$ m$^2$ s$^{-1}$ for the mean diffusion coefficient computed from the pathways (with $\pm 0.35 \times 10^{-5}$ m$^2$ s$^{-1}$ standard deviation) and $1.64 \times 10^{-5}$ m$^2$ s$^{-1}$ ($\pm 0.6 \times 10^{-5}$ m$^2$ s$^{-1}$) for the mean of the maximal diffusion coefficient from flux estimations. Furthermore, as long as particles have been released along a straight line (representing a plane), which was crossed perpendicularly by the *Daphnia*, every run of computation yielded similar results. The diffusion coefficient varied, however, with the degree of spatial averaging, i.e. with the length of the line along which the particles are released. The mean values of $k_{Daph}$ decrease from $1.15 \times 10^{-5}$ m$^2$ s$^{-1}$ to $0.5 \times 10^{-5}$ m$^2$ s$^{-1}$ if the width of released particles increases from the width of one *Daphnia* $D \approx 2.5$ mm to $10 \cdot D$ (Figure 4). The mean diffusion coefficients from flux estimations averaged across the entire cross-section of width $D$ was again $0.8 \times 10^{-5}$ m$^2$ s$^{-1}$ ($\pm 0.4 \times 10^{-5}$ m$^2$ s$^{-1}$).

All hydrodynamic parameters related to the kinetic energy dissipation and scalar transport affected by the tethered and freely swimming *Daphnia* are summarized in Table 1.

**DISCUSSION**

**Comparison between tethered and freely swimming organisms**

In accordance with scaling arguments and numerical simulations (Dabiri 2010; Leshansky & Pismen 2010; Thiffeault & Childress 2010) our results demonstrate that swimming zooplankton organisms create current velocity and density fluctuations at scales, exceeding the size of the organisms by more than one order of magnitude. Major differences regarding the volume of influence, rates of viscous energy dissipation, total dissipated power, and apparent diffusivities could be observed between tethered and freely swimming organisms (Table 1). These findings clearly demonstrate
the necessity for using freely swimming organisms for quantitative and qualitative analyses of the flow fields and fluid transport generated by swimming zooplankton. The stationary flow field and the opportunity to fix and to align organisms within the field of view of the cameras, however, were the reasons for using tethered zooplankton organisms in the majority of related laboratory studies (Videler et al. 2002; van Duren et al. 2005; Goldthwait et al. 2004). As analyzed for copepods by Catton et al. (2007) in greater detail, the differences to freely swimming organisms can be attributed to an unbalanced force, which the tethered organism can impart on the fluid by pushing against the tether. While Catton et al. (2007) observed differences in viscous energy dissipation of a factor of two to four, we found a more severe impact on diffusion coefficients \( \kappa_{\text{Daph}} \), which is nearly \( 1.0 \times 10^{-4} \text{m}^2 \text{s}^{-1} \) for the tethered and one order of magnitude less for the freely swimming organisms. This difference becomes even more pronounced when the volume of influence (i.e. the volume of water for which the diffusion coefficients were estimated) are taken into account.

**Kinetic energy and dissipation rates**

Dissipation rates of kinetic energy, vertical density fluxes and apparent diffusivities observed in the vicinity of freely swimming *Daphnia* are high in comparison to corresponding values found in wind-driven environmental flows (Wüest & Lorke 2003; Ivey et al. 2008). The rate of energy dissipation, however, is nearly identical to the rate of kinetic energy production estimated by Huntley & Zhou (2004) for schooling animals ranging in size from bacteria to whales. The total energy dissipation within the volume of influence of the freely swimming *Daphnia* is one order of magnitude and in case of the tethered *Daphnia* two orders of magnitude larger than the energy dissipation induced by tethered copepods with a body length of about 1 mm (van Duren et al. 2003). In comparison to another study of freely and tethered copepods (Catton et al. 2007), however, the total dissipated power of the tethered *Daphnia* is in the same order of magnitude. Following Catton et al. (2007), the increased dissipation rate in comparison to that measured by van Duren et al. (2003) may result from increased measurement resolution.

**Effect of density stratification**

The observed flow fields induced by the *Daphnia* can be characterized by Reynolds numbers of \( O \sim 10^1 \) and can be considered to be strictly laminar. Moreover, in contrast to turbulent flows, the spatial scales at which kinetic energy is generated by the antenna motion are smaller than the scales at which energy is dissipated. As demonstrated below, in spite of these facts, the effect of stratification can be assessed with surprisingly close correspondence to scaling arguments derived for stratified turbulence.

The effect of density stratification on the observed flow fields and fluxes can be estimated by comparing the kinetic energy in the animal-induced flow with the potential energy of stratification. For stratified turbulence, such comparison is provided by the Ozmidov length scale \( L_O \) \( (L_O = (\varepsilon/N^3)^{0.5}) \) (Lorke & Wüest 2002), which describes the maximum size of (turbulent) vertical fluid motions, before they become damped by buoyancy forces. Using \( \varepsilon = 8 \times 10^{-7} \text{W kg}^{-1} \) and \( N = 0.07 \text{s}^{-1} \) yields \( L_O = 4.8 \text{cm} \), which is in close agreement with the observed vertical dimension of the steady fluid jet produced by the tethered *Daphnia*. In the case of the freely swimming *Daphnia*, \( L_O \) would exceed 7 cm, however, since the dissipation rate is neither homogeneous nor stationary, the concept underlying \( L_O \) is not directly applicable.

In turbulent flows, the effect of stratification is also assessed using the non-dimensional isotropy parameter (Thorpe 2007) or turbulence intensity parameter (Shih et al. 2005) \( I = \frac{\varepsilon}{\mu N^2} \). For the tethered *Daphnia*, \( I \) is approximately 170 \( (\varepsilon = 1 \times 10^{-8} \text{W kg}^{-1} \text{ and } N = 0.07 \text{s}^{-1} \) and hence slightly below the critical value for isotropy \( (I_{\text{crit}} = 200) \) below which the flow field can be expected to be affected by density stratification. For the freely swimming *Daphnia*, \( I \sim 400 \), but again affected by non-stationarity.

Ardekani & Stocker (2010) showed that the effect of stratification on the flow field generated by an object of size \( a \) is predictable via the Rayleigh number,

\[
Ra = a^4 \left( \frac{\partial p}{\partial z} \right) g \eta / \kappa
\]

where \( \eta = 1.3 \times 10^{-3} \text{kg m}^{-1} \text{s}^{-1} \) and diffusivity of salt.
κ = 1 × 10⁻⁹ m² s⁻¹, the flow field of objects with \( a \geq 613 \mu m \) is affected by our density gradient. The length of the investigated *Daphnia* is almost seven times this length scale. Hence, following Ardekani & Stocker (2010), the flow field and fluid transport generated by the freely swimming *Daphnia* is affected by density stratification and vice versa. This finding, however, is contrary to the experimental results of Gries et al. (1999) who observed that the influence of density gradients smaller than 1 kg m⁻³ has negligible influence on the length of the wake of swimming *Daphnia*.

**Diffusivities around freely swimming *Daphnia***

The apparent diffusion coefficient within the volume of influence is comparable in magnitude to turbulent diffusivities found in the interior of lakes (Wüst & Lorke 2003) or of the ocean (Wunsch & Ferrari 2004). According to Shih et al. (2005), the best estimate of eddy viscosity within the energetic regime (\( I > 100 \)), is \( \kappa = 1.5 \nu (\varepsilon/\nu N^2)^{1/2} \sim 2.5 \times 10^{-5} \text{ m}^2 \text{ s}^{-1} \), which is also in close correspondence to the estimated \( \kappa_{\text{Daph}} \) of the freely swimming *Daphnia*.

In the vicinity of the organism, this diffusion coefficient controls the radius of perception of chemical cues, which are known to affect zooplankton swimming and feeding behavior. Assuming that the freely swimming *Daphnia* motion develops a structure which can be described as a vortex ring (Gries et al. 1999) and the third velocity component in the slice is negligible (\( \nu \sim 0 \text{ mm s}^{-1} \)), the estimated diffusion coefficient would be overestimated. The uncertainty associated with the unresolved velocity component, however, is limited to a factor of 1.5, because

\[ k_{2D}/k_{3D} = 6/4 \cdot \langle r_{u,2}^2 / r_{u,3}^2 \rangle = 1.5 \tag{6} \]

if \( \langle r_{u,2}^2 \rangle = \langle r_{u,3}^2 \rangle \), where \( k_{2D} \) and \( k_{3D} \) denote the estimated diffusion coefficient on basis of displacements from two- and three-dimensional velocity samples (Berg 1993). It seems more reasonable, that \( O(\Delta x^2) \approx O(\Delta y^2) \approx O(\Delta z^2) \), because the swimming motion of the *Daphnia* impeded the development of a typical wake structure. Alternatively, \( O(\Delta x^2) \gg O(\Delta y^2, \Delta z^2) \) could result in an underestimation of the diffusion coefficient. This seems to be implausible in our observations, because in our analyses the swimming direction of the *Daphnia* was mainly within the light sheet. Additionally, the low scatter in the estimated diffusion coefficients as a function of time (Figure 3(b)) provides additional indication for sufficient spatial and temporal velocity resolution for determining fluid transport and diffusion.

**Larger-scale implications**

Applying the universal relationship between body mass and typical packing density of organisms provided by Huntley & Zhou (2004) to a typical mass of adult *Daphnia magna* of \( \approx 0.2 \text{ mg} \) (Porter et al. 1982) yields a packing density of \( 7 \times 10^3 \text{ m}^{-3} \) in animal aggregations. Although this value is in the higher range of observed abundances of *Daphnia* in lakes (e.g. Huber et al. 2011), this value corresponds to more than five individuals within the volume of influence of \( 0.08 \text{ cm}^3 \) observed in our experiments and indicates that the estimated diffusivity of \( 1 \times 10^{-5} \text{ m}^2 \text{ s}^{-1} \) could even be exceeded on scales of animal aggregations. Net hauls over vertical distances of a few meters, however, reveal much lower abundances of adult *Daphnia* in lakes with typical values of \( 10^4 \text{ m}^{-3} \) (Huber et al. 2011), corresponding to volume-averaged diffusivities caused by freely swimming individuals of \( 1 \times 10^{-8} \text{ m}^2 \text{ s}^{-1} \). Although this diffusivity is one order of magnitude smaller than the molecular diffusion coefficients of heat (\( \approx 10^{-7} \text{ m}^2 \text{ s}^{-1} \)), it still exceeds the molecular diffusivity of dissolved substances and gases (\( \approx 10^{-9} \text{ m}^2 \text{ s}^{-1} \)). Vertical mass transport within and below the seasonal thermocline was shown to be close to molecular levels using tracer experiments in small lakes (von Rohden & Ilmberger 2007), or by revealing the existence of double-diffusive transport phenomena (Boehrler et al. 2009; Schmid et al. 2004).

The upscaling of the apparent diffusivity, however, is subject to experimental limitations: First our measurements did not resolve the slow process of gravitational adjustment of swimming-induced density inversions and hence no information about the mixing efficiency, which is required to assess the larger-scale impact of the fluid transport, is available. Secondly, it can be expected that the volume of influence and the apparent diffusion coefficient depend strongly on the swimming mode of the *Daphnia* and
therewith on environmental conditions. The rather typical ‘hop and sink’ motion of *Daphnia* (Gorski & Dodson 1996; Gries et al. 1999), for instance, was not observed in our experiments. Instead of the typical hop and sink motion, the freely swimming *Daphnia* were spinning, which might be a panic reaction to the altered light intensity in the laser light sheet. While these limitations should be addressed in more extensive future experiments, the current results can be used to obtain a first order estimate of the larger scale impact of zooplankton swimming.

**CONCLUSION**

In the present study, we have estimated the apparent diffusivity and dissipation rate of kinetic energy in the wake of a *Daphnia* swimming freely in a density gradient to be in the order of $10^{-5} \text{ m}^2 \text{s}^{-1}$ and $10^{-6} \text{ W kg}^{-1}$, respectively. Our experimental results provide strong indication that stratification affects the swimming performance of small zooplankton, which is in accordance with the results of numerical simulations. The well-defined experimental conditions in a first setup with a tethered *Daphnia* have been used to validate the methods for estimating apparent diffusivities resulting from small-scale and low Reynolds number flows generated by the swimming zooplankton organism. The two independent methods are based on virtual particle tracking in the observed flow field, and on direct flux measurements, respectively, and showed remarkably good agreement in both experiments. Because of the higher degree of temporal averaging, enabled by the stationary flow field in the tethered setup, these diffusivity estimates can be considered as robust and their close agreement thus provides proof of the applicability of both methods and underlying measurements. The diffusivity of the tethered setup, however, is clearly overestimated in comparison to that of the freely swimming case and cannot be considered for transport and mixing processes induced by zooplankton in natural waters. Finally, our results thus indicate that biologically-induced fluid transport by zooplankton might potentially be important for vertical mixing in stratified water bodies at sites and during time periods when background mixing is weak and/or zooplankton abundance is high.

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**REFERENCES**


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