J. Phycol. **44,** 1395–1398 (2008)

DOI: 10.1111/j.1529-8817.2008.00603.x

NOTE

VOLVOX BARBERI, THE FASTEST SWIMMER OF THE VOLVOCALES (CHLOROPHYCEAE)[1]

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Volvox barberi **W. Shaw is a volvocalean green alga composed of biflagellated cells. Vovocales with 16 cells or more form spherical colonies, and their largest members have germ-soma separation (all species in the genus *Volvox*). *V. barberi* is the largest *Volvox* species recorded in terms of cell number (10,000–50,000 cells) and has the highest somatic to reproductive cell ratio (S/R). Since they are negatively buoyant, Volvocales need flagellar beating to avoid sinking and to reach light and nutrients. We measured *V. barberi* swimming speed and total swimming force. *V. barberi* swimming speeds are the highest recorded so far for volvocine algae (~600 $\mu m \cdot s^{-1}$). With this speed, *V. barberi* colonies have the potential to perform daily vertical migrations in the water column at speeds of 2–3 $m \cdot h^{-1}$, consistent with what has been reported about *Volvox* populations in the wild. Moreover, *V. barberi* data fit well in the scaling relationships derived with the other smaller *Volvox* species, namely, that the upward swimming speed $V_{up} \propto N^{0.28}$ and the total swimming force $F_S \propto N^{0.77}$ (N = colony cell number). These allometric relationships have been important supporting evidence for reaching the conclusion that as size increases, colonies have to invest in cell specialization and increase their S/R to increase their motility capabilities to stay afloat and motile.**

Key index words: **hydrodynamics; motility; multicellularity; swimming speeds; Volvocales; *Volvox barberi***

Abbreviations: **D, developmental program; ECM, extracellular matrix; F_S, total swimming force; R, colony radius; *Re*, Reynolds number; S/R ratio, somatic to reproductive cell ratio; SVM, Standard *Volvox* Medium; V_{sed}, sedimentation speed; V_{up}, upward swimming speed; η, viscosity of water**

Volvocalean green algae comprise a monophyletic assemblage of lineages featuring varying degrees of complexity in terms of colony size, colony structure, and cell specialization (Kirk 1998). Volvocales have biflagellated cells; colonies with 16 cells or more form spheroids. They range from the unicellular *Chlamydomonas* to colonies made of 4–64 cells with no cellular differentiation (e.g., *Gonium*, *Pandorina*, and *Eudorina*) to multicellular individuals comprising 1,000–50,000 cells with specialization in reproductive and vegetative functions (germ-soma separation), such as *Volvox* (Koufopanou 1994, Solari et al. 2006a). In the multicellular forms, each of the *Chlamydomonas*-like somatic cells is positioned at the surface of the extracellular matrix (ECM), with its two flagella oriented outward, while the germ cells grow on the inside of the colony. Germ-soma separation characterizes the large members of this lineage, and the number of somatic cells per reproductive cell (S/R ratio) increases with colony size (Koufopanou 1994, Solari et al. 2006a). *Volvox* species with germ-soma separation have evolved several times independently, from quite different ancestors with no cellular differentiation (Coleman 1999, Nozaki et al. 1999, 2006, Nozaki 2003).

Volvox barberi belongs to the section *Volvox* in the Volvocales lineage (Fig. 1; Nozaki et al. 2006). In the smaller Volvocales (unicells and undifferentiated colonies), and in several *Volvox* species, reproductive cells do not undergo binary fission; rather, each reproductive cell grows about 2^n-fold in size and then undergoes a rapid, synchronous series of n divisions (within the mother cell wall). This

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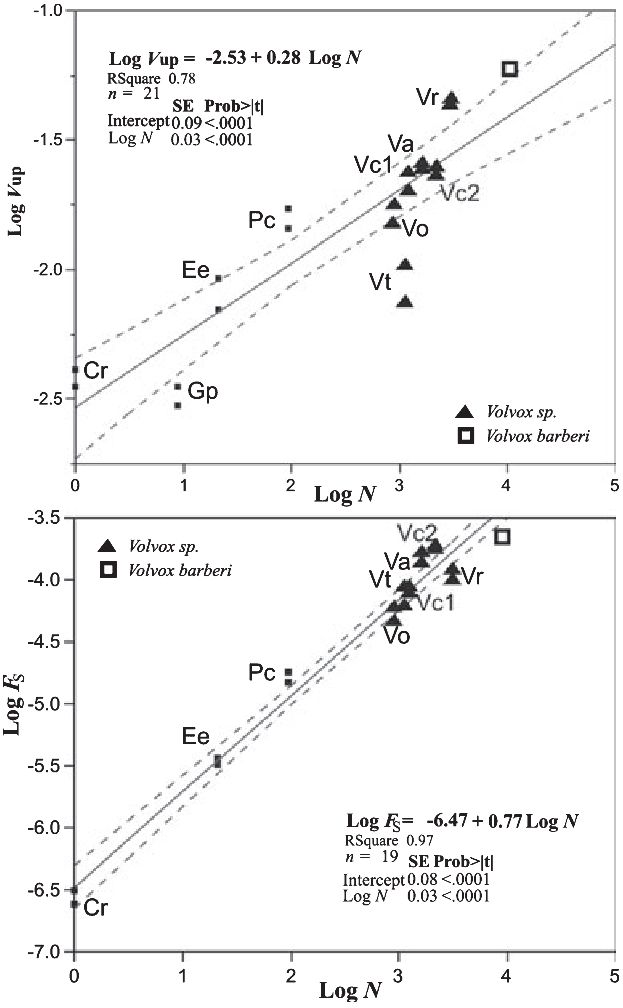

FIG. 1. Allometric analysis of newly hatched colonies: The upward swimming speed (V_{up}) in cm · s^{-1} and total upward swimming force (F_S) in dyne of the colonies as a function of the number of cells (N). *Volvox barberi* data were added to the data published in Solari et al. (2006a). Two measurements are used for the allometric analysis (1 and 3 h after algae having hatched; details in Solari et al. 2006a). *Volvox barberi* was only measured 1 h after algae hatched. Cr: *C. reinhardtii*; Gp: *G. pectorale*; Ee: *E. elegans*; Pc: *P. californica*; Vc1: *V. carteri* grown at 600fc; Vc2: *V. carteri* grown at 1000fc; Vo: *V. obversus*; Vt: *V. tertius*; Va: *V. aureus*; Vr: *V. rousseletii*.

phenomenon is considered the ancestral developmental program in this group (''palintomy''; Developmental Program 1, *D1*; Desnitski 1995). In contrast, in *V. barberi* and other *Volvox* species (e.g., *Volvox aureus*, *Volvox rousseletii*) palintomy is lost; reproductive cells start as small flagellated cells, and during embryonic development, cells grow in between cell divisions (binary fission). This is considered the most derived developmental program (Developmental Program 4, *D4*; Desnitski 1995). Although *D4* reproductive cells start as flagellated cells, their flagella have essentially no motility function since they are reabsorbed before the first cell division. Species in the section *Volvox* retain robust cytoplasmatic bridges that may be involved in the active transfer of nutrients in the adult (Kirk 1998). *V. barberi* is one of the largest (if not the largest) *Volvox* species in terms of cell number (Fig. S1 in the supplementary material). Smith (1944) reported ~50,000 cells; Koufopanou (1994) reported an average of ~6,000 cells.

Volvocales occur in quiet, standing waters of transient vernal puddles or in permanent lakes when thermal stirring stops and the lake becomes stratified (Reynolds 1984, Kirk 1998). Since they are negatively buoyant, these organisms need flagellar beating to avoid sinking and to reach light and nutrients. Recent work shows that in Volvocales the constraints and opportunities of flagellar motility may explain the evolutionary transition from colonies with no cellular differentiation (e.g., *Eudorina*) to larger colonies with germ-soma separation (e.g., *Volvox*). It is hypothesized that the increase in the S/R ratio observed in extant species as colony size increases is due to the need for increased flagellar beating force to keep colonies afloat and motile (Solari et al. 2006a). Moreover, it is argued that the collective flagellar beating of somatic cells in *Volvox* enhances nutrient uptake due to the increased advection generated around the colonies (Short et al. 2006, Solari et al. 2006b). It was observed that *D4* colonies were the fastest swimmers in the volvocine group, especially *Volvox rousseletii*, a species in the section *Volvox* with robust cytoplasmatic bridges, which was recorded swimming at ~500 μm · s^{-1} and had an average of ~3,000 somatic cells (Solari et al. 2006a). Here we report the swimming speed and total swimming force of *V. barberi* colonies. We also investigate how the data of these larger *Volvox* colonies fit in the scaling relationships derived previously with smaller *Volvox* species (Solari et al. 2006a).

V. barberi (Carolina Biological Supply, Burlington, NC, USA) populations were synchronized in Standard *Volvox* Medium (SVM; Kirk and Kirk 1983), 20 mL test tubes with air bubbling, homogeneous cool white light (~140 μmol photons · m^{-2} · s^{-1}), and 16:8 h light (28°C):dark (26°C) cycle. Cell and colony sizes, and the upward swimming speed (V_{up}) and the sedimentation speed (V_{sed}) were measured as detailed in Solari et al. 2006a. Colony trajectories were captured using tracking software (Metamorph, Universal Imaging Corp., Downingtown, PA, USA). Trajectory durations captured for velocity calculations ranged from a minimum of 1 to a maximum of 5 s. Net velocities were used for the analysis.

Volvocales live in a world of Reynolds number Re <1 (Guyon et al. 2001, Solari et al. 2006a). In this ''creeping flow'' or ''Stokes'' regime, motion is dominated by friction, flows are linear, and time is reversible. In this regime, the drag force F on a moving self-propelled sphere can be approximated by the well-known Stokes results $F = 6\pi\eta R\, V_{sed}$ for a solid sphere of radius R (η = viscosity of water).

Within this same framework, the force (F_S) exerted by a colony swimming vertically upward at a specific velocity (V_{up}) balances the sum of the drag force and that of gravity, $F_S = 6\pi\eta R\ (V_{up} + V_{sed})$ (explained in detail in Solari et al. 2006a). By inserting the measured values for V_{up}, V_{sed}, and R into this equation, we can calculate the average upward swimming force of colonies. Table 1 shows *V. barberi* general data, and the specific size, V_{up}, and V_{sed} data of newly hatched synchronized colonies. Clearly, *V. barberi* is the species with the highest number of cells, S/R ratio, and swimming speeds we have recorded, with individuals swimming at velocities of up to 800 $\mu m \cdot s^{-1}$. Moreover, *V. barberi* adds another order of magnitude to the allometric analysis of swimming speed (V_{up}) and force (F_S) as a function of number of cells (N) (Solari et al. 2006a, Fig. 1). *V. barberi* measured data fit well in the scaling relationships previously derived with the other smaller *Volvox* species ($V_{up} \propto N^{0.28}$ and $F \propto N^{0.77}$). Thus, this work lends further support to the conclusions reached previously, namely, that as size increases, colonies have to increase their S/R ratio to increase their motility capabilities to stay afloat and motile (Solari et al. 2006a). Note that higher swimming speeds also correlate with higher advective flows around the colonies, also facilitating nutrient uptake to these larger *Volvox* colonies with higher metabolic demands (Short et al. 2006, Solari et al. 2006b).

It is clear from Figure 1 that species in the section *Volvox* (*V. rousseletii* and *V. barberi*) have higher swimming speeds for their number of cells compared to other *Volvox* species. These species need less flagellar force to counteract drag and gravity and, therefore, can invest more of this force in swimming speed. This phenomenon happens because the colony drag (R) is decreased since the somatic cells are tightly packed in the colony surface due to the retention of cytoplasmic bridges, and because colony mass (i.e., negative gravitational force) is decreased since both somatic and reproductive cells are significantly smaller compared to the other *Volvox* species (Solari et al. 2006a). Another aspect that might contribute to the difference in swimming speeds between the species from the section *Volvox* and the others is that their flagellar apparatuses differ considerably (e.g., *V. rousseletii* vs. *V. carteri*; Hoops 1984). For example, the distance between the two flagella in somatic cells is greater in *V. rousseletii* compared to *V. carteri*; we do not know how these differences influence swimming speeds. We performed multiple additive linear regressions on newly hatched colonies to take into account section *Volvox* species as a nominal factor and found that the exponent of the relation between V_{up} and N becomes 0.24 (SE = 0.03, $P > 0.0001$) and confirmed that section *Volvox* species have on average a significantly higher V_{up} than colonies that do not retain robust cytoplasmatic bridges (estimate = 0.28, SE = 0.11, $P = 0.02$; $n = 21$, $r^2 = 0.84$).

Table 1. Description and data measured for the colonies used in the experiments, grown under the conditions described in the text. Germ cell size for newly hatched colonies is not reported since they are difficult to identify when colonies hatch.

Developmental mode as described by Desnitski (1995)	4
Generation time (h)	48
Hatching time; number of light hours to hatching	4–6
Number of reproductive cells ($n = 10$, SE = 1)	13.5
Number of reproductive cells (median and mode)	14
Number of somatic cells ($n = 10$, SE = 686)	9,919
Somatic to reproductive cell ratio (S/R ratio)	709
Newly hatched colonies	
Colony radius (μm, $n = 10$, SE = 5.8)	169
Somatic cell radius (μm, $n = 10$, SE = 0.2)	2.8
Sedimentation speed V_{sed} ($\mu m \cdot s^{-1}$, $n = 13$, SE = 7.4)	88
Upward swimming speed V_{up} ($\mu m \cdot s^{-1}$, $n = 20$, SE = 32)	611

n, sample size; SE, standard error.

The swimming speeds reached by section *Volvox* species may be an important adaptation to migrate through the water column in search of light and nutrients. Both *V. rousseletii* and *V. barberi* colonies have the potential to perform daily vertical migrations in the water column at speeds of 2–3 $m \cdot h^{-1}$, consistent with what Sommer and Gliwicz (1986) reported about *Volvox* colonies migrating vertically several meters at night in a permanent lake, presumably in search of higher phosphorous concentrations. Even with daughter colonies formed inside ready to hatch, *V. rousseletii* colonies are still able to swim at speeds >300 $\mu m \cdot s^{-1}$, whereas *V. carteri* colonies with daughter colonies inside are not able to swim at all (Solari et al. 2006a).

We thank two anonymous reviewers for comments and improvements on the manuscript. This work was supported in part by NSF grants DEB-0075296 and PHY-0551742 and the Department of Ecology and Evolutionary Biology, University of Arizona. This work was supported in part by the Engineering and Biological Systems program of the BBSRC.

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Supplementary Material

The following supplementary material is available for this article:

Figure S1. (A) *Volvox barberi* daughter colonies hatching from the mother colony. These are ~10,000 cells per colony. Note the high cell density; all the cells retain robust cytoplasmatic bridges. Since the germ cells start as small flagellated cells, they are difficult to differentiate from the somatic cells at this stage (arrows point at germ cells). (B) A colony 1 d after hatching with the daughter colonies developing inside.

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