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# Polychaete Burrowing Behavior in Sand and Mud

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**POLYCHAETE BURROWING BEHAVIOR IN SAND AND MUD**

By

Kevin Terrence Du Clos

B.S. University of California at San Diego, 2007

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Oceanography)

The University of Maine

December, 2012

Advisory Committee:

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## THESIS ACCEPTANCE STATEMENT

On behalf of the Graduate Committee for Kevin Terrence Du Clos I affirm that this manuscript is the final and accepted thesis. Signatures of all committee members are on file with the Graduate School at the University of Maine, 42 Stodder Hall, Orono, Maine.

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## **ACKNOWLEDGEMENTS**

The research presented in this thesis was supported by NSF grant number OCE 0851172 to Peter Jumars and Sara Lindsay.

I would like to thank my committee for providing guidance in planning my experiments and helped me to refine my thesis. I received help from many people in the Marine Science department in Orono, at the Darling Center, and at the CCAR. I had help in the lab from Alex Borsky. I would also like to thank my family and my girlfriend, Brianne, for providing moral support.

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# **POLYCHAETE BURROWING BEHAVIOR IN SAND AND MUD**

By Kevin Terrence Du Clos

Thesis Co-Advisor: Dr. Peter A. Jumars

Thesis Co-Advisor: Dr. Sara M. Lindsay

An Abstract of the Thesis Presented  
in Partial Fulfillment of the Requirements for the  
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December, 2012

Marine sediments are continually reworked by resident organisms that control the ecology, chemistry, and physical structure of these vast systems. For example, the creation of a burrow brings oxygenated water into contact with anoxic sediment, facilitating aerobic respiration and supporting a distinct population of bacteria and meiofauna. Collectively, the effects of infauna on sediments and pore waters are known as bioturbation. Studying the behavior organisms that live beneath the sediment surface (infauna) is crucial to understanding the effects of bioturbation. Infauna can be difficult to study, however, because much their activity cannot be directly observed. The purpose of this thesis is to present the results from a set of experiments conducted to better understand aspects of polychaete burrowing behavior and the small-scale distribution of this behavior, particularly with respect to rigid boundaries (walls).

Chapter 1 focuses on the behavior of two polychaetes, *Allita virens* and *Clymenella torquata* near a rigid wall in sand and mud. The more mobile *A. virens* shows a tendency to burrow near the wall in mud but not in sand. The proposed basis for this difference is the distinct material properties of the two sediment types. Burrowing in mud

occurs by the propagation of cracks. These cracks, and hence burrows, tend to propagate along the surface of walls. In sand, force chains are collections of particles that experience much more stress than the surrounding particles. Stress chains tend to terminate at walls where there high density may inhibit *A. virens* from burrowing. In contrast to *A. virens*, *C. torquata* does not show a significant difference in distance from the wall in sand vs. mud. Because of its limited mobility, *C. torquata* may be less likely to encounter a wall.

In Chapter 2, I report observation of the burrowing behavior of *A. virens* made using particle image velocimetry (PIV). PIV has seen very limited use in studying burrowing behavior, and the method used for this study improves upon past methods by eliminating the need to make observations across a rigid glass wall. I present several calculations made using this method, including the burrowing speed of *A. virens* and the distance of its head from the wall of its enclosure. In addition, I quantify *A. virens*'s effect on the sediment surface including the radial extent of this effect, which could be useful in interpreting the sedimentary record. This method could also be a valuable tool in studying the behavior of a broad range of infaunal organisms.



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## **Chapter 1**

### **THE WALL EFFECT IN MUD AND SAND**

#### **1.1. Introduction**

The term bioturbation refers to the effects of infaunal animals (those that live in sediment) on the sediments in which they live and on the pore water contained within these sediments. Kristensen et al. (2012) define bioturbation as “all transport processes carried out by animals that directly or indirectly affect sediment matrices.” The behaviors that lead to these transport processes include crawling, burrowing, and ventilation. The consequences of bioturbation are myriad. They include: ecological effects, reviewed by Meysman et al. (2006); chemical effects, including increasing the pool of reactive organic matter (Kristensen 2000); and, geological effects, both at the scale of the individual organisms (millimeters to centimeters) and on much larger (meters to kilometers) scales (Murray et al. 2002). The action of burrowing organisms is also a major determinant of the fate of pollutants in coastal environments (Gilbert et al. 1994; Sherwood et al. 2002).

This study focuses on the formation and maintenance of burrows and tubes by polychaete worms in marine sediments. As a first approximation regarding chemical effects, the walls of a burrow are often considered an extension of the sediment-water interface because they increase the surface area available for the diffusion of oxygen, facilitating aerobic respiration (Volkenborn et al. 2007). Without a burrow, diffusion is essentially a one-dimensional process, which occurs vertically across the sediment surface. A burrow allows for diffusion in three dimensions (Aller 1980). The area surrounding a burrow is a unique environment, however, and areas adjacent to burrows provide microniches that support bacterial (Bertics et al. 2010) and meiofaunal (Dittmann

1996) populations distinct from those at both the oxic sediment-water interface and the deeper anoxic sediment.

The subjects of this study, *Allita virens* and *Clymenella torquata*, are representatives of two widely distributed polychaete families, Nereididae and Maldanidae, respectively. Both are commonly studied both in the laboratory and the field. *A. virens* is an omnivorous polychaete (Fauchald and Jumars 1979) commonly found in intertidal mud flats (Larsen 1991) and sand beaches in the Gulf of Maine (Larsen and Doggett 1990), the North Sea (Costello et al. 2001) and elsewhere. *A. virens* builds U-shaped burrows to a depth of about 20 cm that it expands over the course of a few days and maintains as part of a “semi-sessile lifestyle” (Fauchald and Jumars 1979; Miron et al. 1991), generally remaining in its burrow at high tide and emerging at low tide to scavenge for food (Esselink and Zwarts 1989 and personal observations). *C. torquata* is a tube-dwelling, head-down deposit feeder (Fauchald and Jumars 1979) commonly found in dense aggregations in mud and sandy mud. *C. torquata* is sessile, generally maintaining its tube in one place. However, if displaced from its tube, it rapidly reburies and forms a new tube. *C. torquata* also often forms branches at the head end of its tubes and may be able to travel longer distances through tube extension (Fauchald and Jumars 1979).

In laboratory cultures, infaunal organisms often behave differently in enclosures filled with mud than they do in those filled with sand. When *A. virens* is kept in a mud-filled aquarium, it often burrows at the edge of the aquarium, with its U-shaped burrow visible through the aquarium wall (Fig. 1.1). If *A. virens* is kept in the same aquarium filled with sand, however, the worm is generally not seen at the wall. The same pattern of

**Figure 1.1.** *Allita viren*'s burrow at the edge of a transparent aquarium.  
The U-shaped burrow of *Allita virens* as seen through the wall of a cylindrical aquarium filled with mud. Note the oxic sediment, which is lighter than the surrounding sediment, along the length of the burrow.



high concentrations of burrows near walls in mud but not in sand has frequently been observed for various infauna (Jumars unpublished observations). Prior to this study however, the effect of sediment type on infaunal behavior in the presence of a rigid wall, hereafter referred to as the wall effect, had not been tested systematically.

Dorgan et al. (2006) described differences in the material properties of mud and sand that could be used to explain the wall effect. Fractures in mud tend to travel along interfaces, so it may be easier for *A. virens* to burrow at a wall where a “preformed crack” forms as mud separates from the wall in advance of a burrowing organism (Dorgan 2007). Sand is a granular medium, i.e. the bulk properties of sand are determined by the interactions of individual particles. One example of the importance of grains in the material properties of sand is the formation of force chains, which are a network of particles that resist the majority of the force applied to the sediment column (Geng et al. 2008). The high density of force chains at walls, where many force chains terminate, may prevent infaunal organisms from burrowing near a wall in sand.

In this chapter, I test the hypothesis that *A. virens* burrows at rigid boundaries (walls) more often than would be expected by chance in mud but not in sand and to test for the same effect with *C. torquata* whose sessile lifestyle may render the differences in sediment type less important. *C. torquata* often burrows wherever it first lands and rarely relocates its tube unless displaced from the sediment, so differences in the material properties of the sediment in which it burrows may be expected to be relatively unimportant in determining its distribution.

Results from experimental methods that rely on measurements made at the surface of a rigid plate, such as sediment profile imaging or chemical assay using planar

optodes, may be biased because of the wall effect. In addition, if the wall effect were found to extend to the field as well as the laboratory, it would be necessary to refine current ideas about the spatial distribution of infauna. Boulders would be potential hotspots for infauna and thus for bioturbation.

## **1.2. Methods**

*A. virens* were obtained either by collection from the Lowes Cove intertidal mudflat at the Darling Marine Center in Walpole, ME, from the stock maintained at the Center for Cooperative Research and Aquaculture in Franklin, ME, or from commercial sources. The blotted wet weight of *A. virens* at the beginning of the experiments was  $3.9 \pm 0.3$  g (mean  $\pm$  s.e.m.;  $n = 28$ ). All worms used had intact pygidia bearing anal cirri. The pygidium was identified at the end of each experiment to ensure recovery of the entire worm. The weights of worms used for sand and mud experiments did not differ significantly based on a *t*-test ( $p = 0.12$ ). *C. torquata* were collected from an intertidal mudflat near Lubec, Maine. As for *A. virens*, all *C. torquata* had intact pygidia before and after experiments. The blotted wet weight for *C. torquata* at the beginning of the experiments was  $145 \pm 14$  mg (mean  $\pm$  s.e.m.;  $n = 27$ ). The weights of worms for sand and mud experiments were not significantly different based on a *t*-test ( $p = 0.17$ ). All experiments used a single worm per enclosure to avoid complications from inter-individual interactions.

Mud was collected during low tide from the intertidal mud flat in Lowes Cove at the Darling Marine Center in Walpole, Maine. Before use in experiments, mud was homogenized with a paint mixer mounted to an electric drill and sieved through a 2 mm mesh to remove larger solids. The sand used for experiments with *A. virens* was Quikrete



“Play Sand”. Before use, the sand was rinsed with a high volume of fresh water to remove fine particles. *C. torquata* would not burrow in pure sand, likely because the low organic content of the sand is unsuitable for deposit-feeding. To create a sediment in which *C. torquata* would burrow, the sand was supplemented by adding approximately 10% Lowes Cove mud by volume.

Stocks of both worms were maintained in a recirculating seawater system maintained at 16-19°C with a salinity of 32-37 Sp. The same conditions were used for the burrow opening and worm location experiments described below

### **1.2.1. Burrow-Opening Experiments**

A series of experiments was performed in which the distance from the wall of each burrow opening formed by *A. virens* or *C. torquata* was measured. For *A. virens*, a 20.3 cm i.d. was filled with either sand or mud to approximately 15 cm for each worm. For *C. torquata*, a 10.2 cm i.d. pipe was filled to approximately 10 cm for each worm. All pipes were then incubated in recirculating seawater for at least 12 h to allow the sediment to settle and the water at the top of the pipe to clear.

After the addition of one *A. virens* or *C. torquata*, each pipe was incubated in recirculating seawater. A previous study of burrowing behavior notes an “exploration and construction” phase of about 3 days in the process of burrow formation by *A. virens*, after which the burrow morphology stabilizes (Miron et al. 1991). Thus, experiments with *A. virens* were incubated for 7 d to avoid sampling during this period of rapid change. Similarly, experiments with *C. torquata* were incubated for 7 d for consistency. The incubation period was sufficient to allow *C. torquata* to build a tube.

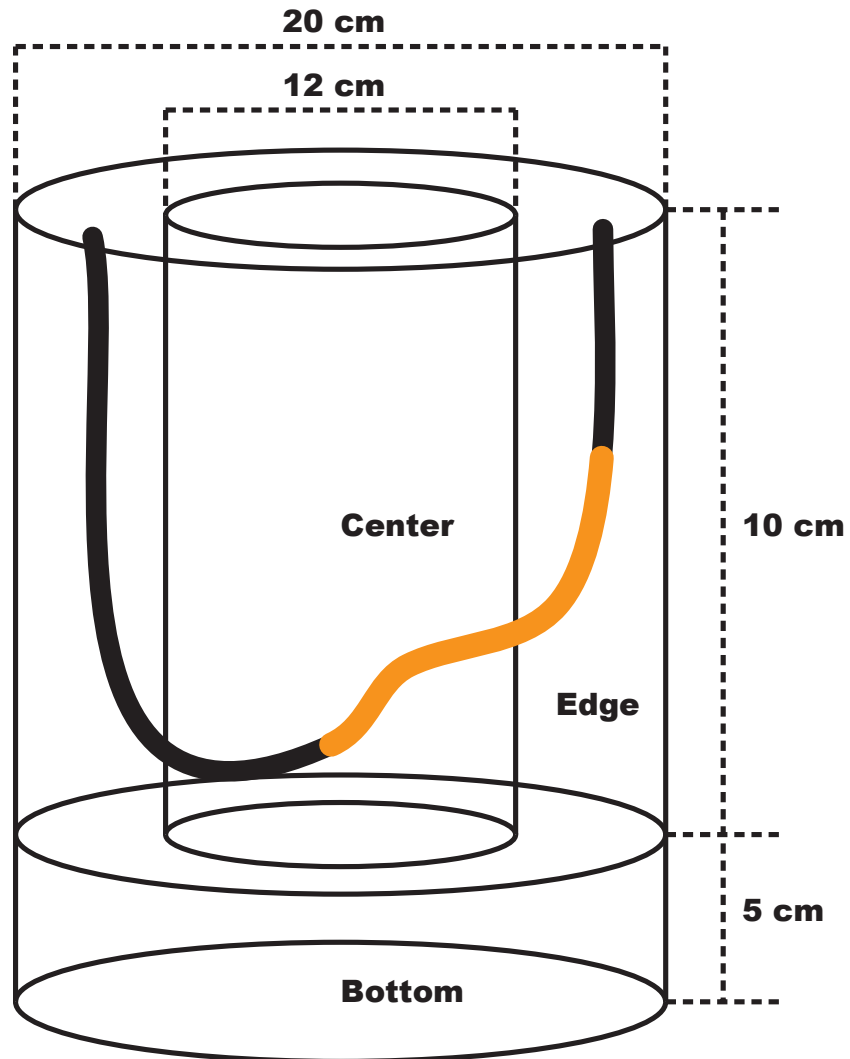
At the end of the incubation period, the surface of mud in each pipe was photographed. Photographs were used to measure the distance from the closest wall to each burrow opening (to the nearest millimeter) using Adobe Photoshop CS5 Extended. Non-parametric statistical methods were used for distance from the wall because the results were not expected to follow a normal distribution. A one-tailed Mann-Whitney (MW) test was performed to compare the median distance from the pipe wall for mud and sand. A significance level of 0.05 was used for this and all subsequent statistical tests. Another one-tailed MW test was performed to compare the median number of burrow openings per pipe for sand and mud.

To compare burrow openings distances between species, the distances were first normalized by dividing by the radius of the pipe. The medians of the normalized values were compared using a Kruskal-Wallis test followed by Dunn's multiple comparison tests between *A. virens* and *C. torquata* for both mud and sand.

### **1.2.2. Worm Location Experiments**

Experiments were then performed to determine the location with respect to the wall, of the worm itself. In the case of *C. torquata*, at the termination of each of the burrow opening experiments (Though *C. torquata* worm locations were sampled from the same experimental pipes used for the burrow location experiments described above, I will refer to worm location "experiments" for convenience.), after removing the water at the top of the pipe with a siphon, sediment in the pipe was divided into inner and outer volumes by pressing a 6.6 cm diameter cylinder into the center of the sediment to the bottom of the pipe. The location (edge or center) of the worm was then recorded. In the case that a worm was cut during sampling and found in multiple sections, the worm's

**Figure 1.2** Sampling scheme for worm-location experiments with *Allita virens*.  
The sampling scheme used for the worm-location experiment with *A. virens*, illustrating the dimensions for each section sampled.



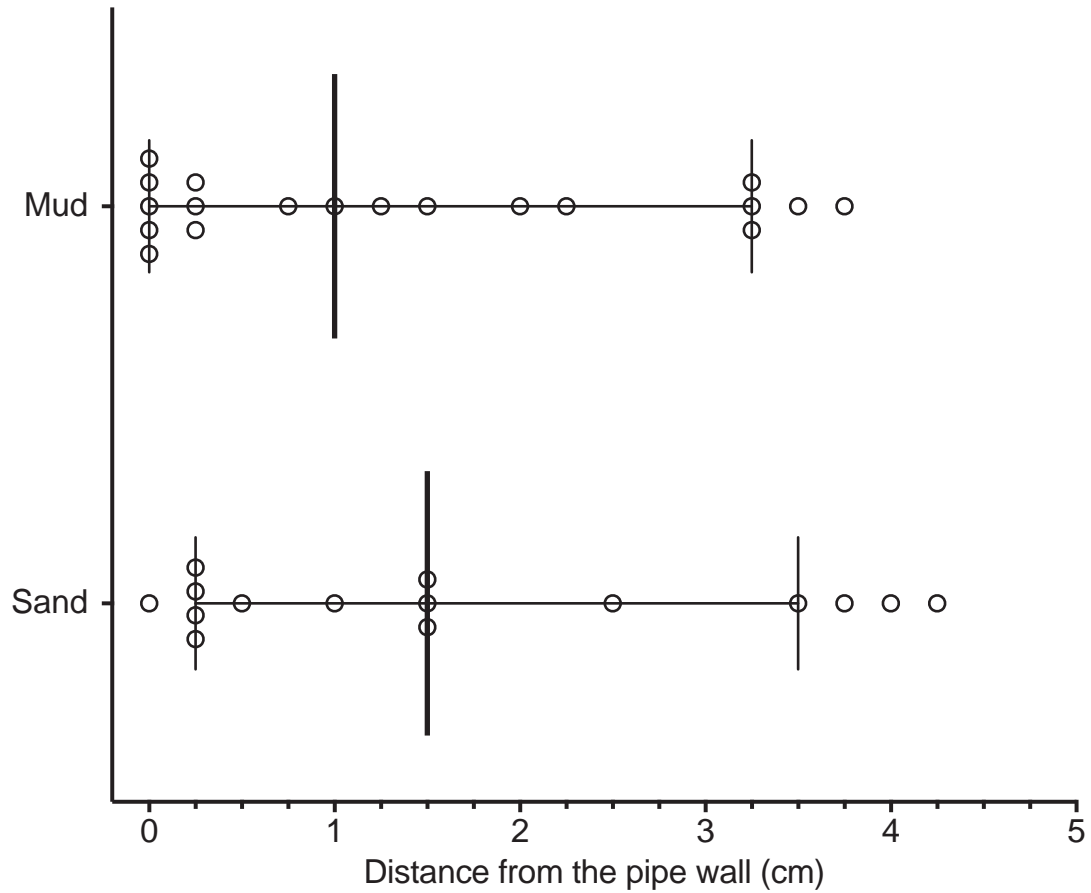
position was scored according to the location of the larger of the two sections (by weight). Assuming the wall has no effect on the distribution of worms in the sediment (the null hypothesis), the likelihood of finding a worm in one of the two sections should be proportional to the area of that section. Thus, a binomial test was performed using the ratio of the area of the outer section to that of both sections combined (0.58) as the expected probability of finding a worm in either section based on chance alone.

I noted during many of the burrow-opening experiments in mud described above that upon reaching the bottom of its container *A. virens* tended to continue to burrow along the bottom of the container, as would be expected if cracks follow a rigid wall. Therefore, the bottom of the pipe was considered as an extension of the wall in determining the location of *A. virens* within the sediment. Pipes (i.d. 20.3 cm), split at 5 cm from the bottom, were used to test whether a worm was at the wall, the center, or the bottom of the pipe (Fig. 1.2). The split pipe sections were sealed with duct tape and band clamps. Pipes were then filled to 15 cm with mud and incubated in flowing seawater with one *A. virens* each. After 7 d, the pipes were sampled as follows. First, the band clamps and duct tape were removed and a metal divider was slid between the split sections of the pipe. Then, with the metal divider still in place, a 12 cm diameter cylinder was pressed down into the center of the pipe. Finally, the pipe was removed, and the position of the worm (bottom, wall, or center) was noted. As described above, the position of a worm found in multiple sections was ascribed to that section in which the largest section was found. Again, a binomial test was performed using the expected probability of finding a worm in the wall or bottom section vs. the entire pipe. In this case, the expected probability was found by dividing the volume of the combined wall and bottom sections

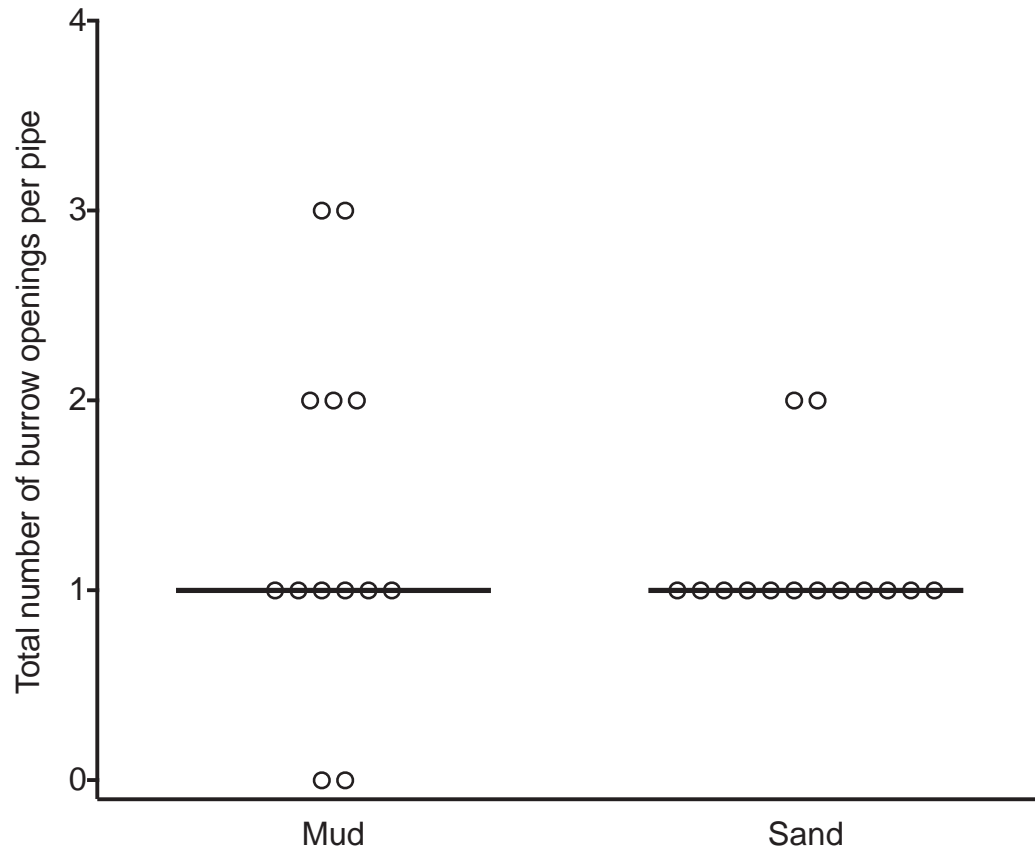




**Figure 1.5.** Burrow-opening wall distances for *Clymenella torquata*. The number of burrow openings per pipe for *C. torquata* in mud and sand. Median and interquartile range are indicated for each sediment type. The difference between groups is not significant ( $p = 0.19$ ) based on a Mann-Whitney test.

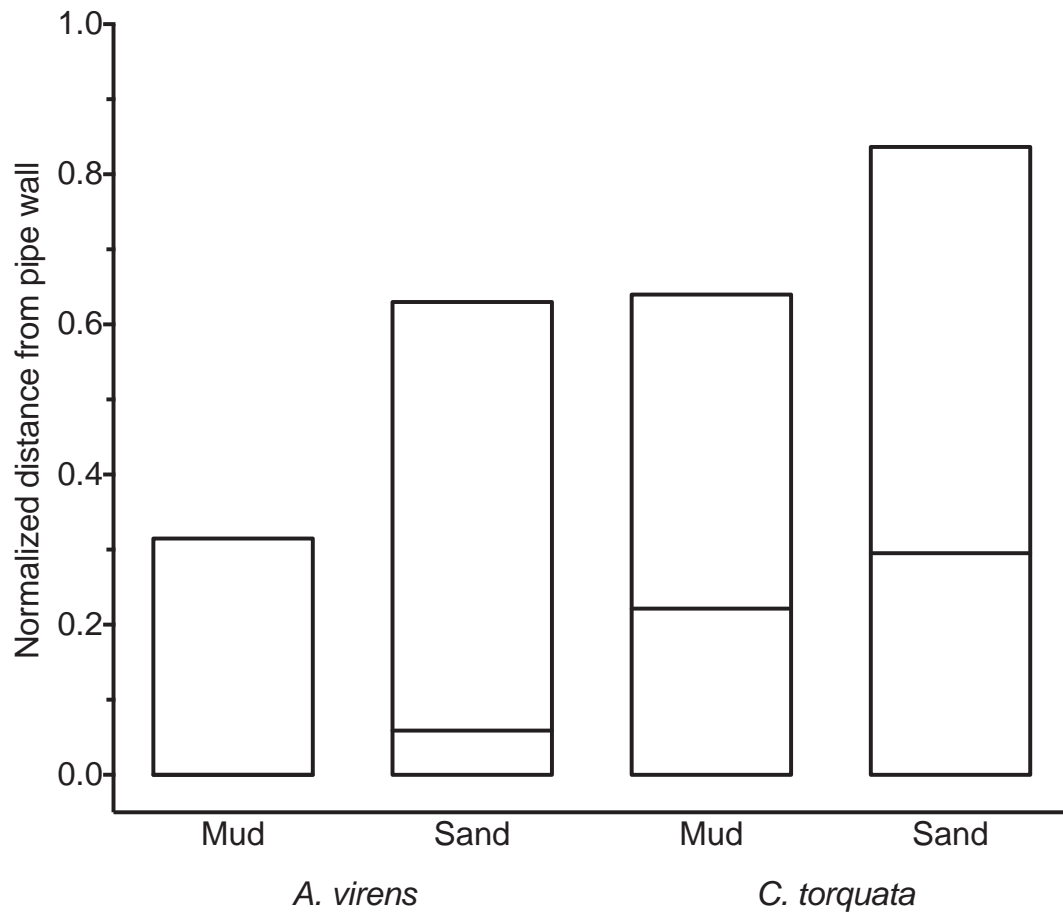


**Figure 1.6.** Number of burrow openings per pipe for *Clymenella torquata*. The median number of burrow openings per pipe for *C. torquata* in mud and sand. The interquartile range for each groups is too narrow to display on the plot. The difference between groups is not significant ( $p = 0.37$ ) based on a Mann-Whitney test.



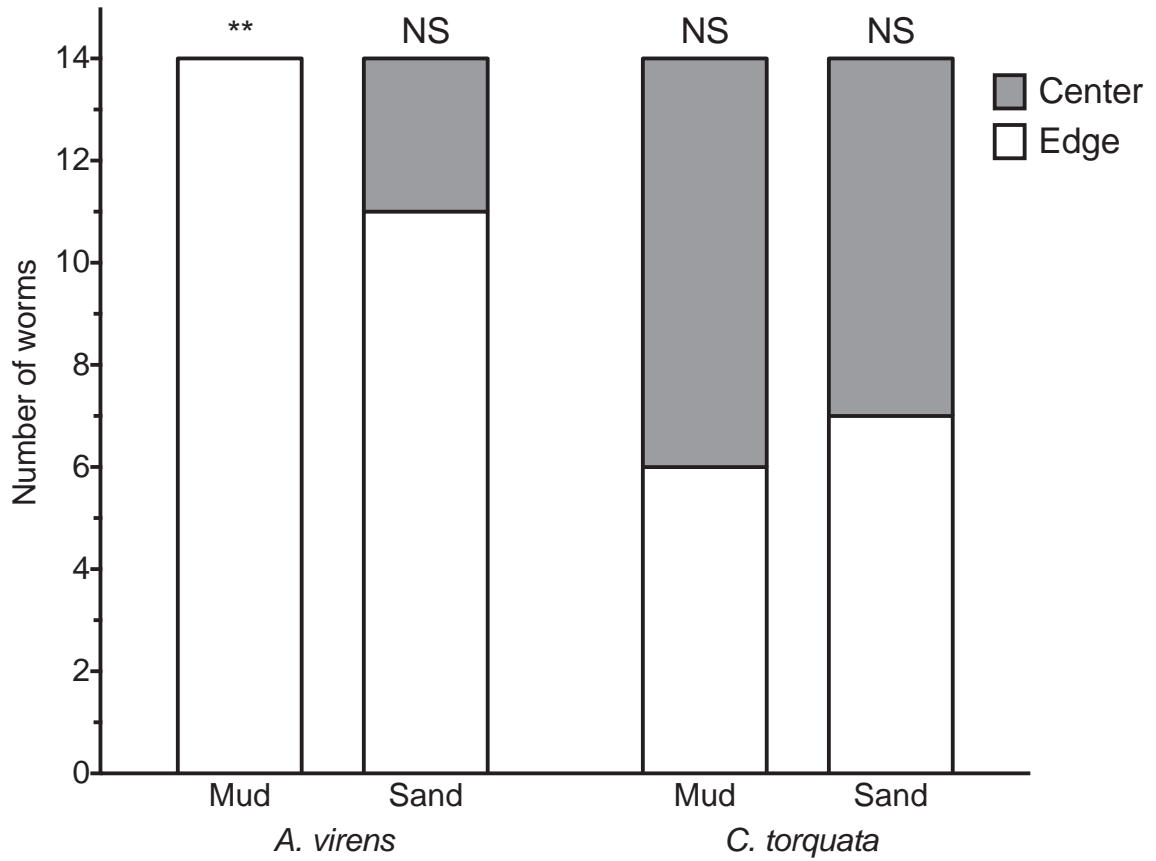


**Figure 1.7.** Normalized wall distances for both species and sediment types. The range and median distance of burrow openings from the wall normalized to the radius of the pipe for both species and sediment types. A value of 0 represents the wall of the pipe, whereas a value of 1 represents the center of the pipe.



**Figure 1.8.** Worm locations for both species and sediment types.

The number of experiments in which a worm was found at the edge or center for each species and sediment type. The ratio of worms found in edge vs. center sections was significantly different from the expected probability only for *Allita virens* in mud ( $p = 0.0074$ ) based on a binomial test.



by the volume of all sections combined (0.67).

### **1.3. Results**

#### **1.3.1. Burrow Opening Experiments**

For the 12 mud-filled pipes in the burrow opening experiments with *A. virens*, a total of 29 burrow openings were counted with a median distance from the wall of 0.0 cm. For the 12 sand-filled pipes, 22 burrow openings were counted with a median distance from the wall of 0.6 cm. The difference between these distances is significant ( $p = 0.0043$ ) based on a MW test (Fig. 1.3). The median total number of burrow openings per pipe was higher for mud (2.0) than sand (1.5). This difference is significant ( $p = 0.026$ ) based on a MW test (Fig. 1.4).

For *C. torquata*, the median distance from the wall for 18 burrow openings in the experiments with mud ( $n = 14$ ) was 1.0 cm. The median distance for the experiments for 16 burrow openings in the burrow openings in the experiments with sand ( $n = 14$ ) was 1.5 cm. These distances are not significantly different ( $p = 0.19$ ) based on a MW test (Fig. 1.5). The median number of burrow openings per pipe was 1.0 for both mud and sand (Fig. 1.6).

The Kruskal-Wallis test indicates significant differences between the four groups (*A. virens* in mud, *A. virens* in sand, *C. torquata* in mud, and *C. torquata* in sand) in distances from the wall (normalized to pipe radius) of burrow openings ( $p = 0.0009$ ) (Fig. 1.7). The differences between species are significantly different for mud but not sand based on a Dunn's multiple comparison test.

### 1.3.2. Worm Location Experiments

For the experiments with *A. virens* in mud, the worm was found in the wall or bottom sections in all 14 enclosures. These results are significantly different from the expected probability (the ratio of the area of the wall and bottom sections to that of all sections combined) of 0.67 ( $p = 0.0074$ ). For sand, the worm was found in the center section in 3 out of 14 enclosures. This result is not significantly different from the expected probability ( $p = 0.57$ ).

In the experiments with *C. torquata* in mud, the worm was in the edge section in 6 out of 14 enclosures. This result is not significantly different from the expected probability of 0.58 (the ratio of the area of the edge section to that of all sections combined) ( $p = 0.29$ ). In experiments in sand, the worm was found in the edge section in 7 out of 14 enclosures. This result is not significantly different from the expected probability ( $p = 0.59$ ). Worm location data for both sediment types and species are plotted in Figure 1.8.

### 1.4. Discussion

In both the burrow-opening and worm location experiments, *A. virens* showed a tendency to burrow near rigid walls in mud. However, *A. virens* did not show the same tendency in either experiment in sand. The difference between *A. virens*' behavior in mud and sand might be attributed, at least in part, to the distinct mechanical properties of the two sediment types.

Although mud and sand are superficially similar materials, there are important mechanical differences between the two. A worm burrows in mud by forming a crack, which continues in advance of the worm's head (Dorgan et al. 2005; Dorgan et al. 2007).

**Figure 1.9.** Forming a crack in mud at a distance from a wall vs. at one.

A schematic showing the location of a crack in mud in half-pipe sections (left) and in detail (right) at a distance from a wall vs. at one. To burrow at a distance from a pipe wall (a), an organism must break the cohesive-adhesive bonds between mud grains. To burrow at the pipe wall (b), an organism must break the adhesive bonds between the mud grains and the wall.

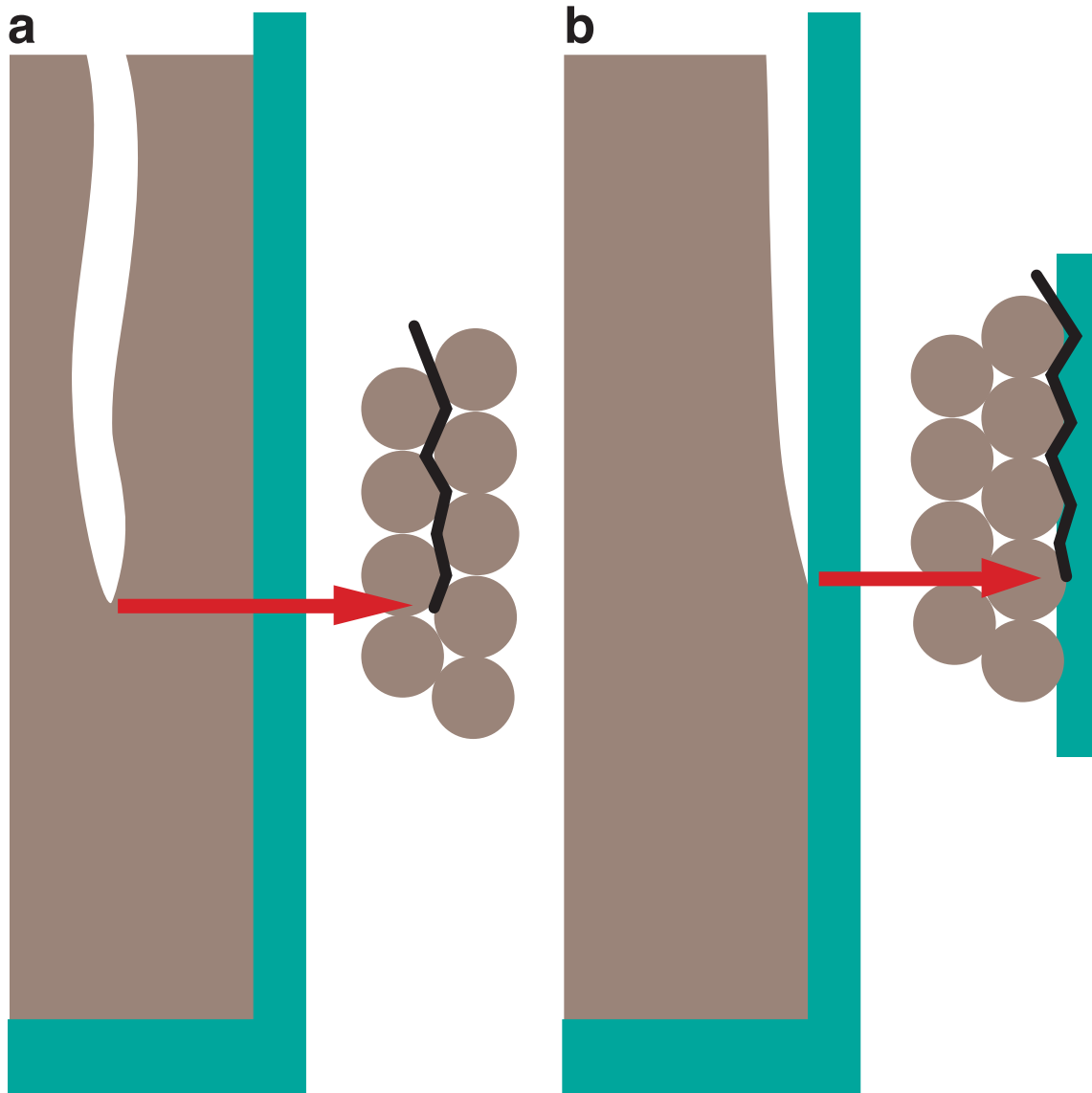


Figure 1.9 depicts the basic mechanics involved in burrowing at a wall vs. farther away from a wall in mud. In mud, to burrow at a distance from a wall, an organism must break the cohesive-adhesive bonds between grains (fig. 1.9a). The cohesive-adhesive forces are stronger in mud than in sand because the smaller particle size of mud leads to larger contact area between grains per unit volume. However, to burrow at a wall an organism overcomes the relatively weaker adhesive forces between grains and the wall (fig. 1.9b). Because burrowing at a wall requires less force than burrowing away from a wall, once a burrowing organism encounters a wall it is likely that it will burrow “downhill” by staying at the wall rather than exerting the greater force required to move away from the wall. In the crack-propagation model of burrowing in mud, a crack forms at the wall in advance of a burrowing organisms (Dorgan 2007; Dorgan et al. 2007). A burrowing organism is likely to follow the existing crack rather than exerting more force to form a crack away from the wall.

Unlike mud, sand is a granular material in which individual particles interact to determine bulk properties of the material. In granular materials, the matrix of the grains resists applied forces. Within this matrix, a subset of grains that make up “force chains” resist a disproportionate fraction of the total load. Force chains are commonly described as any assemblage of three or more particles, each of which exceeds a certain pressure threshold (often the average pressure of the grain assemblage) that approximates a line (e.g., forms an angle larger than  $150^\circ$ ) (Pöschel and Schwager 2005). In experiments and model runs in closed containers, force chains often terminate at the walls of containers, e.g., Geng et al. (2008) in a process known as jamming (e.g., Corwin et al. 2005, Albert et al. 2000). A burrowing organism would, therefore, likely need to break or reorient many

force chains to burrow near the wall of a container. The number of chains that the worm would need to break would increase as the worm descends into deeper sediment, as the concentration of force chains increases with depth because the deeper particles support the weight of overlying particles (Geng et al. 2008). *A. virens* is often observed starting its burrow at a much shallower angle (with respect to the sediment surface) in sand than in mud (personal observation). This behavior may be a method by which a worm can avoid the need to break the force chains present below the surface. Similarly, once the worm has burrowed, it might have difficulty approaching walls due to the jamming that results from the presence of a rigid structure. Based on the results of the burrow-opening experiments, this distance is likely to be less than one centimeter.

These results clarify the burrowing behaviors of *A. virens* observed at the sediment-water interface. In both sand and mud, *A. virens* tends to begin to burrow near the wall of an enclosure, which helps to explain why the burrow openings for both sediment types clustered near the pipe wall. The worm may be able to gain leverage from the wall, making it easier to break the surface. Alternatively or in addition, the worm may simply be following a natural instinct in response to containment. When placed in a round enclosure, such as the pipes used for this study, *A. virens* often crawls around the perimeter of the enclosure several times before beginning its burrow. *A. virens*'s initial burrowing behavior is qualitatively different in mud and sand. When beginning to burrow in mud, the worm burrows nearly perpendicularly to the sediment surface. In sand, the worm burrows at a shallower angle and shovels with its head, loosening the top grains, rather than by crack-propagation (Dorgan et al. 2007). Its behavior in sand may allow the worm to avoid the high concentration of force chains at the wall.

**Figure 1.10.** Burrowing in mud vs. sand.

*Allita virens*'s burrows in mud and sand columns formed in 20.3-cm i.d. pipes, which have been removed. In mud (a), the U-shaped burrow is visible around the perimeter of the column. In sand (b), the worm has started its burrow near the edge of the column and redirected it toward the center of the pipe after reaching about 2 cm deep. Part of the top of the burrow has been dissected away. The direction of the burrow is from left to right.





The divergence in behavior observed at the sediment surface appears to extend to the sediment column below as demonstrated by the results of the worm location experiments. After beginning to burrow at the wall of its enclosure in mud, *A. virens* continues to burrow along the wall, forming a U-shaped burrow that traces the wall's form, as seen in Figure 1.10a. In sand, *A. virens* burrows for a short time near the wall but quickly diverts its burrow away from the wall, probably because of higher densities of force chains at the wall. Figure 1.10b shows a burrow formed by *A. virens* in a pipe filled with sand. The pipe has been removed and the top of part of the burrow has been dissected away to show the form of the burrow near the surface of the sediment column. The burrow begins near the edge of the sediment column but redirects toward the center of the pipe near about 2 cm deep. This commonly observed pattern may result from *A. virens*'s response to the vertical gradient of force chains in the sediment column. While *A. virens* can readily burrow near a wall at the surface, force chains prevent it from doing so at depth. The results from the burrow-opening experiments suggest that the worm may often continue its burrow at about 1 cm from the wall where the force chains are less dense. In other cases, the worm may burrow farther from the wall, although this behavior appears to be less common based on the lower frequency of burrow openings found farther from the pipe wall. The burrowing behavior of *A. virens* in sand, including the distance from the wall at which it burrows, will be further discussed in Chapter 2.

*C. torquata*, unlike *A. virens*, showed no bias for burrowing near the wall in mud or sand. This difference can most easily be explained by the contrasting lifestyles of the two organisms. *C. torquata*, which is not as adept as *A. virens* at crawling on the sediment surface, usually builds its burrow near to where it initially lands (personal observation),

whereas *A. virens* generally crawls along the surface before beginning to burrow, often encountering a wall in the process. In addition, once *C. torquata* has burrowed and formed its tube, it may not move enough to encounter the wall of the aquarium over the course of the experiment. Though it sometimes forms subsurface branches off the primary tube shaft, the tube generally maintains its overall position. The presence of the tube itself may also play an undetermined role in the behavior of *C. torquata* at the wall of the enclosure. The tubes of some polychaetes (e.g. many Terebellids) are often found attached to surfaces, such as boulders, but this was not observed in the present study.

Further work will be needed to determine whether the wall effect extends to the field as well as the laboratory. Several studies have examined differences in invertebrate assemblages based on proximity to boulders. These studies cite differences in organic matter concentration (Motta et al. 2003) and fluid dynamics (Cusson and Bourget 1997) as possible explanations for observed differences. However, neither of these studies was performed on a sufficiently fine spatial scale to detect a wall bias that is likely to operate on the scale of centimeters at most. Past laboratory bioturbation studies using tracers may have missed the wall effect because of their tendency to average horizontally by slicing cores vertically and homogenizing each slice before quantifying tracers. In the future, subsampling cores could provide further information on the small-scale spatial distribution of particle displacement.

Detecting the wall effect in the field and relating the wall effect to bioturbation rates will probably require experiments designed specifically to account for small-scale spatial variation and possibly special techniques for infaunal sampling at the surface of boulders and other obstructions. It should also be noted that adhesion of mud to natural,

rough stone may be far greater than to smooth, man-made surfaces. Results presented in this chapter suggest that mud measurements that rely on proximity to a rigid wall may be biased. At least one study that used sediment profile imaging (SPI) has acknowledged that the effect of the wall may have yielded elevated measures of bioturbation compared to sediment unaffected by the presence of a wall: "The burrow formation rate, observed in this study, may be a liberal estimate since burrow formation at the sediment-camera-faceplate interface is facilitated along the interface." (Sturdivant et al. 2012). Comparing organism densities taken from SPI experiments to those from traditional sampling methods, such as core sampling, may provide a method for testing for the wall effect in the field and could help calibrate results from sediment profile imaging, making the method more widely applicable.

## Chapter 2

### VISUALIZING BURROWING WITH PARTICLE IMAGE VELOCIMETRY

#### 2.1. Introduction

Creative methods are needed to study the behavior of infauna (organisms that live below the sediment surface) because it is not possible to directly observe a burrowing organism in situ. Inferences are often drawn from observations made at the sediment surface, which can lead to false conclusions. For example, the polychaete *Scalibregma inflatum* (Rathke 1843) was named for its balloon-like shape. It has since been revealed, however, that the organism's natural shape is dorsoventrally flattened when below the sediment surface (Dorgan et al. 2007). Some additional information can be gained based on surface traces, e.g., burrow openings and fecal pellets, but traces provide only indirect evidence of subsurface behavior. Burrowing has also been observed at the wall of an aquarium (Herringshaw et al. 2010) and between two plates of lucite (Barnett and Bengough 2009). However, organisms do not necessarily behave the same near a rigid wall as they would farther from the wall, nor do the sediments in which they are burrowing. As an alternative, gelatin has been used as a transparent surrogate for mud because of similarities in the material properties of the two materials. The crack-propagation model for burrowing in mud was developed based on results of burrowing experiments performed in gelatin (Dorgan et al. 2007). However, mud and sand have different material properties, as discussed in Chapter 1, so a different method is needed to visualize burrowing activity in sand. In this chapter, I present the results from a set of experiments in which I used particle image velocimetry (PIV) as a means for studying the

burrowing behavior of infauna and the effect of this behavior on the movement of sand at the sediment surface.

For this study, PIV was used to elucidate the subsurface movement of the polychaete *Allita virens* in the early stages of burrow construction and more specifically to examine the activity of *A. virens* near an enclosure wall. PIV is a technique for analyzing flow at fine spatial and temporal scales (Westerweel 1997). It uses patterns in the displacement of particles to determine flow velocity and velocity-derived parameters (e.g. acceleration, strain, and vorticity) simultaneously at all grid points across the examined field. The method is most commonly used in fluids, often air or water, which are seeded with particles with densities near that of the medium. The same principle, however, has also been extended to the study of granular materials by using the medium itself as the “seed particles” (Lueptow et al. 2000). PIV has been used to visualize soil movements associated with an earthworm burrowing between two sheets of lucite (Barnett and Bengough 2009) and in the development of a burrowing robot designed to mimic a razor clam (Winter et al. 2012). Unlike past studies, the method used here does not rely on photographing particle motion through a transparent and rigid enclosure wall, instead measuring particle motion at the surface of the sand where it is unconstrained by a rigid boundary. Thus, the method can be used to study the movement of surface sediment as well as that of the burrowing organism itself.

Infaunal burrowing speed has not been measured directly, and estimates using indirect methods are rare in the literature. Past studies have measured infaunal burial rates (Tallqvist 2001; Lastra and Dugan 2002; Vanagt and Vincx 2008). However, measurements of burial rates, taken at the sediment surface, may not be representative of

burrowing rates below the surface. One study has examined burrowing speed using time-lapse X-ray analysis to measure the time required for infauna to form burrows (Gingras et al. 2008b), but that study did not examine polychaetes. Dorgan (2007) measured an average burrowing speed of  $0.86 \text{ mm s}^{-1}$  for *A. virens* burrowing in gelatin as an analog for mud. Absent physical evidence for a difference in velocity for burrowing in gelatin vs. sand, the hypothesis for this study was that the burrowing speed measured here would approximate that measured by Dorgan.

As a follow-up to Chapter 1, I also use the results of PIV experiments to explore the behavior of *A. virens* burrowing near a rigid wall in sand. At enclosure walls *A. virens*'s behavior depends on sediment type. Results in Chapter 1 indicate that *A. virens* burrows at a greater distance from the wall in sand than in mud. *A. virens* is likely constrained by the effects of force chains that prevent it from reaching the wall, though the distance at which this effect is important could not be determined using the methods from Chapter 1. In this chapter, PIV was used to measure the mean and minimum distances between a burrowing worm and the wall of its experimental enclosure. The hypothesis was that on average, when burrowing near a wall *A. virens* would at approximately the same distance from the wall as the median distance from the wall of the burrow openings for sand from Chapter 1 (0.6 cm).

Burrowing by polychaetes and other infauna has the dual effects of producing traces that may be preserved as recognizable fossils and disrupting sedimentary structures. The latter process was the initial definition for the term bioturbation, which was later expanded to include effects of animals on present-day sediments and pore water (Kristensen et al. 2012). Both processes are important to the interpretation of the geologic

record, and together they form the basis for the field of ichnology (Ekdale et al. 1984). Polychaetes date to at least the early Cambrian period (Morris and Peel 2008) and are among the most abundant marine metazoans (Fauchald and Jumars 1979). They are thus often subjects for studies that measure the effects of modern organisms on sediments as a means of understanding similar effects on ancient sedimentary structures (e.g., Herringshaw et al. 2010, Gingras et al. 2008a). Data on particle movements at the sediment surface revealed by the PIV experiments performed for this study, particularly the radial extent of surface disturbance, could be useful in interpreting trace fossils by providing parameters for expected extents of trace fossils and disruption of sedimentary structures. PIV also has the potential to provide a means for studying many subsurface behaviors of infauna.

## **2.2. Methods**

Experimental animals (*A. virens*) were obtained and maintained in culture as in Chapter 1. The average blotted wet weight of worms used in the 5 experiments was  $3.22 \pm 0.15$  g (mean  $\pm$  s.e.m.).

PIV experiments were performed in 20.3 cm i.d. pipes. Pipes were filled to 15 cm with playground sand (Quikrete “Play Sand”) then filled with seawater to 5 cm above the surface of the sand and left for at least 12 h before being used in an experiment to allow the sand to settle and the water to clear. Pipes were photographed from directly overhead at intervals of 5 s with a Nikon D5000 camera equipped with an AF Micro Nikkor 60 mm 1:28 D lens.

Images were converted to 8-bit gray scale then preprocessed with contrast-limited adaptive histogram equalization (CLAHE) within PIVlab (Thielicke and Stamhuis 2012)

to improve contrast. PIV analysis was performed using PIVlab, an open-source extension to MATLAB (R2012a; The Mathworks, Inc., Natick, MA). The PIV analysis was performed in three passes with the interrogation area halving between passes, beginning at 64 pixels squared with a shift of 32 pixels. Vector fields were post-processed using standard deviation and local median filters. Vectors that could not be calculated during the initial analysis were interpolated from the surrounding vector field.

The vector fields obtained were used to observe the burrowing behavior of *A. virens* and to calculate its burrowing speed and the distance of its head from the pipe wall. PIVlab was also used to plot velocity magnitude (speed) and divergence (Eq. 2.1). The divergence of a region is a measure of whether that region is a source (positive) or sink (negative) for particles. The velocity magnitude is the scalar velocity of the sediment at a given point.

$$\text{div}(\mathbf{U}) = \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} \quad (\text{Equation 2.1})$$

Validation experiments were performed to estimate the maximum depth below the sediment surface at which *A. virens*'s produces detectable particle motion for the experimental setup used. A 20.3 cm i.d. pipe was prepared as above but with the addition of a 1-cm diam. brass ball attached to a rigid rod extending through a hole in the side of the pipe. The size of ball was chosen to approximate the size of the head of an adult *A. virens*. The brass ball was embedded in the sand at varying depths below the sand's surface. Time-lapse photographs were taken, as described above, as the brass ball was pushed through the sand toward the wall of the pipe. PIV analyses were performed as above. Vector fields were examined for signs of motion at the sediment surface and for



**Table 2.1.** Summary data for PIV analyses.

Results for each experiment with *Allita virens* burrowing in sand and for all experiments combined: mean and Euclidean velocity, median and minimum distances from the wall of the worm's head, mean and maximum sediment displacements, and mean radial disturbance (the distance from the worm's head perpendicular to the worm's movement at which surface sediment movement could be detected).

	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Combined
<b>Burrowing speed (mm s<sup>-1</sup>)</b>	1.44	1.64	1.46	1.70	1.81	1.61
<b>s.e.m. of mean speed</b>	0.23	0.42	0.20	0.20	0.20	0.11
<b>Euclidean speed (mm s<sup>-1</sup>)</b>	1.46	1.51	1.46	1.62	1.68	1.55
<b>Median wall distance (cm)</b>	1.36	0.41	0.69	0.38	0.61	0.57
<b>Minimum wall Distance (cm)</b>	1.29	0.19	0.50	0.14	0.38	0.14
<b>Mean displacement (mm)</b>	0.10	0.19	0.25	0.16	0.08	0.16
<b>Maximum displacement (mm)</b>	0.26	0.45	0.72	0.67	0.43	0.72
<b>Mean radius of disturbance (cm)</b>	3.78	3.82	3.94	2.53	1.57	3.13
<b>s.e.m. of mean radius of disturbance</b>	0.21	0.27	0.27	0.21	0.31	0.22

comparison with vector fields calculated from experimental images. The depths tested were 5 cm, 7 cm, 8 cm, 9 cm, 10 cm, and 12 cm. Additional image sequences were taken for the 10 and 12 cm depth to confirm the results obtained.

General features of the burrowing behavior of *A. virens* in sand were described based on various time-lapse sequences separate from those used for the experiments described below. Images were recorded and analyzed as described above. To make it easier to distinguish the described features, the “smoothn” function (Garcia 2009) was applied to the vector fields and the velocity magnitude and divergence plots shown in the figures.

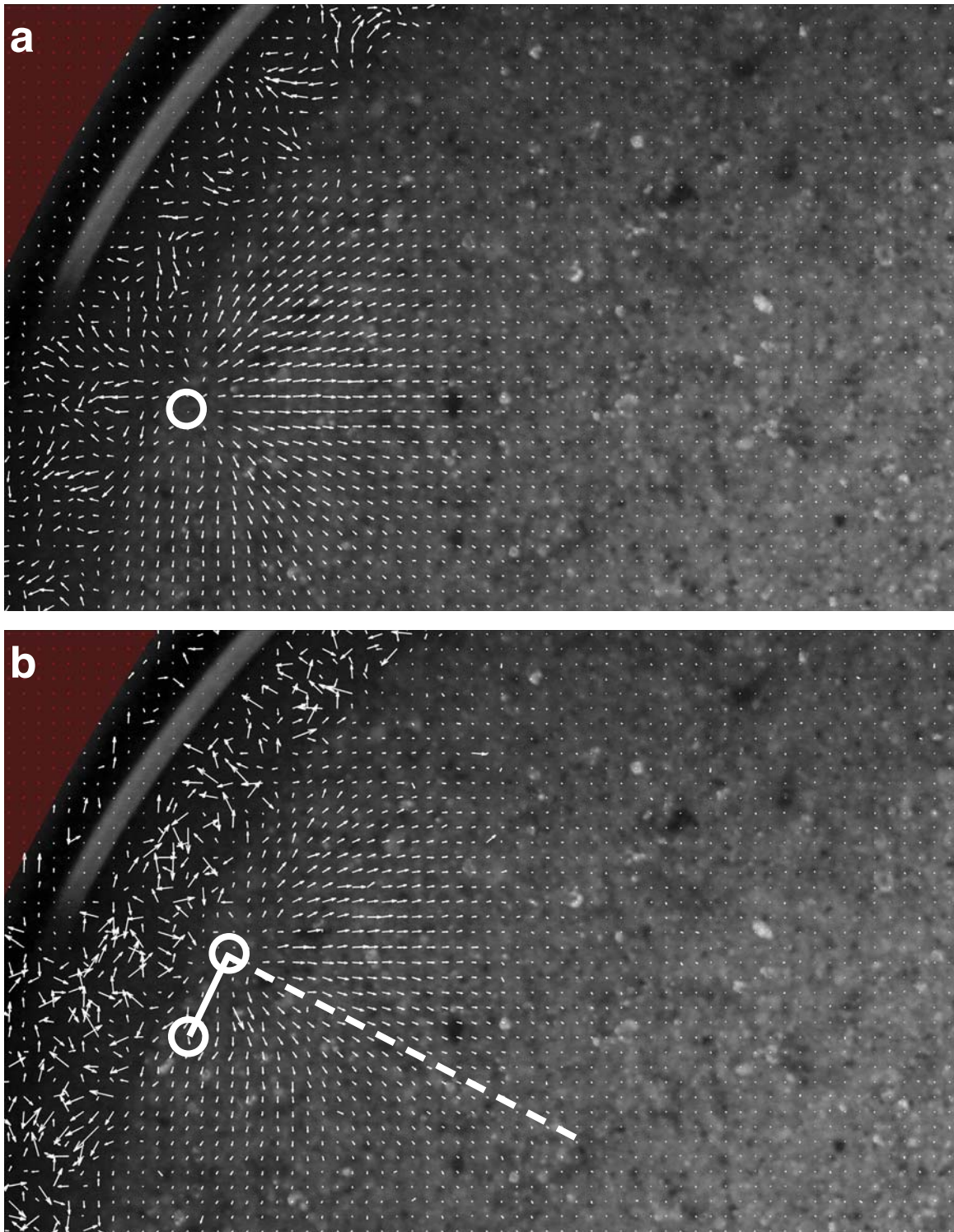
Horizontal burrowing speed (the component of burrowing speed in the horizontal plane) was measured in 5 experiments. For each experiment analyzed, 6 consecutive frames were chosen for which it was possible to determine the location of the worm’s head from the PIV vector map. In all sequences used, *A. virens* was moving for the entire duration of the sequence and was burrowing near the pipe’s wall. Consecutive frames were used to avoid periods of rest in the average velocities calculated. The distance between the worm’s head in consecutive frames was measured with PIVlab, yielding 5 distances for each experiment. Distances were then divided by the inter-frame time interval (5 s) to yield the mean velocity for the time interval between these frames. The mean and standard error of the mean (s.e.m.) of the worm’s velocity were calculated for each experiment and for the combined data from all experiments. A one-way ANOVA compared means between experiments, using a confidence level of 0.05. The mean Euclidean velocity for each experiment was calculated by measuring the distance

between the points in the first and last frames and dividing by the total time interval (25 s).

Using the same 6 frames chosen for the burrowing speed analyses, the distance of the worm's head from the nearest pipe wall was measured with PIVlab. The median wall distance was calculated for each experiment and for the combined data from all experiments.

The radial spatial extent of the surface disturbed by *A. virens*'s burrowing activity was measured from plots of sediment velocity magnitude. Using the same 6 frames used for the burrowing speed and wall distance analyses, the velocity magnitude was recorded along a 5-cm transect of 500 evenly-space points extending toward the center of the pipe, perpendicular to the direction of the worm's travel (based on the position of the worm's head in the previous frame). The method for drawing the transect is illustrated in figure 2.1. The presence of the pipe wall precluded the use of the opposite radial transect. The baseline velocity magnitude for each frame was taken from the average along a similar transect taken distant from the worm. This baseline velocity magnitude was subtracted from the data for each frame. Velocity magnitudes were then converted to displacements by multiplying by the time interval (5 s). The maximum radial distance from the *A. virens*'s head at which it was still possible to detect surface disturbance (radius of surface disturbance) was recorded by noting the first point after the peak displacement at which the displacement was within the s.e.m. of the baseline transect from 0 for at least two consecutive points.

**Figure 2.1.** Diagram of transect used for particle displacement calculations. To calculate particle displacements for a frame (b), the location of the worm's head (open circle) was marked on the vector fields for two successive frames (a and b). A line (solid) was drawn between the two head locations, and particle displacement was measured along a 5-cm transect (dashed) perpendicular to the first line.



### 2.3. Results

In the validation experiments, the movement of the ball could be clearly discerned in PIV analyses of image sequences taken at all depths up to 10 cm. The analyses for the 12 cm sequences revealed some sediment movement, but it was not possible to determine the location of the brass ball from the vector fields. The burrowing activity of *A. virens* observed here can be described as a cycle that comprises three phases: initial entry of *A. virens* into the sediment column, burrowing within the sediment, and emergence by the formation of a new opening to the surface. These three burrowing phases will be considered separately.

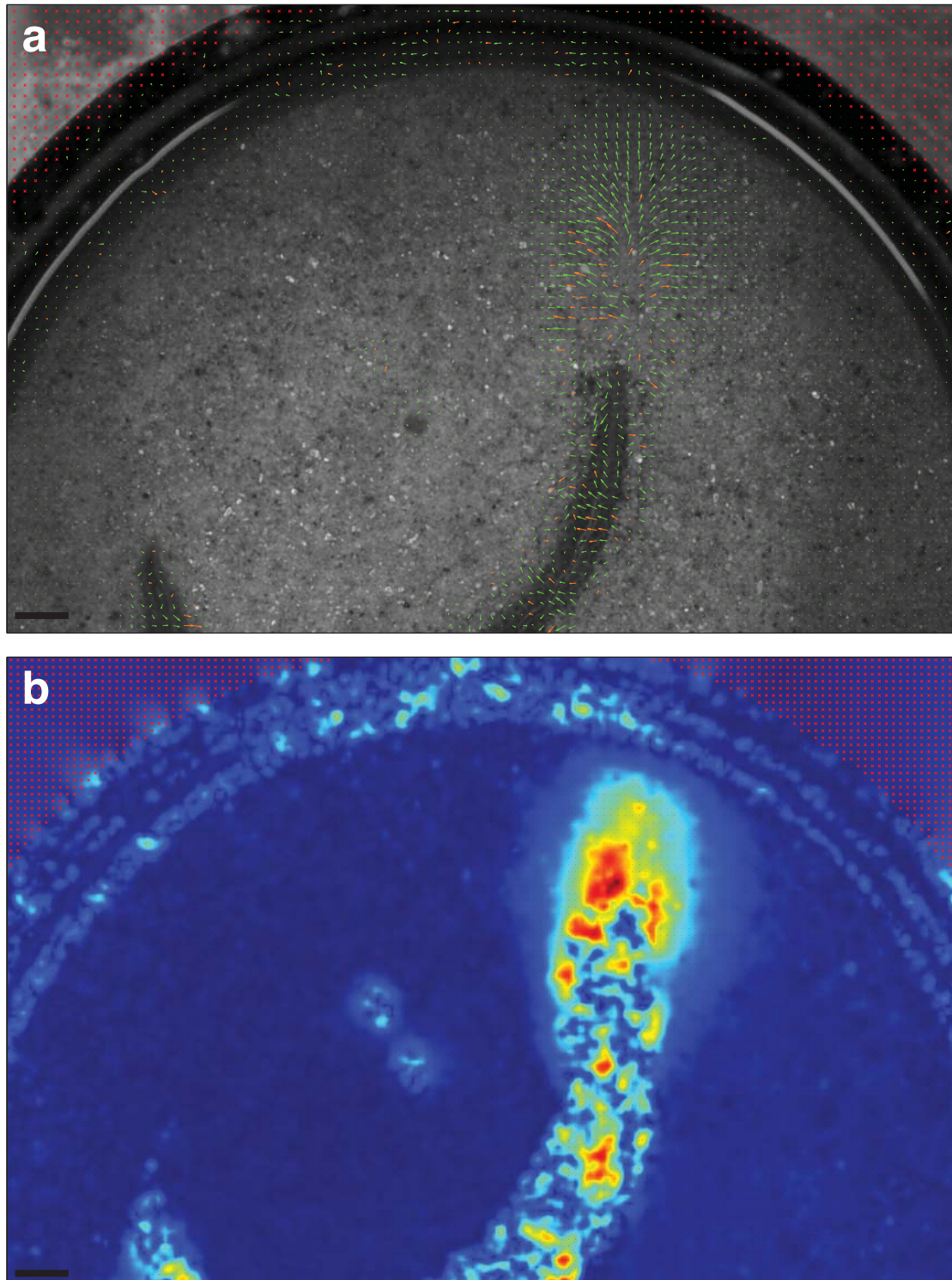
Much of the particle motion in the initial phase of burrowing is obscured by the worm itself. A prominent feature in the vector fields that is visible just after the worm's head has broken the sand's surface is a collection of vectors extending radially outward just ahead of the visible portion of the worm (Fig. 2.2a). The velocity magnitude map of this phase reveals the shape and extent of the area of displaced sediment (Fig. 2.2b).

As the worm continues to burrow, the source region extends along the length of the burrow. The shape of this region is more elongated than that of the region in the initial phase, stretching along the length of the newly formed burrow (Fig. 2.2). Upon approaching a wall, *A. virens* redirects its burrow until it is nearly parallel to the wall (Fig. 2.3) before finally turning back away from the wall and burrowing away in the opposite direction (Fig. 2.4).

As *A. virens* burrows deeper (to approximately 12 cm), it no longer disturbs the surface sufficiently for PIV to detect its movements. The pattern of sediment movement changes from that seen in the early phases of burrowing when *A. virens* burrows toward

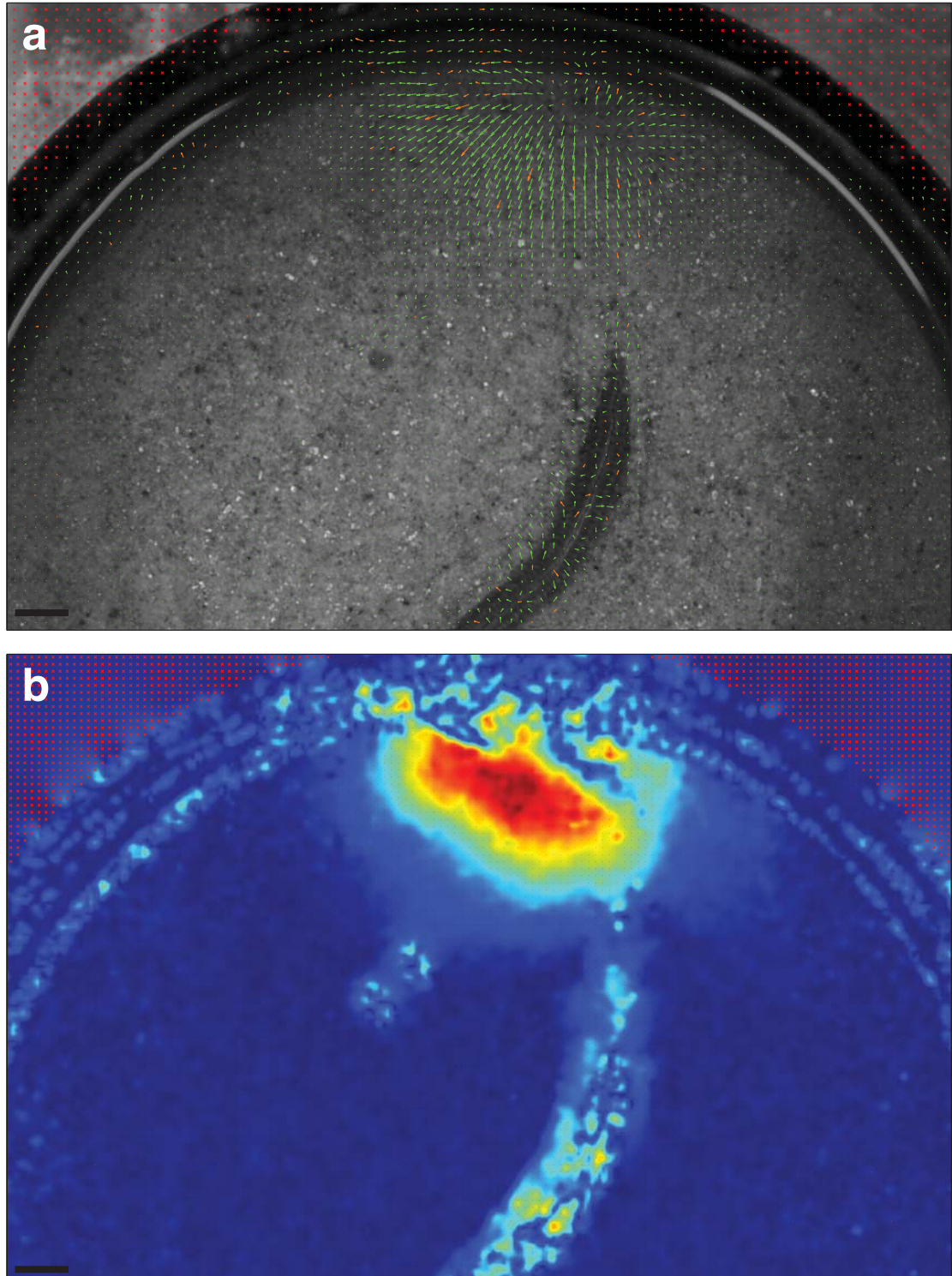


**Figure 2.2.** *A. virens* soon after burrowing below the sediment surface. In the velocity vector field (a), vectors in green were calculated during the initial PIV analysis, whereas those in orange were interpolated from the surrounding vectors in the validation stage. In the velocity magnitude plot, warmer (b) colors represent higher velocities. The scale bar represents 1 cm.



**Figure 2.3.** *A. virens* approaching the wall.

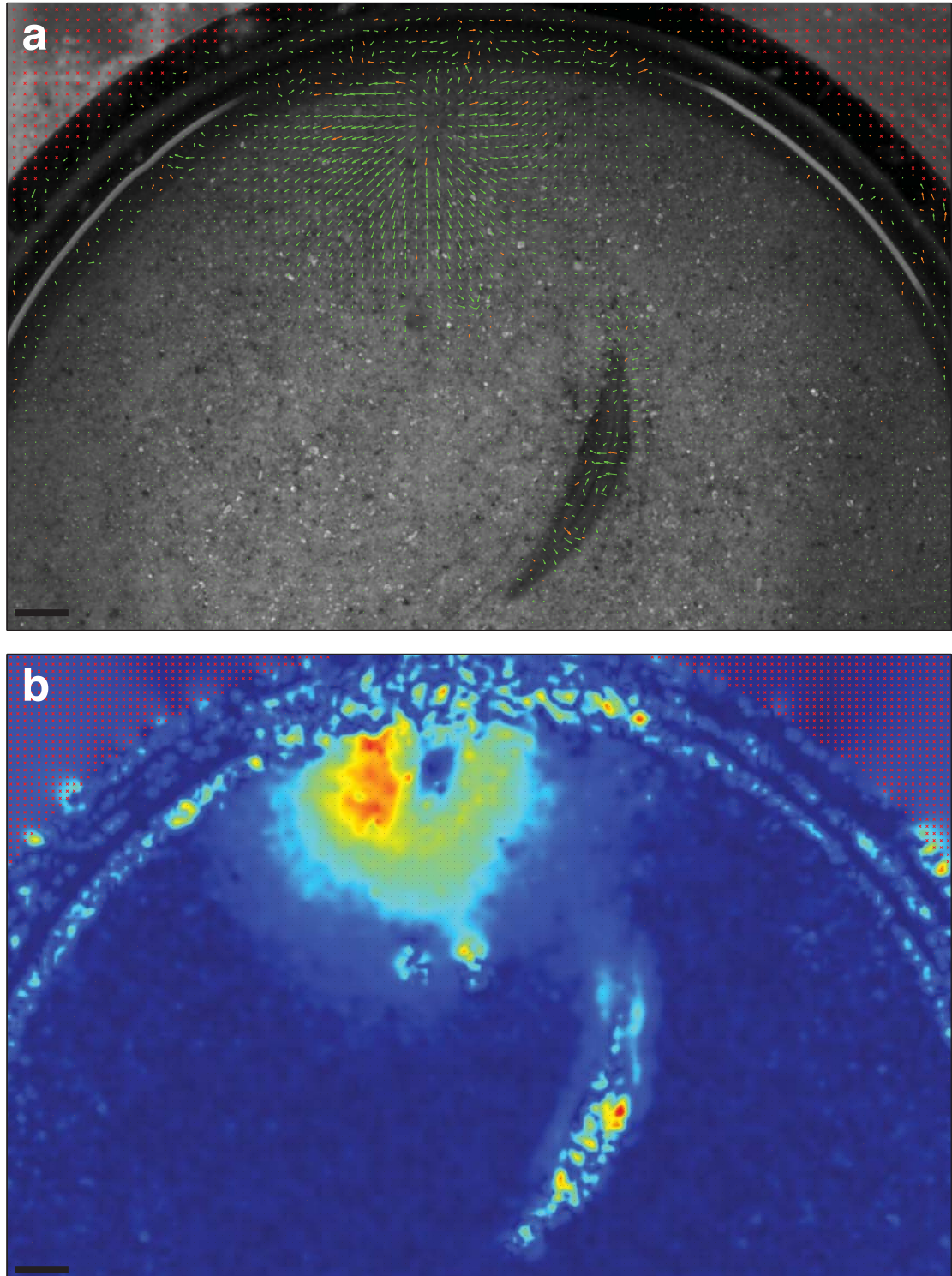
In the velocity vector field (a), vectors in green were calculated during the initial PIV analysis, whereas those in orange were interpolated from the surrounding vectors in the validation stage. In the velocity magnitude plot, warmer (b) colors represent higher velocities. The scale bar represents 1 cm.





**Figure 2.4.** *A. virens* turning away from the wall

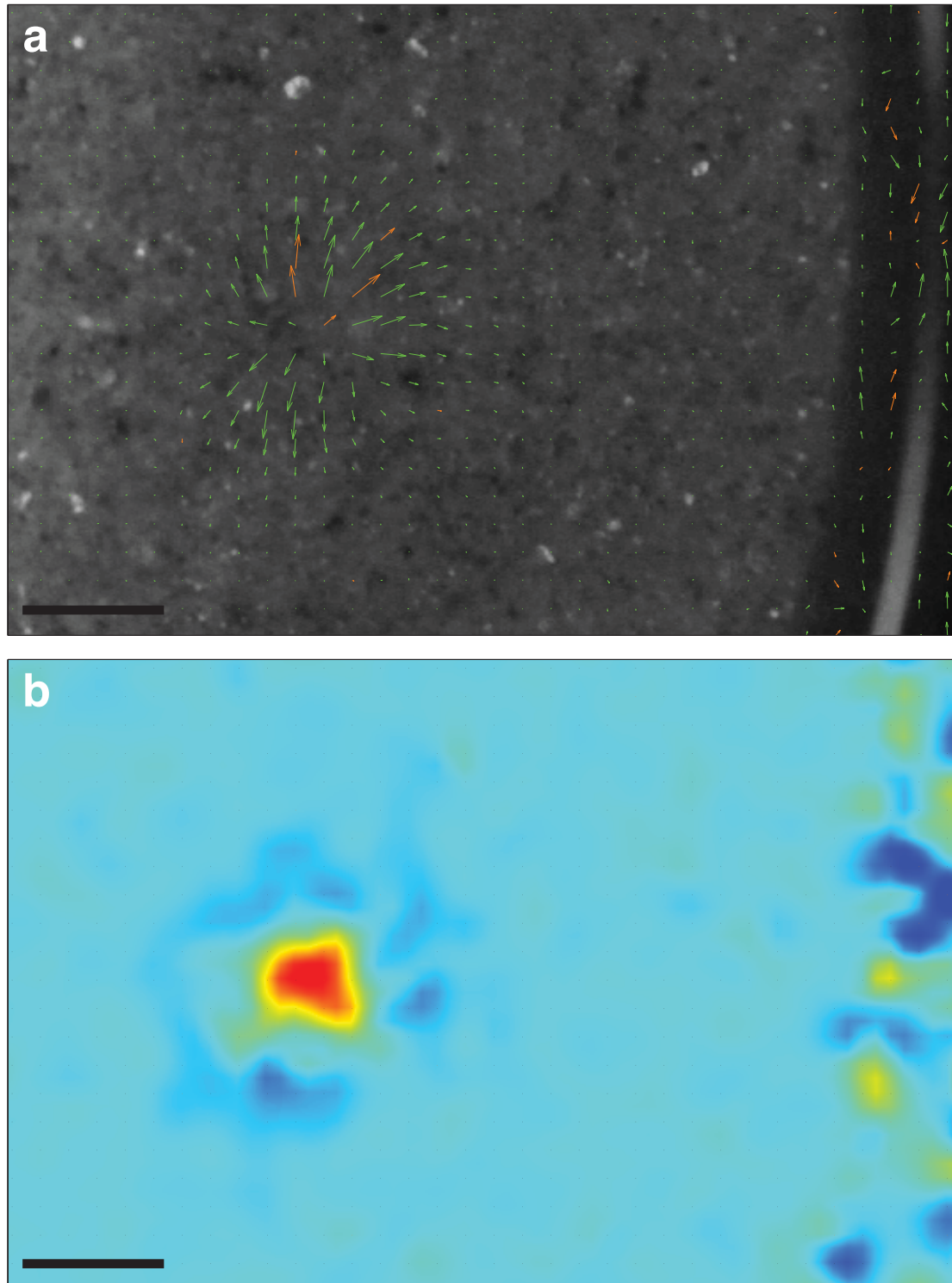
In the velocity vector field (a), vectors in green were calculated during the initial PIV analysis, whereas those in orange were interpolated from the surrounding vectors in the validation stage. In the velocity magnitude plot, warmer (b) colors represent higher velocities. The scale bar represents 1 cm.





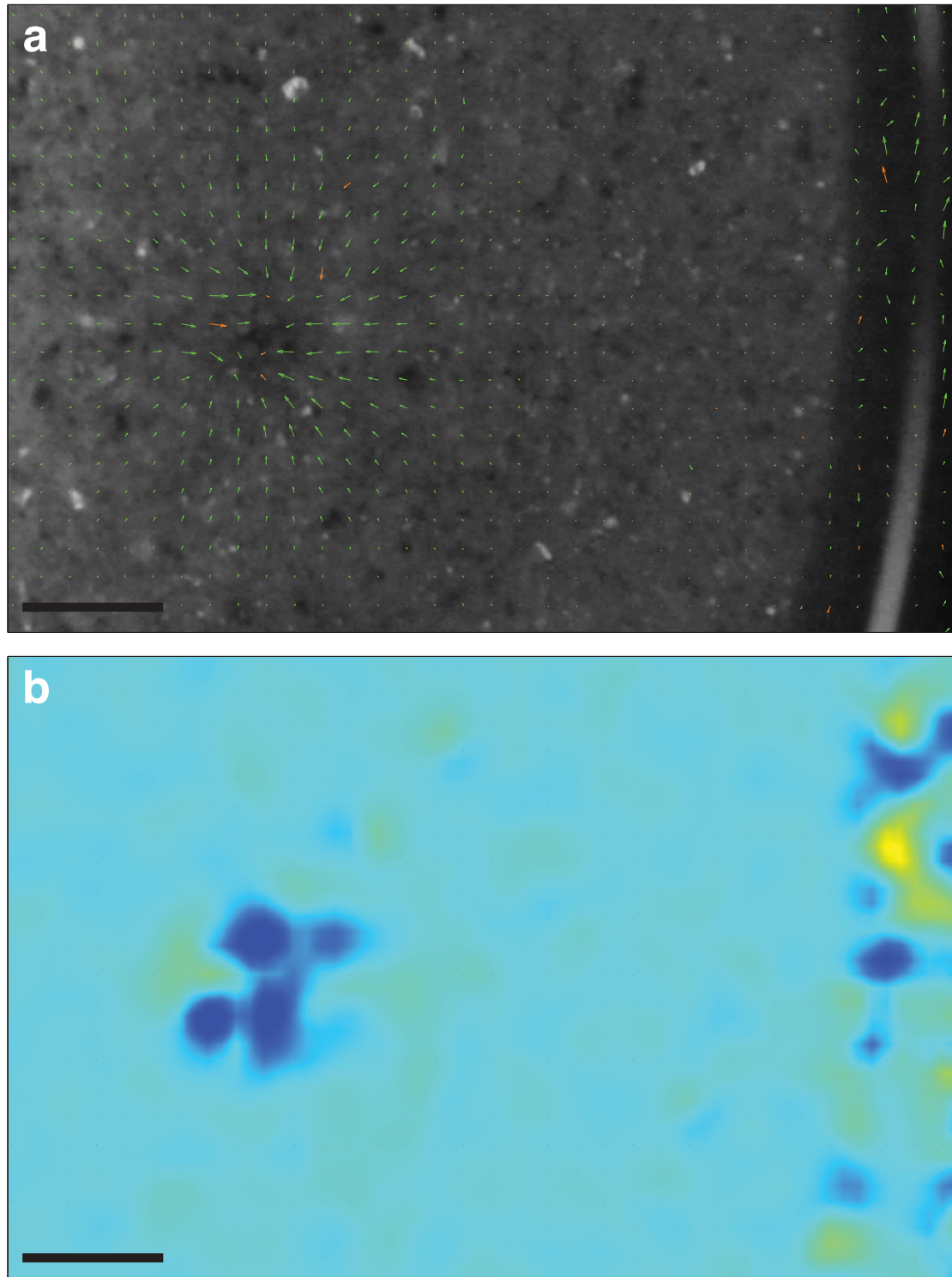
**Figure 2.5.** *A. virens* before emerging from the sediment.

In the velocity vector field (a), vectors in green were calculated during the initial PIV analysis, whereas those in orange were interpolated from the surrounding vectors in the validation stage. In the divergence plot (b), warmer colors represent positive divergence, and cooler colors represent convergence. The scale bar represents 1 cm.



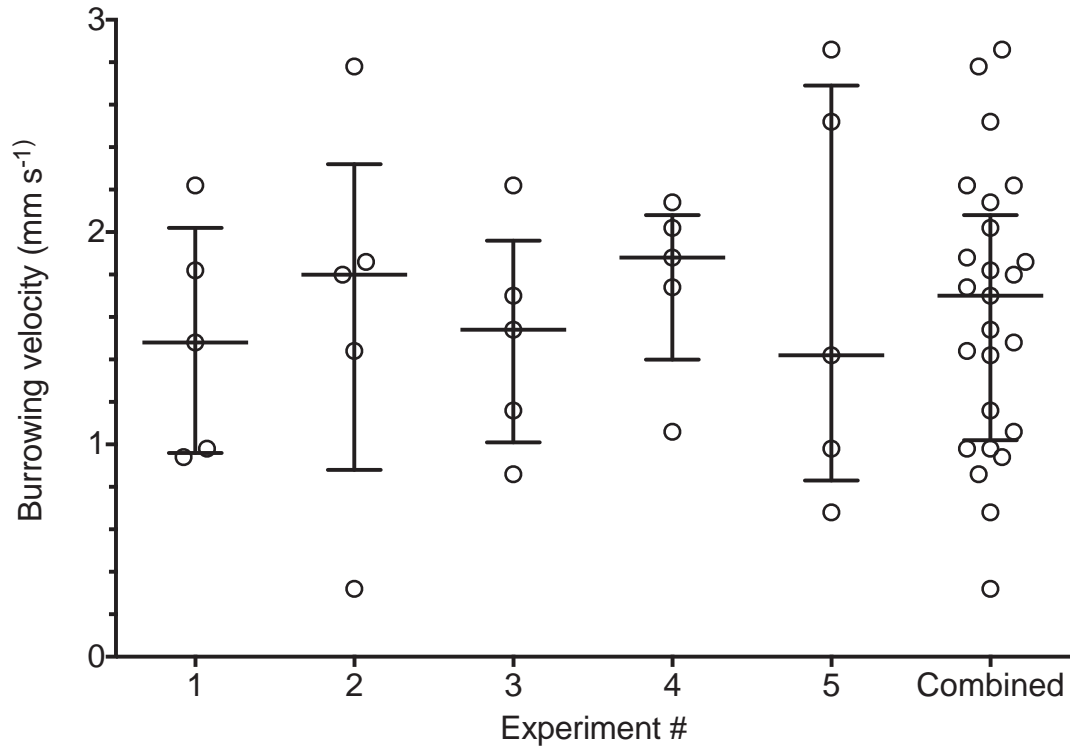
**Figure 2.6.** *A. virens* after emerging from the sediment.

In the velocity vector field (a), vectors in green were calculated during the initial PIV analysis, whereas those in orange were interpolated from the surrounding vectors in the validation stage. In the divergence plot (b), warmer colors represent positive divergence, and cooler colors represent convergence. The scale bar represents 1 cm.



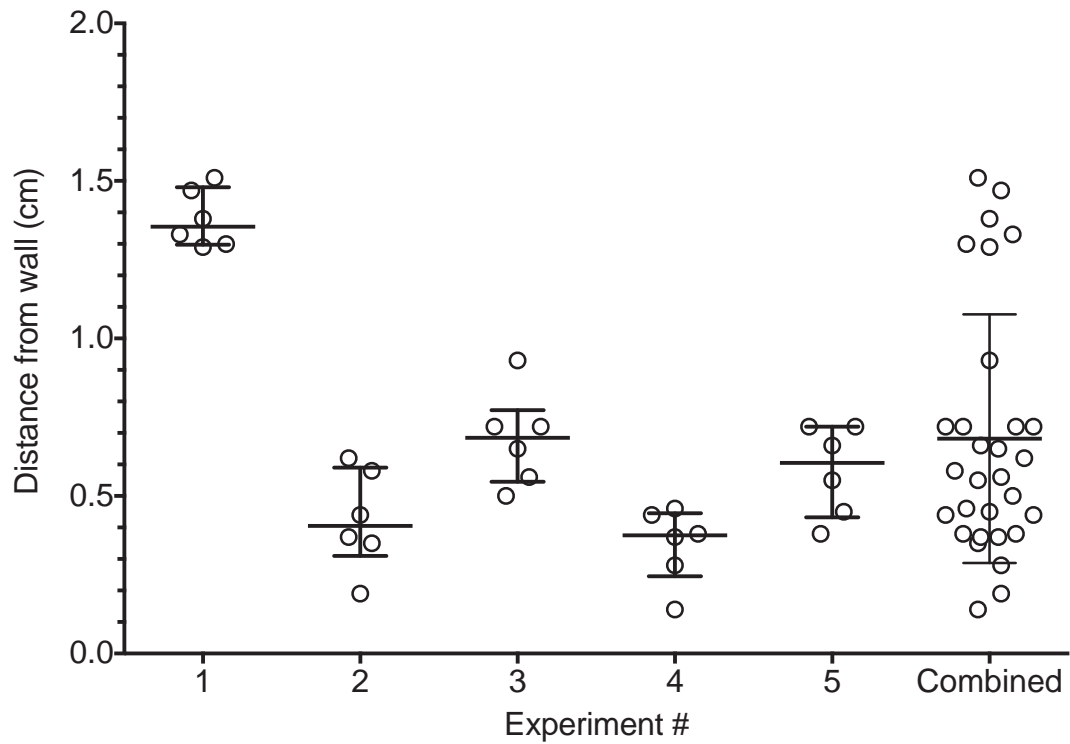
**Figure 2.7.** Burrowing speeds.

Burrowing speeds for each frame with mean and s.e.m. for each of the 5 experiments and for all experiments combined. The results for each experiment consist of five speeds calculated from six frames of a PIV analysis of *Allita virens* burrowing in sand. Means were not statistically significant between experiments ( $p = 0.83$ ).



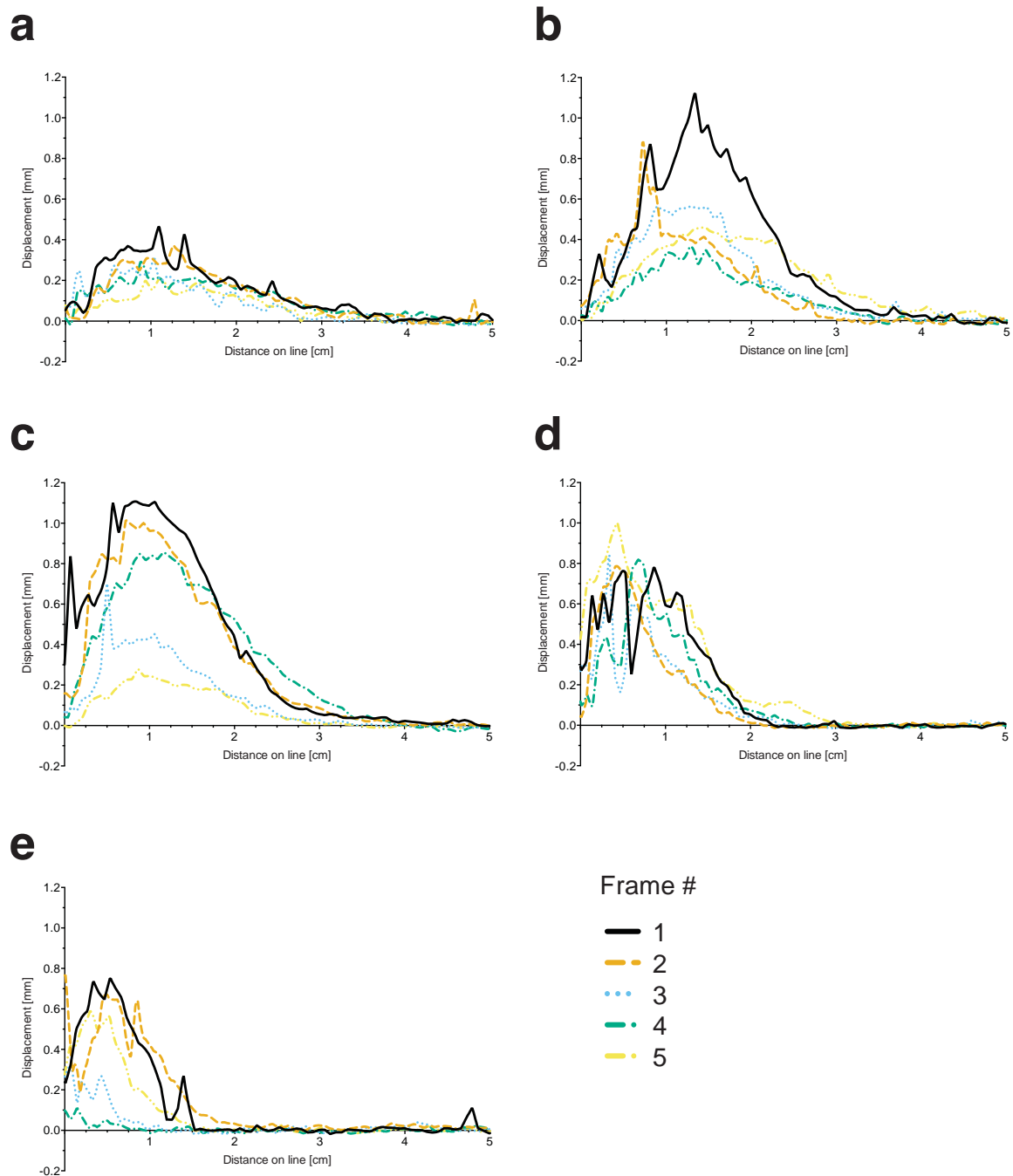
**Figure 2.8.** Wall distances.

The distance of *Allita virens*'s head from the wall for each frame with the mean and s.e.m. for each of the five experiments and for all experiments combined. The results for each experiment consist of six distances calculated from six frames of a PIV analysis of *Allita virens* burrowing in sand.

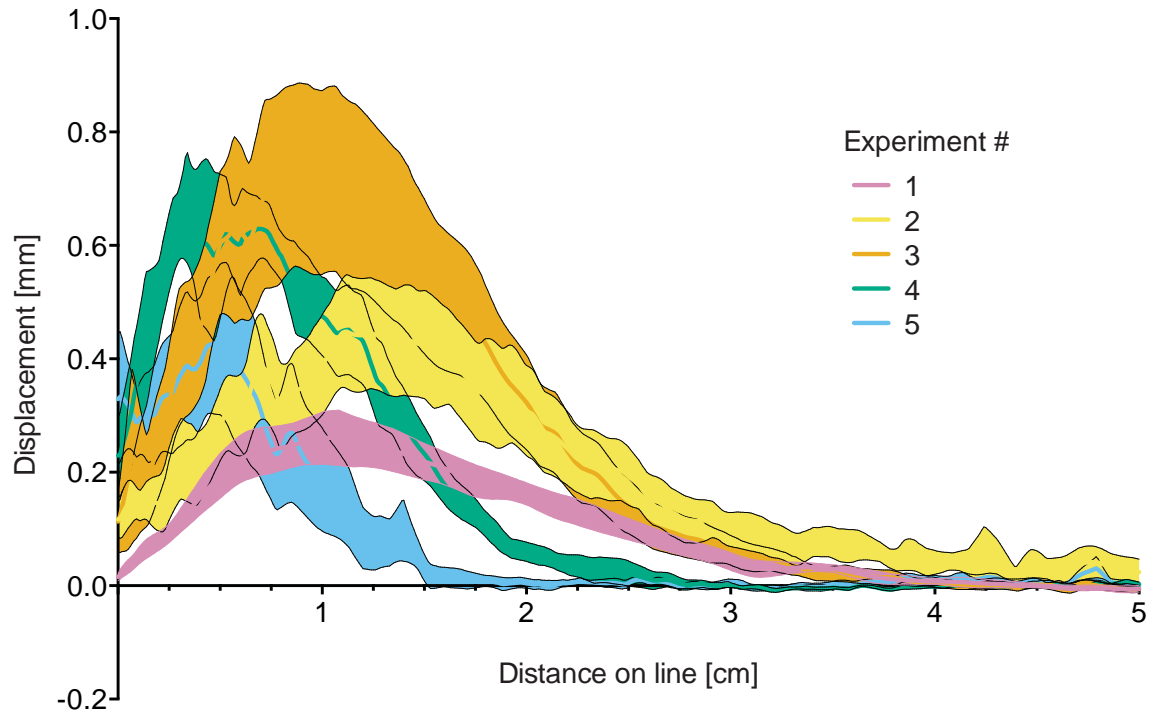


**Figure 2.9.** Sediment displacement for each frame.

Plots of sediment displacement for each frame as a function of radial distance from *A. virens*'s head perpendicular to the direction of burrowing for each experiment (a, b, c, d, and e).

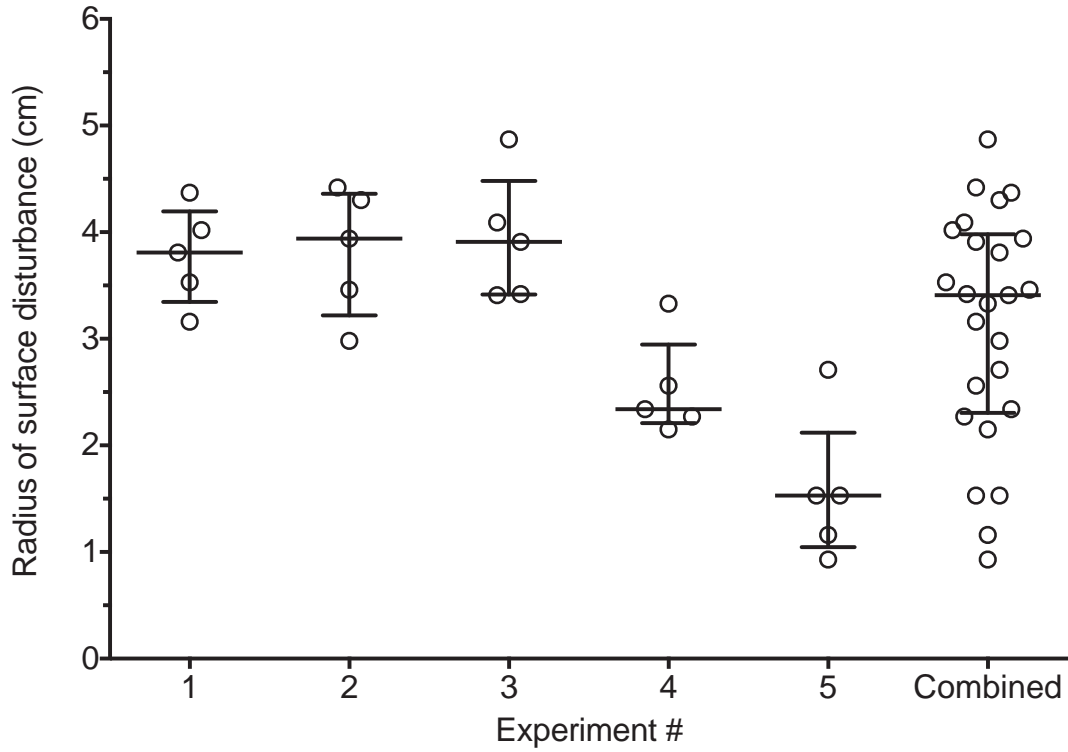


**Figure 2.10.** Averaged sediment displacement for each experiment. Plots of the mean of sediment displacement for all frames in each experiment, as a function of radial distance from *A. virens*'s head, perpendicular to the direction of burrowing. The envelope denotes the s.e.m. for each experiment.



**Figure 2.11.** Radius of disturbance.

The radius of surface sediment disturbance for each frame with mean and s.e.m. for each experiment and for all experiments combined. Each experiment consists of five radius of disturbance calculations from six frames of a PIV analysis of *Allita virens* burrowing in sand.



the sediment surface to form a burrow opening (Figs. 2.5 and 2.6). Just before *A. virens* forms a burrow opening to emerge from the sediment, the vector region compresses longitudinally until it is nearly radial, indicating that the worm is burrowing vertically (perpendicular to the plane of the sediment surface) (Fig. 2.5). As seen in the divergence plot, this region is a sediment source before the worm breaks the sediment surface (Fig 2.5b). However, immediately after the formation of the burrow opening, the region becomes a sink as surrounding particles fall into the newly formed opening (Fig. 2.6b).

Mean burrowing speed for the combined data from all experiments was  $1.61 \pm 0.11 \text{ mm s}^{-1}$  (mean  $\pm$  s.e.m.) (Table 2.1, Fig. 2.7). Means were not significantly different between experiments ( $p = 0.83$ ). Median distance of the worm's head from the wall for all experiments was 0.57 cm. Minimum distance from the wall for all experiments was 0.14 cm (Table 2.1, Fig. 2.8). The mean and maximum sediment displacement for all experiments along the 5 cm transects were 0.16 mm and 0.72 mm respectively (Table 2.2, Figs. 2.9 and 2.10). The radius of disturbance for all experiments was  $3.13 \pm 0.22 \text{ cm}$  (mean  $\pm$  s.e.m.).

## **2.4. Discussion**

The results presented here demonstrate the feasibility of PIV for the observation of infaunal burrowing activity and the application of the method to the measurement of *A. virens*'s burrowing speed, the distance from a rigid wall at which it burrows, and the distance over which it affects the sand's surface. The mean burrowing speed in the horizontal plane of  $1.61 \text{ mm s}^{-1}$  measured in this study is within a factor of two of that measured by Dorgan ( $0.86 \text{ mm s}^{-1}$ ) (Dorgan 2007). The difference may result from the distinct material properties of sand and mud. As discussed in Chapter 1, sand is a granular



medium in which the bulk properties are determined by the accumulation of interactions of individual particles. Albert et al. (2000) characterize the movement of a body through a granular medium as a stick-slip process with stick periods of gradually increasing drag punctuated by slips during which the drag rapidly returns to its previous level. The sticks characterize a jammed state in which force chains resist the motion of the body. A slip occurs when an increase in the interparticle forces at some point in the chain causes a rearrangement of the chain (Albert et al. 2000). A burrowing organism should be able to move faster in sand than in mud because the drag on an object moving through a granular medium is independent of velocity for low velocities (Albert et al. 1999), so the energy constraints on burrowing faster in sand should be less than those in mud.

As discussed in Chapter 1, force chains likely prevent *A. virens* from burrowing close to a rigid wall. The five image sequences examined here show that in sand *A. virens* tends to burrow near but not at rigid walls. *A. virens*'s tendency to burrow near the wall may be a response to containment. The median distance from the wall (0.57 cm) measured from the PIV experiment agrees closely with the median wall distance of 0.6 cm found for the burrow opening experiments in Chapter 1. This value may represent an average minimum distance from the wall at which *A. virens* is still able to resist the net force from force chains that extend to the wall. Shorter wall distances (e.g. the minimum wall distance of 0.14 cm) may result from spatial and temporal variations in the field of force chains. In observations of *A. virens* made using PIV, the worm appears to repeatedly burrow toward the wall of the pipe though it is unable to reach it.

A general pattern of particle movement emerges from examining velocity magnitude fields (Figs. 1b, 2b, and 3b) and radial distance plots (Figs. 8 and 9). Peak

velocities are generally found approximately 0.5 to 1.0 cm from the worm's head.

Moving from the peak away from the worm's head, the decrease in velocity represents a decrease in the influence of the worm's movement on the sediment particles until the velocity returns to baseline levels at the edge of the worm's influence on the sediment surface. The measured velocity reaches a local minimum directly over the worm because vertical particle movement dominates in this region. The vertical component of particle displacement cannot be detected by the methods used here. By looking at the pipe from an pipe, a ridge can be observed immediately above the worm's head.

The extent of *A. virens*'s radial influence on surface sediment (radius of disturbance), which averaged 3.13 cm in this study may be useful in interpreting trace fossils, placing bounds on the area where one can expect to detect the influence of the worm's motion on particle orientation. By doubling the mean radius of influence and multiplying by the mean worm velocity, a value of  $1.01 \text{ mm}^2 \text{ s}^{-1}$  is obtained. This value represents the average area of surface disturbed over time by an individual *A. virens* worm actively burrowing above the depth at which PIV is no longer able to detect its motion (10 - 12 cm). If combined with data on the proportion of time spent burrowing it would be possible to calculate a value for surface sediment disturbed over time, which would be useful in determining rates of surface disturbance for rate studies of biogenic destruction of sedimentary features (e.g., Wheatcroft et al. 1989). Ultimately, the effects of worm size, speed, and depth on surface sediment disturbance will need to be quantified to allow this method to be useful for sedimentary analyses.

In this study, I document the use PIV to study the burrowing behavior of organisms and the movement of sediment in response to burrowing, but the application of

PIV to burrowing could likely be expanded to address many more problems. One obvious direction for future studies is to compare burrowing rates and activities between different taxa of infauna. The best candidate organisms for these studies are likely to be fairly large (upwards of 10 cm), active burrowers, such as mobile deposit feeders. High-resolution images and short time intervals between frames might make the method applicable to smaller organisms as well. As mentioned, quantification of the parameters that affect surface sediment displacement would make this method applicable to a wider range of problems. The most efficient method for determining these parameters would likely be a mechanical, using a rod mounted to a servo motor for example. Three-dimensional PIV techniques have been developed, such as stereo PIV (Westerweel and Nieuwstadt 1991; Willert 1997), which uses two offset cameras, and future studies might use this method to reveal details that two-dimensional PIV is unable to detect. For example, with 3D PIV, it should be possible to measure the height of the disturbed sediment directly above the burrowing animal. The method could also be extended to the field where it might be particularly informative in detecting interactions between individuals. It could also be used to examine infaunal responses to subsurface physical stimuli and chemical cues.

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