Integrative and Comparative Biology
Integrative and Comparative Biology, volume 00, pp. 1–11
https://doi.org/10.1093/icb/icad080
Society for Integrative and Comparative Biology

SYMPOSIUM

Collecting–Gathering Biophysics of the Blackworm *Lumbriculus variegatus*

Harry Tuazon [*], Chantal Nguyen [†], Emily Kaufman[*], Ishant Tiwari [*], Jessica Bermudez[*], Darshan Chudasama [*], Orit Peleg [†,‡,§] and M. Saad Bhamla [*,1]

[*]School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA; [†]BioFrontiers Institute, University of Colorado Boulder, Boulder, CO 80303, USA; [‡]Department of Computer Science, University of Colorado Boulder, Boulder, CO 80309, USA; [§]Santa Fe Institute, Santa Fe, NM 87501, USA

[1]E-mail: saadb@chbe.gatech.edu
Chantal Nguyen, Emily Kaufman and Ishant Tiwari have contributed equally to this work and share second authorship.
From the symposium "Large-scale biological phenomena arising from small-scale biophysical processes" presented at the annual meeting of the Society for Integrative and Comparative Biology virtual annual meeting, January 16–March 31, 2023.

Synopsis Many organisms exhibit collecting and gathering behaviors as a foraging and survival method. Benthic macroinvertebrates are classified as collector–gatherers due to their collection of particulate matter. Among these, the aquatic oligochaete *Lumbriculus variegatus* (California blackworms) demonstrates the ability to ingest both organic and inorganic materials, including microplastics. However, earlier studies have only qualitatively described their collecting behaviors for such materials. The mechanism by which blackworms consolidate discrete particles into a larger clump remains unexplored quantitatively. In this study, we analyze a group of blackworms in a large arena with an aqueous algae solution (organic particles) and find that their relative collecting efficiency is proportional to population size. We found that doubling the population size ($N = 25$–$N = 50$) results in a decrease in time to reach consolidation by more than half. Microscopic examination of individual blackworms reveals that both algae and microplastics physically adhere to the worm's body and form clumps due to external mucus secretions by the worms. Our observations also indicate that this clumping behavior reduces the worm's exploration of its environment, possibly due to thigmotaxis. To validate these observed biophysical mechanisms, we create an active polymer model of a worm moving in a field of particulate debris. We simulate its adhesive nature by implementing a short-range attraction between the worm and the nearest surrounding particles. Our findings indicate an increase in gathering efficiency when we add an attractive force between particles, simulating the worm's mucosal secretions. Our work provides a detailed understanding of the complex mechanisms underlying the collecting–gathering behavior in *L. variegatus*, informing the design of bioinspired synthetic collector systems, and advances our understanding of the ecological impacts of microplastics on benthic invertebrates.

Introduction

Nature contains many organisms that utilize foraging and gathering behaviors to obtain food. Ants, termites, and bees are all organisms that exhibit collective behavior but have a hierarchical social system in place (Haifig et al. 2015; Frank and Linsenmair 2017; Lemanski et al. 2019). Similarly, decorator crabs and assassin bugs are examples of organisms that utilize a gathering behavior to harvest for their survival or hunting, respectively (Brandt and Mahsberg 2002; Thanh et al. 2003). One such organism, the benthic oligochaete *Lumbriculus variegatus*, exhibits this behavior through the formation of particle clusters. They can gather small materials (<1 mm in particle size) in their environments as an individuals or as a collective (Cummins and Klug 1979). Worms are unique in that, unlike ants or termites, they exhibit complex, physically entangled collective behavior without a hierarchy (Nguyen et al. 2021; Ozkan-Aydin et al. 2021; Shishkov and Peleg 2022; Deblais et al. 2023).

Blackworms have been found to actively modify aquatic environments through bioturbation,

Advance Access publication June 27, 2023

which involves reworking and ventilation, and their role as biodiffusors and upward conveyors is well-established (Kristensen et al. 2012; Roche et al. 2016). As oligochaetes, blackworms are categorized as a member of the collecting–gathering functional feeding groups (FFGs), which harvest and feed on fine particles at and below the sediment-water interface (Cook 1969; Cummins and Klug 1979; Wotton 1994; Ilyashuk 1999). Blackworms use an eversible pharynx to feed and have a mucus-lined body wall that aids in lubrication and respiration, although they may also use their tails for oxygenation (Govedich et al. 2010; Timm and Martin 2015; Tuazon et al. 2022). Though there is no direct evidence for blackworms' usage of their mucus body wall for collection, it has been shown that particle capture using mucus is utilized by many other macroinvertebrates, such as the polychaete *Chaetopterus*, larval midges (Chironomidae), and the terebellid *Eupolymnia* (Wotton 1994).

In this work, we quantitatively assess the unexplored mechanism of particle aggregation by blackworms in a debris-filled environment. We first analyze their collective behavior in an algae-laden large arena, followed by a microscopic study of individual worm behavior in a smaller setting. Subsequently, we extend this study to examine blackworm interactions with microplastics, commonly found in their natural habitat (Krause et al. 2021). We supplement our empirical findings with a computational model simulating a worm in a debris field based on van der Waals force, applying a short-range attractive force on the worm to replicate the sticky mucus layer that facilitates particle aggregation.

Materials and methods

Animals

We obtained California blackworms (length 30.2 ± 7.4 mm, diameter 0.6 ± 0.1 mm, and mass 7.0 ± 2.4 mg) and algae (*Chlamydomonos reinhardtii*) from Ward's Science. Worms were reared in a plastic storage box (35 *times* 20 *times* 12 cm) filled with filtered water. They were fed fish food pellets daily, and their water was replaced daily. Worms were kept in water at room temperature (∼21°C) before any experiments. We ensured that the worms used in our experiments were not reused by placing them in a separate container, with each individual worm being used only once to maintain consistency in our results. Institutional animal care committee approval is not required for studies with blackworms.

Data acquisition

In our large arena experiments, we recorded the blackworms' behavior using a Logitech Brio 4K (Taiwan,

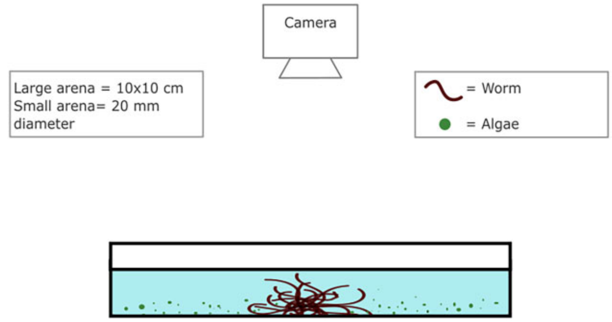

Fig. 1 Experimental Setup Schematic of experimental setup. Large-scale arenas are used for collecting–gathering experiments in a 10 x 10 cm$_2$ Petri dish with 50 mL of filtered water and 10 mg (dry weight) of algae. These experiments are filmed from above using a webcam. Small-scale arenas evaluate a single worm's collecting biophysics using a confocal Petri dish with filtered water and 1 mg (dry weight) of material, either algae or microplastics. These experiments are filmed from above using a microscope camera.

ROC) webcam placed in a photobox with fixed lighting (∼500 lux). The webcam captured frames in TIFF format at a rate of 0.20 FPS for a total duration of two hours, with data analysis focused on the first 90 min. The experimental setup involved adding 10 mg of well-mixed algae to a 10 *times* 10 cm$_2$ Petri dish containing 50 mL of filtered water (Fig. 1). We selected algae due to its abundance as a food source in nature and green color, which is distinguishable for image analysis. Subsequently, worms were distributed into the arena, with variations in population size ($N = 10$, 25, or 50). We selected these populations to provide a range of sample sizes while ensuring optimal visibility and minimizing potential blockage of material from the camera. The mass of algae was measured by drying it using paper towels. Additionally, worms were adequately fed beforehand to minimize algae consumption during the experiments. This 90-min duration was based on the preliminary observation that a population size of 50 worms typically reached its peak collection in around 30 min. Each trial was repeated five times.

For the single worm experiments conducted in the small arena, we utilized a Leica MZ APO microscope (Heerbrugg, Switzerland) equipped with an ImageSource DFK 33UX264 camera (Charlotte, NC, USA). The camera recorded at a frame rate of 30 FPS for one hour with fixed lighting (∼200 lux), with data analysis focused on the first 30 min. Through preliminary trials, we observed that the collecting behavior of individual worms in the small arena reached a relatively stable state within this time frame. The small arena, consisting of a 20-mm glass portion of a 35-mm confocal Petri dish, contained filtered water and the desired test material (algae or microplastics). Similar to algae, we selected microplastics based on their highly contrasting color and their prevalence as a pervasive contaminant in freshwater systems. Smaller and healthier worms (length 17.6 ± 1.8 mm) were randomly selected for these experiments to prevent interference from the arena walls. Healthy worms are defined as having both anterior and posterior segments that are inspected via a microscope. Algae and microplastics (μP, polyethylene microspheres, diameter 68 ± 6 μm, and density 1.35 g/cc) were used as the organic and inorganic materials, respectively, for the test materials. Prior to exposure to the test materials, each worm underwent a one-hour control period in the arena containing only water. In each trial, 1 mg of dry material was well mixed and dispersed into the water in the glass portion of the Petri dish. Each trial was repeated five times.

Data analysis

Images captured from the webcam were processed using ImageJ software (Schindelin et al. 2012). First, noisy elements were removed from the image by subtracting the background. Then, each stack was binarized with the same color threshold settings to isolate only the green colors corresponding to algae. Finally, the total pixel areas were calculated, corresponding to algae over time, by analyzing the stack. To obtain the relative amount of algae collected, the data were normalized by dividing each stack by the maximum threshold area of the entire stack, which we refer to as the relative material collected. Finally, we shifted the curve to offset it to zero percent.

Image stacks from the microscope camera were downsampled to 1 FPS using Adobe™ Premiere Pro for analysis. The same protocol as previously described to isolate and analyze the respective colors of materials was followed here. To estimate a worm's posture, similar thresholding as previously described was performed to segment the worm from the material. A metric called "extent" (r_{ee}) of the worm was defined to estimate the distance between two of the farthest away pixels in the segmented image of a worm. This metric was used to measure how "exploratory" (large r_{ee}) or curled up (lower r_{ee}) the worm was. Using this pipeline, the r_{ee} of a single worm was compared in material versus the same worm in a clean environment.

Results

Blackworms' collective–gathering of algae

We first describe blackworms' collecting–gathering behavior, focusing on algae as the organic particulate material (Fig. 2). We also assess the impact of population size on this behavior. Instances are presented for three population sizes (Fig. 2, top). The camera initially detects only a small amount of finely mixed algae due to its spatial concentration in the arena. As worms move around, the dispersed algae aggregates into a darker green pigment, increasing the relative material collected, which we refer to as the aggregation phase. After this phase, worms consolidate the algae into a singular blob, decreasing the color as they assimilate it and block it from the camera's view (Fig. 2, bottom, and Supplementary Information Fig. 3, top 3 panels). We refer to this as the consolidation phase. Moreover, we observe that for a specific population size, the transition from aggregation to consolidation, if observed, typically occurs at the peak of the curve (represented by red dots in Fig. 2, bottom).

The relative algae collection for $N = 50$ worms reaches a maximum at ∼25 min as they transition from aggregation to consolidation. The reduction in blackworm population within the arena leads to a noticeable delay in the overall collection of algae, as evidenced by the more than two-fold increase in the time required to reach maximum aggregation for $N = 25$ (blue curve) compared to $N = 50$ (orange curve). This suggests that a larger population of blackworms enhances food resource collection and aggregation, possibly due to a greater number of individuals or to social amplification (Amé et al. 2006; Savoie et al. 2023). We note that blackworms weakly smell algae and strongly smell each other, as tested using an olfactometer (see Supplementary Information Figs. 1 and 2). Further reducing the population to $N = 10$ (yellow curve) leads to aggregation but an overall failure to reach the consolidation phase within the 90-min period.

Across these collective–gathering experiments, we observe that during the first ∼30 min of the aggregation period, the collection is similar for all population levels. On individual worms, the algae appears to adhere to the worm's body, coalescing into a larger clump as it moves down onto its tail, which may sometimes appear as a "hook" shape (Fig. 3 algae at t = 0, 3 min, and Supplementary Movie 3). Therefore, we next investigate the potential mechanisms by which a single worm can aggregate material in detail.

Single worm collecting–gathering biophysics

Under microscopic examination in a small arena, we analyze how individual blackworms collect both algae and microplastics. Two distinct aggregation methods are observed: "threading" and "peristaltic" aggregation, which are used by worms when gathering both algae and microplastics (Fig. 1, Supplementary Movies 2 and 3). In "threading" behavior, a worm aggregates material along its body is by passing its anterior

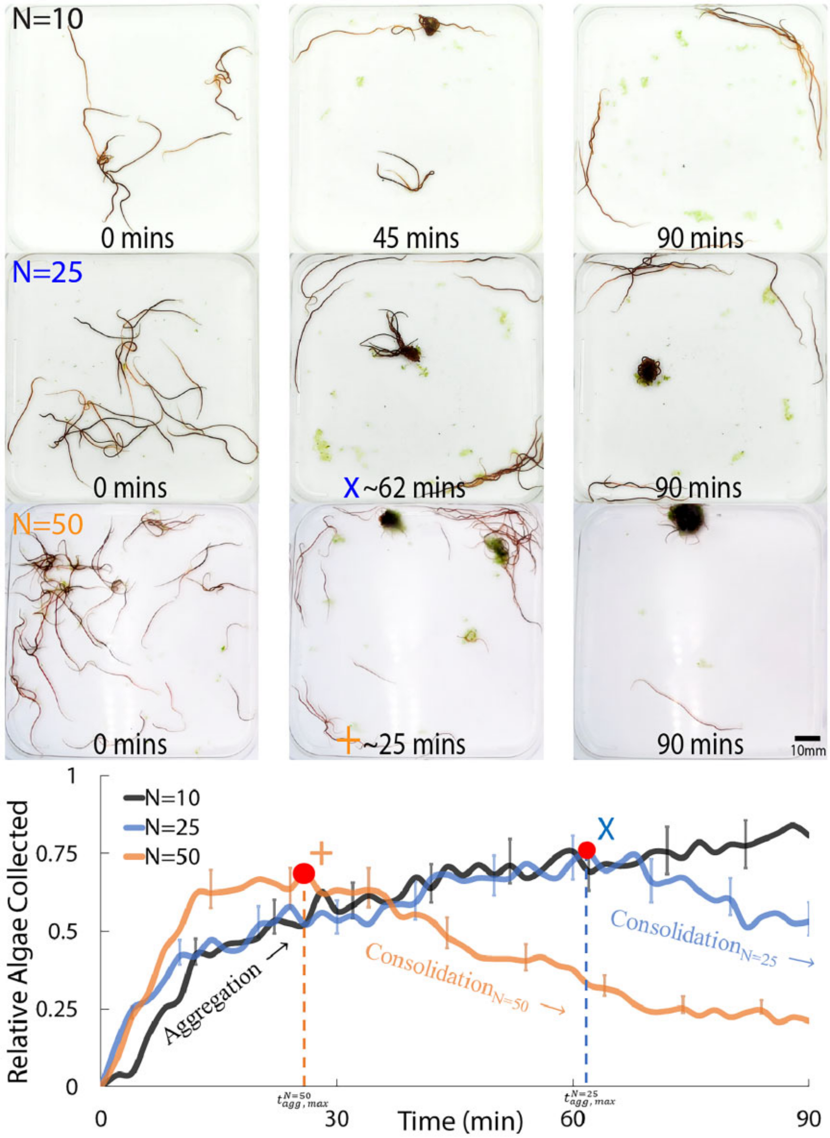

Fig. 2 Collective–gathering of algae by blackworms. (Top) Three blackworm trials with $N = 10, 25,$ and 50 worms collecting 10 mg of algae. The maximum aggregation period for $N = 25$ and 50 are labeled in the graph below. (Bottom) Graph showing the relative algae collected for the three populations over time. The data were obtained by thresholding algae in ImageJ. Each curve represents the average relative algae collected, and the vertical bars show the standard error for $n = 5$ trials. (Supplementary Movie 1).

segments through the clump on its tail (Fig. 3, top panels from t = 3 to 10 mins, and Supplementary Movie 2). In "peristaltic" aggregation, clumps of particles move downwards along the worm's body towards the tail (Fig. 3, middle panels from t = 3 to 10 min, and Supplementary Movie 3). We observe worms using these two methods regardless of the material (algae or microplastics).

When we compare the efficiency of single worm collection to the larger arena behavior, we find similar patterns with algae. We note that unlike algae, which present a green color detection limit due to small size and low concentrations initially, microplastics display a consistent white color, resulting in a plateauing value of material collected over time (Fig. 3, bottom left and Supplementary Information Fig. 3, bottom 2 panels). The dynamic interaction between worm and material shows that algae and microplastics adhere to the worm's entire body and gather at the tail due to external mucus secretions (Fig. 3).

During the microplastic experiments, blackworms did not consume any of the particles. However, prolonged exposure (hours) results in worms consuming some of the particles that are visible inside their digestive tract (Fig. 3, bottom right). Furthermore, the inset image shows that the blackworm's excretion results

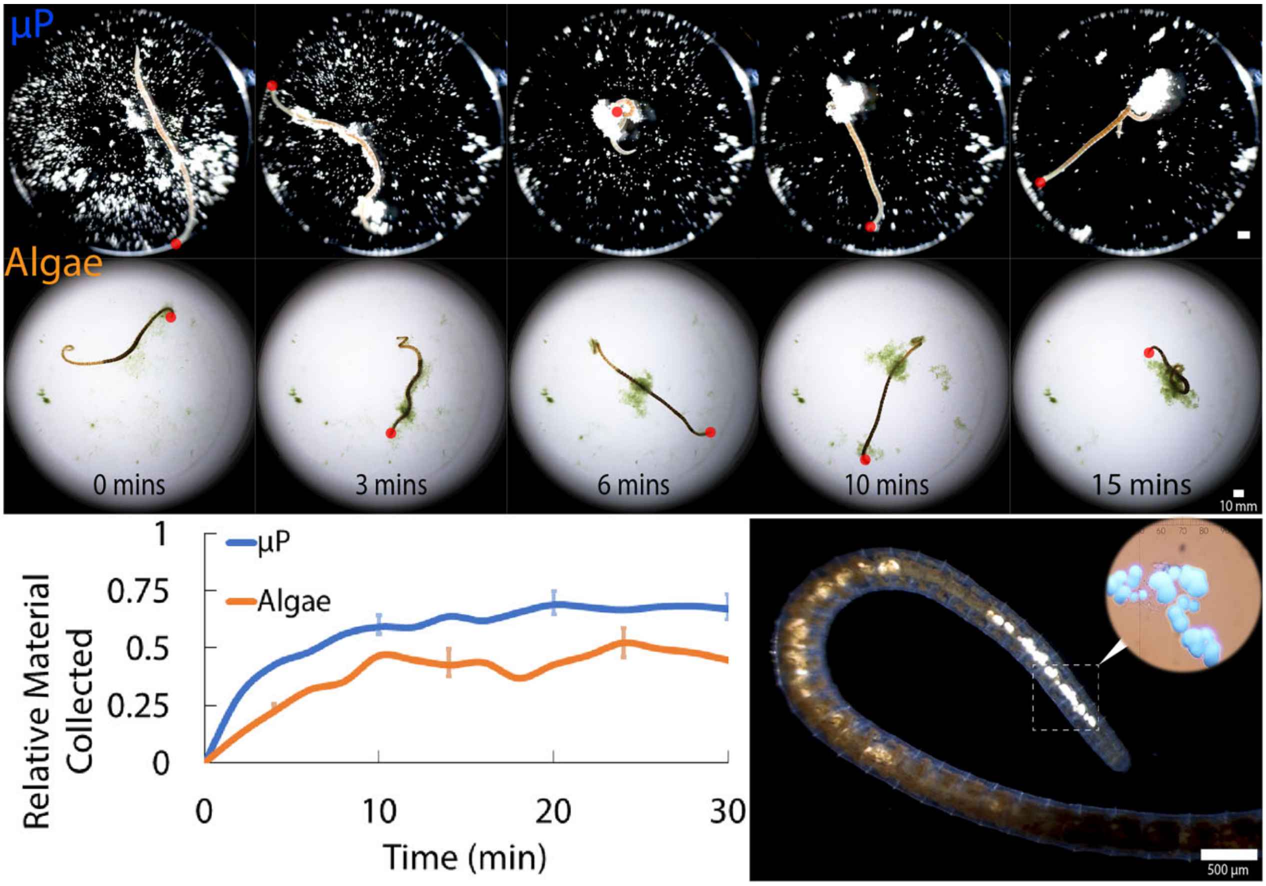

Fig. 3 Single worm collecting microplastic materials. Five instances of a worm collecting 1 mg microplastics (μP). The red dot denotes the worm's head. The top panel shows a single worm collecting microplastics (μP, polyethylene microspheres, diameter 68 $\pm$ 6 μm, and density 1.35 g/cc) into one large clump. Microplastics passively adhere to a worm's body due to externally secreted mucus. At $t = 6$ min, the worm passes through a clump on its tail, consolidating particles along its body (Supplementary Movie 2). The middle panel shows a single worm collecting finely dispersed algae. A small clump moves downward along the worm's body as it moves around (Supplementary Movie 3). The bottom left panel depicts a graph showing the relative material collected from μP (blue curve) and algae (orange curve) over 30 min. The relative material collected is calculated by thresholding each respective color in ImageJ. Each curve represents the average, with the vertical line showing the standard error for $n = 5$ trials. The bottom right panel shows microplastics inside the digestive tract of a blackworm after several hours of exposure. The ingestion of microplastics can lead to enhanced clumping of particles, as shown in the inset where the excretion of the blackworm results in the formation of a cluster of microplastics. (Supplementary Movie 4).

in enhanced microplastic clumping, suggesting that the internal mucus secreted by the worm's body may also play a role in the aggregation of these particles.

We observe that individual worms exhibit relatively less stretched-out movement when the material is in a well-aggregated form around their bodies (Fig. 3, top panel, algae snapshot at 15 min). To quantify the extent of the worm's exploration, we measure the largest distance (r_{ee}) between the two furthest pixels on the worm's body. While the relative material collected shown in large arena (Fig. 2) and small arena (Fig. 3) experiments quantifies the size of the debris cluster, the low-dimensional metric r_{ee} is a measure to estimate the extent and exploration of the worm itself. The evolution of the metric r_{ee} to estimate the extent of the worm is plotted for 30 min of dynamics (Fig. 4, top panels). The black curve in the upper panel is the evolution of r_{ee} for the case of a worm without any algae present in the Petri dish (control), while the blue curve is the same metric in the presence of algae. The videos (see Supplementary Information Movies 2 and 3) show that the worm begins to explore around in the Petri dish. This exploration, in addition to sticky mucus around the body, leads to the clumping of these algae around the worm. It is observed that the worm reduces its exploration after this clumping occurs, which is evident in the reduced r_{ee} in the later stages of the dynamics. Based on previous observations and literature (Timm and Martin 2015; Tuazon et al. 2022), we hypothesized that the reduction in exploration after clumping occurs is due to thigmotaxis, or

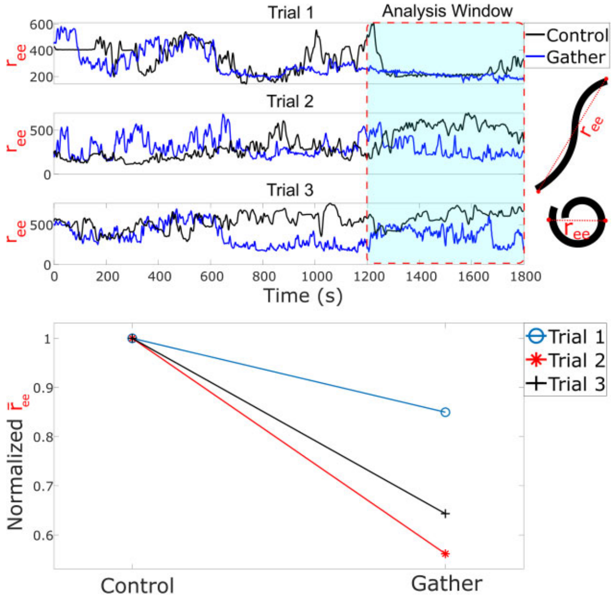

Fig. 4 Estimating worm exploration. (Top) Timeseries of three different worm trials in two scenarios: (1) without any debris (control, in black) and (2) in the presence of well-mixed algae (gather, in blue). (Bottom) Average value of r_{ee} for the last 10-min window in the top panel, normalized with respect to their respective control values.

the worm's natural tendency to move towards physical contact with a surface, which in this case is the clumped material on its body (Patil et al. 2023). The average r_{ee} of the last 10 min of dynamics is plotted for the control and the experiments with debris (Fig. 4, lower panel). This average r_{ee} value for the control experiment is set to unity for each trial for normalization. We found that the average r_{ee} reduces substantially (10–40% reduction, $n = 3$) when there are algae present in the Petri dish. To summarize, our experiments with individual worms show that the uniformly spread particles in the Petri dish begin to clump due to the worm's motion coupled with its sticky mucus on its outer layer. This causes the worm to extend its slender body less and maintain contact with the collected debris. In the next section, we explore the influence of the worm's adhesive properties on collecting efficiency by utilizing an active polymer model of the worm.

Modeling collecting–gathering

Given our observations of worm behavior in collecting and aggregating particles, we develop a computational model to understand further how worms could accomplish this task. We use an active polymer model of worm dynamics to show that collecting–gathering behavior can emerge from only self-propelled movement and short-range attraction to particles (Fig. 5). Our model is similar to the model described in Nguyen et al. (2021): we represent a worm as a self-propelled active polymer subject to spring, bending, and modified Lennard–Jones-type (or van der Waals) interaction potentials. A detailed description of the model can be found in the Supplementary Information. We use many of the same parameters as in Nguyen et al. (2021), experimentally motivated from single blackworm behavioral assays, which are tabulated in Table 1. As in Nguyen et al. (2021), the movement of the worm at each time step is determined by the net force acting on the worm, plus noise, as quantified by a temperature T. Here, the temperature T is set to 0.274 in model units, corresponding to 20°C, following the relation in Nguyen et al. (2021).

The worm is constrained to move within a round arena of a diameter 1.14 times the worm's length, reflecting the parameters of the experiments described in this paper. Additionally, we simulate microplastics as $N_p = 100$ "hard" (represented by an excluded volume potential) particles of size $0.25\pi\sigma^2$ randomly distributed within this arena. The worm experiences short-range attraction to a given particle, modeled using the same interaction potential that governs interactions between monomers of a worm (see Supplementary Information for a mathematical description), and scaled by the worm-particle interaction strength ε_{wp}. This interaction occurs only when the worm is within a short distance (5σ) of a particle. We set the value of the interaction strength $\varepsilon_{wp} = 2$. This value is chosen to ensure efficient gathering: too small of an interaction strength would result in the worm merely passing by particles without collecting them, and too large of a value would restrict the worm's locomotion. We also demonstrate the inefficiency of gathering at low attraction, $\varepsilon_{wp} = 0.1$, and at high attraction, $\varepsilon_{wp} = 15$ (see Supplementary Information Fig. 4).

We characterize the efficiency of the collecting–gathering behavior by quantifying the average particle cluster area as a function of time (Fig. 6). To identify particle clusters, we use the DBSCAN algorithm (Ester et al. 1996), implemented in MATLAB, to define a cluster as any group of five or more particles separated by no >1 particle width. We then compute the convex hull of each cluster and its corresponding area, also in MATLAB. Particles not identified as belonging to clusters from this algorithm are assigned to clusters of size 1 particle area (A_p, see Table 1). We then determine the average area over all clusters and isolated particles.

In experiments, we observe that worms secrete a mucosal substance that not only allows particles to adhere to the worm, but can also bind particles together. To simulate this, we implement an additional attraction between particles, again following a

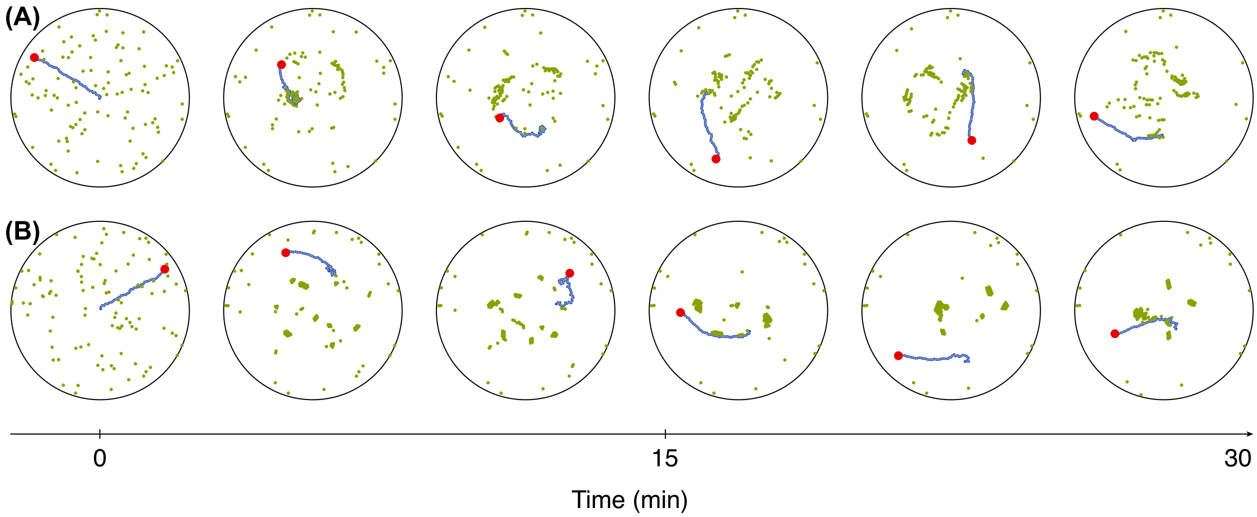

Fig. 5 Active polymer worm model of collecting–gathering behavior. We simulate collecting–gathering behavior in an active polymer model of a worm (blue, with a red dot denoting the head) governed by self-propelled tangential movement and short-range attraction to particles (green). (A) The worm gathers particles into clusters less efficiently in the model with $\varepsilon_{pp} = 0$, i.e. with no attractive force between particles. (B) The worm gathers particles more efficiently in the model with an attractive force between particles with interaction strength $\varepsilon_{pp} = 1$, where the attraction represents the worms' mucosal secretions that bind particles together. (Supplementary Video 5).

Table 1 Parameters used in the active polymer model of collecting–gathering behavior, in both model and real-world units

Parameter	Description	Value(s) (model units)	Value(s) (real units)
N_m	Number of monomers	40	40
σ	Equilibrium distance between monomers	1.189	0.44 mm
L_w	Equilibrium worm length	40σ	17.6 mm
Δt	Time step	1	0.08 s
D	Arena diameter	45.6σ	20 mm
ε	Interaction strength between monomers	1	3.5×10^{-12} J
k_s	Spring constant	5000	0.12 N/m
k_b	Bending stiffness	10	3.5×10^{-11} J/rad
F_{active}	Self-propulsion force magnitude	340	3.2×10^{-6} N
T	Temperature	0.274	20°C
N_p	Number of particles	100	100
A_p	Area of particle	$0.25\pi\sigma^2$	0.15 mm^2
ε_{wp}	Interaction strength between worm and particle	{0.1, 2, and 15}	{0.35, 7, and 52} $\times 10^{-12}$ J
ε_{pp}	Interaction strength between particles	{0, 1}	{0, 3.5} $\times 10^{-12}$ J

The conversion between reduced model units and real units is detailed in the Supplementary Information.

Lennard–Jones-type interaction potential (see Supplementary Information for details) with particle-particle attraction strength $\varepsilon_{pp} = 1$ (Fig. 5B and Supplementary Information Movie 5). This particle-particle attraction results in significantly increased gathering efficiency compared to the model without particle-particle attraction (Fig. 6). These results are obtained for a worm-particle interaction strength $\varepsilon_{wp} = 2$, which produces optimally efficient gathering. We observe significantly reduced efficiency for much lower or higher values of ε_{wp} (See Supplementary Information Fig. 5).

Discussions

Numerous benthic organisms in aquatic environments, including blackworms, modify their surroundings by burrowing and feeding through a process called bioturbation (Kristensen et al. 2012). By burrowing into sediment and keeping their tails above the surface, blackworms ingest sediment from deeper layers and egest it

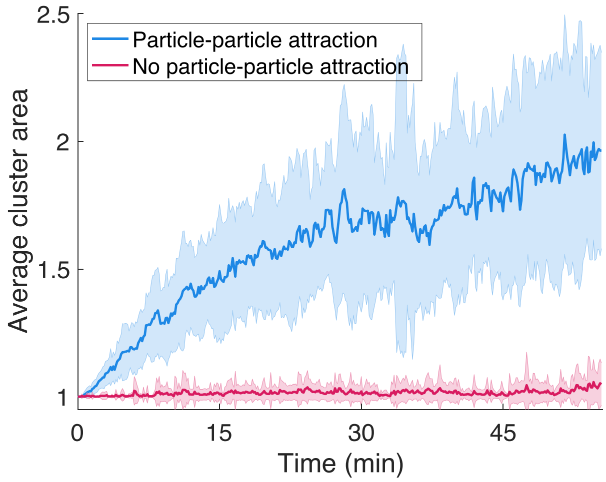

Fig. 6 Gathering efficiency in the active polymer model. The collecting–gathering efficiency of the active polymer worm is quantified by the average area of particle clusters as a function of time, normalized by the area of 1 particle, for the model without particle-particle attraction (pink) and with particle-particle attraction $\varepsilon_{pp} = 1$ (blue). The worm-particle interaction strength is $\varepsilon_{wp} = 2$. An average of over 20 simulations is shown for each case, with the shaded regions representing one standard deviation.

at the surface, making them an upward conveyor. As a biodiffusor, blackworms also alter interfacial sediment through their movement. These behaviors can lead to the formation of significant mounds of sediment (Roche et al. 2016). Blackworms are known to exhibit bioturbating behaviors and are classified as collecting–gathering detritivores based on their feeding behavior. Our results show that the blackworms' collecting–gathering behavior is influenced by population size, with doubling the population resulting in a reduction of time to reach maximum aggregation by at least half. This increased efficiency could be due to a greater number of individuals or better social and chemical signaling, as blackworms may communicate through olfactory cues (see Supplementary Information Fig. 1).

By observing the dynamics of individual worms under a microscope, we are able to gain insight into how blackworms aggregate organic and inorganic particles. Our results demonstrate that worms are capable of effectively collecting particles by using movement and externally secreted mucus, achieving a single clump for both organic and inorganic materials within roughly 10–15 min. We identify two distinct methods of aggregation employed by the worms. The first method, termed "threading" aggregation, involves the worm passing its anterior segments through the clump of material on its tail. This process is clearly observed in the microscope images and Supplementary Movie 2, showing the progressive buildup of material along the worm's body. The second method, known as "peristaltic" aggregation, occurs as the worm's movement causes a small clump of material to move downwards, bringing together any collected particles towards the tail. This process is evident in the microscope images and Supplementary Movie 3. Importantly, we note that these aggregation methods are observed across different types of materials, indicating their consistent usage by the worms.

Our observations suggest that the blackworms' collecting–gathering behavior is not limited to organic matter, and we have also observed them excreting microplastics after prolonged exposure. Although we did not observe them actively feeding on these particles, the latter observation implies that worms consumed microplastics, which is corroborated by other literature that study their physiological effects on blackworms (Beckingham and Ghosh 2017; Scherer et al. 2017; Klein et al. 2021; Silva et al. 2021).

In nature, as blackworms ingest settled detritus, they could also ingest inorganic material that resides in waterbeds, such as microplastics, which can accumulate significantly at the sediment-water interface of benthic zones in freshwater ecosystems (Krause et al. 2021). Consuming microplastics has previously been shown to cause a severe negative impact on their physiology, such as reduced energy reserves, activation of antioxidants and detoxification mechanisms, and an overall reduction of survival (Klein et al. 2021; Silva et al. 2021). Consequently, the presence and accumulation of microplastics in freshwater ecosystems is of growing concern, as these environments are microplastic retention sites that can transport them downstream to oceans and other bodies of water (Krause et al. 2021). The ingestion of microplastics by benthic macroinvertebrates also raises the concern of microplastic transfer across trophic levels. Benthic macroinvertebrates are eaten by benthivorous fish (Winkelmann et al. 2007), which are consumed by piscivores or larger predators (Vander Zanden and Vadeboncoeur 2002), which could lead to the biomagnification of microplastics along the freshwater food chain. Comparatively, it has been shown in marine environments that it is possible for the trophic transfer of microplastics to occur, as seen in the transfer of microplastics from mussels to crabs (Farrell and Nelson 2013). This is concerning not only to the health of marine and freshwater ecosystems, but to the health of humans that consume animals from these water sources, as microplastics have been recently found in the human bloodstream (Leslie et al. 2022). Though the health risks posed to humans by microplastics have not yet been defined, it is hypothesized that as more microplastics are introduced into the environment and become increasingly bioavailable, health risks to humans will become apparent, as they have in a wide range of other species

(Koelmans et al. 2022). The idea that blackworms can aggregate materials through ingestion and excretion has been previously explored for sludge reduction, where blackworms have been shown to ingest waste sludge and excrete it as compact feces, decreasing its sludge volume index by half (Elissen et al. 2006). However, the aggregation via excretion of microplastics by blackworms has not been explored to our knowledge.

Limitations and future outlook

One limitation is that we used a simplified experimental setup compared to the natural environment of blackworms. For instance, we conducted our experiments in a static system, whereas blackworms in the wild may be exposed to flowing currents or predators that could affect their feeding and aggregation behavior. Additionally, we used a relatively small number of replicates in our experiments, which could increase the likelihood of random variation influencing our results. However, we found that our experimental system was robust enough to generate consistent results across experiments. Future studies could use larger sample sizes and different experimental conditions to further investigate the behavior of blackworms. Additionally, consolidation of algae particles was never reached in $N = 10$. Further studies could vary particle concentration and arena size to elucidate if there is a critical population needed to consolidate particles based on particle concentration.

Another limitation of our study is that we focused on only one species of worms, *L. variegatus*, which may not be representative of other worm species or other organisms in general. Additionally, our study focused mainly on the physics and biology of the mechanisms underlying blackworm behavior, rather than the ecological or evolutionary implications of this behavior. Future studies could investigate how the gathering–collecting behavior of blackworms affects their survival and reproduction, as well as how this behavior may have evolved over time.

Finally, we acknowledge that our attempt to analyze the worm's exploration in the microscope studies was limited by the significant blockage caused by the white microplastic particles. Future studies could use alternative methods to evaluate exploration behavior, such as using a different type of particle or color. Despite these limitations, our study provides valuable insights into the behavior of blackworms and opens up avenues for further research into their dynamics as a collecting–gathering organism (Cummins and Klug 1979; Ilyashuk 1999).

Conclusions

We have investigated the collecting–gathering behavior of blackworms using image analysis and simulations, providing new insights into this FFG phenomenon. Our results show that blackworms can efficiently aggregate and consolidate distributed particles into larger clumps using externally secreted mucus on their bodies and movement. This behavior is influenced by population density and the type of material being collected. Our analysis of the extended length of the worm suggests that worms reduce their movement after clumping enough material, potentially due to thigmotaxis. In addition, our simulations have validated the biophysical mechanisms underlying the collecting–gathering behavior of blackworms, demonstrating that this behavior can emerge from self-propelled movement and short-range attraction to particles through secreted mucus. Consequently, we also found evidence that blackworms can collect and consume synthetic materials such as microplastics.

Overall, our findings have implications for the design of synthetic systems inspired by the behavior of blackworms and for understanding the ecological impacts of microplastics. Our study provides new insights into the mechanisms behind collecting–gathering behavior and its potential applications in engineering and environmental science.

Author contributions

H.T. and M.S.B. conceptualized the research. H.T. designed the experiments. H.T., E.K., J.B., and D.C. conducted the experiments, for which H.T., I.T., and J.B performed the analysis. O.P. and C.N. conceptualized and designed the computational model. C.N. performed simulations and analysis. M.S.B. supervised the research. All authors contributed to writing, discussion, and revising the manuscript.

Acknowledgments

We thank members of the Bhamla lab for useful discussions and Dr. Emily Weigel for providing expert feedback related to topics of this work. Finally, we thank NSF Aquatic Ecology summer REU for supporting D.C. Text in this paper was revised using ChatGPT-4.

Funding

H.T. acknowledges funding support from the NSF graduate research fellowship program (GRFP) and Georgia Tech's President's Fellowship. C.N. and O.P. acknowledge funding support from the BioFrontiers Institute at the University of Colorado, Boulder. M.S.B. acknowledges funding support from NIH Grant R35GM142588; NSF Grants MCB-1817334; CMMI-2218382; CAREER IOS-1941933; and the Open Philanthropy Project.

Supporting data

Supplementary data available at *ICB* online.

Competing interests

The authors declare no competing interests.

Data availability statement

Data available upon request.

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