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Leeches Predate on Fast-Escaping and Entangling Blackworms by Spiral Entombment

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Abstract

We investigate how the *Helobdella sp.* freshwater leeches capture and consume *Lumbriculus variegatus* blackworms despite the blackwormâĂŹs ultrafast helical swimming escape reflex and ability to form large tangled âĂŸblobsâĂŹ. We describe a spiral âĂŸentombmentâĂŹ predation strategy, where *Helobdellid* leeches latch onto blackworms with their anterior sucker and envelop them in a spiral cocoon. Quantitative analysis shows that larger leeches succeed more often in entombing prey, while longer worms tend to escape. The rate of spiral contraction correlates with entombment outcomes, with slower rates associated with success. These insights highlight the complex interactions between predator and prey in freshwater ecosystems, providing new perspectives on ecological adaptability and predator-prey dynamics.

Key words: predator-prey, physically entangled collective behavior, worm blobs, blackworms, *Lumbriculus variegatus*, *Helobdella*, leeches, entombment

Predatory Behavior of Helobdella Leeches

Freshwater leeches, particularly *Helobdella* sp., are known for their diverse dietary habits, consuming a variety of prey ranging from oligochaetes to mollusks and insect larvae (Kutschera et al. 2013; Saglam et al. 2018, 2023). As predators in small ponds and lakes, they serve as models for exploring ecological and evolutionary themes, including interspecies competition and niche overlap (Govedich et al. 2010). *Helobdella* leeches, characterized by a pale gray or yellow hue, a flattened head with twin ocular spots, and a body length under two centimeters (Fig.1c, and inset). Unlike macrophagous leeches that ingest prey whole, liquidosomatophagous leeches like *Helobdella* draw nutrients from the bodily fluids of their prey using a specialized proboscis, showing a preference for oligochaetes, as evidenced by oligochaete proteins in their digestive systems (Kutschera et al. 2013; Sawyer 1972, 1986).

The interaction between *Helobdella* leeches and California blackworms, *Lumbriculus variegatus*, exemplifies complex predator-prey relationships in freshwater ecosystems (Fig.1a). *Helobdella* display unique trapping techniques when targeting agile oligochaetes like the blackworm (Fig.1b). Among oligochaetes, *Helobdella* show a preference for blackworms or *T. tubifex* (sludge worms), depending on habitat productivity levels (Young and Ironmonger 1980; Young 1980; Young and Procter 1985).

Among oligochaetes, *Helobdella* species exhibit a preference for blackworms or *T. tubifex* (sludge worms), depending on the productivity levels of their habitats, where higher productivity indicates greater availability of organic material and prey organisms (Young and Ironmonger 1980; Young 1980; Young and Procter 1985).

Blackworms exhibit rapid escape mechanisms through a helical swimming, body shortening gait, and also from protective entangled collectives, or "worm blobs," comprising up to thousands of conspecifics (Patil et al. 2023; Drewes and Fourtner 1989; Drewes 1990). This collective behavior, much larger in size than the leeches themselves, provides a unique perspective on natural selection pressures and biomechanical influences on predatory behaviors (Ozkan-Aydin et al. 2021; Patil et al. 2023; Tuazon et al. 2022, 2023). These robust escape reflexes give blackworms a survival advantage against predators like *Helobdella* leeches. Given these defenses, we ask: How do *Helobdella* leeches capture and consume blackworms despite the blackworms' ultrafast helical swimming escape reflex and ability to form large entangled collectives?

In this study, we describe our discovery of a spiral 'entombment' strategy used by *Helobdella* leeches to overcome the blackworms' active and collective defenses. Unlike their approach to less reactive and solitary prey like mollusks, where leeches simply attach and suck, *Helobdella* leeches employ this spiral entombment strategy for blackworms. We examine the behaviour strategy of this predatory action, exploring how these leeches manipulate their environment to encase and consume a worm. We reveal how the entombment process overcomes the dynamic defenses of worm blobs.

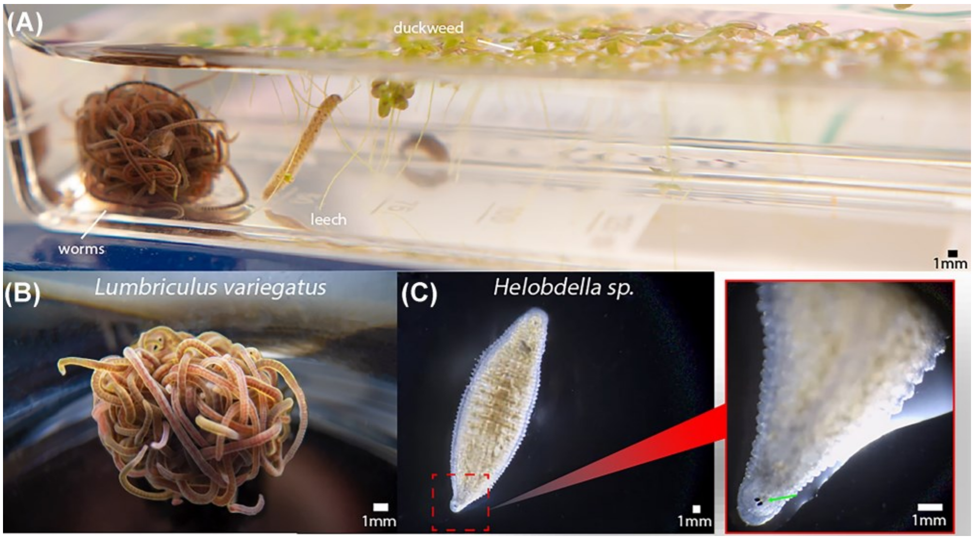

Fig. 1. Interaction Between *Helobdella* sp. Leeches and *Lumbriculus variegatus* blackworms. **(a)** *Helobdella* sp. uses its anterior sucker to explore the area near the roots of duckweed and an entangled worm blob of N 20 *Lumbriculus variegatus* blackworms. **(b)** The prey: California blackworms (*Lumbriculus variegatus*) blob formation. **(c)** The predator: Freshwater leech: *Helobdella* sp. The inset shows the simple eyes (green arrow) of the leech.

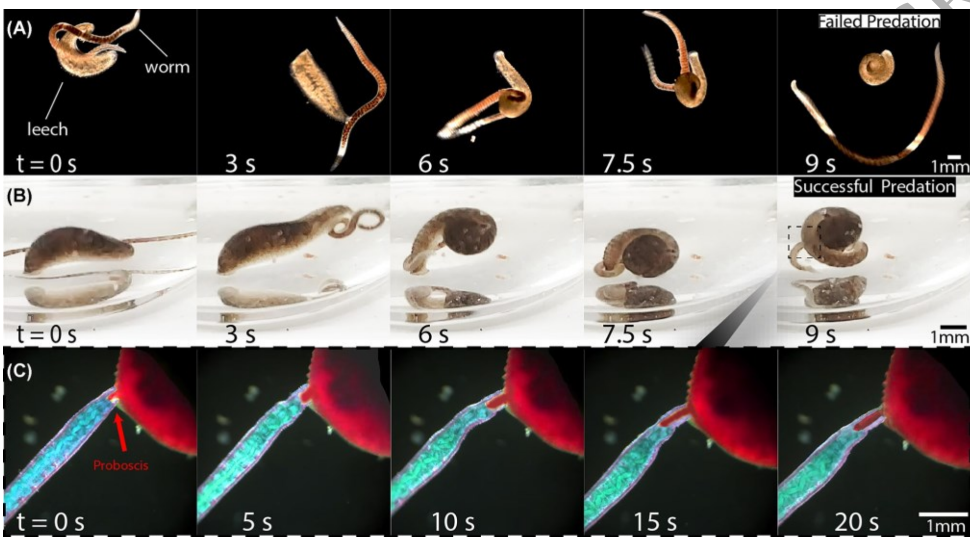

Fig. 2. Predation strategy of *Helobdella* sp. against *Lumbriculus variegatus* **(a)** Sequential frames of a failed predation attempt by a leech from the top perspectives. **(b)** A separate but successful predation attempt from the side view. The time stamps for both (a) and (b) are approximately similar. Initially, the leech secures a position along the worm's body with its anterior sucker at t=0s and proceeds to stretch out at t=3s. In the ensuing entombment phase, spanning t=3s to t=7.5s, the leech performs a series of coordinated ventral folds coupled with a slight medial flexure, actions intended to form a cavity to entrap the worm. Failure to fully ensnare the prey often leads to its escape as shown in t=9s (a). If successfully trapped and immobilized as shown in t=9s (b), **(c)** the leech employs its proboscis (red arrow) to extract the internal contents of the worm. False coloring is added for visual clarity. (Supplementary videos 1 and 2)

Materials and methods

Animals

We sourced California blackworms (*Lumbriculus variegatus*) from Ward's Science, where they often came with freshwater leeches (*Helobdella sp.*) as unintended pests. Both organisms were reared in a plastic box (35 X 20 X 12 cm) with filtered water at room temperature (~ 21 °C). Blackworms were fed tropical fish pellets twice a week and their water was exchanged daily.

Initially, we identified these leeches as *Helobdella stagnalis* based on general morphological features (Govedich et al. 2010). Later, more detailed morphological assessments as described by Saglam, et al. suggested they are more accurately as *Helobdella echoensis*, particularly due to the distinct positioning and shape of the eyes (Fig.1c, inset) (Saglam et al. 2018). In the absence of comprehensive molecular analyses such as DNA or RNA sequencing for identification, we refer to these organisms broadly as *Helobdella* sp. or simply freshwater leeches throughout this paper.

We maintained the rearing conditions for the worms as described in our earlier publications (Tuazon et al. 2022, 2023). We isolated the freshwater leeches from the worms and stored them in refrigerated conditions. Before the experiments, we

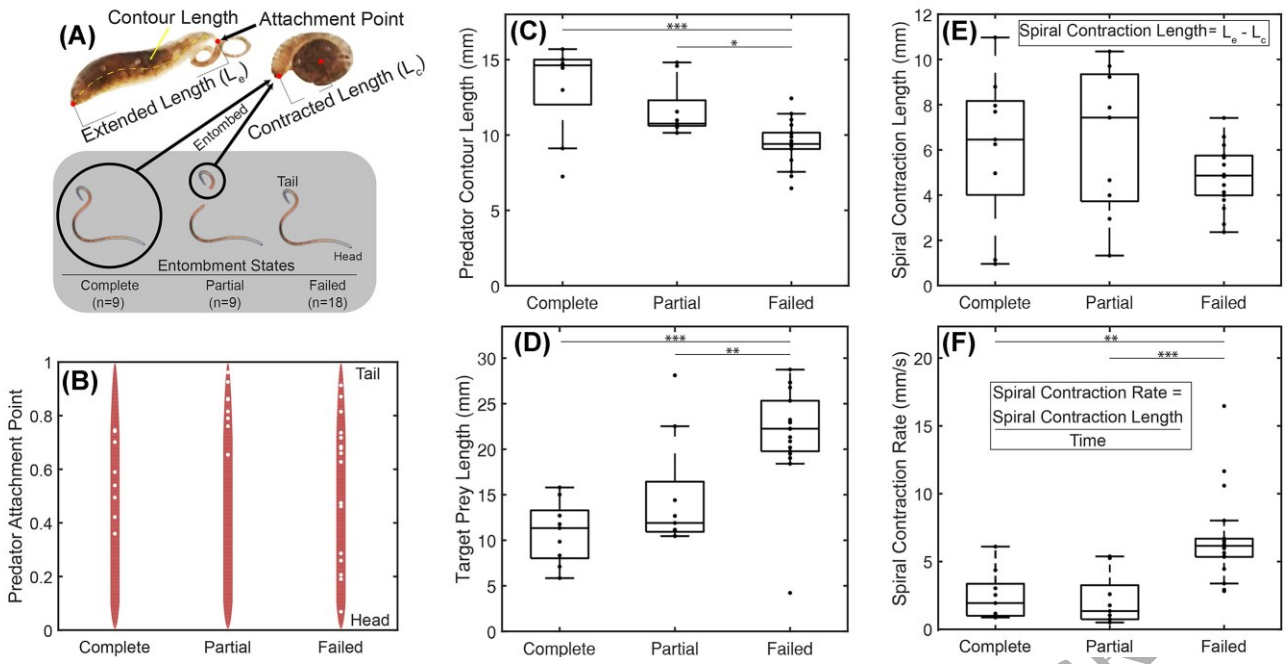

Fig. 3. Quantitative Analysis of Leech Entombment Dynamics (a) Schematic representation of measurement definitions: *Contour Length* measures the length of the leech, incorporating its natural curves. *Extended Length* (L_e) and *Contracted Length* (L_c) are the straight-line distances measured from posterior to anterior sucker before and after contracting, respectively. The *Attachment Point* indicates where the leech's anterior sucker is placed, relative to the worm's body. Entombment outcomes are defined as *Complete* (entire or majority of the worm is entombed, preventing escape), *Partial* (only a part of the worm is entombed due to fission), and *Failed* (worm escapes the attempt). (b) Distribution of attachment points by the leech along the worm's body, showing the preferred regions for each entombment outcome. For partial entombments, attachment points appear to be predominantly near the tail, whereas complete entombments frequently occur nearer to the center of mass. Failed attempts show no consistent pattern. Statistical test indicates no significant differences across the groups (ANOVA p = 0.251) (c) Variation in *Predator Contour Length* across different entombment outcomes, demonstrating that larger leeches are statistically more likely to successfully entomb their prey (ANOVA p = 0.000213) (d) *Target Prey Length* and its impact on entombment outcomes: longer worms have a statistically higher likelihood of escaping, suggesting size as a critical factor in prey defense mechanisms (ANOVA p = 0.000029). (e) *Spiral Contraction Length* ($L_e - L_c$) was measured to explore whether greater contraction displacements could theoretically enhance grasping force. No significant differences were observed across entombment types (ANOVA p = 0.264), suggesting that how much a leech contracts does not directly influence entombment success (f) Dividing *Spiral Contraction Length* over time yield *Spiral Contraction Rate*. Faster contraction rates in failed attempts often result from the leech exerting a constant force as the worm's mass is released during escape, causing a 'whiplash' effect (see Supplementary video 3). Statistical analysis for each panel was conducted using one-way ANOVA followed by Tukey's post-hoc test to assess differences between groups. Significance levels are denoted as: * p < 0.05, ** p < 0.01, and *** p < 0.001.

subjected the freshwater leeches to a starvation period of at least one week.

Microscopy Imaging

We recorded predator-prey interactions using a Leica MZ APO microscope (Heerbrugg, Switzerland) equipped with an Image-Source DFK 33UX264 camera (Charlotte, NC) at 30 FPS. We used a goose neck light to illuminate the arena.

Behavioral Testing

The arena was a 35 mm petri dish containing filtered water, prepared using a reverse osmosis (RO) unit from tap water. Filtered water was treated by adding salts (NilocG Aquatics REKHARB carbonate hardness (KH) booster and NilocG Aquatics REGEN general hardness (GH) booster) to create spring water conditions with a total dissolved solids (TDS) concentration of approximately 50 ppm, as recommended by the manufacturer. For each trial, we randomly selected healthy worms measuring 18.16Âś1.08 mm and a leech measuring 10.89Âś2.53 mm (contour length) and placed them in a 35 mm petri dish with 5mm (height) of filtered water. Each videos are 18 hours long and were run overnight. Before introducing the prey, each leech spent one hour in the arena containing only water as a control period. We repeated each trial n = 9 times, isolating 36 predation attempts.

We defined "entombment" as any action where the leech uses its body to envelop or attempt to envelop a worm. We categorized entombment into three distinct outcomes: "complete entombment", where the leech successfully envelops the entire worm or a major portion of it, effectively immobilizing the prey; "partial entombment", where only a part of the worm is enveloped and often results in the wormâĂŹs fission, allowing the leech to feed on the detached segment; and "failed entombment", where the worm completely escapes the leechâĂŹs grasp. A "successful" predation session was defined as one resulting in either complete or partial entombment, where the leech successfully feeds on the worm. Behaviors were scored based on video analysis, with each entombment attempt documented and categorized according to these criteria.

Data Analysis

For each experiment, recordings were processed and interactions were isolated using Adobe[c] Premiere Pro. The MP4 was then imported into ImageJ for frame-by-frame image analysis.

Statistical Analysis

One-way ANOVA tests followed by TukeyâĂŹs post-hoc tests were employed to compare means between groups and determine statistical significance of the results. Significance levels are denoted as follows: * for p-values ≤ 0.05, ** for p-values ≤ 0.01, and *** for p-values ≤ 0.001.

Spiral Entombment Predation Strategy of Helobdella Leeches

The spiral formation observed during this entombment provides biomechanical advantages such as enhanced grip and control, similar to those seen in elephants, seahorses, chameleons, and various plants and flowers (Porter et al. 2015; Herrel et al. 2013; Takaki et al. 2003). These adaptations are complemented by a series of latching, releasing, and ventilating actions that facilitate respiration in the low-oxygen, detritus-rich environments typical of their habitats (Mann 1956; Milne and Calow 1990).

While blackworms are primarily found along the shallow, stagnant edges of freshwater systems in North America and Europe, *Helobdella* sp. exhibits a more cosmopolitan distribution (Saglam et al. 2018; Kutschera and Weisblat 2015; Timm and Martin 2015; Govedich et al. 2010). *Helobdella* requires substrate attachment via their posterior sucker for successful feeding, indicating a reliance on physical habitat structures for predation.

During predation of a single blackworm, *Helobdella* sp. employs a unique hunting strategy, using its anterior sucker to latch onto a worm and swiftly envelop it in a spiral cocoon (Fig.2b). This “entombment" process often requires multiple attempts and involves meticulous coordination: finding an optimal attachment point with the anterior sucker (t = 0 s), extending through flexure (t = 0 to 3 s), and executing coordinated ventral folding to form a cavity (t = 3 to 9 s). In this cavity, the leech inserts its proboscis into the entombed worm to feed on its internal liquids and tissues through liquidosomatophagy (Fig.2c) (Sawyer 1986).

Quantitative Analysis of Entombment Mechanics

We conducted image analysis to assess physical dimensions and temporal metrics associated with *Helobdella*’s entombment process, focusing on parameters hypothesized to influence predation success. These parameters include the leech’s size, the prey’s size, the initial attachment point of the leech’s anterior sucker relative to the worm’s body, and the dynamics of its contraction related to grip strength. Fig.3a illustrates our methodology for measuring the leechâĂŹs contour length (depicted with yellow dotted lines and measured using ImageJ), along with the straight-line distances for extended length (L_e) and contracted length (L_c).

We categorized entombment outcomes into three states: complete, partial, and failed. A ’complete entombment’ occurs when the leech fully envelops the worm, preventing escape (n=9) (Fig.2b). A ’partial entombment’ happens when the leech captures and feeds on a part of the worm, often resulting in fission (n=9). A ’failed entombment’ describes attempts where the worm escapes entirely (n=18) (Fig.2a). Fig.3b reveals that complete entombments often occur near the worm’s center of mass, partial near the tail, and failed attempts display no consistent pattern.

Transitioning to the impact of predator and prey sizes, Fig.3c and Fig.3d show significant influences on entombment success (ANOVA p-value < 0.001 for both). Larger leeches are more likely to succeed in entombment, while longer worms tend to escape. Tukey’s post-hoc tests reveal significant size differences between complete and failed entombments for predators ($p < 0.001$) and significant differences in prey size between failed and both partial ($p = 0.006326$) and complete entombments ($p < 0.000037$).

Finally, we explore the dynamics of the predator’s spiral entombment. Hypothesizing that larger displacementsâĂŤakin to a spring being stretchedâĂŤmight influence predation success, we find that the spiral contraction length ($L_e - L_c$) does not significantly affect outcomes (p-value = 0.264), suggesting that the extent of contraction alone is not a critical factor (Fig.3e). However, analyzing the rate of spiral contraction, calculated by dividing the contraction length by the time taken, reveals a significant correlation with failed entombments (ANOVA p-value < 0.001), particularly noting that the rates for failed attempts are markedly higher than those for complete (p-value = 0.0014) and partial entombments (p-value = 0.0007) (Fig.3f).

Defensive Mechanisms of Blackworm Blobs

Shifting from the dynamics between a single leech and an individual blackworm to a population of highly entangled worm blobs reveals a change in predation outcomes. In these complex assemblies, we observe defensive maneuvers not just by the targeted worm but by the collective, significantly impeding the leech’s predatory success.

When the leech places its anterior sucker on a worm within the blob, it encounters undulating movements as the worm, while remaining entwined with its conspecifics, vigorously resists (Fig. 4a, t=0 to 0.5s). These movements draw the entire blob towards the predator (t=0.5 to 1s), rather than isolating the prey. Subsequent attempts by the leech to envelop the entire blob through its ventral fold are thwarted by the group’s collective density, presenting an insurmountable barrier and leading to a series of failed predation attempts (Fig. 4a, t > 1.5s).

Fig. 4b captures a successful entombment by the leech, with a worm fully trapped within the formed cavity. The leechâĂŹs entombment forms a spiral that closely follows a Fibonacci pattern (see SI document for more details).

Finally, as shown in Fig. 2c, the leech inserts its proboscis to ingest the internal contents of the prey. These observations indicate that the worm blob’s defensive strategy, through collective undulation and cohesion, challenges the leech’s ability to isolate and entrap prey. This defense mechanism may suggests an co-evolved collective response, perhaps an adaptation driven by persistent predatory pressures from leeches like *Helobdella* sp.

Discussions

Our observations show that *Helobdella* sp., typically effective in feeding on less reactive prey like mollusks or *T. Tubifex*, employ a specialized entombment strategy when preying on blackworms. This adaptation allows the leeches to counter both the blackworms’ escape capabilities and collective entanglement strategy. Unlike *T. Tubifex* worms from the Naididae family, blackworms exhibit robust escape behaviors (see Supplementary video 7). Kutschera and Weisb[illegible] suggests that the dis[illegible] agility and physical prowess of *Helobdella stagnalis* are adapted primarily for feeding, reflecting co-evolutionary pressures that likely extend to other *Helobdella* species interacting with agile prey such as blackworms (Kutschera and Weisblat 2015).

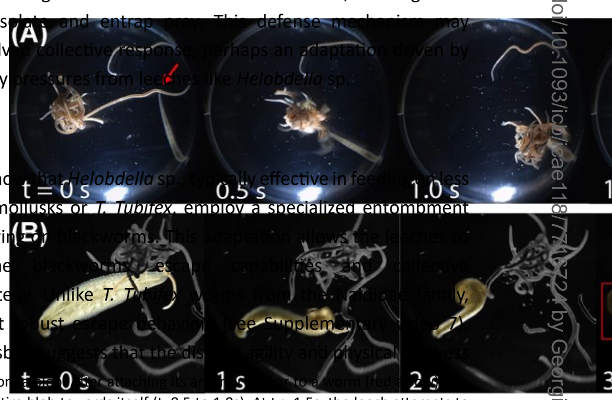

Fig. 4. Predation by *Helobdella stagnalis* on worm blobs. (a) Timelapse of a leech’s failed attempt to trap an individual worm from a blob. After attaching its anterior sucker to a worm (red arrow), the worm undulates violently while maintaining itself entangled with conspecifics (t=0 to 0.5s). This causes the leech to pull the entire blob towards itself (t=0.5 to 1.0s). At t > 1.5s, the leech attempts to execute a ventral fold onto the blob several times but inevitably fails. The inset shows a worm completely trapped inside of the cavity. (b) Successful complete entombment of a worm from an entangled population of (~ 10 worms). (Supplementary videos 4 and 5).

In quantitative analysis, we examine factors hypothesized to influence the success of *Helobdella*’s entombment strategy, including the initial attachment point of the leech on the worm, as well as the sizes of both predator and prey, and the dynamics of the leech’s contraction. Although the location of the leech’s initial contact did not significantly affect the outcome of entombment, we find that complete entombments typically occurred near the worm’s center of mass and partial entombments near the tail, while failed attempts did not show a consistent pattern. Additionally, larger leeches are more successful in entombing prey, while longer worms are more adept at escaping. Finally, while the extent of spiral contraction did not significantly impact entombment outcomes, the rate of contraction was notable. Our analysis revealed that faster contraction rates, which strongly correlated with only failed entombments, were not necessarily due to the leech contracting more rapidly. Instead, these increased rates appear consistent across attempts, suggesting that the leech exerts a constant force during entombment. We interpret the higher contraction rates in failed attempts as a result of the 'whiplash effect,' where the prey's sudden escape dramatically alters the dynamics of the contraction.

Abiotic factors significantly impact these predator-prey dynamics. The dense aggregation of blackworms in blobs may influence local oxygen levels, potentially affecting the respiratory efficiency of the leeches (Tuazon et al. 2022; Savoie et al. 2023; Deblais et al. 2023). *Helobdella* sp., which respire through their skin, sometimes supplement their oxygen intake through undulating movements, a behavior also seen post-feeding or in their parental care practices. During these activities, adult *Helobdella* sp. actively ventilate their young, enhancing the oxygen content around them, which is crucial in low-oxygen environments (Kutschera and Wirtz 2001). We hypothesize that this adaptation could be disadvantageous when a leech attempts to feed within a worm blob, as the collective might reduce the available oxygen, potentially disrupting a feeding leech's ability to aerate (see Supplementary video 6). Alternatively, we propose that the presence of a collective worm blob approaching a feeding leech could trigger a defensive or disruptive behavior in the leech. This response could stem from the physical interference or perceived threat posed by the blob, potentially causing the leech to abandon its feeding to manage the new stimuli.

The habitat preferences of blackworms significantly contribute to their survival strategies. In addition to residing under detritus, studies show that blackworms often inhabit areas beneath macrophytes, such as duckweed (Fig.1a). These plants provide physical shelter, creating a barrier that enhances the worms' protection against predators, and they also improve nutrient conditions (Ohtaka et al. 2011, 2014; Xie et al. 2008; Vanamala Naidu et al. 1981; Cheruvelil et al. 2002). This "interrhizon" or the rhizosphere around plant roots facilitates better oxygenation, supporting the formation of dense worm populations that are less likely to be preyed upon by predators, such as *Helobdella*, which are less frequently found in vegetated areas (Ohtaka et al. 2011; JabłońskaâĂŘBarna 2007; Waters and San Giovanni 2002; Talbot and Ward 1987). This ecological setup highlights the critical role of habitat in shaping the interactions and survival strategies of both blackworms and their leech predators.

Limitations and Future Outlook

Our study better describes the predator-prey dynamics between *Helobdella* and *Lumbriculus variegatus*, but it has some limitations. While we have incorporated both observational and quantitative analysis, the scope of our quantitative exploration was confined primarily to the biomechanical aspects of the entombment strategy. Additionally, our reliance on the general classification of our experimental organisms as *Helobdella* sp. introduces a limitation. Without detailed molecular analysis, we cannot definitively attribute observed behaviors to specific species, potentially affecting the generalizability of our findings across different *Helobdella* species, each of which may exhibit unique predatory strategies and prey preferences. Furthermore, our video data collection faced challenges, particularly in trials involving groups of worms, where the worms' dense, collective movements occasionally obstructed clear views of the leech's actions.

Despite these limitations, our primary goal was to document and analyze the spiral entombment strategy employed by *Helobdella*. In line with this, future research should investigate the feeding behaviors of various liquidosomatophagous species, aiming to understand how these behaviors adapt to diverse environmental pressures and prey characteristics. Additionally, we believe that our work will inspire further studies on active polymer and soft robotic models Nguyen et al. (2021); Prathyusha et al. (2018); Isele-Holder et al. (2015); Prathyusha et al. (2022); Anand et al. (2019); Chelakkot and Mahadevan (2017) to incorporate adaptive, shape-changing behaviors.

Conclusions

Our study described an entombment strategy used by *Helobdella* sp. when preying on blackworms. Our observations highlight a potentially adaptive response to the rapid escape and defensive mechanisms of blackworms, showcasing a complex interaction previously underexplored in aquatic ecosystems. This research could inspire future studies on the prevalence of this behavior across different leech species and its ecological implications. Future studies could explore how variations in environmental conditions, such as oxygenation and vegetation, and prey characteristics influence the evolution and efficacy of these predatory strategies.

Supplementary data

Supplementary data available at *ICB* online.

Competing interests

There is NO Competing Interest.

Author contributions statement

H.T. and M.S.B. conceptualized the research. H.T. and W.D. designed the experiments. H.T., and W.D conducted the experiments, for which H.T., W.D., K.M. performed the analysis. M.S.B. supervised the research. We thank Dr. Ishant Tiwari for assisting with the false coloring figure, Dr. K. R. Prathyusha for fruitful discussion regarding active polymers, and Ivy Li for assisting with the rearing of the worms and duckweed. All authors contributed to writing, discussion, and revising the manuscript.

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