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To move or not to move: taxis responses of the marine acoel *symsagittifera roscoffensis* to different stimuli

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ABSTRACT

Symsagittifera roscoffensis forms a photosymbiotic relationship with the alga *Tetraselmis convolutae* within the intertidal zone. Juveniles lack algal symbionts at birth and acquire them from the environment. Requiring light for photosynthesis, they position themselves within the water column while also balancing the risk of being washed away. To understand their behavioural adaptations, we conducted experiments on their movement in response to algal cues (chemotaxis), light gradients (phototaxis), and mechanical vibrations. Aposymbiotic juveniles showed three times more positive displacement towards algae. Adults exhibited positive phototaxis but retreated from high light intensity. When introduced to a column with a light source, the worms remained just below the surface. In the mechanical vibration experiment, worms only descended when vibrations exceeded a threshold. These findings suggest that S. roscoffensis has chemotactic abilities crucial for acquiring algae and acquires light for photosynthesis while minimizing dispersal risk and photoinhibition, facilitating its life cycle in the intertidal zone.

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Photosymbiosis; intertidal; chemotaxis; phototaxis, physical vibration; model organism

Introduction

The marine acoel worm *Symsagittifera roscoffensis* (von Graff 1891), formerly *Convoluta roscoffensis*) of the Family Convolutidae, was first discovered in Roscoff, France (Geddes 1879) and subsequently observed in Portugal (Carvalho et al. 2013), the Channel Islands (Doonan and Gooday 1982) and South Wales, U.K (Mettam 1979), with the latter considered the northern limit of its known distribution (Mettam 1979; Mcfarlane 1982). It is often found in shallow pools of water (<10 cm deep) at the upper limit of the intertidal zone in dense patches of thousands of individuals (Doonan and Gooday 1982; Bailly et al. 2014). Individuals are easily identifiable by their vivid green colour, due to the presence of the symbiotic microalga *Tetraselmis convolutae* held within the upper epithelium (Bailly et al. 2014), earning it the nickname mint-sauce worm. The worm lacks a true digestive tract and relies entirely on the algal symbionts to provide nutrition through photosynthesis (Bailly et al. 2014; Thomas et al. 2023a). While earlier studies

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focused on its geographical distribution (Mettam 1979; Stoecker et al. 1989), life cycle (Provasoli et al. 1968; Douglas 1985) and ecology (Parke and Manton 1967; Nozawa et al. 1972; Douglas 1983), recent research focus has shifted towards its use as a model organism to study developmental and neural biology (Semmler et al. 2010, Bailly et al. 2014; Sprecher et al. 2015). Nevertheless, the ability of *S. roscoffensis* to acquire an algal symbiont, harness light and maintain its position within the dynamic intertidal zone raises some interesting questions about its behavioural adaptations.

Symsagittifera roscoffensis is hermaphroditic but reproduces by mating. The embryos are encased in a cocoon and emerge as aposymbiotic juveniles, i.e. lacking algal symbionts (Provasoli et al. 1968; Bailly et al. 2014). The juvenile worms must find the algae in the vicinity and establish photosymbiosis within a few days to survive (Bailly et al. 2014, Thomas et al. 2023b), but how the juveniles detect and acquire the algae *in situ* remains unclear. Although *S. roscoffensis* can perceive light with photoreceptors, it lacks eyesight. While no other sensory organs have been described, it is not uncommon for closely related species to have a range of sensory organs that enable it to respond to other stimuli (Pearl 1903; Inoue et al. 2004, 2014). Its inability to swim freely and that it resides in shallow pools of water upon the sand also raises the question: Where may it find algal cells to establish photosymbiosis? We posit that aposymbiotic juveniles can seek out the 'right' algae by sensing the chemicals (positive chemotaxis) from settled algal cells or nearby adult worms.

In the natural environment, the direction to seek light is to move upward. There is the suggestion that the worm's upward movement is linked to high tides (Arboleda et al. 2018), but because the tides shift daily, this would mean *S. roscoffensis* must continuously adjust its movement to align with the tidal cycle. It is also questionable what benefit the worm may gain by responding to high tides at night. Indeed, Arboleda et al. (2018) noted that the alleged tidal migration disappeared when *S. roscoffensis* was kept in the dark, implying that the movement was cued to the light-dark cycle rather than the tidal cycle, but the authors did not provide any further details. Movement in response to light requires the ability to sense light. Photosensitive cells have evolved many times (Burr 1984a; Plachetzki et al. 2005), giving rise to wide-spread phototaxis (positive or negative) in metazoans (Burr 1984b). *Symsagittifera roscoffensis* has photoreceptors, and its positive phototaxis has been reported in the literature (Serôdio et al. 2011; Nissen et al. 2015).

Light intensity at the intertidal zone can reach a very high level especially in a clear summer day, and it is well known that free-living microalgae can suffer photodamage when exposed to excessive light (Straka and Rittmann 2018). Serôdio et al. (2011) proposed that the worms would retreat to avoid excessive light and prevent photoinhibition of the algal symbionts. However, Nissen et al. (2015) disagreed and instead suggested the worms lack the ability to regulate photosynthesis or avoid photoinhibition. In the Channel Islands (Guernsey), population sizes of *S. roscoffensis* were lower in the summer months (Doonan and Gooday 1982). In Wales, we had anecdotal evidence that in the summer months, very few worms were present on the beach surface when the ambient light level reached ~ 2,000 µmol m^{-2} s^{-1}, implicating a negative response to excess light, although detailed data were lacking.

While there are examples of photosymbiosis in sessile invertebrates that are exposed to strong tidal and wave actions, most notably corals, sponges and ascidians,

the detached body of *S. roscoffensis* creates a dilemma: The requirement of light for photosynthesis means the worms must expose themselves in the intertidal zone. At the same time, water motions due to waves, tides and water runoff place the worms in danger of being washed away. During collection in the field, we observed that strong winds continuously agitated the water, but the worms remained positioned just below the water surface unfazed by the agitation. However, as soon as we disturbed the water with our sampling gear, the worms immediately retreated into the sand, similar to that reported by Gamble and Keeble (1904). These observations suggest that *S. roscoffensis* is able to tolerate some level of physical disturbance and continue to photosynthesise within the intertidal zone, but retreats when the physical disturbance crosses some threshold.

To better understand the behavioural adaptations of *S. roscoffensis* to the intertidal environment, we conducted a series of laboratory experiments focusing on chemotaxis, phototaxis and physical disturbance. We tested if *S. roscoffensis* exhibits positive chemotaxis towards an algal chemical signal, and its movements under different light gradients and light intensities. Lastly, we simulated physical vibration and tested if *S. roscoffensis* would respond only to a certain level of disturbance. The results would shed light on how the worm may acquire the essential algal symbionts and the sunlight it needs for photoautotrophy, while avoiding unwanted dispersal in a physically dynamic environment.

Materials and methods

Symsagittifera roscoffensis *collection and master culture*

We collected the worms from a beach in East Aberthaw in South Wales, UK (51° 23' 2.506" N, 3° 22' 28.004" W), in October 2021. The worms were present in the upper limit of the intertidal zone as patches of green on the sand and within small pools of water between rocks. We collected the worms using a plastic pipette, stored them in falcon tubes (50 ml) and returned them to the laboratory within two hours. In the laboratory, the worms were transferred into 300 mL glass containers to establish a master culture. The containers had autoclaved sand collected from the same location; seawater was drawn from Swansea Bay and sterilised by filtration, UV radiation and autoclaving before use (salinity 30, pH 8.1). Inorganic nutrients were added in the form of 0.22 µm-filtered Guillard f/2 medium at 10 ml L^{-1} (f/4 final conc.). The master culture was placed inside an incubator (LMS Model 280NP) set to a temperature of 14.5°C; light was provided by a light panel inside the incubator at an intensity of 69 µmol m^{-2} s^{-1} and a photoperiod of 16 L:8D. One quarter of the seawater was changed every 3 days to remove waste and replenish the nutrients.

Chemotaxis experiments

We harvested cocoons from the adult worms and hatched them in autoclaved seawater to produce 10 aposymbiotic juveniles per trial (Provasoli et al. 1968; Thomas et al. 2023c, 2023b). These were placed at one side of an 8.5 cm wide Petri dish filled to 0.7 cm in

height with seawater. Using a camera (Olympus UC30) attached to a dissecting microscope (Olympus SZX16), we recorded the movement of the worms for 10 min with no stimuli to establish their 'background' movement patterns.

To test chemotaxis, we used freeze-dried adult worms to provide an algal chemical cue. To produce the freeze-dried worms, adult worms full of endosymbiotic algae were taken from the master culture and held at −80°C for 24 h and then placed into a freeze dryer (Edwards Modulyo) 24 h prior to the experiment. We used freeze-drying as a preservation method to maintain an intact algal cell wall (Min et al. 2022), protein, lipids (Aljabri et al. 2023), phenols (Badmus et al. 2019) and carbohydrates (Badmus et al. 2019), such that the end product resembled closely the original chemical characteristics of the algae. A set of 10 aposymbiotic juvenile worms were added to a Petri dish; directly opposite approximately 4 cm away we placed a freeze-dried worm. The movement of the juvenile worms was recorded for 10 min. For negative control, we repeated the experiment replacing the freeze-dried worm with a plastic artificial worm which was blue in colour and made from polyethylene terephthalate and was the approximate size of an adult worm. Each of the treatments (plain seawater, freeze-dried worm, artificial worm) were tested five times, each time with a new Petri dish and a new set of juvenile worms.

Once we had obtained the video footage, the programme AnTracks v1018 was used to analyse the movement of the juvenile worms from their starting positions, in response to freeze-dried worm, artificial worm or plain seawater, and we measured displacement as positive (towards the cue) or negative (away from the cue). We chose to report displacement instead of distance due to the fact that the worm's movement was non-linear, and we were more interested in the net movement towards or away from the cue. We compared the displacement values between treatments using the Kruskal-Wallis and a pair-wise comparison using the Wilcoxon rank sum test with a Bonferroni correction.

Phototaxis experiments

In the first experiment, we used a glass cylinder (1.7–2.3 cm dia.) with 10 cm height of seawater (salinity 30). A single adult worm was placed into the cylinder and allowed to settle to the bottom. Once settled, an LED lamp (Barrian; 6500K) was turned on and focused to illuminate the water column evenly at 69 µmol m^{-2} s^{-1}, and the worm's vertical position was observed continuously for 30 min. The cylinder was left undisturbed throughout the observations, and 50 trials were conducted with a new worm and fresh seawater in each trial.

In the second experiment, the cylinder was placed under a dark cover without light. In the third experiment, the same LED light beam was focused on the surface to create a down-gradient of light. In the fourth experiment, the LED light beam was focused at the bottom to create a reverse light gradient. In each case, the worm's position was recorded either continuously or every 5 min for a total of 30 min. A total of 30 trials were conducted in these latter experiments.

To aid the analysis of the movements and comparison with literature data, we calculated the time each worm spent in the upper (7–10 cm) and lower (0–3 cm) sections of the water column. Time distributions between sections by individual worms were compared using a Mann-Whitney test.

Light intensity experiments

Experiments were conducted to test whether the adult worm would avoid excessive light. We placed 50 worms in a glass jar that contained 2 cm sand as a substrate and 5 cm deep (100 mL) seawater (enriched with f/4) of the same salinity as our master culture. The glass jar was placed inside the LMS incubator at 14.5°C. We placed a Kessil A360X Tuna Sun adjustable LED lamp, with the colour turning knob set to white, at 12.5 cm from the water surface. When using the Tuna Sun, we did not use a spectral controller or the associated Wi-Fi dongle. Using an Apogee quantum light meter, we measured and adjusted the light intensity to 70 μmol m^{-2} s^{-1} at the start of the experiment (Day 0). The photoperiod was kept at 16 L:8D throughout. The number of worms that were present above the sand was counted on Day 3, one hour after the light was turned on. After counting, the light intensity was increased to the next level: 150 μmol m^{-2} s^{-1} on Day 3, 280 μmol m^{-2} s^{-1} on Day 6, 525 μmol m^{-2} s^{-1} on Day 9, 1400 μmol m^{-2} s^{-1} on Day 12, and finally 2500 μmol m^{-2} s^{-1} on Day 15. The counting continued every third day until Day 18. The experiment was then repeated with a new jar and another 50 worms, for a total of three times. At the end of the final experiment, we lowered the light intensity back to 70 μmol m^{-2} s^{-1} for 24 h and recorded the number of worms present above the sand.

For the phototaxis experiments and the light intensity experiments we chose to use 69–70 μmol m^{-2} s^{-1} as this was the light intensity that we used for our culture conditions; previously published data also suggested that photosynthesis remained stable at this intensity (Thomas et al. 2023a).

After confirming that the data were normally distributed by the Kolmogorov-Smirnov test (R studio package DHARMa V4.1.3), we used a non-linear generalised Poisson regression to test if there was a statistical difference in the number of worms that were presented above the sand between the different light intensities.

Behaviour observed under mechanical stimulation

We conducted experiments to study the vertical movement of *S. roscoffensis* in response to physical disturbance in the form of vibrations. A single adult worm was placed inside a glass cylinder (2.3 cm dia.) with 10-cm deep seawater (salinity 30) and allowed to settle to the bottom. A smartphone pre-programmed (mobile application: Vibrator strong) to create a vibration level of 1.764 ms^{-2}. The smartphone was placed (with vibration off) on top of the column. The cylinder was evenly illuminated by an LED lamp and the worm was observed continuously. When it began to move upwards, its vertical movement was tracked and timed. After the worm had reached the surface and stayed there for at least 30 s, the vibration function was turned on and the downward movement of the worm was observed and timed until it reached the bottom. Afterwards, the cylinder was cleaned and refilled with seawater and the experiment was repeated with a new worm, for a total of thirty times. Upward and downward speeds were calculated as vertical distance travelled per second and compared using a Mann-Whitney test.

Vibration intensity experiments

After confirming that the worms responded negatively to vibrations (1.764 ms^{-2}) in the previous experiments, we tested whether there was a threshold level of disturbance before the adult worm would react, by gradually increasing the level of disturbance. We pre-programmed the smartphone to different vibration intensities (m s^{-2}) with different on/off (ms) cycles to create eight levels of disturbance, each lasting 1 min (Table 1). We placed one adult worm into the illuminated cylinder and waited for them to reach the top of the water column as mentioned earlier. The treatment started from disturbance level 1 and increased sequentially to level 8, and we noted the disturbance level that triggered the worm's decent and the descent velocity. Afterwards, the cylinder was cleaned, and the experiment was repeated with a new worm, for a total of ten times. We compared the results between the different disturbance levels using ANOVA; normality was confirmed by the Kolmogorov-Smirnov test (studio package DHARMa V4.1.3).

Results

Effects of chemical cues

In the chemotaxis experiment, the juveniles showed a significantly larger positive displacement towards the freeze-dried worm than towards the artificial worm or plain seawater (Kruskal-Wallis; Chi-squared = 157.29; $p < 0.001$; Figure 1). The juveniles in plain seawater showed only small positive displacement from their starting positions, which increased to 35.9 ± 5.6 mm (accumulative ± s.e.) at the end of the experiment. In the artificial worm treatment, the juveniles showed small but negative displacement (i.e. away from the cue) in the first 450s, then changed to a positive displacement of 30.1 ± 13.4 mm by 600 s. In the freeze-dried worm treatment, the juveniles consistently showed positive displacement, which increased steadily from 19.4 ± 2.9 mm at 75 s to 89.4 ± 14.1 mm at the end of the experiment.

Wilcoxon rank sum test scores indicate significant differences between the artificial worm and plain seawater treatments ($p = 0.0075$), between the freeze-dried worm and plain seawater treatments ($p = < 0.001$), and between the freeze-dried worm and artificial worm treatments ($p = < 0.001$).

Table 1. Vibration intensities and durations used to create different levels of disturbance. The durations that the vibration was turned on and off are given in milliseconds. Total number of worms tested (out of 10 total) showing downward movement and their corresponding speed (s.e. In parenthesis where applicable) are presented.

Setting	Intensity (m s^{-2})	On duration time (ms)	Off duration time (ms)	Number of worms descending	Downward speed (cm s^{-1})
1	0.21	120	1000	0	0
2	0.43	240	875	0	0
3	0.63	360	750	0	0
4	0.84	480	625	0	0
5	1.05	600	500	1	0.16
6	1.27	720	375	1	0.18
7	1.481	840	250	3	0.5 (0.05)
8	1.693	960	125	5	1 (0.5)

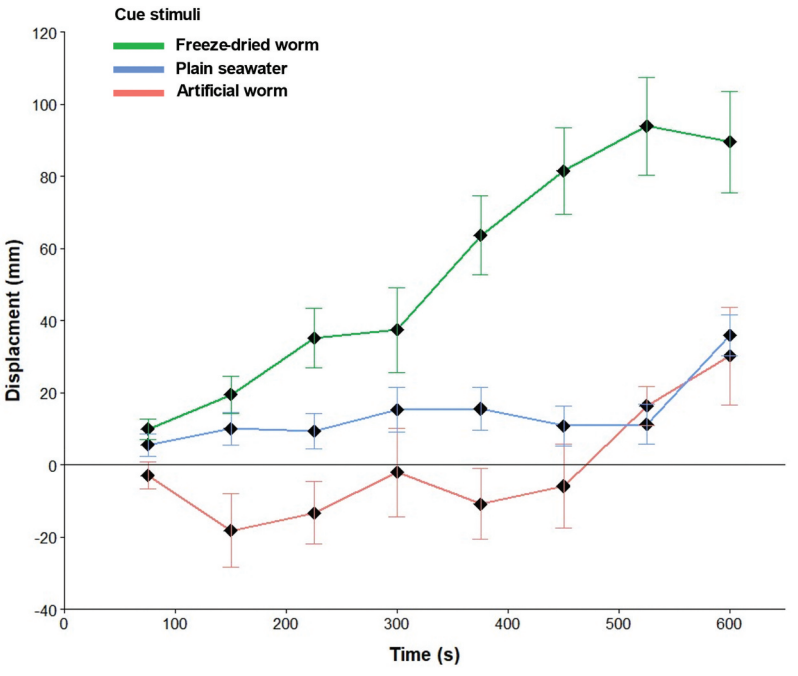

Figure 1. Chemotactic displacement (mm) of *symsagittifera roscoffensis* when presented with plain seawater, a freeze-dried adult worm, and an artificial worm. The overall displacement direction (accumulative mean ± s.e) in each treatment is positive when towards the stimuli and negative when away from the stimuli. $N = 10$ juvenile worms per treatment per replicate. There was a significant overall difference in the displacement values among the treatments (kruskal-Wallis; chi-squared = 157.29; $p < 0.001$).

Effects of light gradient and intensity

In the first experiment with even illumination, the timing of ascent varied between individuals, with some moving upwards after *ca*. 200 s, whereas others remained at the bottom for nearly 700 s before ascending. Some individuals reached and stayed at the surface, whereas others moved up and down repeatedly, and they did not stop for any noticeable amount of time when in transit. On average, the worm spent more time in the upper section (50 ± 0.04%; mean ± s.e.) than the lower (28 ± 0.04%) section of the water column (Mann-Whitney, w = 1752.5 $p = 0.0005$) (Figure 2).

In the second experiment where the cylinder was placed in darkness, 17 of the 30 individuals stayed at the bottom and did not register any vertical movement. Of the other 13 individuals, nine of them travelled up and down multiple times. These 13 individuals spent 46 ± 26% of their time in the upper section of the water column (Figure 3). In the third experiment where the light beam was focused at the surface, only two individuals remained at or close to the bottom the entire time. The other 28 individuals travelled the entire length of the water column and spent on average 64 ± 27% (12 individuals spending >80%) of their time in the upper section (Figure 3). In the fourth experiment where the light beam was focused at the bottom, only three individuals showed any noticeable upward movement and only two of them reached the surface. These three

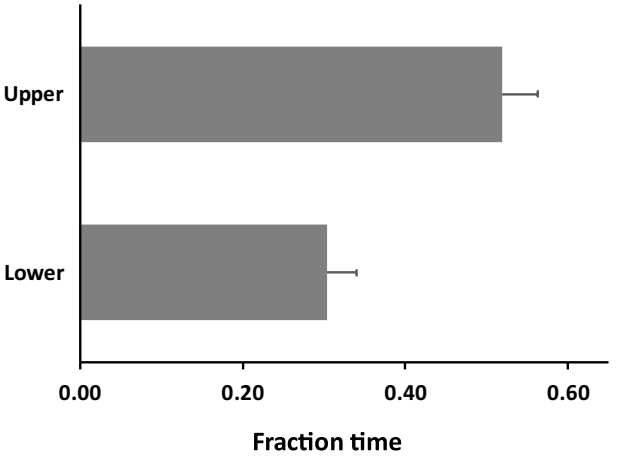

Figure 2. Fraction of time (mean + s.e.) spent by *S. roscoffensis* in the upper (7–10 cm) and the lower (0–3 cm) sections of the water column. Worms were monitored for 30 minutes at 69 µmol m^{-2} s^{-1} in even illumination, n = 50 worms. All of the worms moved. A fraction time of 0.18 was spent in transition between the upper and lower sections and is not included in the graph. There was a significant difference in the time spent between the two sections (Mann-Whitney; w = 1752.5, p = 0.0005).

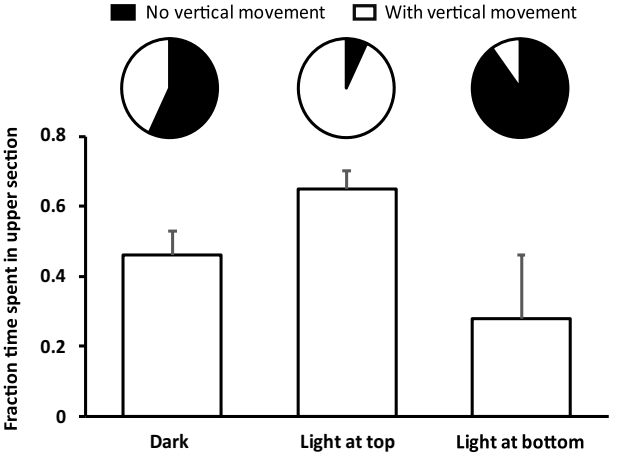

Figure 3. Movement of *S. roscoffensis* under different light conditions. Pie charts show the proportions of worms with or without vertical movement; n = 30 worms. Bar graph shows, for the ones that moved, the fraction of time spent in the upper section of the water column (mean + s.e.).

individuals spent on average 28 ± 31% of their time in the upper section. The other individuals did not register any upward movement and remained at the bottom the entire time (Figure 3).

The next experiment tested the effect of light intensity. At 70 µmol $m^{-2}s^{-1}$, 46.6 ± 1.2 (mean ± s.e.) worms (out of 50) were present above the sand (Figure 4). With the light intensity increasing every 3 days, the number of worms present decreased accordingly: 28.6 ± 4.0 worms at 150 µmol $m^{-2}s^{-1}$, 20.3 ± 3.1 worms at 280

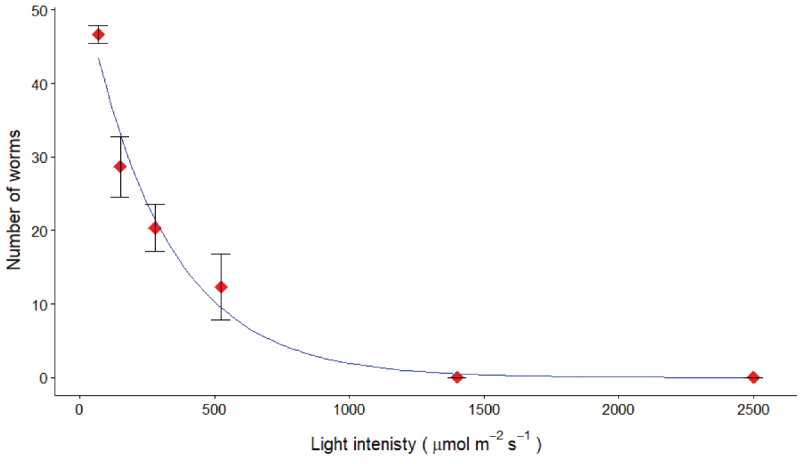

Figure 4. Number of worms present above the sand when exposed to increasing light intensity (mean ± s.E.). $N = 50$ worms per replicate. The results can be described by a non-linear generalised Poisson regression; number of worms = exp (4.0–0.00353 × light intensity), represented by the blue line ($R^2 = 0.92$, Z-value = −9.553, $p < 0.0001$).

µmol $m^{-2}s^{-1}$, 12.3 ± 4.4 worms at 552 µmol $m^{-2}s^{-1}$, and no worms were visible at 1400 µmol $m^{-2}s^{-1}$ and 2500 µmol $m^{-2}s^{-1}$. The results can be described by a non-linear generalised Poisson regression: Number of worms = exp (4.0–0.00353 × light intensity) (Z-value = −9.553, $R^2 = 0.92$, $p < 0.0001$). At the end of the final trial, we decreased the light intensity back to 70 µmol $m^{-2}s^{-1}$ and 23.6 ± 1.4 worms re-emerged after 24 h.

Effects of physical disturbance and vibration intensity

When we tested the effect of vibration, the worms initially ascended at a speed of 0.11 ± 0.01 cm s^{-1} (mean ± s.e.), equivalent to ca. 0.6 body lengths per second (Figure 5). If no disturbance was applied, the worms maintained the position at the top of the water column similar to that presented in Figure 3. At the onset of a vibration of 1.764 ms^{-2}, all of them moved downwards almost immediately in a freefall-like manner, at a speed of 0.66 ± 0.07 cm s^{-1} (***ca.*** 3.4 body lengths per second), significantly faster than the ascent speed (Mann-Whitney, W = 37, p = <0.001) (Figure 5).

In the experiment where we tested increasing level of disturbance, the worms did not respond to disturbance levels 1–4 (Table 1). Disturbance levels 5–6 only triggered descent in one out of 10 trials, at a speed of 0.16–0.18 cm s^{-1} (mean ± s.e.). Disturbance levels 7–8 triggered more responses: Level 7 caused descent in three trials at a speed of 0.5 ± 0.05 cm s^{-1}, whereas level 8 resulted in descent in 5 trials at 1 ± 0.5 cm s^{-1}. There was a significant difference in the number of worms that descended between the different disturbance levels (ANOVA; F-value = 16.95, $p = 0.006$).

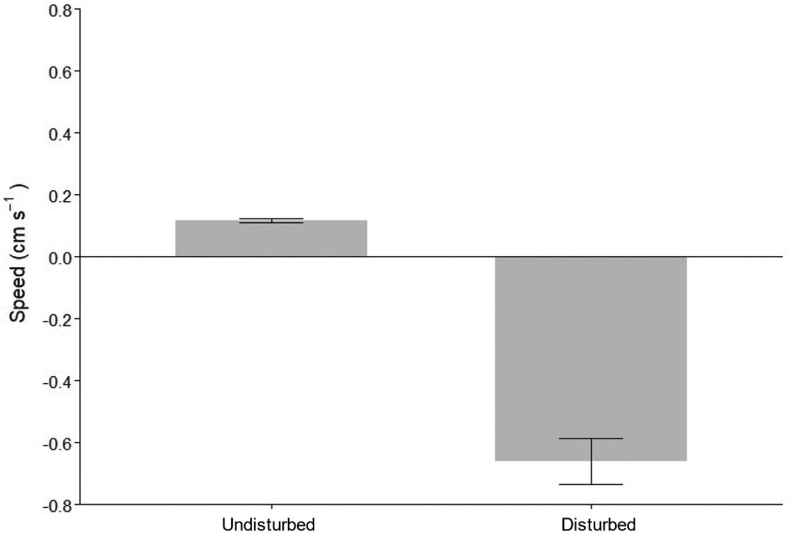

Figure 5. Vertical movement speed (mean ± s.e.) of undisturbed *S. roscoffensis* (upwards; positive values) and after disturbance (downwards; negative values) (Mann-Whitney, W = 37, $p < 0.001$). $n = 30$ worms.

Discussion

The acoel *S. roscoffensis* must find the right algae to establish photosymbiosis. It must also expose and orient itself towards the light, while avoiding unwanted dispersal in the physically dynamic intertidal zone. Using a series of experiments, we aimed to understand the worm's behavioural adaptations to chemical, light, and mechanical stimulations in such an environment.

After birth, the aposymbiotic juvenile must acquire algal symbionts within days to survive. Because the juvenile worm does not display any swimming or filter feeding capabilities that may allow it to capture freely suspended algal cells, we speculated that it likely acquires algal cells that have settled or algae from nearby adult worms (Bailly et al. 2014). In the chemotaxis experiments, juvenile worms showed a larger displacement towards a freeze-dried adult worm than either plain seawater or an artificial worm, suggesting an attraction towards the algal source via chemotaxis. We also repeated the experiment using adult worms full of algal symbionts, but the adult worms showed no movement and remained stationary (data not shown), suggesting that chemotactic response was only present in aposymbiotic juveniles in search of algae, but it is no longer needed once photosymbiosis has been established in the adult worms.

Chemotactic ability is not uncommon in soft bodied marine meiofauna; for instance, planarians use chemotaxis to detect food in the surrounding environment (Inoue et al. 2015). For the aposymbiotic juvenile of *S. roscoffensis*, the most readily available source of algae *in situ* would be the adult worms, each containing over 100,000 alga cells (Bailly et al. 2014; Arboleda et al. 2018). Adults are covered in mucus and during reproduction, eggs rupture from the side wall of the adult's body. It is conceivable that some algal cells get lodged into the mucus as the adults lay eggs (Costello and Costello 1939). As the adults then move around, they shed the mucus,

which may act as an algal source for the juveniles. Chemotactic ability would allow the juveniles to locate the 'right' algae in the vicinity (Provasoli et al. 1968; Bailly et al. 2014). Although the net displacement seems rather modest – less than 90 mm over 10 min in our experiment, this may be sufficient within a dense congregation of *S. roscoffensis* in the field. While we speculate that the juveniles were attracted to the algae contained within the adults, it may be possible that they were attracted to other chemicals from the adults instead. Either way, this behaviour would allow the aposymbiotic juveniles to locate a suitable algal source. Further work should consider comparing algal isolates and algae-free adults to determine the actual source of chemical cue that the juveniles are attracted to.

Previously, Nissen et al. (2015) indicated that adult worms exhibited a clear positive phototaxis, spending 69% or more time in the illuminated side of a Petri dish than in the shade. Notably, their experiment involved the worms moving along a horizontal surface without exposure to potential danger of water motion. In the wild, the light gradient is more vertical than horizontal, and any vertical movement along a water column has to balance the need for light against the risk of unwanted dispersal. In the experiment where we used even illumination, *S. roscoffensis* spent more time in the upper part of the vertical water column. However, by tracking the individual movements, we discovered a considerable amount of variability between individuals, suggesting asynchronous movements within a population. Interestingly, many of them did not stay at the top continuously, but rather they moved up and down repeatedly. Although the worm needs light for photosynthesis, a prolonged stay at the surface may increase the risk of unwanted dispersal by sudden water motion. This may explain why they returned to the bottom repeatedly, perhaps as a safety precaution.

The anterior of *S. roscoffensis* has, in addition to photoreceptors, a statocyst that senses gravity (Bailly et al. 2014). We observed that the worm lied horizontally when at the bottom or just underneath the water surface, but it assumed a vertical posture when in transit, suggesting that it could determine its direction (upwards or downwards) based on the statocyst's orientation. This might explain why the worm didn't pause during transit, but only halted when it encountered a boundary such as the water surface or the bottom substrate, and adopted a horizontal posture, even in the absence of light.

Between our two light gradient experiments, the results reinforced one another in showing that the worm's movement was positively phototactic, individuals were attracted to the light, regardless of its position at either the top or bottom (Figure 3). We initially did not anticipate vertical movement in darkness, but some individuals still moved upwards. *S. roscoffensis* requires light to survive and in nature, the most logical direction to seek light is upwards. Whether an individual should move or not in darkness may depend on its internal state and how strong is the need to search for light. We randomly selected the individuals for the experiments, and prior differences in their photosynthetic history and physiological conditions may contribute to the variations in their vertical movement in the dark. During the course of the experiments and in our culture maintenance, we observed no vertical movement of *S. roscoffensis* that could be linked to tidal cycle, that also has been confirmed by others (Arboleda et al. 2018). Instead, we postulate that the cyclical vertical movement reported in the literature (Keebles 1910; Arboleda et al. 2018) may have been linked to a circadian rhythm induced by a strong phototactic response (Stanton et al. 2022).

Excessive light can harm the algal photosynthetic system, which in turn could be detrimental to the host worm (Androuin et al. 2020). In a recent study, it was observed that at a light intensity of 475 µmol m^{-2} s^{-1}, the worm's photosynthetic oxygen production began to decline after four days, indicating photoinhibition (Thomas et al. 2023a). In this study, we also observed that as light intensity increased, more worms burrowed into the sand, and no worms were visible at the surface at ≥1400 µmol m^{-2} s^{-1}. Some worms re-emerged when the light intensity was decreased. These findings aligned with Doonan and Gooday (1982) who noted a lower number of worms in situ during the summer months when light intensity was highest, and the idea proposed by Serôdio et al. (2011) that *S. roscoffensis* burrows into substrate to avoid excessive light.

In the mechanical stimulation experiment, the worm ascended at an average speed of 0.11 cm s^{-1}, identical to the horizontal speed reported earlier (Nissen et al. 2015). In comparison, all individuals descended ~ 6 times faster in response to physical disturbance. *Symsagittifera roscoffensis* lives in an environment where water movement poses the risk of unwanted dispersal. The worm requires a mate to reproduce despite being hermaphroditic (Bourlat and Hejnol 2009). Therefore, one may posit that the danger of being removed from the habitat and the population outweighs the need for light, and it is necessary for *S. roscoffensis* to descend and secure itself to the bottom as quickly as possible when it senses strong disturbance. However, in the intertidal zone where there can be frequent water movement, overly sensitive reaction to any disturbance could be counter-productive because the worm would be spending energy unnecessarily moving away from the light. We observed in the wild that the worms do not respond to minor agitation in the water. This suggests that they would tolerate background disturbances up to a certain threshold. This was confirmed in our experiment where the worms did not respond to weak vibrations, which allowed the worm to remain near the surface for photosynthesis and avoid unnecessary exertion.

Combining the results from the phototaxis experiments and mechanical stimulation experiments, we propose a 'decision scheme' to describe the response of *S. roscoffensis* to external stimuli such as disturbance and light, moderated by its internal state and a built-in 'safety measure' (Figure 6). This scheme does not imply any conscious thinking by the worm; rather, it illustrates how the different external and internal factors work together to influence the worm's behaviour.

Further work on the chemotactic ability of *S. roscoffensis* should consider identifying the compounds responsible for attracting juveniles. Determining the attractant compound would not only enhance our understanding of what the worms are drawn to but could also facilitate additional experiments involving the detection threshold and associated concentration to which the worms respond. Identifying the attractant could also help us determine the sensory organs involved.

Additional research on how the worms avoid higher light intensity should explore how they achieve photosynthesis during the summer months. For instance, assessing whether the worms shift their photosynthesis to the dawn and dusk periods of the day when light intensity is lower. Understanding how the worms survive during the summer months will contribute to determining population dynamics in these periods.

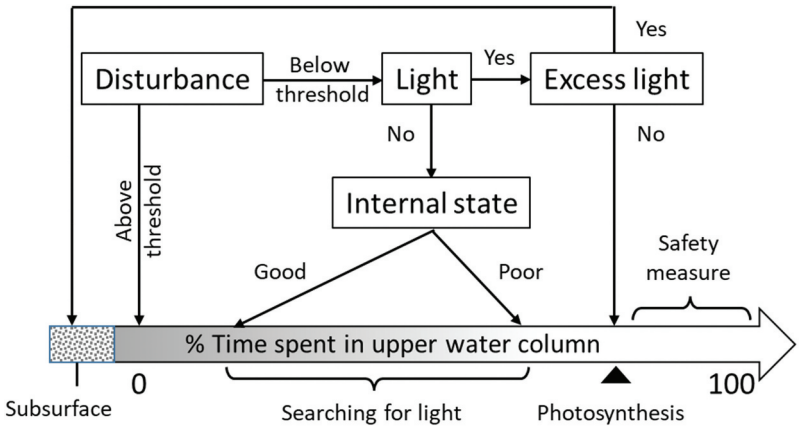

Figure 6. "Behavioural response scheme" showing the influence of external factors (disturbance, light) and internal factors (internal state, built-in safety measure) in determining the vertical movement of *S. roscoffensis*. good internal state refers to an individual with sufficient or excess photoassimilates and therefore having little need for photosynthesis; poor internal state refers to the opposite condition.

Conclusions

Our experimental results describe the behavioural adaptions of *S. roscoffensis* as a photosynthetic acoel living in the dynamic intertidal zone. The aposymbiotic juvenile exhibited chemosensing ability to seek out the algal source (found in adults) for establishing photosymbiosis. The worm showed an intricate balance between positive phototaxis to acquire light by ascending through the water column, and quick descent – at a certain threshold of disturbance – to avoid unwanted dispersal by water movement. The worm also burrowed itself to avoid excessive (harmful) light. Collectively, these behaviours would allow *S. roscoffensis* populations to establish and persist in the intertidal zones, such as those found in South Wales and along the Atlantic coast of continental Europe.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

Data are available upon request to the corresponding author.

Authors contribution

Conceptualization: N.J.T., C.J.C., K.W.T.; Methodology: N.J.T., C.J.C., K.W.T.; Formal analysis: N. J.T.; Investigation: N.J.T.; Resources: K.W.T.; Data curation: N.J.T.; Writing – original draft: N.J.

T., K.W.T.; Writing – review & editing: N.J.T., C.J.C., K.W.T.; Supervision: C.J.C., K.W.T.; Project administration: C.J.C., K.W.T.

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