Environmental constraints on the photosynthetic rate of the marine flatworm *Symsagittifera roscoffensis*

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ABSTRACT

Symsagittifera roscoffensis, an Acoel flatworm that lives within the intertidal zone, was first discovered over a century ago as a "plant animal" due to its symbiotic relationship with the alga *Tetraselmis convolutae*. Although commonly used as a model organism in biomedical research, there is little information regarding its life history or how environmental factors affect this organism. In this study, we investigated the effects of a range of abiotic factors on *S. roscoffensis* by measuring its photosynthetic oxygen production rate under different salinities, temperatures, light intensities, photoperiods, and nutrient concentrations over 6 days. Salinity (20, 30 and 40) had little effect on photosynthetic rate, whereas a temperature of 14 °C yielded significantly higher final oxygen production than 0 and 30 °C. The lowest light intensity (21 μmol m^{-2} s^{-1}), the shortest photoperiod (8 L:16 D) and the intermediate nutrient concentration (f/4) resulted in the highest final oxygen production rates. Our results showed that *S. roscoffensis* had the ability to tolerate and remain photosynthetically active under a wide range of conditions that it is likely to experience within the intertidal zone. Using our experimental data, we estimated that *S. roscoffensis* was able to produce 174% body C d^{-1} via photosynthesis alone, which could explain the total absence of heterotrophic feeding in this organism. The organism appeared to be robust and easy to cultivate, which should open new opportunities for its wider applications.

1. Introduction

Symbiosis occurs within different marine phyla such as Porifera, Cnidaria and Mollusca (Banaszak et al., 1993; Lee et al., 2001; Belda-Baillie et al., 2002). A particular type of symbiosis—photosymbiosis—is rather rare, and perhaps the most well-known example is between corals and Zooxanthellae (*Symbiodinium* spp.). As a result of this symbiotic relationship, corals can harness solar energy and achieve large population densities with minimal feeding (Muscatine, 1990). By comparison, a lesser-known example of photosymbiosis is between the flatworm *Symsagittifera roscoffensis* and its algal symbiont. Unlike corals, which supplement photoassimilates with heterotrophic feeding on suspended food particles, *S. roscoffensis* is completely reliant on its symbionts for nutrition. There are also other stark differences between corals and *S. roscoffensis*. For example, corals are fully submerged, whereas *S. roscoffensis* live in the intertidal zone where it is exposed to strong variations in environmental conditions. While adult corals are sessile organisms, *S. roscoffensis* remains freely mobile and can quickly relocate when disturbed. These characteristics make *S. roscoffensis* a unique

organism that has been previously described as a 'plant animal' (Keebles, 1910).

Symsagittifera roscoffensis, previously known as *Convoluta roscoffensis* (Graff et al., 1891), is an Acoel in the phylum Xenacoelomorpha (previously platyhelminths; ITIS, 2019). The adult is between 2 and 4 mm in length and lacks a defined coelom (Bailly et al., 2014). It has a vivid green colour due to the presence of the symbiotic chlorophyte *Tetraselmis convolutae* (Arboleda et al., 2018; Douglas, 1983a). *Symsagittifera roscoffensis* is hermaphroditic but it reproduces by mating (Provasoli et al., 1968; Douglas, 1983a; Bailly et al., 2014). Newly hatched individuals are aposymbiotic and must acquire the algal symbiont from the environment (Keebles, 1910; Provasoli et al., 1968). The anterior of the body has a mouth-like opening that takes in algae to initiate the symbiotic relationship (Mamkaev and Kostenko, 1991; Arboleda et al., 2018). After the initial acquisition by *S. roscoffensis*, phenotypical changes occur in the algae, such as loss of the cell wall, eyespot and flagella; the algal cells are then incorporated into the upper epidermis of *S. roscoffensis* (Douglas, 1983b). The symbiotic *T. convolutae* provides the host with photoassimilates such as mannitol, glutamic acid and

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lactic acid (Boyle and Smith, 1975), which are then used as precursors to synthesise more complex molecules (Taylor, 1974). The host does not perform any heterotrophic feeding, and it relies entirely upon the symbiont to provide for its nutritional needs; consequently, a true digestive tract is absent in the worm (Jennings, 1971; Mamkaev and Kostenko, 1991; Arboleda et al., 2018; Bailly et al., 2014).

Symsagittifera roscoffensis is capable of regeneration and tissue renewal thanks to the presence of totipotent stem cells (Bely and Sikes, 2010). Accordingly, *S. roscoffensis* has been used extensively as a model organism in biomedical research concerning tissue development and regeneration (Bailly et al., 2014; Dittmann et al., 2018; Reuter et al., 1998). By comparison, information on its natural history, ecophysiology and other potential applications is limited since its discovery at the Station Biologique de Roscoff, Roscoff, France (Geddes, 1879).

The known geographical range of *S. roscoffensis* covers the temperate zone from South Wales (51°23′00.8″N 3°22′27.9″W) to the southern tip of Portugal (37°05′22.2″N 8°11′18.4″W). Bailly et al., (2014) reported that the habitat temperature for the population in Roscoff, France is between 10 and 20 °C, but populations found in the lower and higher latitudes likely experience very different temperatures. For instance, the water temperature at the South Wales site reaches as low as 6 °C and remains <10 °C for much of the winter. The Welsh population can also be exposed to ice and snow occasionally in the winter. Mettam (1979) reported that the South Wales site was inaccessible due to the presence of snow and that the population was still present after the snow thaw. In the summer months, water temperature at the South Wales site can reach up to 30 °C, and *S. roscoffensis* are still present. It appears that the Welsh population of *S. roscoffensis* may have a different thermal preference or tolerance than the other populations.

The algal symbiont requires light for photosynthesis, but photoperiod and light intensity both vary temporally and spatially across the latitudinal range of *S. roscoffensis*. While photoperiod varies by ~5 h seasonally at the lower latitudes such as in Portugal, the population in South Wales experiences a 10 h difference in daylength between summer and winter. The average light intensity also decreases toward the higher latitudes. Therefore, populations at different locations are expected to adapt to different light environments. Surprisingly, there is very limited information on how variations in light intensity and photoperiod affect the photosynthetic activity of *S. roscoffensis* (Androuin et al., 2020).

Unlike the submerged environment, salinity along the intertidal zone will fluctuate due to inundation, evaporation, precipitation, and surface runoff, but information is lacking on how *S. roscoffensis* responds to different salinities. Our own observations at the South Wales site suggest that the salinity can fluctuate between 10, and 30, within a day. Carvalho et al., (2013) showed that *S. roscoffensis* can take up nutrients from the surrounding water to provide for the algal symbiont. Nutrient levels along the shore can vary due to discharge and runoff from land, but it remains unclear how the worm's photosynthetic activity may respond to different ambient nutrient concentrations.

To fill those knowledge gaps and improve our understanding of the basic biology of this organism, we conducted a series of experiments to determine how the different abiotic factors—salinity, temperature, light intensity, photoperiod and nutrient concentration – affect the photosynthetic activity of *S. roscoffensis* collected from South Wales. The results not only enable a better understanding of the natural history and ecophysiology of the organism but may also open possibilities to customise culturing conditions for the organism for different research applications.

2. Methods

2.1. Sample collection and master culture

Symsagittifera roscoffensis was collected from a beach in East Aberthaw in South Wales, U.K. (N 51. 23′ 2.506″ W3. 22′ 28.004″), between late September and early October 2020. It is found in the upper limit of the intertidal zone at low tide as patches of green on the sand, in small rock pools and between pebbles (Fig. 1). The worms were collected with a pipette into test tubes and returned to the laboratory within 2 h. In the laboratory, the worms were transferred into 300-mL glass containers to establish a master culture. The containers held autoclaved sand that was collected from the same location; the seawater was drawn from Swansea Bay and was 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 LMS incubator 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:8 D. One quarter of the water was renewed every 3 days to replenish the nutrients, and the worms were transferred to new containers weekly to avoid build-up of waste and detritus.

2.2. Photosynthetic activity

Photosynthetic activity of the worms was measured as oxygen production through time with a Pyroscience Firesting optical O$_2$ system (Model: FSO2-C4) inside the incubator. For that, the worms were placed inside a 2-mL transparent respiration vial that was sealed air-tight with a lid. The base of the vial had an O$_2$-sensitive patch attached and connected through optic fibre to a Firesting O$_2$ meter, which recorded the dissolved O$_2$ level inside the vial continuously. O$_2$ measurements were always performed during daytime, and each measurement lasted 40 min. A temperature probe connected to the Firesting meter was used to account for any temperature fluctuation during the measurements.

The Firesting system was calibrated using a 2-point calibration with 100% O$_2$-saturated water and a 0% O$_2$ solution (30 g L^{-1} Na$_2$SO$_3$), following the manufacturer's instructions.

2.3. Salinity experiment

In addition to the base seawater described earlier (salinity 30 with added nutrients), solutions of lower (20) and higher salinity (40) were made up by mixing the base seawater with the appropriate amount of deionised water or sea salt. The final salinities were verified by a DD True Seawater Refractometer calibrated following the manufacturer's instructions.

To begin the experiment, worms each ca. 2 mm in length were transferred from the master culture into three sets of five 100-mL containers (200 worms each) with seawater solution of a specific salinity (20, 30 or 40). Temperature, light intensity and photoperiod remained at 14 °C, 69 μmol s^{-1} m^{-2} and 16 L:8 D, respectively throughout the experiment. From each of the 15 treatment containers, a group of 50 worms was randomly selected and placed inside the respiration vial to measure photosynthetic activity. To account for any background O$_2$ changes, a blank measurement was included using water (without worms) from the same treatment container. On Day 3, another group of 50 worms was taken from each treatment container (plus the corresponding blank) to measure photosynthetic activity; the procedures were repeated on Day 6.

2.4. Temperature experiment

To test the effect of different temperatures, the incubator temperature was set to 0, 14 or 30 °C. Sets of five treatment containers were exposed to each of the experimental temperatures. The salinity was kept at 30 and illumination remained at 69 μmol s^{-1} m^{-2} and 16 L:8 D. Oxygen production by the worms (with the corresponding blank) was measured on Day 0, 3 and 6 as described above.

2.5. Light intensity experiment

The amount of light inside the incubator was adjusted to create

Fig. 1. *Symsagittifera roscoffensis.* Left: Dense patches of *Symsagittifera roscoffensis* in the intertidal zone at the South Wales field site. Right: An individual *Symsagittifera roscoffensis* under a light microscope.

different light intensity levels of 21, 69 and 475 μmol m^{-2} s^{-1}, as verified by an Apogee quantum PAR sensor. Temperature was kept at 14 °C, salinity at 30 and photoperiod at 16 L:8 D. Sets of five treatment containers were exposed to each of the light intensity levels and oxygen production by the worms (with the corresponding blank) was measured on Day 0, 3 and 6 as described above.

2.6. Photoperiod experiment

For this experiment, the light intensity level was kept at 69 μmol s^{-1} m^{-2} but the photoperiod was set to 8 L:16 D, 16 L:8 D or 24 L:0 D. Temperature and salinity remained at 14 °C and 30, respectively. Photosynthetic activity of the worms exposed to the different photoperiods was measured in the same manner as described earlier.

2.7. Nutrient experiment

To create different nutrient treatments, Guillard nutrient medium was added to autoclaved base seawater in the amount of 20-, 10- or 5-mL L^{-1} for the equivalent final nutrient level of f/2, f/4 and f/8, respectively. Sets of five treatment containers were set up for each nutrient concentration. Temperature (14 °C), salinity (30) and light (69 μmol s^{-1} m^{-2}; 16 L:8 D) conditions were kept constant for the experiment. Photosynthetic activity of the worms exposed to each nutrient concentration was measured in the same manner as described earlier.

2.8. Respiration rate

To determine the respiration rate of *S. roscoffensis* we randomly selected 50 worms and placed them inside the same respiration vial mentioned above. These were placed inside an incubator under the conditions for our master stock cultures at salinity 30, temperature 14 °C, light intensity 69 μmol m^{-2} s^{-1}, photoperiod 16 L:8 D and nutrient concentration f/4. Net oxygen production rate was obtained by recording data for 40 min with the light turned on and was repeated with the light turned off to obtain the respiration rate. This was replicated on three separate occasions.

2.9. Carbon budget

In order to calculate the daily carbon budget, we used a body water content of 89%, a carbon content of 25% and a wet body weight of 1.6 mg ind^{-1}; from this we calculated the dry weight carbon content. We then calculated the global average oxygen production rate for our master stock cultures at salinity 30, temperature 14 °C, light intensity 69 μmol m^{-2} s^{-1}, photoperiod 16 L:8 D and nutrient concentration f/4 from 0 to

6 days using the oxygen data presented below. The global average was converted to a daily value assuming 12 h of active photosynthesis; converting the daily global average oxygen production to carbon production using a 2.6:1 (g/g) O$_2$-to-C conversion ratio. We used our carbon production per day and our body carbon content to predict a % body C d^{-1} ind^{-1} ratio.

2.10. Data analysis and statistics

Data analysis was conducted in R studio version 1.41717. To avoid initial fluctuations in the oxygen readings due to opening and closing the incubator door, oxygen readings in the first 10 min were excluded from further analysis. The oxygen readings in the subsequent 30 min in both treatments (with worms) and controls (without worms) were fit into a linear model (lm) to generate the respective slopes – the difference between the two slopes represents the O$_2$ production rate by the worms via photosynthesis (expressed in μg O$_2$ h^{-1} ind^{-1}). We calculated the mean and standard error among the five replicates. The same procedures were applied to data from Day 0, 3 and 6 of each of the experiments. ANOVA (aov) with a Tukey's post hoc test for pairwise comparisons were carried out (R package emmeans) to test for putative significant effects due to treatment, time, and their interactions. Graphs were generated using the package tidyvers that included ggplot 2 in R studio.

3. Results

3.1. Salinity

In salinity 20, the oxygen production rate (mean ± SE) on day 0 was at 11.0 ± 3.0 μg O$_2$ h^{-1} ind^{-1}, but it decreased to 8.7 ± 1.8 μg O$_2$ h^{-1} ind^{-1} on Day 6 (Fig. 2). The oxygen production rate in salinity 30 decreased from 10.7 ± 2.5 to 7.8 ± 3.4 μg O$_2$ h^{-1} ind^{-1} over the course of the experiment (Fig. 2). In salinity 40, the oxygen production rate was relatively stable, varying between 9.8 ± 2.8 μg O$_2$ h^{-1} ind^{-1} on Day 0 and 8.6 ± 2.5 μg O$_2$ h^{-1} ind^{-1} on Day 6 (Fig. 2). There was no significant effect due to salinity ($P = 0.977$), time ($P = 0.979$) or their interaction ($P = 0.842$).

3.2. Temperature

Oxygen production rate at 0 °C started at quite a high level of 28.2 ± 5.3 μg O$_2$ h^{-1} ind^{-1} on Day 0 but then decreased rapidly to 5.8 ± 1.3 μg O$_2$ h^{-1} ind^{-1} by Day 6, resulting in an 80% drop over the course of the experiment (Fig. 3). Oxygen production rate was relatively stable but low at 30 °C, varying between 7.9 ± 1.4 and 11.8 ± 3.2 μg O$_2$ h^{-1} ind^{-1} (Fig. 3). Oxygen production rate was also stable at 14 °C and remained
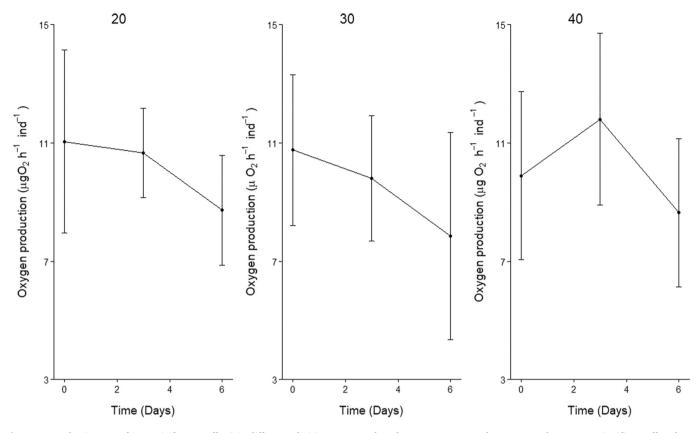
Fig. 2. O₂ production rate of *Symsagittifera roscoffensis* in different salinities. Data are plotted as mean ± SE. Based on ANOVA, there was no significant effect due to salinity, time or interaction between temperature and time ($P > 0.05$). Each data point represents the mean of five replicates, with $N = 50$ individuals per replicate.

higher than the other temperature treatments at the end of the experiment (23.1 ± 3.3 µg O₂ h^{-1}; Fig. 3). There was a significant effect due to temperature ($P < 0.001$), time ($P = 0.045082$) and the interaction between temperature and time ($P < 0.001$).

3.3. Light intensity

The oxygen production rate was highest at 36.3 ± 3.0 µg O₂ h^{-1} ind^{-1} under light intensity 21 µmol m^{-2} s^{-1} on Day 0; despite a drop between Day 0 and Day 3, it rebounded to 31.9 ± 2.9 µg O₂ h^{-1} ind^{-1} on Day 6 (Fig. 4). The oxygen production rate was considerably lower at 69 µmol m^{-2} s^{-1}, and it increased from 11.2 ± 4.0 µg O₂ h^{-1} ind^{-1} to 17.1 ± 5.8 µg O₂ h^{-1} ind^{-1} over the course of the experiment, an increase of 34% (Fig. 4). At 475 µmol m^{-2} s^{-1}, the oxygen production rate varied between 20.3 ± 1.9 and 17.7 ± 2.3 µg O₂ h^{-1} ind^{-1} (Fig. 4). There was a significant effect due to light intensity ($P < 0.001$), time ($P < 0.001$) and the interaction between light intensity and time ($P < 0.001$).

3.4. Photoperiod

Under the 8 L:16 D photoperiod, the oxygen production rate doubled over the course of the experiment, increasing from 16.8 ± 3.4 µg O₂ h^{-1} ind^{-1} on Day 0 to 32.1 ± 2.4 µg O₂ h^{-1} ind^{-1} on Day 6. (Fig. 5). In the 16 L:8 D photoperiod treatment, the oxygen production rate increased by 115%, from 9.7 ± 2.1 to 20.9 ± 2.8 µg O₂ h^{-1} ind^{-1} over the 6-day period (Fig. 5). Under continuous light (24 L), the oxygen production rate began at 24.3 ± 0.4 µg O₂ h^{-1} ind^{-1} and after a slight increase to 31.1 ± 6.6 µg O₂ h^{-1} ind^{-1}, decreased sharply to 12.0 ± 3.3 µg O₂ h^{-1} ind^{-1} at the end of the experiment, representing a 61% drop (Fig. 5). There was no statistically significant effect attributable to photoperiod alone ($P = 0.194$). However, there was a significant effect due to time ($P < 0.001$) and the interaction between photoperiod and time ($P =$

0.00848).

3.5. Nutrients

In the highest nutrient concentration (f/2), the oxygen production rate decreased by 53% from 18.3 ± 3.8 to 8.5 ± 3.7 µg O₂ h^{-1} ind^{-1}, over the 6-day period (Fig. 6). The oxygen production rate in the intermediate nutrient concentration (f/4) remained high over the course of the experiment, varying between 19.9 ± 2.4 and 18.3 ± 2.1 µg O₂ h^{-1} ind^{-1}. In the lowest nutrient concentration (f/8), the oxygen production rate decreased from 20.4 ± 2.7 to 11.4 ± 1.3 µg O₂ h^{-1} ind^{-1}, a 44.1% decrease over the course of the experiment (Fig. 6). There was no significant effect due to nutrient concentration ($P = 0.271$), time ($P = 0.0938$), or their interaction ($P = 0.2512$).

3.6. Respiration rate

In the respiration experiment, under the conditions of salinity 30, temperature 14 °C, light intensity 69 µmol m^{-2} s^{-1}, photoperiod 16 L:8 D and nutrient concentration f/4, an oxygen production rate of 7.9 ± 0.8 was calculated and fell to -3.1 ± 1.3 when placed into the dark. (Fig. 7). There was a significant difference between the oxygen production rate and the respiration rate ($P = 0.00228$).

3.7. Carbon budget

First, we calculated an oxygen production rate of 199.8 µg O₂ d^{-1} ind^{-1} and a wet weight of 1.6 mg ind^{-1}, of which 0.18 mg ind^{-1} is dry weight. With an estimated carbon content of 44 µg C ind^{-1}, and using the oxygen production rate, we determined the daily carbon production rate of 76.8C d^{-1} ind^{-1}. Finally, we calculated the body % carbon content, which gave us *S. roscoffensis*'s body weight to carbon ratio of
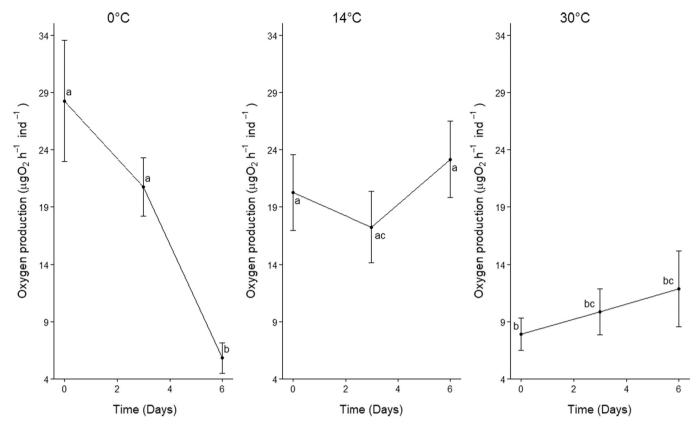
Fig. 3. O_2 production rate of *Symsagittifera roscoffensis* in different temperatures. Data are plotted as mean ± SE. Based on ANOVA there was a significant effect due to temperature, time and interaction between temperature and time (ANOVA; $P < 0.05$). Unshared letters indicate significant differences determined via ANOVA and Tukey's post-hoc tests (Tukey; $P < 0.05$). Each day represents five replicates with N = 50 different individuals involved in each replicate.

174% body C d^{-1} ind^{-1}.

4. Discussion

The intertidal acoel flatworm *Symsagittifera roscoffensis* is a rare example of photosymbiosis where the host is sustained entirely by the algal symbionts. South Wales is considered the northern limit of its geographical range (Bailly et al., 2014). Although the organism has been discovered for over 100 years (Geddes, 1879), much remains unknown about its natural history and ecology. The purpose of this study was to determine how the different abiotic factors—salinity, temperature, light intensity, photoperiod, and nutrient level, affect the photosynthetic activity of the South Wales' population of *S. roscoffensis*.

4.1. Effects of salinity

The oxygen production rate did not vary much with time and salinity, and it remained quite comparable among all treatments, at between 7 and 8 μg O_2 h^{-1} ind^{-1} at the end of the experiment. These results suggest that *S. roscoffensis* was able to cope with the salinity range of 20–40, not unexpected for an organism that lives within the intertidal zone (Bailly et al., 2014). On the beach where we collected our samples, salinity can vary frequently due to evaporation, inundation, sea spray, precipitation and surface runoff, and *S. roscoffensis* must be able to withstand a wide salinity range (Geng et al., 2016). While a salinity of 40 may be extreme, we occasionally saw some of the worms basking in the sun at low tide at the field site and its surrounding salinity could be much higher than 30. Overall, our results showed the ability of *S. roscoffensis* to maintain its photosynthetic activity in a wide salinity range that they likely experience *in situ*.

4.2. Effects of temperature

While there is little literature information on the temperature preference of *S. roscoffensis*, Bailly et al., (2014) suggested an optimal temperature range of 10–20 °C based on the environmental temperature in Roscoff. In our experiments, the oxygen production rate remained stable at 14 °C and the final value was significantly higher than that of other temperature treatments (0 and 40 °C), which seemed to be consistent with Bailey et al.'s suggestion. Interestingly, at 0 °C the oxygen production rate showed a sharp decrease over time whereas a slight increase was observed in 30 °C, suggesting that cold temperature was more stressful to *S. roscoffensis* than warm temperature. While we did not test for a duration longer than 6 days, our results did suggest that *S. roscoffensis* from South Wales was able to cope with a temperature range of 0 to 30 °C. This is not unexpected as the habitat temperature along the South Wales's coast can vary widely depending on tides and seasons. For example, Mettam (1979) reported the presence of the worm after the beach had been sealed off by snow. Our own observations at the field site also suggest that *S. roscoffensis* could experience close to 0 °C and 30 °C during an average year, and we found *S. roscoffensis* in both the coldest and warmest parts of the year, without any sign of ill effects.

4.3. Effects of light intensity

The oxygen production rate appeared steady at 69 μmol m^{-2} s^{-1}, which was the light intensity the worm is accustomed to in the master culture. Surprisingly, the highest oxygen production rate was observed in the lowest light intensity tested (21 μmol m^{-2} s^{-1}). This indicates that 69 μmol m^{-2} s^{-1} light intensity was already too high for the worm. This may be the case as it lives within dense colonies and among pebbles (Fig. 1) that may offer some shading. Franks et al., (2016) showed that
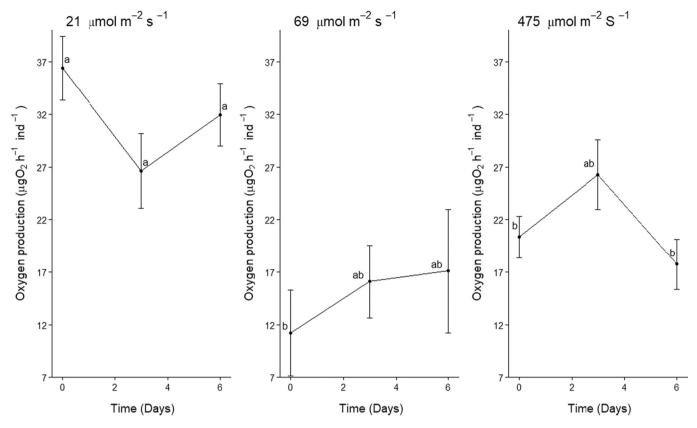
Fig. 4. O$_2$ production rate of *Symsagittifera roscoffensis* in different light intensities. Data are plotted as mean $\pm$ SE. Based on ANOVA there was a significant effect due to light intensity, time and interaction between light intensity and time (ANOVA; $P < 0.05$). Unshared letters indicate significant differences determined via ANOVA and Tukey's post-hoc tests (Tukey; $P < 0.05$). Each day represents five replicates with N = 50 different individuals involved in each replicate.

within an *S. roscoffensis* colony, individuals rotate in a circular motion, which may allow them to photosynthesise in short bursts and avoid over exposure. In contrast, in the highest light level (475 µmol m^{-2} s^{-1}) the oxygen production rate decreased considerably after 3 days, suggesting a stress response. Androuin et al., (2020) found that for *S. roscoffensis* that had been acclimatised to low light – it showed a decrease in oxygen production rate when exposed to light above 200 µmol m^{-2} s^{-1}, akin to a photoinhibition effect. Nevertheless, the worm remained photosynthetically active after 6 days in our experiments, affirming that *S. roscoffensis* was able to cope with the seasonal range of irradiance that it is likely to encounter in situ.

4.4. Effects of photoperiod

Among the tested photoperiods, 8 L:16 D yielded the largest increase and highest final oxygen production rate. This photoperiod is comparable to the shortest winter day in South Wales. Our results suggest that South Wales population of *S. roscoffensis* preferred a shorter daylength, which seemed to agree with our anecdotal field observations where the worm population appeared to be more abundant in the winter months. The 16 L:8 D photoperiod is close to the longest summer day in South Wales, and the measured oxygen production rate remained high toward the end of the experiment although there was a small decrease from Day 3. Data from these two photoperiod treatments support our field observations that *S. roscoffensis* was present in both summer and winter. In contrast, the oxygen production rate of the worm increased slightly first under 24 L, but then decreased sharply toward the end. These observations suggest that while the worm required light for photosynthesis, continuous exposure to light could have a negative effect like photoinhibition.

4.5. Effects of nutrients

By the end of the experiment, the oxygen production rate of *S. roscoffensis* was highest in the f/4 medium. The oxygen production rate was considerably lower in f/8 medium, perhaps reflecting nutrient limitation for the algal symbiont. Interestingly, despite being a standard medium for culturing microalgae including free-living *Tetraselmis* species, f/2 medium resulted in a substantial decrease in the oxygen production rate over the 6-day period. Indeed, when we attempted to use f/2 for the master culture, the worm did not do well and quickly perished. Carvalho et al., (2013) suggested that *S. roscoffensis* can be used to treat nutrient-rich wastewater stream. We would, however, caution against such suggestion because the worm appeared to be stressed when exposed to high nutrient level, perhaps reflecting the fact that *S. roscoffensis* and its algal symbiont from South Wales may have adapted to a lower nutrient concentration than the Portuguese population used in Carvalho et al.,'s study.

4.6. Respiration rate

We determined the oxygen production rate and the respiration rate for *S. roscoffensis* in the light and dark. Using this data, we determine the gross oxygen production rate of 11.1 $\pm$ 2.1 µg O$_2$ h^{-1} ind^{-1}. The gross oxygen production rate is comparable to the net oxygen production obtained for day 1 for the other data sets i.e., salinity, photoperiod, and light intensity. The respiration rate that we detected would have been comprised of respiration from both the algae symbiont and the worm.

4.7. Daily carbon production

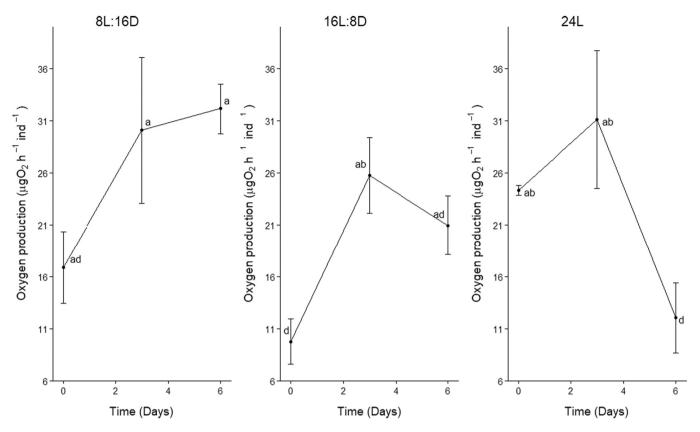We were unable to find information on the dry weight or carbon

Fig. 5. O_2 production rate of *Symsagittifera roscoffensis* in different photoperiods. Data are plotted as mean ± SE. Based on ANOVA there was a no significant effect due to photoperiod (ANOVA; $P > 0.05$). However, there was a significant effect due to time as well as photoperiod and time (ANOVA; $P < 0.05$). Unshared letters indicate significant differences determined via ANOVA and Tukey's post-hoc tests. (Tukey; $P < 0.05$). Each day represents five replicates with N = 50 different individuals involved in each replicate.

content of *S. roscoffensis* in the literature. Nevertheless, we measured the wet weight of our *S. roscoffensis* stock to be 1.6 mg ind^{-1} Assuming that *S. roscoffensis* has the same body water content (89%) and carbon content (25% of dry weight) as algae (Beal et al., 2018), we estimated its dry weight to be 0.18 mg ind^{-1} and a carbon content of 44 μg C ind^{-1}.

Using our experimental results, we estimated the carbon budget of *S. roscoffensis* when exposed to the environmental conditions used in our stock culture, i.e. salinity 30, temperature 14 °C, light intensity 69 μmol m^{-2} s^{-1}, photoperiod 16 L:8 D and nutrient concentration f/4. For each of the environmental parameters, we took the average of the photosynthetic oxygen production rate across the experimental period (Day 0 to Day 6), then calculated a global average photosynthetic oxygen production rate of 16.4 μg O_2 h^{-1} ind^{-1}. We extrapolated this to a daily value assuming 12 h of active photosynthesis per day, giving us a daily rate of 199.8 μg O_2 d^{-1} ind^{-1}. Using a 2.6:1 (g/g) O_2-to-C conversion, this translates to a daily carbon production rate of 76.8 μg C d^{-1} ind^{-1} via photosynthesis, which is equivalent to 174% body C d^{-1} ind^{-1}. While this carbon budget estimation is rather crude and relying on several assumptions, it shows that *S. roscoffensis* has a highly efficient photosynthetic machinery thanks to its algal symbionts, which may explain the absence of the need for heterotrophic feeding in this organism. The high % body C production rate may also allow for the energy and resources it needs to cope with the dynamic and potentially stressful intertidal environment.

4.8. Cultivation method and potential applications

For this study, we established a master culture of *Symsagittifera roscoffensis* in the laboratory. We were, however, unable to find any detailed description of long-term culturing methods for this organism in the literature. Nevertheless, using our simple master culturing protocol (see Methods), we were able to keep a continuous stock of *S. roscoffensis* for over a year. During this time, we did not notice any change in the vivid green colour of the worm that may indicate poor physiological condition. To the contrary, we observed small photosynthetic juveniles appearing in the culture, indicating successful reproduction.

Because *S. roscoffensis* in the wild may experience different and variable conditions, we recommend a gradual acclimatisation of the worms to the culture condition. In our case, we recorded the *in situ* temperature and salinity in the field, and we transported them in ambient seawater back to the laboratory. In the laboratory, we initially set the incubator to the *in situ* temperature then adjusted it by ≤2 °C every 2 days until it reached the desired temperature. Likewise, we adjusted the water salinity incrementally every 2 days until it reached the desired salinity. On one occasion, we accidentally left a container of *S. roscoffensis* in the dark for a month, and afterward we did not notice any mortality or visual change to the size, behaviour, or colour of the worm, suggesting that *S. roscoffensis* is a rather robust organism that can survive an extended period of unfavourable conditions, which should make it fairly easy to cultivate.

5. Conclusion

Our results indicate that *S. roscoffensis* can withstand a range of conditions and still remain photosynthetically active. However, changes to temperature, light intensity, and photoperiod result in significant changes to the oxygen production rate and should be kept within the optimal range in order to maximise oxygen production rate. Moreover, while *S. roscoffensis* has been used as a model organism in biomedical research, its highly efficient photosynthetic ability and the relative
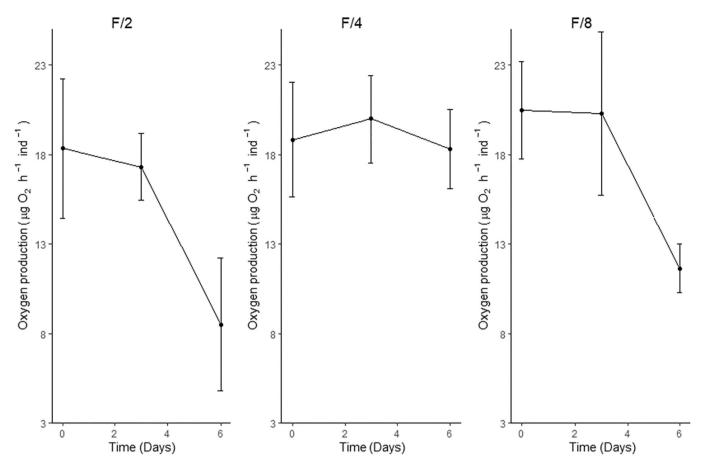
Fig. 6. O₂ production rate of *Symsagitifera roscofensis* in different nutrient concentrations. Data are plotted as mean ± SE. Based on ANOVA nutrient tretments did not have a significant effect on oxygen production between days, neither did the interaction between nutrients and time (ANOVA; $P > 0.05$). Each day represents five replicates, with N = 50 individuals per replicate.
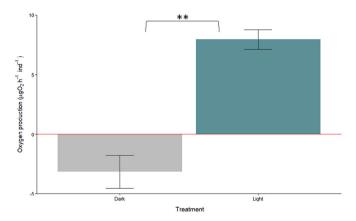

Fig. 7. O₂ production and resperation rate of *Symsagitifera roscofensis*, in the light and dark. Data are plotted as mean ± SE. Based on ANOVA there was a signifcicat difiference in oxygen production between the light and dark tretments (ANOVA; $P < 0.05$). Each treatment represents three replicates, with N = 50 individuals per replicate.

simplicity of its cultivation and maintenance should open other applications/possibilities. For example, the worm has a body size similar to certain life stages of common live-feed organisms (e.g., *Artemia* and copepods) and *Tetraselmis* is generally regarded as a beneficial algal genus for aquaculture (Tulli et al., 2012; Pereira et al., 2020); therefore, *S. roscoffensis* has the potential to be developed into a low-maintenance, high-yield feed for aquaculture. Further research into the biochemical

profile of *S. roscoffensis* under different growth conditions and its palatability to aquaculture species should be considered.

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Data availability

Data will be made available upon request to the corresponding author.

CRediT authorship contribution statement

Nathan J. Thomas: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Christopher J. Coates:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. **Kam W. Tang:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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