Universität Rostock Traditio et Innovatio

Innate behavioural response of *Symsagittifera roscoffensis* under selected environmental conditions

Angeborene Verhaltensreaktion von *Symsagittifera roscoffensis* unter gewählten Umweltbedingungen

Dissertation

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Zusammenfassung

Symbiosen beschreiben das Zusammenleben zweier oder mehrerer Arten. Eine besondere Form der Symbiose stellt hierbei die Symbiose zwischen marinen Organismen und einzelligen Algen dar. Durch die Fähigkeit mancher Organismen, Algen in sich aufzunehmen, sind sie in der Lage, die Vorteile der Photosynthese mit zu nutzen (Photosymbiose). Ein faszinierendes Beispiel ist die Symbiose zwischen dem grüne Wurm *Symsagittifera roscoffensis* und der Grünalge *Tetraselmis convolutae*. Das Vorkommen von *S. roscoffensis* beschränkt sich auf die Gezeitenzone an der Westküste Europas, unter anderem Nordfrankreich und Südengland. Diese Zone ist gekennzeichnet durch eine hohe Variabilität der Umweltfaktoren. Dazu zählen unter anderem Wellenschlag, Nährstoffverfügbarkeit und Lichtklima. Diese Arbeit fokussiert sich auf das Verhalten von *S. roscoffensis* unter verschiedenen Lichtintensitäten sowie die Fähigkeit von *T. convolutae*, sich physiologisch an die Lichtverhältnisse anzupassen. Ein weiterer Aspekt ist das Verhalten von *S. roscoffensis* bei Zugabe verschiedener Nährstoffe.

Über einen Zeitraum von neun Stunden wurde *Symsagittifera roscoffensis* verschiedenen Lichtbedingungen ausgesetzt (Bereich von 90 bis 2200 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Bei hohen Lichtintensitäten zog sich *S. roscoffensis* in den Sand zurück, während Individuen bei niedriger Intensität (90 $\mu mol \cdot m^{-2} \cdot s^{-1}$) auf dem Sand verweilten. Unter einem linearen Lichtgradienten wählten sie zunächst stark beleuchtete Bereiche (bis zu 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$), zogen sich aber im Verlauf der Zeit in schwach beleuchtete Bereiche zurück und sammelten sich am Ende des Experiments im Bereich von $0 – 200\ \mu mol \cdot m^{-2} \cdot s^{-1}$. Eine Verhaltensänderung durch unterschiedliche Lichtfarben (blau, rot und grün) wurde ebenfalls festgestellt, allerdings wurden Bereiche mit rotem Licht gemieden. Da bei rotem Licht deutlich weniger Photonen aufgenommen wurden als unter blau und grün, kann eine abschließende Aussage über das Verhalten von *S. roscoffensis* bei unterschiedlichen Wellenlängen nicht getroffen werden. Zusätzlich zum phototaktischen Verhalten des Wurms, weisen die Veränderungen im absoluten Pigmentgehalt, ein absinkender α-Wert, sowie ein gestiegener Lichtsättigungspunkt (E_K) auf eine physiologische Anpassung der Alge an hohe Lichtbedingungen hin (2200 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Eine andauernde Bestrahlung mit hohen Lichtintensitäten führt allerdings zu Schäden am Photosyntheseapparat der Alge. Dies zeigt sich unter anderem am rapide abfallenden Wert des dunkel Yields (F_v/F_m) bei 2200 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Da Nährstoffe in verschiedenen Stoffwechselvorgängen der Mikroalgen eine wichtige Rolle spielen, müssen sie im Falle einer symbiotischen Verbindung vom Wirt bereitgestellt werden. Ein chemotaktisches Verhalten von *S. roscoffensis* ist jedoch bisher nicht dokumentiert worden. Die Ergebnisse in dieser Arbeit zeigten allerdings keine Veränderung im Verhalten von *S. roscoffensis* gegenüber den getesteten Nährstoffen (*Ulva* sp., *Fucus* sp., *Ceramium* sp., Nitrat, Nitrit, Phosphat, Ammonium und Nitrat + Phosphat).

Summary

The term symbioses describes the association of two or more species. A special form of symbiosis is the association between marine organisms and unicellular algae. Due to the ability of some organisms to host the algae within their body, they are able to use the advantages of photosynthesis (photosymbiosis). A fascinating example of such symbiosis is the green worm *Symsagittifera roscoffensis* and the green algae *Tetraselmis convolutae*. The occurrence of *S. roscoffensis* is limited to the intertidal zone on the west coast of Europe, including northern France and southern England. This zone is characterized by a high variability of environmental factors. These include for example nutrient availability and light climate.

Thus, this thesis investigates the behaviour of *S. roscoffensis* under different light intensities, as well as the ability of *T. convolutae* to adapt its physiology to the light conditions. A further aspect of this thesis is the behavioural response of *S. roscoffensis* towards various nutrients. *Symsagittifera roscoffensis* was exposed to several light intensities (ranging from 90 to 2200 µmol · m^{-2} · s^{-1}) over a period of nine hours. They retreated into the sand when exposed to high light intensities (2200 µmol · m^{-2} · s^{-1}), while individuals exposed to low light intensity conditions (90 µmol · m^{-2} · s^{-1}) stayed above the sand for an extended period. Under a linear light gradient they move into highly illuminated sections (up to 2000 µmol · m^{-2} · s^{-1}), but over time retreat into low illuminated areas and accumulate in the least illuminated section (0 – 200 µmol · m^{-2} · s^{-1}) by the end of the experiment. A change in behaviour due to different light colours (blue, red and green) could also detected with an avoidance behaviour towards red. However, the cross absorbance of photons revealed that were almost no photons reaching the individual worm underneath the red foil, hence a concluding statement on the behaviour of *S. roscoffensis* under different light intensities cannot be made and further data would be desirable. In addition to the phototactic behaviour of the worm, the changes in the absolute pigment content, a decreasing α-value, as well as an increased light saturation point (E_K) indicate a physiological acclimation of the algae to high light conditions (2200 µmol · m^{-2} · s^{-1}). However, continuous irradiation with high light intensities leads to photoinhibition of the photo apparatus. This can be seen, among other things, in the rapidly decreasing value of the dark yield (F_v/F_m) at 2200 µmol · m^{-2} · s^{-1}.

The next step was to investigate the behaviour of *S. roscoffensis* towards nutrients as they play an important role in various metabolic pathways in micro algae. However, a chemotactic

behaviour of *S. roscoffensis* has not yet been investigated. The results in this thesis showed no change in behaviour of *S. roscoffensis* towards the tested nutrients (*Ulva sp*., *Fucus sp*., *Ceramium sp*., nitrate, nitrite, phosphate, ammonium and nitrate + phosphate). Therefore, no evidence for a chemotaxis of *S. roscoffensis* could be documented here.

1 Introduction

1.1 A brief history of symbiosis

Symbiosis is a biological term well known to the public for the fascinating details of interspecific interactions across all kingdoms. The concept of symbiosis was first introduced by the German scientist Anton de Bary as *"the permanent association between two or more specifically distinct organisms, at least during a part of the lifecycle"* (de Bary 1879).

The term symbiosis in the sense of de Bary's definition broadly comprises all forms of a permanent association between two or more species. De Bary differentiated the symbiosis in antagonism (competing with each other), mutualism (beneficial for both partners) and commensalism (individuals have no apparent effect on each partner). Hence, he included parasitism as a form of symbiosis; in fact he stated that parasitism is the most known form of symbiosis (de Bary 1879). Shortly after de Bary's introduction of symbiosis, including parasitism as a subcategory of symbiosis, M. Rees in 1879 (cited in (Toepfer 2011)) clearly excluded parasitism from symbiosis and stated that a symbiotic association is beneficial and more advantageous for the species involved. However, this definition stayed an exception for several years (Hertwig 2017). Over the last decades the definition of symbiosis and all its subcategories continuously changed towards a purely positive meaning of symbiosis (Toepfer 2011). While mutualism and protocooperation are included in the term symbiosis, parasitism stands opposite to it. Yet the terminology of mutualism and protocooperation still differs in meaning across scientists in ecology, zoology and botany. Based on F. Dahl (cited in (Toepfer 2011)) mutualism is often described as an association with organism that do not depend on each other for survival but gain benefits from the partnership. On the other hand, mutualism is described as an interdependency that is mandatory for the survival of the populations. Instead, protocooperation is the form of symbiosis that is advantageous but not obligatory for the maintenance of the species (Odum 1980).

In this thesis I refer to the definition by Schubert (1991) in which protocooperation is defined as a positive interaction between two organisms, which benefits both partners. However, this form of symbiosis is not mandatory for the existence of the participants. For example, the interactions of hermit crabs and sea anemones, in which the sea anemones live on the crabs' shell and offer protection for the hermit crab, in exchange gain substrate and food. Still, both organisms can live separately. Organisms in a mutualistic symbiosis are dependent on each

other and the benefits that they gain through their partner. This form of interaction is mandatory for their existence—the one cannot live without the other. For example, the symbiosis between mycorrhizas and several plants.

The original broadly defined meaning of symbiosis included a wide range of interspecific relations, ranging from parasitism to mutualism. The relation between species in overlapping niche comprises likewise a wide range of intratrophic interactions and are an analogy to the symbiotic associations. It starts with the competition of species over the same limited resource, which eventually leads to competitive exclusion of the weaker competitor and in further consequence to the adaptation of the species to a new ecological niche. It ends with the same phenomenon of a mutualistic coexistence in the form of a protocooperation, as they need to form an alliance to cope in the new niche. All cases in which species form a mutualistic association to ensure their survival are particularly interesting and an example of that are endosymbiotic partnerships.

1.2 Endosymbiosis

In an endosymbiotic association, the symbiont lives within the body of its host. Endosymbiosis often stems from predator-prey associations in which the ingested symbiont was not digested but kept in the host tissue and used, for instance for the photosynthetic ability of the ingested symbiont (Munk 2009). The ingestion of a symbiont within the host tissue for its benefits is also the foundation of the endosymbiotic theory. The endosymbiotic theory or symbiogenesis was first introduced by Schimper in 1883 (cited in (Toepfer 2011)) and further developed by Konstantin Mereschkowski in 1905 (Howe et al. 2008). However, it was Lynn Margulis with her work (Sagan 1967) who was responsible for the wide acceptance of this theory (Toepfer 2011). It describes the origin of eukaryotic cells due to the intake of prokaryotes by eubacterial cells. This early stage of endosymbiosis, which possibly leads to the emergence of plastids, such as mitochondria and chloroplasts, is called primary endosymbiosis. While mitochondria possibly originated from α-proteobacteria (Gray et al. 2001) chloroplasts arose from an early ancestor of the present cyanobacteria (Howe et al. 2008). This endosymbiosis has proven to be so successful for both partners that more complex associations started to evolve and eukaryotic cells with plastids were ingested by another eukaryotic cell (secondary endosymbiosis); for example, Euglenophytes, which arose from an endosymbiosis with a green algae (Vanclová et al. 2017). Whereas Chlorophytes (including terrestrial plants), Glaucophytes, and Rhodophytes have evolved through primary endosymbiosis, all other eukaryotes have at least passed through a secondary endosymbiosis (Gould et al. 2008).

The benefits of those associations are immense as new niches become habitable for species and, as a unity, both partners become more competitive in comparison to single individuals. Thus, more advantaged. The advantages of endosymbiosis seem to be evolutionary so major that tertiary and quaternary endosymbiotic relations are stable. *Mesodinium rubrum* is an example of a quaternary endosymbiosis (Figure 1). This ciliate is capable of photosynthesis due to their ingested plastids, which they obtain by feeding on *Greminigera cryophila* or cryptophyte *Teleaulax amphioxeia,* respectively. These symbionts itself consists of a eukaryotic host and a eukaryotic endosymbiont evolving through secondary endosymbiosis (Fraunholz et al. 1997).

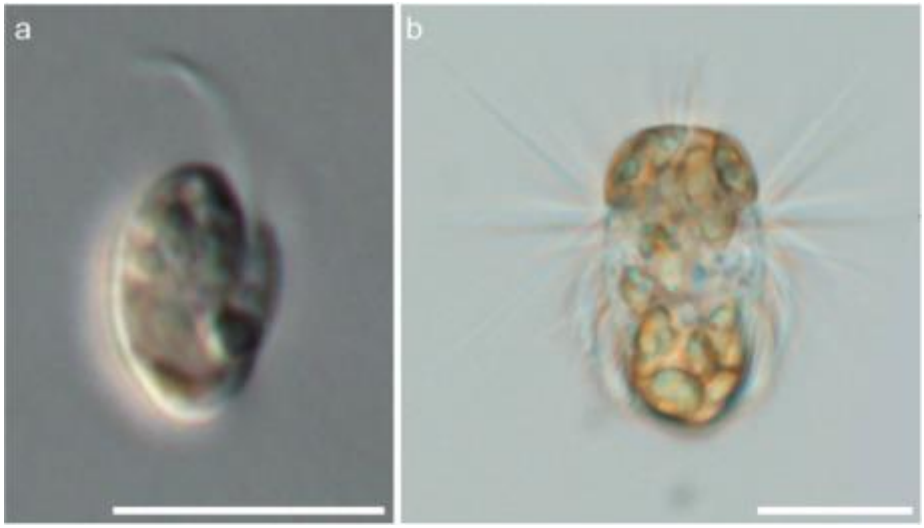

Figure 1: Light micrographs of **a**) *Teleaulax amphioxeia* (scalebar equals 5 µm) and **b**) *Mesodinium rubrum* with ingested *Teleaulax amphioxeia* (scalebars equals 10 µm). Modified from Altenburger et al. (2020).

Besides the benefits deriving from symbiosis, there are also challenges that come with such complex associations. Host and symbiont need to adapt to each other. This becomes more complex with every additional endosymbiotic intake. Both partners of the symbiosis need to be in balance. No one can dominate the other to keep this coexistence mutualistic and stable for all partners involved. Reproduction and growth has to be aligned and thus demands some control mechanisms to be conducted by the host (Muscatine and Pool 1979). For instance, some invertebrate hosts developed mechanisms to regulate the number of symbionts within their body by expulsion of the ingested algae (e.g. Cnidarian-algal symbiosis), by digestion of the symbiotic algae, or by limiting the nutrient supply, which will slow down proliferation of the algal symbiont (Baghdasarian and Muscatine 2000).

Those complex forms of symbiosis are extreme, as each partner can hardly be considered a single individual anymore. Nevertheless, protocooperation and mutualism can extend an

ecologic niche for species that have been displaced of their optimal niche due to intra specific competition. Individuals that have been displaced by competition experience stress and need to find ways to cope in this new habitat. That may lead to a specification, or alternatively protocooperation can become crucial to preserve the existence of the population in that specific place. The colonization of a new habitat based on symbiotic associations becomes especially clear by looking at lichens, a prime example of an endosymbiosis. Lichens are an association consisting of a fungi host (the mycobiont) and photosynthetic partner, like cyanobacteria or green algae (Nash 2001). The occurrence of lichens is widely spread from the tropics to the polar region (Nash 2001), in places with extreme physical variations, no sufficient nutrient supply or an unassured access to water. On the one hand, the heterotrophic mycobiont derives its carbon nutrient from the photosynthetic active partner and on the other hand, the mycobiont protects its symbiont from desiccation for an extended period of time. This symbiosis that sometimes includes more than two species can be considered as a miniature ecosystem (Nash 2001). The very successful symbiosis of lichens gave the mycobiont as well as the photobiont the possibility to expand in areas where they singularly would not be able to inhabit (Nash 2001). Lichens are an example of species that can survive in extreme terrestrial habitats due to their mutualistic association. A pendant to the terrestrial habitat in terms of great physical fluctuations is the sandy beaches of the eulittoral.

1.3 The eulittoral zone

The eulittoral zone comprises the area of a beach that is regularly affected by the tides (Figure 2). It lies dry for a period of time during low tide and is covered with water when the tide comes in. This intertidal zone is eminently affected by great physical variations. The abiotic factors that have an impact on life in intertidal zones include salinity, temperature, availability of sunlight, as well as exposure to wind and wave action. For example, at the shore of Brittany (France) the salinity of the Atlantic is approx. 35 PSU[1]. Since the tidal range exceeds up to 10 m (Pirazolli 2000), the upper beach section lies dry for an extended period of time, strong sun exposure during summer leads to faster evaporation of the water in shallow pools and hence increase the salinity significantly. On the other hand, heavy rainfalls and river influx drastically lower the salinity. Furthermore, the temperature can change drastically within a single day and,

[1]Salinity does not have a unit as it rather represents a ratio. However, for simplicity I use PSU (practical salinity units) in this thesis (Snoeijs-Leijonmalm et al. 2017).

while organisms are buffered when submerged by the water, they are exposed to the temperature (high or low) during low tide.

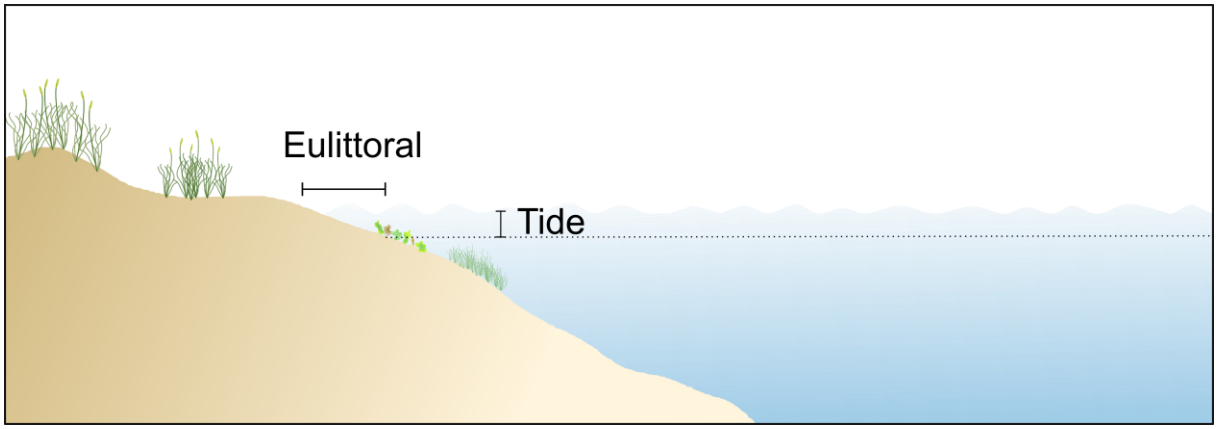

Figure 2: Illustration of the eulittoral zone and the primary zones of the ocean. Eulittoral zone is covered with water during high tide and lies dry during low tide.

The eulittoral zone consists, depending on the geographical area, of hard substrate or a soft bottom and sandy substrate. Whereas in areas where hard substrate is existent the biodiversity is rather rich; sandy and soft-bottom areas in the intertidal zone seem barren (Wittig and Streit 2004).

In particular, sandy beaches provide a harsh and inhospitable environment due to the constantly moving sand particles by turbulent waters and incoming waves. The constant moving of the upper layer makes the sandy intertidal zone almost inhabitable for most epifauna. However, the sandy beaches of the eulittoral zone are not barren at all, but rather inhabited by infauna and therefore not as visible at first sight (Lalli and Parsons 2012). The organisms that live on sandy beaches of the intertidal zone include primary producers such as diatoms and dinoflagellates. Although the diversity of the macrofauna is low in comparison with rocky shores, there are bivalves and polychaetes that can burrow into the sand. These includes larger clams, crustaceans and echinoderms.

A further interesting animal that inhabits the sandy shores of the intertidal zone of Northern France and South England is the green Acoel *Symsagittifera roscoffensis* with its green, photosynthetic endosymbiont *Tetraselmis convolutae*.

1.4 Symsagittifera roscoffensis

Phylum:	Xenacoelomorpha
Order:	Acoela
Family:	Convolutidae
Genus:	*Symsagittifera*
Species:	*S. roscoffensis*

Symsagittifera roscoffensis (v. Graff, 1891 Kostenko & Mamkaev, 1990) is a green flatworm-like animal belonging to the Acoela, a group of mainly free-living species combining the subtaxa Symsagittiferidae and Convolutidae, with *Symsagittifera roscoffensis* belonging to the latter[2]. Due to their flatworm-like appearance, for instance their ciliated body, the lack of body cavities or their hermaphroditic reproduction, Acoela were formerly placed in the taxon of Turbellaria and therefore a representative of the Platyhelminthes. Acoela and Nemertodematida are considered sister groups and are combined to the phylum Acoelomorpha. However, they no longer belong to the phylum of Platyhelminthes, as studies have shown several differences between Acoelomorpha and Platyhelminthes. Instead, they are now positioned as a sister group to all other Bilateria (Mwinyi et al. 2010). For the last few centuries, *S. roscoffensis* has sparked the interest of several scientists who wondered about the origin of its very distinct green colour. Patrick Geddes, a British scientist, has described the evolution of oxygen by captured worms from Roscoff and described the green colour of the animal as “chlorophyll containing cells” (Geddes 1879). A more detailed description of the worms anatomy and nervous system followed by the French scientist Yves Delage (Delage 1886). In his article he wondered about the nature of the symbiont and whether they are “real algae” (Delage 1886). However, due to taxonomic descriptions by Geddes and Delage, Ludwig von Graf found distinct differences in animals from the Adriatic coast and from Roscoff (Brittany, France) and therefore renamed *Convoluta schultzii* in *Convoluta roscoffensis* (Graff and Haberlandt 1891). In 1990 the taxonomy was revised and, due to new techniques in the field of molecular biology, *Convoluta roscoffensis* was changed to *Symsagittifera roscoffensis* (Kostenko and Mamakayev 1990). Representatives of the Acoela are widespread and an abundant group of the maiofauna (Kånneby et al. 2015). *Symsagittifera roscoffensis,* however, is restricted to the intertidal regions on sandy beaches in Portugal, the Channel Islands, and South England as well as on the north shore of France.

[2] Taxonomy based on the World Register of Marine Species (WoRMS) (Tyler et al. 2006-2021.)

Figure 3: Masses of *Symsagittifera roscoffensis* on a beach in Roscoff, France 2019. Photos taken by Hendrik Schubert.

The worms occur in vast colonies (millions of individuals) in shallow pools covered by 2-3 cm of water (Geddes 1879). They appear on these beaches as dark green masses during low tide but disappears into the sand when the tide comes in or if any mechanical disturbances occur. Their dark green appearance is due to the ingestion of *Tetraselmis convolutae*, a green photosynthetic micro algae that is not digested by the worm but kept within the worm's tissue as a photosynthetic endosymbiont (Douglas 1985; Provasoli et al. 1968). In contrast to many other marine photosymbioses of other organisms (e.g. cnidarian and its zooxanthellae), *S. roscoffensis* uses its endosymbiont not only as an additional food source but solely subsists from its endosymbiont (Provasoli et al. 1968).

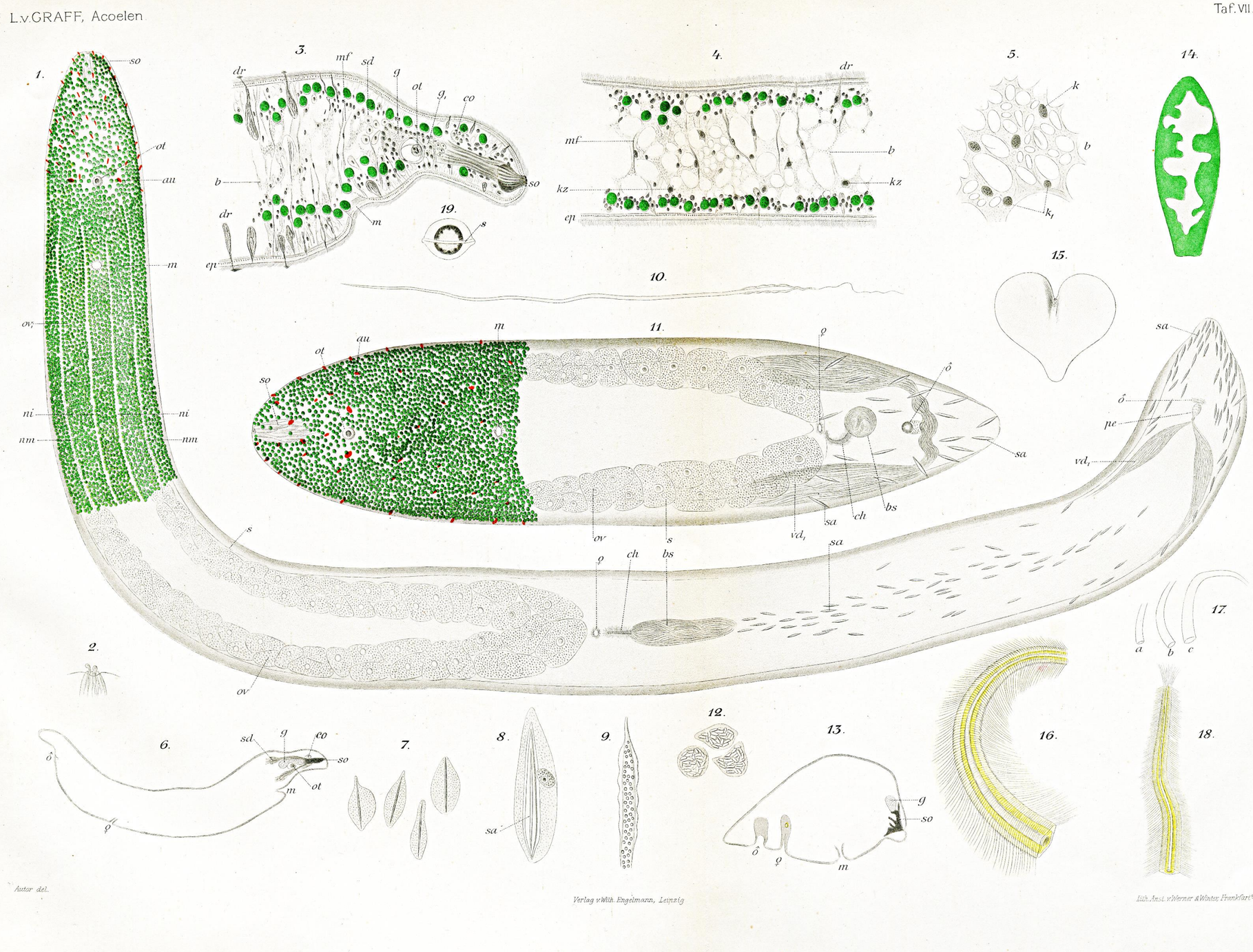

Figure 4: Original drawing of *S. roscoffensis* by Ludwig von Graff, 1891, modified from Graff and Haberlandt (1891).

1 Drawing of *S. roscoffensis* (110 x magnified), endosymbiotic algae exemplary drawn in the anterior part of the worm, **au** photoreceptor. **bs** Bursa seminalis. ch chitinous bursa nozzle (mouthpiece). **m** mouth. **ni** inner longitudinal nerve. **nm** middle longitudinal nerve. ot statocyst/otholit. ov ovarium. **ov**$_{I}$ the front end of the ovarium. **pe** penis. **s** rim of the lateral section bent downwards (ventral). **sa** sagitocyst/rhabdoids. **so** frontal organ (groups of secreting glands). **vd**$_{I}$ sperm in false Vesicula seminalis ♂♀ male and female genitalia pores

2 close up of the frontal organ releasing mucus

3 anterior end of a longitudinal section, **b** parenchyma. **co** anterior commissure of the nerve system. **dr** cutaneous glands. **ep** epithelium. **g** posterior and **g**$_{I}$ anterior part of the brain. m mouth. mf dorsoventral parenchymal muscles. **ot** statocyst/otholit. **sd** frontal gland. **so** mouth of the frontal organ

4 middle part of the body (median longitudinal section), **b** parenchyma. **ep** epithelium. **dr** cutaneous glands. **kz** parenchyma cells. **mf** dorsoventral muscle fibers

5 more magnified parenchyma of drawing number 4, **b** parenchyma joist **k** and **k**$_{I}$ nucleus

6 the outline of the median longitudinal section, abbreviations as in 1 and 3

7 formation cells of the sagitocyst isolated from a living animal

8 formation cell with sagitocyst (**sa**) from a longitudinal section stained with picrocarmine.

9/10 Mature spermatozoon and its front section with the root of the head-flagellum (**9**)

18 mouthpiece of Bursa seminalis, 550 x magnified

19 statocyst/otholith with a central stripe(s)

11 Drawing of *S. schultzei* (former *Concoluta schultzii*), 110x magnified with endosymbiotic algae exemplary drawn in the anterior part of the worm abbreviations as in 1

12 crystalloid cells isolated from living animal

13 the outline of the median longitudinal section, abbreviations as in 1 and 3

14 Individual with dorsally accumulated crystalloid cells (white spot)

15 Shape of an animal with retracted anterior and out stretched lateral parts

16 mouthpiece of Bursa seminalis, 550 x magnified

1.5 Anatomy of *Symsagittifera roscoffensis*

The adult worms are 4-7 mm in size, approximately 550 µm wide and are hermaphrodites (Figure 5). They do not however, self-fertilize one another, but still have to mate with other partners (Arboleda et al. 2018). *Symsagittifera roscoffensis* has a dorsoventrally flattened and ciliated body without a clear separate head and no segmentation. The worms have an anterior region with a central and peripheral nervous system. A feature of *S. roscoffensis* is that they are able to regenerate their entire nervous system within 20 days if the apical part is injured. Furthermore, they have a gravitor-sensor or statocyst in the anterior region, allowing an orientation in space and geotactic behaviour. On each side of the statocyst is a photoreceptor for phototaxis.

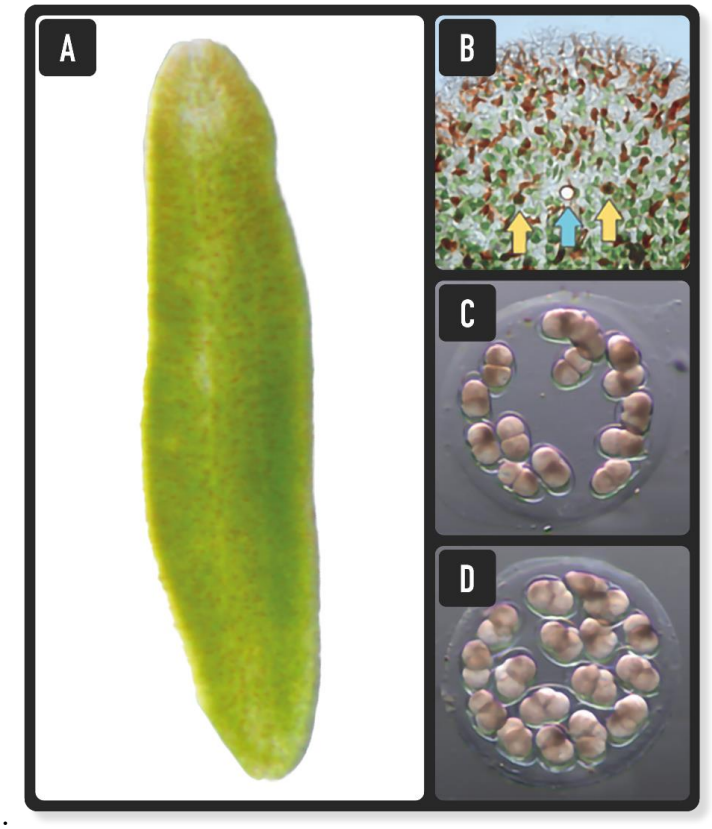

Figure 5: Close up of *S. roscoffensis* **A)** Adult *Symsagittifera roscoffensis* with ingested *Tetraselmis convolutae*. **B)** Apical part of the worm showing the statocyst (blue arrow) surrounded by two photo receptors (yellow arrow). **C)** and **D)** Close up of cocoons containing larvae of *S. roscoffensis*. Modified from Bailly et al. (2014).

They do not possess a circulatory blood system, but obtain their oxygen passively through their tissues and obtain further oxygen from to the photosynthetic active algae *in hospite.* While *S. roscoffensis* does not have a digestive system either, it does have a digestive syncytium and an orifice located ventrally. With that "mouth" larvae of *S. roscoffensis* ingest the green micro

algae *T. convolutae* (Bailly et al. 2014). The number of algal cells per adult vary between 30.000/worm, 70.000/worm up to 121.400/worm, depending on the technics used to estimate the number of endosymbionts per worm (Arboleda et al. 2018).

1.6 Tetraselmis convolutae

Phylum:	Chlorophyta
Class:	Clorodendrophyceae
Family:	Chlorodendraceae
Genus:	*Tetraselmis*
Species:	*T. convolutae*

The Genus Tetraselmis comprises green flagellates that occur in freshwater and marine habitats and have an elliptical to spherical habitus from which two pairs of equally long flagella emerge from the cell (Figure 6). They are unicellular as well as colonial and they form stalks and have one massive chloroplast per cell, with a pyrenoid that is located either centrally or posteriorly. The chloroplast is lobed anterior and can sometimes also be lobed posteriorly. The cell wall (theca) is unique within green algae since it is formed by the fusion of extracellular scales and closely surrounds the cell body. The flagella are also covered in two layers of diamond shaped scales in 24 rows (Borowitzka et al. 2016). Reproduction is ensured asexually during the non-motile, vegetative phase, which is also the most dominant and longest phase; the other one being the flagellate, motile phase. Species of the genus Tetraselmis occur in a variant of habitats. While some are benthic or can be found as plankton, few colonize the sand and some others live in an endosymbiosis. An example of an endosymbiotic Tetraselmis species is *T. convolutae,* the endosymbiont of the Acoela *S. roscoffensis*.

Tetraselmis convolutae lives as a symbiont in *Symsagittifera roscoffensis* and freely in marine waters. Although *S. roscoffensis* is able to establish a symbiosis with closely related species of *Tetraselmis* (Douglas 1985) the potency of the natural symbiont *T. convolutae* is greater in terms of growth and photosynthesis rate than other related species ingested by the worm. Moreover, a symbiosis established with an alien symbiont is replaced with *T. convolutae* if the true symbiont is offered in the medium

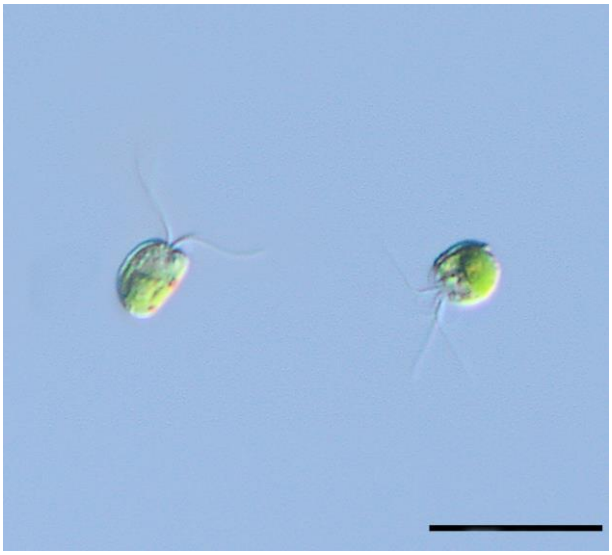

Figure 6: Light micrograph of *Tetraselmis convolutae* (scale bar equals 20 µm) 40 x magnification.

later on (Provasoli et al. 1968). The exact mechanism that leads the replacement of the alien symbiont by *T. convolutae* remain obscure.

1.7 Life cycle, reproduction and establishment of the symbiosis with *Tetraselmis convolutae*

After mating, the partner's spermatozoa fertilize the oocytes and each gravid individual will form a transparent cocoon composed of mucus that each worm abundantly produces. These cocoons contain up to 20 embryos, which are laid in the sand, and after 4-5 days (in laboratory) the translucent larvae hatch. From that moment on they require the photosynthetic algae *T. convolutae* (Figure 7). Although *S. roscoffensis* is able to form a stable symbiosis with closely related species of *T. convolutae*, if the preferred partner is available in the nearby surroundings they prefer to form a monospecific photosymbiotic association (Arboleda et al. 2018). The intake of micro algae is crucial for survival of the animal, as it will fail to survive without a successful symbiosis (Provasoli et al. 1968).

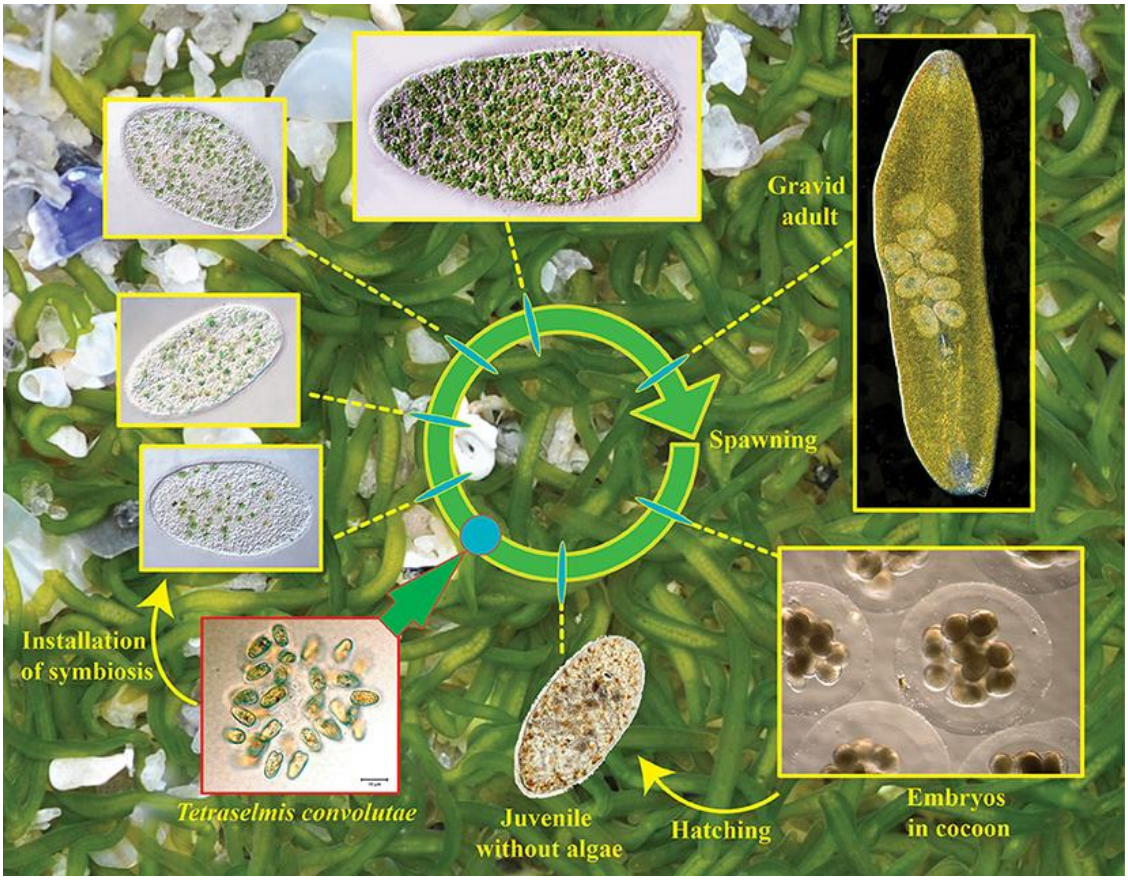

Figure 7: Life cycle of *Symsagittifera roscoffensis* 2.5-3 month. Embryos develop in cocoons. After hatching, the colourless juveniles feed on *Tetraselmis convolutae* and start greening and growing. Illustration from Bailly et al. (2014).

After the intake of *T. convolutae* the algae is vacuolated and transported to the epidermis, where they lay within the animal's cells. When the algae is ingested it loses it theca, flagella and eye spot (Provasoli et al. 1968). Multiplication of algal cells within *S. roscoffensis* by mitosis (Provasoli et al. 1968) are only documented for juvenile individuals and not in adult worms (Arboleda et al. 2018). The exact mechanisms of recognition or repulsion of the symbiont, however, remain unknown.

1.8 Ethology of *Symsagittifera roscoffensis*

Symsagittifera roscoffensis exhibits a wide range of behavioural trades. This includes geotaxis, phototaxis, rheotaxis, an avoidance reaction to high temperature as well as conspecific social behaviour (Franks et al. 2016). A very distinct behaviour is its sensitivity to mechanical stimuli (geotactic behaviour). In a calm environment they appear on the surface and stay above the sand if not disturbed by any mechanical means. If the worms are kept in a petri dish in the laboratory, they will migrate to the bottom if the dish is tilted, and in nature retract themselves into the sand when the tide comes in or if they are disturbed by any kind of vibrations. Phototactic behaviour can be observed in the laboratory as juveniles and adults respond with positive phototaxis towards a white light source. This phototactic behaviour, however, is induced by medium light intensities rather than intensities that are either too high or too low (Gamble and Keeble 1904). Although some studies show a weak negative phototaxis towards red light and positive reaction towards blue and green light (Nissen et al. 2015), the exact behaviour to specific wavelengths is not fully clarified yet. However, all experiments approaching a phototaxis in reference to the light spectrum that have been done to date were conducted without an understanding of the colour perception of the animal but rather with the human perception of the light spectrum.

A rheotaxis as well as a head avoidance is shown by Gamble and Keeble (1904). The authors showed that *S. roscoffensis* reacts positively towards a medium rate current but stick to the ground when the flow rate increases. A response to temperature occurs solely at high temperatures when it becomes life threatening, but they will not react to the temperature if they are driven towards a light source. In that case the study showed that they will move towards the light source and remain there even if the water is gradually heated to the point where it becomes lethal (Gamble and Keeble 1904).

Living on the sandy shore in the intertidal zone contains environmental hazards that may harm both partners of this symbiosis. For instance, the surface layer of the sand is greatly disturbed by incoming tides and seasonal changes lead to variances in temperature ranging from below

zero up to 40 °C, as well as changes in available sunlight, which is absolutely crucial for survival but can also be deadly if reaching a point of photo inhibition. During summertime and low tide they are exposed to high temperatures and high light intensities that can easily reach up to 2000 µmol · m^{-2} · s^{-1} (Sagert et al. 1997). The behavioural repertoire of *S. roscoffensis* may serve as a strategy to cope with such environmental hazards.

To investigate the innate behavioural response of *S. roscoffensis* the following hypotheses are formulated:

Hypothesis 1

The phototactic behaviour of *Symsagittifera roscoffensis* is directly linked to the photocharacteristics of the symbiont *Tetraselmis convolutae*.

In case this hypothesis proves to be applicable, the question arises which parameter causes the host to react.

a. The behaviour of *S. roscoffensis* serves as a protection against photo damage of the symbiont.
b. The host optimizes light conditions by actively moving into areas allowing for efficient photosynthesis.

Hypothesis 2

Irrespective of the phototactic behaviour of the host, the algae is still able and in need for irradiance acclimation of its photo apparatus.

Hypothesis 3

In addition to light and CO_2, macro- and micronutrients are essential components for growth and metabolic pathways in micro algae. In symbiosis, the host must provide nutrients required by *T. convolutae*. Consequently, hypothesis 3 is formulated as:

In addition to phototaxis, chemotactic behaviour is also required by *Symsagittifera roscoffensis* to maintain its endosymbiont *Tetraselmis convolutae*

2 Material and Methods

2.1 Sampling and Culturing *Symsagittifera roscoffensis*

Symsagittifera roscoffensis was found at two samplings sites (Figure 8) on a beach in Roscoff (48°43'11.3"N 4°00'39.3"W) and Plouescat (48°39'21.9"N 4°13'33.3"W) in Brittany, France.

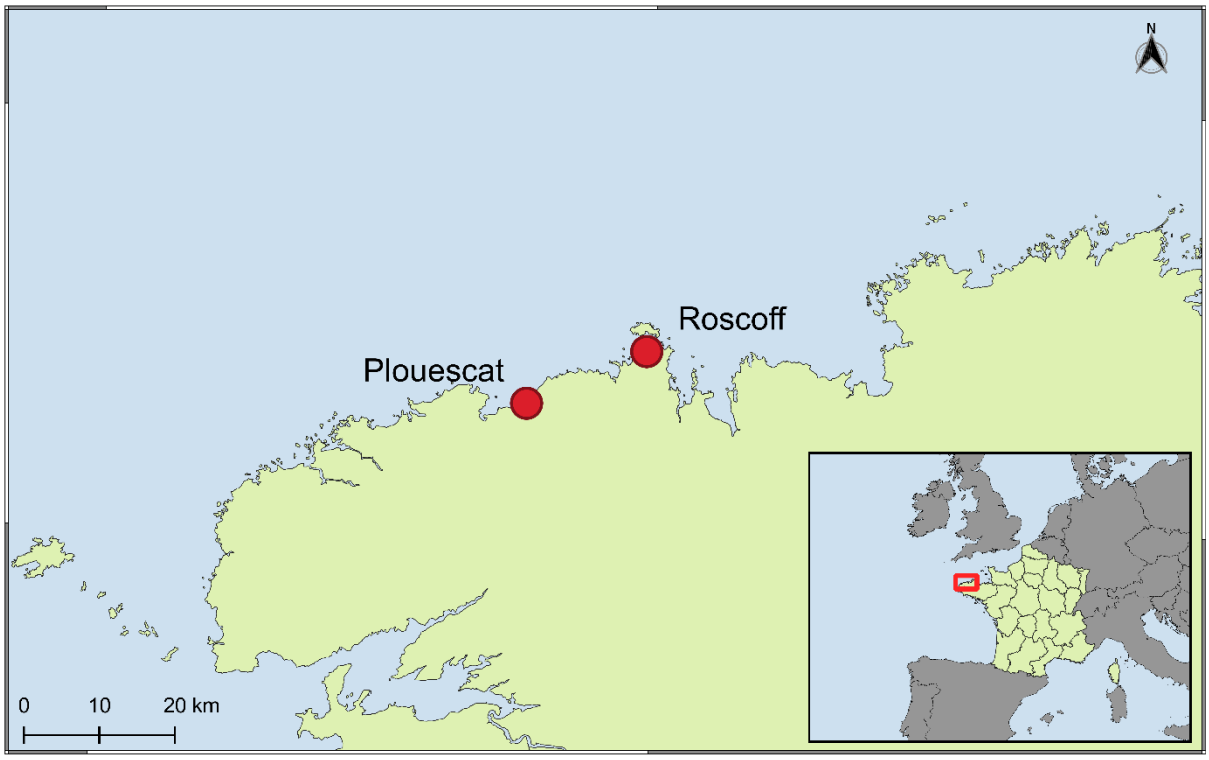

Figure 8: Map of sampling sites in France created with QGIS version 3.16.3 (QGIS Development Team). Map data source is the Database of Global Administrative Areas (GADM).

For the following experiments, animals were collected in Roscoff in October, 2019. Specimens of *S. roscoffensis* were collected on a sandy beach on the upper intertidal zone during low tide. They were placed in wide mouth bottles without sand but covered with seawater and trasported immediately to the laboratory in Rostock. In Rostock, they were cultured in 25 x 20 x 23 cm glass aquariums under natural sunlight and a constant temperature of 15±1 °C. Further steps for culturing *S. roscoffensis* were based on the description by Arboleda et al. (2018). The aquariums were filled with approximately 500 ml artificial sea water (35g/L, Instant Ocean, Blacksburg, VA) enriched with 1 % PES (Provasoli 1968) (Table 1). To ensure a sufficient oxygen supply for *S. roscoffensis*, each aquarium was continuously aired with an air pump (air400 EHEIM

GmbH & Co. KG, Deizisau, Germany). The medium was changed once per week. If Larvae were abundant, they were collected and placed in a separate aquarium and fed with *Tetraselmis convolutae*.

Table 1: Provasoli enriched seawater medium (PES) (Provasoli 1968).

Stock solution		**For 1 L PES**
Disodium DL-ß glycerophosphate * 5 H_2O	(5g / 100 ml Bidest)	8 ml
$NaNO_3$ (autoclave)	(35 g / L Bidest)	120 ml
EDTA		200 ml
– $Fe(NH_4)_2(SO_4)_2$ * 6 H_2O	(1.40 g +	
– Titriplex III	1.32 g / L Bidest)	
Vitamin		2 ml
– Thiamine	(200 mg +	
– Biotin	2 mg +	
– Vitamin B_{12}	4.07 mg / 100 ml Bidest)	
P II trace metal mix		200 ml
– Titriplex III	(5 g +	
– H_3BO_3	5.6 g +	
– $FeCL_3$ * 6 H_2O	240 mg +	
– $MnSO_4$ * H_2O	600 mg +	
– $ZnSO_4$ * 7 H_2O	110 mg +	
– $CoSO_4$ * 7 H_2O	24 mg / L Bidest)	
Bidest		470 ml

The amount of *S. roscoffensis* individuals per aquarium was an important factor of the wellbeing of *S. roscoffensis*. Culturing of *S. roscoffensis* worked best, when number of individuals per aquarium where high enough for the worms to form cluster. However, too many individuals can be counterproductive as well. If a few animals die, the entire aquarium will tilt rather fast. Culturing the worms on sand did not have an impact on the lifespan of *S. roscoffensis* but made the handling more difficult. Also maintaining them with collected Atlantic water instead of artificial seawater did not make any difference in life expectancy. However, the source of light chosen had an effect on the wellbeing of *Symsagittifera roscoffensis*. Spotlight HIT/HST-DE (Sirius, NORKA, Hamburg, Germany) was tested as well as fluorescent lamps (Philips TLD 36W/950, Hamburg, Germany). Individuals cultured under those lamps lost their dark green colour and turned light green to yellow and some individuals appeared deformed. Fluorescent lamp (Osram L36 W, Munich, Germany) and natural sunlight showed almost no deformed worms and the longest life expectancy.

2.2 Culturing *Tetraselmis convolutae*

Tetraselmis convolutae was kindly provided by Central Collection of Algal Cultures (CCAC) from the university Cologne.

Tetraselmis convolutae was cultured in standardized conditions with a 12:12 day/night cycle and 15 °C. They grew in a 100 ml Flask with modified ASP-H medium by McFadden and Melkonian (1986) (Table 2) under fluorescent light (Osram L36 W, Munich, Germany).

Table 2: ASP-H culture medium modified (McFadden and Melkonian 1986). Information for 1 L culture medium. Stock solutions are added to 1000 ml bidest and pH adjusted to 7.7 with NaOH and autoclaved. Vitamins were added after autoclave.

Stock solution		**For 1 L ASPH**
HEPES	238.1 g / L Bidest	3 ml
NaCl		18 g
$MgSO_4 * 7 H_2O$		5 g
KCL	60 g / L Bidest	10 ml
$CaCl_2 * H_2O$	370 g / L Bidest	1 ml
NaNO3	50 g / L Bidest	
$+ K_2HPO_4 * 3 H_2O$	5 g / L Bidest	
$+ NA_2CO_3$	30 g / L Bidest	1 ml
NTA	10 g / L Bidest	
NTA dissolves after addition of NaOH pellets and heating		
Fe-EDTA		1 ml
– EDTA (Titriplex II)	5.22 g / L Bidest	
– $FESO_4 * 7 H_2O$	4.98 g / L Bidest	
– 1 N KOH	54 ml / L Bidest	
EDTA and $FeSO_4$ is heated for 30 min (100 °C); KOH is added to the cooled mixture		
Trace metals		1 ml
– $ZnCl_2$	0.33 g / L Bidest	
– $MnCl_2 * 4 H_2O$	4.3 g / L Bidest	
– $CoCl_2 * 6 H_2O$	0.12 g / L Bidest	
– Na/EDTA (Titriplex III)	25.8 g / L Bidest	
pH of Trace metal solution is adjusted to 7.5 with 10 N NaOH		
H_3BO_3	2.97 g / L Bidest	10 ml
Vitamin		1 ml
– Vitamin B_{12}	0.2 g / L Bidest	
– Biotin	1.0 mg / L Bidest	
– Thiamine-HCL	100 mg / L Bidest	
– Niacinamide	0.1 mg / L Bidest	

2.3 Light acclimation

Photon based irradiance is defined as µmol · m^{-2} · s^{-1}, which is the most applicable unit for photosynthesis. When taking longer time periods into account the applicable term is light dose. Two light levels were tested with intensities of 2200 µmol · m^{-2} · s^{-1} and 90 µmol · m^{-2} · s^{-1} using customary LED lamps (Jansjö LED IKEA, Delft, Netherland). 50 animals per light treatment were placed in a weighing dish filled with fine quartz sand, 0.4 – 0.8 mm (Roth, Karlsruhe, Germany) and 15 °C tempered artificial sea water. Since *Symsagittifera roscoffensis* is very sensitive to any vibrations and to light, both groups were kept at dark for 30 minutes. Light was then turned on over a period of nine hours. Temperature was checked over the entire time of this experiment and kept at 16 ±1 °C. The behaviour of the animals were documented with a camera (α7 Sony, Tokyo, Japan) placed above the dishes and photos were taken in an interval of 30 minutes. The irradiances were measured before the start of the experiment using a PAR- micro sensor LI 250 light meter (LI-COR, Homburg, Germany).

To investigate a correlation among light intensity and the time of disappearing into the sand, five different light intensities were tested: 100, 500, 1000, 1500 and 2200 µmol · m^{-2} · s^{-1}. A total of 50 animals per light treatment were placed in a weighing dish containing fine sand. To begin of the experiment the dish with the animals were carefully placed underneath the lamps and kept at dark for 30 minutes. The temperature was checked regularly and kept at 17 °C. For documentation, the camera was placed directly above the dishes and photos were taken every 30 minutes for a total of nine hours.

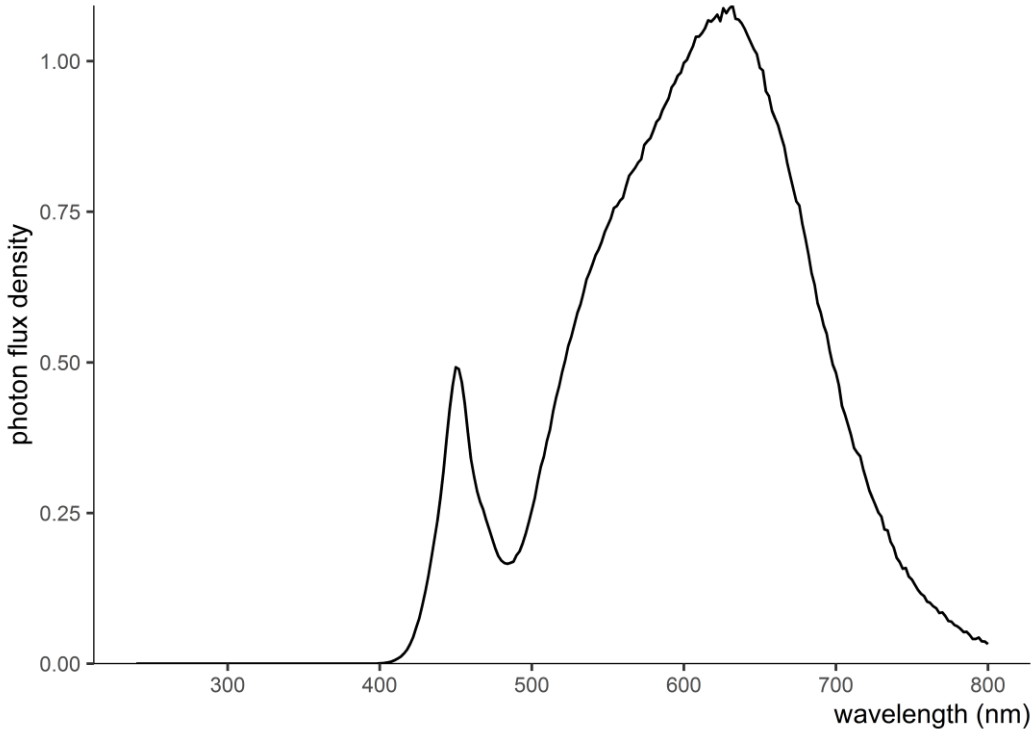

Figure 9: Spectrum of LED lamp (Jansjö LED, IKEA, Delft, Netherland). Spectrum measured with a spectroradiometer (SR9910-V7 IRRADIAN LIMITED, Tranent, Scotland).

2.4 Pigment sampling

Pigment content of *S. roscoffensis* was collected from individuals exposed to two different light regimes, 90 µmol · m^{-2} · s^{-1} and 2200 µmol · m^{-2} · s^{-1}, using LED lamps. An undefined number (approximately 400 per dish) of animals were placed in two weighing dishes without sand and 17 °C tempered artificial seawater. Both groups were kept at dark for 30 minutes. The light was then turned on over a period of nine hours.

For the extraction of the photosynthetic active pigments (carotenoids, chlorophyll a and b), 40 individuals were collected from each weighing dish every hour and placed in a 1.5 ml centrifuge tube. The residual water was carefully removed and the centrifuge tube then filled with 1 ml Dimethylsufid (DMF). The tubes were stored in the fridge at 5 °C overnight. After the incubation period of approximately 12 hours, the samples were centrifuged at 3000 $Umin^{-1}$ for about 1 minute. The supernatant was transferred to a 1 cm UV quartz cuvette (Hellma, Müllheim, Germany).

The extinction was measured in a wavelength range from 350 - 750 nm using a UV/VIS spectrophotometer (UV/VIS spectrometer Lambda 2, PerkinElmer, Waltham, USA). The

calculation for chlorophyll a, b and carotenoids are based on the equations by Wellburn (1994) and are as follows:

$$cla\ a\ (\mu g * ml^{-1}) = \left(11{,}65 * (E_{664} - E_{750}) - 2{,}69 * (E_{647} - E_{750})\right) * \frac{VDMF}{Vextract}$$

$$chl\ b\ (\mu g * ml^{-1}) = (20{,}81 * (E_{647} - E_{750}) - 4{,}53 * (E_{664} - E_{750})) * \frac{VDMF}{Vextract}$$

$$car\ (\mu g * ml^{-1}) = \frac{(1000*(E_{480}-E_{750}))-(0{,}89*(eq.1))-(520{,}2*(eq.2))}{245} * \frac{VDMF}{Vextract}$$

2.5 Fluorescence measurement

The measurement of the quantum efficiency was carried out using the Pulse Amplitude Modulation (PAM) (JuniorPAM Walz GmbH, Effeltrich, Germany).

Bevor every measurement animals were dark adapted for at least ten minutes. For the light curve 12 irradiation steps, each 30 seconds, were chosen: 0, 25, 45, 65, 90, 125, 190, 285, 420, 625, 820, 1150 and 1500 µmol · m^{-2} · s^{-1}. Animals were collected randomly and approximately 15 individuals per sample.

The calculations for the light saturation point (E_K), maximum electron transport rate (ETR_{max}), and for the initial slope of the linear range of a photosynthesis–irradiance (P–I) curve (α) were done with the equations by Platt et al. (1980):

$$ETR = ETR_{mPot}\left(1 - e^{-\frac{\alpha*PPFD}{ETRmPot}}\right) * e^{-\frac{\beta*PPFD}{ETRmPot}}$$

Maximum potential electron transport rate (ETR_{mPot})

PAR Photon flux density (PPFD)

Maximum photochemical quantum yield of photosystem II (yield) and non-photochemical quenching (NPQ) was calculated as follows:

Yield = (Fm-F_0) / Fm (Schreiber et al. 1986)

NPQ = (Fm-Fm') / Fm' (Krause et al. 1982)

2.6 Preference of light intensity

To investigate a light preferential behaviour, approximately 100 individuals were carefully placed into a glass tube of 30 cm (Ø 1 cm), filled with artificial seawater and placed underneath a light gradient created with LED lamps. The glass tube was segmented into six sections, each five cm, with light intensities ranging from 0-200, 200-500, 500-1000, 1000- 1500, 1500-2000 and > 2200 µmol · m^{-2} · s^{-1} (Figure 10). The animals were free to move along the light gradient in both directions. Before the start of the experiment, the worms were dark-adapted for 30 minutes to ensure an equal distribution within the glass tube. The irradiances were measured before the start of the experiment using a PAR- micro sensor LI 250 light meter (LI-COR, Homburg, Germany). Camera was placed above the experimental set-up and photos were taken over a period of eight hours with an interval of 30 minutes.

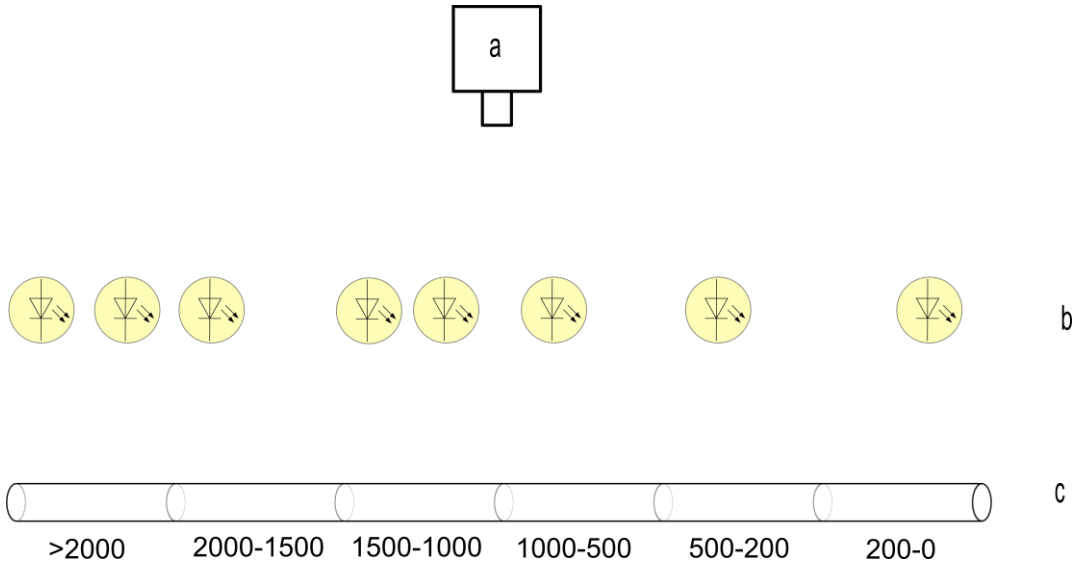

Figure 10: Schematic drawing of the experimental set-up for light intensity preference. **a**) Camera, **b**) LED lamps creating a light gradient, **c**) 30 cm glass tube containing worms and artificial seawater. Glass tube segmented into six sections (each 5 cm), animals are free to move along the light gradient in both directions.

2.7 Preference of light colour

The preference of light spectrum was conducted using a red, blue and green foil as well as a grey foil for the control group (transmission of used foils see Figure 12).

The red, blue and green foils were placed in thirds on the lid of a 14-diameter petri dish. The grey foil was as well split into thirds and placed on the lid of another 14-diameter petri dish (Figure 11).

In total four petri dishes were used for the experiment, three coloured ones and one grey control. 60 worms were placed in each petri dish with artificial seawater and the lids put on top. The

temperature was kept at 15 °C with a cooling pump (Buch & Holm A/S, Herlec, Denmark). A spotlight (SOL 500, Dr. Hönle AG, Gräfeling, Germay) was used as light source positioned above the petri dishes (spectrum see Figure 13). Three different light intensities (10, 200 and 600 µmol · m^{-2} · s^{-1}) were tested, each three hours, starting with the lowest intensity. For documentation a camera was set up beneath the petri dishes taking photos of the worms every 60 minutes. Because the light could not be distributed evenly and differed slightly from the center to the outsides of the experimental set-up, the lids were turned every hour by 120 °. Thus, each colour was in the centre for one hour for each light intensity. The entire experiment run for a total of nine hours.

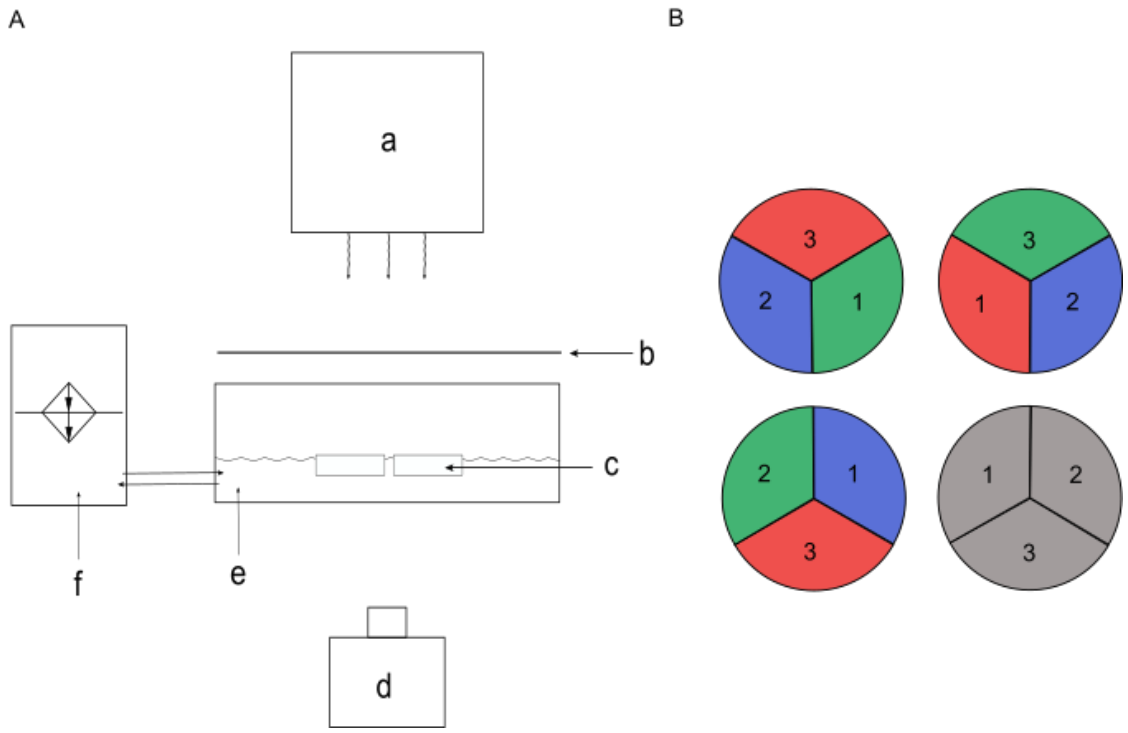

Figure 11: Schematic drawing of the experimental set-up for colour preference. **A a**) light source, **b**) frosted class for light diffusion, **c**) petri dish containing worms and artificial sea water, **d**) camera, **e**) surrounding water for cooling purpose, **f**) cooling pump. **B** Coloured lids of petri dishes. 1 highest light intensity in the centre and 3 lowest intensity outside. Lids were turned once an hour, 120 ° to the right.

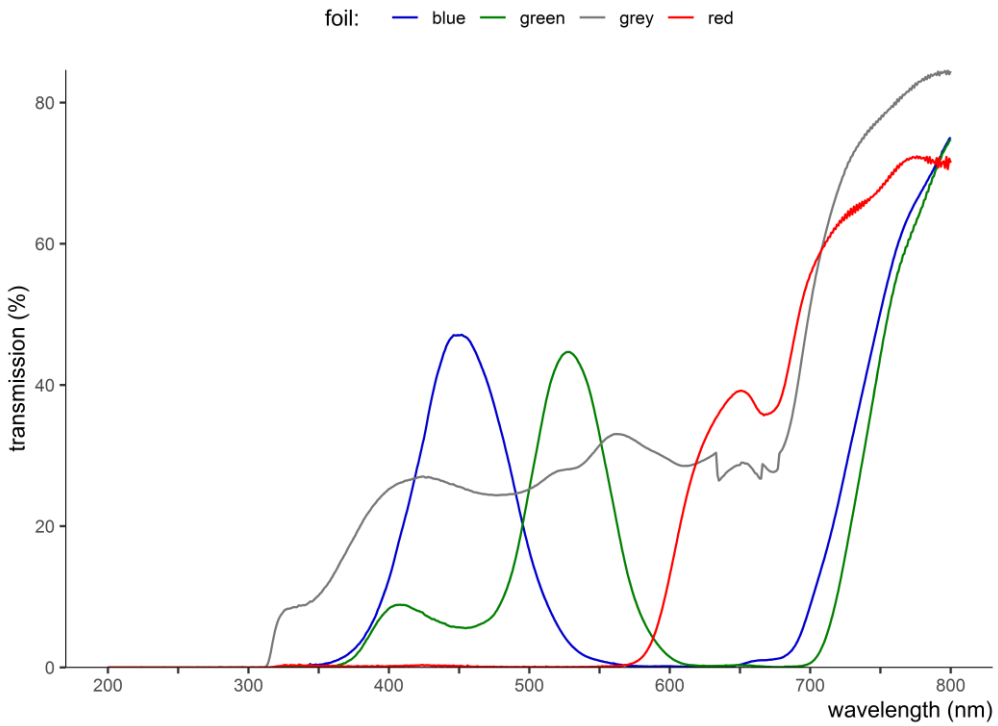

Figure 12: Transmission of red, green, blue and grey foils used for experimental set-up. Transmission (%) was measured with UV/VIS spectrophotometer (Lambda 2, PerkinElmer, Waltham, USA) from 200 nm to 800 nm.

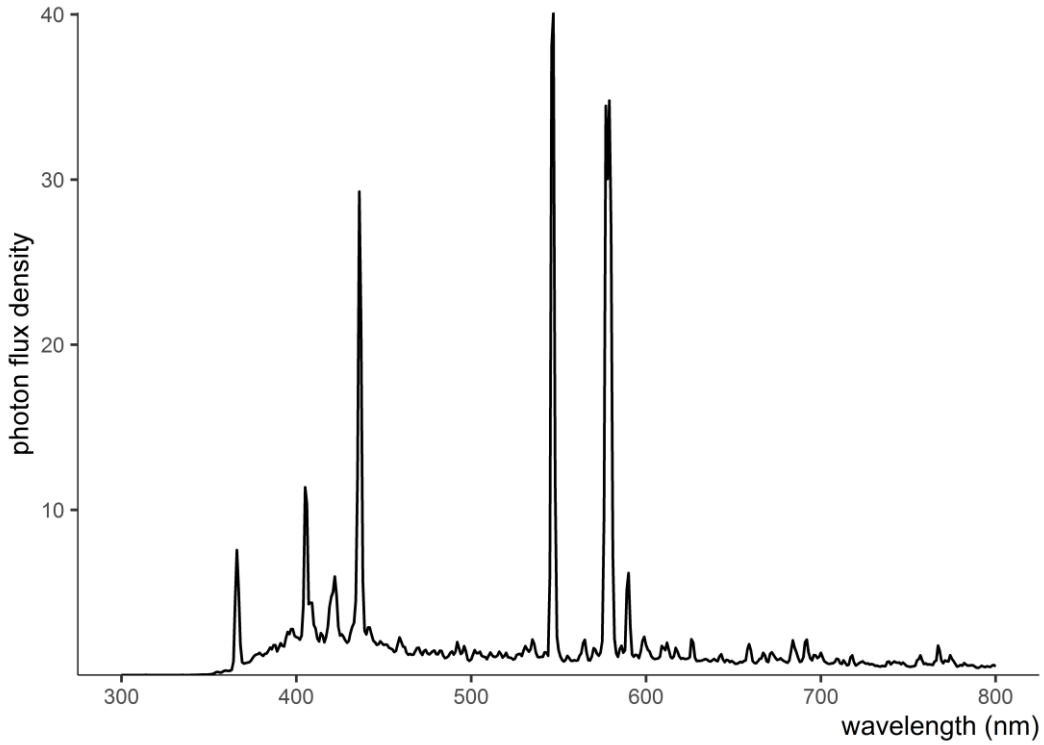

Figure 13: Spectrum of spotlight (SOL 500, Dr. Hönle AG, Gräfeling, Germay) measured with a spectroradiometer (SR9910-V7 IRRADIAN LIMITED, Tranent, Scotland).

2.8 Chemotaxis

To investigate the behaviour of *Symsagittifera roscoffensis* towards a given nutrient source two different experimental designs were conducted. For both experiments filtered Atlantic water with no extra nutrients were used to conduct the experiments.

1. Approximately 100 individuals each were placed in nine petri dishes (Ø 14 cm). Each petri dish contained a different nutrient medium filled in a permeable dialyses membrane (3 ml each). As a nutrient source 800 µmol nitrite (NO_2^-), 800 µmol nitrate (NO_3^-), 800 µmol phosphate (PO_4^{3-}), 800 µmol and nitrate+ phosphate (NO_3^-+PO_4^{3-}), 800 µmol ammonium (NH_4^+) were offered to the animals, as well as aqua dest. serving as a control group. A green algae *Ulva* sp. (1.3 g), a brown algae *Fucus* sp. (1.3 g) and a red algae *Ceramium* sp. (1.3 g) were also given as a nutrient source. The behaviour of the animals was documented using a camera that was placed above the experimental set up. Photos were taken every 30 minutes over a period of nine hours. However, this set up continuously struggled with a strong light gradient within each petri dish that could not be eliminated or reduced. Since *S. roscoffensis* is positive phototactic the data produced is not reliable and thus not included in this thesis. Based on this result a second experiment was designed.

2. Animals were carefully placed into a 30 cm glass tube (Ø 1 cm) containing filtered Atlantic water. The animals were free to move along the glass tube to either side. LED lamps were equally placed along the glass tubes to reduce an uneven light distribution to a minimum. Before the nutrients were added to the glass tubes, the animals were left for 30 minutes to ensure an equal distribution of animals within the tubes. A different nutrient source was added to each glass tube (nine in total). The nutrients used were 800 µmol nitrite, 800 µmol nitrate, 800 µmol phosphate, 800 µmol and nitrate+ phosphate as well as aqua dest.. The algae could not be placed within the glass tubes and were thus incubated in habitat water for several days at 5 °C prior to the experiment. The water was used as a nutrient source instead of the algae itself. All liquids were filled in disposable capillaries (Blaubrand®, Brand GmbH + Co. KG, Wertheim, Germany) and placed carefully into the glass tubes at one side. The glass tube was segmented into six areas, each five cm long (Figure 14). The disposable capillaries were placed in the first segment. The remaining segments were labelled 0-5, 5-10, 10-15 and 20-25 cm.

The camera was set up above the experiment to document the behaviour. Photos were taken in an interval of 30 minutes.

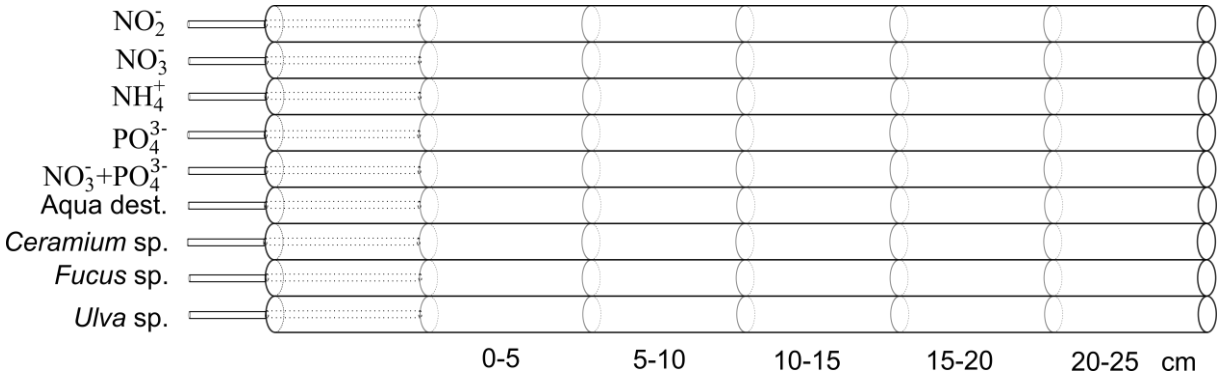

Figure 14: Schematic drawing of the experimental set-up for chemotaxis. Nine glass tubes, each 30 cm long, containing individuals of *S. roscoffensis* and filtered Atlantic Water. Each tube is partitioned into five equally long sections. Disposable capillaries were used to offer the nutrients to the worms and placed inside the glass tubes on one side.

2.9 Statistical analyses

For statistical analyses the program SPSS statistics 27 (IBM, Ehningen, Germany) was used. Data was tested for standard distribution using Shapiro-Wilk-Test and tested for homogeneity using Levene-Test. For further analysis of the data non-parametric test (Wilcoxon rank-sum test) were used and the significance level was set at 0.05.

The graphical representation of the data was carried out with R (R Core Team 2019) and R Studio (Bosten, MA, USA), using ggplot2 (Hadley Wickham 2016). The results are plotted in boxplots (interquartile range 25%- 75%) including median and outliers. The whiskers represent 1.5 times the interquartile range. Line diagrams and bar plots were plotted using mean value and standard deviation.

3 Results

3.1 Photobehaviour

The photobehaviour of *S. roscoffensis* was tested using different light intensities and light colours.

3.1.1 Light avoidance

Figure 15 shows the number of individuals that remain on the sand in dependence of time. Worms that were exposed to 2200 µmol · m^{-2} · s^{-1} disappeared into the sand earlier than those worms that were exposed to 90 µmol · m^{-2} · s^{-1}. While animals treated with 2200 µmol · m^{-2} · s^{-1} started to dig themselves into the sand after 90 minutes, individuals exposed to 90 µmol · m^{-2} · s^{-1} were still abundant by the end of the experiment after nine hours. After 180 minutes animals exposed to 2200 µmol · m^{-2} · s^{-1} were significantly less abundant on the surface compared to animals exposed to 90 µmol · m^{-2} · s^{-1} ($p = 0.029$).

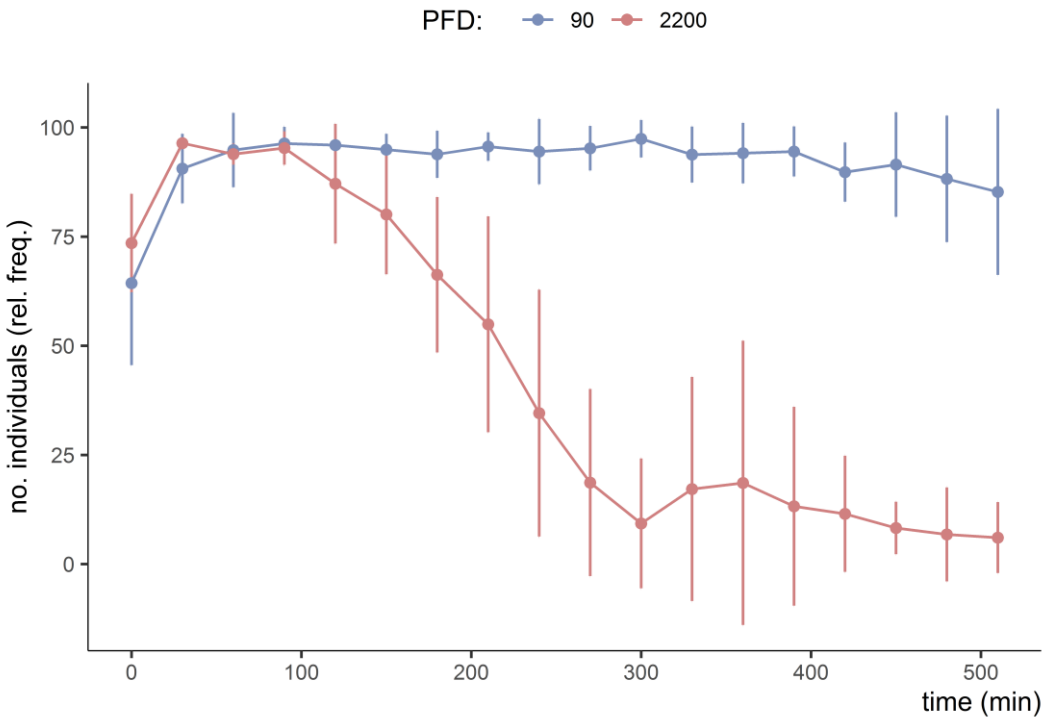

Figure 15: Behaviour of *Symsagittifera roscoffensis* under 90 µmol · m^{-2} · s^{-1} (blue) and 2200 µmol · m^{-2} · s^{-1} (red). Depicted is the number of individuals (relative frequency) on the surface over a period of nine hours. Line diagram is plotted using the mean value and standard deviation. Sample size was n=4 and 50 individuals per replicate.

To test for a light intensity dependent behaviour and for a dose effect of the worm, the following experiment was carried out. The worms were exposed to different light intensities and left with the possibility to dig themselves into the sand to seek protection. In total, five different light intensities ranging from 100 µmol · m^{-2} · s^{-1} to 2200 µmol · m^{-2} · s^{-1} were tested over a period of nine hours. The data shown in Figure 16 A display the time at which 50 % of the animals, in each respective light treatment, disappeared into the sand. 50 % of animals treated with 100 µmol · m^{-2} · s^{-1} dug themselves into the sand after approximately 11 hours of constant light exposure. Animals treated with 400 µmol · m^{-2} · s^{-1} disappeared into the sand after approximately nine hours. Under 900 µmol · m^{-2} · s^{-1} after 6.5 hours and under 1500 µmol · m^{-2} · s^{-1} after approximately six hours. Animals exposed to 2200 µmol · m^{-2} · s^{-1} disappeared first after five hours. The data depicted in Figure 16 B show the calculated light dose at the time that 50 % of the animals disappeared into the sand.
Sample calculation for 100 µmol · m^{-2} · s^{-1} = 6000 µmol · m^{-2} · min^{-1}
797.36 min * 6000 µmol · m^{-2} · min^{-1} = 4784160 µmol · m^{-2}

The received light dose at the specific time differed greatly. Worms treated with 100 µmol · m^{-2} · s^{-1} received 4.78 mol · m^{-2}. Animals that were treated with 400 µmol · m^{-2} · s^{-1} received 14.16 mol · m^{-2} and animals treated with 900 µmol · m^{-2} · s^{-1} were exposed to 23.9 mol · m^{-2}. Worms exposed to 1500 µmol m^{-2} s^{-1} received 36.32 mol · m^{-2} by the time they moved into the sand and worms under 2200 µmol · m^{-2} · s^{-1} were exposed to 40.73 mol · m^{-2}.

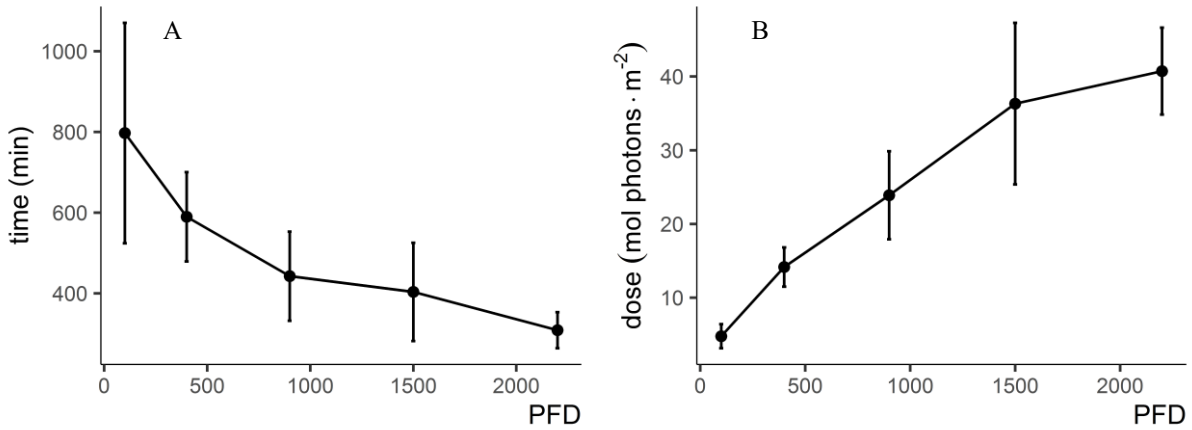

Figure 16: Behaviour of *Symsagittifera roscoffensis* exposed to five different light intensities (100, 400, 900, 1500 and 2200 µmol · m^{-2} · s^{-1}). **A**) Depicted is the time (min) at which 50 % of the individuals retreated into the sand in dependence of photon flux density (PFD). **B**) Shows the received light dose in dependence of the photon flux density (PFD) at the time of disappearance. Line diagram is plotted using the mean value and standard deviation. Sample size was n=5 and 50 individuals per replicate.

3.1.2 Preference of light intensity

To test whether *S. roscoffensis* chooses the optimal light intensity for its symbiont *T. convolutae*, a preference experiment was set up. The animals were exposed to a linear light gradient ranging from 0 – 2200 µmol · m^{-2} · s^{-1} and free to move in both directions. The behaviour of *S. roscoffensis* was documented over time and the results are plotted in Figure 17. To begin of the experiment at time 0, 43.93 % of the worms gathered in segment 0 – 200 µmol · m^{-2} · s^{-1}. From 60 minutes to 150 minutes the maximum accumulation was in high illuminated areas ranging from 500 – 1000 µmol · m^{-2} · s^{-1}, 1000 – 1500 µmol · m^{-2} · s^{-1} and from 1500 – 2000 µmol · m^{-2} · s^{-1}. At the same time the low light area (0 – 200 µmol · m^{-2} · s^{-1}) contained the least individuals (11 %). Starting from 180 minutes the number of individuals in the low illuminated area constantly increased with a maximum of 48.43 % by the end of the experiment at 480 minutes.

Three different points in time (60, 240 and 480 minutes) are exemplarily depicted in Figure 18. There are no significant differences at 60 minutes between the different light intensities but the maximum accumulation of 22.41 % of *S. roscoffensis* are at the range of 1000 – 1500 µmol · m^{-2} · s^{-1} and the least (10.68 %) in the area from 0 – 200 µmol · m^{-2} · s^{-1}. At 240 minutes the maximum of individuals (21.14 %) gather at 1000 – 1500 µmol · m^{-2} · s^{-1} and the least (10.01 %) are at the highest light intensity of > 2000 µmol · m^{-2} · s^{-1}. By the end of the experiment (480 minutes) 48 % of *S. roscoffensis* accumulated in the range of 0 – 200 µmol · m^{-2} · s^{-1}.

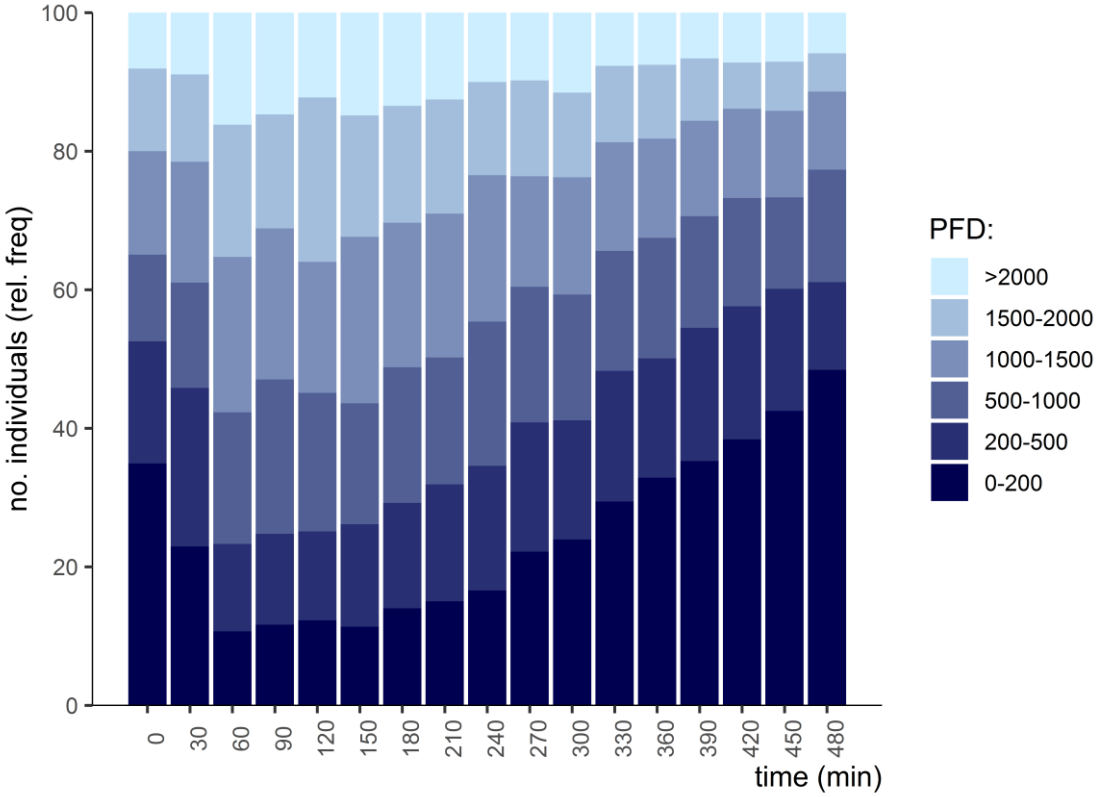

Figure 17: Distribution pattern of *Symsagittifera roscoffensis* exposed to a light gradient ranging from 0 to 2000 µmol · m^{-2} · s^{-1}. The diagram shows the number of individuals (rel. freq.) in dependence of the time under the light gradient (segmented into six sections). The stack bar is plotted using the mean value. Sample size was n=6 and approximately 100 individuals per replicate.

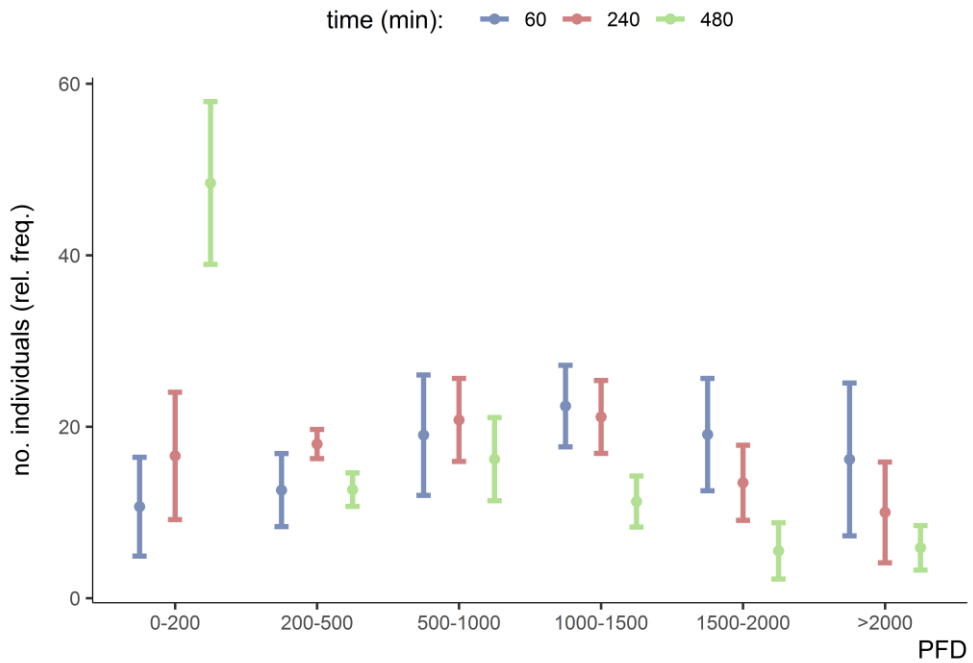

Figure 18: Distribution pattern of *Symsagittifera roscoffensis* exposed to a light gradient ranging from 0 to over 2000 µmol · m^{-2} · s^{-1} at three selected points in time. Depicted is the number of individuals (rel. freq.) in dependence of the respective light segments at three selected time points (60, 240 and 480 min). The line diagram is plotted using the mean value and standard deviation. Sample size was n=6 and approximately 100 individuals per replicate.

3.1.3 Preference of light colour

To investigate whether the worms prefer a particular light colour the animals were exposed to red, blue and green light. The position of the colour had a significant influence in all three light intensities on the number of individuals. Under low light conditions (Figure 19) only the control group in position one (closest to the light source) differs significantly compared to control group in position two regarding the number of individuals. Under high light intensities all colours in position one differ significantly compared to their respective colour in position two and three, with the highest number of individuals always being in position one.

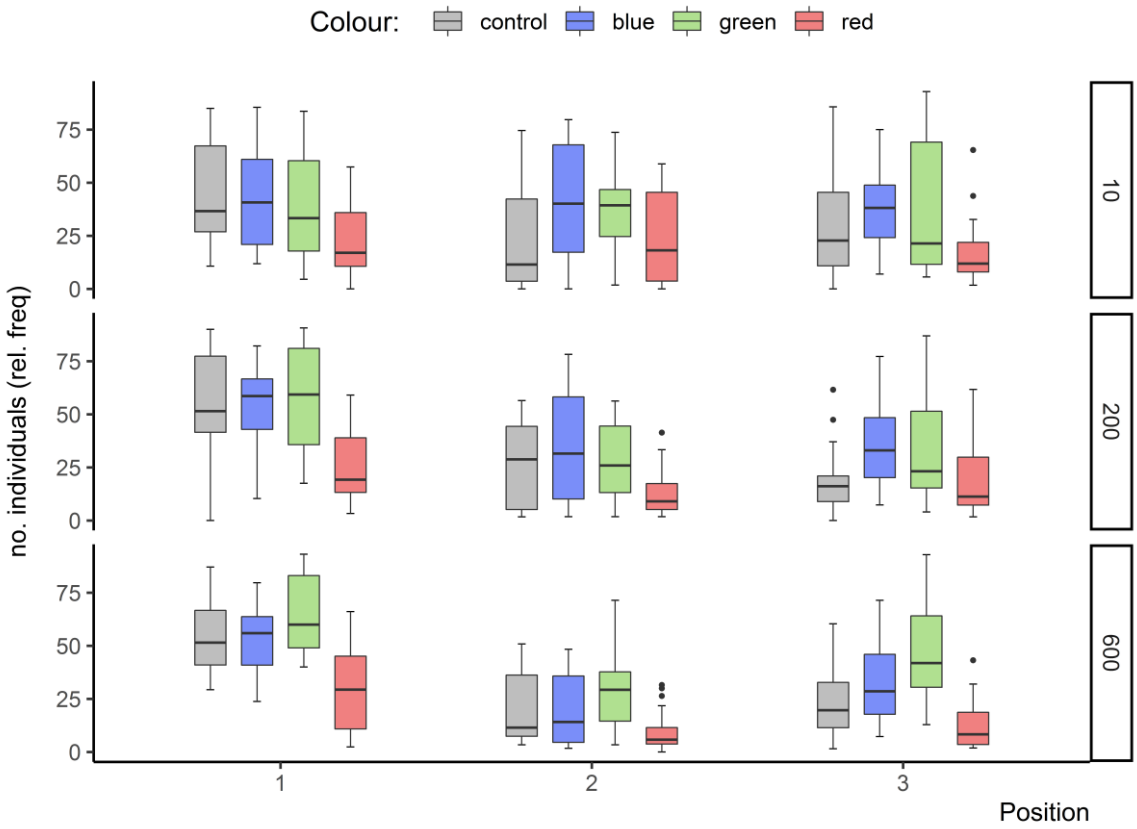

Figure 19: Distribution pattern of *Symsagittifera roscoffensis* under different light colours. Depicted is the number of individuals (relative frequency) in dependence of the position of the coloured foils and grouped by colour as well as three different light intensities: 10, 200 and 600 µmol · m^{-2} · s^{-1}. Boxplots include median, outliers (points) and whiskers (1.5 times of the interquartile range). Sample size was n=7 and 60 individuals per replicate.

However, the colour of the light does matter and *S. roscoffensis* prefers blue and green light to red. Under low light conditions (10 µmol · m^{-2} · s^{-1}) were significantly less worms under red light compared to blue light and control. It is a similar result in position three with significantly less animals under red light in comparison to blue and green light. This pattern is repeated for 200 µmol · m^{-2} · s^{-1} and 600 µmol · m^{-2} · s^{-1}. While there is no significant difference among

blue, green and control, red light has significantly less individuals compared to all other groups in the first position. In the second position, red differs significantly to blue light and to green but not to the control group. The number of individual's also significantly smaller under 600 µmol · m^{-2} · s^{-1} compared to the number of individuals under blue and green light regardless of the position the red foil was on.

Depicted in Figure 20 is the incidence irradiance and the cross absorbance of the coloured foils in the range from 300 – 800 nm. To obtain the incidence irradiance, the light spectrum (Figure 13) and the transmission of each foil (Figure 12) were calculated. The cross absorbance was obtained by calculating the incidence irradiance with the absorption spectrum of the adult animal containing *T. convolutae*. Under the blue foil *S. roscoffensis* absorbed 82 µmol · m^{-2} · s^{-1}, under the green foil 23 µmol · m^{-2} · s^{-1} and under the red foil 6.7 µmol · m^{-2} · s^{-1}.

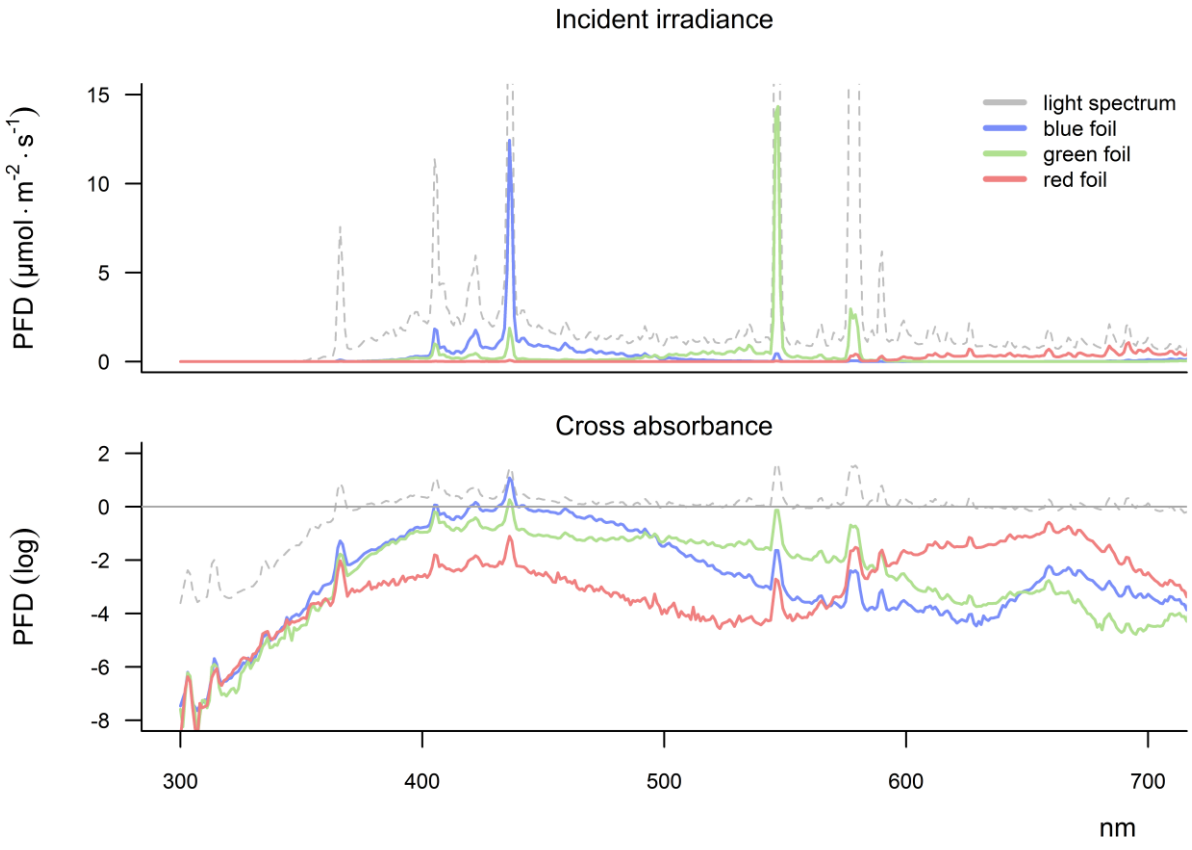

Figure 20: Incident irradiance and cross absorbance for experimental set up of light colour preference. The upper diagram shows the incident irradiance, which was calculated using the emission spectrum of the light source (Figure 13) and the transmission of the coloured foils (Figure 12). The lower diagram shows the cross absorbance (logarithmic scale) which was calculated with the absorption spectrum of *S. roscoffensis* combined with the incidence irradiance shown in the upper panel.

The efficiency of which light is available for photosynthesis differs among the different wavelength (Emerson and Lewis 1943). The quantum yield declines at wavelength below 400 nm as well as greater than 680 nm (Evans 1987). Thus, the photosynthetically active radiation is defined from 400 to 700 nm (Evans 1987). The cross absorbance and the efficiency, in the range from 405 - 685 nm, of chlorophyll a and carotenoids under the blue, green and red foil is presented in Table 3. The calculations for the absorption of the respective pigment and their efficiency are based on Schubert (1989).

Table 3: Absorbed photons ($\mu mol \cdot m^{-2} \cdot s^{-1}$) under the blue, green and red foil in the range of 405-685 nm. Total cross absorbance, absorbed by chlorophyll a (Chl), absorbed by carotenoids (Car). Total amount of photons available for photosynthesis from chlorophyll a and carotenoids. Absorbed photons available for photosynthesis by chlorophyll a (Echl) and carotenoids (Ecar). Calculations for the efficiency (Echl and Ecar) are based on the quantum efficiency of 0.85 for chlorophyll a and 0.25 for carotenoids (Schubert 1989).

		blue	green	red
Cross absorbance	**Total**	54.39	15.61	6.63
	Chl	16.74	6.57	6.43
	Car	37.65	9.04	0.20
Photosynthetic efficiency	**Total**	22.13	4.05	2.10
	Echl	14.23	4.05	2.06
	Ecar	7.91	1.87	0.04

3.2 Photophysiology

A behavioural response to light is one way for the host to provide its symbiont with the optimal light condition and protection for photoinhibition and permanent damage under high irradiances. The high motility of the worm may allow the host to protect *T. convolutae* against photoinhibition when exposed to high irradiances for a prolonged period of time. Nevertheless, it might still be in favour for the algae to undergo changes in photophysiology as well to optimize photosynthesis and avoid photo damage. In their natural habitat, they are exposed to changes in available sunlight ranging on a timescale from hours to seconds.

The changes in photophysiology are typically achieved by an increase in carbon fixation rates or by the activation of the xanthophyll cycle, which allows the dissipation of excess light energy into heat (Müller et al. 2001). Since an acclimation to light can be seen in the changes of pigment content and changes in photosynthesis rate, the following two experiments were conducted.

3.2.1 Pigment content

Chlorophyll a, b and Carotenoids were extracted and measured for animals treated with 90 µmol · m^{-2} · s^{-1} and 2200 µmol · m^{-2} · s^{-1} over a period of nine hours (Figure 21). Table 4 presents the pigment content per individual worm bevor the experiment started.

Table 4: Mean and standard deviation (SD) of absolute pigment content for chlorophyll a (chl a), chlorophyll b (chl b) and carotenoids (car) per individual worm before light treatment. N is the number of samplings. Each sampling contained 40 individuals. Calculations by Wellburn (1994).

	n	mean		SD
Chl a	8	0.05	±	0.01
Chl b	8	0.03	±	0.01
Car	8	0.02	±	0.00

The content of chl a, chl b and car for animals treated with 90 µmol · m^{-2} · s^{-1} as well as 2200 µmol · m^{-2} · s^{-1} decreases within the first four hours and then continuously increases, reaching a maximum after seven hours for 90 µmol · m^{-2} · s^{-1} and after six hours for 2200 µmol · m^{-2} · s^{-1}. For chl a and chl b the only detectable significant difference between 90 µmol · m^{-2} · s^{-1} and 2200 µmol · m^{-2} · s^{-1} is after six hours with a p- value of 0.004 and 0.04 respectively. For extracted carotenoids significant difference were found after six ($p = 0.002$) and nine ($p = 0.05$) hours. However, after eight hours many animals had died under 2200 µmol · m^{-2} · s^{-1}, which made it difficult to collect enough individuals for pigment extraction and resulted in less replicates. Therefore, the graphs for pigment content (Figure 21) and the ratio of the pigments (Figure 22) represent eight instead of nine hours.

The ratio from chl a to chl b and the ratio from chl a + chl b to car in dependence of the time is depicted in Figure 22. There are no changes over time, however, the chl a / chl b as well as the chl a + chl b / car ratio differ significantly ($p = 0.000$) between 90 µmol · m^{-2} · s^{-1} and 2200 µmol · m^{-2} · s^{-1}.

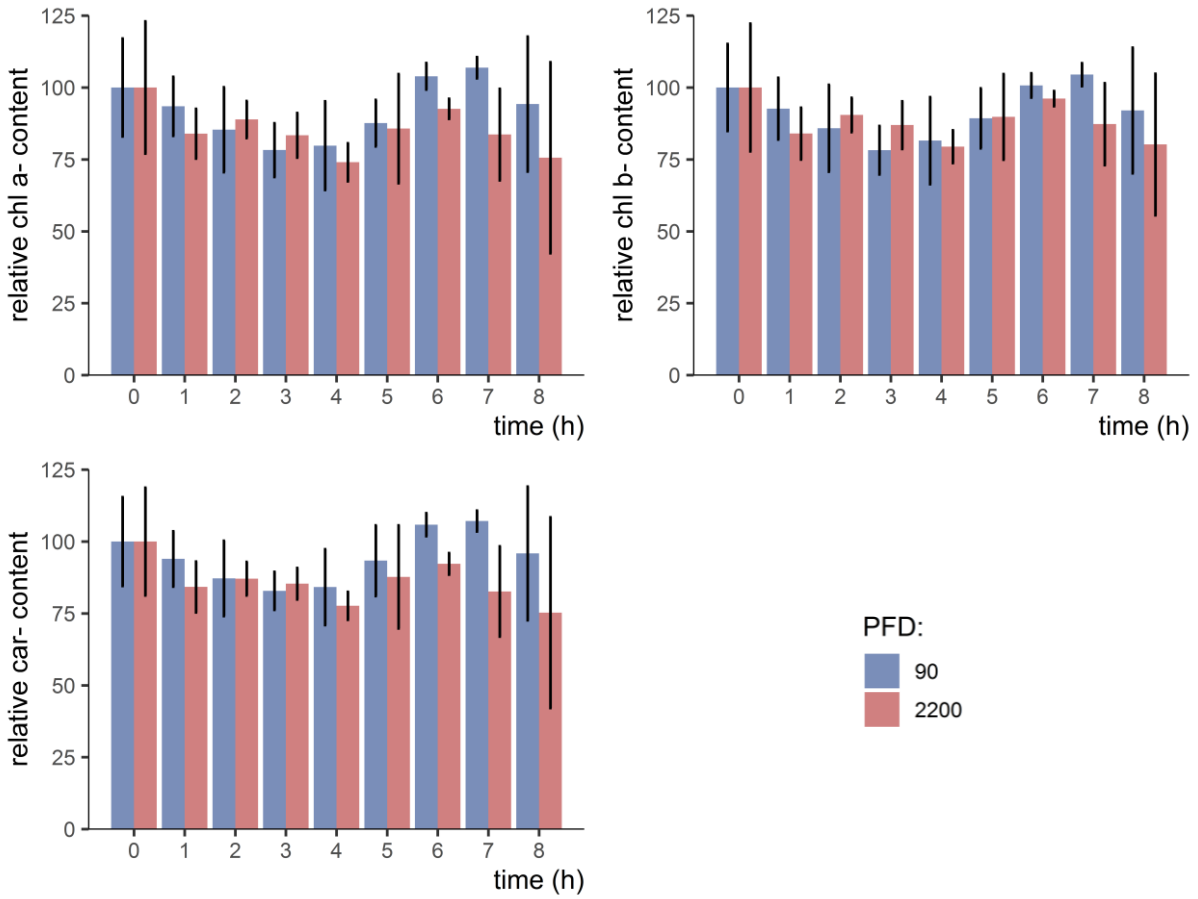

Figure 21: Pigment content of ingested *Tetraselmis convolutae* by *Symsagittifera roscoffensis*. Depicted is the content of chl a, chl b and car over a period of eight hours and constant light exposure to 90 µmol · m^{-2} · s^{-1} (blue) and 2200 µmol · m^{-2} · s^{-1} (red). The content in relation to the mean of the start values (time point 0). Bar plot is plotted using the mean value and standard deviation. Sample size was n=4 and 40 individuals per replicate.

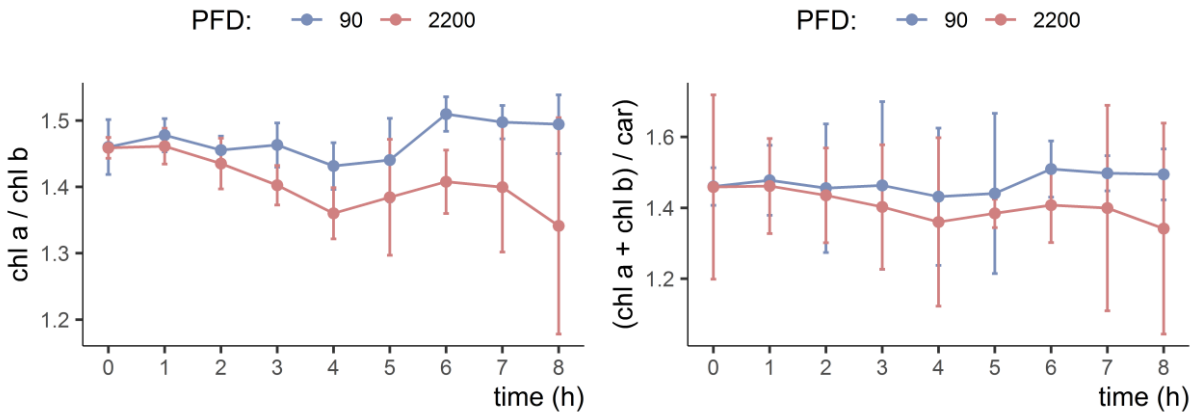

Figure 22: Ratio of chlorophyll a to b for ingested *Tetraselmis convolutae* by *Symsagittifera roscoffensis* and ratio of chlorophyll a and b to carotenoids. Animals were exposed to 90 µmol · m^{-2} · s^{-1} (blue) and 2200 µmol · m^{-2} · s^{-1} (red). The line diagram is plotted using the mean value and standard deviation. Sample size was n=4 and 40 individuals per replicate.

3.2.2 Fluorescence measurement

The Pulse-Amplitude-Modulation (PAM) is a non-destructive way to measure the quantum efficiency of *T. convolutae* without harming the host.

The dark_yield (F_v/F_m) was measured over nine hours for animals treated with 90 and 2200 µmol · m^{-2} · s^{-1} (Figure 23). While the yield for 90 µmol · m^{-2} · s^{-1} is consistent over time, the yield measured for animals treated with 2200 µmol · m^{-2} · s^{-1} already decreases significantly after the first hour of irradiance and continuously decreases until reaching the lowest value of 0.03 after nine hours.

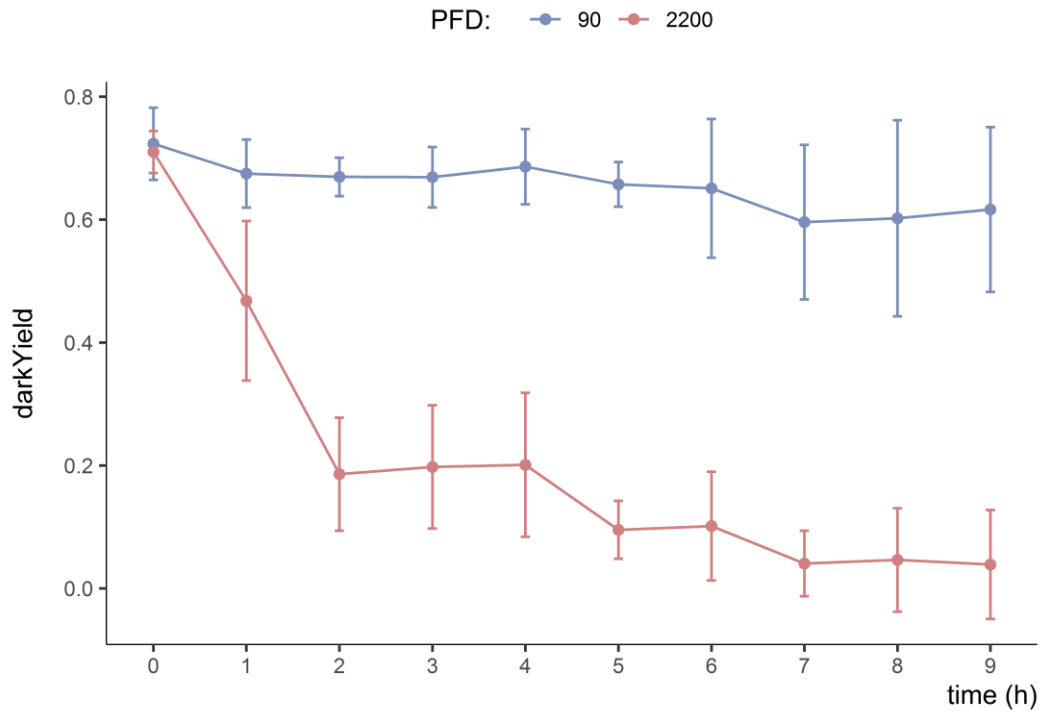

Figure 23: Dark-yield of *Symsagittifera roscoffensis*. Animals were exposed to 90 µmol · m^{-2} · s^{-1} (blue) and 2200 µmol · m^{-2} · s^{-1} (red). Depicted is the yield of dark-adapted individuals in dependence of the time (hours). The line diagram is plotted using the mean value and standard deviation. Sample size was n=8.

The initial slope of the linear range of a photosynthesis–irradiance (P–I) curve (α) is depicted in Figure 24. The diagram shows α in dependence of the time grouped by light intensity. The value for 2200 µmol · m^{-2} · s^{-1} differs significantly compared to α for 90 µmol · m^{-2} · s^{-1} ($p = 0.000$). However, there was no significant difference to begin of the experiment at 0 minutes.

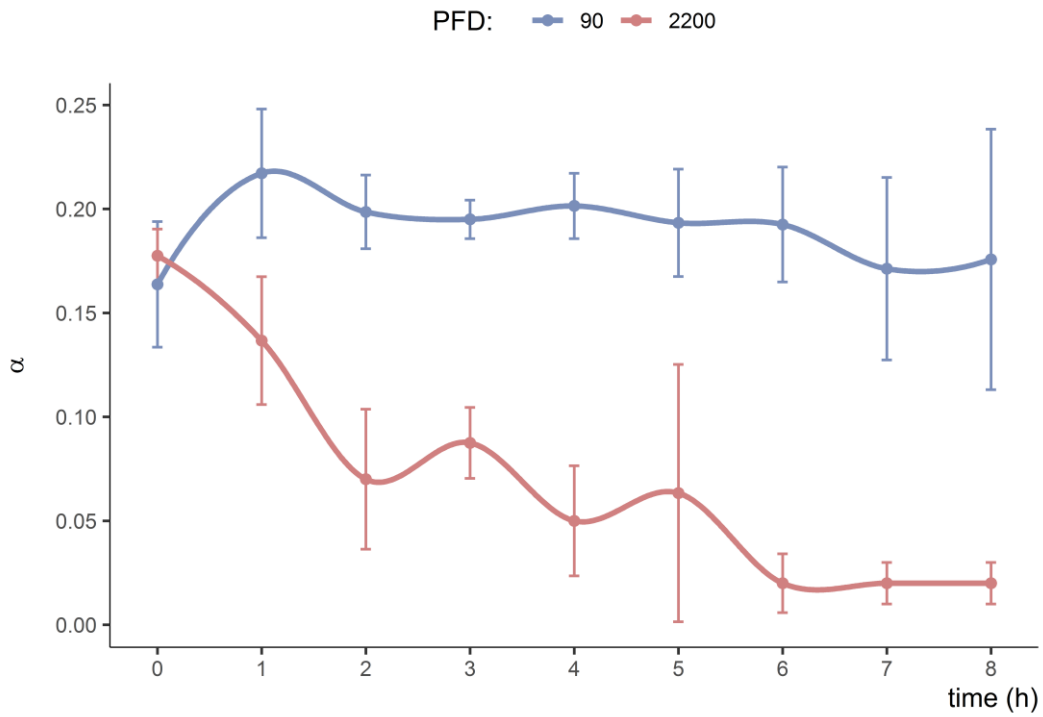

Figure 24: Progress of alpha (α) of *Symsagittifera roscoffensis*. Animals were exposed to 90 µmol · m^{-2} · s^{-1} (blue) and 2200 µmol · m^{-2} · s^{-1} (red) over a period of eight hours. The line diagram is plotted using the mean value and standard deviation. Sample size was n=8.

The light saturation point (E_K) for animals treated with 90 µmol · m^{-2} · s^{-1} and 2200 µmol · m^{-2} · s^{-1} is shown in Figure 25. The groups differ significantly after the first hour of the experiment ($p = 0.002$). Median for 90 µmol · m^{-2} · s^{-1} is 262.31 and for 2200 µmol · m^{-2} · s^{-1} significantly higher with 370.39. The value for the light saturation point (E_K) to begin of the experiment is at an average of 355 µmol · m^{-2} · s^{-1} (90 µmol · m^{-2} · s^{-1}) and 389 µmol · m^{-2} · s^{-1} (2200 µmol · m^{-2} · s^{-1}).

Figure 26 shows the development of the Non-photochemical fluorescence quenching (NPQ) over the period of nine hours. Used for data analysis is the median of the NPQ curve for each measurement. Animals under 90 µmol · m^{-2} · s^{-1} differ significantly ($p = 0.000$) compared to animals treated with 2200 µmol · m^{-2} · s^{-1} with a median of 0.18 for 90 µmol · m^{-2} · s^{-1} and 0.05 for 2200 µmol · m^{-2} · s^{-1}.

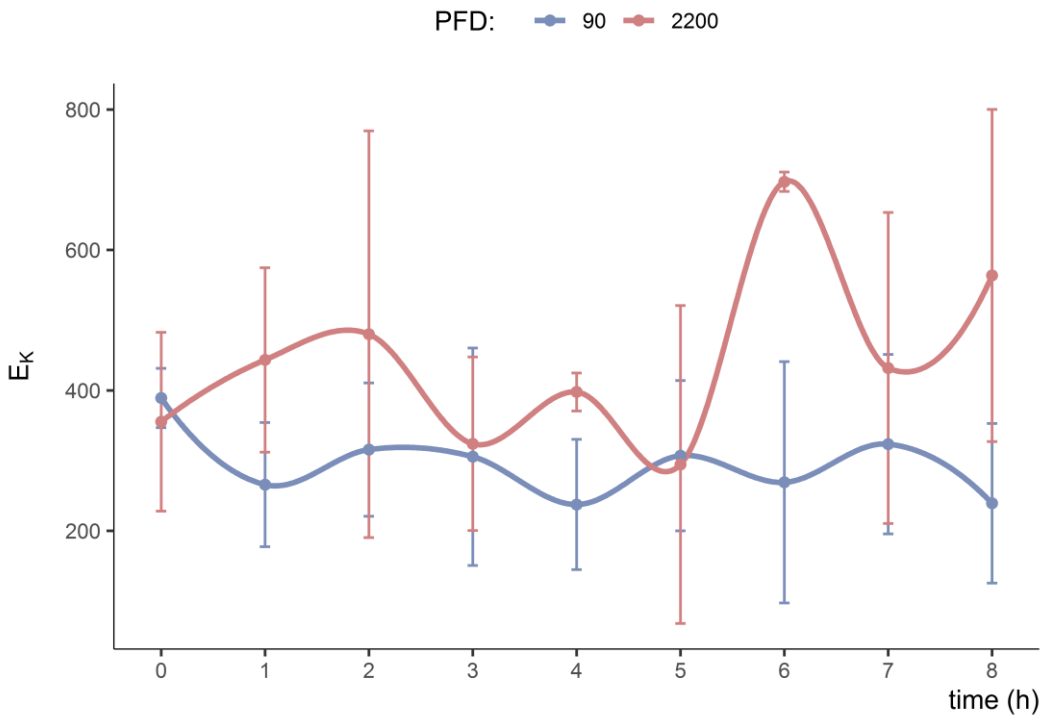

Figure 25: Light saturation point of *Symsagittifera roscoffensis*. Animals were exposed to 90 µmol · m^{-2} · s^{-1} (blue) and 2200 µmol · m^{-2} · s^{-1} (red) over a period of eight hours. The line diagram is plotted using the mean value and standard deviation. Sample size was n=8.

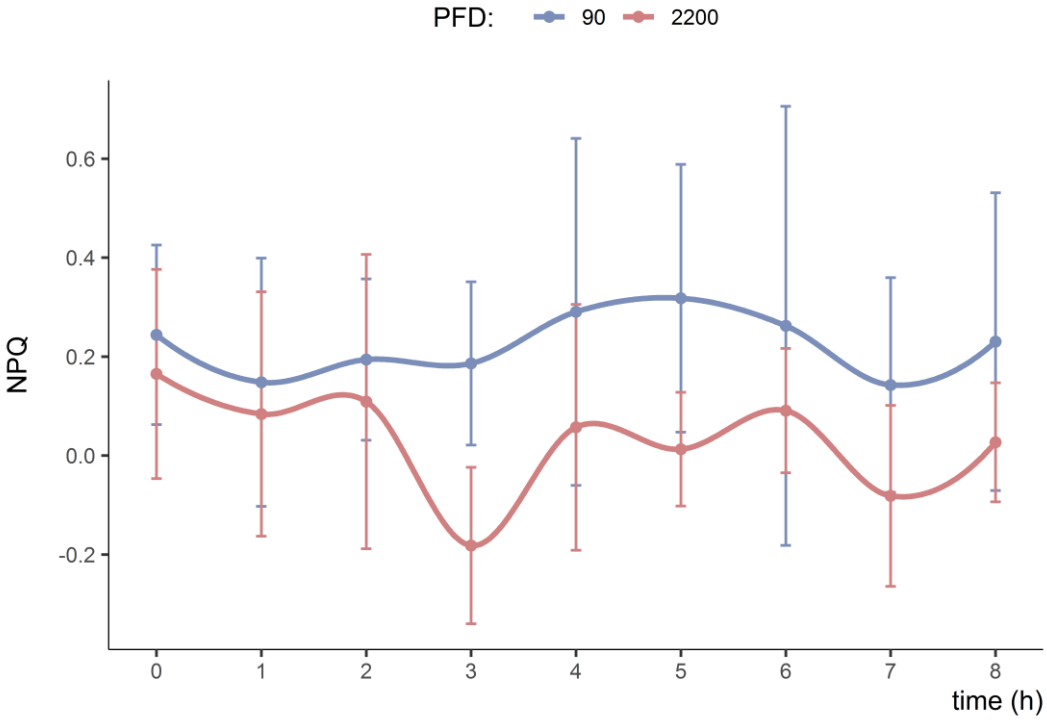

Figure 26: Non-photochemical fluorescence quenching (NPQ) of *Symsagittifera roscoffensis*. Animals were exposed to 90 µmol · m^{-2} · s^{-1} (blue) and 2200 µmol · m^{-2} · s^{-1} (red) over a period of eight hours. The line diagram is plotted using the mean value and standard deviation. Sample size was n=8.

3.3 The role of nutrients

In addition to light as the main energy source, macro- and micronutrients are essential for micro algae as they play important roles in numerous functional and regulatory mechanisms. While some nutrients are required in higher concentrations (macronutrients), other are required in much lower concentrations (micronutrients). Micronutrients include, among many others Iron (Fe), Manganese (Mn) or Zinc (Zn), they are key elements to photosynthetic pathways (Fe), electron transport and maintenance of the chloroplast membrane structure (Mn) or they are components of enzymes and other proteins (Zn) (Quigg 2016). Phosphorous (P) and Nitrogen (N) are examples for macronutrients and are needed in high concentrations but can be limited in natural environments and therefore limit the growth of the micro algae. In the cell, P is often bound as PO_4^{3-} and involved in several metabolic pathways. It is a fundamental element to ATP, DNA, Proteins as well as some enzymes. The intake of N by the organisms is often in form of NO_2^- and NO_3^- as well as NH_4^+ and it plays a role in N-containing metabolites, the synthesis of proteins and enzymes or as a component of chlorophyll (Pareek 2010).

For micro algae living in symbiosis, the host must provide those nutrients. In the natural habitat of *S. roscoffensis* and *T. convolutae* in Northern France, accumulations of *S. roscoffensis* were observed near washed up macro algae residues. However, a chemotaxis has not yet been investigated.

Figure 27: Accumulation of *Symsagittifera roscoffensis* on a beach in Roscoff, France, 2019. Photos taken by Hendrik Schubert.

3.3.1 Chemotaxis

A possible chemotaxis was tested using 800 µmol of NO_2^-, NO_3^-, NH_4^+ , PO_4^{3-}, NO_3^-+PO_4^{3-} , *Ulva* sp., *Fucus* sp., C*eramium* sp. and aqua dest. as control medium. The nutrients were

presented on one side of a glass tube containing *S. roscoffensis*. Each tube was equally divided into five segments, each 5 cm long (0 - 5, 5 - 10, 10 - 15, 15 - 20, 20 - 25 cm). The animals were free to move within all segments over the entire time during the experiment.

Figure 28 presents the results for *Ulva* sp., *Fucus* sp., C*eramium* sp. When comparing each segment over the time of one, four and eight hours, the middle segment (10 - 15 cm) had significantly more individuals after four and eight hours than to begin of the experiment (one hour) when *Fucus* sp. was given as a nutrient source. For *Ceramium* sp. and *Ulva* sp. no significant differences were found for either segment over the period of eight hours.

Comparing the differences between the segments at the respective time of one hour, four and eight hours more significant differences were found.

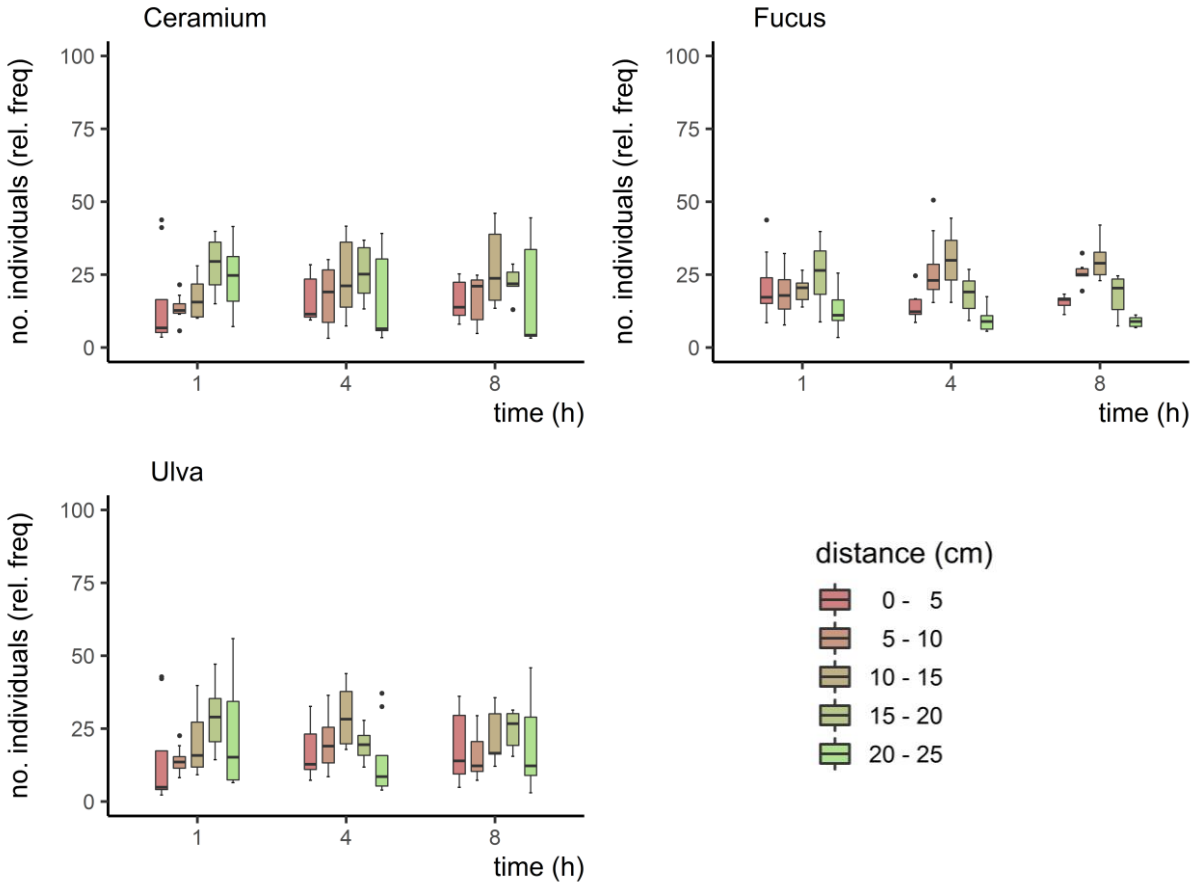

Figure 28: Distribution of *Symsagittifera roscoffensis* exposed to *Ceramium* sp, *Fucus* sp. and *Ulva* sp.. Depicted is the number of individuals (relative frequency) depending on the time (hours). Each glass tube is segmented in five equal sized sections. The legend shows each segment of the glass tube starting from the segment closest to the nutrient source (0-5 cm) to the segment furthest away (20-25 cm). Boxplots include median, outliers (points) and whiskers (1.5 times of the Interquartile range). Sample size was n=3 and approximately 100 individuals per replicate.

With *Ceramium* sp. as nutrient source (Figure 28) there are significantly more individuals of *S. roscoffensis* in the segments 15 - 20 cm and 20 - 25 cm (further way from the nutrient source) compared to 0 - 5 cm and 5 - 10 cm (closest to nutrient source) after one hour, but none after four and eight hours. For *Fucus* sp. (Figure 28), no difference can be found after one hour but after four and eight hours the highest number of individuals are in the middle segments (10 - 15 and 15 - 20 cm) and significantly less individuals closest to the source (0 - 5 cm) and the furthest away (20 - 25 cm). With *Ulva* sp. as source the only significant differences were found after four hours in the middle segment (10 - 15 cm) containing significantly more individuals compared to the segment closet to the source (0 - 5 cm) and the segment furthest away (20 - 25 cm). These differences however were not significant anymore after eight hours.
Figure 29Figure 28 presents the results for the control groups (aqua dest.), NO_3^- and NO_2^-.

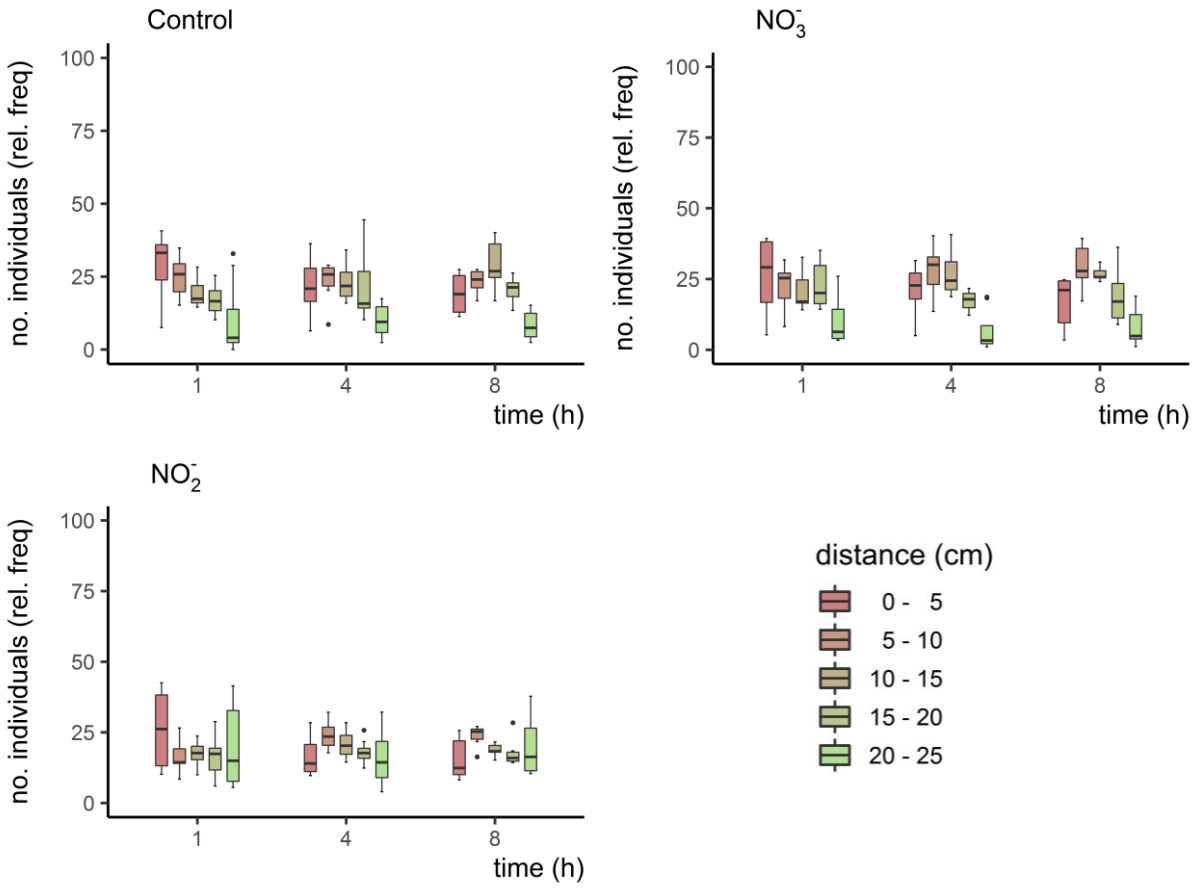

Figure 29: Distribution of *Symsagittifera roscoffensis* exposed to aqua dest. (Control), nitrate (NO_3^-) and nitrite (NO_2^-). Depicted is the number of individuals (relative frequency) depending on the time (hours). Each glass tube is segmented in five equal sized sections. The legend shows each segment of the glass tube starting from the segment closest to the nutrient source (0-5 cm) to the segment furthest away (20-25 cm). Boxplots include median, outliers (points) and whiskers (1.5 times of the Interquartile range). Sample size was n=3 and approximately 100 individuals per replicate.

For NO_3^- and the control group, the last segment (20 - 25 cm) had at all times significantly less individuals compared to all other segments. For the control, the segment closest to the source (0 - 5 cm) had the highest number of individuals at the start of the experiment with a mean over 30 %. This decreased over time and after eight hours the middle segment (10 -15 cm) contained the most worms with a mean of approximately 28 %. Likewise, the middle segments (10 - 15 cm) and (15 -20 cm) of NO_3^- contained the highest number of individuals after four and eight hours and differ significantly towards the last segment (20 - 25 cm). There are no significant differences between the segments with NO_2^-. While there are no significant differences between the time point of one hour, four hours and eight hours for each respective segment for NO_3^- and the control group, segment 10 - 15 cm differs significantly with an increase of individuals after four and eight hours compared to one hour with NO_2^- as medium (Figure 29). Figure 30Figure 28 presents the results for PO_4^{3-}, $NO_3^-+PO_4^{3-}$ and NH_4^+.

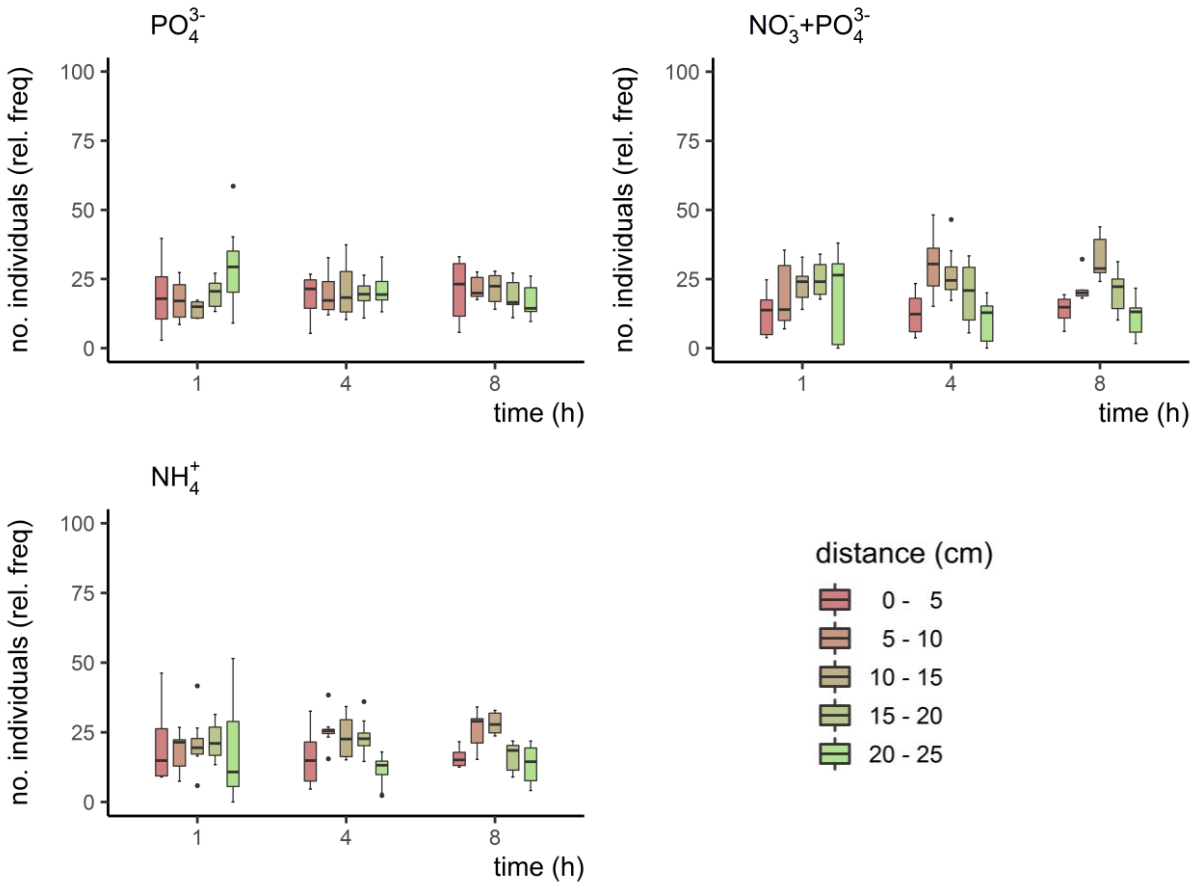

Figure 30: Distribution of *Symsagittifera roscoffensis* exposed to phosphate (PO_4^{3-}), nitrate together with phosphate ($NO_3^-+PO_4^{3-}$) and ammonium (NH_4^+). Depicted is the number of individuals (relative frequency) depending on the time (hours). Each glass tube is segmented in five equal sized sections. The legend shows each segment of the glass tube starting from the segment closest to the nutrient source (0-5 cm) to the segment furthest away (20-25 cm). Boxplots include median, outliers (points) and whiskers (1.5 times of the Interquartile range). Sample size was n=3 and approximately 100 individuals per replicate.

Comparing each segment 0 - 5, 5 -10 , 10 - 15, 15 - 20, 20 - 25 cm respectively over the time of one, four and eight hours, segment 5 - 10 cm had significantly more individuals after four and eight hours compared to the start of the experiment (one hour) when NH_4^+ was given as a nutrient source. For PO_4^{3-} and $NO_3 + PO_4$ no significant differences were found for any segment over the period of eight hours (Figure 30). The nutrients NH_4^+ and NO_3^-+PO_4^{3-} show a similar pattern, with the last segment (20 - 25 cm) containing the smallest number of individuals. For both nutrients, the segment furthest away (20 - 25 cm) differs significantly compared to the middle segments (5 - 10 and 10 - 15 cm) after four and eight hours. There are no significant differences between the segments for PO_4^{3-}.

4 Discussion

The symbiosis between the Acoela *Symsagittifera roscoffensis* and the micro algae *Tetraselmis convolutae* has been the foundation of many studies over the last century. This thesis aims to elucidate the behaviour of the host *S. roscoffensis* and its ability to react to the needs of its photosynthetic symbiont under light stress and its availability to nutrients. Consequently, the work discussed in this thesis focusses on two aspects: phototaxis and chemotaxis.

4.1 Photobehaviour

The habitat of *S. roscoffensis* is the sandy shore of the upper eulittoral which is characterized by highly variable abiotic conditions and a tidal range up to 10 m in Roscoff (Pirazolli 2000). The sandy, soft bottom eulittoral is a harsh environment for potoautotrophs. Wind action and incoming waves regularly disturb the habitat and thus the organisms are easily washed away by the tide. This circumstance is also reflected in the available literature. The diversity of phototroph organisms on rocky shores, hard bottom shores of the intertidal zone can be seen in numerous studies (Quadir et al. 1979; Stefan et al. 2016; Guillaumont et al. 1993), the literature on photoautotrophs for the sandy habitats is sparse. For the micro algae *T. convolutae*, the endosymbiotic association with *S. roscoffensis* offers protections against these disturbances, as the worm is able to seek protection underneath the sand if necessary. For the worm however, the symbiotic relation with a photsynthetic active algae this creates a conflict. On the one hand, *S. roscoffensis* needs to prevent being washed away by the incoming tide and on the other hand expose its symbiont to light for a sufficient photosynthesis. However, the light climate is highly variable as well and can vary in long term-fluctuations (e.g. weather and diurnal changes), as well as short-term fluctuations due to wave action or clouds (Sagert et al. 1997). Thus, *S. roscoffensis* needs to find a way to expose its symbiont to enough light for a sufficient photosynthesis but also protect it against too high illumination which can possible lead to photoinhibition and permanent damage of the photo apparatus (Dau 1994). The question arises which trigger lead to a reaction of the worm.

The results represented in Figure 15 show a photophobic behaviour of *S. roscoffensis* towards high light intensities. Animals exposed to 2200 µmol · m^{-2} · s^{-1} retreated into the sand after 90 minutes of constant light exposure. Individuals that were treated with a low light intensity of 90 µmol · m^{-2} · s^{-1}, however, did not show a reaction to light and were still abundant by the end

of the experiment after eight hours. The results presented here are in good agreement with a statement proposed by Doonan, S.A. and Gooday, G.W. (1982). They suggested that *S. roscoffensis* retreats into the sand to seek protection not only against mechanical stimuli but also under conditions of high light intensities. A light induced avoidance behaviour is already known in other organisms, depending on photosynthesis, that are constantly exposed to fluctuating irradiance (Serôdio et al. 2006; Serôdio et al. 2011) for instance, the light induced movement response in some ciliates, that have installed an endosymbiosis with a green micro algae of the genus *Chlorella* (Reisser and Häder 1984). The coloured ciliate *Stentor coervelus* expresses a shift motility in response to an alteration in light levels (increase or decrease). For some ciliates (e.g. *Paramecium bursaria*) their light response behaviour changes drastically when they have installed the endosymbiosis with the green micro algae *Chlorella* sp. compared to aposymbiotic ciliates (Niess et al. 1981). However, they do show a response to light even without the algae. The symbiotic partner enhances these movements and a response can be observed already under low light intensities, thus indicating an intrinsic reaction of the ciliate, which is possibly linked to the photosynthesis of the algae partner (Reisser and Häder 1984). In case of *S. roscoffensis*, the light induced behaviour can only be observed for *S. roscoffensis* associated with *T. convolutae*. In order to discuss whether the light induced movement behaviour of *S. roscoffensis* is induced by the algal symbiont or an intrinsic reaction of the worm, more surveys are necessary. All experiments are based on adult worms containing the algal symbiont. For further insight the experiments need to be repeated with aposymbiotic larvae (within the first days after hatching) or with DCMU, a photosynthetic inhibitor which blocks the electron transfer chain of *Tetraselmis convolutae* (Serôdio et al. 2006).

In consequence of this result, the question arises whether this photobehaviour of *S. roscoffensis* is directly depending on the light intensity or whether the behaviour is linked to the light dose. The results of the experiments concerning intensity dependent behaviour and the calculated light dose are represented in Figure 16 A and B, respectively. Five different intensities were tested with the outcome that the time *S. roscoffensis* spends on the surface decreases with increasing photon flux density (PFD). However, the received dose (Figure 16 B), at which 50 % of the worms have disappeared into the sand, is the highest for individuals exposed to the highest light intensity and decreases with decreasing intensity. The time of retraction is therefore not dependent on the dose but rather linked to the light intensity (ranging from 4.78 mol $\cdot$ m^{-2} for animals exposed to 100 µmol $\cdot$ m^{-2} $\cdot$ s^{-1} and 40.73 mol $\cdot$ m^{-2} for animals exposed to 2200 µmol $\cdot$ m^{-2} $\cdot$ s^{-1}).

The photophobic reaction of *S. roscoffensis* to high light intensities leads to the assumption that the two photoreceptors on the apical part of the worm not only sense the direction of the light source but also the light intensity. However, how does *S. roscoffensis* determine the light intensity that the algae needs, especially since the perception of the worm does not necessarily have to correspond to that of the algae. Instead, the host might react to chemical cues released by the symbiont in response to light stress. Here two possibilities come into consideration: a) a response of the worm to exudates released by the algae during the Calvin-cycle of the photosynthesis or b) a response to reactive oxygen species likewise produced by the algae. However, a reaction to exudates seems unlikely here, because the formation of exudates is dependent on the light dose. The amount of exudates produced is linked to the energy available in the form of ATP and NADPH, which is formed during the light reaction of the photosynthesis. The worm's photophobic behaviour is, however, a reaction to light intensity and not to light dose (Figure 16), which would be expected if exudates from the algae were the cause for the worm's behaviour. The behavioural response of *S. roscoffensis* to light intensity is therefore more suitable to reactive oxygen species as they are depending on the light intensity to which the algae were exposed to. Reactive oxygen species (ROS) are produced when the energy of the incoming light is higher than the need for ATP/NADPH of the photosynthetic metabolism. An excess of light energy leads to overly excited chlorophyll molecules at PS II (Heldt and Piechulla 2015). These excited chlorophyll molecules enter the triplet state, formatting thus singlet oxygen, which can cause damage to the photo apparatus (Photoinhibition). However, a response of *S. roscoffensis* to either of those cues causes a late reaction to high light intensities. This late reaction of the host to increasing light intensities may already lead to photoinhibition or even permanent damage of the algae's photo apparatus and is thus lethal for the worm as well. This assumption is supported by the circumstance that the observed mortality rate of *S. roscoffensis* during the experiment was higher for individuals under high light intensity conditions compared to those individuals that were exposed to low intensities[3]. Furthermore, the population of free living *S. roscoffensis* decreases over the summer months, which also may be an indication that the long exposure to the high intensities during summer months are too high for the algae and *S. roscoffensis* reacts too late to the needs of its symbiont due to a delayed communication pathway.

[3] A mortality was observed for animals exposed to 900 µmol · m^{-2} · s^{-1} and higher after the worms retreated into the sand. This might also be due to a more difficult gas exchange underneath the sand or the light still passing through the substrate and thus still reaching the worms.

Nevertheless, *S. roscoffensis* does choose the optimal light intensity if presented with a light gradient when they are free to move either way (Figure 17). Over a period of eight hours *S. roscoffensis* opted for high illuminated areas in the range of 500 – 1000, 1000 – 1500 and 1500 – 2000 µmol · m^{-2} · s^{-1} within the first 150 minutes. After that time the amount of individuals moving into the low illuminated area (0 – 200 µmol · m^{-2} · s^{-1}) constantly increased until 48.43 % of the worms gathered in the lowest illuminated segment. Although, *S. roscoffensis* shows a change in spatial distribution of the in dependence to light intensity over time, a delayed response of the host to the needs of the algae cannot be ruled out here. *Symsagittifera roscoffensis* collected in Portugal prefers light intensities of approximately 150 µmol · m^{-2} · s^{-1} and tolerated intensities up to 400 µmol · m^{-2} · s^{-1} (Serôdio et al. 2011). However, the light-saturation parameter E_K is similar for *S. roscoffensis* collected in France (average 355 µmol · m^{-2} · s^{-1} and 389 µmol · m^{-2} · s^{-1}) and for *S. roscoffensis* collected in Portugal (average 250 µmol · m^{-2} · s^{-1}). Slight deviations in the preference of light intensity might be caused by different sampling sites. *Symsagittifera roscoffensis* is able to form a stable symbiosis with different species of the subgenus Tetraselmis. This is, for example, the case on the beach of South England (Douglas 1985). Thus, it may be possible that the worms collected in Portugal have a different but closely related symbiont. However, the study by Serôdio et al. (2011) and the results discussed here are nevertheless comparable and the results are in good agreement to one another. Serôdio et al. (2011) suggest a photobehaviour adapted to the needs of the symbiont *T. convolutae*, which is supported by the data displayed in this thesis.

While light intensity plays an important role in the behaviour of *S. roscoffensis*, as too high intensities damage the algae, exposing the worms to different wavelength might trigger a different behavioural response. Under all three light intensities, significantly more individuals of *S. roscoffensis* accumulated under blue and green light but avoided red light. This avoidance behaviour was also observed despite the red foil being in position one (highest light intensity). These results are supported by Nissen et al. (2015). They tested the amount of time one individual spent under one out of three light colours. They also found a preference of *S. roscoffensis* towards blue light and avoidance towards red. This behaviour is in contradiction to preference of *T. convolutae*, showing the highest photosynthetic rate for red light (Nissen et al. 2015) which is generally the optimum wavelength for green algae (Butler 1978; Emerson and Lewis 1943). The behaviour of *S. roscoffensis* to choose blue/green light over red, despite of the preference of its symbiont to red light, might be because of the structure of the host´s eye. The eyes of *S. roscoffensis* consist of two ocelli on each side of the statocyst and have a different structure than the typical turbellarian ocellus (Yamasu 1991). Each ocellus is

constructed of nerve cells and a cup-shaped pigment cell that contains platelets within a vacuole that presumably act as reflectors and only one type of receptor cell (Yamasu 1991). Furthermore, supposedly all photoreceptors originated from one common ancestor and are based on the pigment rhodopsin. In their study Nissen et al. (2015) suggested that it is very likely that *S. roscoffensis* only expresses one type of receptor cell and is therefore not able to detect red light.

Nevertheless, the amount of photons available for photosynthesis differ greatly among the coloured foils (Table 3). The pigments absorbing in the range of blue light are to a large extent carotenoids, while the absorption at wavelength greater than 520 nm are mainly due to chlorophyll (Emerson and Lewis 1943). The main role of carotenoids however, is the protection of the photo apparatus. The probability of which the absorbed photon by carotenoids is passed onto photosynthesis is much smaller than compared to photons absorbed by chlorophyll a (Schubert 1989; Emerson and Lewis 1943). But, even when taking into account that the photon based efficiency for short-wavelength light is lower than for long-wavelength light the differences among the foils are still too big. This may have been the impulse for the worms to opt for blue and green light instead of red. Therefore, the data shown here is not sufficient to make assumptions concerning the behaviour of *S. roscoffensis* towards light of different wavelength.

4.2 Photophysiology

The high motility (average of 1.1 mm $\cdot$ s^{-1}) and photophobic behaviour of *S. roscoffensis* is an effective way to protect the photosynthetic symbiont from photoinhibition or damage of the photo apparatus under light stress. However, in a highly variably habitat, such as the habitat of *S. roscoffensis* and *T. convolutae*, long-term and especially short-term adaptions by the photosynthetic partner are important. After all, 10 minutes of exposure to high irradiance (above light saturation point) on a bright sunny day, can already be damaging to photosystem II (Dau 1994). Those adaptions include the synthesis and degeneration of proteins and pigments associated with the photo apparatus. The changes in absolute pigment content for chlorophyll a (chl a), chlorophyll b (chl b), and carotenoids (car) are an indicator of light induced acclimation by photoautotrophs (Falkowski and LaRoche 1991). For example in the green algae *Chlorella fusca* photo pigments were degraded under ongoing high light conditions (Senge and Senger 1990). Therefore, an acclimation to high light intensities by changing the morphology of the algae is likely to happen for *T. convolutae* as well.

The absolute chlorophyll content (chl a and b) for ingested *T. convolutae* in *S. roscoffensis* exposed to 90 and 2200 µmol · m^{-2} · s^{-1} (Figure 21), decreases over the span of four hours, but then show an increase until reaching a tipping point at approximately six to seven hours, after which the content drops rapidly. This is the case for *S. roscoffensis* exposed to both, low light and high light conditions. Studies by Schäffer (2007) show a constant decrease in pigment content for chl a and chl b for *S. roscoffensis* exposed to 253 µmol · m^{-2} · s^{-1} and 128 µmol · m^{-2} · s^{-1}, whereas the content for animals treated with 64 µmol · m^{-2} · s^{-1} and lower did not show any changes. However, the low light intensity in the study presented here was installed at 90 µmol · m^{-2} · s^{-1}, thus comparatively high and both groups showed a degradation of chl a as well as chl b pigments. A decrease in absolute pigment content is not necessarily solely based on pigment degradation, but can also be based on the expulsion of the endosymbiont. Examples of this can be found in corals that have dinoflagellates as an endosymbiont within. Corals expulse the symbiont under stress situations, such as changes in temperature, salinity or light, a phenomenon known as coral bleaching (Fujise et al. 2014; Hoegh-Guldberg and Smith 1989). Nevertheless, corals are able to re-establish or recover a symbiosis if allowed by the environmental conditions (Douglas 2003). In case of *S. roscoffensis* a reuptake of algae seems unlikely. A reestablishment of the symbiosis has only been documented in juveniles and not in adult individuals (Provasoli et al. 1968). Moreover, the ventral opening and the syncytium forms back after initially installing the symbiosis (Schäffer 2007) [4], thus preventing a reuptake of new algae. An expulsion of *T. convolutae* by *S. roscoffensis* has only been documented in the laboratory for individuals exposed to high levels pCO_2 (Dupont et al. 2012). However, this was only the case for super-saturated CO_2 medium. The authors showed that *Symsagittifera roscoffensis* rapidly expulsed its symbiont and appeared almost white. Besides that, there are no other cases documented for *S. roscoffensis* expulsing its symbiont and hence the decrease is possibly based on degradation of pigments and not due to the expulsion of the algae.

The content of car shows a similar pattern as described for chl a and chl b (Figure 21). Carotenoids are a group of pigments that can be divided into carotenes and their oxidized form the xanthophylls (Goodwin 1960). Their roles within the cells are manifold (Krinsky 1971). However, in this theses only two of them are discussed, the role as accessory pigment (e.g. Owens et al. 1987; Mandelli 1972) and their protective function (Foote et al. 1970a; Foote et

[4] The degeneration of the mouth and syncytium of *S. roscoffensis* is among others cited in Schäffer (2007). However, a reliable primary source could not be found.

al. 1970b). For a better utilisation of the spectral range the antennae hold, next to chlorophyll molecules, carotenoids. For higher plants and green algae, these carotenoids are mainly xantophylls, including lutein, violaxanthin, neoxanthin as well as carotene (β-carotene). They have the ability to channel absorbed light energy into photosynthetic reactions by extending the absorption spectrum of the algae into the blue-green range. The second fundamentally important role is the protection of photosynthesis from triplet excitation or singlet oxygen. A prolonged exposure to higher irradiance leads to an excess energy level when the algae is light saturated. This excess leads to a decrease in the pH-level within the cell and activates the enzyme violaxanthin de-epoxidase. The enzyme reduces violaxanthin to antheraxin and then to zeaxanthin. This cycle is reversible and the reverse reaction is performed by the enzyme zeaxanthin epoxidase and takes place within minutes (Ralph et al. 2002). For *S. roscoffensis* treated with 90 µmol · m^{-2} · s^{-1} and 2200 µmol · m^{-2} · s^{-1} the measured car content decreased within the first hours in the experiment and increased after five hours of constant light exposure. However, the content of car completely breaks down towards the end. With increasing light irradiance it would be expected that the chl a content decreases and car increases, which would lead to a decrease in the chl a + chl b to car ratio. But the ratio (Figure 22) is significantly higher for animals treated with 2200 µmol · m^{-2} · s^{-1} compared to animals treated with 90 µmol · m^{-2} · s^{-1}. The changes in pigment content as well as the decreasing α-value (Figure 24) show that there is an acclimation to high intensities. Nevertheless, 2200 µmol · m^{-2} · s^{-1} is too high for ingested *T. convolutae* and thus results in an irreversible damage of the photo apparatus. This is also supported by the value for F_v/F_m (dark-yield) which shows a rapid decline for *S. roscoffensis* treated with 2200 µmol · m^{-2} · s^{-1} already after the first hour of light exposure (Figure 23). This decline of F_v/F_m upon light exposure is a common occurrence for photoinhibition in the cell (Falkowski et al. 1994). There was also no increase in car content for *S. roscoffensis* treated with high light intensity in the study (Schäffer 2007) and no significant signs for acclimation upon light stress for *S. roscoffensis* in the study of Serôdio et al. (2011).

4.3 Chemotaxis

Light is the main energy source for photosynthetic active organisms, but other resources also play an important role in numerous physiological pathways or have an effect on the photosynthesis rate. Phytoplankton productivity is often limited by nutrient availability, predominately by nitrogen and phosphorous (Young and Beardall 2005). For example, the starvation of nitrogen reduces the efficiency of photosynthesis (Kolber et al. 1988) and lead to a decline in maximum quantum yield (Welschmeyer and Lorenzen 1981). For algae in an

endosymbiotic association, the host provides those nutrients needed by its endosymbiont. If taking into consideration that *S. roscoffensis* may react to reactive oxygen species (ROS) produced by its endosymbiont *T. convolutae* under light stress, further insights on the behaviour of *S. roscoffensis* towards the availability of nutrients appears to be of particular interest.

The formation of ROS is, among other factors, due to high light intensities. If the energy of the incident light is higher than the need for NADPH and ATP of the photosynthetic metabolism, it comes to an excess reduction of the components of the photosynthetic electron transport. Overly excited chlorophyll molecules at the PS II enter the triplet state, forming thus more aggressive singlet oxygen (Heldt and Piechulla 2015).

Despite the broad behavioural repertoire of *S. roscoffensis*, a chemotaxis has not been documented so far. *Symsagittifera roscoffensis* appears on beaches in South Portugal (Praia de Galé and Olhos da Água). They occur abundantly in areas with submarine groundwater discharge rich in nutrients, especially nitrogen (Carvalho et al. 2013). A study by Carvalho et al. (2013) shows that the assimilation rate of NO_3-N is directly dependent on the amount available to the worm in the substrate. The higher the concentration of NO_3-N in the surrounding substrate, the higher is the assimilation rate. Therefore, light plays an important role, as the uptake of NO_3- N can only be measured during light period. This leads to the conclusion that it is the algae that is responsible for the assimilation rate and not the worm itself (Carvalho et al. 2013). The assimilation rate has also been documented for other green algae, e.g. *Chlorella vulgaris* (Jeanfils et al. 1993).

A further influential source on the photosynthesis rate is carbon, which has also been documented for in *S. roscoffensis* with an increase of the net photosynthesis rate under high light intensity and with the addition of HCO_3^-. The photosynthesis rate increased 110 % after two days acclimation period and 150 % after ten days (Schäffer 2007). Inorganic carbon (e.g. CO_2 or HCO_3^-) is an important parameter, as it is needed in high amounts to allow high rates of photosynthesis. The intake of inorganic carbon, however, comes with a high effort for marine phytoplankton, although solved carbon is found in high amounts within the ocean. The cause for that is the enzyme RubisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase). The primary carboxylating enzyme has a low affinity to CO_2 but a high susceptibility to O_2. However, most marine phytoplankton have thus developed another way to increase the CO_2 concentration within the cell. The CO_2-concentrating mechanism (CCM) involves the uptake of CO_2 and HCO_3 and increases the pace of which HCO_3 is converted to CO_2 by an enzyme called carbonic anhydrase (CA). In case of the symbiont of *S. roscoffensis* it has been shown

that *T. convolutae* (free living and in symbiosis) also exhibits a pyrenoid, which is a component of CCM (Parke and Manton 1967).

Additionally, *S. roscoffensis* accumulates on beaches near algae residues in Roscoff, France (Figure 27), which may further indicate an ability of *S. roscoffensis* to move towards areas high in nutrients to provide for its symbiont. Nevertheless, the results in this thesis (Figure 28-29) show no signs of a possible chemotaxis of *S. roscoffensis*. Neither the macro algae residues (*Fucus* sp., *Ceramium* sp, *Ulva* sp.), nor the chemical compounds (e.g. NO_3^-, NH_4^+ or PO_4^{3-}) used for the experiment trigger any change in the behaviour of *S. roscoffensis.* Thus, a chemotaxis could not be experimentally verified in this thesis. However, although a light gradient within the experimental set up was reduced to a minimum it could not be completely eliminated. Therefore, it cannot be ruled out that the phototaxis may have overshadowed a chemotaxis if present. Furthermore, the observed accumulation of individuals of *S. roscoffensis* near algae residues on beaches in Roscoff may also be due to the formation of a specific microbiome. It has been postulated that the symbiosis of *S. roscoffensis* and *T. convolutae* is not only between the two organisms, but possibly involve a third partner (Bailly et al. 2014), a bacterial community that is abundant on the outside of the worm. Nonetheless, further data on this point would be desirable.

4.4 Conclusion and future research

The green worm *Symsagittifera roscoffensis* and its endosymbiont *Tetraselmis convolutae* live in the intertidal zone of West Europe a habitat characterized by highly variable light intensities. In particular, the high intensities during summer months and low tide present the duo with the challenge of adapting to those potentially damaging irradiances. For the photosynthetic active endosymbiont, it is crucial that *S. roscoffensis* reacts to the needs of the algae to protect it from light damage. The results presented in this study show that indeed under high light intensities *S. roscoffensis* seeks protection by moving underneath the sand. This occurs significantly earlier for animals treated with 2200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ compared to animals treated with 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$, thus showing a photophobic behaviour for high light intensities. This negative phototaxis is directly linked to the level of photon flux density. A range of five different intensities were tested, ranging from 100 to 2200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. *Symsagittifera roscoffensis* disappeared faster into the sand the higher the light intensity the worms were exposed to. The calculated dose, however, differs greatly between the different intensities, thus the observed behaviour is not in response to the specific light dose, but depending on the level of PFD. When presented with a light gradient and free movement in

either direction, *S. roscoffensis* showed first a positive phototaxis and opted for segments of high intensities reaching from 500 up to 2000 µmol · m^{-2} · s^{-1}. However, after five hours of constant light exposure a photophobic behaviour of the host could be observed and the amount of individuals within the lowest segment (0 - 200 µmol · m^{-2} · s^{-1}) increased significantly until 48.45 % accumulated in the low illuminated area at the end of the experiment. The observed behaviour of *S. roscoffensis* over time under constant light exposure does not however, exclude a delayed communication between host and symbiont as the results show solely a change in distribution over time. This cannot be observed under different light colours. Despite red light being the optimal wavelength for the photosynthesis rate of green algae, like *T. convolutae*, the worms opted for green and blue light instead. Under all three light intensities tested, red light was constantly avoided by *S. roscoffensis*. However, as the experimental set-up did not allow an equal amount of photons reaching the animal under all three light colours, the reason for a preference of *S. roscoffensis* towards a certain part of the light spectrum cannot be answered here. Therefore, a repetition of the experiment using a different set-up or light source would be desirable. In addition to the animals light induced behaviour, the algae itself is able to undergo physiological adaptation to cope with increasing irradiances itself. The decrease of the α-value and changes in pigment content show a clear acclimation to an increase in light intensity. Nevertheless, *T. convolutae* cannot adapt to high irradiance for a prolonged period of time. The sudden decrease of F_v/F_m is a common sign for photoinhibition (Falkowski et al. 1994) and chl a, chl b and car decreased greatly towards the end of the experiment. Therefore, the light induced behaviour of the worms is an additional step of photoprotection and *S. roscoffensis* does react to the needs of its symbiont, although the communication between host and symbiont may be delayed. The reaction of *S. roscoffensis* to a light regime may be caused by ROS as these are produced by the algae under light stress. This circumstance and the observed occurrence of *S. roscoffensis* near macro algae residues and on nutrient rich beaches made an investigation of a chemotaxis of *S. roscoffensis* particular interesting. However, *S. roscoffensis* did not show any signs of a chemotactic behaviour. Independent of the nutrients chosen for the experiment, non-triggered any chance in behaviour towards the nutrients. Hence, the data presented here does not support an ability to react to the availability of nutrients by *S. roscoffensis*.

In summary, the data presented here confirm the first hypothesis. The phototactic behaviour of *Symsagittifera roscoffensis* is directly linked to the photocharacteristics of the symbiont *Tetraselmis convolutae*. The hypothesis consist of two sub points:

A) The behaviour of *S. roscoffensis* serves as a protection against photo damage of the symbiont.

As the results show, *S. roscoffensis* does react to high irradiances and seeks protections underneath the sand. Thus, protecting its symbiont from photo damage.

B) The host optimizes light saturation by actively moving into areas of optimal light conditions.

Symsagittifera roscoffensis does opt for high light intensities, but moves into areas with low light intensities after an extended period of time. A wavelength dependent behaviour cannot be answered on the basis of the collected data.

The second hypothesis can also be confirmed. Next to the phototactic behaviour of the host, the algae acclimates to high light intensities. However, the algae can only acclimate to high irradiance for a certain amount of time and thus suffers photo damage after a long exposure to high irradiances. Moreover, the algae does not acclimate to light intensities lower than their E_K.

The third hypothesis however, has to be declined. In addition to phototaxis, a chemotactic behaviour is not available to *S. roscoffensis* based on the collected data in this thesis.

The results discussed here have contributed to a better understanding of the innate behaviour of *S. roscoffensis* and its response to environmental factors, such as the access to light and nutrients. A chemotaxis of *S. roscoffensis* towards nutrients has been shown to be unlikely and the results of *S. roscoffensis´* behaviour towards different light conditions complemented existing studies. However, the symbiosis of *Symsagittifera roscoffensis* and *Tetraselmis convolutae* still raises many questions. For example, studies on the worm's perception of wavelength have focused on the human perception of the light. It is not clear whether the animal is capable of distinguishing the light spectrum, as the eyes of Acoela are not complex and might thus not be able to detect different light colours. Therefore, further experiments conducting the perception of wavelength of *S. roscoffensis* are still interesting. Further surveys concerning the behavioural response to chemical cues are also still desirable. Since *S. roscoffensis* is able to choose *T. convolutae* as the preferred symbiont, it is very likely that there are mechanisms for recognition (Douglas 1985). They might be attributed to the outer structure of the algae and closely related species or there may be chemical cues possibly released by the algae that serve the worms as a distinguishing feature.

Although further small-scale surveys would be informative and desirable, the next big field of research is at the molecular level. It is known that *S. roscoffensis* ingests the algae with its ventral opening and from there the algae passes the syncytium, losing its theca, flagella and eyespot and then reaches a sub-epidermal position in the worm (Bailly et al. 2014). However,

the exact mechanisms remain unknown until today. Moreover, it is still not completely clarified how *S. roscoffensis* controls its symbiont within its body. The ability of *S. roscoffensis* to either seek sunlight or seek protection from it might help the worm to control the increase of algal cells in its body, as light provides the energy needed for the photosynthetic algae to multiply. However, it is possible that there are more factors, which play a role in controlling the growth of *T. convolutae*. That may be either by various behavioural responses or at a molecular level. For other photosymbiosis, like cnidarian-zooxanthellae symbiosis, it is known that the host is able to expulse or digest its symbiont if necessary (Provasoli et al. 1968) but nothing of this sort has been observed for *S. roscoffensis*. Furthermore, it has been suggested by Bailly et al. (2014) that the symbiosis is not simply between *S. roscoffensis* and *T. convolutae* but that there is also a third partner involved a specific bacterial community. To my knowledge, nothing of the nature of these bacteria has yet been described.

Even though the nature of the symbiosis has been researched for centuries, the green Acoela *Symsagittifera roscoffensis* and its endosymbiont *Tetraselmis convolutae* remain nevertheless an exciting and promising system for further research and is far from understood.

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Declaration of Authorship

I hereby declare that I have written the thesis submitted today independently and have not used any other sources or aids other than those indicated, and that I have clearly marked the citations. The copies of this thesis are completely identical in word and picture.
Furthermore, I declare that the illustrations are prepared by myself or are clearly labelled otherwise.

Rostock, 30.07.2021