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# MASTERARBEIT

Titel der Masterarbeit

**„Termination of the *Candidatus Thiobios  
zoothamnicoli Zoothamnium niveum* symbiosis  
under oxic conditions“**

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*Wie sie dich mitreißt  
die Melodie des Meeres  
dich überspült mit Glück  
dich einhüllt  
in den weißen Schaum  
wacher Träume  
dich treibt zum Weiter-  
und Weitergehen.*

*A. Schnitt*



## Abstract

Chemosynthetic symbioses often influence the ecology, physiology and evolution of host and symbiont and are therefore of major interest. The cultivation and maintenance of thiotrophic symbioses involving an animal host is known to be extremely difficult and was not successful till nowadays. In contrast, the cultivation of *Zoothamnium niveum* (Ciliophora, Oligohymenophora) a colonial, peritrich ciliate that is obligate associated with its ectosymbiont *Candidatus Thiobios zoothamnicoli* was successful over several generations. The giant ciliate can be found at oxic-anoxic interfaces in sulfide-rich habitats in shallow waters. To date despite intensive search *Z. niveum* never has been detected without its symbiont in nature. For a thiotrophic symbiosis, the cessation of sulfide flux stresses and ultimately threatens the survival. However, whether the host, the symbiont or the association survives or not has not been studied in many systems. Therefore the focus of this work is to find out what happens to this symbiotic association when sulfide ceases. To simulate this situation, the association was monitored under oxic stagnant conditions and a variety of symbiont parameters were compared with the *in situ* population. Furthermore, we cultivated this symbiosis under oxic flow-through conditions starting with swimmers to compare the outcomes. Colonies of *Z. niveum* were dehydrated after different time points and analyzed with a scanning electron microscope. This study revealed that sulfide starvation under oxic conditions led to the breakdown of the symbiotic association between *Z. niveum* and *Cand. Thiobios zoothamnicoli*. Under oxic stagnant conditions the symbiosis was terminated through the death of the host after three days, while under oxic flow-through conditions an aposymbiotic host was observed after seven days. Over the different time points morphological changes as well as a decrease of fitness of the symbiont was monitored on microzooids as well as on swimmers.

*Key words:* chemosynthetic symbioses, thiotrophic, ciliate, cessation of sulfide flux, scanning electron microscope, breakdown of symbiotic association



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## 1. Introduction

The evolutionary success of chemosynthetic symbioses is evident from a wide range of animal groups, with at least seven animal phyla (Dubilier et al., 2008). In the oceans, numerous chemosynthetic symbioses involving chemoautotrophic sulfide-oxidizing (thiotrophic) bacteria and invertebrates can be found. Their diversity and prevalence of habitats is enormous (Dubilier et al., 2008; Bright et al., 2014), including hot vents along the axis of midoceanic ridges, cold seeps of the deep sea and continental slope sediments (Paull et al., 1984; Suess et al., 1985) and shallow-water habitats, such as sheltered sediments in inter- and subtidal zones (Bright et al., 2014). However, the spatially and temporally complex chemoclines of the mentioned habitats are challenging as chemical conditions often significantly vary within a few millimeters within a few seconds. In such unstable environments, productive communities of protists and animals have been shown to often rely on thiotrophic microbes. For thiotrophic symbioses, sources and sufficient transport mechanisms of both, reduced sulfide and oxygen are essential (Vopel et al., 2005).

In general, research on thiotrophic symbioses is challenging, as many of the natural habitats are difficult to reach, such as the deep sea. Furthermore, organisms are extremely difficult to maintain under artificial conditions, or even to culture (Bright et al., 2014). So far, the symbiotic association between the giant colonial ciliate *Zoothamnium niveum* and the sulfide-oxidizing ectosymbionts named *Candidatus Thiobios zoothamnicoli* was found to be the only thiotrophic symbiosis possible to cultivate under laboratory conditions for more than one generation (Rinke et al., 2007). For the first time, Hemprich and Ehrenberg described *Z. niveum* in 1831. The ciliate is completely covered with *Cand. Thiobios zoothamnicoli*. The symbionts contain inclusions of elemental sulfur, which is an intermediate storage product in the oxidation process of reduced sulfur species. The white color of the ciliate resulting from the inclusions (Maurin et al., 2010) lead to the descriptor "niveum" (latin for "white") in the original species description. In the early 90's then Jörg Ott rediscovered this species in the mangrove islands of Belize in the (Bauer-Nebelsick et al., 1996a). *Cand. Thiobios zoothamnicoli* was also found to live in symbiosis in several other habitats with similar morphology (Bauer-Nebelsick et al., 1996a) making this symbiont particularly interesting for experimental studies. In addition, this species a

suitable for experiments concerning its size, occurrence and short life span of about 11 days (Ott et al., 1998; Rinke et al., 2007).

Ectosymbioses is a field of special interest due to the evolution of mutualistic relationships between the organisms, as hosts and symbionts are similar to their closest non-symbiotic relatives in morphology, physiology and behavior. Further research on the above mentioned ciliate might give more detailed information about functional aspects concerning cooperation and evolution for chemolithoautotrophic symbioses as well as possible reconstructions of a scenario, how the relationship evolved (Ott, 1996).

During the last years, studies on the behavior of this symbiosis under different chemical conditions were performed. However, the main focus was set on the fitness of the host depending on sulfide concentration. To gain more information about the morphology and fitness of the thiotrophic symbiont *Candidatus Thiobios zoothamnicoli*, Drexel (Diploma thesis, 2013) carried out experiments monitoring both, the host and its symbiont. The study included *in situ* as well as *in vivo* specimens, treated under optimal conditions and sulfide starvation.

In this thesis the termination of the symbioses under sulfide starvation was investigated in more detail following Drexel's approach. *Zoothamnium niveum* never has been observed without its symbiont in nature. However, the sulfide supply from rotting material in nature is fundamentally limited and someday the chemical gradient will be depleted. Hence, the question arises how such a scenario affects the symbiotic association. In particular, the influence of sulfide starvation on morphology and fitness of *Candidatus Thiobios zoothamnicoli* as well as the possible break down of the symbiotic association is of major interest. To resolve the mentioned questions, sulfide starvation studies were performed under oxic stagnant conditions and oxic flow-through conditions.

The first experiment focuses on morphology and fitness of symbionts attached to colonies and swarmers of *Zoothamnium niveum* under oxic stagnant conditions. Symbionts are transmitted vertically on swarmers to the next generation accomplishing the asexual reproduction. So far, no studies focusing on symbionts attached to swarmers exist. However, the vertical transmission of symbionts to the next generation has to be regarded as a crucial step of the maintenance of this symbiotic association. Hence, the influence of sulfide starvation on symbionts attached to swarmers is of major interest. Main research objectives in this context are the morphological and fitness parameters of the symbionts under sulfide starvation and possible differences between symbionts on swarmers and microzooids under oxic stagnant conditions.

In the second experiment, new colonies were cultivated from swimmers under oxic flow-through conditions. The actual cultivation of an aposymbiotic host is of major scientific interest, as the symbioses between host and symbiont is mandatory. Further research objectives were possible changes in morphology and fitness of the symbiont without sulfide supply under oxic flow-through conditions.

## 1.1. *Zoothamnium niveum*

### 1.1.1. *General*

*Zoothamnium niveum* is a giant marine ciliate, which belongs to a colonial ciliate genus of Peritrichida (Oligohymenophora). It was described for the first time more than hundred years ago in the Red Sea (Hemprich & Ehrenberg, 1931). The genus *Zoothamnium* contains about 60 described species native in different aquatic habitats including freshwater and marine systems as well as benthic and pelagic areas (Ott et al., 1998). *Z. niveum* is unique due to the giant size and the typical bell-shaped microzooids (Bauer-Nebelsick et al., 1996a). The eukaryotic cell colony can reach a size of up to 1.5 cm and is therefore the largest representative of this genus (Bauer-Nebelsick et al., 1996a,b; Ott et al., 1998; Vopel et al., 2005).

*Zoothamnium niveum* is obligatorily associated with the ectosymbiotic, chemoautotrophic, sulfide-oxidizing bacterium *Candidatus Thiobios zoothamnicoli* (Bauer-Nebelsick et al., 1996a,b). Except for the adhesive disc and the basal noncontractile part of the stalk, the giant ciliate is entirely covered with ectosymbionts (Ott et al., 2004). The symbionts give the colony the typical white color for which this species was called “niveum” (Hemprich & Ehrenberg, 1831; Bauer-Nebelsick et al., 1996b). On the most basal parts of the ciliate many different kinds of microbes overgrow the remaining symbionts (Bauer-Nebelsick et al., 1996a). Food vacuoles of *Z. niveum* revealed only bacteria with the same characteristic ultrastructure as their symbionts, indicating that the host nourishes on its symbionts (Bauer-Nebelsick et al., 1996a). Comparing growth rates of aposymbiotic host with those covered with *Cand. Thiobios zoothamnicoli*, a trophic relationship was suggested (Bauer-Nebelsick et al., 1996b; Ott et al., 1998; Vopel et al., 2001).

### 1.1.2. Morphology

The feather-like colonies consist of a basal adhesive disc and a central stalk with alternating branches. In addition, second fans can be established. Except for the proximal end of the stalk and the adhesive disc, a contractile spasmoneme runs through the entire colony. Hence, it is able to contract and expand rapidly (Bauer-Nebelsick et al., 1996b).

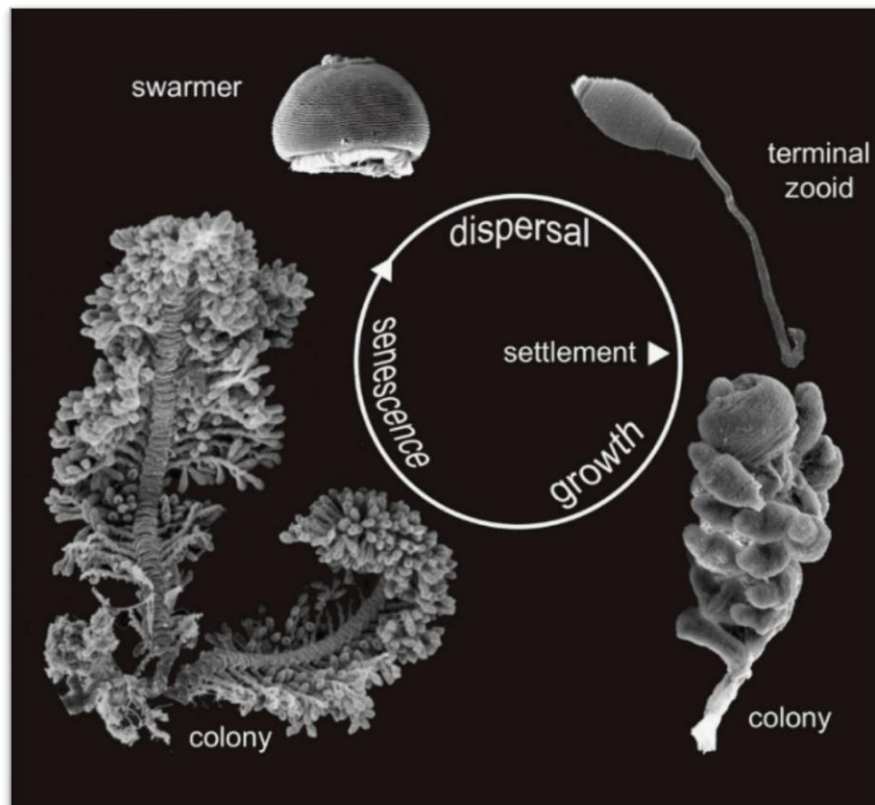
*Zoothamnium niveum* consists of three different cell morphotypes on the alternated branches: microzooids, macrozooids and terminal zooids (Bauer-Nebelsick et al., 1996b). Studies by Kloiber et al. (2009) revealed that the DNA synthesis is restricted to the terminal zooids and macrozooids. Two different subtypes of terminal zooids can be distinguished. The top terminal zooid on the tip of the stalk and the terminal zooids at the tips of the alternated branches. The terminal zooids built up new microzooids whereas the top terminal zooid generates new terminal zooids and initiates the formation of new branches. Limitations in proliferation capacity lead to a maximum number of 20 microzooids (zooids= single feeding cell) that can be found on one branch. Consequently the number of branches of a colony is equivalent to the divisions of the top terminal zooid (Rinke et al., 2007). The youngest parts of a colony are located at the top, the oldest ones at the bottom. As the colony grows, the division rate of the top terminal zooid decreases, but remains nonzero (Kloiber et al., 2009).

Microzooids are produced by the terminal zooids at the tip of each branch. The feeding microzooids show typical digestive structures, such as an oral ciliature and a cytopharynx. Food is ingested by filter feeding. Usually the cytopharynx also contains bacteria similar to the ectosymbionts (Bauer-Nebelsick et al., 1996b).

Macrozooids develop on the base of the branches and are the dispersal stages. They are capable of leaving the mother colony as swarmers for asexual reproduction (Bauer-Nebelsick et al., 1996a). In large colonies with more than 50 branches about 15 macrozooids are generated, whereas smaller colonies only produce about 6. The macrozooids do not have food vacuoles or a cytopharynx, but have a fully developed oral ciliature. Microzooids can vary highly in size (20-150  $\mu\text{m}$ ), but no correlations between the size of the macrozooids and the development of the somatic girdle was reported (Bauer-Nebelsick et al., 1996a). As no digestive structures are developed, Bauer-Nebelsick et al. (1996b) concluded that they are nourished by the microzooids.

### 1.1.3 Lifecycle

Swarmers leave the mother colony as soon as their somatic girdle, a circular row of cilia, is fully developed (Bauer-Nebelsick et al., 1996b). After dispersal and settlement, the swarmer builds up a new colony (see Fig. 1). Experiments revealed that for the settlement of a swarmer a concentration of about  $250\text{-}300\ \mu\text{mol L}^{-1}\Sigma\text{H}_2\text{S}$  and about  $200\ \mu\text{mol L}^{-1}$  oxygen are required (Vopel et al., 2005). In contrast to these values, *Zoothamnium niveum* was observed to colonize sunken wood with only about  $100\ \mu\text{mol L}^{-1}\Sigma\text{H}_2\text{S}$  (Laurtent et al., 2009). The new colony initially consists of a single cell, the terminal zooid. Due to longitudinal fission the terminal zooid produces new terminal zooids, branches with microzooids and macrozooids (Bauer-Nebelsick et al., 1996a,b). The colony's growth phase is followed by the senescence phase. Subsequently new swarmers are released and the life cycle of *Z. niveum* is completed. From observations of the disappearance of the colonies in a natural environment the life expectancy of a colony was estimated to approximately three weeks (Ott et al., 1998).



**Figure 1.** Life cycle of *Zoothamnium niveum*; not in scale (Bright et al., 2014).

#### **1.1.4. Behavior**

In typical habitats, the chemical environment of *Zoothamnium niveum* is characterized by concentration gradients for oxygen and sulfide established between the degrading material on which it grows and the sulfide-free, oxic seawater above. However, these concentration gradients are subsequently varied by the contraction and expansion of the ciliate, as well as the filter feeding of the microzooids (Ott et al., 1998).

Due to the small size of the colonies, Reynolds numbers can be considered small for slowly moving objects. Therefore the seawater approximately sticks to the colony at rest. A rapid contraction movement increases the Reynolds number significantly and sticking is avoided (Vopel et al., 2002). Hence, the colony can get into contact with sulfidic water located at the bottom of the colony (Vopel et al., 2005). The actual contraction speed was measured to be up to  $520 \text{ mm s}^{-1}$ . The subsequential expansion is about 700 to 1000 times slower which leads to smaller Reynolds number and corresponding pumping of sulfid into the oxygenated zone. The contraction and expansion of the colony has a periodicity of 1.7 min on average. Due to the different surface area, the total time required for one contraction cycle increases with size of the colony. For colonies with ten and 33 branches, Vopel et al. (2002) measured contraction times of 2.6 ms and 4.2 ms, respectively. The corresponding expansion times were determined to 1.4 sec and 4.2 sec (Vopel et al., 2002).

Directly after the expansion of the colony, microzooids resume filter feeding by beating their oral cilia (Vopel et al., 2002). The generated currents transport sulfide and oxygen saturated seawater towards the microzooids. On the one hand, this host-created chemical environment is assumed to be beneficial for the chemoautotrophic, sulfide-oxidizing ectosymbionts (Vopel et al., 2001; 2002). On the other hand, the rapid bunching of the microzooids and stalk contraction during the contraction movement are assumed to lead to enough shear stress to detach some of the ectosymbionts (Vopel et al., 2005). Suspended ectosymbionts can enter the feeding current of the host (Vopel et al., 2002). As food vacuoles only contain bacteria that show the same characteristic ultrastructure as *Candidatus Thiobios zoothamnicoli* (Bauer–Nebelsick et al., 1996b), it has been assumed that *Zoothamnium niveum* is also nourished by its own symbiont.

## 1.2. *Candidatus Thiobios zoothamnicoli*

### 1.2.1. *General*

*Candidatus Thiobios zoothamnicoli* is an ectosymbiont with a specific 16S rRNA phylotype and a cell wall that is typical for gram-negative bacteria (Rinke et al., 2006). It was re-discovered in the 90s leading to the redescription of *Zoothamnium niveum* by Bauer-Nebelsick et al. (1996a,b). The Thiobios group is dominated by free-living bacteria that habitate in shallow waters at tropical temperatures (Bright et al., 2014). This ectosymbiont is known to cluster with thiotrophic free-living bacteria and other symbiotic Gammaproteobacteria. For the first time, this symbiotic association has been evolved in the Thiobios group in *Z. niveum*. Later it was also evolved in the archaea *Gigantothauma karukerense* (Muller et al., 2010).

Rinke et al. (2006) performed 16rRNA gene sequence analysis that revealed highest sequence similarity between two Gammaproteobacteria and *Candidatus Thiobios zoothamnicoli*. On one hand 94.5 % sequence similarity was found to the free-living sulfur-oxidizing bacterial strain ODIII6, a monophyletic group inhabiting shallow-waters and hydrothermal vents of the Mediterranean Sea. On the other hand, 93.1 % sequence similarity was found to the endosymbiont from the scaly snail gastropod of the Indian Ocean Ridge (Rinke et al., 2006).

*Candidatus Thiobios zoothamnicoli* was shown to be a sulfide-oxidizing chemolithoautotroph bacterium based on the presence of the CO<sub>2</sub>-fixing key enzyme ribulose-1.5-bisphosphate carboxylase/oxygenase (RuBisCo) of the Calvin-Benson-Bassham cycle that catalyzes the assimilated CO<sub>2</sub> to organic carbon (Ott et al., 1998; Rinke et al., 2009). Furthermore, Rinke et al. (2007) found genes encoding enzymes that are typically for inorganic carbon (RuBisCO-*cbbL*) and sulfide metabolism (*dsrAB*, *apsA*).

It is well known that thiotrophic bacteria need a reduced sulfide source acting as electron donor. Hydrogen sulfide or thiosulfates are the prevalent sources in the environment (Rinke et al., 2009). Typical electron acceptors are oxygen and sometimes nitrates (Rinke et al., 2007). The oxidation of sulfide delivers electrons that are used for energy transformation via the respiratory chain and for fixation of carbon dioxide. The actual mechanism for bacterial sulfide oxidation can follow several pathways though (Friedrich et al., 2005). As an intermediate product, elemental sulfur (S<sub>8</sub>) is stored in membrane-bound vesicles leading to the white color of *Candidatus Thiobios zoothamnicoli*. For a time period of about 4 h, the elemental sulfur from the reservoir can be used

as electron acceptor. Its continuous depletion can be observed as fading of the white color (Ott et al., 1998). However, in the case of a symbiosis with *Zoothamnium niveum*, the movement of the host enables the bacteria to frequently resume their chemoautotrophic activity (Ott et al., 1998).

### **1.2.2. Morphology**

*Candidatus Thiobios zoothamnicoli* is a pleomorphic species. In symbiosis with *Zoothamnium niveum* it occurs in two different morphological forms. Rod shaped symbionts can be found on the stalk, branches, terminal zooids, macrozooids and the aboral parts of the microzooids. Coccid formed symbionts can be found on the aboral part of the microzooid. A series of intermediate shapes between both morphotypes on the oral and aboral part of the microzooids was noted (Bauer-Nebelsick et al., 1996b). No strict order can be observed and in some cases a pseudo-multilayer can be formed. However, the latter does not provide direct contact to the host's surface for each bacterium (Bauer-Nebelsick et al., 1996a).

### **1.2.3. Transmission**

In general, two different transmission modes are distinguished. In horizontal transmission, each generation takes up its symbionts from the environment. In vertical transmission, symbionts are transferred directly to the next host generation, have co-evolved with their hosts and do not occur free-living in the environment (Bright & Bulgheresi, 2010).

In the case of *Candidatus Thiobios zoothamnicoli*, however, none of the strict definition fits. On the one hand, swimmers that leave the mother colony are totally covered with ectosymbionts. Based on the fact that an ectosymbiotic partner is covering also the asexually produced propagules, Bright et al. (2014) suggested the vertical transmission in the ancestral mode of transmission. On the other hand, strictly vertically transmitted symbionts have co-evolved with their hosts and do not occur free-living in the environment (Bright & Bulgheresi, 2010). The release of symbionts due to sloppy feeding or due to host death may support a free-living population from which host populations could be reinfected. As potentially other microbes from the surrounding environment could replace *Cand. Thiobios zoothamnicoli*, vertical transmission may not be the only possible kind of transmission. However, the possibility of additional



horizontal transmission in this symbiosis must be investigated in the future, as it might have influences on the dynamics and the demography of the symbiont population (Vrijenhoek, 2010).

### 1.3. Benefits and costs of this symbioses

In mutualistic relationship, benefits and costs are implemented for both partners. However, the benefits must exceed the costs. For the initiation of a mutualistic symbiotic association, byproduct benefits are considered to be of high relevance (Sachs et al., 2011). The latter describe benefits without costs for one symbiotic partner. Such benefits occur automatically as a self-serving act of the symbiotic partner (Hauert et al., 2006). To gain more insight into costs and benefits of symbioses, comparisons between host and symbionts fitness are required. Therefore data from *in situ* and cultured ciliates that are cooperating and defecting must be compared (Buston & Balshine, 2007). However, the conduction of such experiments is extremely challenging, so that direct evidence is scarce (Bright et al., 2014).

The symbiosis with *Zoothamnium niveum* brings many obvious benefits for the bacteria such as frequent movement through the oxic/sulfidic chemocline providing substrates for sulfide oxidation and carbon fixation. Roy et al. (2009) investigated with a combination of experimental and numerical methods the constraints on sulfide uptake by the symbionts on the ciliate. Their numerical models showed that *Cand. Thiobios Zoothamnicoli* can reach a 100 times larger sulfide uptake in association with *Zoothamnium niveum* compared to bacteria living on flat surfaces such as microbial mats. Furthermore, symbionts have a competition-free habitat with optimal conditions for sulfide oxidation and carbon fixation, compared to flat surfaces. Both benefits caused the selection advantage leading to competitive dominance of the bacterial cells on the host (Roy et al., 2009; Ott, 1996; Ott et al., 2004; Stewart et al., 2005; Cavanaugh et al., 2006; Dublier et al., 2009, Bright et al., 2014).

For the host, the major benefit from the symbiosis is being nourished on the symbionts. *Zoothamnium niveum* benefits directly from the symbiont's organic carbon, which is translocated to him (J.M Volland pers. comm.). The host surface of *Z. niveum* is almost entirely covered with *Candidatus Thiobios zoothamnicoli* indicating mechanisms developed for specific colonization. The host seems to be able to control the position and arrangement of its ectosymbionts. It is assumed that the host is also able to control certain regions of its body, the growth of the bacterial cells and division rates of the symbiont (Ott, 1996). Senescent and the most basal parts of the

ciliates are susceptible to microbial fouling. Other microbes occur and can overgrow or replace *Cand. Thiobios Zoothamnicoli* (Bauer-Nebelsick et al., 1996a;b; Bright et al., 2014). Another major benefit for the host may be the detoxification of sulfide (Oeschger & Vetter, 1992).

The major cost of the symbioses for the symbionts is the nourishment of the host. However, the actual share in nourishment of symbionts is currently studied. Cultivation experiments showed that the host fitness (measured as host growth and life span) decreased when symbionts were absent or forced to defect (Bright et al., 2014). Rinke et al. (2007) showed that the symbiont is not able to fix carbon under oxic culture conditions without sulfide. In this case the host can only be nourished by digestion of its symbionts and filter feeding, indicating that a high percentage of food is provided by the symbionts (Bright et al., 2014).

The costs for the host have not been investigated in detail yet. Possible contributions include the bearing of the ectosymbionts during the whole life cycle (Bright et al., 2014; Genkai-Kato & Yamamura, 1999) and the regulation of the bacterial community. However, the giant size of *Zoothamnium niveum* indicates that the benefits from the symbionts must exceed the costs (Bright et al., 2014).

#### **1.4. Occurrence and Habitat**

A widespread occurrence of *Zoothamnium niveum* is observed in shallow subtidal waters from subtropical, tropical and temperate regions (Bauer-Nebelsick et al., 1996a,b; Ott et al., 1998; Rinke et al., 2006; 2007). Biogeographic provinces of the Caribbean Sea (Bauer-Nebelsick et al., 1996a; Clamp & Williams, 2006; Laurent et al., 2009), the Atlantic Ocean (Clamp & Williams, 2006; Wirtz, 2008), the Mediterranean Sea (Rinke et al., 2007; Wirtz, 2008), the Red Sea (Hemprich & Ehrenberg, 1838), and the Pacific Ocean (Kawato et al., 2010) were described as habitats in the literature.

In tropical and subtropical regions *Zoothamnium niveum* colonizes mangrove peat (Lovelock et al., 2011) as well as sunken wood and leaves of mangroves (Bauer-Nebelsick et al., 1996a; Clamp & Williams, 2006; Laurent et al., 2009). In temperate regions this species has a habitat on whale falls (Kawato et al., 2010), sunken wood (Bright M., personal observation) and sea grass debris of *Posidonia oceanica* (Rinke et al., 2007; Wirtz, 2008; Bright et al., 2014). However, the ultimate depth limit of this species has not been investigated yet. No data indicate the occurrence of this symbiosis in deep waters, where they potentially could colonize drifted sunken wood and

whale falls (Bright et al., 2014). The ciliate is described as a pioneer colonizer. The colonies appear when sulfide exposure starts to occur (Laurent et al., 2013). *Z. niveum* can occur strongly aggregated in large groups of more than hundred colonies on a 1 m<sup>2</sup>, as found on mangrove peat walls in the Caribbean Sea. Small patches of colonies usually consist of either small, young colonies or large, senescent ones. In contrast, large patches can contain colonies of all sizes and ages (Ott et al., 1998).

*Zoothamnium niveum* lives in a highly dynamic microenvironment in terms of sulfide and oxygen concentrations (Bright et al., 2014). The tidal cycle causes large-scale fluctuations in sulfide concentration with a maximum during high tide and a minimum during low tide (Laurent et al., 2009). Furthermore, the flow speed of the water can change the sulfide concentration significantly (Vopel et al., 2005). In general, concentration can change from sulfide to nearly fully oxygenated seawaters within less than 1 hour, indicating an unstable and sensitive chemical environment (Laurent et al., 2009).

### **1.5. Artificial cultivation of *Zoothamnium niveum* and its symbionts**

*Zoothamnium niveum* and its symbionts were successfully cultivated under artificial laboratory conditions. Best results were observed for cultivations in a flow-through respirometer system under stable conditions. Due to the continuous flow of all chemicals, the environmental conditions for both partners were changed, breaking the host's control over the access to the needed chemicals. Experiments revealed that under optimal artificial conditions (24-25°C, salinity 40, pH 8.2, ~ 200 μmol L<sup>-1</sup> O<sub>2</sub>, 3-33 μmol L<sup>-1</sup> Σ H<sub>2</sub>S, flow rate 100 ml h<sup>-1</sup>) the colonies increased by an order of magnitude within only 1 week. The mean life span of the colonies was measured to be 11 days. In contrast, without external sulfide source under oxic conditions the life span was reduced to about 7 days (Rinke et al., 2007).

The symbiont's morphology changes dramatically with environmental conditions. In natural habitats the chemical gradient leads to more coccid shaped cells on the oral part of the ciliate and more rod-shaped symbionts on the aboral part. In experiments conducted by Rinke et al. (2007) the missing gradient of sulfide resulted in uniform rod-shaped symbionts on the entire host. This observation confirmed the hypothesis, that the ciliary beating of the microzooids has a significant influence on the symbiont's performance (Vopel et al., 2005). Furthermore, measurements of other parameters considering fitness and morphology of the symbiont indicated that on oral parts

of the microzooids the fitness of symbionts was higher under the optimal cultivation conditions in the laboratory compared to *in situ* populations. In contrast, no differences for the fitness of the lower part of the microzooids could be observed (Rinke et al., 2007).

## 1.6. Research objectives

In the past decades, many studies were addressed to the fitness and the behavior of this symbiotic association exposed to different chemical conditions. However, the main observable was the fitness of the host. The morphology and fitness of *Candidatus* Thiobios zoothamnicoli have been studied under *in situ* and optimal conditions only. As sulfide is in nature not an endless source, someday the chemical gradient leaking from rotting material is depleted. Literature points out that a sulfide source can support growth of *Zoothamnium niveum* for about three weeks until the source is depleted (Ott et al., 1998). Hence, the question arises how such a scenario affects the symbiotic association. In particular, the influence of sulfide starvation on morphology and fitness of *Cand.* Thiobios zoothamnicoli as well as the possible break down of the symbiotic association is of major interest.

As *Zoothamnium niveum* never has been detected without its symbiont in nature, several major research objectives arise defining the first goal of this thesis. What happens to the symbiotic association when sulfide ceases? What influence does sulfide starvation have on morphology and fitness of *Candidatus* Thiobios zoothamnicoli? Does the symbiotic association break down and if yes, how does that happen?

The second goal of this work is the investigation of the morphology and fitness of symbionts that are attached to swarmers. This is of major interest as asexual reproduction is accomplished through the swarmers and symbionts are transmitted vertically to the next colony. The performed experiments will gain information about processes that also occur in nature. What happens to symbionts attached to swarmers under sulfide starvation? Will sulfide starvation change their morphological and fitness parameters? Are significant differences between symbionts on swarmers and those on microzooids observable under oxic stagnant conditions? Furthermore, possible differences in results from experiments under oxic stagnant conditions and oxic flow-through conditions are of major interest for any further study. Additionally, investigations will be addressed to the question if cultivation of an aposymbiotic host is possible.

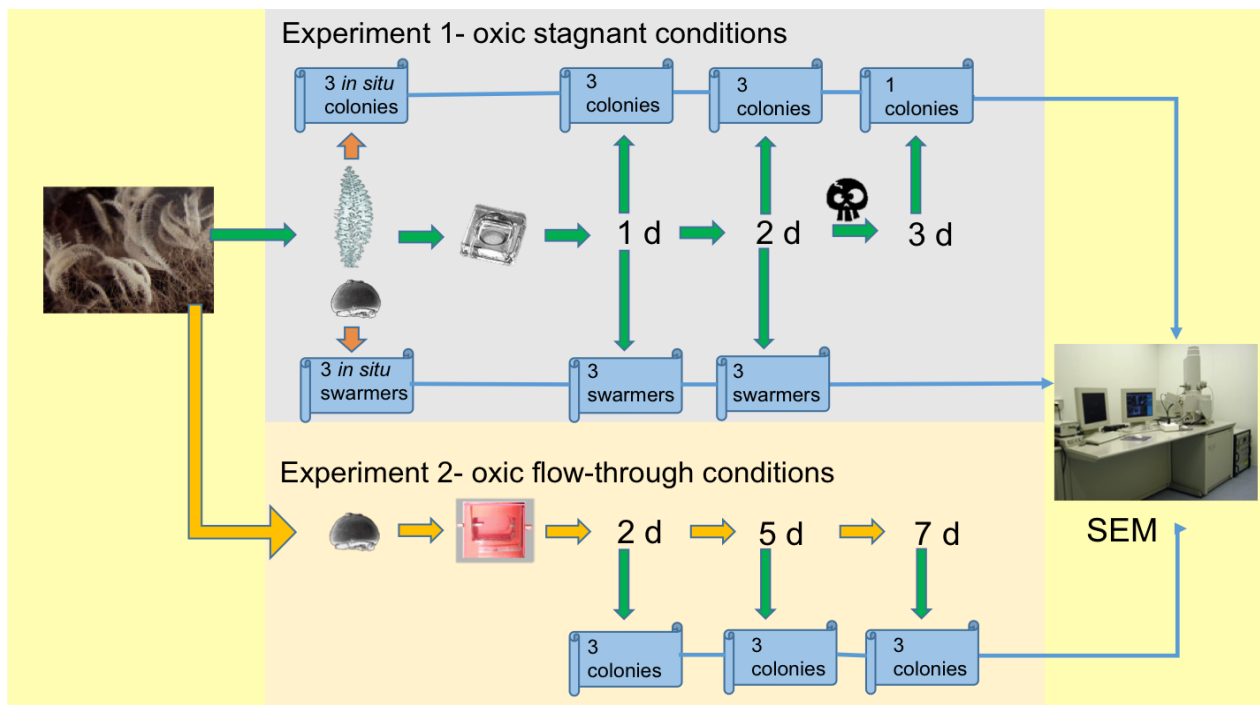
## 2. Material and Methods

### 2.1. Sample Collection

*Zoothamnium niveum* was collected on sunken wood by snorkeling at a depth of about 1 m in the Sv. Jernej canal, Piran, Slovenja in October 2012 and July 2013. Submerged in water the colonies were transported into the laboratory and separated under a dissecting microscope from the wood by cutting them at the lower part of the stem with a MicroPoint™ Scissor. Afterwards they were rinsed twice using 0.2 µm-filtered seawater to remove debris. Subsequently the colonies were placed into flow-through respirometer chambers or into embryo dishes, where they were maintained throughout the experiments (Drexel, diploma thesis 2013).

### 2.2. Experimental set up

Two different experiments were conducted to gain more information about the maintenance and the breakdown of the symbioses under artificial conditions (see Fig. 2).



**Figure 2.** Overview experimental set up experiment 1 and experiment 2

For the first experiment, freshly collected colonies of *Zoothamnium niveum* were maintained in oxic seawater under stagnant conditions for 3 days (upper section of Fig. 2). Filtered seawater was exchanged every day. As the colonies were cut of their substrates they were free floating in the embryo dishes. At different time points (*in situ*, 1 day, 2 days), three sample colonies each were taken. After 3 days, one sample colony of *Z. niveum* was taken although the host had already died. Swarmer released from the *in situ* colonies were taken at different time points (*in situ*, 1 day, 2 days). All samples were fixed and prepared for scanning electron microscope (SEM) measurements.

For the second experiment, swarmer of *Zoothamnium niveum* were transferred into respirometer chambers and exposed to a pulse of sulfide for 1 h to ensure settlement and growth of the colony. The growth under oxic flow-through conditions with 50 ml/h flow  $262 \pm 8 \mu\text{mol l}^{-1} \text{O}_2$ , pH  $8.1 \pm 0.1$ ,  $22.6 \pm 0.9^\circ\text{C}$  and a salinity of  $34.5 \pm 0.6$  was monitored after two, five and seven days (Drexel, diploma thesis 2013). However, each measurement corresponds to a different cultivation at very similar conditions. At each time step, three sample colonies were taken, fixed and prepared for SEM.

### 2.3. Preparation of SEM samples

To avoid contraction of the colony, *Zoothamnium niveum* samples were cooled down prior to fixation. Therefore the sample colonies were put into an embryo dish filled with 2.5 ml of 0.2  $\mu\text{m}$ -filtered seawater and exposed to  $-20^\circ\text{C}$  for 9.5 min using a freezer (according to Rinke et al., 2007; Drexel, diploma thesis 2013). Samples were fixed with 2.5 ml of Trump's fixative before the freezing point was reached (2.5 % glutaraldehyde, 2 % paraformaldehyde in 0.1 M sodium cacodylate buffer 1100 mOsm, pH 7.2, filtered with a 0.2  $\mu\text{m}$ -filter prior to usage). The samples then were rinsed in cacodylate buffer, dehydrated up to 70% ethanol and stored until further treatment (Drexel, diploma thesis 2013). During sample preparation it was observed that colonies threatened under the oxic stagnant conditions remained white whereas many colonies of the oxic-flow trough conditions lost their white color during incubation. The samples were further processed upon delivery to the laboratory in Vienna. Samples were dehydrated using an ascending ethanol series (one run at 80 % ethanol for 5 min, one run at 90 % ethanol for 5 min) and finally ethanol was exchanged with 100% acetone (one run at 100 % acetone for 5 min). The samples were placed in a mixture of acetone/hexamethyldisilazane (HDMS) (1:1) for 15 min

followed by pure HDMS for 30 min including with one exchange of liquid. Afterwards, the samples were air-dried overnight, placed on a stab and covered with gold using an Agar Sputter Coater Agar 108 for 250 seconds.

#### 2.4. Observation with SEM

*Zoothamnium niveum* samples were investigated using a Philips XL 20 scanning electron microscope operated at an acceleration voltage of 20kV. For each colony sample pictures of 15 microzooids were taken at 2000x magnification. For each swarmer as many pictures as necessary were taken to cover the entire swarmer, overlapping the borders of individual pictures to be able to reconstruct the whole surface area. Analysis of all pictures was performed using the Gimp 2.8 (GNU Image Manipulation Program) software and ZEN lite 2012 (ZEISS) software.

#### 2.5. Image analyses with ZEN lite 2012

First off, oral and aboral parts were distinguished dividing the microzooid along of the pictures, plotting a centerline perpendicular to the long axis. Starting from a cell located in the middle of the oral or aboral part and continuously adding surrounding cells following a clockwise spiral pattern, a collection of cells was defined for each part (see Fig. 3c). One collection included up to 70 symbionts. For all symbionts, length and width was measured. The measured data of the symbionts was recorded in a excel sheet, merging all the necessary information.

Approximating the shape of the bacterial cells as hemisphere-capped cylinders (van Veen & Paul, 1979), the cell volume is given as

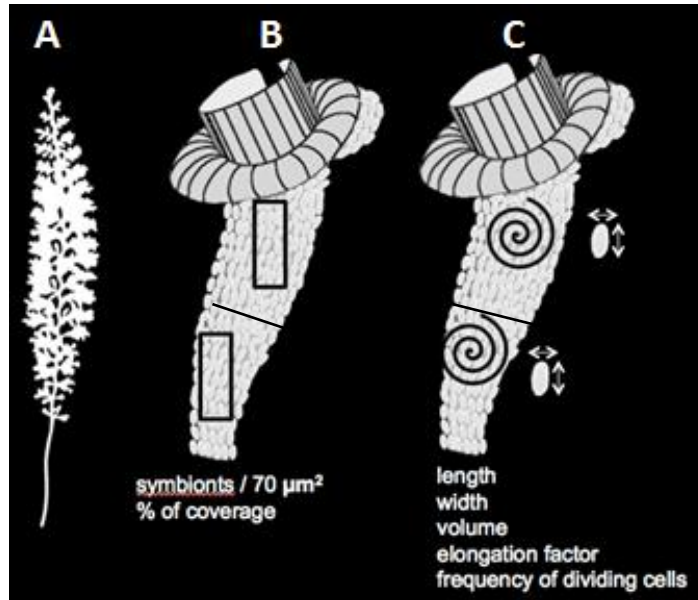
$$V = \pi w^2(l - w) + \frac{4}{3} \pi \left(\frac{w}{2}\right)^3$$

with  $w$  the width and  $l$  the length. The Elongation Factor (EF) is defined as the ratio of length to width and provides important information about the form of the cells. An EF value of 1 is considered as coccoid-shaped whereas  $EF > 1.5$  is considered as rod-shaped (Sunamara et al., 2004).

The frequency of dividing cells (FDC) was determined as average value for each microzooid. It is defined as

$$\text{FDC} = \frac{\overline{N_{\text{div}}}}{\overline{N_{\text{tot}}}} \times 100$$

with  $\overline{N_{\text{div}}}$  and  $\overline{N_{\text{tot}}}$  the average number of dividing and total cells, respectively. Symbionts showing an invagination but not a clear intervening zone between the cells were considered as dividing cells (Hagström et al., 1979; Drexel, diploma thesis 2013).



**Figure 3.** Sample analysis. (A) Single colony of *Zoothamnium niveum*, 15 microzooids were analyzed per colony. (B) Analyses with Gyp 2.8 software: Single microzooid at 2000x magnification.  $70 \mu\text{m}^2$  rectangular frames were placed at oral and aboral parts to determine host surface coverage and symbiont density. (C) Analyses with ZEN lite software: Microzooid at 2000x magnification. Up to 70 symbionts were measured in length and with in a spiral patterns at oral and aboral parts to determine length, width, volume, EF and FDC. (Modified from Bauer-Nebelsick et al., 1996a)

The evaluation procedure for the swarmers was identical to that for the microzooids, beside from the fact that at 2000x magnification several pictures had to be recorded to cover the entire swarmer. Three swarmers per time step were analyzed. During the analysis the swarmer was divided into an upper and a lower part.

According to natural differences such as size of the colonies and number of microzooids per colony, slightly different amounts of microzooids per treatment and experiments have been analyzed. Details are given in Tab. 1.



**Table. 1:** Overview of the analyzed microzooids and swarmer in Experiment 1 and the microzooids of experiment 2

EXPERIMENT 1				EXPERIMENT 2						
VIAL	t	colonies	microzooids	VIAL	t	swarmer	VIAL	t	colonies	microzooids
#498	<i>in situ</i>	1	15	1882	<i>in situ</i>	3 swarmer	2171	2 days	1	8
		2	15					2	6	
		3	15					3	5	
2283	1 day	1	15	2283	1 day	3 swarmer	2153	5 days	1	12
		2	15					2	15	
		3	15					3	15	
2338	2 days	1	15	2338	2 days	3 swarmer	#693	7 days	1	15
		2	15					#696	2	15
		3	15					#699	3	15
2421	3 days	1	15							

## 2.6. Image analyses with Gymp 2.8 software

According to the procedure described in the previous section, oral and aboral parts of the microzooids were distinguished. To gain detailed information about the coverage of the symbiont on the host, as well as the number of symbionts in the specific areas of the oral and aboral part, two  $70 \mu\text{m}^2$  rectangular frames were defined at the end of oral and aboral part (see Fig. 3b). To determine the total number of symbionts in the frame (symbiont density) only cells that were completely located in the frame, as well as cells that extended beyond the right and upper border of the frame were taken into account. To calculate the ratio of coverage all of the cells were circularly marked. Subsequently the percentage of coverage was determined as ratio between marked and total number of pixels (Drexel, diploma thesis 2013). During a few analyses a small fraction of the frame was covered with dirt, so that not all of the cells in the  $70 \mu\text{m}^2$  rectangular frame were visible. In these cases, the covered amount of pixels were measured, as well as the potential hidden bacteria estimated and considered for further analyses.

## 2.7. Statistical data analysis

All statistical evaluations were performed using the software IBM SPSS Statistics 22. Differences between symbionts located at the oral parts of the microzooids and symbionts located at the aboral parts, as well as the pooled symbionts from upper and lower part together were tested for statistical significance using the entire data recorded. Within each group, length, width, volume, density, EF, FDC and host surface coverage were chosen as relevant parameters. As the modified Shapiro-Wilk test of normality ( $p < 10^{-3}$ ) did not reveal normal distributions for the mentioned parameters, non-parametric statistics had to be applied for further analysis.

The post hoc tests in the analysis of unequal variance (ANOVA) with a 99 % CI were used to test for significant differences among the three colonies of the same treatment. Under *in situ* conditions, frequency of dividing cells, elongation factor, width and surface coverage did not show significant differences between the aboral and oral part of the microzooid. For these parameters a Wilcoxon signed-rank test was conducted to investigate statistically significant differences between the aboral and oral part of the microzooids.

The 2 days and 3 days old colonies were considered and tested with the post hoc test in the analysis of unequal variances (ANOVA) with a 99% CI as one group, due to the high variances of the measured parameters. The post hoc test revealed no significant differences between the volume and FDC between the oral and aboral part. For these parameters a Wilcoxon signed-rank test was performed to investigate statistically significant differences.

A Spearman's rank-order correlation was run for data from *in situ* conditions and the 1 and 2 days group to determine the relationship between the different parameters.

### 3. Results

#### 3.1. Experiment 1

##### 3.1.1. *Symbiont behavior on microzooids (in situ)*

The analyzed symbionts revealed the following information on morphology, density, host surface coverage and fitness (estimated by FDC) (see Tab. S1-S3; Fig. S1.1; 1.2- S7.1; 7.2).

Average values for length and width of symbionts located at the oral part were determined to 1805.32 nm ( $\pm 144.54$ ) and 866.58 nm ( $\pm 99.11$ ), respectively. The volume was determined to  $0.92 \mu\text{m}^3$  ( $\pm 0.25$ ). The EF was calculated to 2.16 ( $\pm 0.23$ ). Hence, the symbionts can be considered rod-shaped (Sunamara et al., 2004). The Spearman correlation test revealed a correlation between length and width ( $r_s = 0.586$ ,  $P < 0.01$ ) (see Tab. S4). Both, the length and width are each positively correlated with the volume ( $r_s = 0.751$ ,  $P < 0.01$ ;  $r_s = 0.960$ ,  $P < 0.01$ ). The EF is negatively correlated with the width ( $r_s = -0.666$ ,  $P < 0.01$ ) but not with the length. The host surface coverage on the oral part was determined to 87.96 % ( $\pm 3.14$ ) with 48.62 ( $\pm 10.42$ ) cells per  $70 \mu\text{m}^2$ . The FDC on the oral part of the microzooid was determined to 14.67 % ( $\pm 2.11$ ). The Spearman correlation revealed that the symbiont density was negatively correlated with the symbiont volume ( $r_s = -0.438$ ,  $P < 0.01$ ). Furthermore, the FDC was correlated with the host surface coverage ( $r_s = -0.371$ ,  $P < 0.05$ ). The host surface coverage was not correlated with any other parameter.

On the aboral part of the host symbionts were 1649.38 nm ( $\pm 192.18$ ) long and 586.98 nm ( $\pm 107.83$ ) wide. The volume of the ectosymbionts was  $0.42 \mu\text{m}^3$  ( $\pm 0.21$ ). The cell had an EF of 2.93 ( $\pm 0.37$ ) therefore the symbionts can be considered rod-shaped according to Sunamara et al. (2004). The Spearman correlation test revealed a strong correlation between the length and the width ( $r_s = 0.674$ ,  $P < 0.01$ ), as well as the volume ( $r_s = 0.866$ ,  $P < 0.01$ ) (Tab. S5). The host surface coverage was 89.80 % ( $\pm 2.88$ ) with 74.11 ( $\pm 14.95$ ) cells per  $70 \mu\text{m}^2$ . The measured FDC was on the aboral part 11.78 % ( $\pm 1.93$ ). Spearman correlation showed that the symbiont density was negatively correlated with the volume ( $r_s = -0.705$ ,  $P < 0.01$ ), width ( $r_s = -0.594$ ,  $P < 0.01$ ) and length ( $r_s = -0.721$ ,  $P < 0.01$ ) (see Tab. S5). It has to be noted that the host coverage did not correlate with any parameter.

Pooling the symbionts from the upper and lower parts, overall the cells were 1727.35 nm ( $\pm 186.37$ ) long and 726.78 nm ( $\pm 174.26$ ) wide, with an EF of 2.54 ( $\pm 0.49$ ). The volume was calculated with  $0.67 \mu\text{m}^3$  ( $\pm 0.34$ ). The Spearman correlation test revealed that the length of the symbionts is positively correlated with the width ( $r_s = 0.659$ ,  $P < 0.01$ ), and the volume of the cells ( $r_s = 0.750$ ,  $P < 0.01$ ) and weakly negatively correlated with the EF ( $r_s = -0.255$ ,  $P < 0.05$ ) (see Tab. S6). The host surface coverage of the whole microzooid was 88.98 % ( $\pm 3.01$ ) with an FDC of 13.22 % ( $\pm 2.48$ ). The FDC was depending on the size of the symbionts as shown by moderate positive correlations with width ( $r_s = 0.489$ ,  $P < 0.01$ ) and volume ( $r_s = 0.457$ ,  $P < 0.01$ ) and a negative moderate correlation to the EF ( $r_s = -0.497$ ,  $P < 0.01$ ). A total of 13.22 ( $\pm 2.48$ ) cells were detected on  $70 \mu\text{m}^2$ . Spearman correlation revealed that higher numbers of symbionts lead to higher host surface coverage ( $r = 0.266$ ,  $P < 0.05$ ) and less dividing cells ( $r_s = -0.436$ ,  $P < 0.01$ ). The number of symbionts was negatively correlated with the length of the cells ( $r_s = -0.639$ ,  $P < 0.01$ ), width ( $r_s = -0.793$ ,  $P < 0.01$ ) and volume ( $r_s = -0.810$ ,  $P < 0.01$ ).

Comparing the symbiont populations on the upper part of the microzooids of the three *in situ* colonies, the Tamhane posthoc test revealed that the three populations were similar in width, EF, host surface coverage, volume and FDC, while significant differences between replicates were present in symbiont length and density. Further, also the symbiont populations on the lower parts of the microzooids were not significantly different in width, EF, host coverage and FDC. Significant differences in length, volume and density were detected. Therefore, the Wilcoxon signed-rank test was conducted only for the parameters, which I could pool to test for differences between upper and lower part populations. The symbionts on the oral part were significantly wider ( $866.58 \text{ nm} \pm 99.11$ ) than on the aboral part ( $586.98 \text{ nm} \pm 107.83$ ), but exhibited a lower host surface coverage ( $87.96 \% \pm 3.14$ ;  $89.80 \% \pm 2.88$ ) and a higher FDC ( $14.67 \pm 2.11$ ;  $11.78 \pm 1.93$ ). Although with relatively high within variability of length ( $1805.0 \text{ nm} \pm 144.54$ ;  $1649.38 \text{ nm} \pm 192.18$ ), volume ( $0.92 \mu\text{m}^3 \pm 0.25$ ;  $0.42 \mu\text{m}^3 \pm 0.21$ ) and density ( $48.62 \pm 10.42$ ;  $74.11 \pm 14.95$ ) in upper and lower part populations each, overall symbionts on the upper part tended to be larger with a higher volume and accordingly less density compared to those on the lower part.

### 3.1.2. *Symbiont behavior on swarmers (in situ)*

The analyzed symbionts revealed the following information on morphology, density, host surface coverage, and fitness (estimated by FDC) (see Tab. S1-S3; Fig. S1.3; 1.4 –S7.3; 7.4).

Average values for length and width of symbionts located at the oral part were determined to 2021.19 nm ( $\pm 466.32$ ) and 621.41 nm ( $\pm 75.13$ ), respectively. The volume was determined to  $0.6 \mu\text{m}^3$  ( $\pm 0.32$ ). The EF was calculated to 3.26 ( $\pm 0.37$ ). Hence, the symbionts can be considered rod-shaped (Sunamara et al., 2004). The symbiont coverage on swarmers of the upper part was measured with 67.13 % ( $\pm 27.96$ ) with a symbiont density of 55.35 ( $\pm 24.44$ ) cells per  $70 \mu\text{m}^2$ . The FDC of the symbionts was determined to 4.92 ( $\pm 3.93$ ).

On the lower part of the swarmer the symbionts were 2311.91 nm ( $\pm 609.06$ ) long and 594.89 nm ( $\pm 61.57$ ) wide. The calculated volume of the symbionts was  $0.63 \mu\text{m}^3$  ( $\pm 0.29$ ). The measured cells had an EF of 3.90 ( $\pm 0.81$ ). On the lower part the coverage of the swarmers was measured with 83.97 % ( $\pm 17.46$ ) with 68.37 ( $\pm 20.94$ ) symbionts on a surface area of  $70 \mu\text{m}^2$ . The FDC on the oral part of the microzooid was determined to 5.49 ( $\pm 3.99$ ).

Considering the whole swarmer, pooling the symbionts from the upper and the lower part, overall cells were 2170.48 nm ( $\pm 556.65$ ) long and 607.78 nm ( $\pm 68.86$ ) wide, with an EF of 3.59 ( $\pm 0.71$ ). The calculated volume was determined to  $0.62 \mu\text{m}^3$  ( $\pm 0.30$ ). The total coverage of the swarmers was 71.89 % ( $\pm 26.41$ ) with 55.23 cells per  $70 \mu\text{m}^2$  ( $\pm 21.97$ ). The FDC of the symbionts was determined to 5.21 ( $\pm 3.91$ ).

The variability between all individual swarmers was very high, therefore no statistical tests were conducted. Although with relatively high within variability of length (2311.91 nm  $\pm$  609.06; 2021.19 nm  $\pm$  466.32) and width (594.87 nm  $\pm$  61.57; 621.41 nm  $\pm$  75.13) in upper and lower part populations each, overall symbionts tended to be slightly larger and thinner on lower parts.

The measured EF values on the lower part of the swarmers (3.90  $\pm$  0.81) tended to be slightly higher than on the upper part of the swarmers (3.26  $\pm$  0.37). The volume of the symbionts was comparable on oral part ( $0.60 \mu\text{m}^3 \pm 0.32$ ) and aboral part ( $0.63 \mu\text{m}^3 \pm 0.29$ ). However, the coverage of the lower part (83.97 %  $\pm$  17.46) was higher than on the upper part (67.13 %  $\pm$  27.96), also the number of cells per  $70 \mu\text{m}^2$  was higher on the lower part (68.37  $\pm$  20.94) than on the upper part (55.35  $\pm$  24.44). The FDC tended to be slightly higher on the lower part (5.49  $\pm$  3.99) compared to the upper part (4.92  $\pm$  3.93).

### 3.1.3. Symbiont behavior on microzooids (1d, 2d, 3d)

All colonies from the time points 1 day and 2 days were considered for statistical tests as one group (1-2 days), due to the high variances between the measurements. The analyzed symbionts revealed the following information on morphology, density, host surface coverage and fitness (estimated by FDC) (see Tab. S1-S3; Fig. S1.1; 1.2-S7.1; 7.2).

Average values for length and width of symbionts located at the oral part were determined to 2177.31 nm ( $\pm 311.63$ ) and 788.24 nm ( $\pm 141.95$ ), respectively. The volume was determined to  $0.96 \mu\text{m}^3$  ( $\pm 0.37$ ). The EF was calculated to 2.90 ( $\pm 0.64$ ). Hence, the symbionts can be considered rod-shaped (Sunamara et al., 2004). The Spearman correlation test revealed a correlation between length and volume ( $r_s = 0.551$ ,  $P < 0.01$ ), EF ( $r_s = 0.603$ ,  $P < 0.01$ ) and coverage ( $r_s = 0.5222$ ,  $P < 0.01$ ) (see Tab. S7). The host surface coverage was on the oral part 52.69 % ( $\pm 31.06$ ) with 27.06 ( $\pm 18.27$ ) cells per  $70 \mu\text{m}^2$ . The FDC on the oral part of the microzooid was determined to 6.85 ( $\pm 4.87$ ). The Spearman correlation revealed that symbiont density was positively correlated with the coverage ( $r_s = 0.854$ ,  $P < 0.01$ ), length of the symbionts ( $r_s = 0.379$ ,  $P < 0.01$ ), EF ( $r_s = 0.587$ ,  $P < 0.01$ ) and negatively correlated with the width of the symbionts ( $r_s = -0.332$ ,  $P < 0.01$ ). The coverage was positively correlated with the length of the symbionts ( $r_s = 0.522$ ,  $P < 0.01$ ) and with the EF ( $r_s = 0.495$ ,  $P < 0.01$ ). It is to note that the FDC was not correlated with any other parameter.

On the aboral part of the microzooids symbionts were 2044.28 nm ( $\pm 256.18$ ) long and 667.90 nm ( $\pm 136$ ) wide. The volume of the cells was  $0.64 \mu\text{m}^3$  ( $\pm 0.25$ ) with an EF of 3.31 ( $\pm 0.70$ ) indicating rod-shaped bacteria. The conducted Spearman correlation test showed a correlation between length and coverage ( $r_s = 0.281$ ,  $P < 0.05$ ), EF ( $r_s = 0.674$ ,  $P < 0.01$ ) and volume of the cells ( $r_s = 0.349$ ,  $P < 0.01$ ) (see Tab. S8). The host surface coverage was after 1 – 2 days 65.13 % ( $\pm 24.26$ ) with 46.95 ( $\pm 21.33$ ) cells per  $70 \mu\text{m}^2$  and a FDC of 4.15 ( $\pm 3.82$ ). The Spearman correlation revealed that the density of *Cand. Thiobios zoothamnii* was correlated with the coverage ( $r_s = 0.782$ ,  $P < 0.01$ ), length of the cells ( $r_s = 0.508$ ,  $P < 0.01$ ), EF ( $r_s = 0.680$ ,  $P < 0.01$ ) and negatively correlated with the width of the cells ( $r_s = -0.496$ ,  $P < 0.01$ ). The coverage of the host was positively correlated with the length of the cells ( $r_s = 0.479$ ,  $P < 0.01$ ) and the EF ( $r_s = 0.412$ ,  $P < 0.01$ ). The FDC was positively correlated with the width of the cells ( $r_s = 0.225$ ,  $P < 0.05$ ) and negatively correlated with the EF ( $r_s = -0.268$ ,  $P < 0.05$ ).

After 3 days symbionts on the oral part of the microzooid were 1279.66 nm ( $\pm 429.35$ ) long and 716.17 nm ( $\pm 210.59$ ) wide. The volume of the symbionts was  $0.59 \mu\text{m}^3$  ( $\pm 0.74$ ) with an EF of 1.92 ( $\pm 0.40$ ). The coverage of the host was only 6.68 % ( $\pm 6.37$ ) with 5.29 ( $\pm 4.46$ ) cells per  $70 \mu\text{m}^2$ . The FDC of the ectosymbionts was after 3 days 0.42 ( $\pm 1.61$ ).

On the aboral part of the three days old colonies the symbionts were 1141.57 nm ( $\pm 271.17$ ) long and 594.89 nm ( $\pm 120.22$ ) wide. The volume of the cells was  $0.27 \mu\text{m}^3$  ( $\pm 0.10$ ) with an EF of 2.03 ( $\pm 0.49$ ). The coverage of the host was 4.52 % ( $\pm 3.35$ ) with an FDC of 0.33 ( $\pm 1.29$ ).

Pooling the symbionts from the aboral and oral part, overall the cells had a length of 2110.79 nm ( $\pm 292.18$ ) and a width of 728.07 nm ( $\pm 151.17$ ) after 1-2 days. The volume of the cells was  $0.81 \mu\text{m}^3$  ( $\pm 0.35$ ) with an EF of 3.10 ( $\pm 0.70$ ). The Spearman correlation test revealed that the length was correlated with the coverage ( $r_s = 0.252$ ,  $P < 0.01$ ), EF ( $r_s = 0.543$ ,  $P < 0.01$ ) and with volume ( $r_s = 0.475$ ,  $P < 0.01$ ) (see Tab. S9). The coverage of the host was 58.37 % ( $\pm 28.76$ ) with 36.14 ( $\pm 22.03$ ) cells per  $70 \mu\text{m}^2$  with an FDC of 5.50 ( $\pm 4.57$ ). The Spearman correlation test showed that the symbiont density correlated with all measured parameters, it was positively correlated with the coverage ( $r_s = 0.785$ ,  $P < 0.01$ ), length ( $r_s = 0.272$ ,  $P < 0.01$ ) and EF ( $r_s = 0.636$ ,  $P < 0.01$ ). Furthermore negative correlations were found between the symbiont density and the width ( $r_s = -0.539$ ,  $P < 0.01$ ), volume ( $r_s = -0.344$ ,  $P < 0.01$ ) and the FDC ( $r_s = -0.236$ ,  $P < 0.01$ ).

Pooling the symbionts from the oral and aboral part of the microzooid after 3 days together, it revealed that the average length was 1210.62 nm ( $\pm 358.20$ ) long and 657.95 nm ( $\pm 180.62$ ) wide. On the whole microzooid the volume of the symbionts was  $0.44 \mu\text{m}^3$  ( $\pm 0.55$ ) with an EF of 1.97 ( $\pm 0.43$ ). The coverage of the host was 5.48 % ( $\pm 4.79$ ) with an FDC of 0.38 ( $\pm 1.43$ ). Due to the fact that only one microzooid was analyzed after 3 days, no statistical tests were conducted.

Comparing the symbiont populations on the oral parts of the three microzooids after 1-2 days, the Tamhane post hoc test revealed that the three populations were similar in width, FDC and volume. High variability between the replicates was present in symbiont length, EF, coverage and density. On the aboral part the volume and FDC of the three populations were similar, high variability between lengths, width, EF, host surface coverage, symbiont density and FDC were found. Therefore the Wilcoxon signed-rank test was conducted only for volume and FDC, which were pooled to test for differences between the oral and aboral part populations. The symbionts on the oral part of the microzooid were significantly more voluminous ( $0.96 \mu\text{m}^3 \pm 0.37$ ) than on

the aboral part ( $0.64 \mu\text{m}^3 \pm 0.25$ ). Furthermore the FDC was significantly higher on the oral part ( $6.85 \pm 4.87$ ) than on the aboral part ( $4.15 \pm 3.82$ ).

#### **3.1.4. Symbiont behavior on swarmers (1d, 2d)**

All colonies from the time points 1 day and 2 days are considered as one group (1-2 days) due to the high variances between the measurements. The analyzed symbionts revealed the following information on morphology, density, host surface coverage and fitness (estimated by FDC) (see Tab. S1-S3; Fig. S1.3; 1.4 –S7.3; 7.4).

On the upper part of the swarmers the symbionts were 2098.34 nm ( $\pm 259.76$ ) long and 578.86 nm ( $\pm 56.88$ ) wide. The volume was determined to  $0.52 \mu\text{m}^3$  ( $\pm 0.09$ ) and the EF to 3.74 ( $\pm 0.73$ ). The coverage of the swarmer was determined with 26.38 % ( $\pm 30.18$ ) and 224.95 ( $\pm 31.96$ ) cells per  $70 \mu\text{m}^2$ , respectively. The FDC was determined to 1.92 ( $\pm 2.17$ ).

On the lower part of the swarmer bacteria were 1920.01 nm ( $\pm 296.22$ ) long and 608.69 nm ( $\pm 73.66$ ) wide. The volume was calculated with  $0.41 \mu\text{m}^3$  ( $\pm 0.19$ ) and an EF of 3.21 ( $\pm 0.61$ ). The host surface coverage was 23.83 % ( $\pm 29.27$ ) with 21.89 ( $\pm 29.89$ ) cells per  $70 \mu\text{m}^2$ . The FDC was determined to 1.66 ( $\pm 1.79$ ).

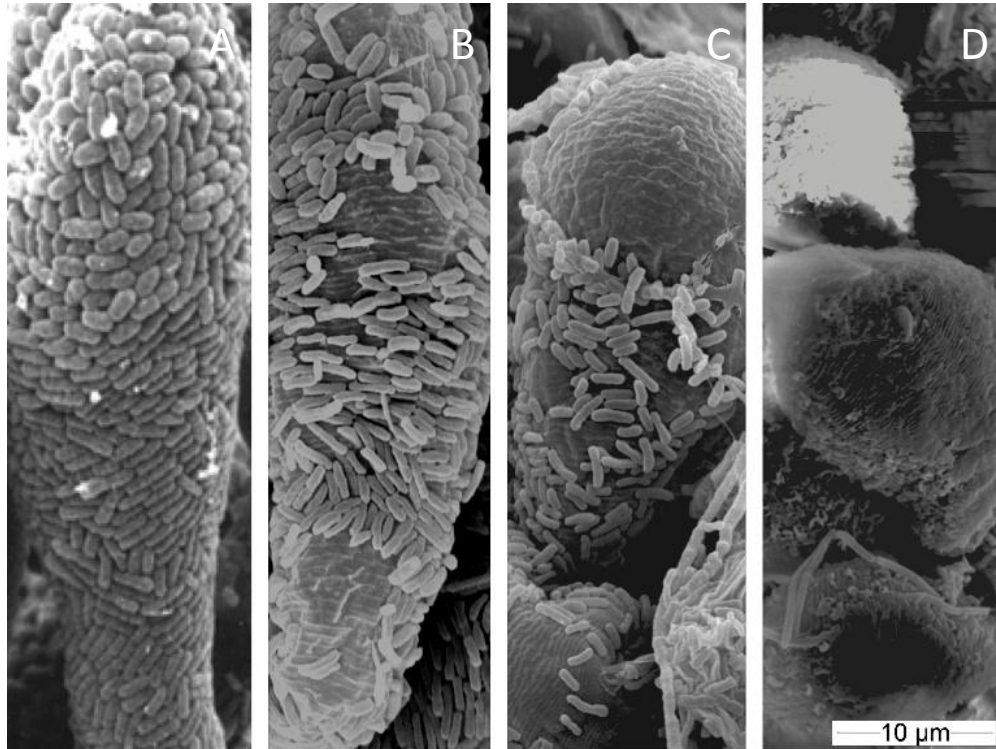
Pooling upper and lower part of the swarmer together, the overall length of symbionts was determined to 2067.48 nm ( $\pm 271.98$ ) with a width of 587.47 nm ( $\pm 62.92$ ), respectively. The overall volume of symbionts was  $0.49 \mu\text{m}^3$  ( $\pm 0.13$ ) with an EF of 3.64 ( $\pm 0.73$ ). The total coverage of the swarmer was calculated with 25.16 % ( $\pm 29.67$ ) with 23.49 ( $\pm 30.63$ ) cells per  $70 \mu\text{m}^2$ . The FDC was determined to 1.91 ( $\pm 2.14$ ).

The variability between the individual colonies of the samples was very high, therefore it is to note that no statistical tests were conducted for comparisons between the upper and lower part of the swarmer. Comparing the symbiont populations of the three swarmers in the group 1-2 days, symbionts on the upper part and lower part were comparable in all measured parameters.



### 3.1.5. Comparison between symbionts of microzooids (*in situ*, 1d, 2d, 3d)

Morphological changes as well as fitness parameters of the symbionts were measured during the analyses of the SEM pictures of the whole microzooid. During the *in situ* situation the host was totally covered with its ectosymbiont. After three days the death of the host was observed, it is to note that at this time point only one individual colony was monitored (see Fig. 4).



**Figure 4.** SEM observation of microzooids of *Zoothamnium niveum* showing the monolayer of bacteria covering the host cell during different time points, scale bar 10  $\mu\text{m}$ . (A) *in situ*. (B) 1 day. (C) 2 days. (D) 3 days.

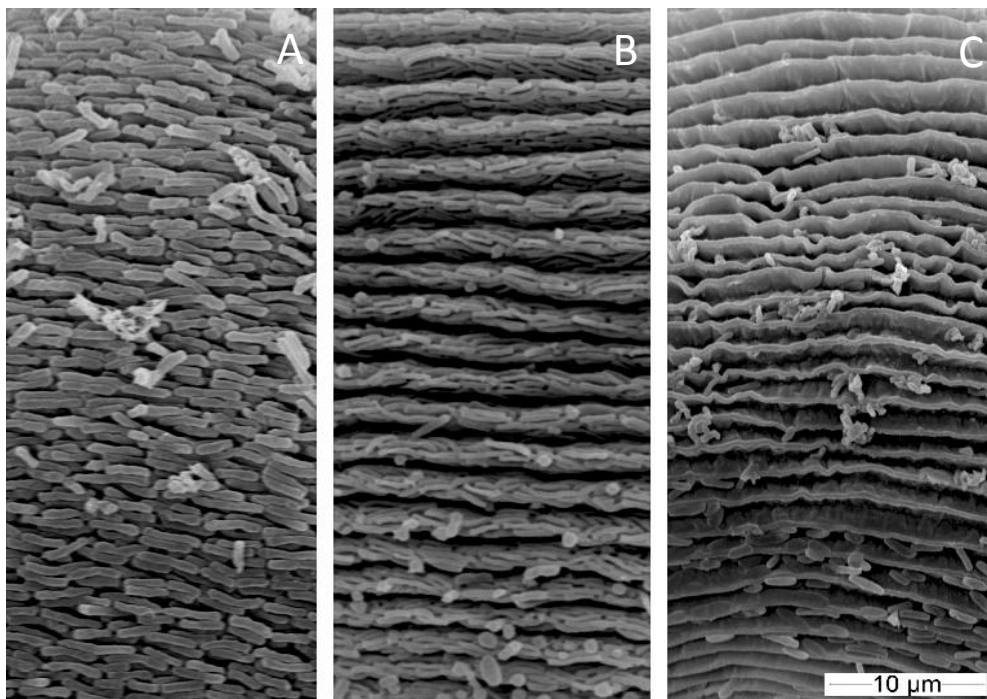
Comparing the whole microzooids of the *in situ* and the 1-2 days old microzooids it revealed that overall the cells were longer ( $2110.79 \text{ nm} \pm 292.18$ ) after 1-2 days than cells of the colonies from the *in situ* treatment ( $1727.35 \text{ nm} \pm 186.37$ ) (see Fig. S8.1). The width did not change between both treatments ( $726.78 \text{ nm} \pm 174.26$ ;  $728.07 \text{ nm} \pm 151.17$ ) (see Fig. S9.1). Also the volume of bacterial cells after 1-2 days ( $0.81 \mu\text{m}^3 \pm 0.35$ ) was slightly higher compared to the symbionts of the *in situ* situation ( $0.67 \mu\text{m}^3 \pm 0.34$ ) (see Fig. S11.1) The calculated EF values of bacterial cells were slightly higher after 1-2 days ( $3.10 \pm 0.70$ ) compared to the *in situ* situation ( $2.54 \pm 0.49$ ), indicating that after 1-2 days symbionts get more rod-shaped (Sunamara et al., 2004) (see Fig. S10.1). However, the host surface coverage was higher during the *in situ* situation

( $88.98\% \pm 3.01$ ) with more symbionts per  $70\ \mu\text{m}^2$  ( $61.37 \pm 18.12$ ) compared to time ship 1-2 days ( $58.37\% \pm 28.76$ ;  $36.14 \pm 22.03$ ) (see Fig. S12.1; 13.1). Also the FDC was decreasing from the *in situ* situation ( $13.22 \pm 2.48$ ) to the time point 1-2 days ( $5.50 \pm 4.57$ ) (see Fig. S14.1).

It was to detect that after 3 days the cells were even smaller ( $1210.62\ \text{nm} \pm 358.20$ ) and thinner ( $657.96\ \text{nm} \pm 180.62$ ) compared to the measurements of the *in situ* situation and 1-2 days old colonies. Also the volume ( $0.44\ \mu\text{m}^3 \pm 0.55$ ) and the EF ( $1.97 \pm 0.43$ ) were the lowest values detected during the different time points. Additionally the host surface coverage ( $5.48\% \pm 4.79$ ) and the number of symbionts per  $70\ \mu\text{m}^2$  ( $5.20 \pm 4.06$ ) showed a drastically decrease over the different time points. Nearly no dividing cells were detected ( $0.38 \pm 1.43$ ) after three days.

### 3.1.6. Comparison between symbionts on swarmer (*in situ*, 1d, 2 d)

Morphological changes as well fitness parameters of the symbionts were measured during the analyses of the SEM pictures of the whole swarmer. During the *in situ* situation the swarmer was totally covered with symbionts. After two days only some symbionts were remaining on the swarmer (see Fig. 5).



**Figure 5.** SEM observation of swarmer cells of *Zoothamnium niveum* showing the monolayer of bacteria covering the host cell during different time points, scale bar  $10\ \mu\text{m}$ . (A) *in situ*. (B) 1 day. (C) 2 days.

Comparing the whole swarmer of the *in situ* and the 1-2 days old swarmers it revealed that overall the cells were slightly longer ( $2170.48 \text{ nm} \pm 556.65$ ) and wider ( $607.78 \text{ nm} \pm 68.86$ ) during the *in situ* situation than after 1-2 days ( $2067.48 \text{ nm} \pm 271.98$ ;  $587.47 \text{ nm} \pm 62.92$ ) (see Fig S8.2; 9.2). Also the volume of the symbionts decreased slightly from the *in situ* situation ( $0.62 \mu\text{m}^3 \pm 0.30$ ) to the 1-2 days old swarmers ( $0.49 \mu\text{m}^3 \pm 0.13$ ) (see Fig. S11.2). The determined EF values of bacterial cells were comparable between the *in situ* situation ( $3.59 \pm 0.71$ ) and the 1-2 days old swarmers ( $3.64 \pm 0.73$ ), indicating rod- shaped bacteria during all measurements (Sunamara et al., 2004) (see Fig. S10.2). Also the host surface coverage decreased drastically from the *in situ* situation ( $71.89 \% \pm 26.41$ ) with more symbionts per  $70 \mu\text{m}^2$  ( $55.23 \pm 21.97$ ) to time ship 1-2 days ( $25.16 \% \pm 29.67$ ;  $23.94 \pm 30.63$ ) (see Fig. S12.2; 13.2). Furthermore the FDC was decreasing from the *in situ* situation ( $5.21 \pm 3.91$ ) to the time point 1-2 days ( $1.91 \pm 2.14$ ) (see Fig. S14.2).

### **3.1.7. Comparison between microzooids and swarmers (*in situ*, 1d, 2d, 3d)**

Comparing symbionts from the microzooids and swarmers *in situ* revealed that overall the cells were longer ( $2170.48 \text{ nm} \pm 556.65$ ) and slightly thinner ( $607.78 \text{ nm} \pm 68.86$ ) on swarmers than on microzooids ( $1727.35 \text{ nm} \pm 168.36$ ,  $726.77 \text{ nm} \pm 174.26$ ). The calculated EF values of bacterial cells were lower ( $2.54 \pm 0.49$ ) on the microzooids than the EF values of the swarmers ( $3.59 \pm 0.71$ ), indicating that swarmers were covered with more rod- shaped bacteria (Sunamara et al., 2004). The host surface coverage of the microzooids ( $88.98 \% \pm 3.01$ ) was higher than on the swarmers ( $71.89 \% \pm 26.41$ ) with slightly more cells on microzooids per  $70 \mu\text{m}^2$  ( $61.37 \pm 18.12$ ) than on swarmers ( $55.23 \pm 21.97$ ). The FDC on microzooids ( $13.22 \pm 2.48$ ) was higher compared to the FDC of the swarmers ( $5.21 \pm 3.91$ ).

Comparing the time point 1-2 days of the microzooids and swarmers it was to detect that symbionts were slightly longer on the microzooid ( $2110.79 \text{ nm} \pm 292.18$ ) than on swarmers ( $2067.48 \text{ nm} \pm 271.98$ ). The cells were also wider on the microzooids ( $728.07 \text{ nm} \pm 151.17$ ) with a higher cell volume ( $0.81 \mu\text{m}^3 \pm 0.35$ ) compared to the swarmers ( $587.47 \text{ nm} \pm 62.92$ ;  $0.49 \mu\text{m}^3 \pm 0.13$ ). Focusing on the total host surface coverage it was to detect that it was higher on the whole microzooids ( $58.37 \% \pm 28.76$ ) than on the whole swarmers ( $25.16 \% \pm 29.67$ ). Also the number of cells per  $70 \mu\text{m}^2$  was higher on microzooids ( $61.37 \pm 18.12$ ) than on

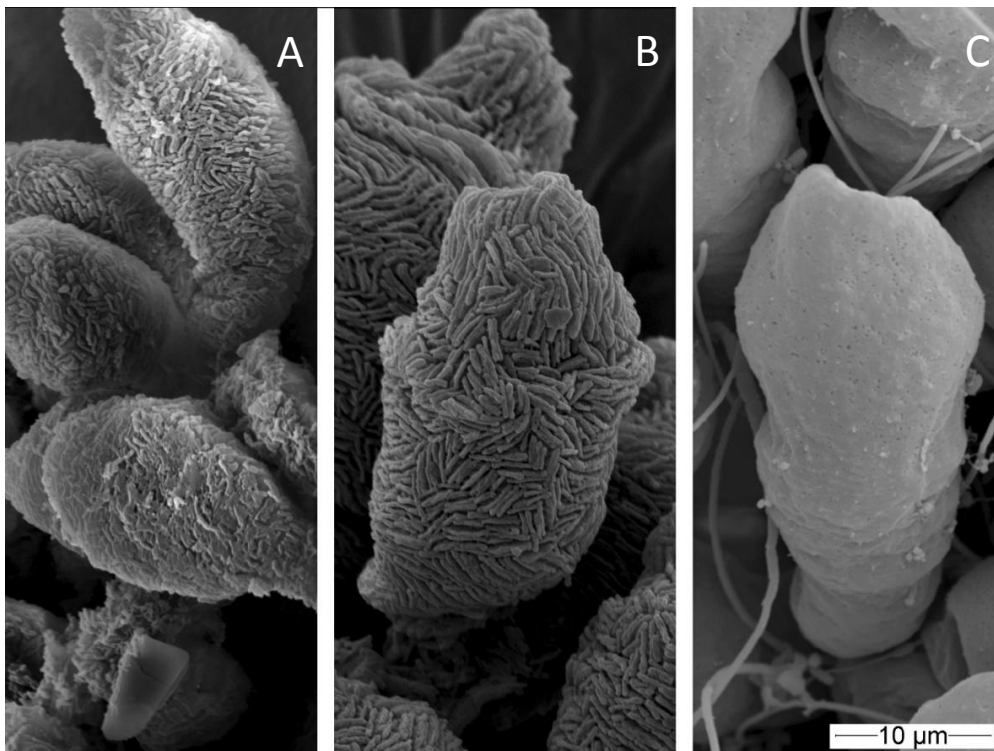
swarmers ( $23.49 \% \pm 30.63$ ), as well as the FDC was higher on microzooids ( $13.22 \pm 2.48$ ) compared to swarmers ( $1.91 \pm 2.14$ ).

After 3 days, only symbionts on the microzooid were measured but not on the swarmers, therefore no comparisons between microzooids and swarmers were conducted.

## 3.2. Experiment 2

### 3.2.1. Symbiont behavior on microzooids (2d, 5d, 7d)

Morphological changes and fitness parameters of the symbiont were measured during the analyses of the SEM pictures of the whole microzooid. During the *in situ* situation the microzooid was totally covered with symbionts while after three days the host was aposymbiotic but still viable (see Fig. 6).



**Figure 6.** SEM observation of microzooids of *Zoothamnium niveum* showing the monolayer of bacteria covering the host cell during different time points, scale bar 10  $\mu\text{m}$ . (A) 2 days. (B) 5 days. (C) 7 days.

Symbionts analyzed revealed the following information on morphology, density, host surface coverage and fitness (estimated by FDC) (see Tab. S1-S3; Fig. S1.5; 1.6-S7.5; 7.6).

After 2 days the symbionts on the oral part of the host were 1463.68 nm ( $\pm 205.31$ ) long and 472.88 nm ( $\pm 63.16$ ) wide. The calculated volume of the symbionts was  $0.25 \mu\text{m}^2$  ( $\pm 0.08$ ) with an EF of 3.18 ( $\pm 0.20$ ) indicating rod-shaped symbionts (Sunamara et al., 2004). The host surface coverage of the colonies was 75.09 % ( $\pm 18.36$ ) with 112.32 ( $\pm 47.51$ ) cells per  $70 \mu\text{m}^2$ . The measured FDC of the symbionts was 3.73 ( $\pm 2.58$ ).

On the aboral part of the microzooids symbionts were 1439.65 nm ( $\pm 173.78$ ) long and 478.94 nm ( $\pm 66.36$ ) wide. The calculated volume of *Candidatus Thiobios zoothamnicoli* was  $0.25 \mu\text{m}^3$  ( $\pm 0.08$ ) with an EF of 3.10 ( $\pm 0.28$ ). The host surface coverage was 76.33 % ( $\pm 15.26$ ) with 120.16 cells ( $\pm 44.28$ ) per  $70 \mu\text{m}^2$ . The FDC was determined to 2.73 ( $\pm 3.42$ ).

Pooling symbionts from the oral and the aboral part together, considering the total microzooids, after 2 days cells were 1429.25 nm ( $\pm 208.69$ ) long and 475.91 nm ( $\pm 64.02$ ) wide. The average volume of cells on the whole microzooids was  $0.25 \mu\text{m}^3$  ( $\pm 0.08$ ) with an EF of 3.14 ( $\pm 0.24$ ). The host surface coverage was 75.71 ( $\pm 16.97$ ) with 115.35 ( $\pm 45.70$ ) cells per  $70 \mu\text{m}^2$ . The FDC was determined to 3.23 ( $\pm 3.01$ ).

The variability between individual colonies, which were measured was very high, therefore no statistical tests were conducted for the different time points. Comparing aboral and oral part of the microzooid after 2 days no difference between symbionts length and width on oral part and aboral part were visible. The volume ( $0.25 \mu\text{m}^2$ ) and the EF ( $3.18 \pm 0.20$ ;  $3.10 \pm 0.28$ ), of both parts was similar, indicating rod-shaped bacteria on both parts (Sunamara et al., 2014). Also in terms of coverage and symbiont density no differences were detected. The FDC of the oral part was slightly higher ( $3.73 \pm 2.58$ ) than on the aboral part ( $2.73 \pm 3.42$ ).

After 5 days the measured symbionts on the oral part of the host were 1742.35 nm ( $\pm 119.56$ ) long and 449.68 nm ( $\pm 39.32$ ) wide. The volume of the bacterial symbionts was calculated with  $0.27 \mu\text{m}^3$  ( $\pm 0.06$ ) with an EF of 3.99 ( $\pm 0.28$ ) indicating rod-shaped cells (Sunamara et al., 2014). Considering the host surface coverage 79.91 % ( $\pm 18.96$ ) were covered with 103.70 ( $\pm 18.87$ ) cells per  $70 \mu\text{m}^2$ . Dividing cells were only observed on one colony with an average FDC of 0.14 ( $\pm 0.53$ ).

Considering the aboral part of the host after 5 days, symbionts were 1736.87 nm ( $\pm 170.77$ ) long and 444.96 nm ( $\pm 56.58$ ) wide. Cells had a volume of  $0.26 \mu\text{m}^3$  ( $\pm 0.10$ ) and a calculated EF of 4.02 ( $\pm 0.40$ ) considering that the symbionts were rod-shaped (Sunamara et al., 2014). The measured host surface coverage was 84.13 % ( $\pm 7.97$ ) with 116.54 ( $\pm 14.97$ ) cells per  $70 \mu\text{m}^2$ . Only on one microzooid dividing cells were detected with an FDC of 0.03 ( $\pm 0.22$ ).

Pooling symbionts from oral and aboral part together, after 5 days symbionts were 1739.57 nm ( $\pm 146.84$ ) long and 447.29 nm ( $\pm 48.59$ ) wide. The calculated volume of symbionts on the whole microzooid was  $0.27 \mu\text{m}^3$  ( $\pm 0.08$ ) with an EF of 4.01 ( $\pm 0.34$ ). The host surface coverage was 80.92 % ( $\pm 16.99$ ) with 106.85 ( $\pm 18.71$ ) cells per  $70 \mu\text{m}^2$ . Only on one colony of *Zoothamnium niveum* dividing cells on the oral and aboral part were detected, pooling them together the FDC was 0.09 ( $\pm 0.40$ ).

Comparing upper and lower part from the 5 days old colonies, no differences between length (1742.35 nm  $\pm$  119.56; 1736.87 nm  $\pm$  170.77) and width (449.68 nm  $\pm$  39.32; 444.96 nm  $\pm$  56.58) of symbionts were detected. Also the EF on both parts was comparable (3.99  $\pm$  0.28; 4.02  $\pm$  0.40) indicating rod-shaped bacteria for both parts of the microzooids (Sunamara et al., 2014). The host surface coverage was slightly higher on the aboral (84.13 %  $\pm$  7.97) part compared to the oral part (79.91 %  $\pm$  18.96) with slightly more cells per  $70 \mu\text{m}^2$  on the aboral part (116.54  $\pm$  14.97; 103.38  $\pm$  18.87). The FDC was only measured on one colony, hence no differences between oral and aboral part were detected (0.03  $\pm$  0.22; 0.14  $\pm$  0.53).

After 7 days it was observed that the host was still viable but aposymbiotic. Therefore, no analyses on *Cand. Thiobios zoothamnicoli* was conducted.

Differences between the different time points on the whole microzooid revealed that after 5 days symbionts were larger (1739.57 nm  $\pm$  146.84) and slightly thinner (447.29 nm  $\pm$  48.59) than after 2 days (1429.25 nm  $\pm$  208.69; 475.91  $\pm$  64.02). The EF was slightly higher after 5 days (4.01  $\pm$  0.34) than after 2 days (3.14  $\pm$  0.24). However the FDC was higher after 2 days (3.23  $\pm$  3.01) than after 5 days (0.09  $\pm$  0.4). At 7 days no symbionts were detected on the host which was aposymbiotic but still viable.

### 3.2.2. Comparison swarmer and microzooids (*in situ*, 2d)

The collected swarmers under the *in situ* situation are comparable with the collected swarmers, which were transferred into respirometer chambers to grow the colonies of experiment 2. To gain information about changes in morphology, density, host surface coverage and fitness from the swarmers to the colonies, those two groups were compared. Due to high variability between the values no statistical tests were conducted.

It was to detect that on the upper part of the swarmers the symbiont was larger (2021.19 nm  $\pm$  466.32) and wider (621.41 nm  $\pm$  75.13) as the symbionts on the oral part of the

microzooids ( $1463.68 \text{ nm} \pm 205.31$ ;  $472.88 \text{ nm} \pm 63.16$ ). Also the volume of the symbionts on swarmer was higher ( $0.60 \mu\text{m}^3 \pm 0.32$ ) compared to the volume of bacterial cells on the microzooids ( $0.25 \mu\text{m}^3 \pm 0.08$ ). However, the host surface coverage of the microzooids was slightly higher ( $75.09 \% \pm 18.36$ ) compared to the swarmer ( $67.13 \% \pm 27.96$ ). Also the numbers of cells per  $70 \mu\text{m}^2$  was higher on microzooids as on swarmer ( $112.32 \pm 47.51$ ;  $55.35 \pm 24.44$ ). The FDC on swarmer was slightly higher as on microzooids ( $4.92 \pm 3.93$ ;  $3.73 \pm 2.58$ ).

Comparing the aboral part of the microzooids with the lower part of the swarmer it was seen that symbionts were longer ( $2311.91 \text{ nm} \pm 609.06$ ) and wider ( $594.87 \text{ nm} \pm 61.57$ ) on swarmer than on microzooids ( $1439.65 \text{ nm} \pm 173.78$ ;  $478.94 \text{ nm} \pm 66.36$ ). Cells were more voluminous on swarmer ( $0.63 \mu\text{m}^3 \pm 0.29$ ) than on microzooids ( $0.25 \mu\text{m}^3 \pm 0.08$ ), as well as the swarmer had higher EF values ( $3.90 \pm 0.81$ ) than the microzooids ( $3.10 \pm 0.28$ ), indicating more rod-shaped bacteria on swarmer (Sunamara et al., 2014). On the lower part of the swarmer the host surface coverage was higher ( $83.97 \% \pm 17.46$ ) with less cells per  $70 \mu\text{m}^2$  ( $68.37 \pm 20.94$ ) than on the aboral part of the microzooids ( $76.33 \% \pm 15.26$ ;  $120.16 \pm 44.28$  cells per  $70 \mu\text{m}^2$ ). On the aboral part of the microzooid less dividing cells were counted ( $2.73 \pm 3.42$ ) as on the swarmer ( $5.49 \pm 3.99$ ).

Pooling symbionts from the oral and aboral part together, a comparison between symbionts on the *in situ* swarmer and symbionts on the 2 days old colonies revealed that they were larger ( $2170.48 \text{ nm} \pm 556.65$ ) and wider ( $607.78 \text{ nm} \pm 68.86$ ) on the swarmer compared to those of the microzooids ( $1429.25 \text{ nm} \pm 208.69$ ;  $475.91 \pm 64.02$ ) (see Fig S8.3; 9.3). Bacterial symbionts from swarmer were more voluminous ( $0.62 \mu\text{m}^3 \pm 0.30$ ) than on microzooids ( $0.25 \mu\text{m}^3 \pm 0.08$ ) (see Fig. S11.3). Also the EF was higher on swarmer ( $3.59 \pm 0.71$ ) compared to the EF of the microzooids ( $3.14 \pm 0.24$ ) (see Fig. S10.3). It is interesting to note that the host surface coverage of the swarmer was only slightly lower ( $71.89 \% \pm 26.41$ ) as on microzooids ( $75.57 \pm 16.97$ ) (see Fig. S12.3). However the number of cells per  $70 \mu\text{m}^2$  was higher on microzooids ( $115.35 \pm 45.70$ ) as on swarmer ( $55.23 \pm 21.97$ ), but the FDC was higher on swarmer ( $5.21 \pm 3.91$ ) as on microzooids ( $3.23 \pm 3.01$ ) (see Fig. S13.3; 14.3).

## 4. Discussion

The symbioses between *Zoothamnium niveum* and *Candidatus Thiobios zoothamnicoli* was proven to be an excellent model system. First off, sample colonies from natural habitats are easily accessible. Furthermore, laboratory scale experiments benefit from the fast growing of the hosts and their short lifecycle. Additionally, manipulation of the symbiosis is possible due to the rapid response of both partners to changes in chemical conditions. In this work two different studies were performed without sulfide supply, leading to the breakdown of the symbiotic association. Firstly fresh collected colonies were cut of their substrate and maintained free floating under oxic stagnant conditions and prepared for further SEM analysis after different time frames. Secondly colonies grown from swarms under oxic flow-through conditions were prepared for further SEM analysis after different time frames. Under oxic stagnant conditions, a monitoring of the chemical parameters such as pH, salinity, O<sub>2</sub> concentration and temperature was not provided. As evaporation of seawater was avoided, the salinity and pH can be assumed as constant. In contrast to the first experiment, chemical parameters at the second experiment were established as in natural habitats. The investigation of morphology and fitness parameters of the hosts and symbionts under different oxic conditions lead to a more complex understanding of this symbiotic association. Major research objectives as defined in Sec. 1 were achieved.

### 4.1. Host

In the first experiment the symbiosis was maintained under oxic stagnant conditions. Although the host died after three days still some remaining symbionts were observed. Unfortunately the actual age of the collected colonies remains unknown. In contrast to the observations under oxic stagnant conditions, under oxic flow-through conditions the host was still alive after seven days. However, the host was aposymbiotic and it remains unknown how long the host would have survived. These results indicate that sulfide is required for a successful symbiotic association of *Zoothamnium niveum* and *Candidatus Thiobios zoothamnicoli*. Previous studies revealed that the minimum sulfide requirement for this symbiotic association under steady in vivo conditions is 3 to 33  $\mu\text{mol l}^{-1}$   $\Sigma\text{H}_2\text{S}$  in normoxic seawater (Rinke et al., 2007). Literature points out that the life span of colonies cultured under optimal conditions is about 11 days (Rinke et al., 2007).



The first experiment presented in this work was conducted under oxic stagnant conditions with natural occurring prokaryotes in the seawater. The host gains energy for growth and reproduction through digestion of free-living microbes and its own symbionts. However, the latter were not performing carbon fixation any more. Therefore I hypothesize that the host digested more symbionts under sulfide starvation to nourish himself. This is in agreement with the decreasing number of ectosymbionts over the different time steps. According to that the death of the host after three days might be explained by starvation.

In the second experiment an aposymbiotic host was grown under sulfide starvation for the first time. After seven days the aposymbiotic host was still viable and able to proliferate. Enhanced growth was observed indicating that energy uptake was successful under the artificial oxic flow-through conditions. It is assumed that host survival was based on digestion of symbionts and free-living microbes. Due to the constant inflow of fresh seawater, the amount of free-living microbes available was higher than in the first experiment. However, for aposymbiotic hosts significantly slower growth rates and less overall size was observed compared to symbiotic hosts (personal communication with J.M. Volland). This observation is in agreement with literature assuming that *Zoothamnium niveum*'s growth speed and giant size is based on its symbiotic association. Reported values for growth rate and maximum size of aposymbiotic hosts reared from aposymbiotic swarmers are about 10 % of those for colonies in symbiotic association (Ott et al., 2004). Hence, an aposymbiotic host may be overcompeted by symbiotic hosts and therefore not occur in nature.

The dead host was found to be overgrown by other microbes indicating the loss of homeostasis between partners. However, it is not known yet whether the control of the homeostasis is derived from *Zoothamnium niveum* or the symbionts (personal communication with M. Bright). For many microbial symbiotic associations the control of homeostasis is assumed to be host-controlled (Douglas, 2010; Brooks & Richards, 1955; Ruby & Assato, 1993; Whitehead & Douglas, 1993). Some hosts are known to restrict the surface areas on which symbionts can grow and proliferate. Furthermore, there are indications that some hosts are also in control of their symbiont's abundance and distribution (Douglas, 2010). Assuming similar control mechanisms for *Z. niveum* the above-mentioned observations can be explained.

A significantly reduced lifetime of the hosts in the first experiment was observed. Under stagnant normoxic conditions changes in chemical parameters during the day cannot be excluded. Oxygen concentration, however, may have decreased due to microbes metabolizing oxygen

(Kampell & Chapelle, 2000). Furthermore, the water temperature might have varied slightly. Another main difference between the two experiments is the different surround of the colonies. Hence, the access of oxygen and other chemical parameters of the colonies might have been different. In summary, the potentially unstable chemical conditions and artificial surrounding of the first experiment might have caused a decrease in lifetime and fitness of the host.

#### **4.2. Symbiont**

In both experiments a continuous decrease of host surface coverage and symbiont density was observed for the microzooids. Remaining symbionts were observed after three days and five days in the first and second experiment, respectively. Any of the replicates showed natural variations in the behavior of the symbionts. In the second experiment the decrease in host surface coverage and symbiont density lead to a complete breakdown of the symbiotic association between the fifth and seventh day. The explanation is given by the fact that obligate sulfide-oxidizing bacteria will turn inactive without any sulfide supply (Rinke et al., 2007). This is supported by the fact that in both experiments, the FDC was found to decrease to approximately zero after three and five days for the first and second experiment, respectively. Therefore, symbionts cannot recolonize a host under oxic stagnant conditions.

Besides reduction of the symbiont population due to possible feeding of the host, the decrease in symbiont density is also caused by the growth of the host as detected in the second experiment. Due to the inactivity of the symbionts, new host tissue was not recolonized. Unfortunately, the host growth was not measured in this work. In future studies, however, an addition monitoring of the size of the microzooids could reveal new information about the major contribution on density decrease.

Under oxic stagnant conditions, active symbionts were detected after several days. This is highly unexpected. In previous studies vesicles filled with elemental sulfur were identified in the symbionts using Raman microspectroscopy (Maurin et al., 2010). This elemental sulphur storage is known to keep up metabolism for roughly 4 hours (Ott et al., 1998). However, this time span is an order of magnitude too small to explain the observations. Furthermore, the colonies in the experiment did not lose their white color, indicating that the internal sulphur storage was not metabolized.

A possible explanation for remaining cell activity is the switchover to a heterotrophic metabolism under sulfide starvation. This has been hypothesized for other organisms, such as the trophosome sulphur-oxidizing symbionts of the large tubeworm *Riftia pachyptila* (Markert et al., 2007; Robidart et al., 2008). However, details on possible non-sulfur-based metabolisms for *Candidatus Thiobios zoothamnocoli* are not known yet. Hence, a metagenomic and functional analyses of this symbiont could give a deeper understanding of the symbiosis with *Zoothamnium niveum* and explain if a switch to heterotrophy under sulfide starvation is possible. However, assuming that *Z. niveum* could switch to heterotrophy, the deactivation of the symbionts under oxic flow-through conditions cannot be explained and needs to be investigated.

During the experiments changes in morphological parameters of the symbionts were detected. Measured morphological changes in length, width, volume and EF can be interpreted as a response to stress (Neidhardt et al., 1990). Furthermore, correlations between the growth rate and other parameters such as cell size (Neidhardt et al., 1990) and morphology (Young, 2006) are known for other bacteria. Here, the main stressor is the absence of sulphide prohibiting sulfide oxidation and carbon fixation, which might ultimately lead to morphological changes. Additionally, the harvesting and incubation of the colonies have to be considered as stress factors.

#### **4.3. Symbioses under oxic stagnant conditions**

In the first experiment, the host's surface coverage and the density of the symbionts on microzooids was found to decrease with increasing time. It is hypothesized that both observations are caused by sulphide starvation over longer time periods. The bacterial cells are not provided with the required sulphide for sulphide oxidation and carbon fixation and turn inactive. The correspondingly reduced supplement of fixed carbon results in lower growth rates and lower fitness of the host. This ultimately causes a threat for the survival of both partners. However, some symbionts may adapt their metabolism like described in Chapter 4.2 but the share in active bacteria is rather small.

Considering the oral part of the microzooids, significantly smaller host surface coverage and symbiont density was observed than on the aboral part under *in situ* conditions. This gradual change can be explained by different chemical microenvironments determined by the natural contraction and expansion behavior of the host. As suggested by other authors, high shear stress

during stalk contraction, cell shrinkage, bunching of the zooids and beating of the cilia can result in detachment of symbionts. The latter are likely to be ingested and digested by the host. The impact of the oral ciliature is stronger on oral parts compared to aboral parts. Therefore more symbionts are detached and digested on the oral part (Vopel et al., 2002; Bauer-Nebelsick et al., 1996a,b). In any case, the loss of symbionts is larger than the rate of cell proliferation that leads to a decrease in host surface coverage and symbionts density.

A similar decrease in symbiont density and host surface coverage was also detected on swarmers with natural variation between the replicates. I suppose that this is related to sulphide starvation and the suboptimal cultivation environment provided by the petri dishes. During their motile phase, however, the swarmers stay in an arrested cell cycle (Kloiber et al., 2009). The possibility of an arrested cell cycle in swarmers is supported by a molecular study of another ciliate (Markmann-Mulisch et al., 1999; Kloiber et al., 2009). Hence, it is assumed that they do not feed on their symbionts and therefore the feeding pressure on symbionts cannot be the explanation of decreasing symbiont populations.

Furthermore, dilution of the symbiont population due to growth of the host can be excluded, as swarmers do not grow after their release. The observations for swarmers cannot be sufficiently explained by any of the theories working for microzooids, indicating that the underlying mechanism of symbiont loss due to sulphide starvation might be more complex. Hence, more research on swarmers is urgently required to be able to draw a full picture of this symbiotic association.

The FDC was taken as an indicator for symbiont fitness. Under oxic stagnant conditions, the FDC of the symbionts on the microzooids decreased drastically after 1-2 days and converged to zero after 3 days. The same observation was found on swarmers. It is assumed that sulphide starvation prohibits proliferation of the bacterial symbionts on microzooids and swarmers. As the incubation of swarmers and microzooids in petri dishes implies a stressful situation, it could be possible that symbionts turned inactive. Therefore, their elemental sulphide storage remained unused explaining the invariant white color of the analyzed colonies. Nevertheless, the FDC was not suppressed immediately for all symbionts.

Obviously some of the symbionts were able to adapt to the sulphide starvation scenario. The question arises from which substances some symbionts gained their energy for cell division? One possibility could be that individual symbionts can use alternative internal storage compounds, which ensure cell metabolism over a certain time period. Many different chemical compounds

might act as storage compounds in bacteria, including intracellular polysaccharides such as glycogen as well as starch and lipids such as poly-P-hydroxybutyrate (Wilkinson, 1959).

Another hypothesis for continuous metabolic cell activity is the usage of other chemical sources provided by the actual environment. An alternative reduced sulphur source is thiosulfate. Studies of Rinke et al. (2006) revealed that there is evidence that the free-living bacterial strain ODIII6 and *Candidatus* Thiobios zoothamnicoli have a similar physiological background. Interestingly, the strain ODIII6 was observed to oxidize thiosulfate in culture (Kuever et al., 2002). Rinke et al. (2002) conducted experiments treating colonies of *Zoothamnium niveum* with 1 mM thiosulfate and observed that the colonies remained white, but only survived for 15 to 27 hours. The white color of the colonies indicates that the symbionts internal storage of elemental sulphur was not depleted. This experiment might indicate that *Cand.* Thiobios zoothamnicoli is able to use thiosulfate as a reduced sulphur species for sulphur-oxidation and thus autotrophic fixation in organic carbon. Thiosulfate was not measured during our experiments (Drexel diploma thesis, 2013). However, the question remains why only some of the symbionts remain active. Hence, further studies are required to gain a comprehensive explanation.

Under *in situ* conditions it was observed that the oral part of the microzooids had significant higher FDC values than the aboral part, but lower symbiont density. This might be explained by the different availability of free host surface that has to be recolonized by the symbionts. It is assumed, that more symbionts are detached from the oral part due to higher impact of the microzooids ciliature. Accordingly, more host surface coverage must be recolonized. These results are in agreement with previous studies, observing higher FDC on the oral part of the microzooids under optimal cultivation conditions (Rinke et al., 2007; Drexel diploma thesis, 2013). In general, non-zero FDC values can only be observed as long as enough energy sources are provided to the symbionts to maintain their metabolic activity. Under the *in situ* situation this requirement is fulfilled.

The mechanism for maintenance of the ectosymbiotic association could involve processes such as quorum sensing. Quorum sensing describes the regulation of gene expression in response to fluctuations in cell-population density (Miller & Bassler, 2001). Furthermore, gram-negative bacteria are known to use quorum-sensing communication to regulate physiological activities, processes as symbioses, virulence, biofilm formation etc. (Miller & Bassler, 2001). This mechanism can presumably explain newly built host surface is immediately covered by symbionts and free host surface was never detected as observed in previous studies performed

under sulphidic conditions (Rinke et al., 2007). However, quorum sensing might only be possible as long as enough energy sources are provided to maintain metabolic activity of the bacterial cells. Under the *in situ* situation this requirement is fulfilled.

#### **4.4. Symbioses under oxic flow-through conditions**

In the second experiment it was observed that host surface coverage and density did not decrease till the fifth day. Subsequently, a sharp decrease in host surface coverage was detectable in the data till the host was aposymbiotic after seven days. In contrast to the first experiment, many analysed colonies lost their white colour indicating the usage of their internal sulphur storage for chemosynthesis and metabolic activity. This might be related to the lower stress level of the bacteria due to more stable chemical parameters under oxic flow-through conditions. However, the elemental sulphur storage of the symbionts is depleted after roughly four hours. Afterwards symbionts are not able to maintain their metabolic activities and to recolonize free host surface. This is also indicated by the decreasing FDC values after the fifth day. As the host continuously ingests some of its symbionts, the surface coverage and density decrease until the host is aposymbiotic. For the first time the cultivation of such aposymbiotic hosts was established under laboratory conditions.

Regarding length, width and volume of the symbionts on oral and aboral part of the microzooids no noticeable changes were found. In contrast to the first experiment, the absence of sulphide during cultivation resulted in morphologic uniform shaped cells. This can be explained by the absence of chemical gradient leading to different morphological shapes. As described in the previous chapters, the natural movements of the host cause different chemical microenvironments for the oral and aboral part of the microzooid (Rinke et al., 2007). However, established stable flow-through conditions avoided chemical gradients. Therefore uniformly shaped cells were found all over the microzooid.

## 5. Conclusion

For a thiotrophic symbiosis, the absence of sulfide stresses the symbiotic partners and ultimately threatens the survival. However, whether the host, the symbiont or the association survives or not has not been studied in many systems. This study demonstrated that sulfide starvation under oxic conditions leads to the breakdown of the symbiotic association between *Zoothamnium niveum* and *Candidatus Thiobios zoothamnicoli*.

Under stagnant normoxic conditions the symbiosis was terminated by the death of the host. Some of the symbionts at least remained on the dead host. Morphological changes as well as a decrease in fitness of symbionts were observed. For long incubation times the symbiont density, the host surface coverage and the FDC were significantly decreased on microzooids. The same trends were found for symbionts that were attached to swarmer. Since swarmer are not nourished by the symbionts the results indicate an influence of sulphide starvation on the vertical transfer of symbionts to the next generation. Further studies should be addressed to the possibility of aposymbiotic swarmer and colonies and their reinfection. For some symbionts cell activity was also verified under sulfide starvation. Different possible adaptation mechanisms of symbiont's metabolic activity were discussed. Further investigations are necessary to explain which metabolic processes are involved to obtain cell division and growth of the symbionts under sulphide starvation.

Under oxic-through conditions the symbiosis was successfully cultured starting from swarmer. The hosts survived for seven days and proliferated. This was a major break through as controlled cultivation is an essential prerequisite for any further systematic study on this symbiosis. Under these conditions, the symbiosis was terminated by the loss of symbionts. For the first time the successful cultivation of an aposymbiotic host was presented. However, it is still unknown if the rejection of symbionts is controlled by the host or induced by the symbionts. This should be investigated in future studies. Furthermore, the cultivation of aposymbiotic hosts provide detailed studies on pre- and post-infection mechanisms. The fact that aposymbiotic hosts have not been found in nature so far is interesting. Hence, slow growing aposymbiotic hosts probably cannot compete against symbiotic colonies.

In the future more experiments under sulfide starvation have to be conducted to increase the sample number and to be able to make appropriate conclusions about the termination of this symbiotic association under sulphide starvation. Furthermore, the cultivation of colonies from

swarmers, which are collected from the field and then kept under oxic stagnant conditions, could give more information about the possible life span of the colonies. Further investigations with  $^{14}\text{C}$  incubations could provide new knowledge regarding the feeding pressure on symbionts.

The cultivation of an aposymbiotic host provides the basis for new studies on this symbiotic association. Further studies with aposymbiotic hosts, which are exposed to the toxic sulfide, should be conducted to determine how fitness of the host is affected by the presence of the symbionts (Bright et al., 2014). Furthermore, reinjection experiments of aposymbiotic hosts could reveal if strict vertical transmission is indeed the only transmission mode, or if a mixed transmission mode is present in this symbiotic association.



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## 8. Supplementary Information

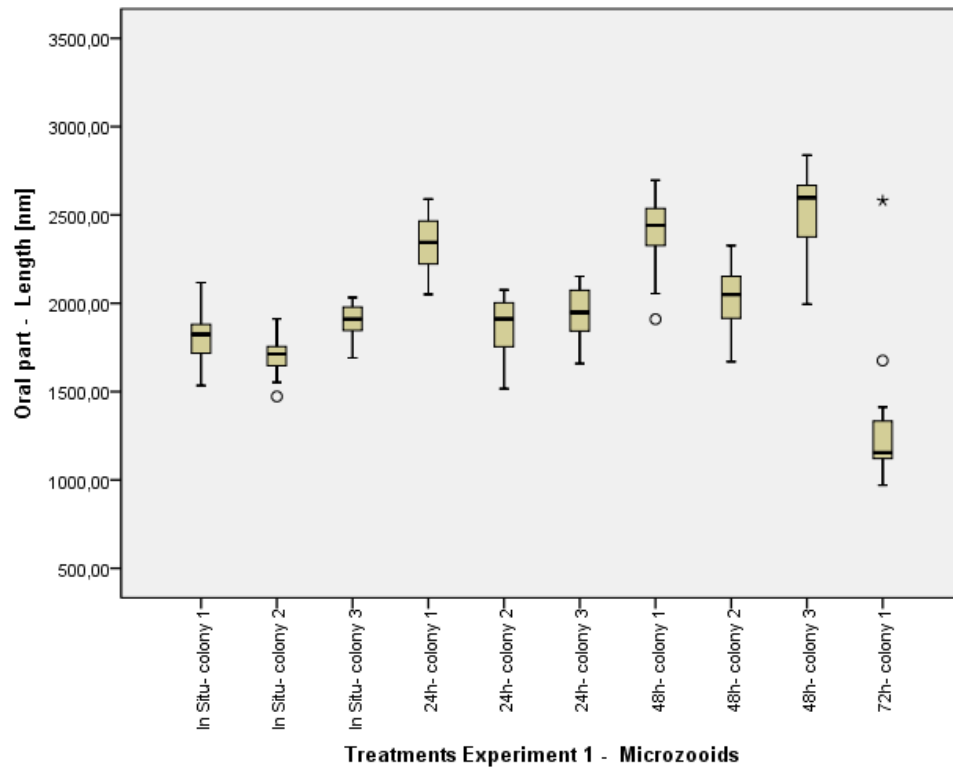


Figure S1.1. Length of *Cand. Thiobios zoothamnicoli* per treatment on the oral parts of the microzooids under oxic stagnant conditions.

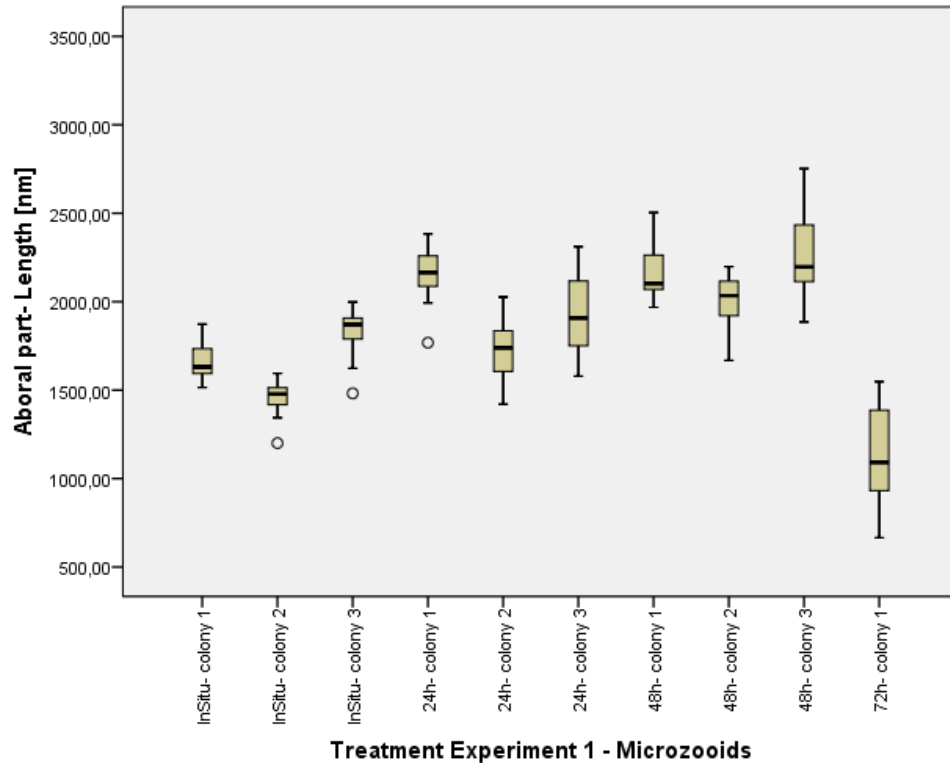
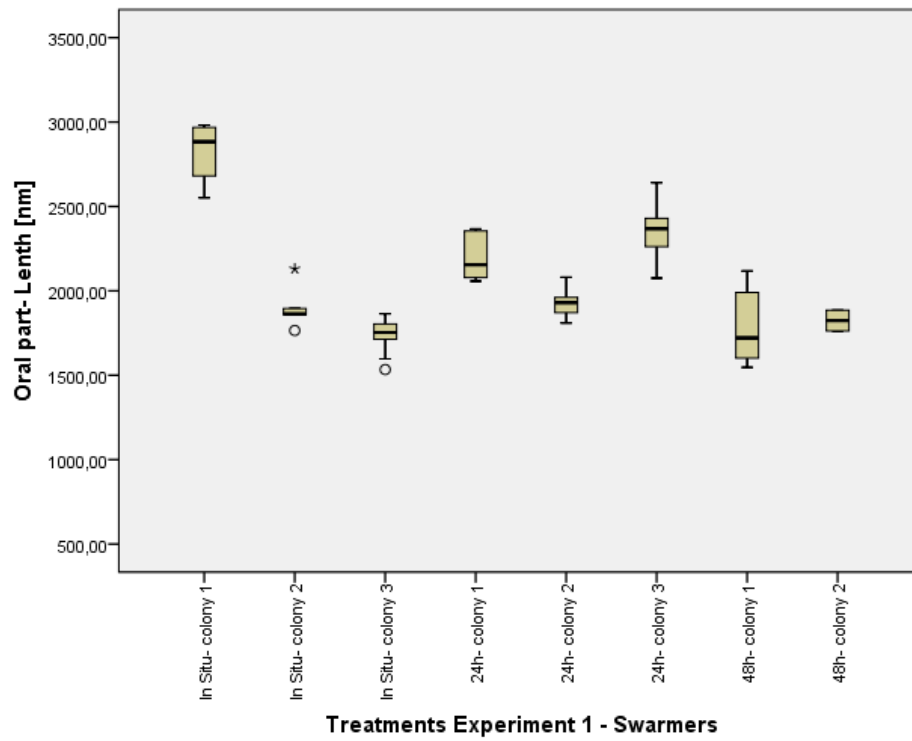
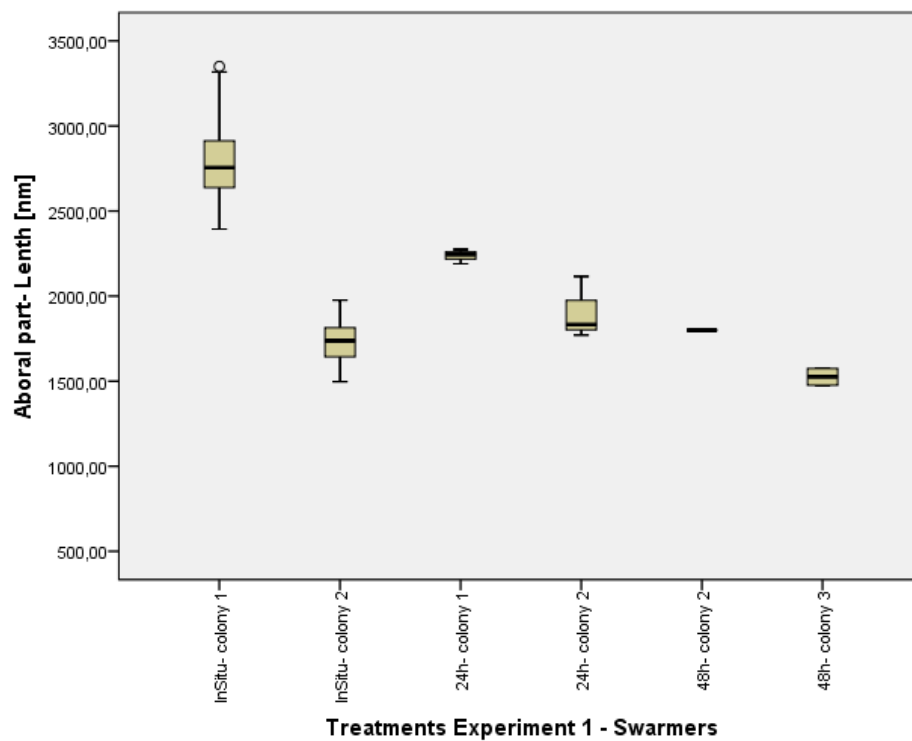


Figure S1.2. Length of *Cand. Thiobios zoothamnicoli* per treatment on the aboral parts of the microzooids under oxic stagnant conditions.



**Figure S1.3.** Length of *Cand. Thiobios zoothamnici* per treatment on the oral parts of the swarmers under oxic stagnant conditions.



**Figure S1.4.** Length of *Cand. Thiobios zoothamnici* per treatment on the aboral parts of the swarmers under oxic stagnant conditions.



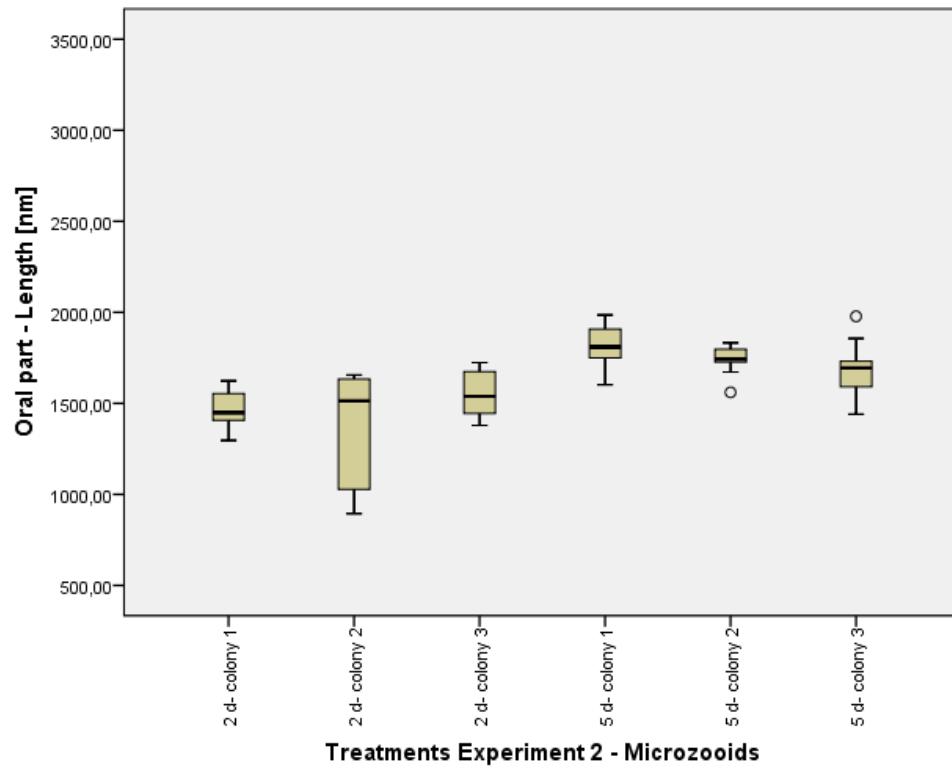


Figure S1.5. Length of *Cand. Thiobios zoothamnici* per treatment on the oral parts of the microzooids under oxic flow-through conditions.

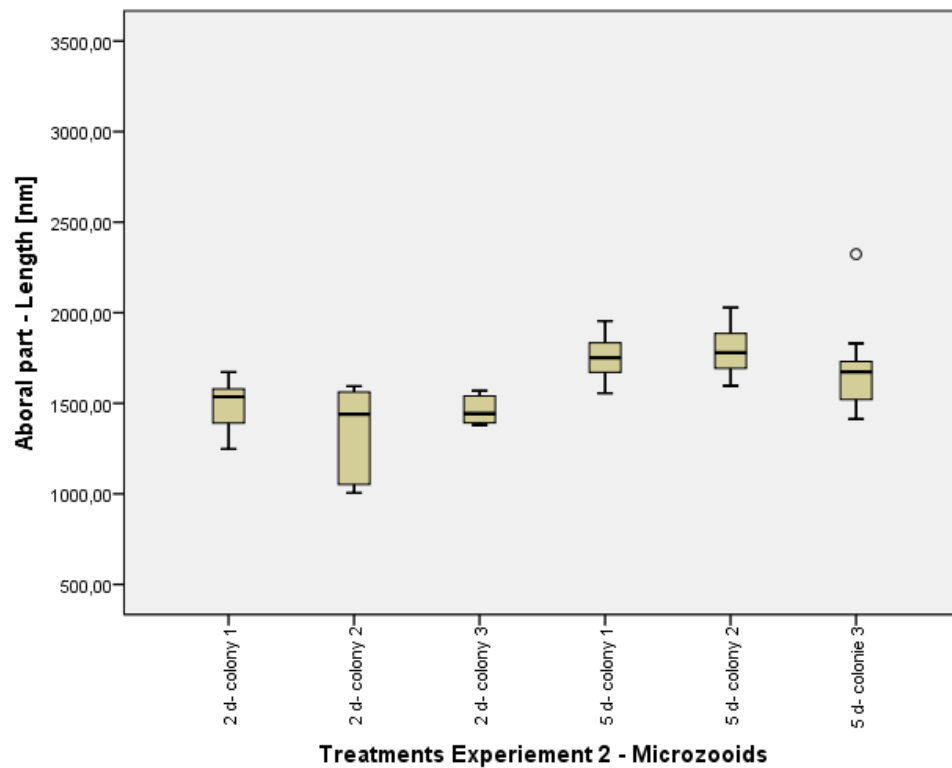


Figure S1.6. Length of *Cand. Thiobios zoothamnici* per treatment on the aboral parts of the microzooids under oxic flow-through conditions.

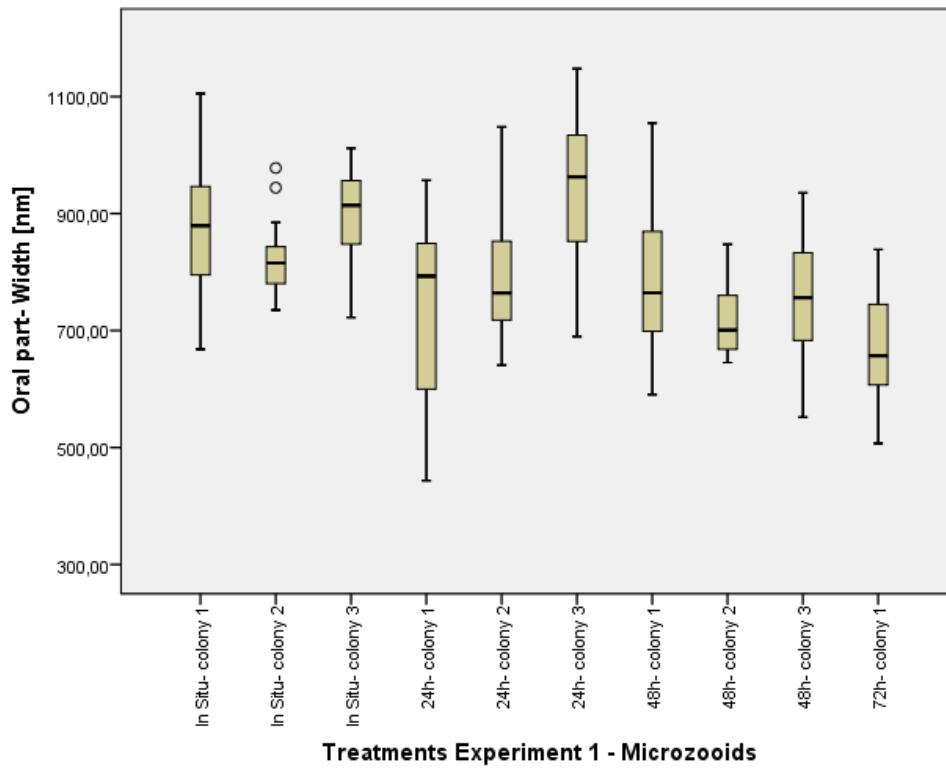


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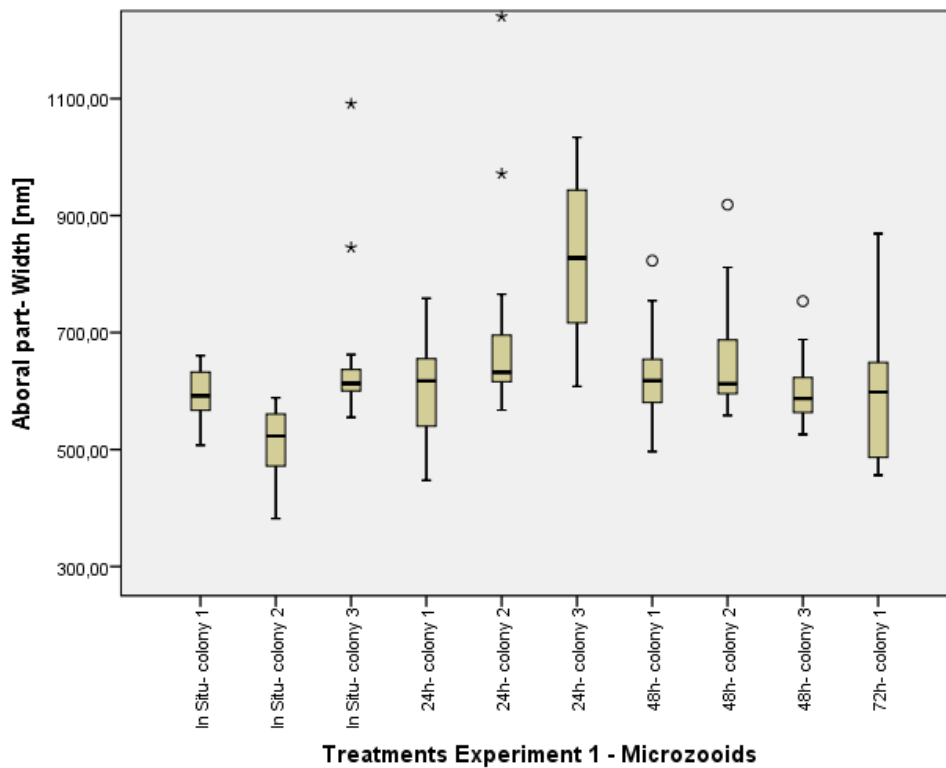


Figure S2.2. Width of *Cand. Thiobios zoothamnicoli* per treatment on the aboral parts of the microzooids under oxic stagnant conditions.

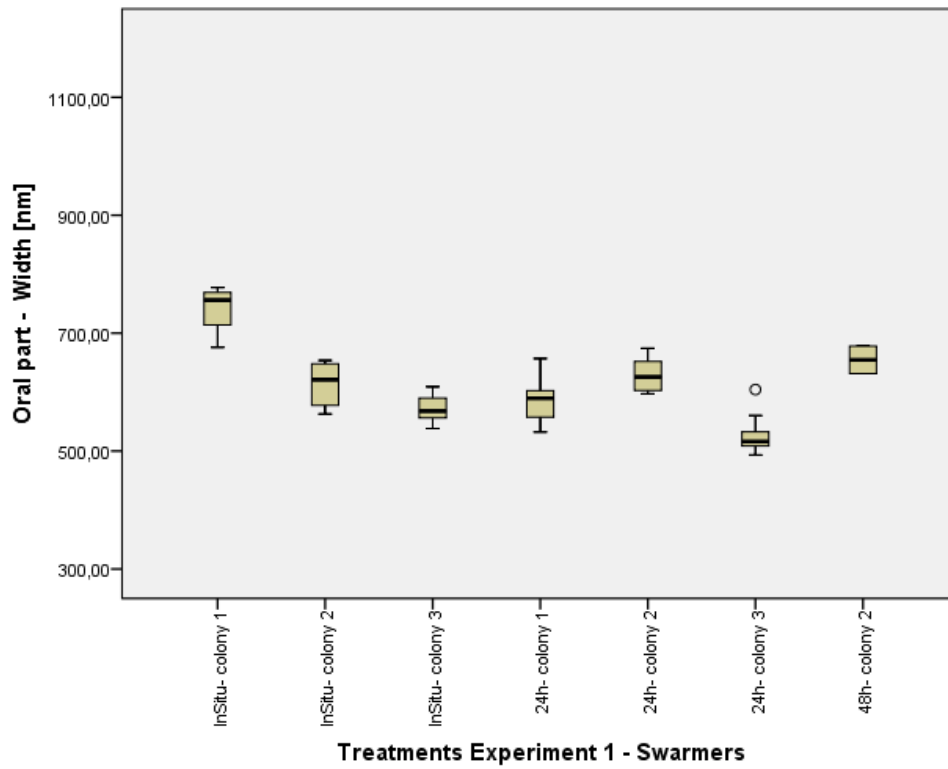


Figure S2.3. Width of *Cand. Thiobios zoothamnici* per treatment on the oral parts of the swimmers under oxic stagnant conditions.

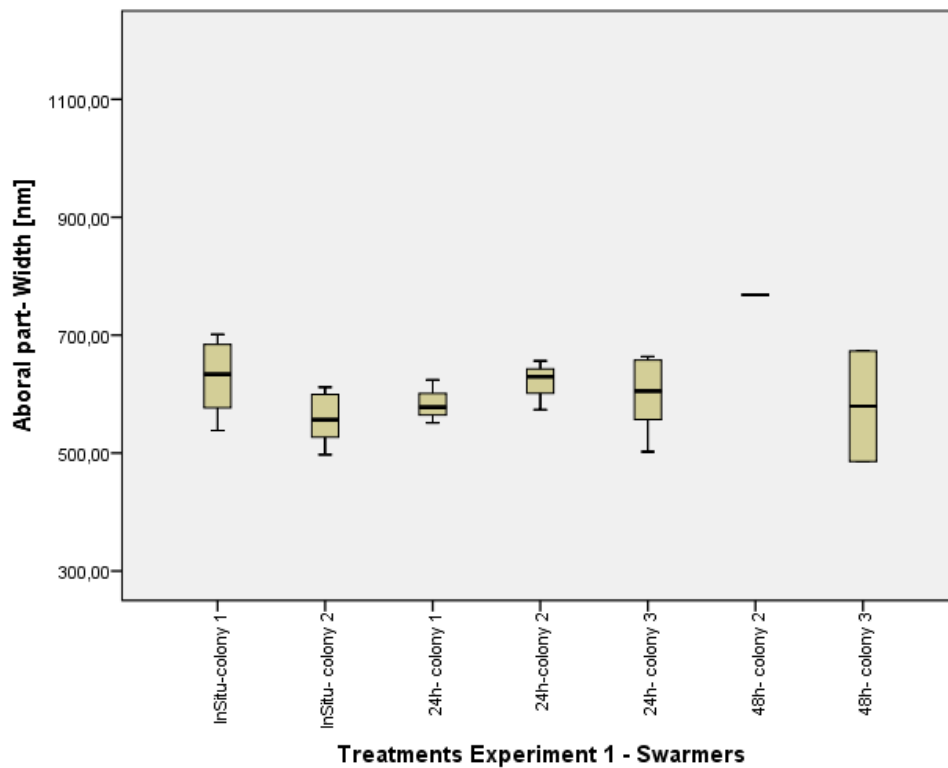


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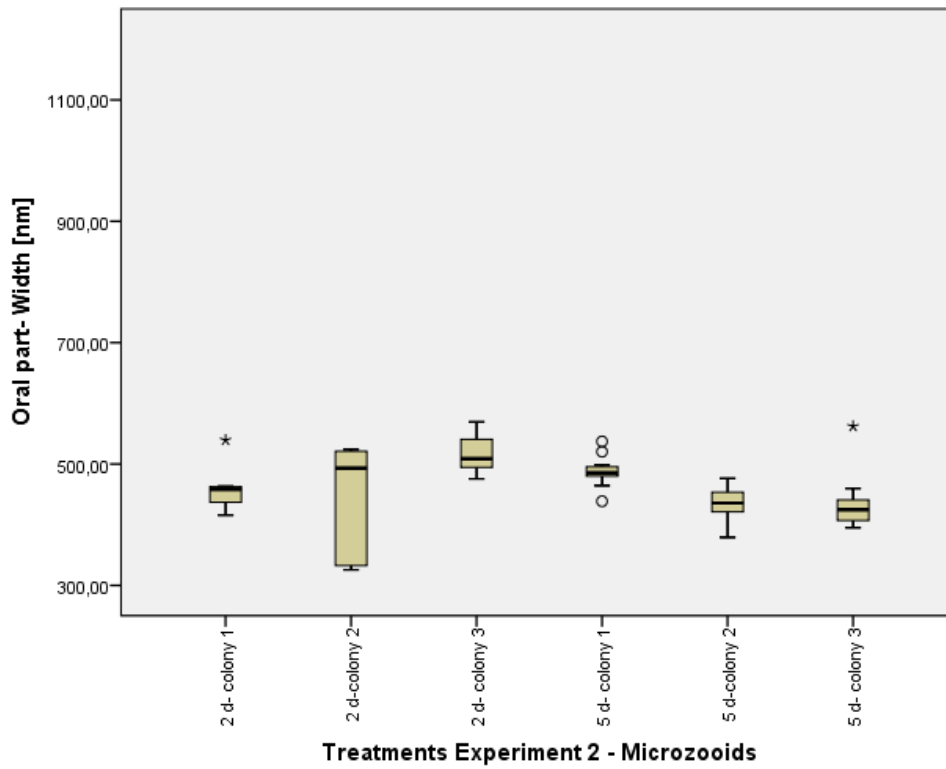


Figure S2.5. Width of *Cand. Thiobios zoothamnici* per treatment on the oral parts of the microzooids under oxic flow-through conditions.

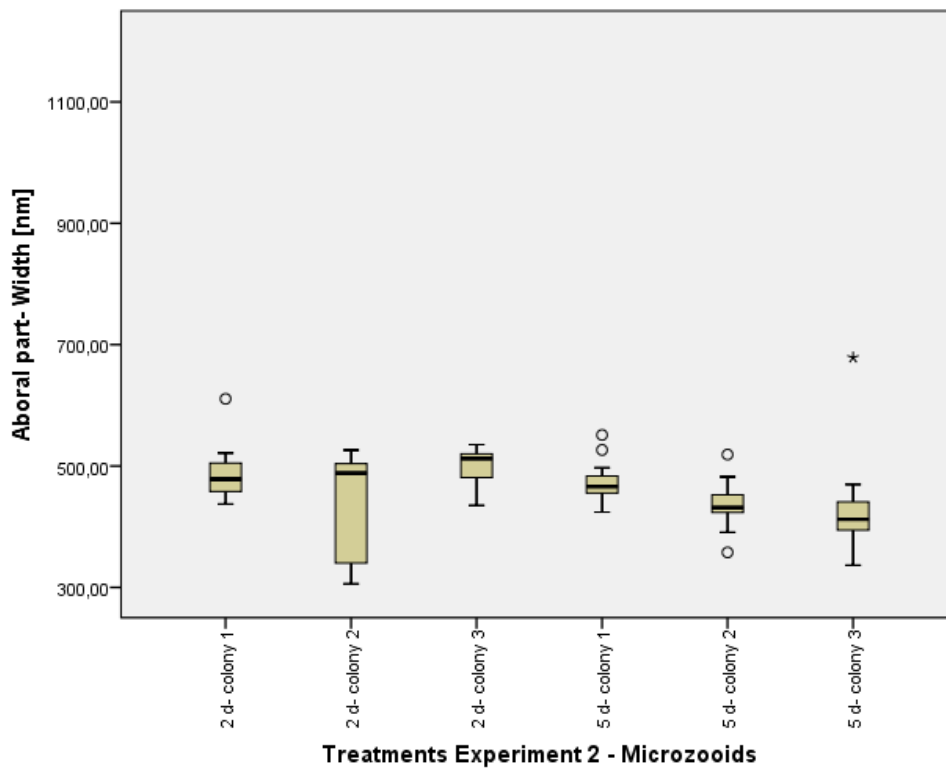
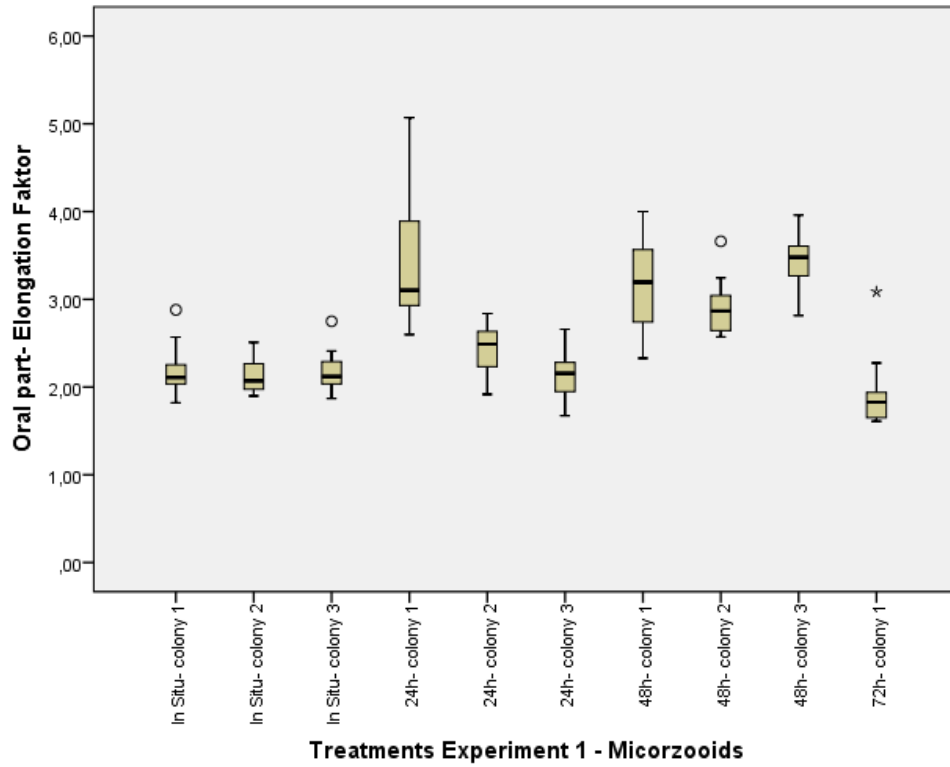
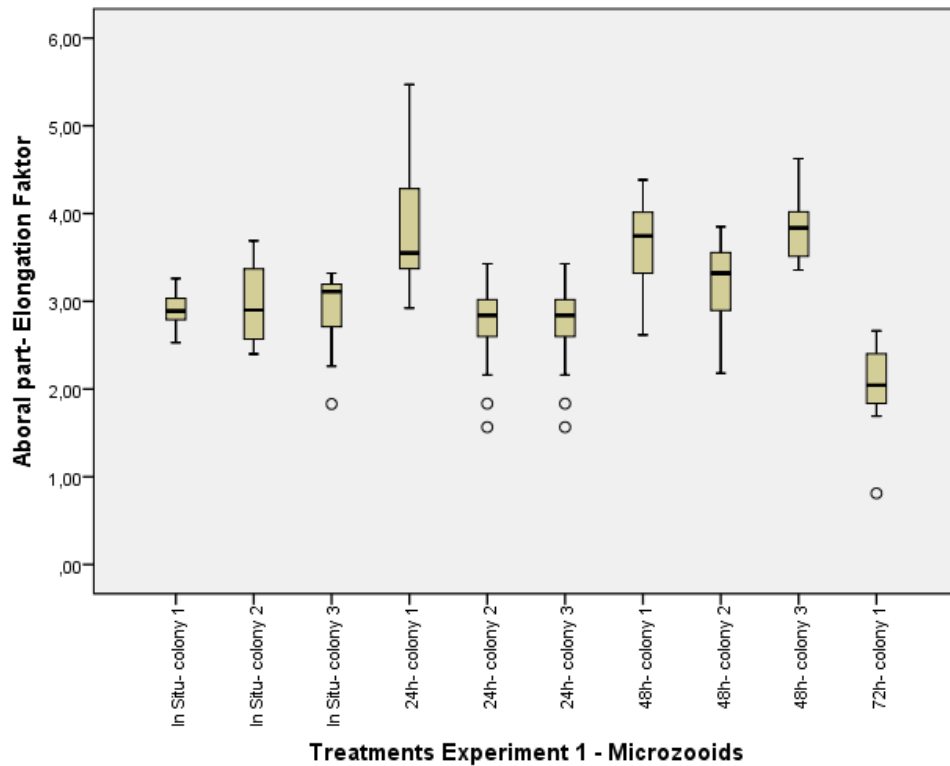


Figure S2.6. Width of *Cand. Thiobios zoothamnici* per treatment on the aboral parts of the microzooids under oxic flow-through conditions.



**Figure S3.1.** Elongation Factor of *Cand. Thiobios zoothamnocoli* per treatment on the oral parts of the microzooids under oxic stagnant conditions.



**Figure S3.2.** Elongation Factor of *Cand. Thiobios zoothamnocoli* per treatment on the aboral parts of the microzooids under oxic stagnant conditions.

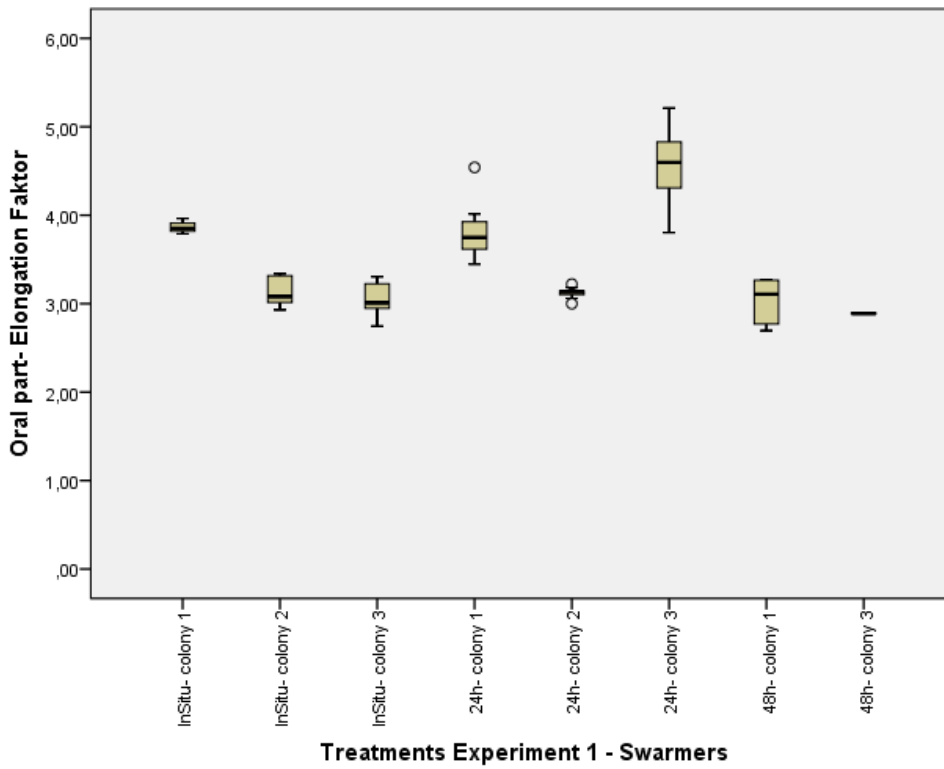


Figure S3.3. Elongation Factor of *Cand. Thiobios zoothamnicoli* per treatment on the oral parts of the swarmers under oxic stagnant conditions.

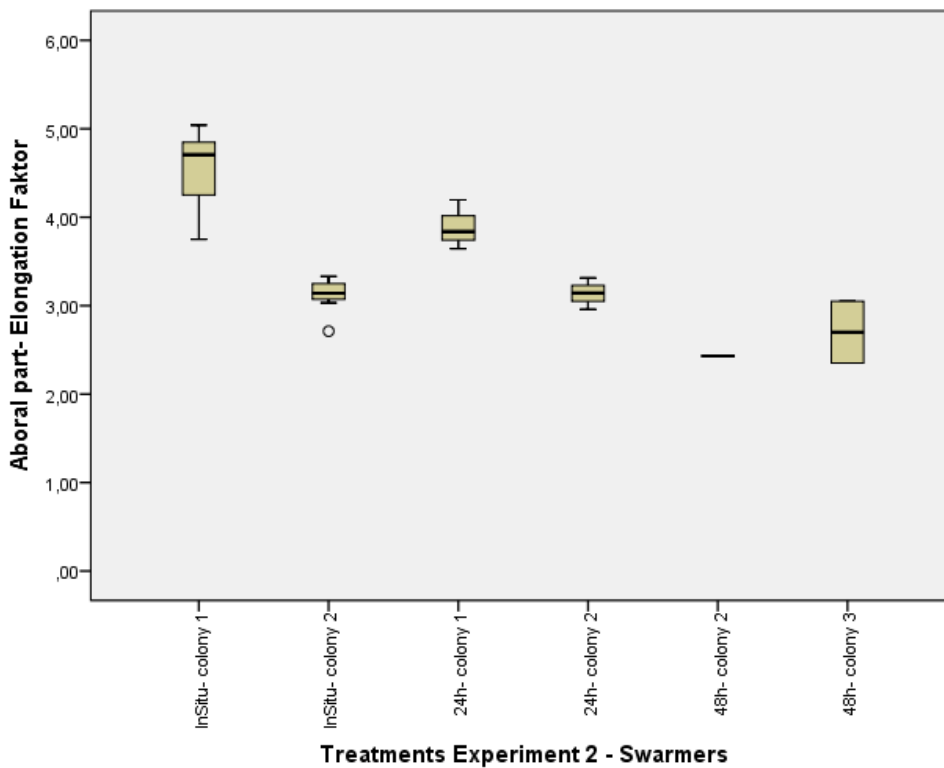
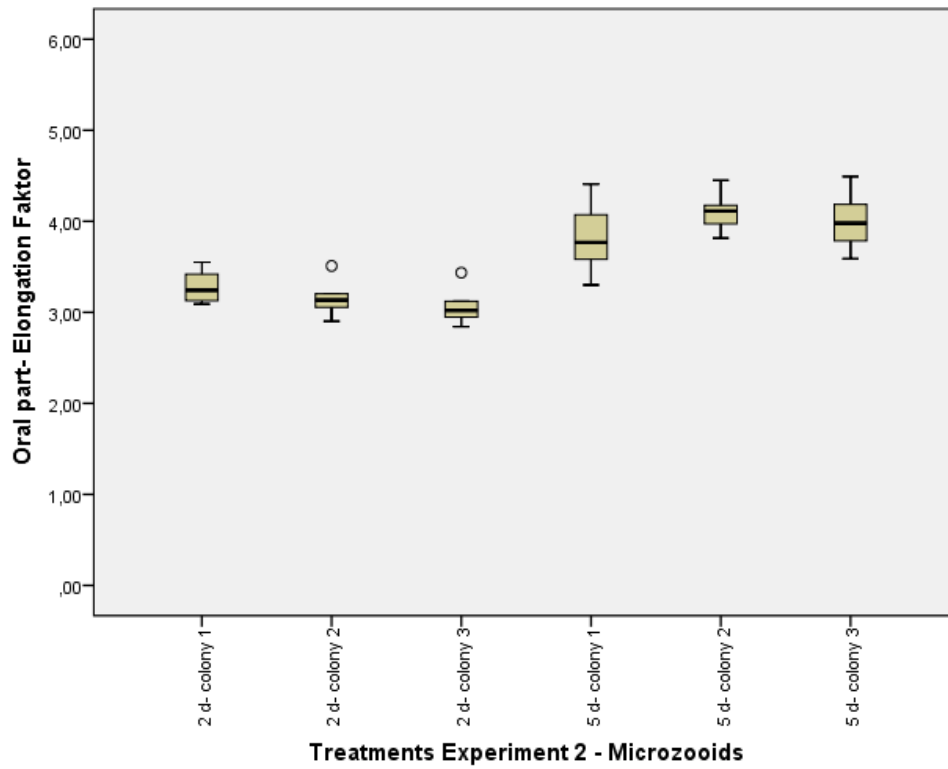
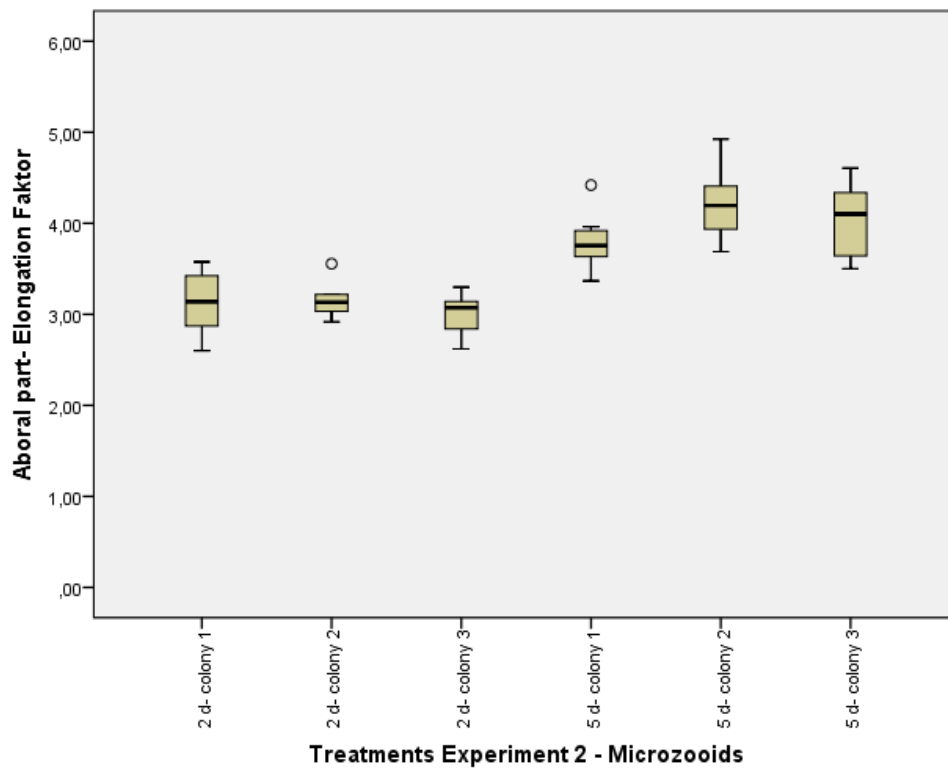


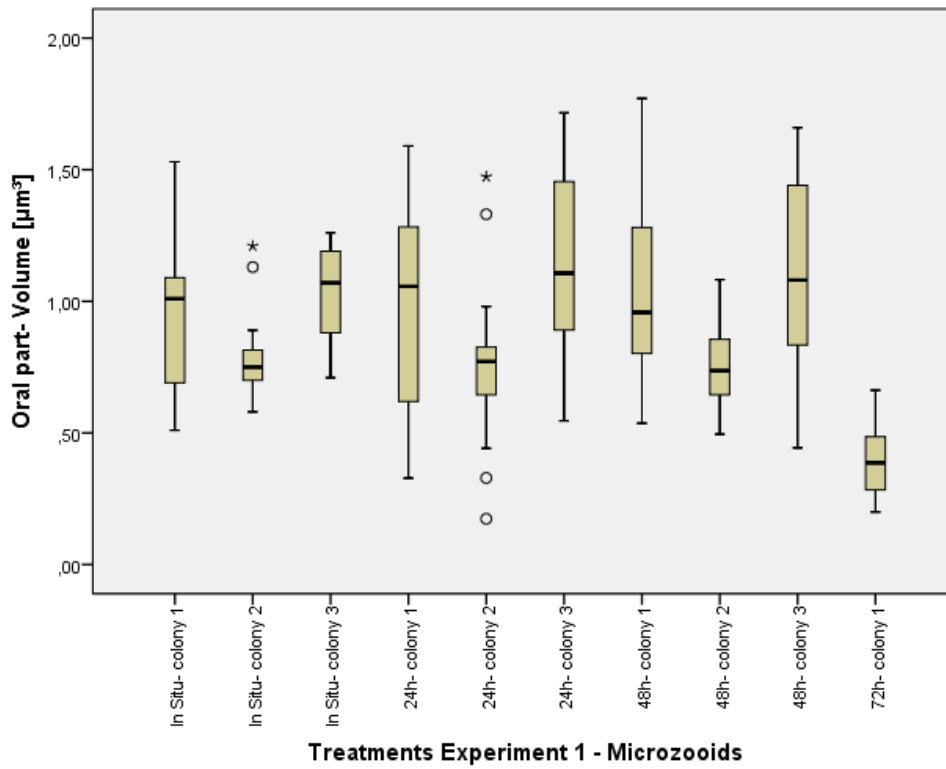
Figure S3.4. Elongation Factor of *Cand. Thiobios zoothamnicoli* per treatment on the aboral parts of the swarmers under oxic stagnant conditions.



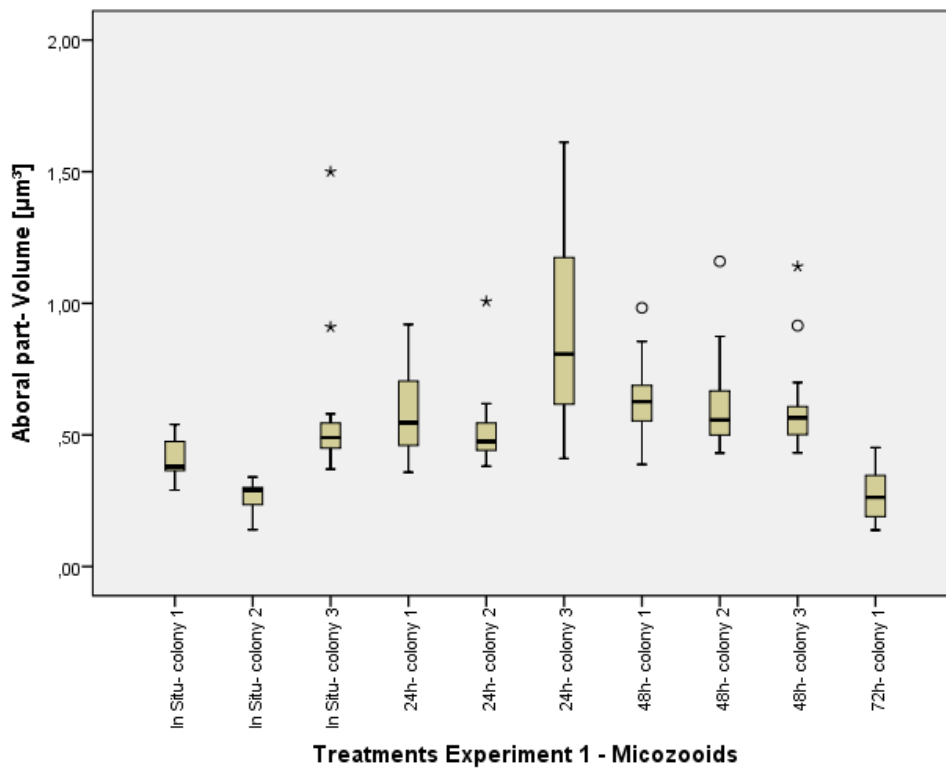
**Figure S3.5.** Elongation Factor of *Cand. Thiobios zoothamnicoli* per treatment on the oral parts of the microzooids under oxic flow-through conditions.



**Figure S3.6.** Elongation Factor of *Cand. Thiobios zoothamnicoli* per treatment on the aboral parts of the microzooids under oxic flow-through conditions.

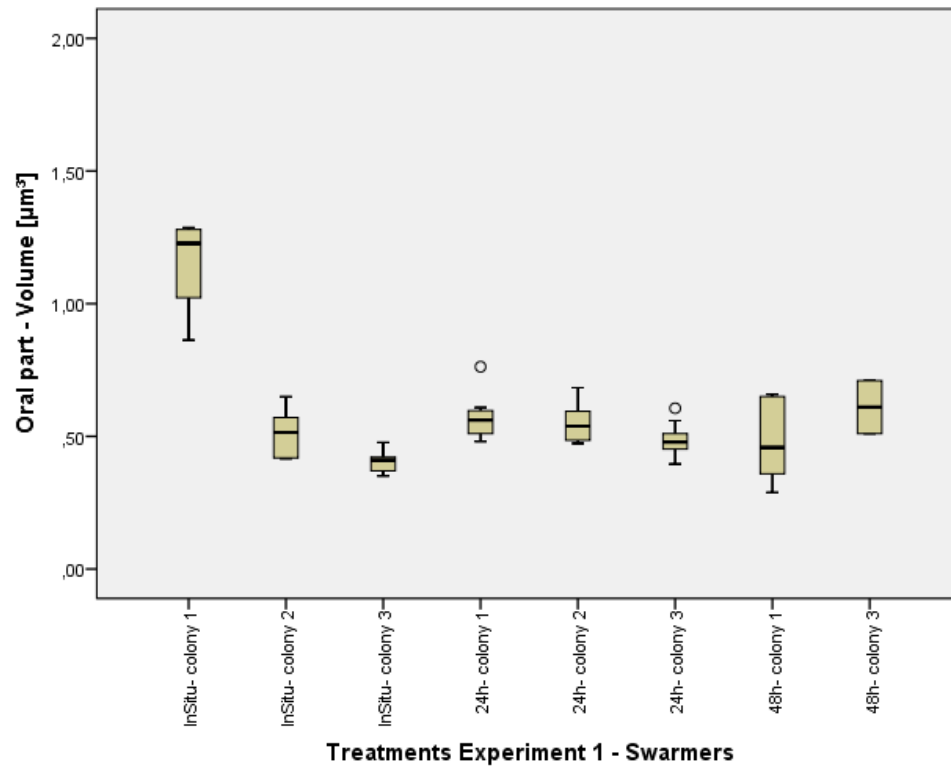


**Figure S4.1.** Volume of *Cand. Thiobios zoothamnicoli* per treatment on the oral parts of the microzooids under oxic stagnant conditions.

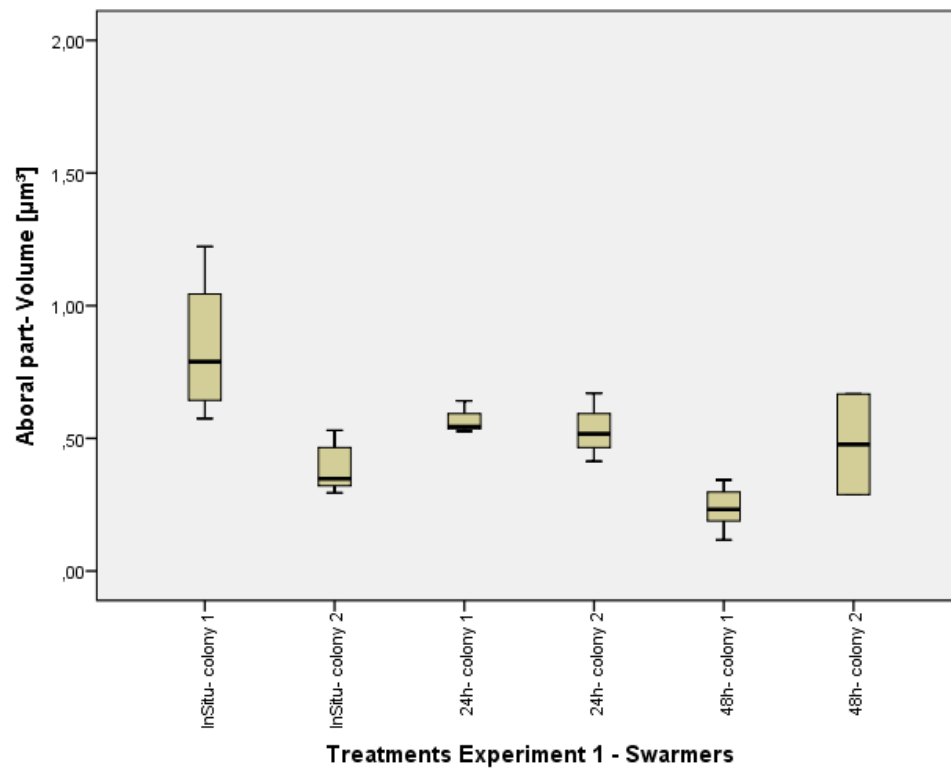


**Figure S4.2** Volume of *Cand. Thiobios zoothamnicoli* per treatment on the aboral parts of the microzooids under oxic stagnant conditions.





**Figure S4.3.** Volume of *Cand. Thiobios zoothamnici* per treatment on the oral parts of the swarmers under oxic stagnant conditions.



**Figure S.4.4.** Volume of *Cand. Thiobios zoothamnici* per treatment on the aboral parts of the swarmers under oxic stagnant conditions.

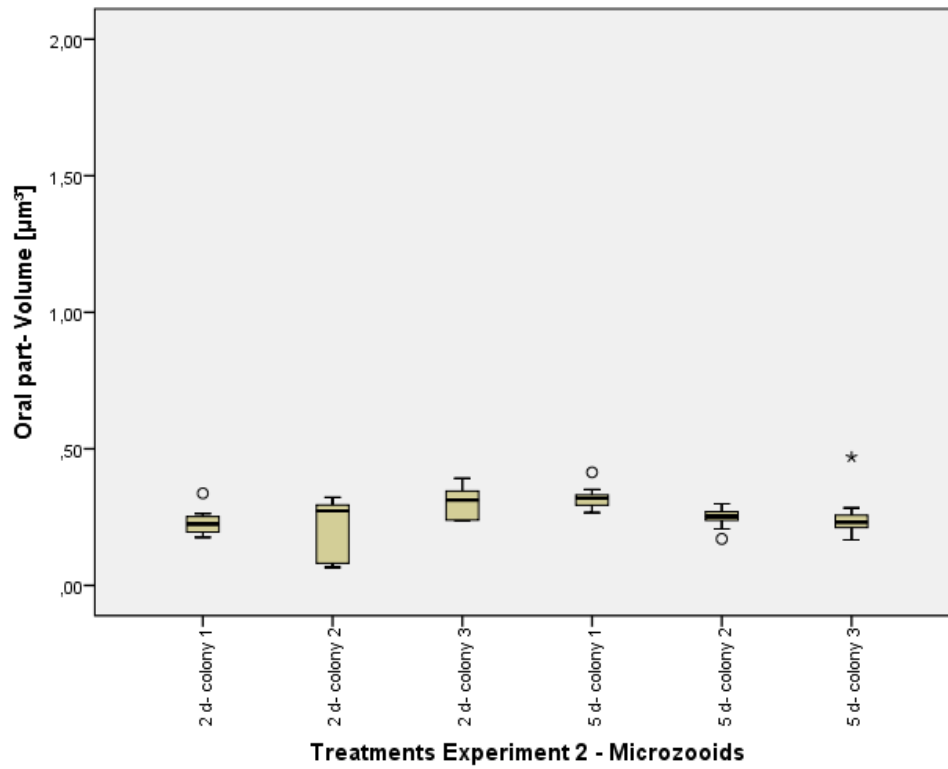


Figure S4.5. Volume of *Cand. Thiobios zoothamnici* per treatment on the oral parts of the microzooids under oxic flow-through conditions.

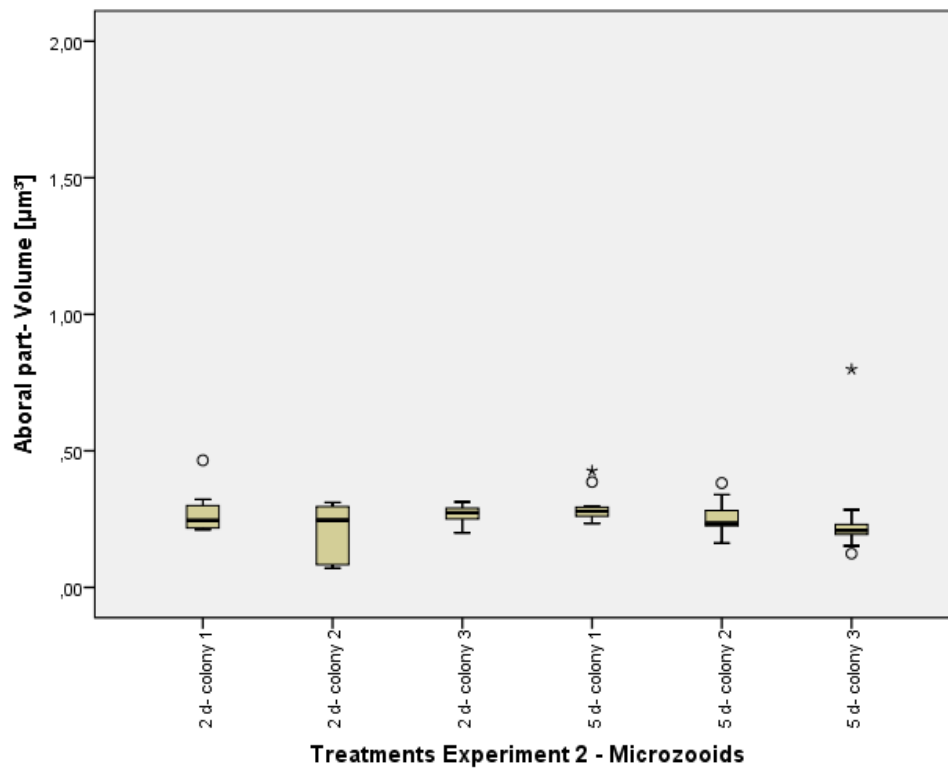


Figure S4.6. Volume of *Cand. Thiobios zoothamnici* per treatment on the aboral parts of the microzooids under oxic flow-through conditions.

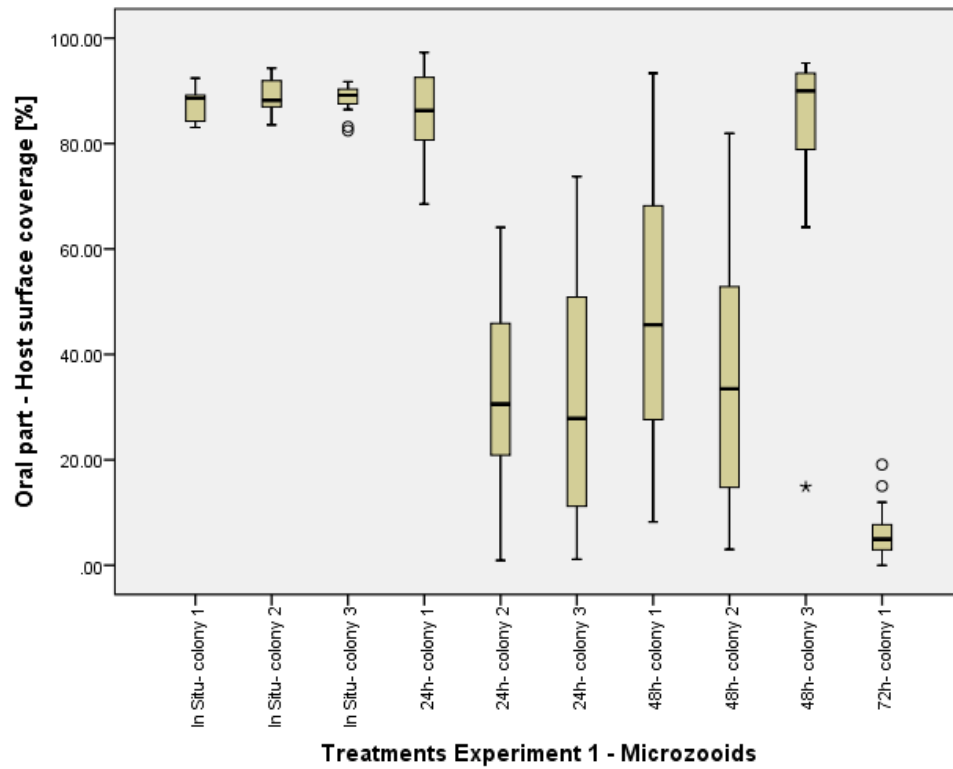


Figure S5.1 Host surface coverage of *Cand. Thiobios zoothamnici* per treatment on the oral parts of the microzooids under oxic stagnant conditions.

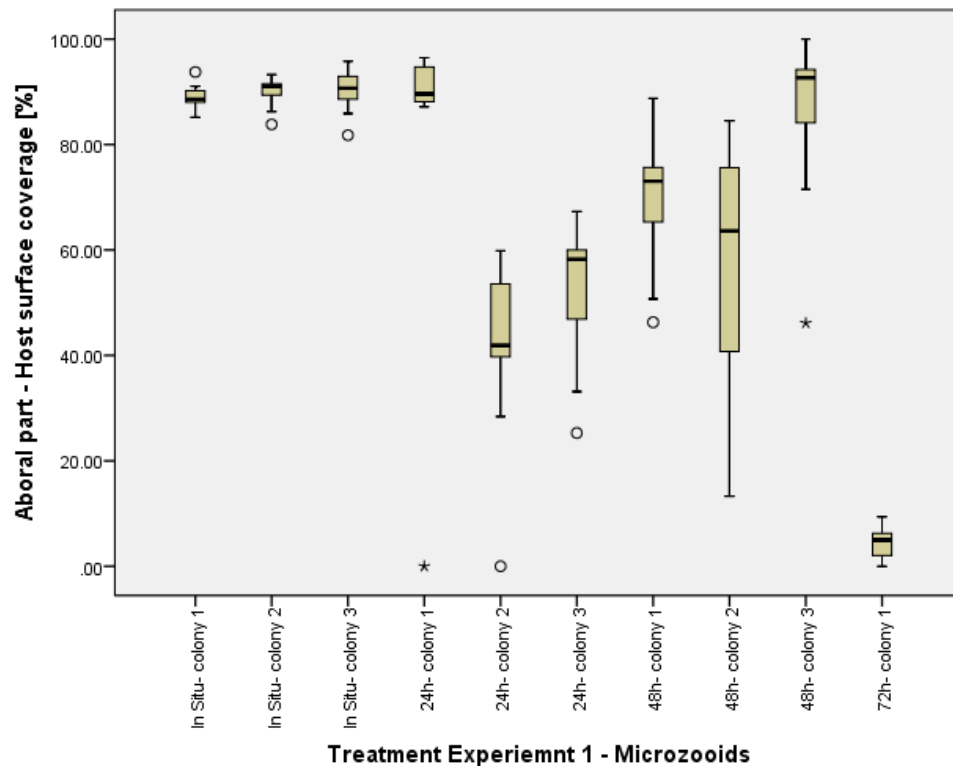
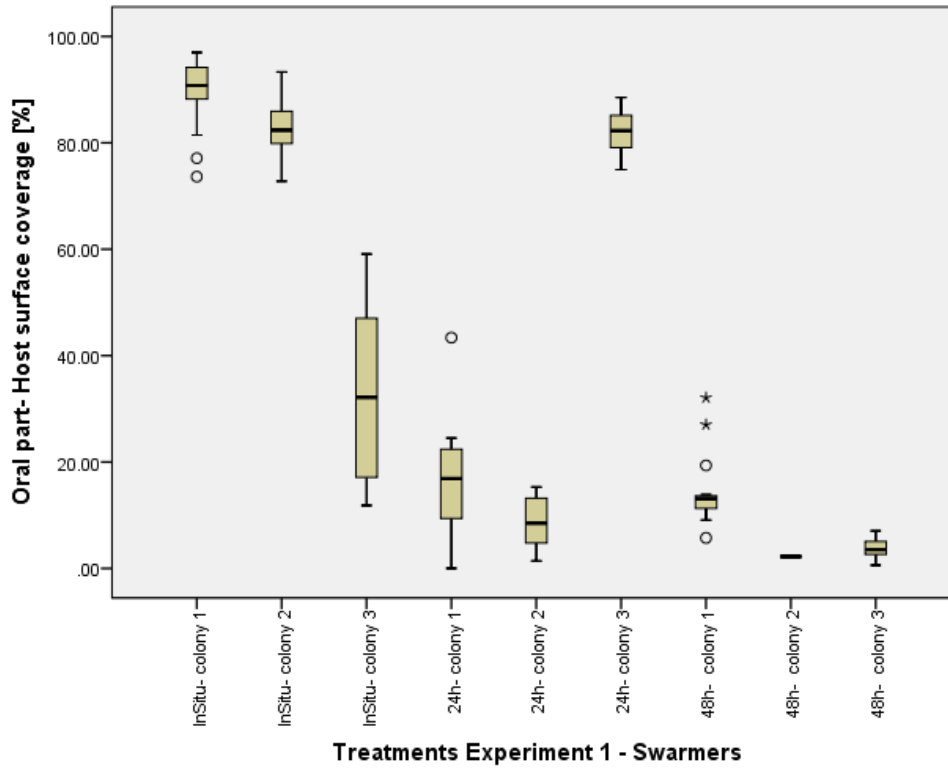
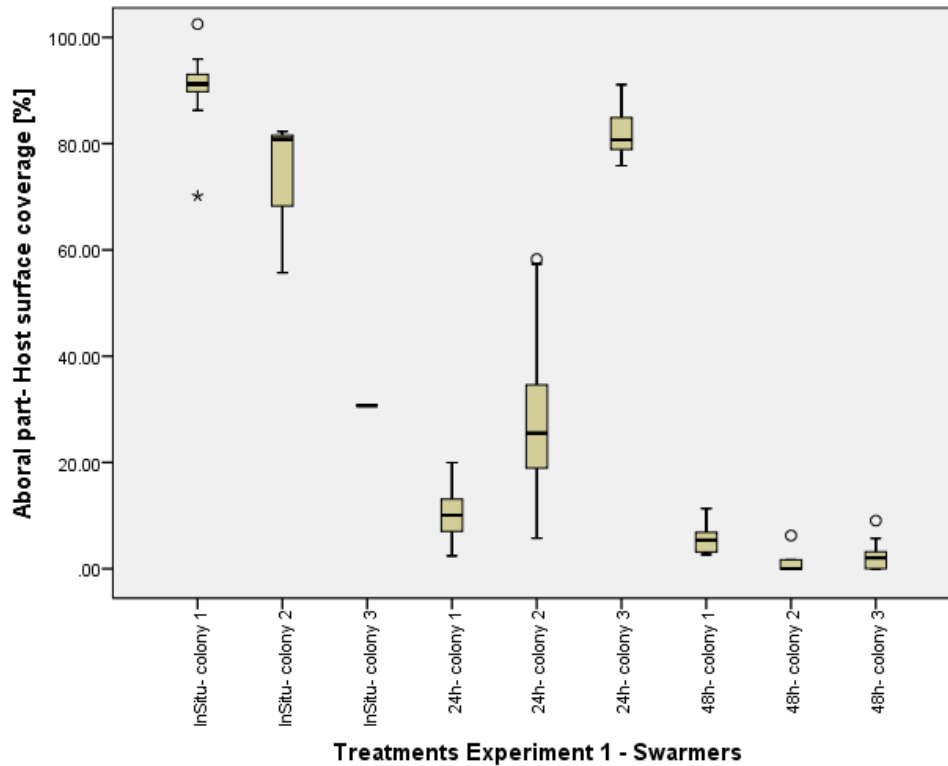


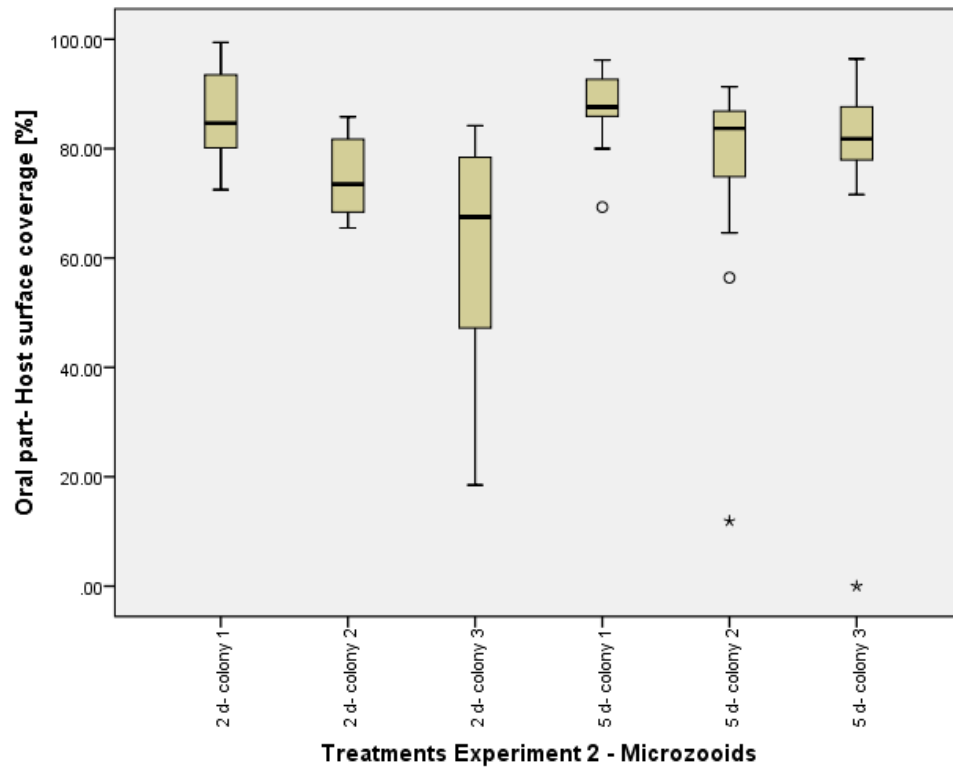
Figure S5.2. Host surface coverage of *Cand. Thiobios zoothamnici* per treatment on the aboral parts of the microzooids under oxic stagnant conditions.



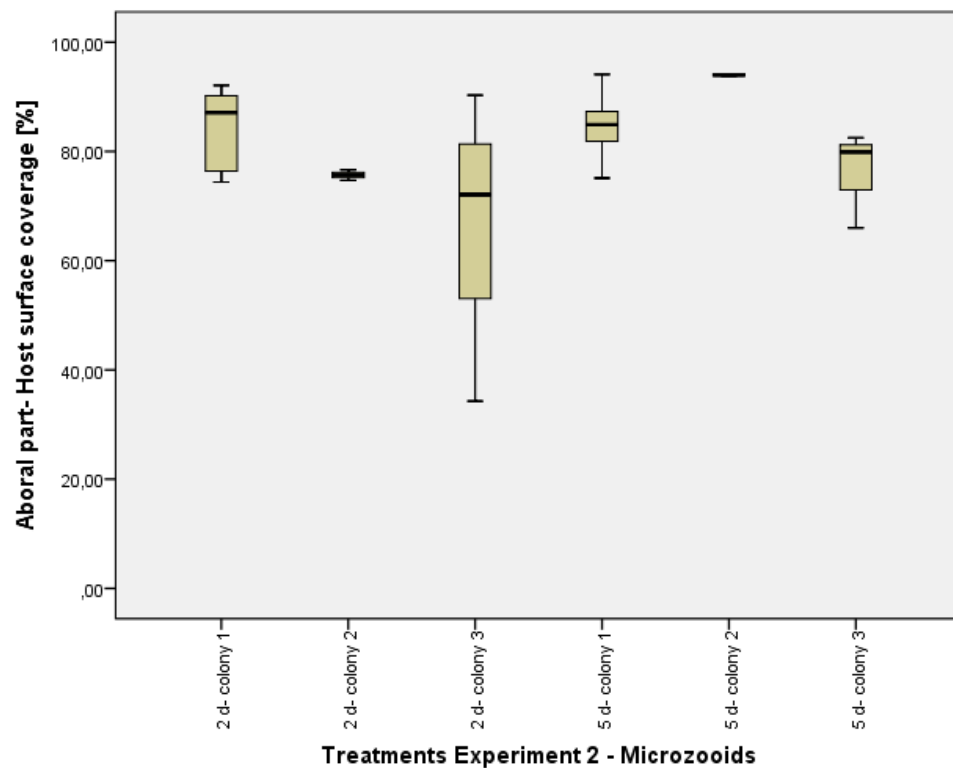
**Figure S5.3.** Host surface coverage of *Cand. Thiobios zoothamnicali* per treatment on the oral parts of the swarmers under oxic stagnant conditions.



**Figure S5.4.** Host surface coverage of *Cand. Thiobios zoothamnicali* per treatment on the aboral parts of the swarmers under oxic stagnant conditions.



**Figure S5.5.** Host surface coverage of *Cand. Thiobios zoothamnificoli* per treatment on the oral parts of the microzooids under oxic flow-through conditions.



**Figure S5.6.** Host surface coverage of *Cand. Thiobios zoothamnificoli* per treatment on the aboral parts of the microzooids under oxic flow-through conditions.

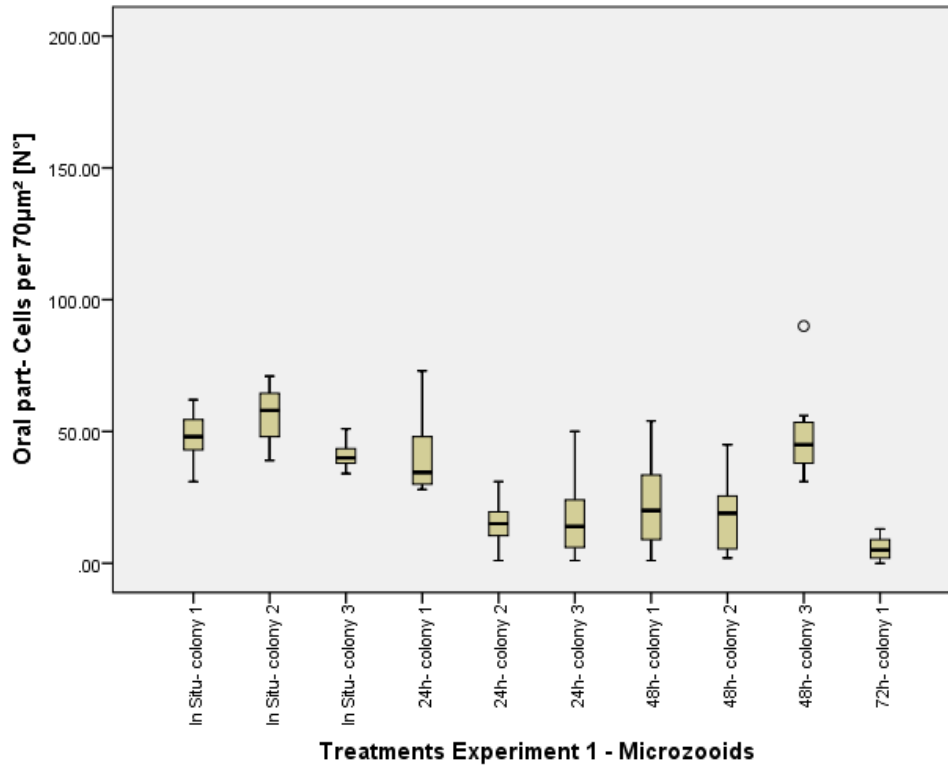


Figure S6.1. Cells of *Cand. Thiobios zoothamnici* per 70 µm<sup>2</sup> per treatment on the oral parts of the microzooids under oxic stagnant conditions.

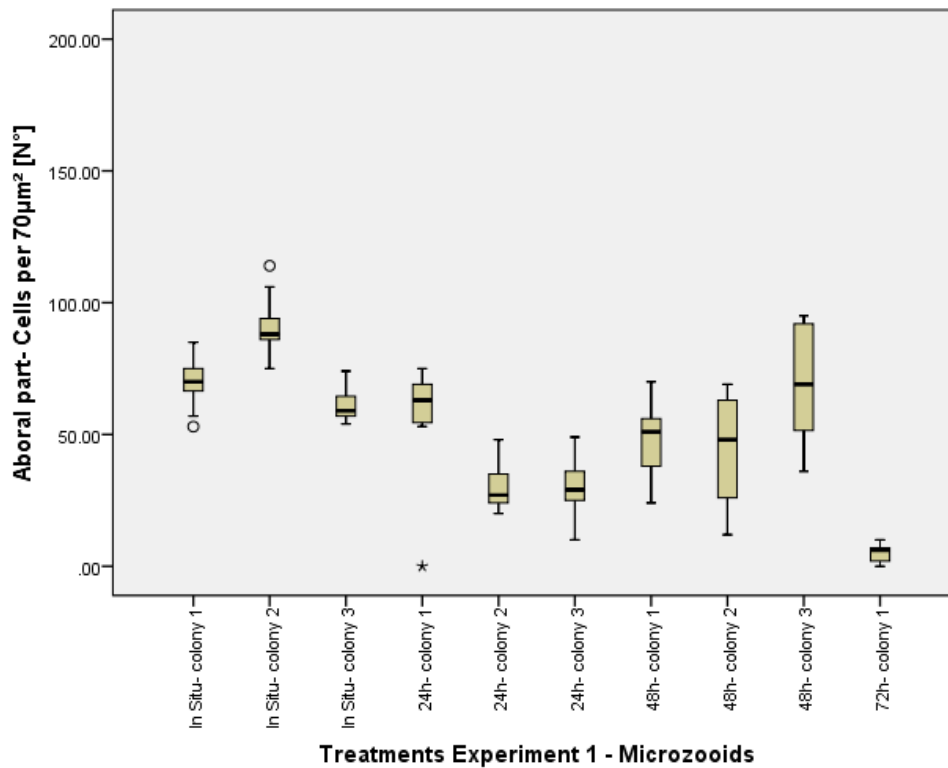
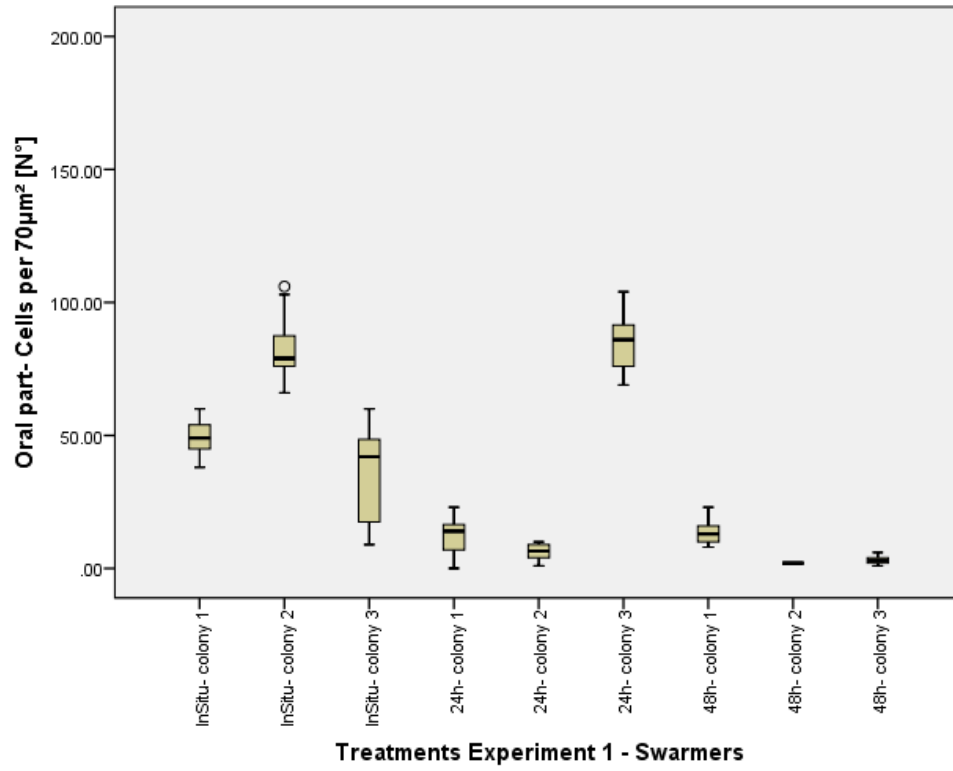
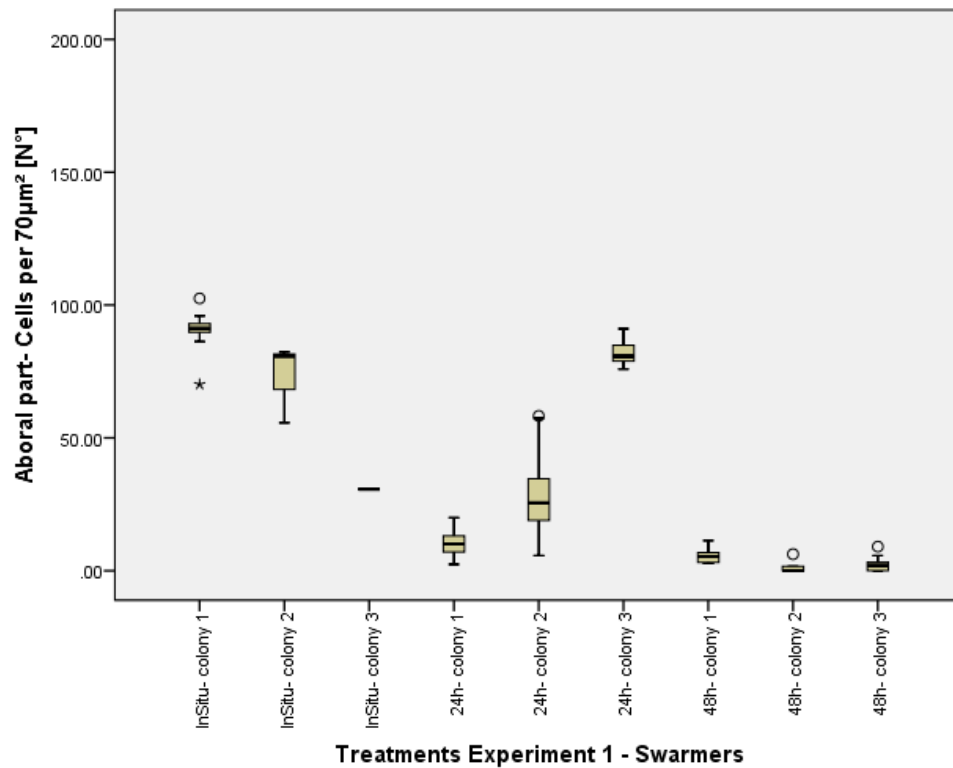


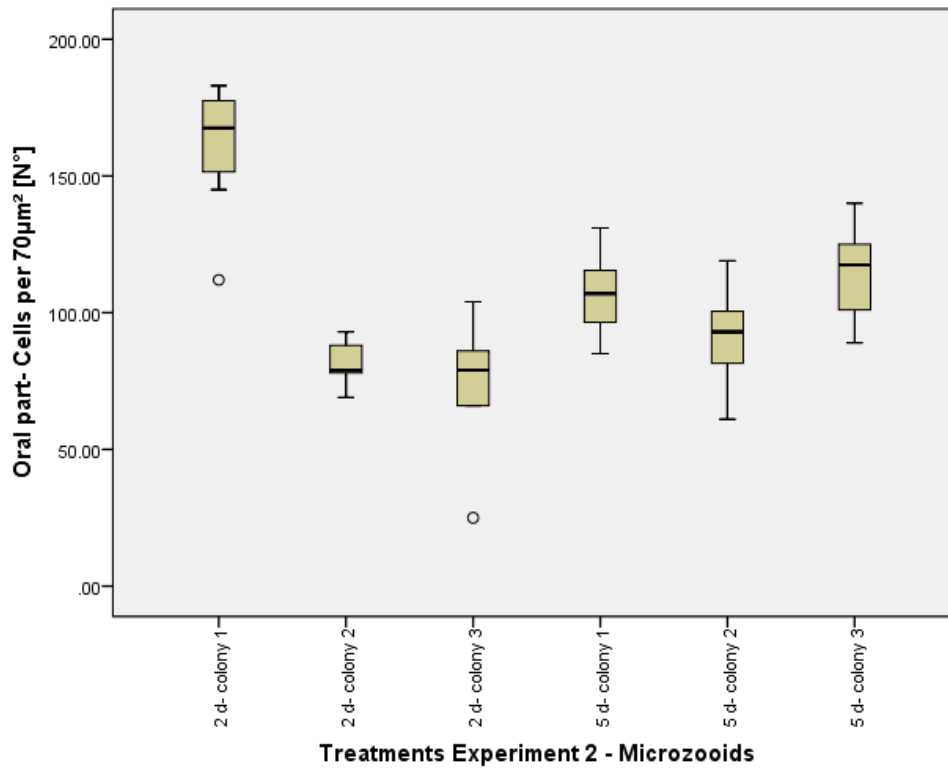
Figure S6.2. Cells of *Cand. Thiobios zoothamnici* per 70 µm<sup>2</sup> per treatment on the aboral parts of the microzooids under oxic stagnant conditions.



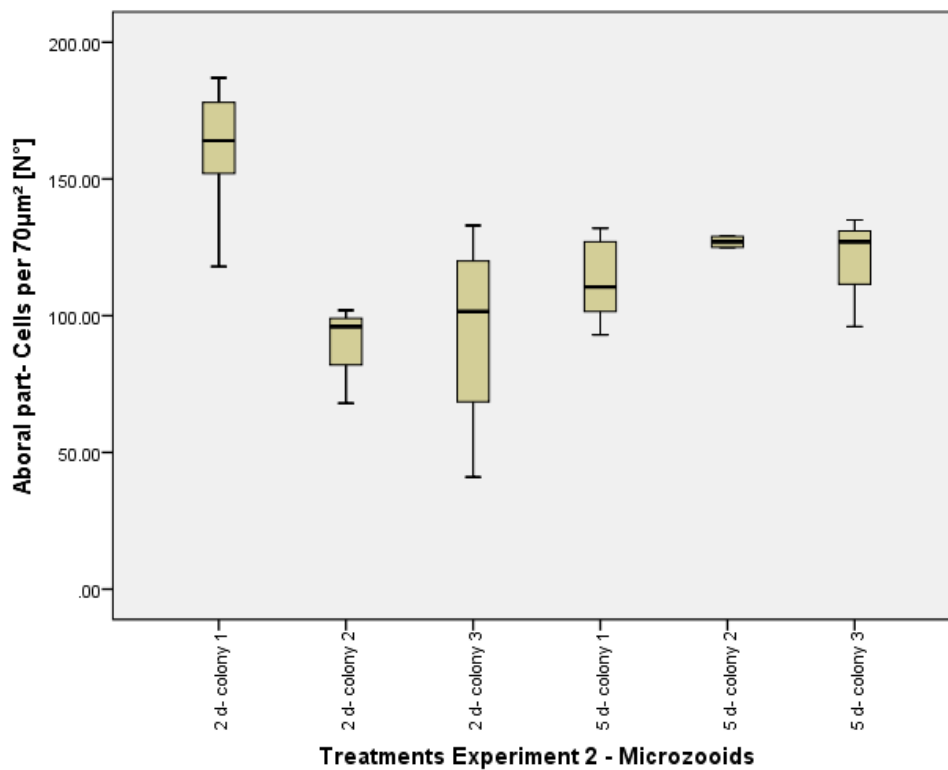
**Figure S6.3.** Cells of *Cand. Thiobios zoothamnicoli* per 70  $\mu\text{m}^2$  per treatment on the oral parts of the swarmers under oxic stagnant conditions.



**Figure S6.4.** Cells of *Cand. Thiobios zoothamnicoli* per 70  $\mu\text{m}^2$  per treatment on the aboral parts of the swarmers under oxic stagnant conditions.



**Figure S6.5.** Cells of *Cand. Thiobios zoothamnici* per 70 µm<sup>2</sup> per treatment on the oral parts of the microzooids under oxic flow-through conditions.



**Figure S6.6.** Cells of *Cand. Thiobios zoothamnici* per 70 µm<sup>2</sup> per treatment on the aboral parts of the microzooids under oxic flow-through conditions.



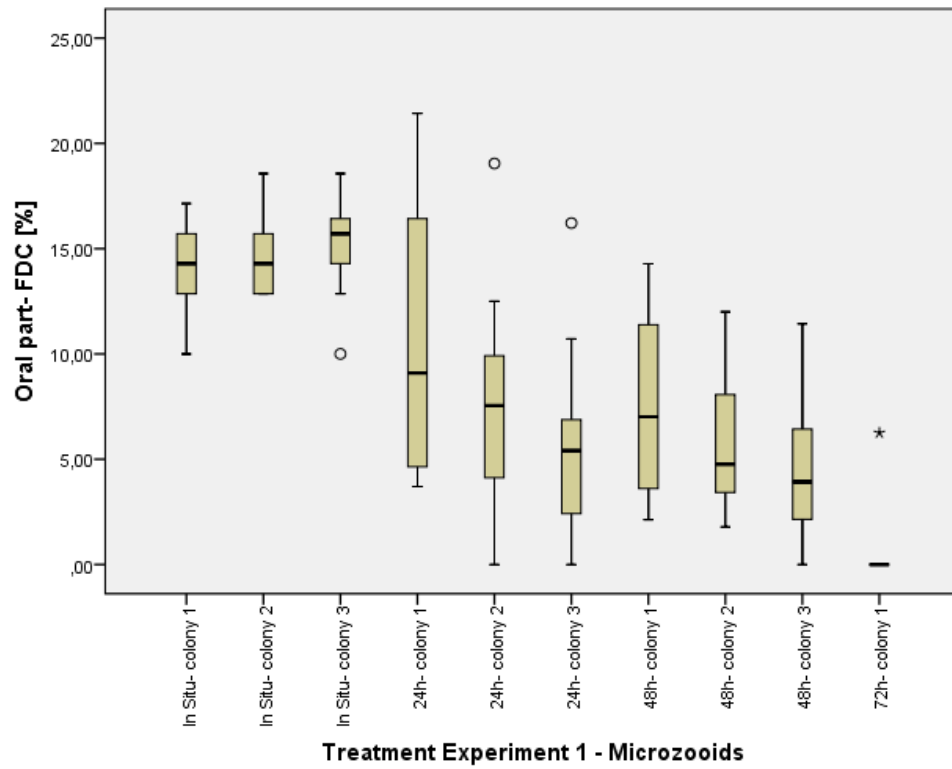


Figure S7.1. FDC of *Cand. Thiobios zoothamnici* per treatment on the oral parts of the microzooids under oxic stagnant conditions.

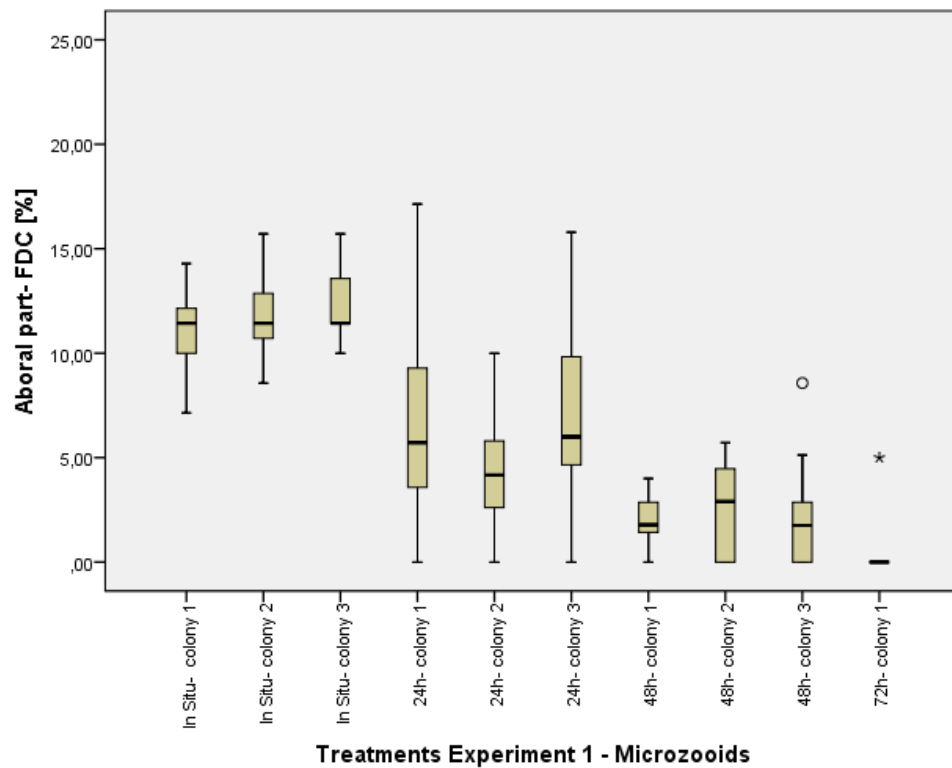


Figure S7.2. FDC of *Cand. Thiobios zoothamnici* per treatment on the aboral parts of the microzooids under oxic stagnant conditions.

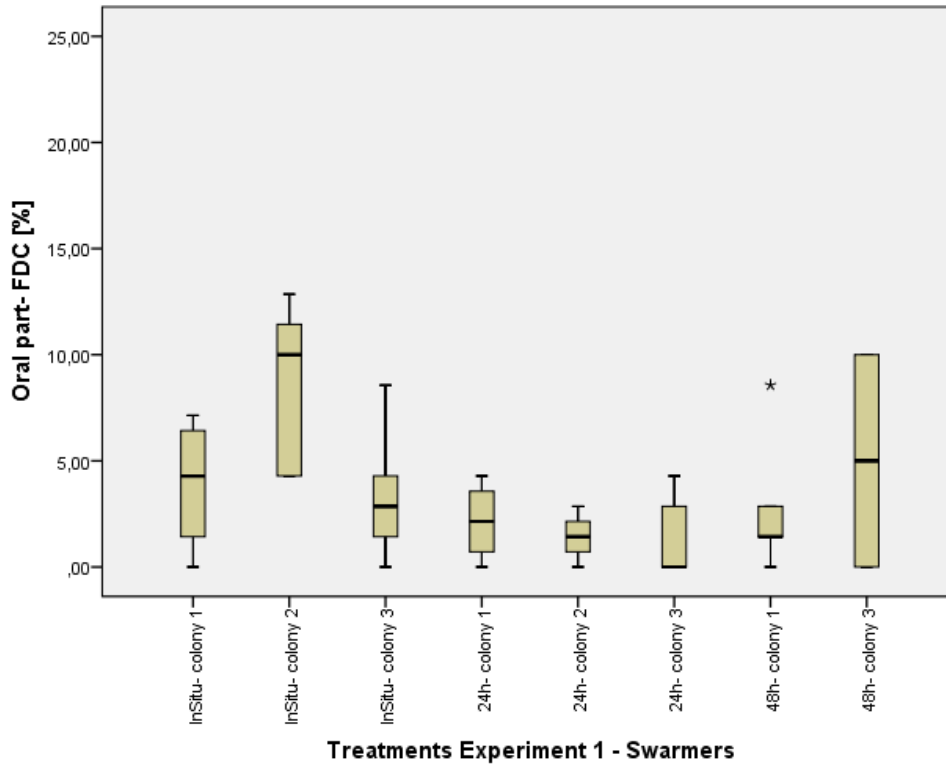


Figure S7.3. FDC of *Cand. Thiobios zoothamnicoli* per treatment on the oral parts of the swarmer under oxic stagnant conditions.

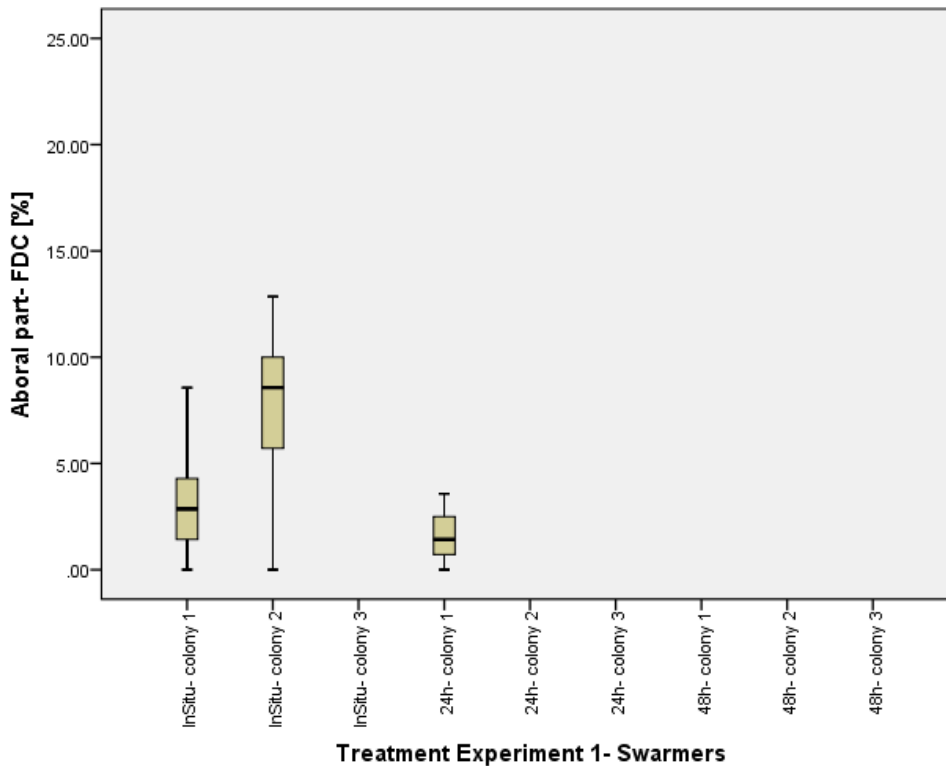


Figure S7.4. FDC of *Cand. Thiobios zoothamnicoli* per treatment on the aboral parts of the swarmer under oxic stagnant conditions.

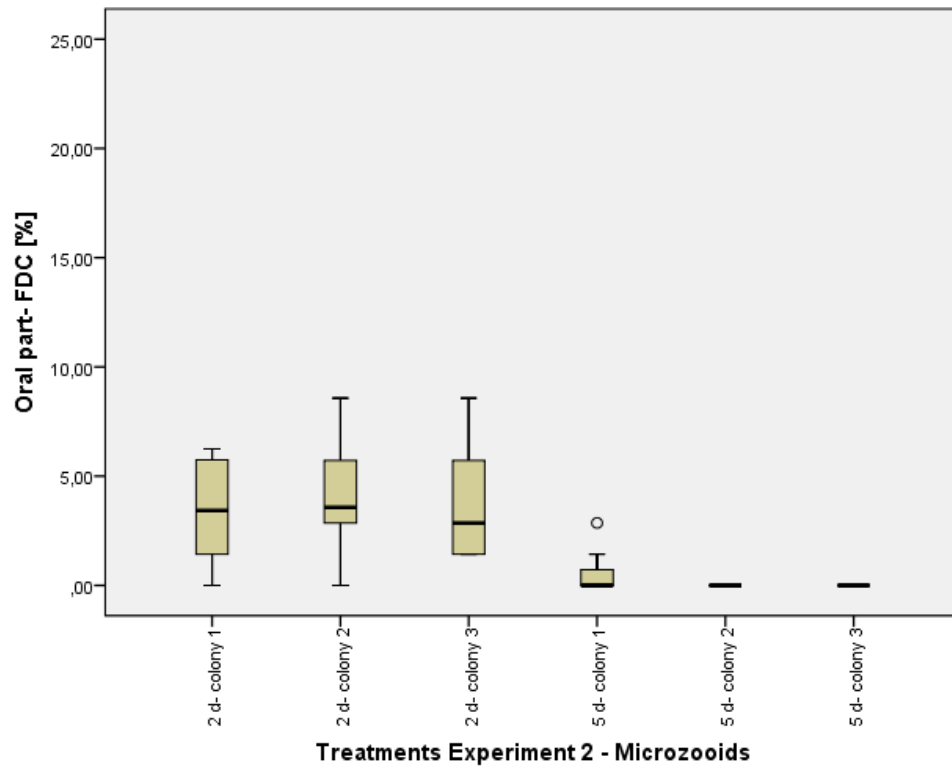


Figure S7.5. FDC of *Cand. Thiobios zoothamnici* per treatment on the oral parts of the microzooids under oxic flow-through conditions.

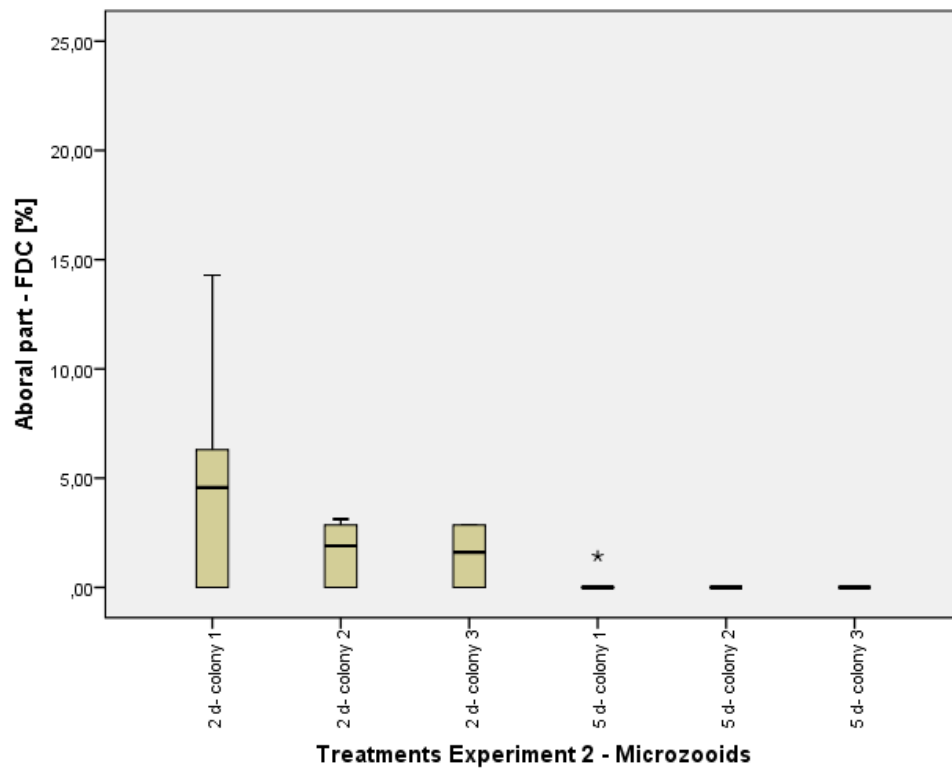
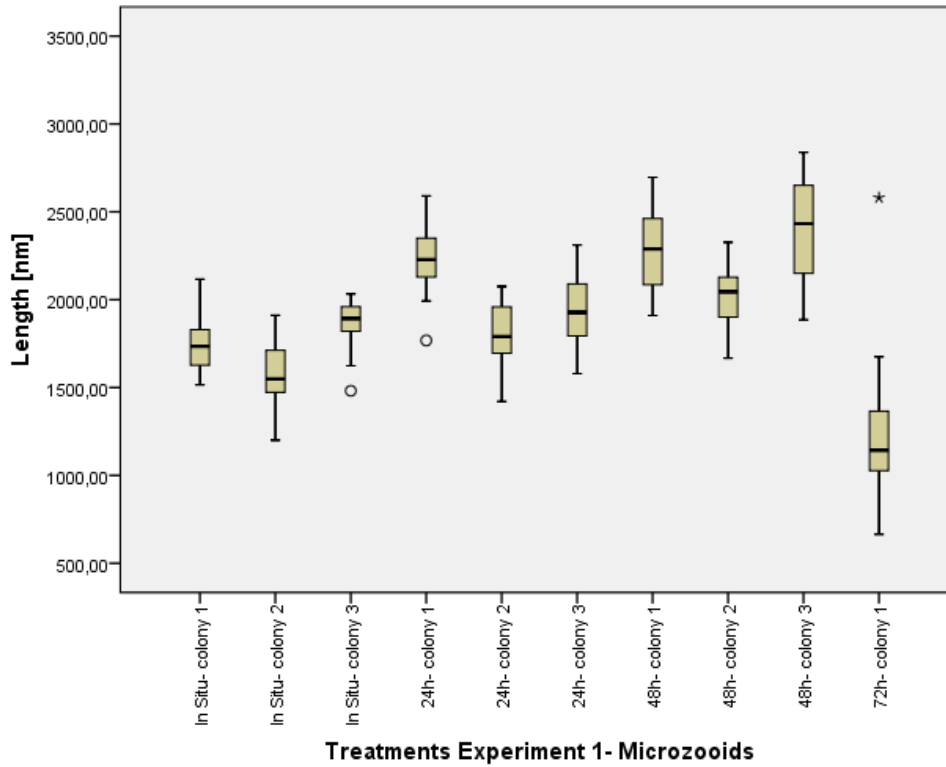
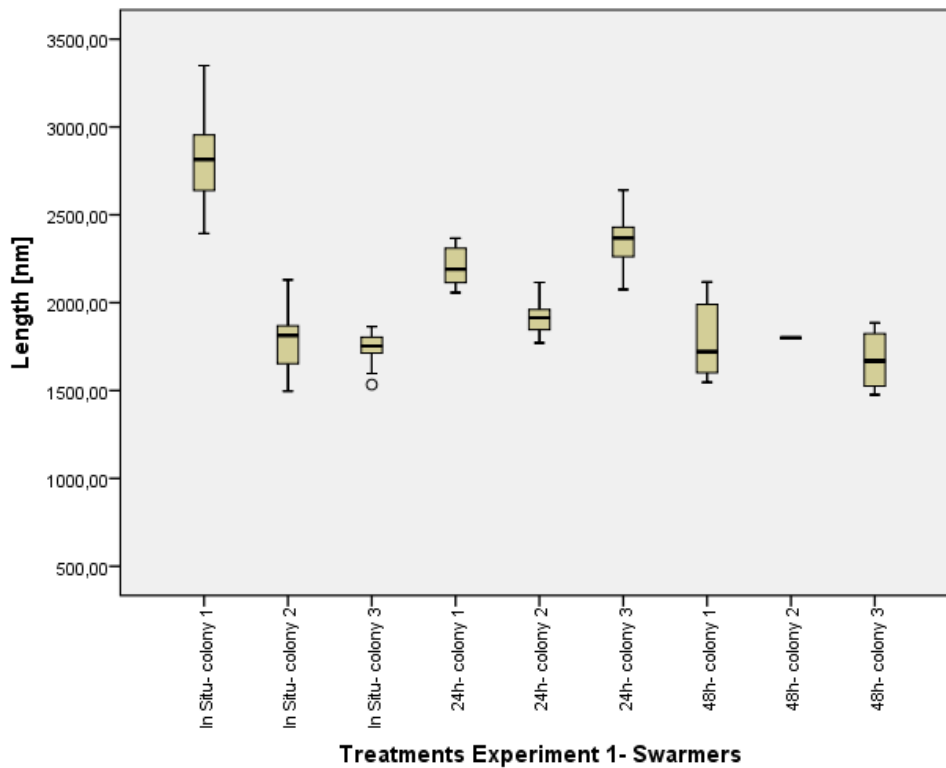


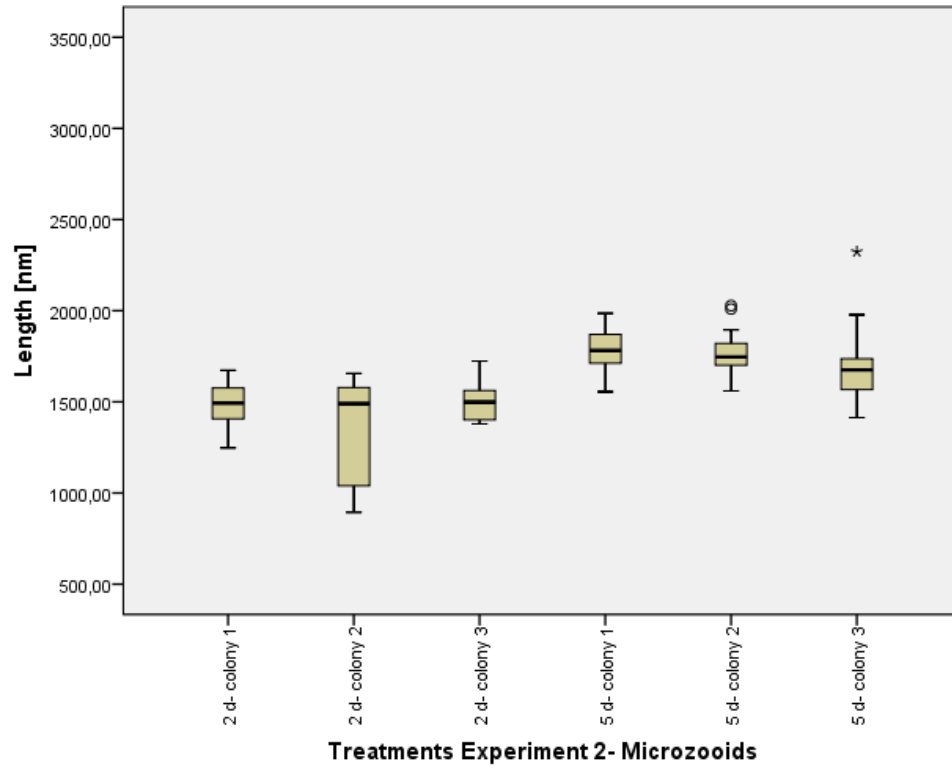
Figure S7.6 FDC of *Cand. Thiobios zoothamnici* per treatment on the aboral parts of the microzooids under oxic flow-through conditions.



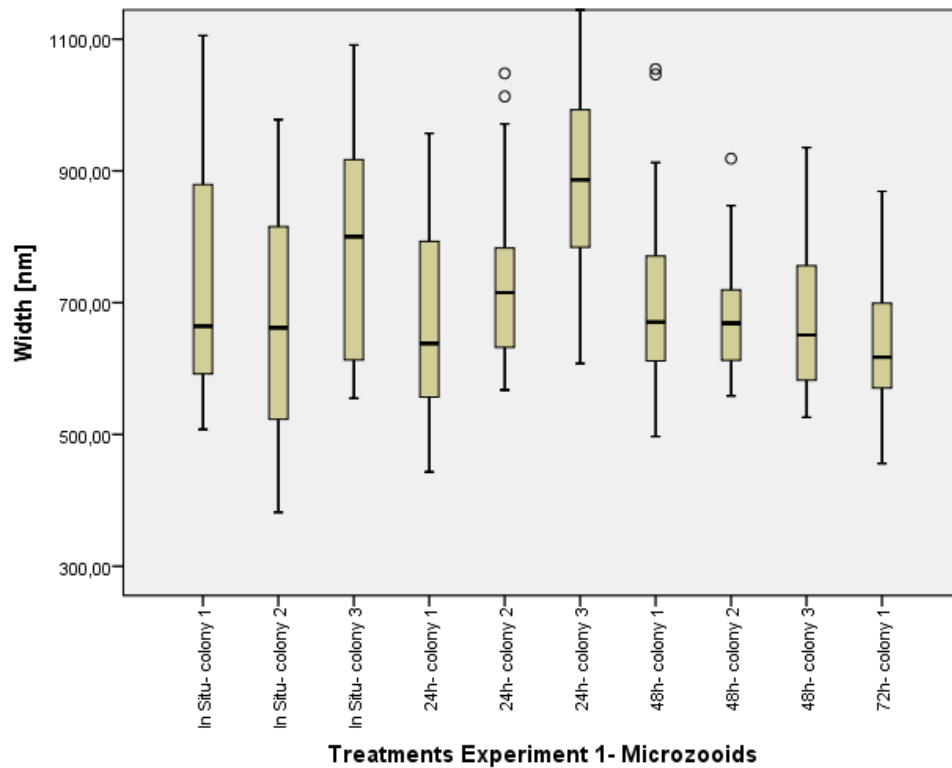
**Figure S8.1.** Length of *Cand. Thiobios zoothamnici* per treatment on the whole microzooids under oxic stagnant conditions.



**Figure S8.2.** Length of *Cand. Thiobios zoothamnici* per treatment on the whole swarmer under oxic stagnant conditions.



**Figure S8.3.** Length of *Cand. Thiobios zoothamnici* per treatment on the whole microzooid under oxic flow- through conditions.



**Figure S9.1.** Width of *Cand. Thiobios zoothamnici* per treatment on the whole microzooids under oxic stagnant conditions.

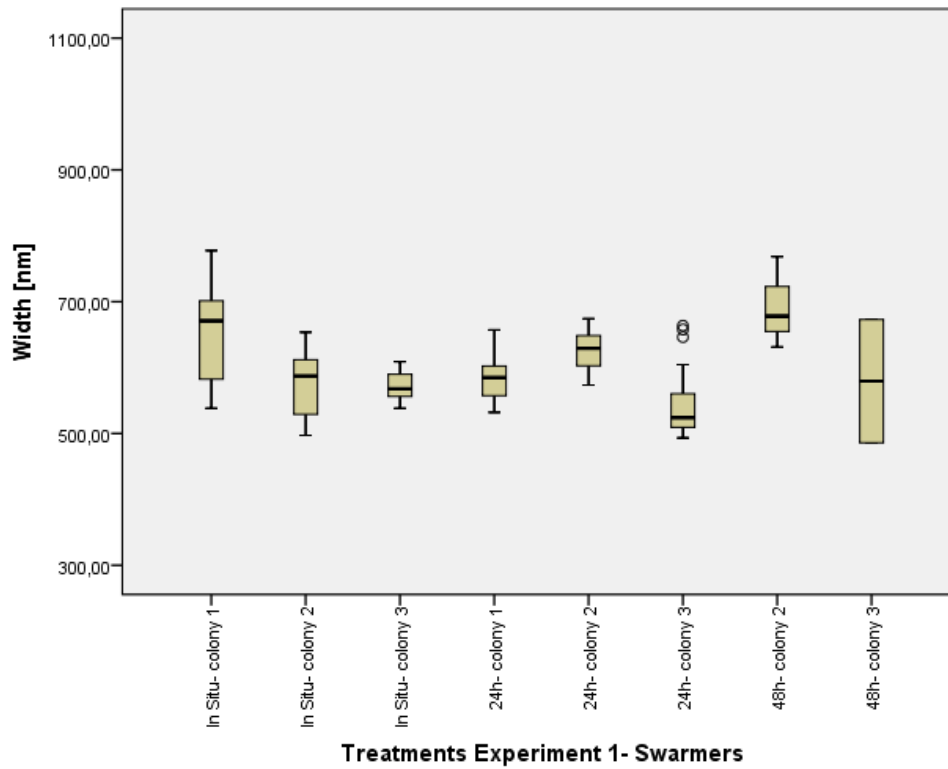


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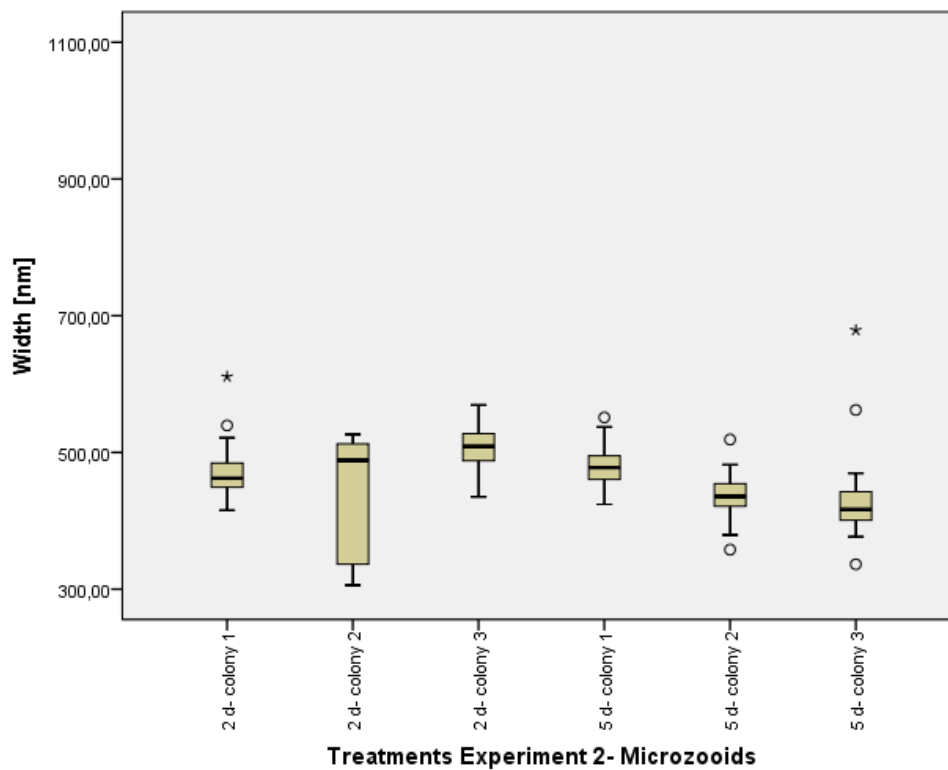
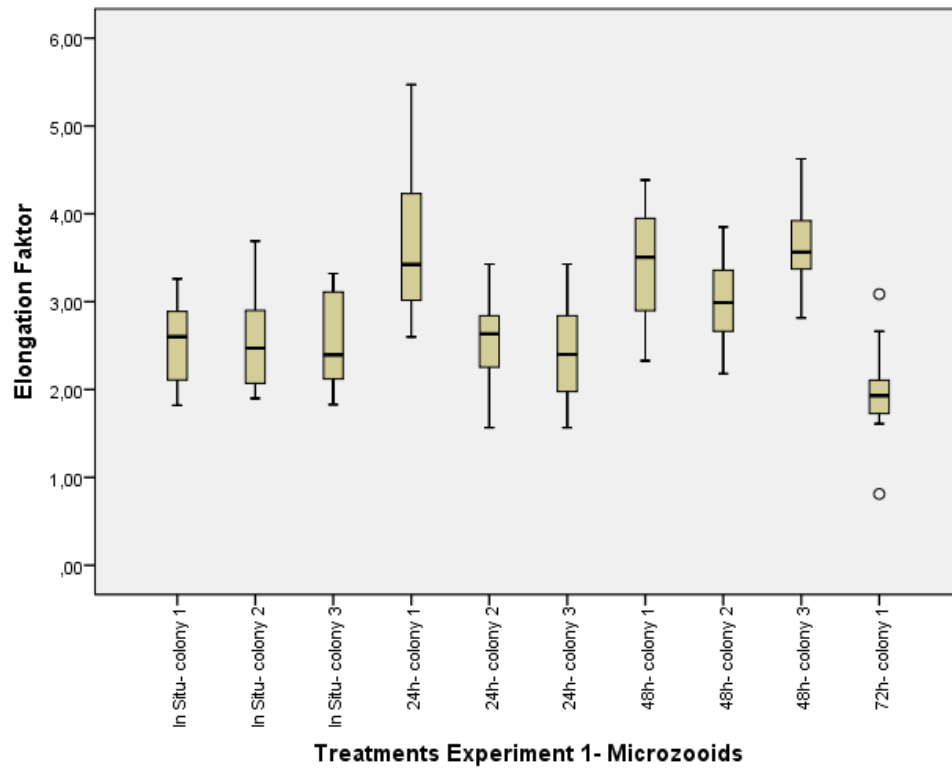
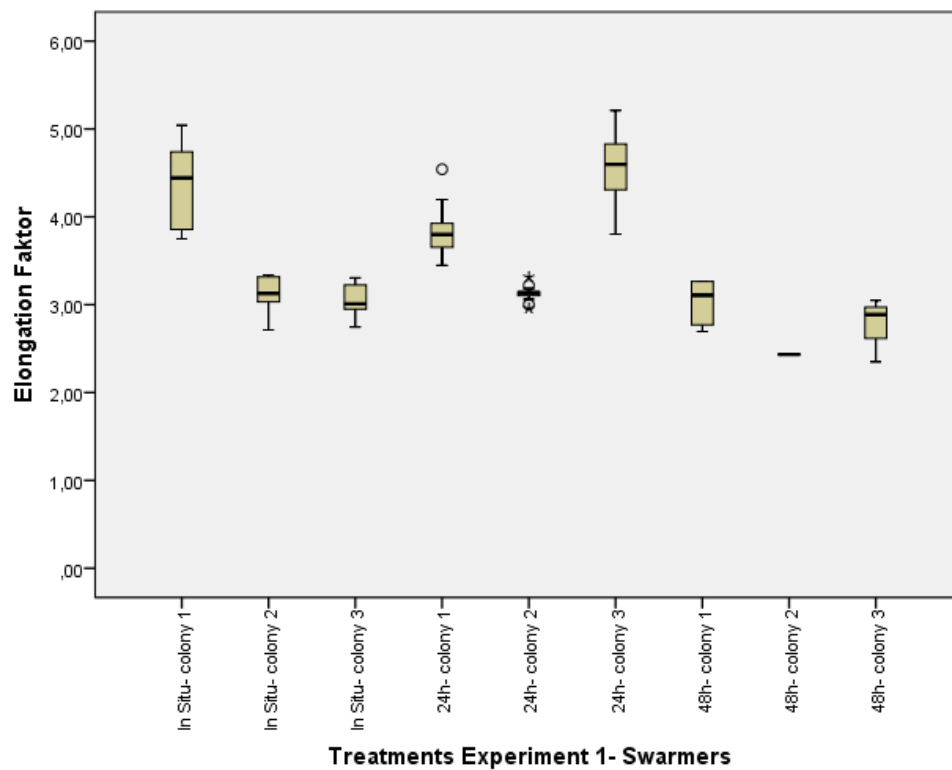


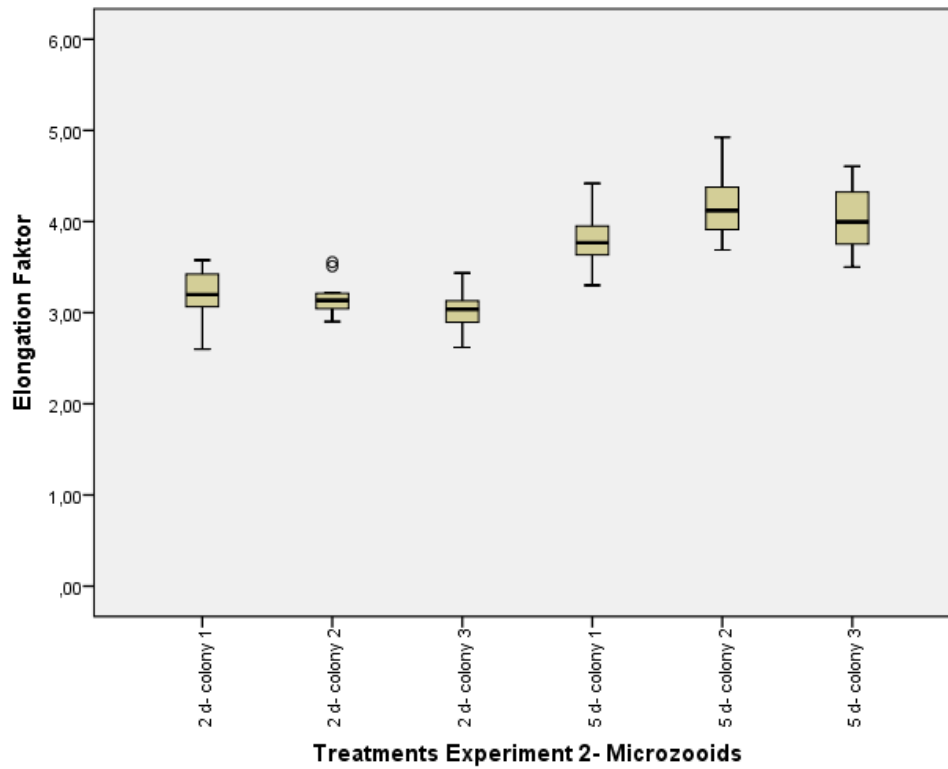
Figure S9.3. Width of *Cand. Thiobios zoothamnici* per treatment on the whole swarmer under oxic flow-through conditions.



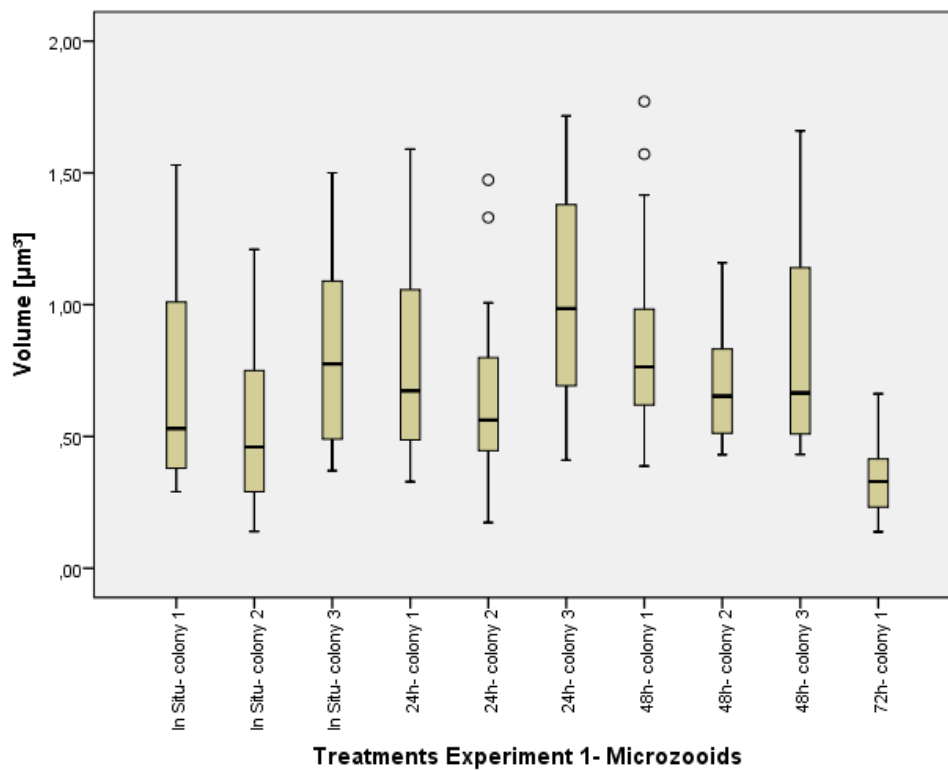
**Figure S10.1.** Elongation Factor of *Cand. Thioibios zoothamnici* per treatment on the whole microzooid under oxic stagnant conditions.



**Figure S10.2.** Elongation Factor of *Cand. Thioibios zoothamnici* per treatment on the whole swarmer under oxic stagnant conditions.

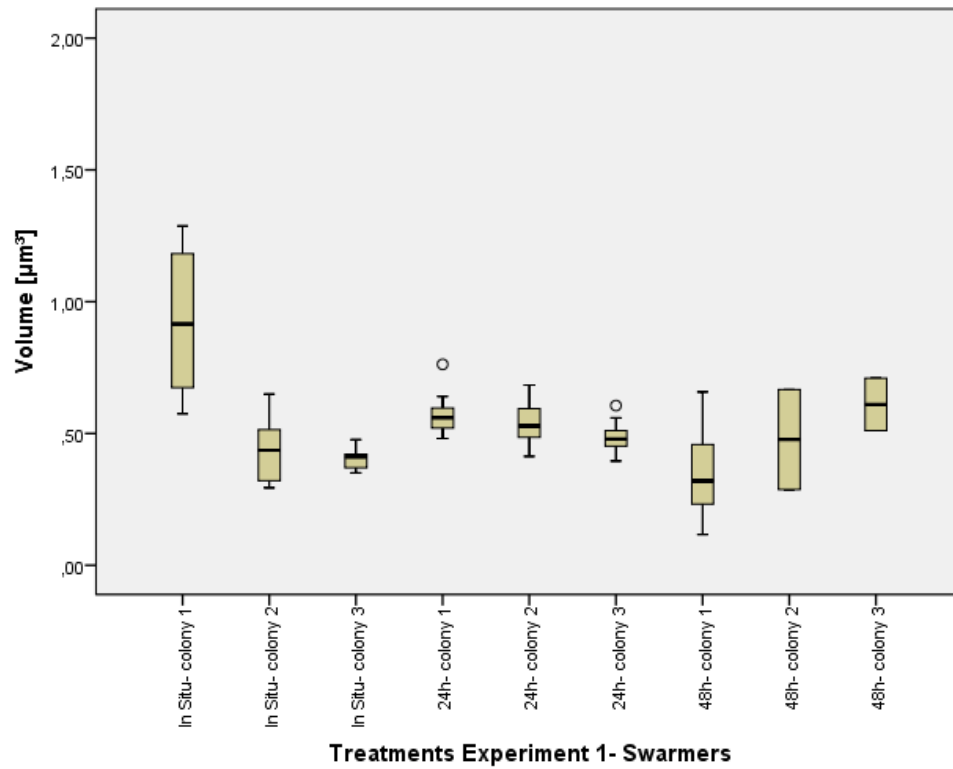


**Figure S10.3.** Elongation Factor of *Cand. Thiobios zoothamnici* per treatment on the whole microzooid under oxic flow-through conditions.

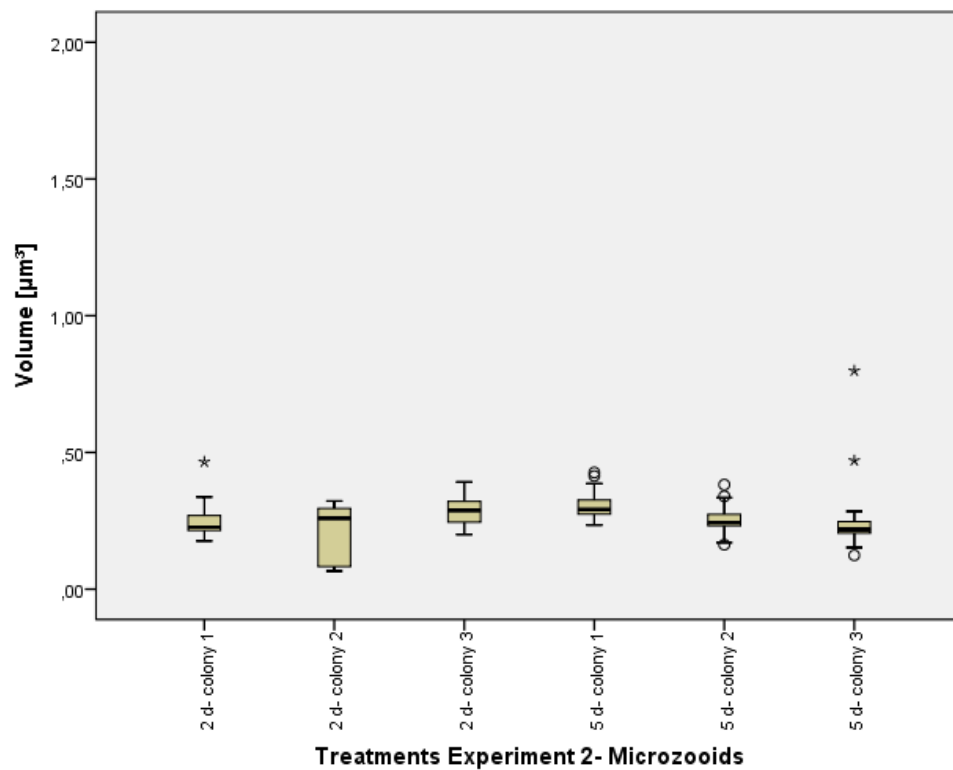


**Figure S11.1.** Volume of *Cand. Thiobios zoothamnici* per treatment on the whole microzooid under oxic stagnant conditions.

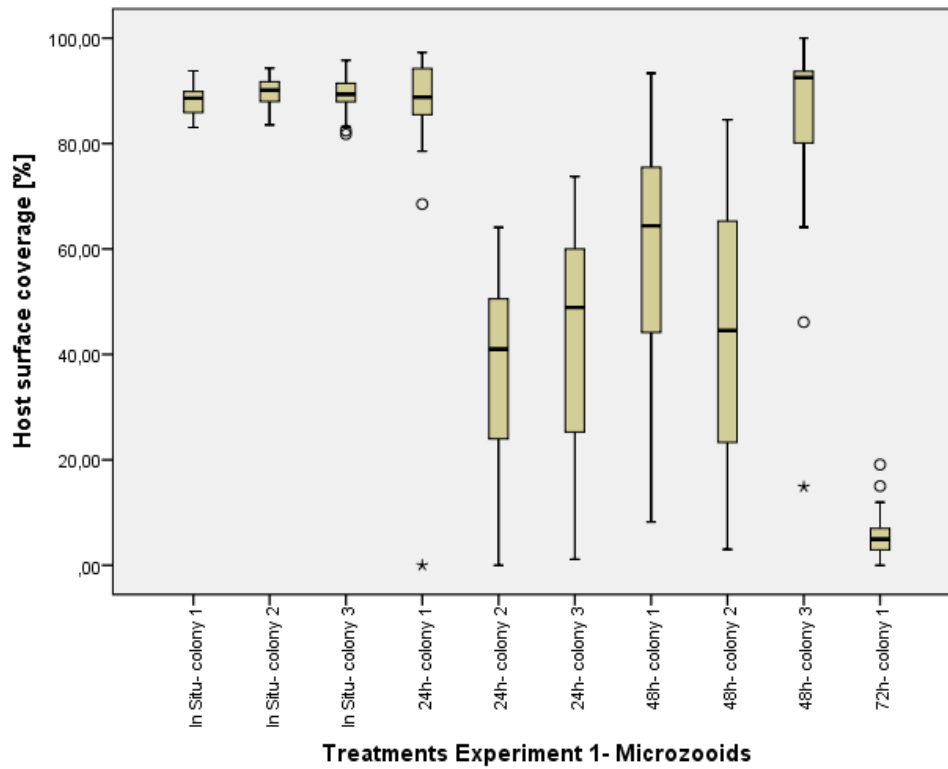




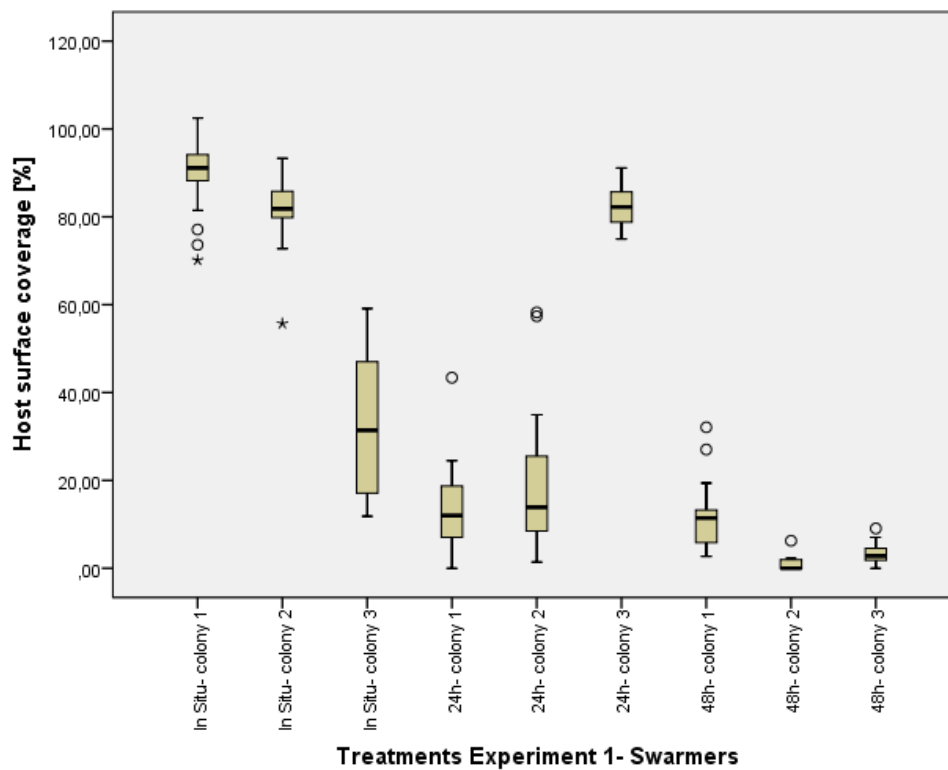
**Figure S11.2.** Volume of *Cand. Thioobios zoothamnicoli* per treatment on the whole swarmer under oxic stagnant conditions.



**Figure S11.3.** Volume of *Cand. Thioobios zoothamnicoli* per treatment on the whole microzooid under oxic stagnant conditions.



**Figure S12.1.** Host surface coverage of *Cand. Thiobios zoothamnici* per treatment on the whole microzooid under oxic stagnant conditions.



**Figure S12.2.** Host surface coverage of *Cand. Thiobios zoothamnici* per treatment on the whole swarmer under oxic stagnant conditions.

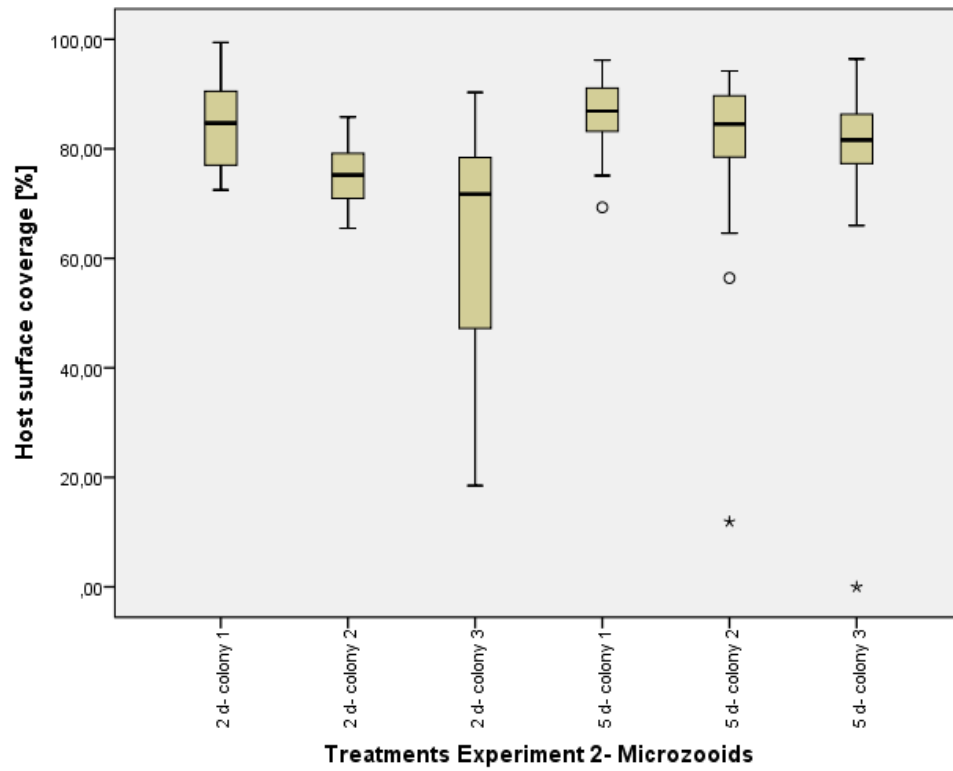


Figure S12.3. Host surface coverage of *Cand. Thiobios zoothamnici* per treatment on the whole microzooid under oxic flow-through conditions.

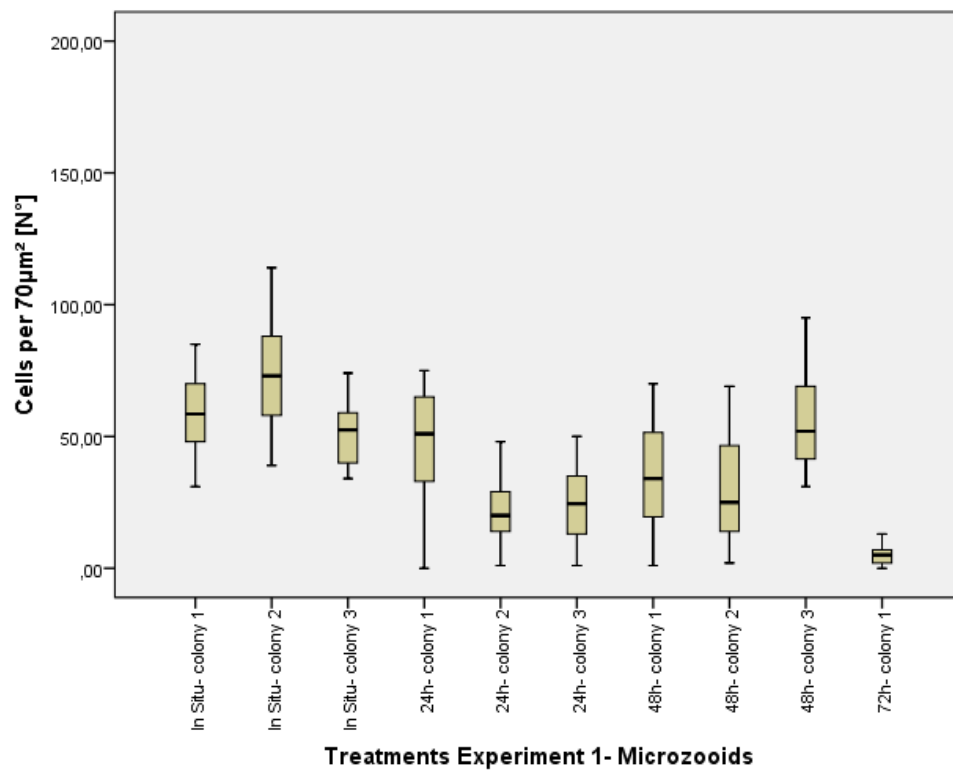
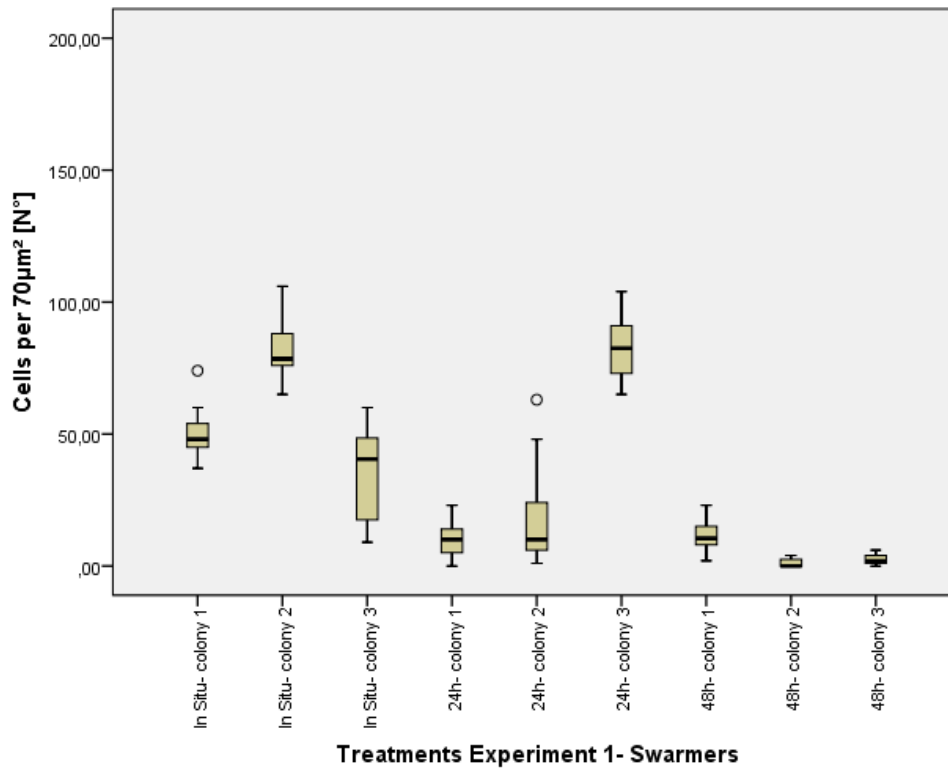
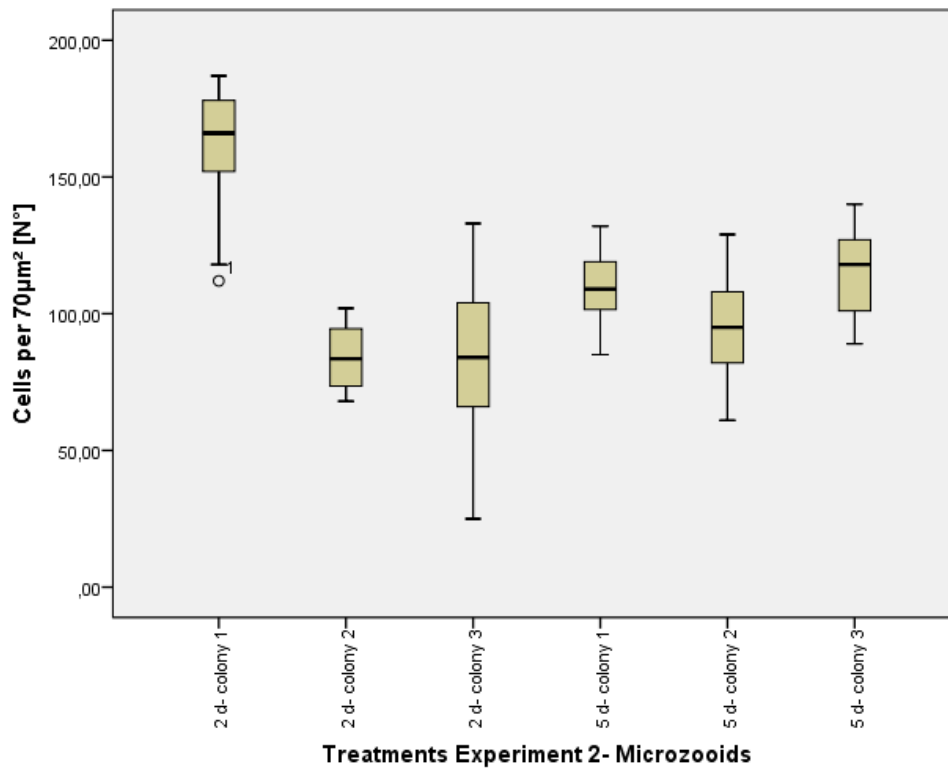


Figure S13.1. Cells of *Cand. Thiobios zoothamnici* per 70  $\mu\text{m}^2$  per treatment on the whole microzooids under oxic stagnant conditions



**Figure S13.2.** Cells of *Cand. Thiobios zoothamnicoli* per 70  $\mu\text{m}^2$  per treatment on the whole swarmer under oxic stagnant conditions.



**Figure S13.3.** Cells of *Cand. Thiobios zoothamnicoli* per 70  $\mu\text{m}^2$  per treatment on the whole microzooid under oxic flow-through conditions.

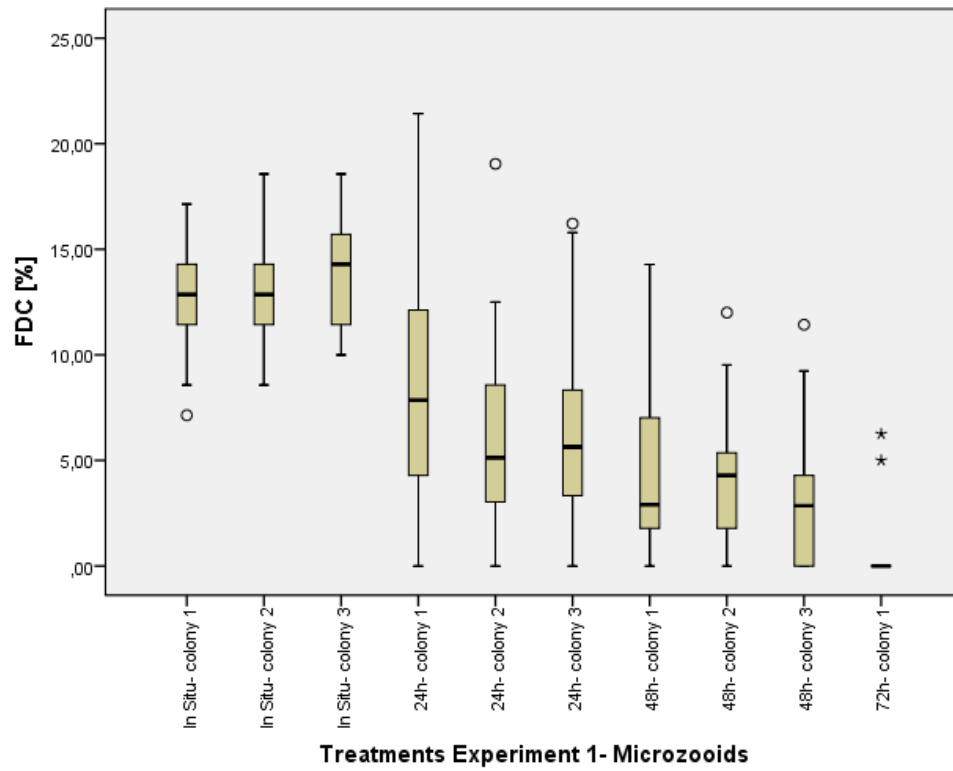


Figure S14.1 FDC of *Cand. Thiobios zoothamnici* per treatment on the whole microzooids under oxie stagnant conditions.

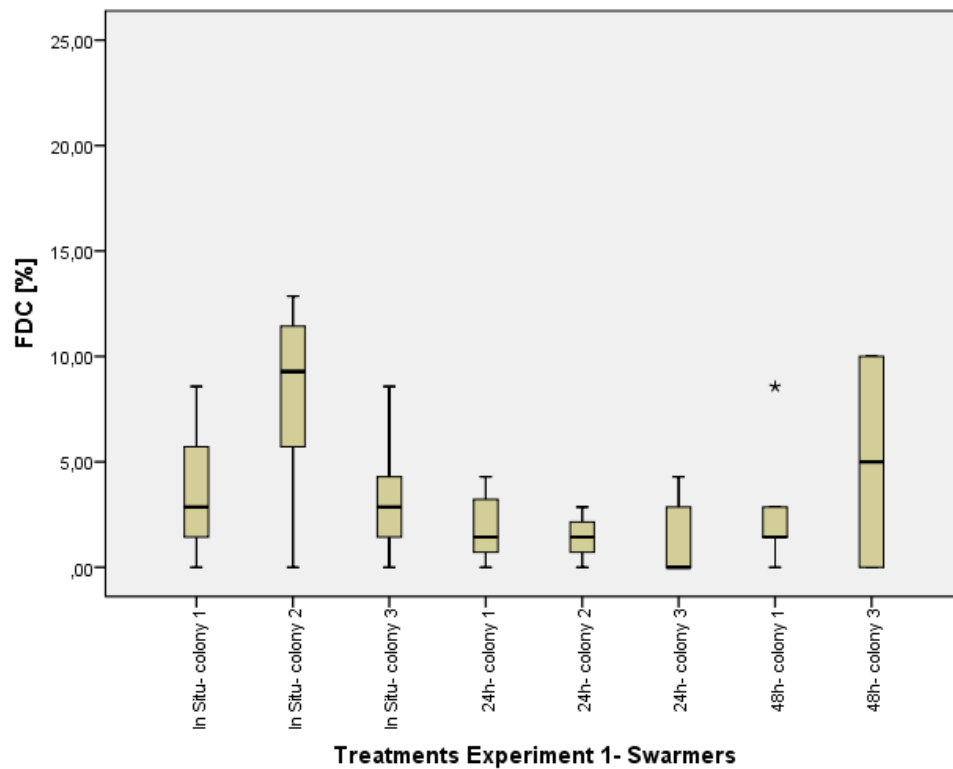


Figure S14.2. FDC of *Cand. Thiobios zoothamnici* per treatment on the whole swarmer under oxie stagnant conditions.

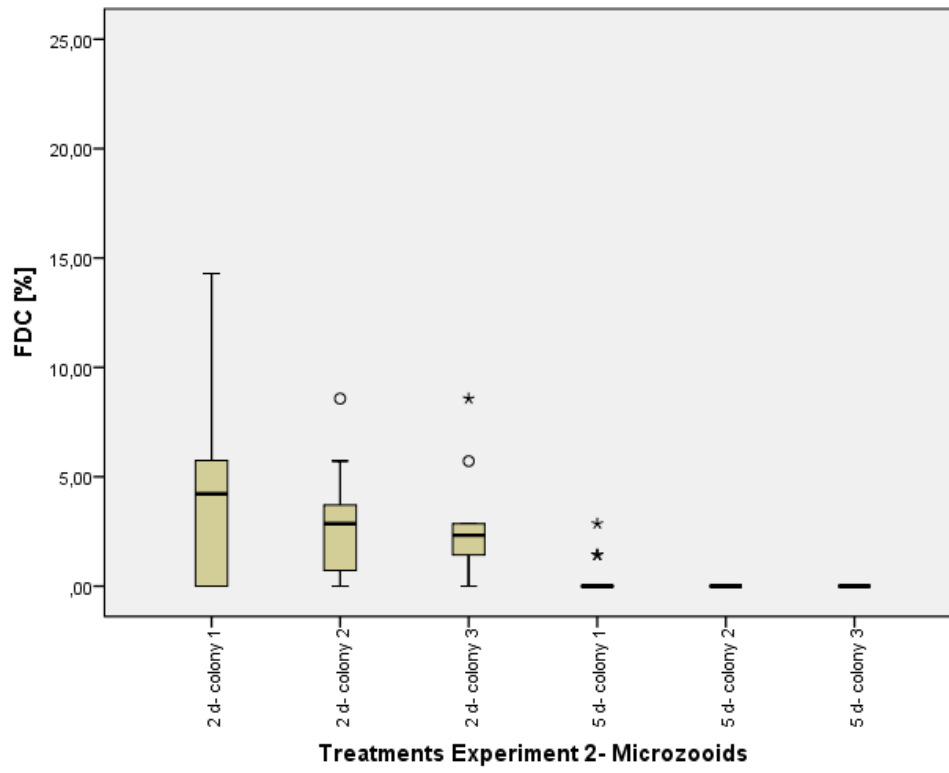


Figure S14.3. FDC of *Cand. Thiobios zoothamnicoli* per treatment on the whole microzooid under oxic flow-through conditions.

**Table S1:** Measured parameters between all treatments on the oral part of the microzooids/ swarmers.

Oral Part		Experiment 1					Experiment 2		
		Microzooids			Swarmers		Microzooids		
Parameters	Values	<i>in situ</i>	<i>1/2 days</i>	<i>3 days</i>	<i>in situ</i>	<i>1/2 days</i>	<i>2 days</i>	<i>5 days</i>	<i>7 days</i>
length [ $\mu\text{m}$ ]	mean value	1805.32	2177.31	1279.66	2021.19	2098.34	1463.68	1742.35	
	standard deviation	144.54	311.63	429.35	466.32	259.76	205.31	119.559	nv
	maximum	2116.86	2839.02	2580.56	2981.47	2641.13	1723.44	1985.67	nv
	minimum	1472.51	1516.69	970.84	1533.52	1546.66	894.09	1440.68	nv
	no of measured values	45	90	12	18	43		41	0
width [ $\mu\text{m}$ ]	mean value	866.58	788.24	716.17	621.41	578.86	472.88	449.68	nv
	standard deviation	99.11	141.95	210.59	75.13	56.88	63.16	39.32	nv
	maximum	1105.32	1147.71	1337.16	777.45	678.00	569.43	562.17	nv
	minimum	667.96	443.20	507.02	538.16	493.28	325.55	379.16	nv
	no of measured values	45	90	13	18	37	20	41	0
volume [ $\mu\text{m}^3$ ]	mean value	0.92	0.96	0.59	0.60	0.52	0.25	0.2669	nv
	standard deviation	0.25	0.37	0.74	0.32	0.09	0.08	0.06	nv
	maximum	1.53	1.77	3	1.29	0.76	0.39	0.47	nv
	minimum	0.51	0.17	0.2	0.35	0.29	0.07	0.17	nv
	no of measured values	45	90	13	18	43	20	41	0
Elongation Factor	mean value	2.16	2.90	1.92	3.26	3.74	3.18	3.99	nv
	standard deviation	0.23	0.64	0.40	0.37	0.73	0.20	0.28	nv
	maximum	2.88	5.07	3.09	3.96	5.21	3.55	4.49	nv
	minimum	1.82	1.67	1.61	2.75	2.70	2.84	3.30	nv
	no of measured values	45	90	13	18	43	20	41	0
N° of cells [per $70\mu\text{m}^2$ ]	mean value	48.62	27.06	5,29	55.35	24.95	112.32	103.70	nv
	standard deviation	10.42	18.27	4.46	24.44	31.96	47.51	18.87	nv
	maximum	71	90	13	106	104	183.00	140	nv
	minimum	31	1	2	9	0	25	61	nv
	no of measured values	45	87	14	43	71	19	40	0
FDC [%]	mean value	14.67	6.85	0.42	4.92	1.92	3.73	0.14	nv
	standard deviation	2.11	4.87	1.61	3.93	2.17	2.58	0.53	nv
	maximum	18.57	21.43	6.25	12.86	10	8.57	2.86	nv
	minimum	10	0	0	0	0	0	0	nv
	no of measured values	45	90	15	18	43	20	42	0
Host surface coverage [%]	mean value	87.96	52.69	6.68	67.13	26.38	75.09	79.91	nv
	standard deviation	3.14	31.06	6.37	27.96	30.18	18.36	18.96	nv
	maximum	92.84	97.30	19.09	97	88.51	99.40	96.40	nv
	minimum	82.46	0.91	0	11.84	0	18.50	0.00	nv
	no of measured values	27	87	11	43	71	19	41	0

**Table S2:** Measured parameters between all treatments on the aboral part of the microzooids/ swarmers.

Aboral Part		Experiment 1					Experiment 2			
		Microzooids			Swarmers		Microzooids			
Parameters	Values	<i>in situ</i>	1/ 2 days	3 days	<i>in situ</i>	1/ 2 days	2 days	5 days	7 days	
<b>length [<math>\mu\text{m}</math>]</b>	mean value	1649.38	2044.28	1141.57	2311.91	1920.01	1439.65	1736.87	nv	
	standard deviation	192.18	256.18	271.17	609.06	296.22	173.78	170.77	nv	
	maximum	1998.37	2752.16	1548.29	3349.68	2273.95	1672.49	2323.48	nv	
	minimum	1200.18	1420.18	665.42	1496.81	1476.51	1005.04	1413.87	nv	
	no of measured values	45	90	12	19	9	20	42	0	
<b>width [<math>\mu\text{m}</math>]</b>	mean value	586.98	667.90	594.89	594.87	608.69	478.94	444.96	nv	
	standard deviation	107.83	136.00	120.22	61.57	73.66	66.36	56.58	nv	
	maximum	1090.96	1240.26	869.18	701.16	768.40	610.69	678.77	nv	
	minimum	381.87	447.52	455.95	497.20	485.71	305.67	336.41	nv	
	no of measured values	45	90	12	19	15	20	42	0	
<b>volume [<math>\mu\text{m}^3</math>]</b>	mean value	0.42	0.64	0.27	0.63	0.41	0.25	0.26	nv	
	standard deviation	0.21	0.25	0.10	0.29	0.19	0.08	0.10	nv	
	maximum	1.50	1.61	0.45	1.22	0.67	0.47	0.80	nv	
	minimum	0.14	0.36	0.14	0.29	0.12	0.07	0.12	nv	
	no of measured values	45	90	12	19	14	20	42	0	
<b>Elongation Factor</b>	mean value	2.93	3.31	2.03	3.90	3.21	3.10	4.02	nv	
	standard deviation	0.37	0.70	0.49	0.81	0.61	0.28	0.40	nv	
	maximum	3.69	5.47	2.66	5.04	4.20	3.58	4.93	nv	
	minimum	1.83	1.57	0.81	2.71	2.35	2.60	3.37	nv	
	no of measured values	45	90	12	19	9	20	42	0	
<b>N° of cells [per 70<math>\mu\text{m}^2</math>]</b>	mean value	74.11	46.95	5.09	68.37	21.89	120.16	116.54	nv	
	standard deviation	14.95	21.33	3.70	20.94	29.28	44.28	14.97	nv	
	maximum	114	95	10	106	100	187	135	nv	
	minimum	53	0	0	37	0	41	93	nv	
	no of measured values	45	73	11	19	65	12	13	0	
<b>FDC [%]</b>	mean value	11.78	4.15	0.33	5.49	1.66	2.73	0.03	nv	
	standard deviation	1.93	3.82	1.29	3.99	1.79	3.42	0.22	nv	
	maximum	15.71	17.14	5	12.86	3.57	14.29	1.43	nv	
	minimum	7.14	0	0	0	0	0	0	nv	
	no of measured values	45	90	15	19	3	20	42	0	
<b>Host surface coverage [%]</b>	mean value	89.80	65.13	4.52	83.97	23.83	76.33	84.13	nv	
	standard deviation	2.88	24.26	3.35	17.46	29.27	15.26	7.97	nv	
	maximum	95.80	100	9.40	100	91.11	92.10	94.20	nv	
	minimum	81.78	0	0	30.67	0	34.30	66.00	nv	
	no of measured values	45	73	11	17	65	12	13	0	



**Table S3:** Measured parameters between all treatments on the whole microzooids/ swarmers.

Total Microzooid		Experiment 1					Experiment 2		
		Microzooids			Swarmers				
Parameters	Values	<i>in situ</i>	<i>1/ 2days</i>	<b>3 days</b>	<i>in situ</i>	<i>1/ 2days</i>	<b>2 days</b>	<b>5 days</b>	<b>7 days</b>
<b>length [µm]</b>	mean value	1727.35	2110.79	1210.62	2170.48	2067.48	1429.25	1739.57	
	standard deviation	186.37	292.18	358.20	556.65	271.98	208.69	146.84	nv
	maximum	2116.86	2839.02	2580.56	3349.68	2641.13	1723.44	2323.48	nv
	minimum	1200.18	1420.18	665.42	1496.81	1476.51	894.09	1413.87	nv
	no of measured values	90	180	24	37	52	28	83	0
<b>width [µm]</b>	mean value	726.78	728.07	657.95	607.78	587.47	475.91	447.29	nv
	standard deviation	174.26	151.17	180.62	68.86	62.92	64.02	48.59	nv
	maximum	1105.32	1240.26	1337.16	777.45	768.40	610.69	678.77	nv
	minimum	381.87	443.20	455.95	497.20	485.71	305.67	336.42	nv
	no of measured values	90	180	25	37	52	40	83	0
<b>volume [µm]</b>	mean value	0.67	0.81	0.44	0.62	0.49	0.25	0.27	nv
	standard deviation	0.34	0.35	0.55	0.30	0.13	0.08	0.08	nv
	maximum	1.53	1.77	3.00	1.29	0.76	0.47	0.80	nv
	minimum	0.14	0.17	0.14	0.29	0.12	0.07	0.12	nv
	no of measured values	90	180	25	37	57	40	83	0
<b>Elongation Factor</b>	mean value	2.54	3.10	1.97	3.59	3.64	3.14	4.01	nv
	standard deviation	0.49	0.70	0.43	0.71	0.73	0.24	0.34	nv
	maximum	3.69	5.47	3.09	5.04	5.21	3.58	4.93	nv
	minimum	1.82	1.57	0.81	2.71	2.35	2.60	3.30	nv
	no of measured values	90	180	25	37	52	40	83	0
<b>N° of cells [per 70µm<sup>2</sup>]</b>	mean value	61.37	36.14	5.20	55.23	23.49	115.35	106.85	nv
	standard deviation	18.12	22.03	4.06	21.97	30.63	45.70	18.71	nv
	maximum	31.00	95	13.00	106.0	104	187	140	nv
	minimum	114	0	0	9	0	25	61	nv
	no of measured values	90	160	25	60	136	31	53	0
<b>FDC [%]</b>	mean value	13.22	5.50	0.375	5.21	1.91	3.23	0.09	nv
	standard deviation	2.48	4.57	1.43	3.91	2.14	3.01	0.40	nv
	maximum	18.57	21.43	6.25	12,86	10	14.29	2.86	nv
	minimum	7.14	0	0	0	0	0	0	nv
	no of measured values	90	180	30	37	46	80	84	0
<b>Host surface coverage [%]</b>	mean value	88.98	58.37	5.48	71.89	25.16	75.57	80.92	nv
	standard deviation	3.09	28.76	4.79	26.41	29.67	16.97	16.99	nv
	maximum	95.80	100	19.09	100	91.11	99.40	96.40	nv
	minimum	81.78	0	0	11.84	0	18.5	0.0	nv
	no of measured values	90	160	25	60	136	31	54	0

**Table S4:** Parameters of the oral parts of the microzooids tested against each other under *in situ* conditions.

	# symbionts	coverage	Length	Width	EF	Volume	FDC
coverage	0.198		0.054	-0.051	0.080	-0.042	0.371*
length	-0.421**	0.054		0.586**	0.127	0.751**	-0.059
width	-0.422**	-0.051	0.586**		-0.666**	0.960**	-0.183
EF	0.190	0.080	0.127	-0.666**		-0.475**	0.232
volume	-0.438**	-0.042	0.751**	0.960**	-0.475**		-0.198
FDC	0.109	0.371*	-0.059	-0.183	0.232	-0.198	

*n*=45. \*\*\* < 0.001, \*\*<0.01; \*<0,05

**Table S5:** Parameters of the aboral parts of the microzooids tested against each other under *in situ* conditions.

	# symbionts	coverage	Length	Width	EF	Volume	FDC
coverage	0.021		-0.175	-0.048	-0.149	-0.081	0.168
length	-0.721**	-0.175		0.674**	0.209	0.866**	-0.028
width	-0.594**	-0.048	0.674**		-0.488**	0.939**	0.151
EF	.00	-0.149	0.209	-0.488**		-0.236	-0.247
volume	-0.705**	-0.081	0.866**	0.939**	-0.236		0.040
FDC	-0.165	0.168	-0.028	0.151	-0.247	0.040	

*n*=45. \*\*\* < 0.001, \*\*<0.01; \*<0,05

**Table S6:** Parameters of the oral and aboral parts of the microzooids tested against each other under *in situ* conditions.

	# symbionts	coverage	Length	Width	EF	Volume	FDC
coverage	0.266*	0.113 <sup>1</sup>	-0.131	-0.211*	0.170	-0.210*	0.059
length	-0.639**	-0.131	0.566** <sup>1</sup>	0.659**	-0.255*	0.750**	0.200
width	-0.793**	0.211*	0.659**	0.213 <sup>1</sup>	-0.877**	0.986**	0.489**
EF	0.632**	0.170	-0.255*	-0.877**	-0.877** <sup>1</sup>	-0.812**	-0.497**
volume	-0.810**	-0.210*	0.750**	0.986**	-0.812**	0.339* <sup>1</sup>	0.457**
FDC	-0.436**	0.059	0.200	0.489**	-0.497**	0.457**	0.169 <sup>1</sup>

*n*=90; *x*<sup>1</sup> = *n* of 45; \*\*\* < 0.001; \*\*<0.01; \*<0,05

**Table S7:** Parameters of the oral parts of the microzooids tested against each other under 24h and 48 h conditions.

	# symbionts	coverage	Length	Width	EF	Volume	FDC
coverage	0.854**		0.303**	-0.156	0.415**	0.025	0.137
length	0.379**	0.522**		0.161	0.603**	0.551**	0.089
width	-0.332**	-0.113	0.161		-0.657**	0.854**	0.127
EF	0.587**	0.495**	0.603**	-0.657**		-0.258*	-0.045
volume	-0.08	0.184	0.551**	0.854**	-0.258*		0.123
FDC	-0.117	0.010	0.089	0.127	-0.045	0.123	

*n*=90; *x*<sup>1</sup> = *n* of 45; \*\*\* < 0.001; \*\*<0.01; \*<0,05

**Table S8:** Parameters of the aboral parts of the microzooids tested against each other under 24h and 48 h conditions.

	# symbionts	coverage	Length	Width	EF	Volume	FDC
coverage	0,782**		0.281*	0.083	0.066	0.224	-0.007
length	0,508**	0.479**		-0.191	0.674**	0.349**	-0.104
width	-0,496**	-0.160	-0.191		-0.799**	0.443**	0.225*
EF	0.680**	0.412**	0.674**	-0.799**		-0.345**	-0.268*
volume	-0.156	0.168	0.349**	0.753**	-0.345**		0.083
FDC	-0.253	0.014	-0.104	0.225*	-0.268*	0.083	

$n=90$ ;  $x^1 = n \text{ of } 45$ ; \*\*\* < 0.001; \*\*<0.01; \*<0,05

**Table S9:** Parameters of the oral and aboral part of the microzooids tested against each other under 24h and 48 h conditions.

	# symbionts	coverage	Length	Width	EF	Volume	FDC
coverage	0,785**		0.252**	-0.138	0.309**	0.001	0.040
length	0,272**	0.475**		0.075	0.543**	0.475**	0.059
width	-0.539**	-0.166*	0.075		-0.766**	0.846**	0.276**
EF	0.636**	0.443**	0.543**	-0.766**		-0.403**	-0.217**
volume	-0,344**	0.089	0.475**	0.846**	-0.403**		0.243**
FDC	-0,236**	-0.021	0.059	0.276**	-0.217**	0.243**	

$n=90$ ;  $x^1 = n \text{ of } 45$ ; \*\*\* < 0.001; \*\*<0.01; \*<0,05

## Zusammenfassung

*Zoothamnium niveum* ist eine Art der einzelligen Wimperntierchen. Viele Einzelzellen bilden zusammen eine komplexe Kolonie, welche eine Größe von 1,5 cm erreichen kann. Der Ciliat ist ein Pionierbesiedler und seine natürlichen Lebensräume befinden sich in Flachwasserzonen von tropischen bis hin zu gemäßigten Zonen in der Nähe von austretendem Sulfid. *Z. niveum* lebt in Symbiose mit dem sulfid-oxidierenden chemolithoautotrophen Bakterien der Art *Candidatus Thiobios zoothamnicoli*, aus der Gruppe der Gammaproteobacteria. Dies ist die einzige bekannte thiotrophe Symbiose, die über mehrere Generationen kultiviert werden kann.

Im natürlichen Lebensraum sind die Ausströmungen von Sulfid begrenzt, daher wird in dieser Arbeit der Frage nachgegangen was mit den symbiotischen Partnern passiert wenn kein Sulfid verfügbar ist. Wie wirkt sich das fehlende Sulfid auf messbare Parameter der Morphologie und Fitness der Symbionten aus? Bleibt die Symbiose unter diesen Bedingungen erhalten oder zerbricht sie? Ist es möglich neue Kolonien aus Schwärmern ohne Zugabe von Sulfid zu züchten?

Um eine Antwort auf diese Fragen zu finden, wurden *in situ* Proben von Mikrozooiden und Schwärmern von *Z. niveum* ohne Zugabe von Sulfid kultiviert, dabei wurden zwei experimentelle Ansätze verfolgt. Zum einen wurden Kolonien und Schwärmer unter sauerstoffreichen stagnierenden Bedingungen gehalten, zum anderen wurden Kolonien in Inkubationskammern mit ständigem Wasserdurchfluss herangezüchtet.

Die Ergebnisse des ersten Experiments zeigten, dass unter sauerstoffreichen stagnierenden Bedingungen die Fitness der Symbionten auf den Mikrozooiden mit der Zeit abnahm. Des Weiteren wurden morphologische Veränderungen der Symbionten beobachtet. Nach drei Tagen starb der Wirt, welcher noch vereinzelt Symbionten auf der Wirtsoberfläche aufwies. Auch auf den Schwärmern war eine Abnahme der Symbiontendichte, sowie eine geringere Fitness der verbleibenden Symbionten festzustellen. Nach meinem Wissen wurden bisher keine Studien über Morphologie und Fitness der Symbionten auf Schwärmern durchgeführt. Für thiotrophe Symbiosen stellt fehlendes Sulfid einen großen Stressor dar, welcher schlussendlich das Überleben beider Partner gefährdet. Mit dem ersten Experiment wurde gezeigt, dass fehlendes Sulfid über längeren Zeitraum zum Tod des Wirts führt und somit die Symbiose zerbricht. Das zweiten Experiment zeigte, dass es auch ohne die Zugabe von Sulfid möglich ist, neue Kolonien aus Schwärmern heranzuzüchten. Der Wirt überlebte dieses

Experiment nicht nur für 7 Tage, sondern zeigte auch noch Teilung, allerdings wurde er zwischen 5-7 Tagen aposymbiotisch – auch hier zerbrach die Symbiose.

Weitere Studien werden in Zukunft zeigen, ob *Candidatus Thiobios zoothamnicoli* eigenständig zurück in die Wassersäule gelangen und sich dort teilen kann. Ob die Symbionten eigenständig den Wirt verlassen haben, um nach einem günstigeren Habitat zu suchen oder ob der Wirt die Symbionten abgestoßen hat, da die Kosten zu hoch sind einen nicht Kohlenstoff-fixierenden Symbionten zu tragen, werden zukünftige Studien zeigen müssen.

## CURRICULUM VITAE

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### Personal Information

Name: Julia Kesting

Nationality: German, Spanish

### Professional Background and Education

2012 - 2015	MSc in Ecology with main focus on Marine Biology University of Vienna, Austria
2007 - 2011	Bachelor in Coastal Zone Management Van Hall Larenstein Institute of Applied Sciences, The Netherlands
07/2010 - 08/2010	Graduate Summer Program Fisheries Ecology Reykjavík University, Iceland
09/2009 - 02/2010	Erasmus Exchange semester in Marine Living Resources, Aquaculture and Fisheries Wageningen University, The Netherlands
2005 - 2007	Professional education as Biological-Technical Assistant Berufskolleg Olsberg, Germany
2002 - 2005	Upper-stream Secondary School Kolleg Bergkloster Bestwig, Germany

### Research Activities / Internships

2014- 2015	Master Thesis at the Department of Limnology and Bio-Oceanography University of Vienna, Austria Title “Termination of the <i>Candidatus</i> Thiobios zoothanicoli <i>Zoothamnium niveum</i> symbiosis under oxic conditions“
01/2015 - 03/2015	Employed at the Department of Limnology and Bio- Oceanography University of Vienna, Austria
05/2014	2 <sup>nd</sup> Symbiomics Field Workshop Hydra Institute for Marine Science, Fetovaia, Italy
10/2013	Course on submicroscopical anatomy and preparatory techniques in electronmicroscopy University of Vienna, Austria
2012	Marine biological field course on Mediterranean fauna and flora Center for Marine Research, Rovinj, Croatia

2012	Advanced marine ecological field course Royal Netherlands Institute for Sea Research (NIOZ), Texel, The Netherlands
2011	Data collection in the Hauraki Gulf for the Bachelor Thesis Massey University, New Zealand Title “The occurrence and prevalence of body scars, skin lesions and skin abnormalities related to anthropogenic impacts towards the common dolphin ( <i>Delphinus sp.</i> ) of the Hauraki Gulf, New Zealand”
09/2010 – 01/2011	Project internship in the working group Ecology of Marine Mammals and Birds Research and Technology Centre Westcoast (FTZ), Christian – Albrecht University Kiel, Germany Project “Click communication patterns of harbour porpoises ( <i>Phocoena phocoena</i> ) in the wild”
05/2009 - 07/2009	Assistance with field and laboratory experiments Royal Netherlands Institute for Sea Research (NIOZ), Curaçao, Dutch Caribbean Project “What is the net dissolved organic matter release by different species of dominant benthic reef algae?”
02/2009 – 05/2009	Assistance with field and laboratory experiments Center for Tropical Marine Ecology (ZMT), Curaçao, Dutch Caribbean Project “ Does the brown algae <i>Lobophora variegata</i> response to changes in nutrients and light in a coral reef ecosystem?”
02/2007 - 04/2007	Internship at the department of Microbiology B. Braun AG, Melsungen, Germany

### **Skills and Qualifications**

It-Skills:	Microsoft Office, SPSS, basic knowledge of R, basic knowledge of GIS
Languages:	German, Spanish mother tongues (bilingual) Dutch, Fluent spoken and written English, Fluent spoken and written French, basic knowledge