Ecology of Giant, Sulfur-Oxidizing *Thioploca* Bacteria in Great Lakes Sediments

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ABSTRACT

Microorganisms play a key role in regulating the cycling of carbon, oxygen, nitrogen, sulfur, and other important elements in aquatic ecosystems. *Thioploca* is a giant, filamentous bacteria that oxidizes sulfide and reduces nitrate, coupling the nitrogen and sulfur cycles in its benthic habitats. *Thioploca* can achieve high abundances in marine sediment where it is known to alter nitrogen and sulfur dynamics by removing toxic sulfide and recycling fixed nitrogen back into the sediment and water column. *Thioploca* can also achieve high abundances in freshwater sediments; however, its distribution and biogeochemical function are poorly understood in freshwater environments, making it difficult to determine how it impacts elemental cycling in these habitats. To analyze *Thioploca* abundance, factors affecting its distribution, and its biogeochemical function in the Great Lakes, I quantified *Thioploca* biomass and water column and sediment characteristics at 33 sites that spanned a gradient of depth and trophic conditions in the Apostle Islands region of Lake Superior and Green Bay in Lake Michigan. Sediment cores were also collected at eight of my study sites to analyze vertical *Thioploca* biomass distribution and sediment chemistry.

Thioploca was common in both the Apostle Islands and Green Bay and reached biomasses of up to 250 g/m^2 wet weight at some sites. While PCA and logistic regression analysis indicated that *Thioploca* may be more likely to be present under eutrophic conditions, *Thioploca* was also common and abundant at some oligotrophic sites in the Apostle Islands. *Thioploca* was more abundant in fine-grained than coarse-grained sediment, suggesting *Thioploca* distribution may be linked to depositional areas of lakes.

At most sites, *Thioploca* was most abundant in the top 5 cm of sediment. Ammonia profiles in some sediment cores appear to indicate possible ammonia consumption in sediment layers with *Thioploca*, which suggests these freshwater *Thioploca* may interact with benthic nitrogen cycling differently than marine species of *Thioploca*. My results, along with other reports from the Great Lakes, suggest that freshwater *Thioploca* may be widespread throughout the Great Lakes. At the abundances observed, *Thioploca* is likely significantly influencing nitrogen and sulfur cycling in these areas, although many questions remain about *Thioploca*'s biogeochemical functioning in freshwater environments, including how it achieves high biomass in low sulfur environments, whether it reduces nitrate to ammonia or N_2, and whether it promotes the recycling of fixed nitrogen or acts as a fixed nitrogen sink.

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CHAPTER 1: *Thioploca* Literature Review

Thioploca, a genus of large, benthic, sulfur-oxidizing bacteria, has collected a few different nicknames since it was first described in 1907 (Lauterborn 1907). *Thioploca* has been called "gunpowder bacteria" for its ability to accumulate nitrate and sulfur (Teske & Nelson 2006). Its conspicuous braided filaments inspired the name "spaghetti bacteria" among researchers, and South American fishermen dubbed *Thioploca*, which comes up in their bottom trawls, "estopa" meaning waste cotton in Spanish (Teske & Nelson 2006).

Chilean and Peruvian fisherman are familiar with *Thioploca* because thick mats of it cover an area of approximately 10,000 km^2 off the Pacific coast of Chile and Peru, forming what is probably the world's largest community of visible bacteria (Jørgensen & Gallardo 1999). High abundances of *Thioploca* have also been found in some freshwater and brackish ecosystems including the Laurentian Great Lakes (Maier 1980, Dermott & Legner 2002, Høgslund et al. 2010). In regions where it achieves high abundance, *Thioploca* is thought to exert an important influence on benthic carbon, nitrogen, and sulfur cycling (Jannasch 1995, Teske & Nelson 2006). Among the large, sulfur-oxidizing bacteria, *Thioploca* have garnered particular interest for their unique morphology, high abundances, and biogeochemical influence.

Diversity

Thioploca is a genus of giant, filamentous, sulfur-oxidizing bacteria within the Gammaproteobacteria (Jørgensen & Gallardo 1999). *Thioploca* is closely related to *Beggiatoa* and *Thiomargarita*, two other genera of large sulfur-oxidizing bacteria (Teske

& Nelson 2006, Salman et al. 2011). *Thioploca* can be found in marine, freshwater, and brackish sediments, although the marine species are morphologically and phylogenetically different from the freshwater and brackish species (Teske & Nelson 2006, Salman et al. 2011). Based on phylogenetic analysis, it has been suggested that the marine *Thioploca* species be grouped under a new genus *Candidatus* Marithioploca, separate from the freshwater species (Salman et al. 2011).

Different *Thioploca* species are distinguished primarily by the width of their trichomes (filaments composed of chains of cells). Two marine species are officially recognized: *T. chileae* (12 – 20 µm diameter trichomes) and *T. araucae* (30 – 43 µm diameter trichomes) (Maier & Gallardo 1984). A smaller species of marine *Thioploca* (*T. marina*: 2.5 – 5 µm diameter trichomes) has also been observed but has not been officially recognized (Jørgensen & Gallardo 1999). Three different species of freshwater/brackish *Thioploca* have been described: *T. schmidlei* (5 – 9 µm diameter trichomes), *T. ingrica* (2 – 4.5 µm diameter trichomes), and *T. minima* (1 – 2 µm diameter trichomes) (Lauterborn 1907, Wislouch 1912, Jørgensen & Gallardo 1999). *Thioploca ingrica* is the only species that has been reported in North America and is the most commonly reported freshwater *Thioploca* species (e.g., Maier 1980, Nishino et al. 1998, Dermott & Legner 2002). *T. ingrica* from Lake Biwa and Lake Constance have been shown to be genetically closely related (Kojima et al. 2003)

Morphology

Thioploca, meaning "sulfur braid," was named for its unique morphology (Lauterborn 1907). *Thioploca* trichomes live together in gelatinous polysaccharide sheaths, with each sheath containing up to 100 trichomes that sometimes twist around one another and appear braided (Fig. 1; Lauterborn 1907, Schulz et al. 1996, Teske & Nelson 2006). Filaments of different *Thioploca* species can inhabit the same sheath in populations where multiple species are present (Jørgensen & Gallardo 1999). The trichomes are uniseriate and are made of cylindrical disk-shaped cells (Lauterborn 1907). *Thioploca* trichomes have tapered terminal cells (Lauterborn 1907). Outside of these sheaths, *Thioploca* trichomes are essentially morphologically indistinguishable from the trichomes of the closely related *Beggiatoa* (Salman et al. 2011). *Thioploca* sheaths can be up to 20 cm long and 500 µm in diameter (Jørgensen & Gallardo 1999). Individual trichomes range from 2 – 5 cm in length and 2.5 – 80 µm in diameter (Teske & Nelson 2006). *Thioploca* sheaths have striations, along which filamentous epibiotic bacteria can be found (Fukui et al. 1999).

Thioploca is conspicuous in ecosystems where it is abundant. Marine *Thioploca* communities have been described as looking like "white lawns" or "slimy grass" where the trichomes protrude from the sediment (Gallardo 1977, Rosenberg et al. 1983). Fishermen have reported *Thioploca* clinging to nets and bottom trawls off the coast of South America and in Lake Biwa (Nishino et al. 1998, Jørgensen & Gallardo 1999). *Thioploca* filaments can be seen with the naked eye in split-core examinations of sediment with low densities of *Thioploca* (Høgslund et al. 2010).

Physiology and Biogeochemistry

  Thioploca oxidizes sulfide and reduces nitrate, coupling the nitrogen and sulfur

cycles in its benthic habitats (Jørgensen & Gallardo 1999). *Thioploca* is microaerophilic,

or possibly preferentially anaerobic, although aerobic metabolism has been detected in

marine *Thioploca* (Jørgensen & Gallardo 1999, Teske & Nelson 2006, Høgslund et al.

2009). A full understanding of *Thioploca* energy and carbon metabolism remains elusive

because *Thioploca* has not yet been successfully grown in pure culture (Jørgensen &

Gallardo 1999).

Nitrate Vacuoles and Migration

  Marine *Thioploca* cells have large vacuoles (up to 80% of the cell volume) in

which they can store nitrate at concentrations exceeding 0.5 M (or up to 20,000 times the

ambient concentration) (Fossing et al. 1995). The vertically oriented sheath structure of

Thioploca allows the trichomes to migrate up and down in the sediment between upper

sediment layers with nitrate and lower sediment layers with sulfide (Huettel et al. 1996).

The vacuoles in *Thioploca* act as an "anaerobic lung" and allow *Thioploca* to transport

the nitrate downward in the sediment to layers with abundant sulfide where *Thioploca*

oxidizes the sulfide and reduces the stored nitrate (Fossing et al. 1995, Otte et al. 1999).

This strategy gives *Thioploca* a competitive advantage over other sulfur oxidizers in

sediment where nitrate and sulfide pools do not overlap (Høgslund et al. 2010). Although

large marine *Beggiatoa* also have nitrate vacuoles, *Beggiatoa* filaments are less vertically

mobile than *Thioploca*, so *Beggiatoa* requires either simultaneous or alternating presence

of sulfide and oxygen or nitrate (Jørgensen & Gallardo 1999, Teske & Nelson 2006). Nitrate vacuoles have also been identified in freshwater *Thioploca* from Lake Baikal but were lacking in *Thioploca* from other freshwater and brackish environments (Zemskaya et al. 2001a, Høgslund et al. 2010). However, freshwater and brackish *Thioploca* that lack conspicuous vacuoles can still accumulate nitrate intracellularly, although at much lower concentrations than marine species (Kojima et al. 2007, Høgslund et al. 2010).

Marine *Thioploca* demonstrate a positive chemotactic response to nitrate, nitrite, and low sulfide concentrations and a negative chemotactic response to oxygen (>15 µM) and high sulfide concentrations (≥150 µM) (Huettel et al. 1996, Zopfi et al. 2001). Marine *Thioploca* filaments move 1 – 3 µm/second (up to 10 cm/day), and freshwater *Thioploca* from Lake Baikal have been measured moving up to 8.18 µm/second (Jørgensen & Gallardo 1999, Zemskaya et al. 2001a). This migration behavior causes *Thioploca* to orient themselves vertically in the sediment, forming mats that are several cm thick and can extend more than 20 cm down into the sediment (Huettel et al. 1996). *Beggiatoa*, on the other hand, are generally limited to a thin layer at the oxic/anoxic interface (Teske & Nelson 2006). However, in some freshwater ecosystems it has been suggested that *Thioploca* may be able to obtain nitrate from other microbes or to take it up around worm burrows, reducing the necessity of migration up to nitrate-rich sediment layers and aiding in survival in low nitrogen systems (Zemskaya et al. 2001b, Høgslund et al. 2010, Chernitsyna et al. 2016).

Thioploca *and Nitrogen Cycling*

In the oxygen minimum zone off the Pacific coast of South America, extensive *Thioploca* mats are thought to contribute significantly to global nitrogen cycling (Jannasch 1995). Marine *Thioploca* reduce nitrate to ammonia, causing ammonia to accumulate at high rates in those sediment layers (Thamdrup & Canfield 1996, Otte et al. 1999). The ammonia produced by *Thioploca* can then be recycled into the water column, assimilated by other bacteria, or reoxidized to nitrate (Farias et al. 1996, Farias 1998, Otte et al. 1999). Thus, marine sediments with abundant *Thioploca* can become nitrogen sources rather than denitrifying nitrogen sinks, like most marine sediments (Thamdrup & Canfield 1996, Teske & Nelson 2006, Brandes et al. 2007). The ammonia produced by *Thioploca* may be enough to significantly impact primary productivity in the overlying water (Zopfi et al. 2001, Sommer et al. 2016). Large populations of marine *Beggiatoa* also reduce nitrate to ammonia and can have similar biogeochemical impacts (Graco et al. 2001).

Anammox bacteria have recently been discovered living in a consortium with marine *Thioploca* in the Soledad basin at the Mexican Pacific Margin (Prokopenko et al. 2013). These anammox bacteria promote fixed nitrogen loss from the sediment and account for more than half of the benthic N_2 production, counteracting the production of ammonia by *Thioploca*. (Prokopenko et al. 2013). Associations with anammox bacteria are not ubiquitous among *Thioploca* populations, as a study of the Chilean *Thioploca* did not reveal N_2 production by anammox bacteria (Høgslund et al. 2009). However, ammonia produced by *Thioploca* and released from the sediment can contribute

significantly to pelagic anammox activity, thus indirectly contributing to fixed nitrogen loss in the water column (Dale et al. 2016, Sommer et al. 2016).

While marine *Thioploca* reduces nitrate to ammonia, recent genomic and proteomic evidence suggests that freshwater *Thioploca* species may reduce nitrate to dinitrogen gas (Otte et al. 1999, Kojima et al. 2015). Genetic analysis of *Thioploca* from Lake Okotanpe found genes necessary for respiratory nitrate reduction to dinitrogen gas, but not the necessary genes for dissimilatory nitrate reduction to ammonia (Kojima et al. 2015). If freshwater *Thioploca* species reduce nitrate to N_2, they may contribute to nitrogen loss from the sediment. However, in Lake Baikal high ammonia concentrations have been associated with *Thioploca*-inhabited sediments, and anammox bacteria have been discovered in the sheath community of *Thioploca* in Lake Baikal, suggesting these freshwater *Thioploca* may be producing ammonia (Chernitsyna et al. 2016). If these freshwater *Thioploca* are producing ammonia, they may be indirectly enabling nitrogen loss from the sediment through a consortium with anammox bacteria (Chernitsyna et al. 2016). Recent genome sequencing of *Thioploca ingrica* also revealed multiple potential nitrogen assimilation pathways, indicating *T. ingrica* is capable of assimilating nitrate, ammonia, N_2, and organic nitrogen (Kojima et al. 2015).

Thioploca *and Sulfur Cycling*

Thioploca oxidizes sulfide first to elemental sulfur and then to sulfate (Otte et al. 1999). *Thioploca* trichomes contain numerous elemental sulfur inclusions with concentrations of 200-300 µmol/cm^3, which act as a stored energy reserve (Jørgensen &

Gallardo 1999). These sulfur globules are visible even in *Thioploca* from low sulfur

freshwater environments (Fig. 1; Kojima et al. 2007).

Very high phytoplankton productivity in marine upwelling areas with abundant

Thioploca leads to high organic matter sedimentation (Jørgensen & Gallardo 1999). This

organic matter supports the reduction of sulfate to sulfide by bacteria like *Desulfonema*,

resulting in high sulfate reduction rates in the sediment (Jørgensen & Gallardo 1999).

These sulfate reducers provide sulfide that can be utilized by *Thioploca* (Jørgensen &

Gallardo 1999). The upper layers of *Thioploca*-inhabited sediment have sulfate reduction

rates up to 1500 nmol/cm^3/day, which are some of the highest rates measured in marine

sediment (Thamdrup & Canfield 1996, Ferdelman et al. 1997, Jørgensen & Gallardo

1999).

In marine environments *Thioploca* helps detoxify the sediment of sulfide and

reduces sulfide flux into the water column (Jørgensen & Gallardo 1999, Teske & Nelson

2006). Dale et al. (2016) hypothesized that *Thioploca* can decrease the frequency of

sulfidic events in the Peruvian oxygen minimum zone by continuing to oxidize sulfide

with intracellularly stored nitrate, even when nitrate is depleted in the bottom water.

Although sulfate reduction rates in marine sediment with *Thioploca* are very high,

Thioploca can oxidize sulfide rapidly and efficiently enough that sulfide concentrations

in the sediment remain low (5 – 50 µM), and there is little depletion of sulfate in the top

several cm of sediment (Thamdrup & Canfield 1996, Ferdelman et al. 1997, Jørgensen &

Gallardo 1999). Most studies suggest *Thioploca* accounts for 20-30% of the sulfide

oxidation in marine sediment where it is present, although estimates go as high as 91%

(Otte et al. 1999, Zopfi et al. 2001). *Thioploca* likely moves the sulfide layer lower in the sediment, which helps it outcompete other sulfur-oxidizers which require overlapping pools of oxygen or nitrate and sulfide (Huettel et al. 1996).

Filamentous, sulfate-reducing *Desulfonema* bacteria have been frequently found in marine *Thioploca* sheath communities, which could provide sulfide to *Thioploca* directly and may help explain how *Thioploca* oxidizes sulfide so efficiently in sediments with low sulfide concentrations in the pore water and thus low sulfide flux into *Thioploca* sheaths (Fukui et al. 1999, Jørgensen & Gallardo 1999, Teske et al. 2009). Freshwater *Thioploca*-inhabited sediments have much lower sulfate reduction rates than marine sediments, and there are concomitantly fewer sulfate reducers present (Nishino et al. 1998, Zemskaya et al. 2001b, Kojima et al. 2007). It is unknown how high biomasses of *Thioploca* survive in these low sulfur environments (Nemoto et al. 2011). Freshwater *Thioploca* could potentially also benefit from associations with sulfate-reducing bacteria, or they may be utilizing electron donors other than sulfide (Nemoto et al. 2011). Open questions remain about the functioning of freshwater *Thioploca* in sediments with low sulfide supply.

Thioploca *and Carbon Cycling*

Marine and freshwater *Thioploca* species can use both inorganic and organic carbon sources. *Thioploca* is thought to be a facultative chemolithoautotroph, a concept that was first suggested based on Winogradsky's research on *Beggiatoa* (Otte et al. 1999, Teske & Nelson 2006). Marine *Thioploca* take up CO_2 and acetate at similar rates (Otte

et al. 1999). Marine species are also able to incorporate carbonate, amino acids, glucose,

and glycine as sources of carbon (Maier & Gallardo 1984, Otte et al. 1999). Marine

Thioploca utilizing acetate as a carbon source has an estimated biomass doubling time of

21 to 52 days (Otte et al. 1999, Schulz et al. 2000). Freshwater *Thioploca* is also capable

of incorporating both bicarbonate and acetate (Høgslund et al. 2010, Kojima et al. 2015).

Although methanotrophs have been observed on the outside of *Thioploca* sheaths in Lake

Baikal, and methylotrophy has been suggested for marine *Thioploca*, it appears that

neither marine nor freshwater *Thioploca* species are capable of incorporating methane

(Jørgensen & Gallardo 1999, Kojima et al. 2015).

Thioploca *and Phosphorus Cycling*

Beggiatoa and *Thiomargarita*, other large sulfur-oxidizing bacteria closely related

to *Thioploca*, have the capacity to accumulate high concentrations of polyphosphate

(Schulz et al. 1999, Schulz & Schulz 2005, Brock & Schulz-Vogt 2011). *Thiomargarita*

and *Beggiatoa* can also release phosphate into the water column under anoxic and

sulfidic conditions, which could increase primary productivity and contribute to

eutrophication (Schulz & Schulz 2005, Brock & Schulz-Vogt 2011, Dale et al. 2013).

Genetic analysis of *Thioploca ingrica* revealed genes involved in the formation of

polyphosphate granules (Kojima et al. 2015). However, polyphosphate accumulation was

not detected in marine or freshwater *Thioploca* by Høgslund et al. (2009) or Nemoto et

al. (2011). Large phosphate releases have been observed from sediment with *Thioploca*,

but they were determined not to be related to *Thioploca* and were attributed instead to

deposition and mineralization of organic detritus (Holmkvist et al. 2010). Although polyphosphate accumulation has not been observed in *Thioploca*, there is not yet enough evidence to conclusively dismiss it.

Biogeochemical Significance of Thioploca

In areas where it is abundant, *Thioploca* can alter sediment biogeochemistry in several important ways. The extensive *Thioploca* mats inhabiting the oxygen minimum zone of the Pacific coast of South America are thought to contribute significantly to global nitrogen cycling (Jannasch 1995). Marine *Thioploca* turn the sediment into a source rather than a sink of fixed nitrogen, potentially supporting carbon fixation by primary producers in the water column (Teske & Nelson 2006, Gallardo et al. 2013). Marine *Thioploca* also remove significant amounts of sulfide from sediment with very high sulfate reduction rates, detoxifying the sediment of sulfide and potentially reducing the frequency of sulfidic events (Jørgensen & Gallardo 1999, Dale et al. 2016). The impact of freshwater *Thioploca* on sediment biogeochemical dynamics is so far unclear. It remains to be determined if freshwater *Thioploca* mats act as a source or a sink of fixed nitrogen and if they support anammox activity like the marine species. It is also unclear how *Thioploca* achieves high biomasses in freshwater sediments with low sulfate reduction rates and low sulfide concentrations and how freshwater *Thioploca* may interact with sulfate reducing bacteria.

Distribution

Marine Distribution

Marine *Thioploca* form extensive mats (up to 1000 g/m^2 wet weight) along 3000 km off the coast of Chile and Peru, extending over an area of 10,000 km^2 (Gallardo 1977, Jørgensen & Gallardo 1999). These mats form in the oxygen minimum zone under the Peru-Chile Subsurface Countercurrent where high organic productivity in the water column causes oxygen depletion in the bottom water (Gallardo 1977, Jørgensen & Gallardo 1999, Teske & Nelson 2006). These conditions produce organic-rich sediments with very high sulfate reduction rates where *Thioploca* can take advantage of high concentrations of nitrate and sulfide in the bottom water and sediment (Gallardo 1977, Jørgensen & Gallardo 1999, Teske & Nelson 2006). Most of the research on *Thioploca* has focused on the South American marine populations. *Thioploca* has also been found in similar upwelling regions off the coasts of Pakistan, Namibia, and Baja California (Gallardo et al. 1998, Schmaljohann et al. 2001, Prokopenko et al. 2006, Prokopenko et al. 2013), as well as in association with cold seeps in the Gulf of Mexico, where sulfate reduction is driven by geogenic methane flux (Robinson et al. 2004).

Marine *Thioploca* biomass fluctuates seasonally, with biomass decreasing in winter when there is more oxygen in the overlying water and increasing in summer when oxygen is depleted, and organic matter deposition is high (Gallardo et al. 1995). *Thioploca* can become scarce during other deep-water oxygenation events, such as during El Niño, when influxes of nutrient-poor water decrease primary production in the water column, and oxygen concentrations increase in the bottom water (Gutiérrez et al. 2008,

Gallardo et al. 2013). Strong oxygen deficiency in the bottom water, and concomitant

nitrate depletion, as can occur during La Niña, can also cause *Thioploca* populations to

decrease (Gutiérrez et al. 2008).

Freshwater Distribution

Although *Thioploca* was first described in a freshwater system (Lake Constance;

Lauterborn 1907), freshwater microbiologists have paid relatively little attention to the

genus. *Thioploca* has been reported in three of the Laurentian Great Lakes, Lake Baikal,

and several lakes in Japan and central Europe (Maier & Preissner 1979, Maier 1980,

Nishino et al. 1998, Zemskaya et al. 2001a, Dermott & Legner 2002, Dermott et al. 2005,

Nemoto et al. 2011). *Thioploca* has also been found in brackish fjords and lakes in

Denmark and in one tropical lake in Cambodia (Høgslund et al. 2010, Nemoto et al.

2012). Although freshwater and brackish *Thioploca* abundances are often low compared

to marine populations, *Thioploca* in eastern Lake Ontario and the Bay of Quinte form

mats comparable in wet weight to some marine sites (Fossing et al. 1995, Schulz et al.

1996, Dermott & Legner 2002, Dermott et al. 2005).

Despite the high biomasses of *Thioploca* reported from some freshwater

ecosystems, few studies have explored the distribution and biogeochemistry of freshwater

Thioploca populations, and its ecological niche is not well understood. Marine *Thioploca*

distribution patterns suggest that *Thioploca* should be more abundant in areas of low

oxygen concentrations in the water column and sediment with high organic content and

high sulfate reduction rates. This hypothesis has gained some support from a study in

Lake Biwa that suggests increased sulfate reduction caused by eutrophication may

increase *Thioploca* abundance (Nishino et al. 1998). Dermott et al. (2005) also suggested

that recent increases in *Thioploca* in Lake Ontario could be related to increased sediment

organic content related to invasion of dreissenid mussels and a decline in native

bioturbating *Diporeia* amphipods.

Freshwater *Thioploca* abundance often seems to be patchy both spatially and

temporally. In Lake Baikal, Lake Okotanpe, and Danish brackish water, *Thioploca*

biomass has been observed to vary considerably over small areas, sometimes by an order

of magnitude within 1 meter (Zemskaya et al. 2001a, Høgslund et al. 2010, Nemoto et al.

2011). Freshwater *Thioploca* populations can also appear and disappear unpredictably

and sometimes quite rapidly. Of three locations in Lake Constance that had previously

supported abundant *Thioploca*, Maier and Preissner (1979) found only sparse *Thioploca*

in one location and no *Thioploca* in the other two locations. Similarly, *Thioploca*

populations observed in Lake Erie in 1960 declined to below detection by 1980 (Maier

1980). At one location in eastern Lake Ontario, *Thioploca* biomass increased by three

orders of magnitude over five years (Dermott & Legner 2002). This patchiness makes it

difficult to estimate the total *Thioploca* biomass in a system at a given time and thus

difficult to determine its biogeochemical impacts.

Questions remain about the controls of freshwater *Thioploca* abundance and its

ecological niche. Understanding the conditions under which freshwater *Thioploca* is

likely to be abundant and understanding the causes of its spatial and temporal patchiness

are important to determining the extent to which freshwater *Thioploca* influences

biogeochemical cycling in the sediments it inhabits. Questions also remain about *how* freshwater *Thioploca* interacts with sediment biogeochemistry, even though at the abundances observed it is likely to be involved in the cycling of large portions of the nitrogen and sulfur in the sediment. It is currently unclear how freshwater *Thioploca* affects nitrogen cycling. Does it produce ammonia and recycle it back into the water column, potentially supporting phototrophic primary production, like marine *Thioploca*? Or does freshwater *Thioploca* reduce nitrate to N_2 and act as a sink for fixed nitrogen? Is freshwater *Thioploca* associated with anammox activity? It is also unknown how freshwater *Thioploca* survives in sediments with low sulfate reduction rates and sulfide concentrations compared to marine sediments inhabited by *Thioploca* (Nemoto et al. 2011). Does freshwater *Thioploca* live in consortia with sulfate reducing bacteria? Can freshwater *Thioploca* utilize electron donors other than sulfide? A better understanding of the biogeochemical functioning of freshwater *Thioploca* is crucial to understanding its impacts on the sediments it inhabits. In my work, I address some of the uncertainties about freshwater *Thioploca* abundance, distribution, and biogeochemical significance.

CHAPTER 2: Ecology of Giant, Sulfur-Oxidizing *Thioploca* Bacteria in Great Lakes Sediments

INTRODUCTION

Microorganisms play a key role in regulating Earth's biogeochemical cycles (Paerl & Pinckney 1996, Strom 2008). In freshwater and marine sediments, microbes mediate fluxes of carbon, oxygen, phosphorus, nitrogen, sulfur, and other elements with important consequences for ecosystem function (Falkowski et al. 2008, Wasmund et al. 2017). Large sulfur-oxidizing bacteria, including *Thioploca* and *Beggiatoa*, have garnered particular interest not only because of their conspicuousness, but also because they are active in coupling the nitrogen and sulfur cycles in their benthic habitats (Mußmann et al. 2007, Wasmund et al. 2017). *Thioploca* in particular can have a significant influence on biogeochemical dynamics in benthic communities where it can form extensive mats and dominate benthic habitats (Teske & Nelson 2006).

Thioploca's unique morphology and physiology contributes to its biogeochemical impact. *Thioploca* are giant, filamentous, sulfur-oxidizing bacteria (Jørgensen & Gallardo 1999, Teske & Nelson 2006). *Thioploca* cells form trichomes, or filaments, that live together in polysaccharide sheaths, with each sheath containing up to 100 trichomes (Lauterborn 1907, Schulz et al. 1996, Teske & Nelson 2006). *Thioploca* oxidizes sulfide and reduces nitrate, coupling the carbon, nitrogen, and sulfur cycles in its benthic habitats (Jørgensen & Gallardo 1999). *Thioploca* trichomes are motile and can migrate vertically in their sheaths in response to oxygen, nitrate, and sulfide concentrations in order to

utilize nitrate and sulfide in sediments where nitrate and sulfide pools do not overlap spatially (Huettel et al. 1996). *Thioploca* can store nitrate intracellularly in large vacuoles at up to 0.5 M (or up to 20,000 times the ambient concentration), which allows *Thioploca* to take up nitrate in surface sediment layers and transport it to deeper sediment layers to oxidize sulfide (Fossing et al. 1995, Schulz et al. 1996, Teske & Nelson 2006). *Thioploca* oxidizes sulfide first to elemental sulfur, which can be stored as sulfur globules in *Thioploca* trichomes and serve as an energy reserve (Jørgensen & Gallardo 1999, Otte et al. 1999). The elemental sulfur can then be further oxidized to sulfate (Otte et al. 1999).

Marine *Thioploca* takes advantage of high concentrations of nitrate and sulfide in the bottom water and sediment to form extensive mats (up to 1000 g/m^2 wet weight) along the coast of Chile and Peru (Gallardo 1977, Jørgensen & Gallardo 1999, Teske & Nelson 2006). These mats form in the oxygen minimum zone where high organic productivity in the water column causes oxygen depletion in the bottom water (Gallardo 1977, Jørgensen & Gallardo 1999, Teske & Nelson 2006). These conditions produce organic-rich sediments with high nitrate concentrations and with very high rates of sulfate reduction to sulfide (Gallardo 1977, Jørgensen & Gallardo 1999, Teske & Nelson 2006). Most of the research on *Thioploca* has focused on these South American marine populations.

Although *Thioploca* was first described in a freshwater system, Lake Constance, relatively little is known about the freshwater species (Lauterborn 1907). *Thioploca* has been reported in three of the Laurentian Great Lakes, Lake Baikal, and several lakes in Japan and central Europe (Maier & Preissner 1979, Maier 1980, Nishino et al. 1998,

Zemskaya et al. 2001a, Dermott & Legner 2002, Dermott et al. 2005, Nemoto et al. 2011). *Thioploca* has also been found in brackish fjords and lakes in Denmark (Høgslund et al. 2010). Although freshwater and brackish *Thioploca* abundances are often low compared to marine populations, *Thioploca* in eastern Lake Ontario and the Bay of Quinte form mats comparable in wet weight to some marine sites, and *Thioploca* biomass can be several times greater than the biomass of benthic macroinvertebrates (Fossing et al. 1995, Schulz et al. 1996, Dermott & Legner 2002, Dermott et al. 2005). *Thioploca* biomass is also high relative to total benthic bacterial biomass (Schallenberg & Kalff 1993).

Despite reports of high *Thioploca* biomass from some freshwater habitats, few studies have explored the abundance and distribution of freshwater *Thioploca* populations (Dermott & Legner 2002). Marine *Thioploca* distribution patterns suggest that *Thioploca* should be more abundant in sediment with high organic matter content, high sulfate reduction rates, and low oxygen in the bottom water. Results from Lake Biwa provide support for this hypothesis by showing that increased sediment sulfate reduction associated with eutrophication may increase *Thioploca* abundance (Nishino et al. 1998). Dermott et al. (2005) also suggested that recent increases in Great Lakes *Thioploca* are related to increased sediment organic content associated with the invasion of dreissenid mussels and declines in abundance of native *Diporeia* amphipods. Understanding the ecological niche of freshwater *Thioploca*, including what controls freshwater *Thioploca* abundance and how much *Thioploca* is present in freshwater ecosystems is essential to understanding the effects of freshwater *Thioploca* on biogeochemical cycling in the

sediments it inhabits. However, only a few studies have quantified *Thioploca* biomass in freshwater ecosystems, and the drivers of freshwater *Thioploca* abundance remain poorly understood.

In areas where it is abundant, *Thioploca* can have important effects on sediment biogeochemistry. For example, the extensive *Thioploca* mats inhabiting the oxygen minimum zone of the Pacific coast of South America are thought to contribute significantly to global nitrogen cycling (Jannasch 1995). Marine *Thioploca* reduce nitrate to ammonia; thus, marine sediments with abundant *Thioploca* can become nitrogen sources rather than denitrifying nitrogen sinks (Thamdrup & Canfield 1996, Otte et al. 1999, Teske & Nelson 2006). However, recent genomic and proteomic evidence suggests that freshwater *Thioploca* species may have different effects on nitrogen cycling than marine species, reducing nitrate to dinitrogen gas instead of to ammonia, which would have the opposite effect of removing fixed nitrogen from the sediment (Kojima et al. 2015). Determining whether freshwater *Thioploca* recycles fixed nitrogen or acts as a sink for fixed nitrogen is important for understanding how freshwater *Thioploca* affects benthic nitrogen cycling.

Thioploca also impacts benthic sulfur cycling. In marine sediments with very high sulfate reduction rates, *Thioploca* contributes significantly to sulfide reoxidation, helping to detoxify the sediment of sulfide (Jørgensen & Gallardo 1999). Marine *Thioploca* mats significantly reduce sulfide flux into the water column and may decrease the frequency of sulfidic events (Teske & Nelson 2006, Dale et al. 2016, Sommer et al. 2016). Freshwater *Thioploca* mats, however, can be found in systems with 1000 times less sulfide in the

sediment than in marine sediment with *Thioploca* (Ferdelman et al. 1997, Fakhraee et al. 2017). In these freshwater ecosystems it is unknown how *Thioploca* survives with a very low supply of sulfide compared to marine ecosystems, and determining how freshwater *Thioploca* achieves high abundances in low sulfur environments is important to understanding their biogeochemical function (Nemoto et al. 2011).

The majority of research on *Thioploca* has been done on the marine species, and the ecological niche and biogeochemical function of the marine species are much better understood than those of the freshwater *Thioploca* species. However, *Thioploca* can grow to high abundances in freshwater environments, and recent studies suggest that freshwater *Thioploca* may have a different biogeochemical function than marine *Thioploca*, potentially affecting the nitrogen and sulfur cycling in sediments in different ways than the marine species. Yet, relatively little research has been done to determine the ecological niche and biogeochemical impacts of freshwater *Thioploca*. To address some of the uncertainties about freshwater *Thioploca* abundance, distribution, and biogeochemical function I studied the distribution and aspects of the biogeochemistry of *Thioploca* at 33 sites spanning a wide range of environmental conditions in the Apostle Islands area of Lake Superior and in Green Bay (Lake Michigan). I addressed the following questions: 1) How much *Thioploca* is present in the Apostle Islands and Green Bay? 2) What drives *Thioploca* distribution in these regions? 3) How does *Thioploca* interact with sediment chemistry, especially with regards to nitrogen and sulfur? Based on marine *Thioploca* distribution patterns, I hypothesized that *Thioploca* would be more abundant in more eutrophic sites and sites with higher sediment organic carbon, nitrogen,

and sulfur content. I also expected that vertical sediment profiles would show evidence of

ammonia production in layers with *Thioploca*, because marine *Thioploca* reduces nitrate

to ammonia.

METHODS

Field Methods

Study sites

33 sites in the Apostle Islands (Lake Superior) and Green Bay (Lake Michigan) were sampled between June 17 and June 28, 2019 aboard the R/V Kingfisher. Sites were chosen to span a gradient of depths – from 9 to 85 m. Sites also spanned a gradient of trophic status – from highly eutrophic sites in southern Green Bay to oligotrophic sites in the Apostle Islands (Fig. 2, Table 1).

At each site, water was collected from approximately 0.5 m below the surface and frozen for total phosphorus (TP) analysis. Secchi depth was measured as an indicator of water clarity, and a CTD (EXO2 Multiparameter Sonde, YSI, Yellow Springs, OH) profile was taken to measure temperature, oxygen, and chlorophyll concentrations throughout the water column.

Ponar sampling

At each site, a petite ponar sampler (sampling area 0.0231 m^2) was used to collect triplicate sediment samples for *Thioploca* biomass quantification, surface sediment organic carbon, nitrogen, and sulfur analysis, and visual assessment of sediment coarseness. The sediment texture in the ponar samples was examined visually and broadly categorized as fine-grained (mud, soft clay, etc.) or coarse-grained (sand, mix of sand and mud, etc.). A small sample (1-2 cm^3) for sediment CNS analysis was also collected from each site by carefully scooping off a spoonful of sediment from the top

centimeter of one ponar sample and freezing the sample in a Whirl-Pak bag until analysis. Sediment samples were rinsed through a 600 µm sieve, and retained material was preserved in 7% formalin for quantification of *Thioploca* biomass.

Sediment core collection and processing

   At a subset of sites (5 in the Apostle Islands, 3 in Green Bay, Fig. 2), triplicate sediment cores were collected with a gravity corer (80 cm long, 7 cm diameter core tubes). Sediment cores were capped and stored upright in core liners in a dark cooler in lake water until return to the lab for *Thioploca* biomass quantification and sediment and porewater chemical analyses. Care was taken not to disturb the core samples. Sediment cores were processed within 12 hours of collection.

   In the lab, one core from each site was extruded and sliced down to $10 – 20$ cm at 0.5 to 1 cm intervals for *Thioploca* biovolume quantification. The sediment slices were rinsed through a 600 µm sieve and retained material was preserved in 7% formalin. A second replicate core from each site was extruded and sliced at 0.5 to 1 cm intervals in a glove bag under a N_2 atmosphere for extraction of pore water for ammonia and sediment CNS analysis. Pore water was extracted by centrifuging the samples at 4000 rpm for 10 minutes in 50 mL centrifuge tubes. The supernatant was decanted and frozen. The residual sediment was also frozen for sediment CNS analysis. The final replicate core from each site was micro-profiled in 1 to 5 mm increments for sediment oxygen concentrations using a Unisense OX-N O_2 microsensor (1.1 mm sensor tip diameter; Unisense A/S, Aarhus, Denmark) mounted on a micromanipulator.

Sample Analyses

Water column nutrient analysis

Total phosphorus was measured in the surface water samples using the persulfate digestion method (APHA et al. 2012b). Triplicate samples were digested with a potassium persulfate solution and analyzed on a Seal AQ400 Discrete Analyzer (Mequon, WI). Standard solutions were also prepared using the same method with a stock phosphorus solution. Chlorophyll α was measured fluorometrically in-situ with a EXO2 Multiparameter Sonde (YSI, Yellow Springs, Ohio) and was averaged over the epilimnion (Table 1).

Ponar sample processing

While measuring wet weight is a common method of quantifying *Thioploca* biomass, it can be time-consuming and imprecise, especially for samples with very small amounts of *Thioploca*. To determine whether image analysis can provide a more efficient way of estimating *Thioploca* biomass, I measured *Thioploca* biomass in two ways – as wet weight and as area covered in a standardized image – and compared the results (Fig. 3).

Thioploca wet weight was determined in all ponar samples. The formalin-preserved material that was retained following sieving was rinsed with de-ionized (DI) water. *Thioploca* filaments were then sorted from debris and other material in the samples. In these rinsed samples, *Thioploca* tended to clump together into tufts that could be seen unmagnified. The tufts of *Thioploca* were removed from the rest of the sample,

and each *Thioploca* tuft was cleaned with tweezers under a dissecting microscope at 30 – 70x magnification to remove most of the other non-*Thioploca* material to the extent that was possible in a reasonable amount of time. The cleaned *Thioploca* was then transferred to a 10 cm diameter petri dish with DI water, and tweezers were used to spread the *Thioploca* into a thin layer. Samples with too much *Thioploca* to fit in one petri dish were divided into two petri dishes.

The petri dish with *Thioploca* was placed on a piece of white paper, and a standardized photograph was taken from directly above the petri dish. Using ImageJ software (Rasband 1997) each photograph was cropped around the petri dish to a square the same width as the petri dish. The photographs were then converted to 8-bit format, and *Thioploca* was measured by adjusting the color threshold to highlight and measure the percent of image area covered by *Thioploca*. The wet weight of the cleaned *Thioploca* tufts was also measured after dabbing off excess water on Kimwipes and weighing the clumps to the nearest 0.001 g. The relationship between percent image area covered by *Thioploca* and wet weight of *Thioploca* was best fit with a 2nd order polynomial model (Fig. 3). The *Thioploca* from one site, GB12, was particularly sticky and tended to adhere to the Kimwipes, which prevented the excess water from being completely removed. For those samples, wet weight was estimated from percent image area using the 2nd order polynomial equation.

Sediment core processing

Vertical distribution of *Thioploca* biovolume was determined in the sediment core slices by taking photomicrographs of preserved *Thioploca* at 75x magnification under a dissecting microscope. The formalin-preserved material from each core slice was rinsed with DI water and transferred to a Sedgewick Rafter counting slide. Each sample was carefully scanned through under a dissecting microscope, and a photomicrograph was taken of any view that included *Thioploca*. The length of the *Thioploca* bundles in each image was traced using ImageJ software, measuring the length of the traced lines.

Three different analysts worked on tracing the images. To account for observer bias, all observers regularly measured identical sets of randomly selected "quality control" images. There was good agreement among the analysts based on Pearson correlation coefficients (Fig. 4, Analyst 1 vs. 2: r = 0.96, Analyst 1 vs. 3: r = 0.94, Analyst 2 vs. 3: r = 0.96), however *Thioploca* length measurements varied among analysts for high density images. Variation in determination made by different analysts increased with increasing *Thioploca* abundance in the samples. A threshold of 54 mm of *Thioploca* per image was determined as the divergence point where *Thioploca* length measurements differed by observer (Fig. 4, average divergence between analysts for images with < 54 mm *Thioploca* = 1.3 mm, average divergence between analysts for images with > 54 mm *Thioploca* = 15.0 mm). Based on that threshold, low density images (less than 54 mm of *Thioploca* based on initial observation) were measured by one analyst, and photomicrographs with more than 54 mm of *Thioploca* visible (as

measured by the initial observer) were measured by three different analysts, and the mean of the three measurements was used.

In addition to length, average bundle width for each core slice was determined by measuring 20 random bundles from each core slice at 250x magnification in order to calculate *Thioploca* bundle biovolume. *Thioploca* biovolume (mm^3 *Thioploca* / cm^3 sediment) was calculated with the following equation, assuming the bundles to be cylindrical.

$$Thioploca\ biovolume$$
$$= Thioploca\ bundle\ length\ (mm)$$
$$* \left(\frac{Average\ Thioploca\ bundle\ width\ (mm)}{2}\right)^2 * \pi$$

Sediment pore water analysis

Pore water, extracted at 0.5 to 1 cm intervals from each core, was filtered through a .22 µm membrane syringe filter. Ammonia was measured using a benchtop phenate method (APHA 2012a). A small amount of each pore water sample was mixed with a phenol solution, a sodium nitroprusside solution, and an oxidizing solution to produce indophenol upon reacting with the ammonia in the pore water. Absorbance at 640 nm was measured in a spectrophotometer (Genesys 30 Visible Spectrophotometer, Waltham, MA). Standard solutions were also prepared using the same method with a stock ammonium solution. Duplicates were included every ten samples.

Sediment CNS content analysis

Bulk sediment from cores sliced at 0.5 to 1 cm intervals was freeze dried for 72 hours and homogenized with a mortar and pestle (rinsed with water and methanol between samples). 25 to 60 mg of homogenized sediment was measured into silver capsules (10 x 10 mm Elemental Microanalysis silver capsules) with approximately 50 µL of MilliQ water, and the samples were placed in a fumigator with a beaker of 12 M HCl for 6 to 8 hours to remove inorganic carbon. The samples were then dried completely on a hot plate at approximately 50ºC for 6 to 8 hours. The silver capsules were carefully folded shut with blunt forceps and then encased in tin capsules (9 x 10 mm Costech tin capsules) and again folded shut with blunt forceps. Standards were prepared from Costech BBOT and Leco Proximate + Coal Reference Material Part No. 502-442. The standards did not require fumigation and were measured directly into tin capsules (5 x 9 mm Costech tin capsules). The sediment samples were analyzed for organic carbon, nitrogen, and sulfur (as % element by weight) with an Elementar Vario EL Cube CHNS elemental analyzer (Ronkonkoma, NY). Duplicates were included every ten samples.

Data Analysis and Statistical Methods

All statistical analyses were done in R (version 3.6.3) and were based on site-averages for each variable. To explore a potential relationship between *Thioploca* presence and combined sediment and water quality parameters, principal components analysis (PCA) was performed including surface water TP, Secchi depth, and surface sediment percent organic carbon, nitrogen, and sulfur as predictor variables. The PCA

was based on a correlation matrix to standardize the data because the predictor variable units are not comparable. A logistic regression was performed to explore the probability of *Thioploca* presence/absence at different values of PCA component 1 using the glm function in R with a binomial distribution. Lower values of PCA component 1 indicate more eutrophic conditions (higher TP and sediment CNS and lower Secchi depth). A Welch's t-test was performed to test whether average *Thioploca* wet weight was the same in coarse- and fine-grained sediment. Pearson correlation coefficients were calculated to compare the *Thioploca* length measurements by the three different analysts.

Dissolved oxygen uptake was calculated using the oxygen profiles from the sediment cores by the method in Malkin et al. (2017). Calculations were based Fick's First law of diffusion, using the following equations:

$$DOU = (-\frac{\delta C_{O2}}{\delta x})(\emptyset)(D_S)(1000)$$

$$D_S = \frac{(\emptyset)(D_O)}{(1 - 2(ln\emptyset))}$$

where DOU is dissolved oxygen uptake (mmol/m^2/day), $\frac{\delta C_{O2}}{\delta x}$ is the oxygen concentration gradient just below the sediment water interface (µM/mm) (assumed linear and calculated using at least five points), $\emptyset$ is porosity in the top 1 cm of sediment (v/v), D_O is the oxygen diffusion coefficient (m^2/day), and D_S is the oxygen diffusion coefficient corrected for tortuosity in muddy sediment. Ammonia flux was calculated in the same way as dissolved oxygen uptake but using ammonia concentration gradients from the steepest part of the curve and ammonia diffusion coefficients. Oxygen and ammonia

diffusion coefficients were obtained from the *marelac* package in R (version 2.1.10,

Soetaert et al. 2012).

RESULTS

***Thioploca* abundance and environmental conditions**

Thioploca was observed at 16 of the 33 sites sampled (8 of the 20 sites in the Apostle Islands and 8 of the 13 sites in Green Bay). At the sites where it was present, average *Thioploca* wet weight ranged from 0.79 – 157 g *Thioploca* (m^2 sediment)$^{-1}$ in the Apostle Islands and from 1.2 – 28 g *Thioploca* (m^2 sediment)$^{-1}$ in Green Bay (Fig. 2). Within sites *Thioploca* density varied considerably with the highest variability occurring at the highest density sites (Table 2).

PCA on site water quality and sediment parameters (Secchi depth, surface water total phosphorus, and sediment organic carbon, nitrogen, and sulfur) efficiently summarized site environmental characteristics with 85% of the variation explained by the first two components (Fig. 5). Lower values of PCA components 1 and 2 correspond to higher TP, higher sediment CNS, and lower Secchi depths, all indicators of more eutrophic conditions. The PCA showed some evidence of separation between sites with and without *Thioploca* along a eutrophication gradient, with more of the *Thioploca*-present sites falling at lower values of PCA components 1 and 2, and more of the *Thioploca*-absent sites occurring at higher values of PCA components 1 and 2. A logistic regression on *Thioploca* presence and PCA component 1 showed a nearly significant pattern that *Thioploca* is more likely to be present in more eutrophic conditions (Fig. 6; Predicted logit of *Thioploca* presence = -.4093*PCA component 1 + -0.8968, odds ratio = 0.664, z = -1.92, p = 0.056). However, the pattern was not very strong, as some sites with

Thioploca were at the low end of the TP and sediment CNS range and the high end of the
Secchi depth range observed at the sites sampled.

Thioploca was present at 12 of the 13 sites that had fine-grained sediment and at
only 4 of the 20 sites that had coarse-grained sediment. A Welch's t-test revealed that the
mean *Thioploca* wet weight was higher in fine-grained sediment (t = -2.331, df = 12.035,
p = 0.038). The *Thioploca* wet weight was 30.5 ± 46.0 g/m^2 for sites with fine-grained
sediment (mean $\pm$ standard deviation (SD)) and 0.7 ± 2.2 for sites with coarse-grained
sediment (Fig. 7).

***Thioploca* vertical distribution and sediment chemistry**

Thioploca was present in 7 of the 8 sediment cores that were analyzed for
Thioploca biovolume and porewater and sediment chemistry. *Thioploca* was most
abundant in the top 5 cm of sediment in all the cores except at the GB12 site (the most
eutrophic site cored), where the *Thioploca* biovolume was relatively consistent from 1.5
to 9.5 cm deep in the sediment (Fig. 8). In the cores from the Apostle Islands, *Thioploca*
was present only in a 2 – 3 cm thick layer, while in the cores from Green Bay, the
Thioploca formed 5 – 10 cm thick layers. In cores with *Thioploca*, oxygen extended up to
approximately 2 cm deep in the sediment. In most of the cores, the sharp decrease in
oxygen occurred near the top of the layer where *Thioploca* became abundant. The
dissolved oxygen uptake rates (DOU) were low and relatively similar at most of the sites
(~2.7 – 3.7 mmol/m2/day), although the DOU was higher for the GB31 site (7.04
mmol/m^2/day) and lower for the A23 site (1.08 mmol/m^2/day), the only cored site that

didn't have *Thioploca* (Table 3). Some of the cores, most notably the GB12 core, showed

a distinct increase in porewater ammonia beginning at the bottom of the *Thioploca* layer.

The core with the highest *Thioploca* biovolume (A32) also had particularly high

porewater ammonia concentrations below the layer of *Thioploca*.

There was no clear correlation between *Thioploca* biovolume and percent organic

carbon, nitrogen, or sulfur in the sediment (Fig. 9). The two cores with the highest

Thioploca biovolume (A32 and GB12) spanned the range of some of the highest and

lowest values for sediment organic carbon, nitrogen, and sulfur in my samples. The GB12

and GB42 cores showed an increase in sulfur beginning at the bottom of the *Thioploca*

layer, however that pattern is not consistent across the other cores.

DISCUSSION

The high abundances of *Thioploca* reported from freshwater systems (Table 2) suggest that it may be biogeochemically significant to the cycling of sediment carbon, nitrogen, and sulfur. Yet, freshwater *Thioploca* is relatively understudied compared to its marine counterpart, and the drivers of freshwater *Thioploca* abundance and its potential influence on sediment chemistry remain enigmatic. The goal of this study was to shed light on the abundance, distribution, and biogeochemical function of freshwater *Thioploca* in two nearshore areas in the Great Lakes. Data from sites spanning a large trophic status gradient revealed high abundances of *Thioploca* at both oligotrophic and eutrophic locations. *Thioploca* was most abundant in the top few centimeters of fine-grained sediment, and vertical chemical profiles revealed interesting patterns consistent with ammonia consumption in some sediment layers with abundant *Thioploca*. The presence of high quantities of *Thioploca* in the low sulfur environment of the Great Lakes also raises questions about the metabolism of *Thioploca* in these environments.

Methodological Recommendations

There is currently no standard method for quantifying *Thioploca* biomass, which makes it difficult to compare biomass data from different studies. *Thioploca* biomass is most commonly measured as wet weight in g/m^2 (e.g., Schulz et al. 1996, Dermott & Legner 2002) or as biovolume (e.g., Høgslund et al. 2010), which can be converted to wet weight assuming a density of 1 g/cm^3. However, some studies measure only trichome lengths (e.g., Nishino et al. 1998) or measure wet weight as mg *Thioploca* / kg sediment

(Zemskaya et al. 2001a). I utilized both wet weight and biovolume methods in this study and summarize here what I have learned regarding the advantages and disadvantages of each method.

The most common method used for estimating freshwater *Thioploca* biomass involves measuring trichome biovolume under a microscope. Sediment is rinsed through a sieve, the retained material is preserved (often in 6-10% formalin), and *Thioploca* trichome or bundle length and width are measured under a microscope (Nishino et al. 1998, Nemoto et al. 2011). A minor variation involves preserving whole sediment instead of sieved material, mixing the sediment, and microscopically examining a small amount of diluted sediment (Høgslund et al. 2010). *Thioploca* biomass can also be measured as wet weight by rinsing sediment through a sieve, separating the *Thioploca* from other material, blotting to remove excess water, and weighing (Dermott & Legner 2002, Kojima et al. 2007). I used the wet weight method to measure larger amounts of *Thioploca* from ponar samples, and the biovolume method to measure smaller amounts of *Thioploca* from sediment core slices.

At most sites with *Thioploca*, the *Thioploca* was visible with the naked eye in the sieved sediment, even at the sites with the lowest densities. Høgslund et al. (2010) also noted that low densities of *Thioploca* could be detected by split-core examination with the naked eye. This indicates that visual inspection of sediment is an effective way to detect *Thioploca* presence. A 600 µm sieve was sufficient to retain *Thioploca* filaments. Formalin (7%) worked well for preservation. Individual trichomes were intact and easily identifiable after up to 15 months of storage.

I used the biovolume method to quantify *Thioploca* biomass for vertical

distribution in the sediment cores, although I measured the *Thioploca* by tracing the

bundles on photomicrographs using ImageJ software rather than measuring them directly

under a microscope. This photomicrographic method worked well and reduced the

amount of time the analyst had to spend looking through a microscope. It also allowed for

easy comparison between the measurements of different analysts. *Thioploca* tends to

clump together when sediment is rinsed through a sieve, which can be convenient for

separating *Thioploca* from other material. However, it can be difficult to separate the

trichomes again under a microscope, and the *Thioploca* in some of the photomicrographs

resembled a bowl of spaghetti, making it difficult to trace overlapping *Thioploca* bundles

accurately. Preserving and examining whole sediment samples in the manner of

Høgslund et al. (2010) rather than examining material retained on a sieve could help

avoid this problem of separating tangled clumps of *Thioploca.*

I used the wet weight method to quantify *Thioploca* biomass from ponar samples,

and I also compared it with photographic analysis of biomass. There was some

subjectivity involved in both methods. Removing excess water by dabbing *Thioploca*

tufts with Kimwipes worked well for most samples, but a few *Thioploca* samples were

very sticky, and the excess water could not be completely removed without losing

Thioploca that stuck to the Kimwipes. The photographic method was also somewhat

imprecise. I spread the *Thioploca* into a thin layer before photographing it, but the

thinness of the layer was subjective to a certain degree. Using both techniques allowed

me to intercompare and cross-verify that both approaches provide similar results (Fig. 3).

It was useful to use both methods for cases where the excess water could not be effectively removed from very sticky *Thioploca* samples.

Both the biovolume and wet weight methods have advantages. For samples that contained at least 0.2 – 0.3 g of *Thioploca*, the wet weight method works well and is much less time consuming than measuring biovolume. The photographic method can be useful as a backup for samples where measuring wet weight is problematic, but naturally could not be used alone without determining the relationship between wet weight and image area covered by *Thioploca*. The biovolume method is labor intensive and time consuming, but it is a more precise method of measuring *Thioploca* than wet weight, and it works better for very small amounts of *Thioploca*. For studies with high quantities of fairly large samples where precision is not critical, I recommend the wet weight method. However, for studies with small amounts of *Thioploca* or where precision is important, I recommend measuring biovolume.

Thioploca **Abundance and Distribution**

Thioploca was widespread across the Apostle Islands and Green Bay and reached densities of up to 250 g/m^2 wet weight. The amounts of *Thioploca* at my study sites spanned the range of *Thioploca* biomass observed in other parts of the Great Lakes and in other freshwater ecosystems and at some sites were even comparable to biomasses observed in marine systems, in which *Thioploca* significantly impacts nitrogen and sulfur cycling (Table 4). My *Thioploca* biomass estimates were also similar to, and at several sites considerably higher than, *Beggiatoa* biomass in systems where it is thought to

contribute significantly to sediment carbon and sulfur cycling (Jørgensen 1977, Mußmann et al. 2003) (Table 4). At the site with the most abundant *Thioploca* (A32), carbon in *Thioploca* accounted for approximately 1.5% of the total sediment organic carbon. The *Thioploca* abundances at my sites were also high compared to the total benthic bacterial biomass observed in other lake sediments. For sites with *Thioploca*, the average amount of carbon in *Thioploca* biomass was 1.1 g C/m^2 in the Apostle Islands and 0.34 g C/m^2 in Green Bay. At the site with the highest *Thioploca* abundance (A32), there was approximately 4.4 g C/m^2 from *Thioploca* biomass. To compare, a study of several lakes in Quebec and Ontario found average sediment bacterial biomass carbon to be 5.0 g C/m^2 (Schallenberg & Kalff 1993), and the total sediment bacterial biomass carbon in an oligotrophic lake in Germany was only 1.5 g C/m^2 (Bergtold & Traunspurger 2005). These data suggest that *Thioploca* in the Apostle Islands and Green Bay constitute a large portion of the total benthic bacterial biomass.

It is worth noting that marine *Thioploca*, freshwater *Thioploca*, and *Beggiatoa* wet weights are not perfectly comparable due to differences in morphology. Large nitrate vacuoles in marine *Thioploca* and *Beggiatoa* can constitute 80% of the cell volume, but freshwater *Thioploca* have only much smaller or no nitrate vacuoles (Fossing et al. 1995, Høgslund et al. 2010). *Thioploca* wet weight also includes the sheath material, and the proportion of the total wet weight that is sheath versus trichomes can vary (Schulz et al. 1996, Gallardo et al. 2013). Nevertheless, the high abundances of *Thioploca* I observed strongly suggest that *Thioploca* in the Apostle Islands and Green Bay (and other

freshwater environments where it achieves high biomass) have significant, but not yet well understood, effects on sediment biogeochemistry.

One goal of this study was to identify controls on freshwater *Thioploca* abundance and distribution, particularly with regard to eutrophication and sediment organic matter content. Based on the physiology of *Thioploca* and the biogeographic distribution of marine *Thioploca* and *Beggiatoa*, I expected to find more *Thioploca* in eutrophic, high sediment organic content environments. However, there was abundant *Thioploca* at oligotrophic sites, and only a weak (and not statistically significant) relationship between *Thioploca* presence and water column and sediment indicators of eutrophication.

The ecological niche of marine *Thioploca* is much better understood than that of freshwater *Thioploca*. Marine *Thioploca* are found in sediments below upwelling regions with very high phytoplankton productivity, which receive high organic matter sedimentation (Jørgensen & Gallardo 1999, Teske & Nelson 2006). This results in oxygen-depleted, nitrate-rich bottom water and very high sulfate reduction rates in the sediment, providing an ideal habitat for the microaerophilic, sulfide-oxidizing *Thioploca* (Jørgensen & Gallardo 1999). The most extensively studied marine *Thioploca* mats are located in the oxygen minimum zone off the Pacific coast of South America, but *Thioploca* is also found in similar upwelling regions off the coasts of Pakistan and Namibia (Gallardo et al. 1998, Jørgensen & Gallardo 1999, Schmaljohann et al. 2001, Teske & Nelson 2006). Marine *Thioploca* is intolerant of high oxygen concentrations,

leading to decreased abundance when the water becomes oxygenated in the winter or during other oxygenation events, like El Niño (Schulz et al. 2000, Teske & Nelson 2006).

Marine *Beggiatoa*, which are closely related to *Thioploca*, also inhabit organic-rich sediments in oxygen-poor, eutrophic coastal environments, and they can also be found in a variety of sulfide-rich environments including cold sulfide seeps and hydrothermal vents (Nelson et al. 1989, Sassen et al. 1993, Teske & Nelson 2006). Marine *Beggiatoa* is particularly abundant, forming mats several centimeters thick, in the sulfide-emanating sediment of deep-sea hydrothermal vents of the Guaymas Basin in the Gulf of California (Nelson et al. 1989). Freshwater *Beggiatoa* is also found in a variety of environments including sulfur springs, puddles, wetlands, and lake sediment (Teske & Nelson 2006). There has been particular interest in the ability of *Beggiatoa* to detoxify sediments of hydrogen sulfide, for example when growing in association with rice roots in sulfate-enriched rice paddies (Joshi & Hollis 1977). The suboxic zone in the sediment created by *Beggiatoa* and other filamentous sulfur-oxidizing bacteria can also delay euxinia after oxygen is depleted in bottom waters (Seitaj et al. 2015). *Beggiatoa* can only traverse much smaller spatial separations of electron donors and acceptors (0.5 – 2 cm) compared to *Thioploca* (which can traverse distances of 2 – 8+ cm), and thus they require either simultaneous or alternating presence of oxygen or nitrate and sulfide (Teske & Nelson 2006). *Thioploca*'s sheath structure, however, allows *Thioploca* to migrate vertically in the sediment and transport nitrate, which allows *Thioploca* to occupy sediment where nitrate and sulfide are physically separate (Jørgensen & Gallardo 1999).

The ecological niche of freshwater *Thioploca* is not yet well understood. Based on marine observations, higher freshwater *Thioploca* abundances might be expected in more eutrophic systems or systems with higher sulfate reduction rates or sediment organic matter content. However, my results and the results of other studies suggest the drivers of freshwater *Thioploca* distribution and the biogeochemical function of freshwater *Thioploca* may be different than those of marine *Thioploca*. Presence of *Thioploca* in freshwater systems has been linked to a variety of environmental conditions, but a broad understanding of the controls on *Thioploca* abundance has been elusive. Observations of *Thioploca* in Lake Biwa suggest that *Thioploca* might be more abundant under eutrophic conditions, since high organic matter availability and low oxygen levels could increase sulfate reduction rates and sulfide availability to *Thioploca* (Nishino et al. 1998, Graco et al. 2001). This would be consistent with marine *Thioploca* and *Beggiatoa*, which inhabit organic-rich, oxygen-poor sediments with high sulfate reduction rates (Jørgensen & Gallardo 1999, Teske & Nelson 2006). Marine *Beggiatoa* has even been hypothesized to contribute to eutrophication by increasing benthic ammonia production and potentially releasing large amounts of phosphate (Graco et al. 2001, Dale et al. 2013).

In contrast, *Thioploca* is also found in deep, offshore regions of Lake Baikal where the water column is not eutrophic, and the water is well oxygenated (Zemskaya et al. 2009). Lake Baikal *Thioploca* populations are often associated with cold methane seeps or low temperature vents that release sulfate- and chloride-enriched fluids (Zemskaya et al. 2001a, Zemskaya et al. 2009, Chernitsyna et al. 2016). In Lake Erie and Lake Ontario, recent increases in *Thioploca* abundance have been attributed to increased

sediment organic content and decreased sediment oxygen penetration depth resulting from the establishment of invasive dreissenid mussels and declines in abundance of native *Diporeia* amphipods (Dermott & Legner 2002, Dermott et al. 2005). *Thioploca* in Danish brackish water is associated with river outlets (that provide nitrate and organic matter) and worm burrows that allow oxygen and nitrate to extend into sediment layers that also have sulfidic spots (Høgslund et al. 2010).

In my study there was some indication of a correlation between *Thioploca* presence and eutrophic conditions (higher water turbidity, TP, and sediment CNS) in the Apostle Islands and Green Bay, but the relationship was not statistically significant. In addition, *Thioploca* was most abundant in the Apostle Islands, which are well within the oligotrophic range, indicating that freshwater *Thioploca* is able to survive in environments with low nutrient concentrations and low sediment organic matter content. It is important to note that there is not always a direct relationship between sediment CNS content and water column trophic status in aquatic systems (Pusceddu et al. 2009). In the nearshore Apostle Islands region of Lake Superior, and in other oligotrophic lakes, allochthonous organic carbon inputs can contribute to sediment organic matter content, increasing sediment CNS even where the water column is oligotrophic (Ask et al. 2009). Organic matter can also accumulate in low spots or depositional areas, a process which is not directly related to water column trophic status (Rea et al. 1981, Cole & Weigmann 1983). Potentially eutrophication could be an indirect driver of *Thioploca* presence and abundance, being related to other sediment characteristics that might be more direct drivers of *Thioploca* abundance like sediment grain size or oxygen concentration.

Marine *Thioploca* mats are associated with high sediment organic carbon content (~13 – 17% LOI), although marine *Thioploca* has also been observed in sediment with as little as ~2 – 4 % organic carbon (Huettel et al. 1996, Holmkvist et al. 2010). In the Apostle Islands and Green Bay, surface sediment organic carbon content was not related to *Thioploca* biomass (Fig. 10), nor was there a clear relationship between sediment organic carbon and *Thioploca* biomass among other freshwater ecosystems with *Thioploca*. Compared to other sites with *Thioploca* in the Great Lakes, the sediment organic carbon and nitrogen values in the Apostle Islands and Green Bay are in a similar range (~0.1 – 9% C, ~0.03 – 0.9% N) (Dermott et al. 2005). However, the highest *Thioploca* abundances in the Apostle Islands occurred at one of the sites with the lowest sediment organic carbon values observed (~1%), while a similar amount of *Thioploca* was measured in the Bay of Quinte with approximately 7% sediment organic carbon (Table 4) (Dermott et al. 2005). Lake Okotanpe had considerably greater sediment organic carbon than the Great Lakes (~14 – 23 % LOI), but only very small *Thioploca* biomass (Table 4) (Nemoto et al. 2011). In freshwater systems, sediment organic carbon content alone does not appear to be an indicator of *Thioploca* abundance.

Marine *Thioploca* also inhabits sediments with very high sulfate reduction rates, which provide abundant sulfide that is used as an energy source by *Thioploca* (Ferdelman et al. 1997). However, sulfate reduction rates also do not appear to be good predictors of freshwater *Thioploca* abundance. Sulfate reduction rates in Lake Superior (0.11 – 1.4 nmol/cm^3/day; Fakhraee et al. 2017) are considerably lower than those in Lake Biwa (11 – 28 nmol/cm^3/day; Kojima et al. 2007), but *Thioploca* biomass is higher in the Apostle

Islands than in Lake Biwa (Table 4). Sulfate reduction rates in both these lakes are much lower than those in *Thioploca*-dominated marine sediment (170 – 4700 nmol/cm^3/day), even though the highest *Thioploca* biomasses observed in the Apostle Islands are within the range of marine *Thioploca* biomass (Ferdelman et al. 1997). However, sulfate reduction rate measurements from Great Lakes sediments with *Thioploca* are lacking, and more studies of sulfate reduction in freshwater sediment with *Thioploca* are necessary to understand sulfur dynamics in *Thioploca*-inhabited freshwater sediment.

The most consistent pattern observed in my data was a negative relationship between *Thioploca* biomass and sediment particle size. *Thioploca* occurred more frequently and was present in higher quantities in fine-grained (muddy) sediment compared to coarse-grained (sandy) sediment. Fine-grained sediment is often associated with depositional areas, whereas coarse-grained sediment indicates erosional areas, suggesting that *Thioploca* is more prevalent in depositional areas (Rea et al. 1981). *Thioploca* has also been observed in depositional areas of Lake Michigan and Lake Huron, where the water column is oligotrophic (unpublished observations, T. Ozersky). Depositional areas and fine-grained sediment are also associated with higher organic matter content (Cole & Weigmann 1983, Evans et al. 1990). In this study, fine-grained sediment did have a higher organic carbon content in the surface sediment (3.5 ± 2.6 %C (mean $\pm$ SD)) than coarse-grained sediment (1.5 ± 1.5 %C), which was a statistically significant difference based on a Welch's t-test (t = -2.55, df = 17.42, p = 0.020). However, there does not seem to be a direct relationship between *Thioploca* abundance and sediment organic matter content in the Great Lakes (Fig. 10), so it is unclear why

Thioploca was more prevalent in fine-grained sediment in my samples. The organic carbon content of the sediment could be a factor, but it appears that other factors must also be involved in making *Thioploca* more abundant in fine-grained sediment. Small marine *Beggiatoa* have been found to be restricted to muddy sediments due to being unable to move through the rigid structure of sandy sediments (Jørgensen 1977). *Thioploca* also tended to be absent from sandy sites in Lake Ontario (Dermott & Legner 2002). However, *Thioploca* in Danish brackish water did not show a relationship with sediment texture and was found in organic-rich sediment of a variety of textures, from fine silt to peat and sand (Høgslund et al. 2010). It is possible that oxygen penetration depth could be deeper in sandy sediment, which could make a less suitable habitat for *Thioploca*.

Elucidating the ecological niche of freshwater *Thioploca*, including the patterns in *Thioploca* biomass is made especially difficult by the high small-scale variability in *Thioploca* presence and biomass. At some of the sites I sampled, *Thioploca* biomass varied by an order of magnitude between replicate ponar samples collected meters apart. This patchiness is consistent with observations in Lake Baikal, Lake Okotanpe, and Danish brackish water (Zemskaya et al. 2001a, Høgslund et al. 2010, Nemoto et al. 2011). *Thioploca* also has a tendency to appear and disappear unpredictably in freshwater ecosystems, including the Great Lakes (Maier & Preissner 1979, Maier 1980, Dermott & Legner 2002). For example, *Thioploca* was reported in Lake Erie in 1960, but that population had declined to below detection by 1980 (Maier 1980), and at a site in Lake Ontario, *Thioploca* biomass increased by three orders of magnitude in five years

(Dermott & Legner 2002). This spatial and temporal patchiness of *Thioploca* in

freshwater ecosystems makes it difficult to discern patterns of *Thioploca* distribution and

suggests larger scale studies may be needed to confidently determine the ecological niche

of freshwater *Thioploca* and the complicated drivers of *Thioploca* presence and

abundance.

Thioploca Biogeochemistry

This study also attempted to further our understanding of how freshwater

Thioploca interacts with sediment nitrogen dynamics and how *Thioploca* survives in low

sulfur environments. Both marine and freshwater *Thioploca* utilize nitrate as an electron

acceptor, reducing it either to ammonia or to N_2 (Teske & Nelson 2006). These two

processes (dissimilatory nitrate reduction to ammonia (DNRA) vs. complete

denitrification to N_2) are likely to have opposite effects on nitrogen cycling – either

removing fixed nitrogen (production of N_2) or recycling fixed nitrogen back into the

water column (production of ammonia) (Burgin & Hamilton 2007). Marine *Thioploca*

has been observed to produce ammonia, which accumulates in the surface layers

(Thamdrup & Canfield 1996, Otte et al. 1999). Marine *Thioploca* mats can also

contribute ammonia to the water column, making the sediment a nitrogen source rather

than sink (Farias et al. 1996, Farias 1998). Similarly, ammonia production by *Beggiatoa*

has been hypothesized to contribute to the long-term eutrophication of Concepción Bay

by recycling fixed nitrogen back into the water column (Graco et al. 2001). However,

Thioploca associated with anammox bacteria can contribute to nitrogen loss from

sediment by production of N_2 (Prokopenko et al. 2013). It is unclear whether freshwater

Thioploca reduce nitrate to ammonia or N_2. High concentrations of ammonia have also

been associated with *Thioploca* habitats in Lake Baikal, but recent genomic and

proteomic analysis suggests freshwater *Thioploca* may reduce nitrate to N_2 (Kojima et al.

2015, Chernitsyna et al. 2016).

The ammonia profiles in some of my sediment cores (notably GB12 and possibly

A32) are more consistent with consumption rather than production of ammonia by

Thioploca. *Thioploca* has been found in consortia with anammox bacteria in freshwater

and marine systems (Prokopenko et al. 2013, Chernitsyna et al. 2016), and significant

anammox activity has recently been reported in Lake Superior (Crowe et al. 2017), so it

is possible that anammox bacteria could also be responsible for the ammonia

consumption apparent in these cores. It is also conceivable that these *Thioploca* could be

utilizing ammonia rather than sulfide as an electron donor in these low sulfur

environments. Another possibility is that removal of sulfide through sulfide oxidation by

Thioploca could promote nitrification, as it does in marine sediments, although the

sulfide concentrations in these freshwater sediments may be too low to reduce

nitrification (Joye & Hollibaugh 1995).

It is also possible that the *Thioploca* are consuming ammonia as a nitrogen source

(i.e., nutrient), rather than metabolizing it as an energy source. Ammonia inputs into the

sediment come from production within the sediment and upward flux from lower

sediment layers. Upward ammonia flux was estimated at $0.17 – 0.44$ mmol/m^2/day at my

sites in the Apostle Islands and Green Bay. Assuming ammonia production to be driven

by respiration of deposited phytoplankton in the sediment at Redfield ratios, ammonia

production can be estimated as DOU/16. Thus, total estimated ammonia input (upward

flux plus production in the sediment) at my sites ranged from 0.2 – 0.5 mmol/m^2/day. We

can also estimate nitrogen assimilation by *Thioploca* based on growth rates and nitrogen

content, which have been estimated for marine *Thioploca*. Ferdelman et al. (1997)

calculated a CO_2 uptake rate of 2400 nmol CO_2/g/day of wet *Thioploca* biomass for

marine *Thioploca*. Otte et al. (1999) calculated a CO_2 uptake rate that was about three

times lower. I used the higher rate in order to be conservative in my estimates. At the

average *Thioploca* density in the Apostle Islands (~40 g/m^2 wet *Thioploca* biomass), the

CO_2 uptake rate would be 0.096 mmol CO_2/m^2/day. Marine *Thioploca* is approximately

1.9% nitrogen by dry weight after the removal of the excess nitrate stored in the vacuoles

(Thamdrup & Canfield 1996). Approximating *Thioploca* stoichiometry as CH_2O, the

nitrogen assimilation rate of *Thioploca* should be approximately 0.005 mmol N/m^2/day.

This nitrogen assimilation rate is two orders of magnitude lower than the estimates of

ammonia production in these sediments, suggesting that assimilation of ammonia by

Thioploca as a nitrogen source is not great enough to account for the depletion of

ammonia observed in some of my sediment cores.

Although there is some evidence of a potential interaction between *Thioploca* and

ammonia in the Great Lakes, the nature of that interaction is still unknown. To determine

whether these freshwater *Thioploca* mats are acting as a source or sink for nitrogen,

further studies are needed to investigate whether *Thioploca* is reducing nitrate to

ammonia or N_2. A better bioenergetic understanding of the nitrogen dynamics of

freshwater *Thioploca* would also require measurements of freshwater *Thioploca* growth rates and stoichiometry which do not currently exist. Analysis of the microbial communities associated with *Thioploca* in the Great Lakes is also necessary to identify potential interactions with anammox bacteria. Measurements of nitrate and nitrite profiles in the sediment could also help us better understand these processes. I collected and analyzed pore water samples to measure nitrate and nitrite concentrations, but I did not consider the results trustworthy enough to report. My results indicated that both nitrate and nitrite were below the detection limit for nearly all the samples, which was surprising. Li and Katsev (2014) measured porewater nitrate concentrations around 50 µM in sediment from several sites in Lake Superior. However, I did not have enough pore water to repeat the measurements to confirm the results.

Another important unknown about the biogeochemistry of freshwater *Thioploca* is how it survives and accrues high biomasses in low sulfur environments. *Thioploca* is understood to utilize sulfide as an electron source in marine sediment (Teske & Nelson 2006). This strategy makes sense in the sulfide-rich oxygen minimum zones that marine *Thioploca* species inhabit. However, those marine systems have 1000 times more sulfide in the sediment than the Great Lakes (Ferdelman et al. 1997, Fakhraee et al. 2017). This raises questions of how high abundances of *Thioploca* survive in Great Lakes sediment with low sulfide availability. Maximum observed depth-integrated sulfate reduction rates in Lake Superior sediment are approximately 0.2 mmol SO_4/m^2/day (Fakhraee et al. 2017). The energy available to microbial metabolism from sulfate reduction is approximately 4 kJ/mol SO_4, and if approximately 1000 kJ is required per mol of carbon

synthesized, then microbes can synthesize approximately 0.004 mol C-biomass per mol SO_4 (McCollom & Amend 2005, Smeaton & Van Cappellen 2018). If the stoichiometry of *Thioploca* is approximated as CH_2O, then carbon accounts for approximately one third of *Thioploca* dry weight, and *Thioploca* dry weight is approximately 8.45% of *Thioploca* wet weight (Dermott & Legner 2002). Thus, *Thioploca* could synthesize approximately 0.34 mg of wet weight biomass/m^2/day at my Lake Superior sites. I measured an average of approximately 40 g/m^2 *Thioploca* wet weight in the Apostle Islands. At this estimated rate, it would take over 300 years to accumulate the biomass of *Thioploca* I observed.

These order-of-magnitude level approximations are based on the energy from sulfate reduction, rather than sulfide oxidation, which could provide an order of magnitude more energy. And, the cycle of sulfate reduction and sulfide oxidation could be happening dozens of times within the sediment, providing more energy to the *Thioploca* than is being approximated here from only the diffusion and initial reduction of sulfate in the sediment. Marine *Thioploca* are able to take up sulfide efficiently enough to keep porewater sulfide concentrations less than 1µm (Jørgensen & Gallardo 1999). This has been attributed to rapid recycling of sulfide between *Thioploca* and sulfate-reducing bacteria inhabiting the *Thioploca* sheaths (Jørgensen & Gallardo 1999). A similar association with sulfate-reducers could also be benefitting these freshwater *Thioploca*.

However, this could also be an indication that sulfide may not be the only electron source utilized by these freshwater *Thioploca*. Potential metabolic pathways for freshwater *Thioploca* could include oxidation of sulfide or elemental sulfur with nitrate or

oxygen, or oxidation of ammonia with nitrate or oxygen, all of which could provide more energy than sulfate reduction and could potentially be energetically feasible (Table 5). These bioenergetic estimates could be improved by measurements of sulfate reduction rates and sulfide concentration from sediments inhabited by *Thioploca* in the Great Lakes. Analysis of microbial communities associated with Great Lakes *Thioploca* could also reveal potential consortia with sulfate reducing bacteria, such as *Desulfonema* (Fukui et al. 1999). Further investigation is needed to determine whether *Thioploca* is able to use other electron donors in low sulfur environments.

Conclusions

Further studies of freshwater *Thioploca* ecology and metabolism are necessary to understand the interactions of *Thioploca* with its environment. How does *Thioploca* survive in such low sulfur environments and grow to biomasses comparable with marine *Thioploca* populations? Do freshwater *Thioploca* reduce nitrate to ammonia or N_2? Do they live in association with anammox bacteria? Are they recycling ammonia back into the water column or removing nitrogen from the sediment as N_2? A deeper understanding of *Thioploca* metabolism and growth patterns in freshwater ecosystems is necessary to understand how *Thioploca* interact with biogeochemical cycling in these systems.

The abundant *Thioploca* I found in the Apostle Islands and Green Bay are among only a few instances of *Thioploca* reports in the Great Lakes (Maier 1980, Dermott & Legner 2002, Dermott et al. 2005). However, my results and previous reports suggest that *Thioploca* is widespread and locally abundant in the Great Lakes and possibly other

freshwater systems. *Thioploca* is clearly able to survive and achieve high biomass in the Great Lakes under low nutrient conditions, high water column oxygen concentrations, low sediment organic content, and low sulfate reduction rates. These findings suggest that *Thioploca* could be present and active in a much wider variety of environmental conditions than previously expected. At the quantities observed, *Thioploca* is likely contributing significantly to carbon, nitrogen, and sulfur cycling in some areas of the Great Lakes, but there are many open questions remaining about the biogeochemical functioning of *Thioploca* in low sulfur environments.
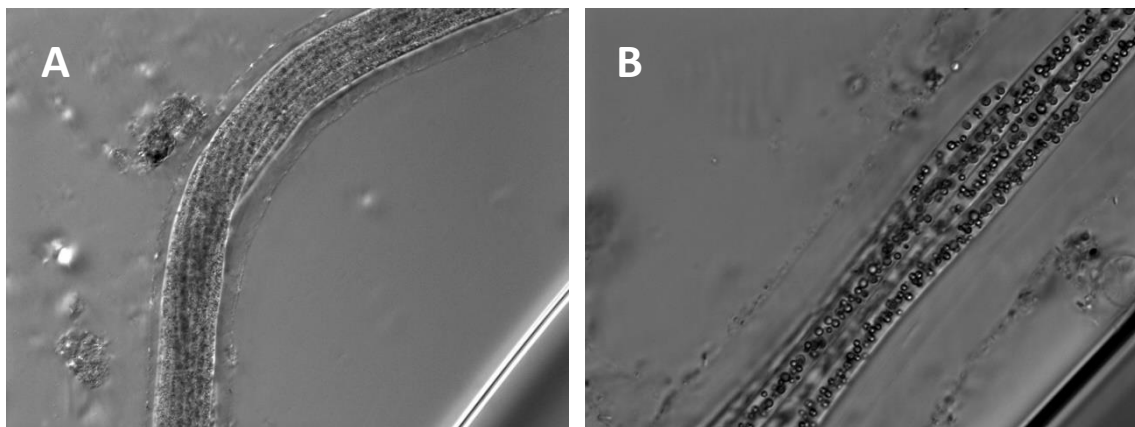
FIGURES AND TABLES

Figure 1. Photomicrographs of *Thioploca* from the Apostle Islands region of Lake Superior. A) 40x magnification, multiple braided trichomes visible. B) 100x magnification, visible sulfur globules. Courtesy of Sairah Malkin.
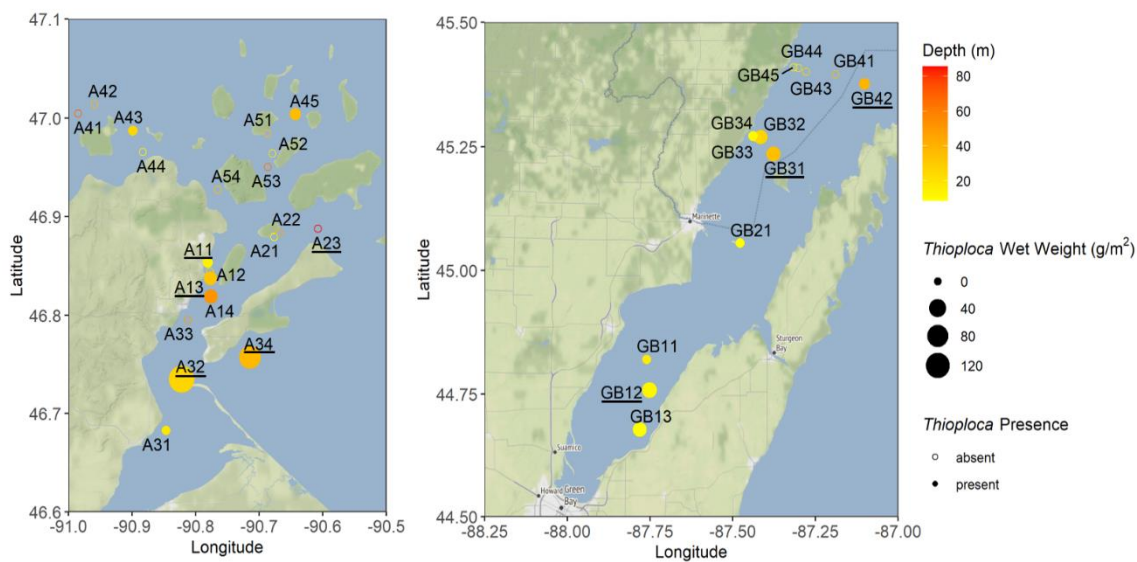

Figure 2. Water column depth (m), *Thioploca* wet weight (g/m^2), and *Thioploca* presence in the Apostle Islands (left) and Green Bay (right) during June 2019. Sediment cores were collected at underlined sites.
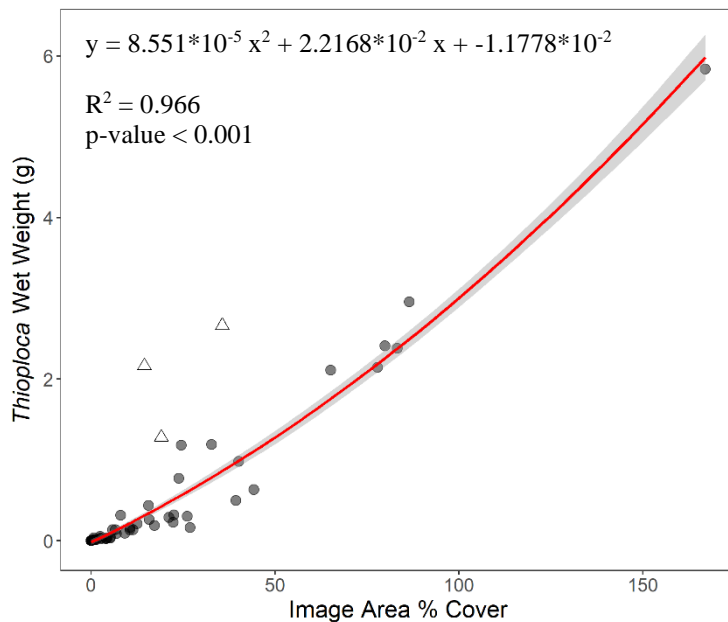
Figure 3. Percent area covered by *Thioploca* in a standardized image compared with *Thioploca* wet weight. Triangular points indicate GB12 data not included in regression model.
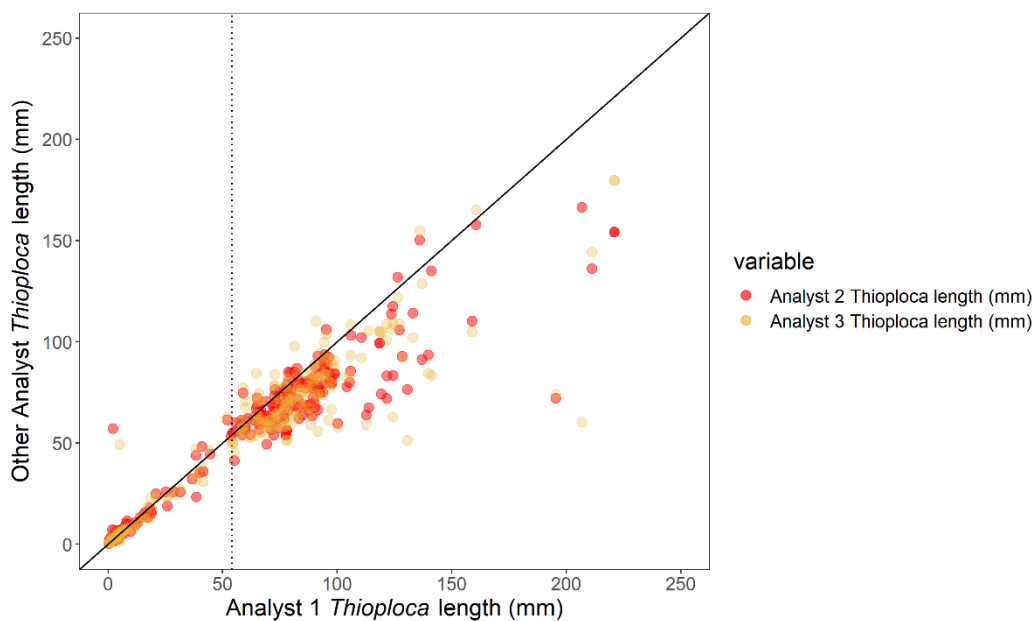

Figure 4. Comparison of *Thioploca* bundle length measurements by the three different analysts. Dotted line indicates where measurements began to diverge. Pearson correlation coefficients: Analyst 1 vs. 2: r = 0.96, Analyst 1 vs. 3: r = 0.94, Analyst 2 vs. 3: r = 0.96.
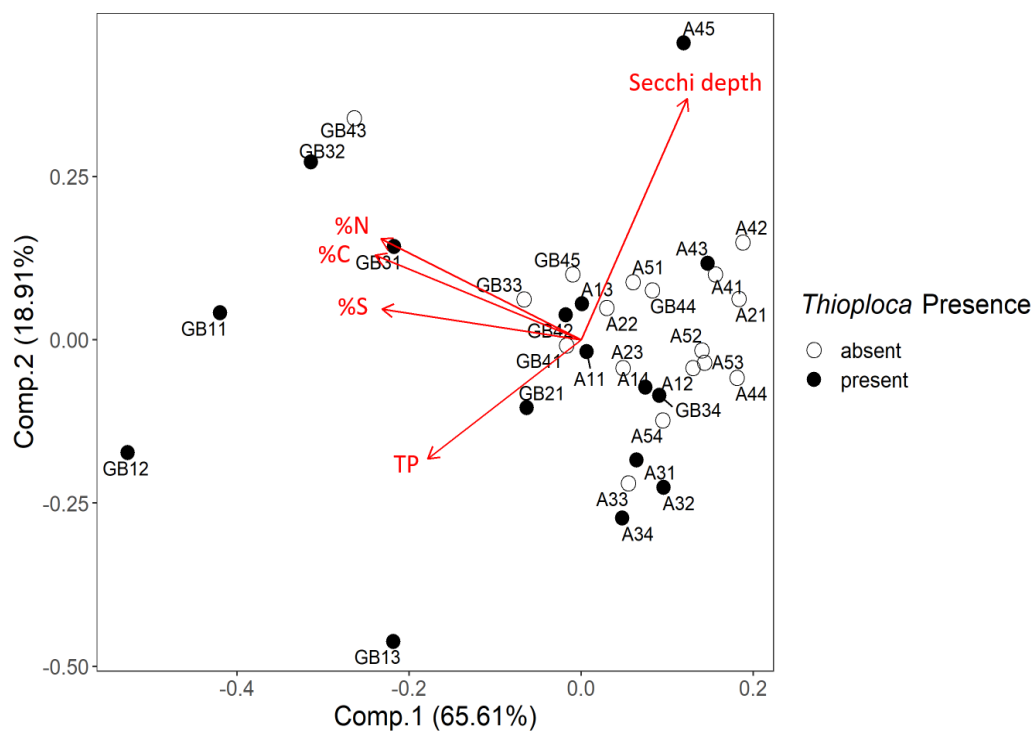
Figure 5. PCA components 1 and 2 including Secchi depth (m), surface water total phosphorus (TP) (µg/L), and sediment organic carbon, nitrogen, and sulfur (%) as predictors. Sites with *Thioploca* present are indicated by closed circles, and sites without *Thioploca* are indicated by open circles.
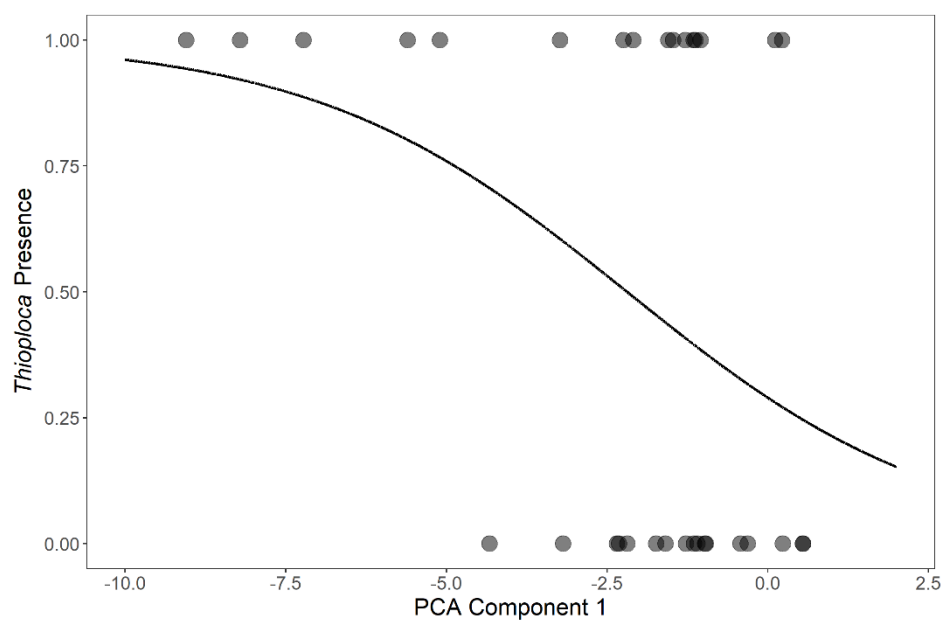
Figure 6. Logistic regression of the probability of *Thioploca* presence at different values of PCA Component 1.
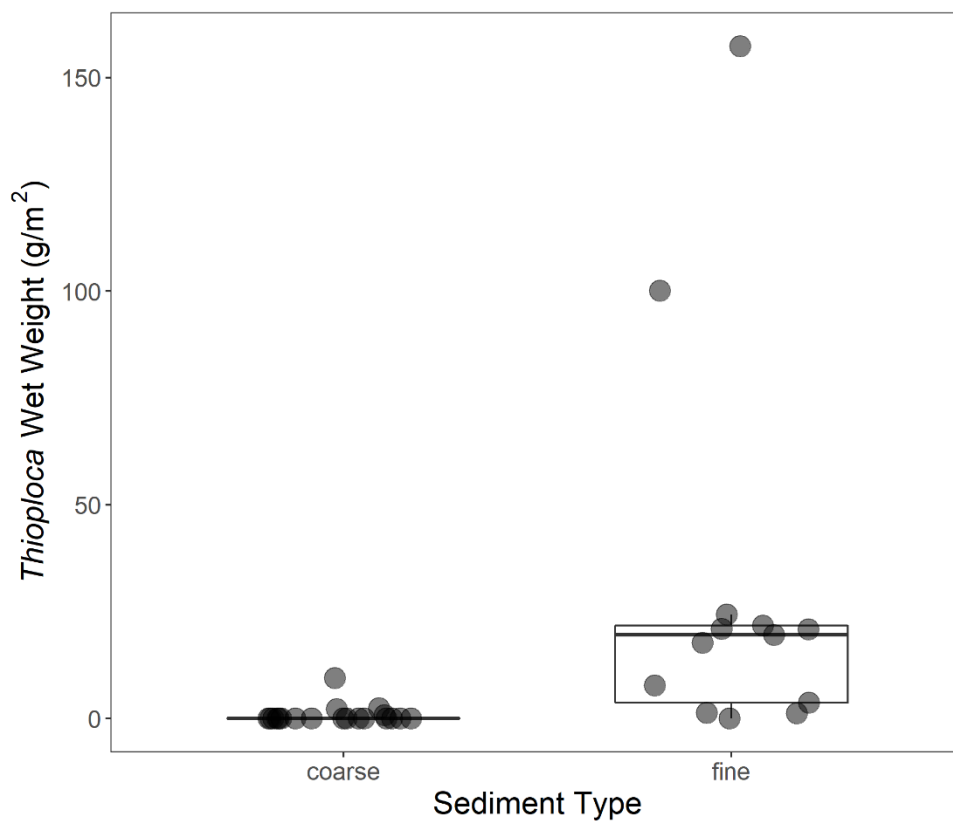
Figure 7. *Thioploca* wet weight (g/m^2) by sediment texture. Sediment was categorized as fine-grained or coarse-grained based on field observations.
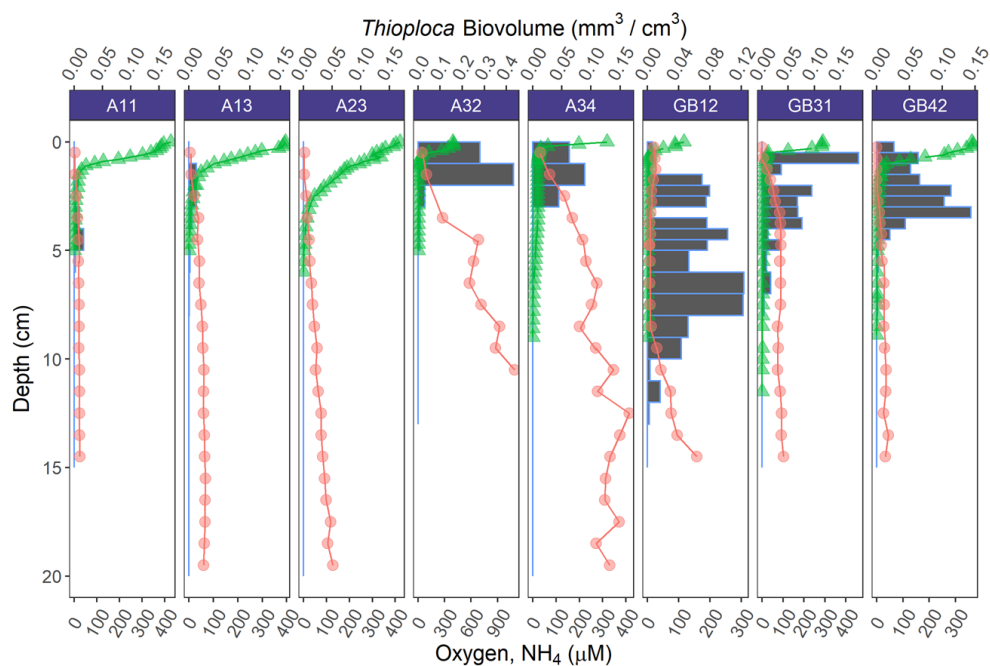
Figure 8. *Thioploca* biovolume (mm³ *Thioploca* / cm³ sediment as filled, grey bars) and porewater oxygen (triangles) and ammonium (circles) concentrations (μM) in sediment cores collected in June 2019. Note that the panels have different x-axis scales.
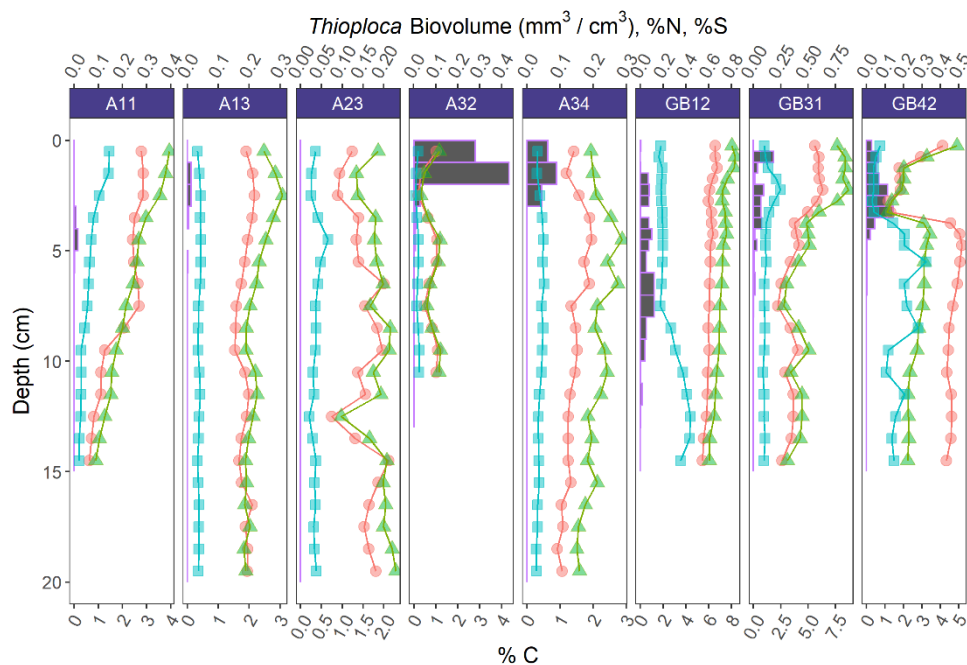
Figure 9. *Thioploca* biovolume (mm³ *Thioploca* / cm³ sediment as filled, grey bars) and sediment organic carbon (circles), nitrogen (triangles), and sulfur (squares) (as % of dry weight) in sediment cores collected in June 2019. Note that the panels have different x-axis scales.
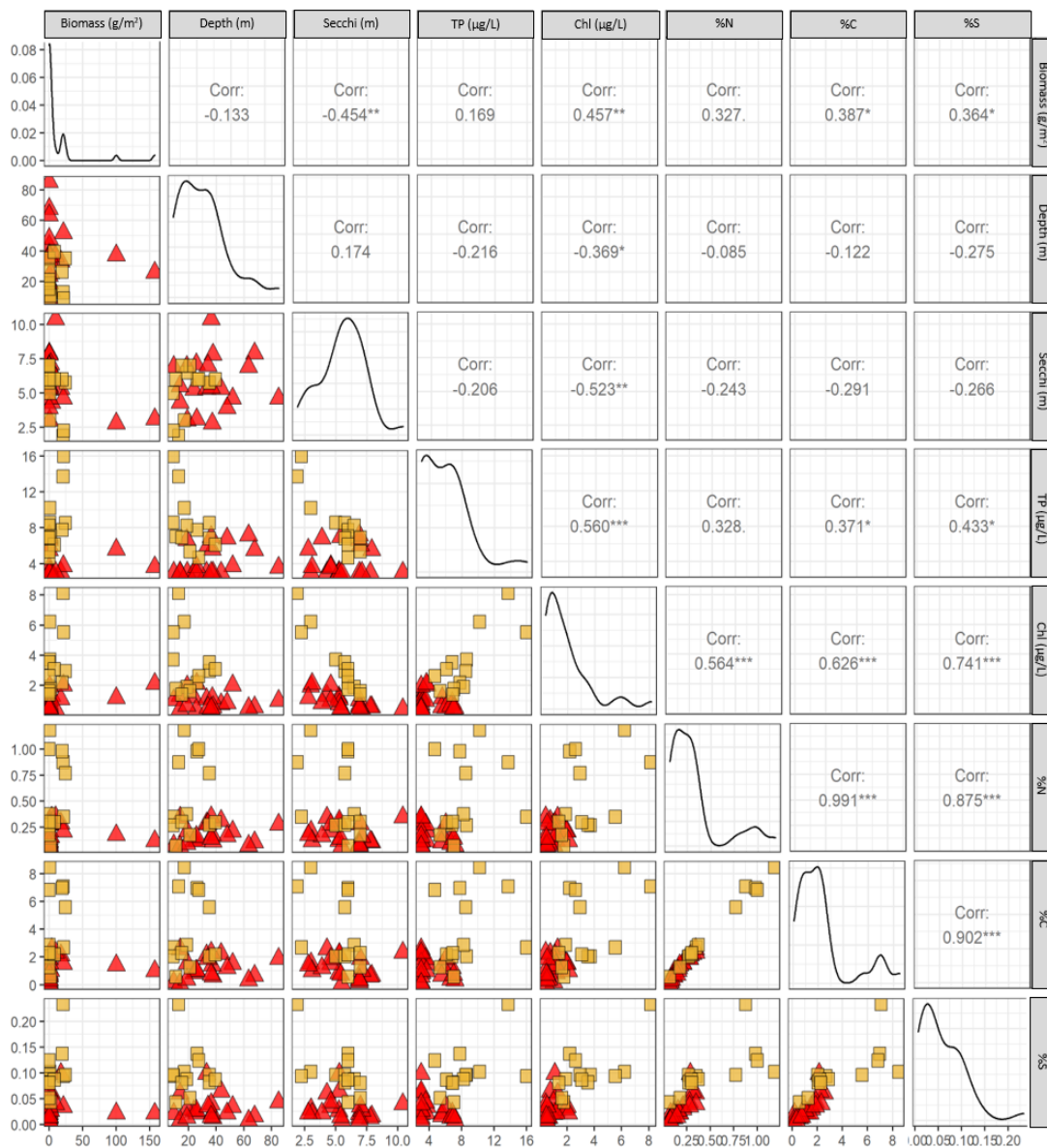
Figure 10. Spearman correlation coefficients and density plots for *Thioploca* biomass (average, g/m^2), depth (m), Secchi depth (m), total phosphorus in surface water (TP) (µg/L), sediment organic carbon, nitrogen, and sulfur content (%), and chlorophyll in the epilimnion (µg/L). Apostle Islands sites indicated by triangles; Green Bay sites indicated by squares.

Table 1. Location, water column depth (m), epilimnion Chlorophyll (µg/L), and types of samples collected at each site.

| Site	Coordinates	Depth (m)	Chlorophyll (µg/L)	Ponars	Cores
A11	46°51.220' N, 90°46.876' W	13.8	1.32	X	X
A12	46°50.66' N, 90°46.663' W	23.8	1.22	X	
A13	46°50.248' N, 90°46.621' W	34.1	1.1	X	X
A14	46°49.130' N, 90°46.544' W	52.1	2.01	X	
A21	46°52.79' N, 90°40.618' W	11.3	1.03	X	
A22	46°53.035' N, 90°40.128' W	45.1	0.76	X	
A23	46°53.274' N, 90°36.489' W	85.2	0.99	X	X
A31	46°40.980' N, 90°50.815' W	18.9	1.94	X	
A32	46°44.082' N, 90°49.307' W	25.9	2.11	X	X
A33	46°47.728' N, 90°48.753' W	47.9	0.85	X	
A34	46°45.433' N, 90°42.883' W	37.2	1.18	X	X
A41	47°00.284' N, 90°59.101' W	67.7	0.56	X	
A42	47°00.833' N, 90°57.584' W	37.5	0.56	X	
A43	46°59.236' N, 90°53.942' W	25.6	0.4	X	
A44	46°57.944' N, 90°53.007' W	13.7	0.44	X	
A45	47°00.226' N, 90°38.567' W	36.3	0.44	X	
A51	46°59.089' N, 90°41.239' W	34.1	0.54	X	
A52	46°57.857' N, 90°40.784' W	18.6	0.66	X	
A53	46°57.036' N, 90°41.209' W	63.4	0.51	X	
A54	46°55.650' N, 90°45.936' W	36.3	0.47	X	
GB11	44°49.221' N, 87°45.681' W	17	6.22	X	
GB12	44°45.450' N, 87°45.106' W	12.8	8.13	X	X
GB13	44°40.671' N, 87°46.888' W	9	5.54	X	
GB21	45°3.312' N, 87°28.670' W	8.8	3.72	X	
GB31	45°14.098' N, 87°22.611' W	35.1	2.95	X	X
GB32	45°16.109' N, 87°24.872' W	26.2	2.2	X	
GB33	45°16.050' N, 87°25.737' W	18.6	1.9	X	
GB34	45°16.278' N, 87°26.385' W	10.7	1.77	X	
GB41	45°23.731' N, 87°11.374' W	35.1	3.53	X	
GB42	45°22.564' N, 87°06.135' W	39.3	3.08	X	X
GB43	45°24.024' N, 87°16.733' W	27.4	2.62	X	
GB44	45°24.500' N, 87°18.183' W	21	1.63	X	
GB45	45°24.591' N, 87°19.007' W	15.2	1.4	X	

Table 2. *Thioploca* wet weight (g/m^2) (mean ± standard deviation), sediment texture (coarse- or fine-grained), Secchi depth (m), total phosphorus in surface water (µg/L), and sediment organic carbon, nitrogen, and sulfur content (as % of dry weight) for all sites sampled.

Site	*Thioploca* Wet Weight (g/m^2) (mean ± SD)	Coarse or Fine-grained	Secchi Depth (m)	Surf. TP (µg/L)	Sed % C	Sed % N	Sed % S
A11	3.7 ± 2.2	fine	4.3	<3.00	2.56	0.34	0.06
A12	0	coarse	5.25	<3.00	0.85	0.12	0.03
A13	17.7 ± 14.2	fine	5.3	<3.00	2.13	0.29	0.1
A14	21.0 ± 20.5	fine	4.6	<3.00	1.51	0.21	0.03
A21	0	coarse	6.9	<3.00	0.17	0.07	0.02
A22	0	coarse	5.3	<3.00	2.23	0.31	0.06
A23	0	fine	4.6	<3.00	1.87	0.27	0.04
A31	0.8 ± 0.4	coarse	3	<3.00	1.54	0.21	0.03
A32	157.4 ± 84.6	fine	3.1	<3.00	0.98	0.12	0.02
A33	0	coarse	3.9	5.52	1.21	0.16	0.02
A34	100.1 ± 6.3	fine	2.8	4.99	1.41	0.17	0.02
A41	0	coarse	7.9	5.2	0.65	0.1	0.02
A42	0	coarse	7.8	<3.00	0.6	0.11	0.01
A43	2.2 ± 3.3	coarse	7.1	<3.00	1.02	0.16	0.03
A44	0	coarse	5.5	<3.00	0.08	0.06	0.01
A45	9.5 ± 2.8	coarse	10.4	<3.00	2.31	0.35	0.04
A51	0	coarse	7	6.03	1.86	0.28	0.04
A52	0	coarse	6.9	6.63	0.37	0.09	0.01
A53	0	coarse	6.9	7.18	0.33	0.07	0.01
A54	0	coarse	5.4	7	0.75	0.14	0.02
GB11	1.2 ± 0.7	fine	3	10.21	8.48	1.19	0.1
GB12	20.9 ± 10.5	fine	1.9	13.73	7.07	0.88	0.23
GB13	21.7 ± 26.3	fine	2.25	16.01	2.69	0.35	0.09
GB21	1.3 ± 0.1	fine	5	8.59	2.04	0.27	0.1
GB31	24.3 ± 23.4	fine	5.75	8.52	5.58	0.77	0.1
GB32	19.6 ± 8.9	fine	6	7.77	6.98	0.98	0.14
GB33	0	coarse	6.5	8.23	2.88	0.37	0.09
GB34	2.4 ± 4.1	coarse	6	7.04	0.57	0.07	0.04
GB41	0	coarse	5.75	6.82	2.04	0.27	0.08
GB42	7.7 ± 4.8	fine	6	6.11	2.18	0.3	0.09
GB43	0	coarse	6	5.5	6.84	1	0.12
GB44	0	coarse	7	3.46	1.27	0.18	0.05
GB45	0	coarse	7	6.91	2.29	0.3	0.08

Table 3. Total *Thioploca* biovolume and dissolved
oxygen uptake rates (DOU) at each site cored

Site	(mm³/cm²)	DOU (mmol/m2/d)
A11	0.028	4
A13	0.032	2.74
A23	0.000	1.08
A32	0.760	4.37
A34	0.197	4.74
GB12	0.664	3.08
GB31	0.323	7.04
GB42	0.320	3.54

Table 4. *Thioploca* and *Beggiatoa* biomass comparison across freshwater, brackish, and marine ecosystems.

	Beggiatoa	range (g/m^2)	
Apostle Islands (values from individual ponar samples, not site averages)	*Thioploca*	0.5 – 250 (mean: 39.0)	This study
Green Bay (values from individual ponar samples, not site averages)	*Thioploca*	0.5 – 93 (mean: 12.4)	This study
Lake Superior - Batchawana Bay	*Thioploca*	10	Dermott et al, 2005
Lake Erie - Eastern	*Thioploca*	12	Dermott et al, 2005
Lake Ontario - Eastern	*Thioploca*	83	Dermott et al, 2005
Lake Ontario - Eastern	*Thioploca*	0 – 206	Dermott and Legner 2002
Bay of Quinte (connected to eastern L. Ontario)	*Thioploca*	206	Dermott et al, 2005
Lake Biwa	*Thioploca*	12 – 50[1]	Kojima, Nakajima, Fukui 2007
Lake Okotanpe	*Thioploca*	0.003 – 0.13[2]	Nemoto, Kojima, Fukui 2011
Hjarbæk Fjord (Denmark, brackish)	*Thioploca*	33.8 ± 14.3 (mean ± SD)	Høgslund, Nielsen, Nielsen 2010
Marine – Chile	*Thioploca*	Up to 1000	Gallardo 1977
Marine - Chile, Bay of Concepción	*Thioploca*	202 – 774	Fossing et al., 1995
Marine - Chile, Bay of Concepción	*Thioploca*	100 – 800	Schulz et al. 1996
Marine - Chile, Bay of Concepción	*Thioploca*	Up to 100	Holmkvist et al. 2010
Marine - Chile, Bay of Concepción	*Thioploca*	356.4 ± 329.3	Huettel et al. 1996
Marine - Chile, Bay of Concepción	*Thioploca*	Up to 31.9	Carrasco, Gallardo, Baltazar, 1999
Marine - Chile, Bay of Concepción	*Thioploca*	Up to 26[3]	Neira, et al. 2001
Marine - Central Chilean Coast	*Thioploca*	66.0 – 83.7	Gallardo et al. 2013
Marine - Central Chilean Coast	*Thioploca*	12.66	Gallardo et al. 1995
Marine - Central Peruvian Coast	*Thioploca*	Up to 131.9	Gutiérrez, et al., 2008

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[1] Values estimated from Fig. 4 using webplotdigitizer, dry weight converted to wet weight using regression from Dermott & Legner 2002

[2] Values estimated from Fig. 1 using webplotdigitizer

[3] Values estimated from Fig. 8 using webplotdigitizer

Marine - Peruvian Coast	*Thioploca*	Up to 107.6	Rosenberg, et al. 1983
Limfjorden (Denmark, brackish)	*Beggiatoa*	5 – 20 (average, max: 48)	Jørgensen 1977
Arctic marine (Svalbard)	*Beggiatoa*	1.13 – 3.36	Jørgensen, et al. 2010
Limfjorden (Denmark, brackish)	*Beggiatoa*	14 – 16	Mußmann et al. 2003
Dangast (intertidal mud flat, German Wadden Sea)	*Beggiatoa*	0.6 – 1	Mußmann et al. 2003
Lake Chiprana (Spain, hypersaline)	*Beggiatoa*	10.1 – 11.5	Hinck et al. 2007
Barents Sea (Håkon Mosby Mud Volcano)	*Beggiatoa*	3.8	Lichtschlag et al., 2010

Table 5. Potential metabolic pathways for freshwater *Thioploca* including ΔG° (kJ/mol) at standard conditions, ΔG (kJ/mol) calculated as ΔG = ΔG° + RTlnQ at typical conditions in Lake Superior (Li & Katsev 2014, Fakhraee et al. 2017), rate of flux for sulfide (assuming all sulfate is reduced to sulfide), elemental sulfur, or ammonia (mmol/m²/day), power available to *Thioploca* (kJ/m²/day), and the time (years) required to accumulate 40 g/m² *Thioploca*, the average abundance of *Thioploca* observed in the Apostle Islands. The estimated power required by *Thioploca* for anabolism, based on the CO₂ uptake rates of marine *Thioploca* is 0.1 kJ/m²/day.

Reaction	ΔG° (kJ/mol)	ΔG (kJ/mol)	Rate of flux (mmol/m²/day)	Power (kJ/m²/day)	Time to accumulate 40 g/m² *Thioploca* (years)
$NO_3^- + HS^- + H^+ + H_2O \rightarrow NH_4^+ + SO_4^{2-}$ [1]	-487.6	-450	0.2	0.09	28
$8NO_3^- + 5HS^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2$ [1]	-768.4	-550	0.2	0.11	23
$HS^- + 2 O_2 \rightarrow SO_4^{2-} + H^+$ [2]	-732.6	-730	0.2	0.15	18
$6NO_3 + 5S^0 + 2H_2O \rightarrow 3N_2 + 5SO_4^{2-} + 4H^+$ [3]	-516.2	-600	0.2	0.12	22
$S^0 + 1.5 O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$ [2]	-504.8	-550	0.2	0.11	23
$NH_4^+ + 3/2 O_2 \rightarrow NO_2^- + 2H^+ + H_2O$ [4]	-273	-330	0.3	0.10	26
$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+$ [5]	-297	-280	0.3	0.08	31

[1]Dolfing & Hubert 2017
[2]Kelly 1999
[3]Fossing et al. 1995
[4]Campos et al. 2019
[5]van de Graaf et al. 1995

REFERENCES

American Public Health Association (APHA), American Water Works Association (AWWA), & Water Environment Federation (WEF) (2012). 4500-NH$_3$. In L. Bridgewater & E.W. Rice (Eds.), *Standard methods for the examination of water and wastewater*, (22nd ed., pp. 4-148 – 4-164). Washington, DC: American Public Health Association.

American Public Health Association (APHA), American Water Works Association (AWWA), & Water Environment Federation (WEF) (2012). 4500-P Phosphorous. In L. Bridgewater & E.W. Rice (Eds.), *Standard methods for the examination of water and wastewater*, (22nd ed., pp. 4-148 – 4-164). Washington, DC: American Public Health Association.

Ask, J., Karlsson, J., Persson, L., Ask, P., Byström, P., & Jansson, M. (2009). Whole-lake estimates of carbon flux through algae and bacteria in benthic and pelagic habitats of clear-water lakes. *Ecology*, *90*(7), 1923-1932. https://doi.org/10.1890/07-1855.1

Bergtold, M., & Traunspurger, W. (2005). Benthic production by micro-, meio-, and macrobenthos in the profundal zone of an oligotrophic lake. *Journal of the North American Benthological Society*, *24*(2), 321-329. DOI: 10.1899/03-038.1

Brandes, J. A., Devol, A. H., & Deutsch, C. (2007). New developments in the marine nitrogen cycle. *Chemical Reviews*, *107*(2), 577-589. https://doi.org/10.1021/cr050377t

Brock, J., & Schulz-Vogt, H. N. (2011). Sulfide induces phosphate release from polyphosphate in cultures of a marine *Beggiatoa* strain. *The ISME Journal*, *5*(3), 497-506. https://doi.org/10.1038/ismej.2010.135

Burgin, A. J., & Hamilton, S. K. (2007). Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Frontiers in Ecology and the Environment*, *5*(2), 89-96. https://doi.org/10.1890/1540-9295(2007)5[89:HWOTRO]2.0.CO;2

Campos, J. L., Dumais, J., Pavissich, J. P., Franchi, O., Crutchik, D., Belmonte, M., ... & Val del Río, Á. (2019). Predicting accumulation of intermediate compounds in nitrification and autotrophic denitrification processes: A chemical approach. *BioMed research international*, *2019*. DOI: 10.1155/2019/2051986

Carrasco, F. D., Gallardo, V. A., & Baltazar, M. (1999). The structure of the benthic macrofauna collected across a transect at the central Chile shelf and relationships

with giant sulfur bacteria *Thioploc*a spp. mats. *Cahiers de Biologie Marine*, *40*(2), 195-202. https://doi.org/10.21411/CBM.A.738BE8F8

Chernitsyna, S. M., Khal'zov, I. A., Khanaeva, T. A., Morozov, I. V., Klimenkov, I. V., Pimenov, N. V., & Zemskaya, T. I. (2016). Microbial community associated with *Thioploca* sp. sheaths in the area of the Posolsk Bank methane seep, southern Baikal. *Microbiology*, *85*(5), 562-569. https://doi.org/10.1134/S0026261716050027

Cole, R. A., & Weigmann, D. L. (1983). Relationships among zoobenthos, sediments, and organic matter in littoral zones of western Lake Erie and Saginaw Bay. *Journal of Great Lakes Research*, *9*(4), 568-581. https://doi.org/10.1016/S0380-1330(83)71929-5

Crowe, S. A., Treusch, A. H., Forth, M., Li, J., Magen, C., Canfield, D. E., Thamdrup, B., & Katsev, S. (2017). Novel anammox bacteria and nitrogen loss from Lake Superior. *Scientific Reports*, *7*(1), 1-7. https://doi.org/10.1038/s41598-017-12270-1

Dale, A. W., Bertics, V. J., Treude, T., Sommer, S., & Wallmann, K. (2013). Modeling benthic–pelagic nutrient exchange processes and porewater distributions in a seasonally hypoxic sediment: evidence for massive phosphate release by *Beggiatoa*? *Biogeosciences*, *10*(2), 629-651. https://doi.org/10.5194/bg-10-629-2013

Dale, A. W., Sommer, S., Lomnitz, U., Bourbonnais, A., & Wallmann, K. (2016). Biological nitrate transport in sediments on the Peruvian margin mitigates benthic sulfide emissions and drives pelagic N loss during stagnation events. *Deep Sea Research Part I: Oceanographic Research Papers*, *112*, 123-136. https://doi.org/10.1016/j.dsr.2016.02.013

Dermott, R., & Legner, M. (2002). Dense mat-forming bacterium *Thioploca ingrica* (Beggiatoaceae) in eastern Lake Ontario: implications to the benthic food web. *Journal of Great Lakes Research*, *28*(4), 688-697. https://doi.org/10.1016/S0380-1330(02)70614-X

Dermott, R., Munawar, M., Carou, S., Bonnell, R., & Niblock, H. (2005). Is sediment or pseudofaeces toxicity responsible for the decline of the amphipod *Diporeia hoyi* in Lakes Erie and Ontario? *Aquatic Ecosystem Health & Management*, *8*(1), 11-20. https://doi.org/10.1080/14634980590927373

Dolfing, J., & Hubert, C. R. (2017). Using thermodynamics to predict the outcomes of nitrate-based oil reservoir souring control interventions. *Frontiers in microbiology*, *8*, 2575. DOI: 10.3389/fmicb.2017.02575

Evans, K. M., Gill, R. A., & Robotham, P. W. J. (1990). The PAH and organic content of sediment particle size fractions. *Water, Air, and Soil Pollution*, *51*(1), 13-31. https://doi.org/10.1007/BF00211500

Fakhraee, M., Li, J., & Katsev, S. (2017). Significant role of organic sulfur in supporting sedimentary sulfate reduction in low-sulfate environments. *Geochimica et Cosmochimica Acta*, *213*, 502-516. https://doi.org/10.1016/j.gca.2017.07.021

Falkowski, P. G., Fenchel, T., & Delong, E. F. (2008). The microbial engines that drive Earth's biogeochemical cycles. S*cience*, *320*(5879), 1034-1039. https://doi.org/10.1126/science.1153213

Farias, L. (1998). Potential role of bacterial mats in the nitrogen budget of marine sediments: the case of *Thioploca* spp. *Marine Ecology-Progress Series*, *170*, 291-292. https://doi.org/10.3354/MEPS170291

Farías, L., Chuecas, L. A., & Salamanca, M. A. (1996). Effect of coastal upwelling on nitrogen regeneration from sediments and ammonium supply to the water column in Concepcion Bay, Chile. *Estuarine, Coastal and Shelf Science*, *43*(2), 137-155. https://doi.org/10.1006/ecss.1996.0062

Ferdelman, T. G., Lee, C., Pantoja, S., Harder, J., Bebout, B. M., & Fossing, H. (1997). Sulfate reduction and methanogenesis in a *Thioploca*-dominated sediment off the coast of Chile. *Geochimica et Cosmochimica Acta*, *61*(15), 3065-3079. https://doi.org/10.1016/S0016-7037(97)00158-0

Fossing, H., Gallardo, V. A., Jørgensen, B. B., Hüttel, M., Nielsen, L. P., Schulz, H., Canfield, D. E., Forster, S., Glud, R. N., Gundersen, J. K., Küver, J., Ramsing, N. B., Teske, A., Thamdrup, B. & Ulloa, O. (1995). Concentration and transport of nitrate by the mat-forming sulphur bacterium *Thioploca*. *Nature*, *374*(6524), 713-715. https://doi.org/10.1038/374713a0

Fukui, M., Teske, A., Aßmus, B., Muyzer, G., & Widdel, F. (1999). Physiology, phylogenetic relationships, and ecology of filamentous sulfate-reducing bacteria (genus *Desulfonema*). *Archives of Microbiology*, *172*(4), 193-203. https://doi.org/10.1007/s002030050760

Gallardo, V. A. (1977). Large benthic microbial communities in sulphide biota under Peru–Chile Subsurface Countercurrent. *Nature*, *268*(5618), 331-332. https://doi.org/10.1038/268331a0

Gallardo, V. A., Carrasco, F. D., Roa, R., & Cañete, J. I. (1995). Ecological patterns in the benthic macrobiota across the continental shelf off central Chile. *Ophelia*, *40*(3), 167-188. https://doi.org/10.1080/00785326.1995.10430583

Gallardo, V. A., Fonseca, A., Musleh, S. S., & Espinoza, C. (2013). Extrapolations of standing-stocks of big bacteria in Humboldt Eastern Boundary Current ecosystem (HEBCE). *Oceanography*, *1*(110), 2. https://doi.org/10.4172/2332-2632.1000110

Gallardo, V. A., Klingelhoeffer, E., Arntz, W., & Graco, M. (1998). First report of the bacterium *Thioploca* in the Benguela ecosystem off Namibia. *Journal of the Marine Biological Association of the United Kingdom*, *78*(3), 1007-1010. https://doi.org/10.1017/S0025315400044945

Graco, M., Farías, L., Molina, V., Gutiérrez, D., & Nielsen, L. P. (2001). Massive developments of microbial mats following phytoplankton blooms in a naturally eutrophic bay: Implications for nitrogen cycling. *Limnology and Oceanography*, *46*(4), 821-832. https://doi.org/10.4319/lo.2001.46.4.0821

Gutiérrez, D., Enríquez, E., Purca, S., Quipúzcoa, L., Marquina, R., Flores, G., & Graco, M. (2008). Oxygenation episodes on the continental shelf of central Peru: Remote forcing and benthic ecosystem response. *Progress in Oceanography*, *79*(2-4), 177-189. https://doi.org/10.1016/j.pocean.2008.10.025

Hinck, S., Neu, T. R., Lavik, G., Mussmann, M., De Beer, D., & Jonkers, H. M. (2007). Physiological adaptation of a nitrate-storing *Beggiatoa* sp. to diel cycling in a phototrophic hypersaline mat. *Applied and Environmental Microbiology*, *73*(21), 7013-7022. https://doi.org/10.1128/AEM.00548-07

Høgslund, S., Nielsen, J. L., & Nielsen, L. P. (2010). Distribution, ecology and molecular identification of *Thioploca* from Danish brackish water sediments. *FEMS Microbiology Ecology*, *73*(1), 110-120. https://doi.org/10.1111/j.1574-6941.2010.00878.x

Høgslund, S., Revsbech, N. P., Kuenen, J. G., Jørgensen, B. B., Gallardo, V. A., Van de Vossenberg, J., Nielsen, J. L., Holmkvist, L., Arning, E. T., & Nielsen, L. P. (2009). Physiology and behaviour of marine *Thioploca*. *The ISME Journal*, *3*(6), 647-657. https://doi.org/10.1038/ismej.2009.17

Holmkvist, L., Arning, E. T., Küster-Heins, K., Vandieken, V., Peckmann, J., Zabel, M., & Jørgensen, B. B. (2010). Phosphate geochemistry, mineralization processes, and *Thioploca* distribution in shelf sediments off central Chile. *Marine Geology*, *277*(1-4), 61-72. https://doi.org/10.1016/j.margeo.2010.08.011

Huettel, M., Forster, S., Kloser, S., & Fossing, H. (1996). Vertical migration in the sediment-dwelling sulfur bacteria Thioploca spp. in overcoming diffusion limitations. *Applied and Environmental Microbiology*, *62*(6), 1863-1872. https://aem.asm.org/content/62/6/1863.short

Jannasch, H. W. (1995). Life at the sea floor. *Nature*, *374*(6524), 676-677. https://doi.org/10.1038/374676a0

Jørgensen, B. B. (1977). Distribution of colorless sulfur bacteria (*Beggiatoa* spp.) in a coastal marine sediment. *Marine Biology*, *41*(1), 19-28. https://doi.org/10.1007/BF00390577

Jørgensen, B. B., Dunker, R., Grünke, S., & Røy, H. (2010). Filamentous sulfur bacteria, *Beggiatoa* spp., in arctic marine sediments (Svalbard, 79°N). *FEMS Microbiology Ecology*, *73*(3), 500-513. https://doi.org/10.1111/j.1574-6941.2010.00918.x

Jørgensen, B. B., & Gallardo, V. A. (1999). *Thioploca* spp.: filamentous sulfur bacteria with nitrate vacuoles. *FEMS Microbiology Ecology*, *28*(4), 301-313. https://doi.org/10.1111/j.1574-6941.1999.tb00585.x

Joshi, M. M., & Hollis, J. P. (1977). Interaction of *Beggiatoa* and rice plant: detoxification of hydrogen sulfide in the rice rhizosphere. *Science*, *195*(4274), 179-180. https://doi.org/10.1126/science.195.4274.179

Joye, S. B., & Hollibaugh, J. T. (1995). Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. *Science*, *270*(5236), 623-625. DOI: 10.1126/science.270.5236.623

Kelly, D. P. (1999). Thermodynamic aspects of energy conservation by chemolithotrophic sulfur bacteria in relation to the sulfur oxidation pathways. *Archives of Microbiology*, *171*(4), 219-229. DOI: 10.1007/s002030050703

Kojima, H., Nakajima, T., & Fukui, M. (2007). Carbon source utilization and accumulation of respiration-related substances by freshwater *Thioploca* species. *FEMS Microbiology Ecology*, *59*(1), 23-31. https://doi.org/10.1111/j.1574-6941.2006.00201.x

Kojima, H., Ogura, Y., Yamamoto, N., Togashi, T., Mori, H., Watanabe, T., Nemoto, F., Kurokawa, K., Hayashi, T., & Fukui, M. (2015). Ecophysiology of *Thioploca ingrica* as revealed by the complete genome sequence supplemented with proteomic evidence. *The ISME Journal*, *9*(5), 1166-1176. https://doi.org/10.1038/ismej.2014.209

Kojima, H., Teske, A., & Fukui, M. (2003). Morphological and phylogenetic characterizations of freshwater *Thioploca* species from Lake Biwa, Japan, and Lake Constance, Germany. *Applied and Environmental Microbiology*, *69*(1), 390-398. https://doi.org/10.1128/AEM.69.1.390-398.2003

Lauterborn, R. (1907). Eine neue Gattung der Schwefelbakterien (*Thioploca schmidlei* nov. gen. nov. spec.). *Berichte der Deutschen Botanischen Gesellschaft, 25*(5), 238-242. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1438-8677.1907.tb06615.x

Li, J., & Katsev, S. (2014). Nitrogen cycling in deeply oxygenated sediments: Results in Lake Superior and implications for marine sediments. *Limnology and oceanography*, *59*(2), 465-481. DOI: 10.4319/lo.2014.59.2.0465

Lichtschlag, A., Felden, J., Brüchert, V., Boetius, A., & De Beer, D. (2010). Geochemical processes and chemosynthetic primary production in different thiotrophic mats of the Håkon Mosby Mud Volcano (Barents Sea). *Limnology and Oceanography*, *55*(2), 931-949. https://doi.org/10.4319/lo.2010.55.2.0931

Maier, S. (1980). Growth of *Thioploca ingrica* in a mixed culture system. *The Ohio Journal of Science, 80*(1), 30-32. https://kb.osu.edu/handle/1811/22669

Maier, S., & Gallardo, V. A. (1984). *Thioploca araucae* sp. nov. and *Thioploca chileae* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *34*(4), 414-418. https://doi.org/10.1099/00207713-34-4-414

Maier, S., & Preissner, W. C. (1979). Occurrence of *Thioploca* in Lake Constance and Lower Saxony, Germany. *Microbial Ecology*, *5*(2), 117-119. https://doi.org/10.1007/BF02010502

Malkin, S. Y., Seitaj, D., Burdorf, L. D., Nieuwhof, S., Hidalgo-Martinez, S., Tramper, A., Geeraert, N., De Stigter, H., & Meysman, F. J. R. (2017). Electrogenic sulfur oxidation by cable bacteria in bivalve reef sediments. *Frontiers in Marine Science*, *4*, Article 28. https://doi.org/10.3389/fmars.2017.00028

McCollom, T. M., & Amend, J. P. (2005). A thermodynamic assessment of energy requirements for biomass synthesis by chemolithoautotrophic micro-organisms in oxic and anoxic environments. *Geobiology*, *3*(2), 135-144. https://doi.org/10.1111/j.1472-4669.2005.00045.x

Mußmann, M., Hu, F. Z., Richter, M., De Beer, D., Preisler, A., Jørgensen, B. B., Glöckner, F. O., Amann, R., Koopman, W. J. H., Lasken, R. S., Janto, B., Hogg, J., Stoodley, P., Boissy, R., & Ehrlich, G. D. (2007). Insights into the genome of

large sulfur bacteria revealed by analysis of single filaments. *PLOS Biology*, *5*(9), e230. https://doi.org/10.1371/journal.pbio.0050230

Mußmann, M., Schulz, H. N., Strotmann, B., Kjær, T., Nielsen, L. P., Rosselló-Mora, R. A., Amann, R. I., & Jørgensen, B. B. (2003). Phylogeny and distribution of nitrate-storing *Beggiatoa* spp. in coastal marine sediments. *Environmental Microbiology*, *5*(6), 523-533. https://doi.org/10.1046/j.1462-2920.2003.00440.x

Neira, C., Sellanes, J., Soto, A., Gutierrez, D., & Gallardo, V. A. (2001). Meiofauna and sedimentary organic matter off Central Chile: response to changes caused by the 1997–1998 El Niño. *Oceanologica Acta*, *24*(3), 313-328. https://doi.org/10.1016/S0399-1784(01)01149-5

Nelson, D. C., Wirsen, C. O., & Jannasch, H. W. (1989). Characterization of large, autotrophic *Beggiatoa* spp. abundant at hydrothermal vents of the Guaymas Basin. *Applied and Environmental Microbiology*, *55*(11), 2909-2917. https://aem.asm.org/content/55/11/2909.short

Nemoto, F., Kojima, H., & Fukui, M. (2011). Diversity of freshwater *Thioploca* species and their specific association with filamentous bacteria of the phylum *Chloroflexi*. *Microbial Ecology*, *62*(4), Article 753 (2011). https://doi.org/10.1007/s00248-011-9916-6

Nemoto, F., Kojima, H., Ohtaka, A., & Fukui, M. (2012). Filamentous sulfur-oxidizing bacteria of the genus *Thioploca* from Lake Tonle Sap in Cambodia. *Aquatic Microbial Ecology*, *66*(3), 295-300. https://doi.org/10.3354/ame01578

Nishino, M., Fukui, M., & Nakajima, T. (1998). Dense mats of *Thioploca*, gliding filamentous sulfur-oxidizing bacteria in Lake Biwa, central Japan. *Water Research*, *32*(3), 953-957. https://doi.org/10.1016/S0043-1354(97)00227-3

Otte, S., Kuenen, J. G., Nielsen, L. P., Paerl, H. W., Zopfi, J., Schulz, H. N., Teske, A., Strotmann, B., Gallardo, V. A., & Jørgensen, B. B. (1999). Nitrogen, carbon, and sulfur metabolism in natural *Thioploca* samples. *Applied and Environmental Microbiology*, *65*(7), 3148-3157. https://doi.org/10.1128/AEM.65.7.3148-3157.1999

Paerl, H. W., & Pinckney, J. L. (1996). A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. *Microbial Ecology*, *31*(3), 225-247. https://doi.org/10.1007/BF00171569

Prokopenko, M. G., Hammond, D. E., Berelson, W. M., Bernhard, J. M., Stott, L., & Douglas, R. (2006). Nitrogen cycling in the sediments of Santa Barbara Basin and Eastern Subtropical North Pacific: Nitrogen isotopes, diagenesis and possible

chemosymbiosis between two lithotrophs (*Thioploca* and Anammox) — "riding on a glider". *Earth and Planetary Science Letters*, *242*(1-2), 186-204. https://doi.org/10.1016/j.epsl.2005.11.044

Prokopenko, M. G., Hirst, M. B., De Brabandere, L., Lawrence, D. J. P., Berelson, W. M., Granger, J., Chang, B. X., Dawson, S., Crane, E. J., III, Chong, L., Thamdrup, B, Townsend-Small, A., & Sigman, D. M. (2013). Nitrogen losses in anoxic marine sediments driven by *Thioploc*a–anammox bacterial consortia. *Nature*, *500*(7461), 194-198. https://doi.org/10.1038/nature12365

Pusceddu, A., Dell'Anno, A., Fabiano, M., & Danovaro, R. (2009). Quantity and bioavailability of sediment organic matter as signatures of benthic trophic status. *Marine Ecology Progress Series*, *375*, 41-52. https://doi.org/10.3354/meps07735

Rea, D. K., Owen, R. M., & Meyers, P. A. (1981). Sedimentary processes in the Great Lakes. *Reviews of Geophysics*, *19*(4), 635-648. https://doi.org/10.1029/RG019i004p00635

Robinson, C. A., Bernhard, J. M., Levin, L. A., Mendoza, G. F., & Blanks, J. K. (2004). Surficial hydrocarbon seep infauna from the Blake Ridge (Atlantic Ocean, 2150 m) and the Gulf of Mexico (690–2240 m). *Marine Ecology*, *25*(4), 313-336. https://doi.org/10.1111/j.1439-0485.2004.00034.x

Rosenberg, R., Arntz, W. E., de Flores, E. C., Flores, L. A., Carbajal, G., Finger, I., & Tarazona, J. (1983). Benthos biomass and oxygen deficiency in the upwelling system off Peru. *Journal of Marine Research*, *41*(2), 263-279. https://doi.org/10.1357/002224083788520153

Salman, V., Amann, R., Girnth, A. C., Polerecky, L., Bailey, J. V., Høgslund, S., Jessen, G., Pantoja, S., & Schulz-Vogt, H. N. (2011). A single-cell sequencing approach to the classification of large, vacuolated sulfur bacteria. *Systematic and Applied Microbiology*, *34*(4), 243-259. https://doi.org/10.1016/j.syapm.2011.02.001

Sassen, R., Roberts, H. H., Aharon, P., Larkin, J., Chinn, E. W., & Carney, R. (1993). Chemosynthetic bacterial mats at cold hydrocarbon seeps, Gulf of Mexico continental slope. *Organic Geochemistry*, *20*(1), 77-89. https://doi.org/10.1016/0146-6380(93)90083-N

Schallenberg, M., & Kalff, J. (1993). The ecology of sediment bacteria in lakes and comparisons with other aquatic ecosystems. *Ecology*, *74*(3), 919-934. DOI: 10.2307/1940816

Schmaljohann, R., Drews, M., Walter, S., Linke, P., von Rad, U., & Imhoff, J. F. (2001). Oxygen-minimum zone sediments in the northeastern Arabian Sea off Pakistan: a

habitat for the bacterium *Thioploca*. *Marine Ecology Progress Series*, *211*, 27-42. https://doi.org/10.3354/meps211027

Schulz, H. N., Brinkhoff, T., Ferdelman, T. G., Mariné, M. H., Teske, A., & Jørgensen, B. B. (1999). Dense populations of a giant sulfur bacterium in Namibian shelf sediments. *Science*, *284*(5413), 493-495. https://doi.org/10.1126/science.284.5413.493

Schulz, H. N., Jorgensen, B. B., Fossing, H. A., & Ramsing, N. B. (1996). Community structure of filamentous, sheath-building sulfur bacteria, *Thioploca* spp., off the coast of Chile. *Applied and Environmental Microbiology*, *62*(6), 1855-1862. https://aem.asm.org/content/62/6/1855.short

Schulz, H. N., & Schulz, H. D. (2005). Large sulfur bacteria and the formation of phosphorite. *Science*, *307*(5708), 416-418. https://doi.org/10.1126/science.1103096

Schulz, H. N., Strotmann, B., Gallardo, V. A., & Jørgensen, B. B. (2000). Population study of the filamentous sulfur bacteria *Thioploca* spp. off the Bay of Concepcion, Chile. *Marine Ecology Progress Series*, *200*, 117-126. https://doi.org/10.3354/meps200117

Seitaj, D., Schauer, R., Sulu-Gambari, F., Hidalgo-Martinez, S., Malkin, S. Y., Burdorf, L. D., Slomp, C. P, & Meysman, F. J. (2015). Cable bacteria generate a firewall against euxinia in seasonally hypoxic basins. *Proceedings of the National Academy of Sciences*, *112*(43), 13278-13283. https://doi.org/10.1073/pnas.1510152112

Smeaton, C. M., & Van Cappellen, P. (2018). Gibbs Energy Dynamic Yield Method (GEDYM): Predicting microbial growth yields under energy-limiting conditions. *Geochimica et Cosmochimica Acta*, *241*, 1-16. https://doi.org/10.1016/j.gca.2018.08.023

Soetaert, K., Petzoldt, T., and Meysman, F. J. R. (2012). *Package 'Marelac': Tools for Aquatic Sciences*. R: CRAN library.

Sommer, S., Gier, J., Treude, T., Lomnitz, U., Dengler, M., Cardich, J., & Dale, A. W. (2016). Depletion of oxygen, nitrate and nitrite in the Peruvian oxygen minimum zone cause an imbalance of benthic nitrogen fluxes. *Deep Sea Research Part I: Oceanographic Research Papers*, *112*, 113-122. https://doi.org/10.1016/j.dsr.2016.03.001

Strom, S. L. (2008). Microbial ecology of ocean biogeochemistry: a community perspective. S*cience*, *320*(5879), 1043-1045. https://doi.org/10.1126/science.1153527

Stumm, W., & Morgan, J. J. (1996). Appendixes: Thermodynamic Data. *Aquatic chemistry: Chemical equilibria and rates in natural waters* (pp. 976 – 1004). John Wiley & Sons, Inc.

Teske, A., Jørgensen, B. B., & Gallardo, V. A. (2009). Filamentous bacteria inhabiting the sheaths of marine *Thioploca* spp. on the Chilean continental shelf. *FEMS Microbiology Ecology*, *68*(2), 164-172. https://doi.org/10.1111/j.1574-6941.2009.00659.x

Teske A., & Nelson D. C. (2006). The genera *Beggiatoa* and *Thioploca*. In: M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The Prokaryotes* (pp. 784-810). Springer. https://doi.org/10.1007/0-387-30746-X_27

Thamdrup, B. O., & Canfield, D. E. (1996). Pathways of carbon oxidation in continental margin sediments off central Chile. *Limnology and Oceanography*, *41*(8), 1629-1650. https://doi.org/10.4319/lo.1996.41.8.1629

Van de Graaf, A. A., Mulder, A., de Bruijn, P., Jetten, M. S., Robertson, L. A., & Kuenen, J. G. (1995). Anaerobic oxidation of ammonium is a biologically mediated process. *Applied and environmental microbiology*, *61*(4), 1246-1251. DOI: 10.1128/aem.61.4.1246-1251.1995

Wasmund, K., Mußmann, M., & Loy, A. (2017). The life sulfuric: microbial ecology of sulfur cycling in marine sediments. *Environmental Microbiology Reports*, *9*(4), 323-344. https://doi.org/10.1111/1758-2229.12538

Wislouch, S. M. (1912). *Thioploca ingrica* nov. spec. *Berichte der Deutschen Botanischen Gesellschaft*, *30*, 470-474. https://www.biodiversitylibrary.org/item/27152#page/475/mode/1up

Zemskaya, T. I., Chernitsyna, S. M., Dul'tseva, N. M., Sergeeva, V. N., Pogodaeva, T. V., & Namsaraev, B. B. (2009). Colorless sulfur bacteria *Thioploca* from different sites in Lake Baikal. *Microbiology*, *78*(1), 117-124. https://doi.org/10.1134/S0026261709010159

Zemskaya, T. I., Namsaraev, B. B., Dul'tseva, N. M., Khanaeva, T. A., Golobokova, L. P., Dubinina, G. A., Dulov, L. E., & Wada, E. (2001). Ecophysiological characteristics of the mat-forming bacterium *Thioploca* in bottom sediments of the Frolikha Bay, northern Baikal. *Microbiology*, *70*(3), 335-341. https://doi.org/10.1023/A:1010463613498

Zemskaya, T. I., Namsaraev, B. B., Dul'tseva, N. M., Khanaeva, T. A., Golobokova, L. P., & Dulov, L. E. (2001). Spatial distribution of various physiological groups of bacteria in the region of underwater thermal vents in Frolikha Bay, northern Baikal. *Microbiology*, *70*(3), 374-376. https://doi.org/10.1023/A:1010428000295

Zopfi, J., Kjær, T., Nielsen, L. P., & Jørgensen, B. B. (2001). Ecology of *Thioploca* spp.: Nitrate and sulfur storage in relation to chemical microgradients and influence of *Thioploca* spp. on the sedimentary nitrogen cycle. *Applied and Environmental Microbiology*, *67*(12), 5530-5537. https://doi.org/10.1128/AEM.67.12.5530-5537.2001