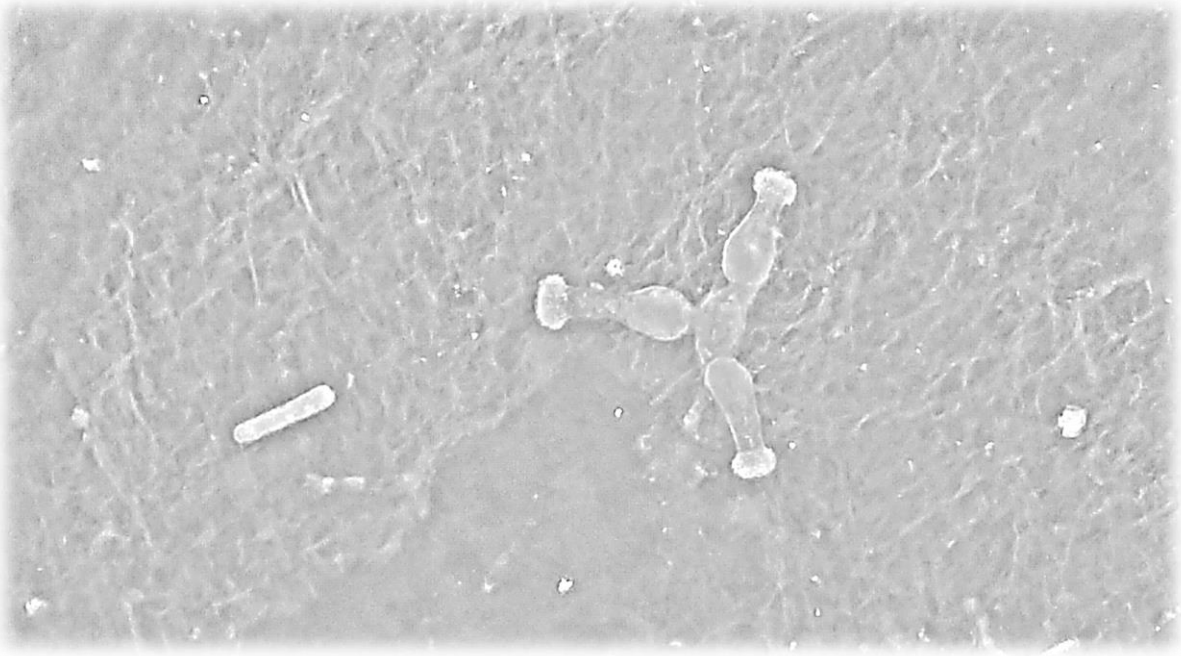
Hidden Neozoans in Macrozoobenthos – The Polyp Stage of the Freshwater Jellyfish *Craspedacusta sowerbii*



Dissertation der Fakultät für Biologie
der Ludwig-Maximilians-Universität München

Ramona Ursula Klotz

München 2022

Diese Dissertation wurde angefertigt
unter der Leitung von **Prof. Dr. Herwig Stibor**
an der **Fakultät für Biologie**
der **Ludwig-Maximilians-Universität München**

Erstgutachter: **Prof. Dr. Herwig Stibor**
Zweitgutachter/in: **Prof. Dr. Martin Heß**

Tag der Abgabe: 07.03.2022
Tag der mündlichen Prüfung: 18.07.2022

Acknowledgements

First, I extend my gratitude to my supervisor Prof. Dr. Herwig Stibor for giving me the freedom to follow my own style during my project but still providing me all support whenever I needed it.

A huge thank you also for the Evangelisches Studienwerk e.V. for providing me a full scholarship for my work.

I am also very grateful for the help and support of Dr. Sabine Gießler during all my scientific work in the lab as well as during planning, analysing and writing.

Special thanks also for my fellow Ph.D. candidates Yuanyuan Wang and Nora Kipferler for offering help whenever needed and for making long office hours feeling shorter.

Deep gratitude also for Dr. Katrin Schachtl for introducing me to the world of *Craspedacusta sp.* firsthand and for offering support throughout the whole time I have been working with jellyfish.

I also thank all members of the Aquatic Ecology group for providing open doors and ears at all times.

Many thanks also to Dr. Guillaume Marchessaux for providing me the distribution data of *Craspedacusta sp.* in France.

I am also thankful for the help of my internship and bachelor students in the field or in the laboratory.

Special thanks also for all the volunteers following my calls to report jellyfish sightings.

Furthermore, I am deeply thankful for all my friends enduring all ups and downs of my Ph.D. project with me.

Finally, I offer my deepest gratitude to my family for their endless support throughout my whole life.

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Summary

Craspedacusta sowerbii is a freshwater jellyfish species that has been invading freshwater almost all over the world. In marine environments, which are usually associated with jellyfish, increasing jellyfish observations are related to eutrophication, temperature increases and habitat degradation. Reports of jellyfish observations in freshwater have also been increasing over recent decades. The question arises as to whether population dynamics of freshwater jellyfish are affected by similar factors than observed in marine systems. Difficulties in studying freshwater jellyfish are related to the fact that most scientific interest is focused on the easily visible medusa stage. However, in the complex life cycle of *C. sowerbii* medusae play only a minor role. The inconspicuous polyp stage is much more important, as it is present throughout the year and is almost exclusively responsible for reproduction. The polyp stage is therefore crucial for a successful invasion of new habitats, and for current and future distribution patterns of this species. In my thesis I investigated the following aspects: (1.) Distribution patterns of medusae and polyps of *C. sowerbii*, (2.) Factors affecting the growth of polyps of *C. sowerbii* (chemical environment, such as nitrate and biocides) (3.) Technical aspects of polyp handling during monitoring and experimental analyses.

With a “Citizen Science” project and a literature research, recent distribution patterns of *C. sowerbii* medusae in Germany were revealed and evaluated. Analyses of the distribution patterns show that rivers probably act as important pathways for distribution. To determine how well the observation of easily detectable medusae reflect the “real” distribution of the species, including the polyp stage, the distributions of both life stages were analysed in lakes in Upper Bavaria. The analysis revealed that the polyp stage is approximately twice as abundant as the medusa stage; many more lakes than previously thought are therefore inhabited by this species. Additional comparison of lakes inhabited by only the polyp stage with lakes inhabited by both polyps and medusae show a clear difference in mean altitude—lakes inhabited by polyps and medusae are located at considerably lower altitudes. This could reflect a predicted influence of temperature on the development of medusae.

The distribution of polyps of *C. sowerbii* is affected by environmental parameters. Recently, the importance of parameters related to the chemical constitution of freshwater environments has gained increased attention. Nitrate is seen as an abundant and common threat to freshwater organisms; laboratory experiments on the effect of nitrate on the growth of the polyp population of *C. sowerbii* were therefore performed. The results of these experiments show that nitrate decreases the growth of polyp populations. This decrease was observable under both acute and chronic exposure to nitrate. A comparison with field data supports the potential importance of nitrate as shown in the laboratory. In two rivers in Upper Bavaria with differing nitrate concentrations, polyp distributions were quantified. In the river with higher nitrate concentrations the number of polyps was much lower than in the river with lower nitrate concentrations.

Additionally, experiments with certain agricultural pesticides known to enter freshwater systems by run-off were performed. Results show that the polyp stage of *C. sowerbii* is sensitive to at least some pesticides, also potentially affecting species distribution.

As mentioned above, the polyp stage of *C. sowerbii* is studied less frequently than its medusa stage. Detection in the field of this highly inconspicuous stage is extremely difficult, and needs some experience. Experiments on staining the polyp stage show that neutral red is able to permeate and stain the species, showing a clear visible red colour in the polyp stage. This staining does not seriously harm the polyps and no higher mortality of polyps with staining was observed. After collecting polyps in the field, it is possible to successfully maintain and grow them in the laboratory and use individuals for further experiments. The necessary medium changes during cultivation have no influence on the reproduction of polyps, allowing experiments that need controlled environmental parameters that can only be achieved by regular growth medium changes. During such experiments polyps have to be transferred between culture and experimental environments and injuries of the fragile polyps can occur. However, my results show that almost 100% of the polyps used in experiments regenerated within 24 to 96 hours, even after heavy injuries. This demonstrates the high regeneration capabilities many

cnidarian species are known for. My results provide important knowledge for planning and conducting further experiments investigating the ecology of the polyp stage of *C. sowerbii*.

In summary, my results help to understand recent and future distribution patterns of this highly mobile, invasive species. It became clear that the inconspicuous and under-investigated polyp stage of the jellyfish is key to understanding the dynamics of *C. sowerbii*. My findings show that *C. sowerbii* is much more broadly distributed than originally thought from medusa observations. Additionally, temperature plays an important role for medusae production, hence increased abundances, and increasing food web effects, of *C. sowerbii* medusa with ongoing Global Change can be predicted.

1. Introduction

1.1. Invasion processes

Species distributing outside their native range is a natural process (Lodge, 1993; Moyle & Light, 1996). Increasing population numbers along with increasing demands for resources cause a pressure to migrate, resulting in an expansion of range. Changing environmental conditions can also cause organisms to migrate towards new habitats.

The whole process of migration and invading a new habitat consists of several steps (Figure 1). Each step contains barriers an invading species has to overcome to get to the next one (Williamson & Fitter, 1996; Blackburn et al., 2011; Jeschke & Pyšek, 2018). The first step is to actually reach a new habitat.

This dispersal can happen actively or passively. Active dispersal results from a species' own strength/locomotion, and passive dispersal by natural vectors such as water current, wind, or transportation by other migrating species. However, with increasing human globalization and mobility, migration pathways have become more numerous (Hulme, 2009; Amano et al., 2016). The demand for globally connected waterways for transportation has opened new possibilities for the distribution of aquatic species. As active dispersal is often also accompanied by passive dispersal of associated smaller species, both dispersal pathways are affected by the increasing human demand for higher connectivity.

For marine ecosystems, the Suez Canal connecting the Mediterranean with the Red Sea is a prominent example. With this connection, non-indigenous species were suddenly able to move between the Mediterranean and Red Seas (Agur & Safriel, 1981). Nowadays, non-indigenous species play a dominant role in communities in eastern parts of the Mediterranean. The recent change in composition of indigenous and non-indigenous species might also have been caused and supported by climate change, but without the possibility of migration via the Suez Canal this composition would not have been possible in the first place (Albano et al., 2021).

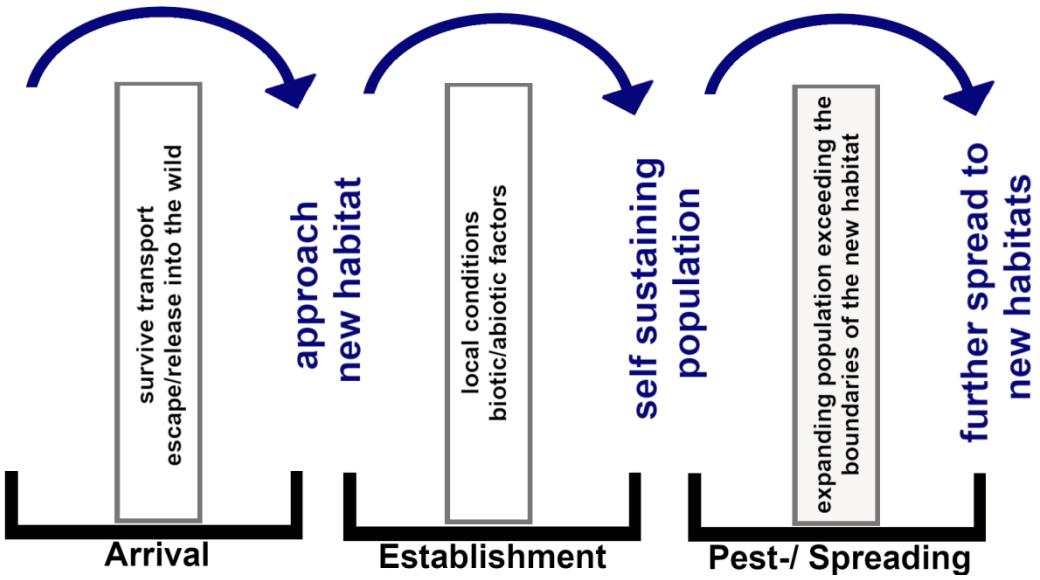


Figure 1: The three stages of invasion processes (after Blackburn et al., 2011; Jeschke & Pysek, 2018 and Williamson & Fitter, 1996).

Increased numbers of goods transported globally have also increased possible vectors for passive transportation. Whether arriving in new habitats as commodities or accidentally, more arriving alien species increase the possibility for a successful invasion (Hulme et al., 2008; Keller et al., 2011). While vertebrate species are most often imported intentionally, invertebrates are mostly imported as contamination or by stowaway transport (Hulme et al., 2008). Many countries target a close examination of unintentionally introduced species, but this is often impossible considering the large amount of imported goods. Additionally, the import of alien species is sometimes not directly connected to the commodities itself. For example, transportation in the ballast water of ships or on the surface of ships' hulls is common and difficult to prevent. The variety of pathways for invading a new habitat are therefore often hard to fully identify and understand.

Surviving travel and the escape or release into the wild are important processes in the first step (arrival) of an invasion. If these barriers are overcome the invading species must next confront the local conditions in the exotic environment.

This next step—establishment—is achieved by successful survival and reproduction in the new habitat. These two barriers are dependent on the local conditions. There are numerous hypotheses in invasion biology that aim to predict the factors influencing the success of an invasion (Enders et al., 2020). Factors like propagule pressure, availability of all necessary species-specific niche conditions, presence/absence of enemies, presence/absence of mutualists, traits of the competing non-native species, biodiversity of the new habitat, and many others play a role in the establishing process. To which degree each hypothesis can be used for a certain invasion process is extremely difficult to decide, and is dependent on many different and case-specific circumstances (Enders et al., 2020). The establishment step can be considered complete when a species has built up a self-sustaining population in a new habitat (Blackburn et al., 2011). Most invasions by alien species merely add one more component to the existing community composition in a habitat (Lodge, 1993; Karatayev et al., 2009) and the invasion process stops at this point. However, some invaders might also get to the third and last stage, which is the pest or spreading stage (Williamson & Fitter, 1996; Blackburn et al., 2011). In this stage, the species has to experience conditions that allow for reproduction in such high numbers that a further strong dispersal away from the new habitat is possible (spreading stage) or negative consequences for the new habitat occur (pest stage).

There exist several opportunities for an invasive species to have a negative influence on an ecosystem. Most obviously are the direct consequences on local communities if alien species are diminishing or even displacing native species. This is the case with the Asian Ladybird species, *Harmonia axyridis,* for example, which was originally introduced to North America and Europe for biological pest control and is now more abundant than some native ladybird species (Roy & Wajnberg, 2008; Vilcinskas et al., 2013). In these cases, communities are affected directly by competitive replacement or predation.

If the invading species not only affects one target species but also other species in the local food web, it is called an indirect effect. One prominent example of this is the lionfish (*Pterois volitans*). While the lionfish is a carnivorous species, it negatively influences the coral reefs in its new habitat by preying on the herbivorous parrotfish, which itself consumes seaweed that would otherwise outcompete the

corals. In this way the lionfish indirectly affects the coral community in its new habitat (Albins & Hixon, 2013). Consequences for human life can also occur. For example, alien species can threaten human health and economics. One example of an invader causing negative consequences in the new environment is the ctenophore *Mnemiopsis leidyi.* Originating from the western Atlantic Ocean, it became established in the Black Sea around 1980. Massive jellyfish blooms occurred and as a consequence the Black Sea zooplankton community was strongly affected. By competing with fish for zooplankton prey in the Black Sea, the invasive jellyfish affected the fish communities in this area. Consequently, jellyfish had a serious impact on the local fishing economy (Ivanov et al., 2000).

Predictions about possible consequences of an invader are commonly made by comparing the current state of the invasion process with other regions with similar climatic and ecological conditions where the alien species has already invaded. In Europe, several risk assessment protocols are in use for different species groups in order to evaluate potential negative consequences of invasion processes (Roy et al., 2014).

1.2. General characteristics of *Craspedacusta sp.*

The jellyfish *Craspedacusta sowerbii* is a cosmopolitan freshwater inhabitant (Dumont, 1994; Fritz et al., 2007). Originating in China (Dumont, 1994) it was first identified in Europe in 1880. As there are no original European freshwater medusae, *C. sowerbii* occupies a completely new functional guild. The first specimens were found in the Botanical Gardens in London in a tank for ornamental plants (Lankester, 1880). The first report in Germany was in 1905, when it was observed in the Botanical Gardens in Munich (Boecker, 1905). Nowadays sightings of jellyfish are reported all over the world, with the obvious exception of Antarctica (Dumont, 1994). The most often observed medusa stage (Duggan & Eastwood, 2012) occurs at the end of summer in all kinds of standing water. It could potentially also occur in flowing water, but since it cannot withstand stronger currents, detection in such bodies of water is unlikely. Single individuals are often overlooked as they are almost transparent.

Mass occurrences—so called jellyfish blooms—are common and draw public attention. In such cases densities of 1,000 individuals per m^2 are possible (Jankowski & Ratte, 2001).

A mature medusa is about 2 cm in size and has several rings of tentacles of varying length. The tentacles are spotted with nematocytes, which the medusa uses to hunt for prey. In their lifespan of about 30-40 days (Folino-Rorem et al., 2016) medusae prey on zooplankton in the pelagic area of lakes. As medusa densities might be very high, swarms can diminish zooplankton communities by local predation (Jankowski & Ratte, 2001). As a tactile hunter *C. sowerbii* is able to catch a large variety of prey. Such generalistic behaviour enables them to survive in regions with various zooplankton communities. If a prey organism comes in contact with the nematocytes, the nematocyst is fired into the prey and paralyses it. In the lifecycle of *C. sowerbii* (Figure 2) the medusa stage is the only one capable of sexual reproduction (Dejdar, 1934). However, outside its native range, male and female medusae are rarely found in the same habitat (Lytle, 1960; Deacon & Haskell, 1967; Lundberg et al., 2005). This kind of reproduction therefore seems to play a minor role in population building and distribution.

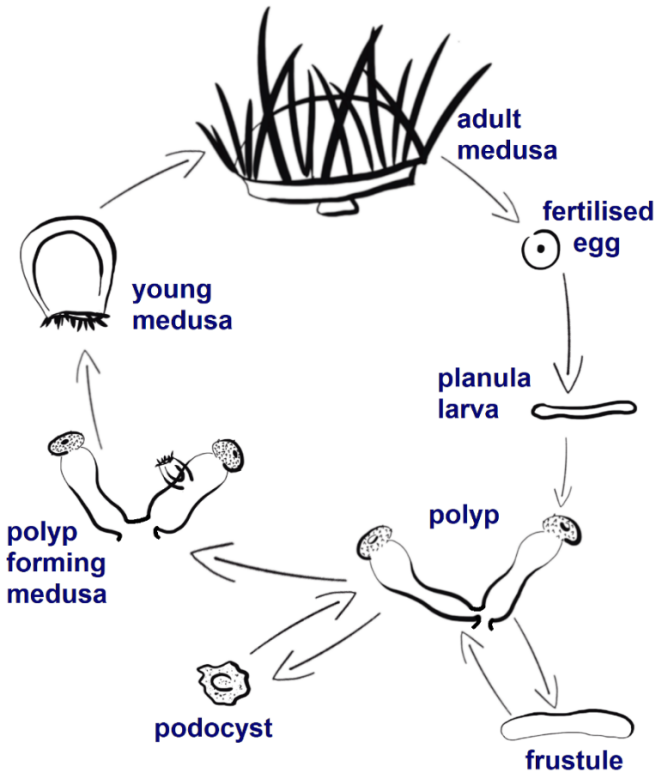


Figure 2: Schematic life cycle of *Craspedacusta sowerbii* (modified after (Lundberg et al., 2005))

Besides the medusa stage there is only one other mobile life stage. The so-called frustule stage is about 1-2 mm in size and rod shaped (Dejdar, 1934). It moves in a crawling motion. As it is not able to take on energy by feeding, its range is limited by its initial energy content (Kuhl, 1947). The frustule has two polar ends with defined front and back directions (Dejdar, 1934; Kuhl, 1947). This stage is not able to reproduce beyond a strong ability to regenerate injuries, even to the point of splitting into two parts (McClary, 1961). The frustule stage is, like most other life stages, dependent on aquatic environments, but if it dehydrates slowly it is able to morph into the endurance state of *C. sowerbii* (Dejdar, 1934). This state, the so-called podocyst, measures about 1 mm and is roughly disc shaped. With its chitinous cover it is resistant to drought and hot or cold temperatures (Dejdar, 1934; Reisinger, 1957). In this form *C. sowerbii* can remain dormant for up to 40 years (Bouillon & Boero, 2000).

All these stages of *C. sowerbii* (medusa, frustules and podocysts) originate from the most important life stage: the polyp stage. This stage measures 1–5mm and is sessile in benthic regions of all kinds of freshwater (Dejdar, 1934). Polyps occur in colonies of up to seven individuals, the most common size being two. The shape of a polyp is highly variable by compression or elongation. The morphology can generally be differentiated in a basal part, which is connected to other individuals and the ground. The cylindrical body narrows at the neck before widening to the head region again. The head region is spotted with nematocytes surrounding the mouth opening. As in the medusa stage, the nematocytes shoot nematocysts to catch prey if they are triggered. In this way, polyps feed on as many prey organisms as they are able to catch and hold, as long as the prey fits in the mouth opening. In this way the polyp is also very generalistic regarding its food sources. With a constant level of food supply the polyp stage is able to propagate rapidly. It reproduces asexually by budding off frustules or medusae. The frustules then build new polyp colonies, while the medusae act most often as a so-called reproductive "dead end". Given its fast method of asexual reproduction, its generalistic feeding, and its tolerance of a broad range of environmental conditions (for example temperature), the polyp stage seems like the most important life stage of *C. sowerbii* in terms of distribution and establishment (Acker & Muscat, 1976; Duggan & Eastwood, 2012).

Morphological analyses (based on the medusa stage) in several countries revealed a broad variety of *Craspedacusta* morphotypes (Payne, 1924; Bouillon & Boero, 2000). However, recent molecular data show that this morphological variety can be classified into three different species (Fritz et al., 2009; Lewis et al., 2012). Recent studies in Germany on the molecular data of the polyp stage indicate the existence of two different haplotypes, with one subgroup each (Schachtl, 2019). The haplotypes cannot be differentiated by eye. As the assignment of the haplotypes to the actual species is not finished yet, in this thesis I will refer to all individuals as *C. sowerbii.*

1.3. Distribution of *Craspedacusta sp.*

As active dispersal is unlikely, since the reproducing life stage of *C. sowerbii* is sessile, passive transportation seems to be the means of choice for distribution. Until now there have only been vague assumptions about the possible ways of passive spreading. The first observed occurrences in tanks of botanical gardens point towards transportation along with intentionally introduced tropical ornamental plants as a convincing pathway for the first introduction to Europe. But since *C. sowerbii* was soon found in natural waters without intentionally introduced plants, there must have been further dispersal means after these first establishments, probably via different vectors. A dispersal of polyps, frustles or podocysts with biological vectors like migratory birds, fish, big mammals or crayfish is highly probable on both a local and regional scale (Dumont, 1994; Arbačiauskas & Lesutienė, 2005; Lundberg et al., 2005). Large-scale distributions are also possible, especially via avian transportation. For large scale distribution there seem to be other realistic vectors besides biological ones. Non-biological vectors such as inland shipping activities or passive drift in rivers or streams also seem plausible.

As rivers are generally seen as good invasion routes for alien species (Lytle, 1960; Bij de Vaate et al., 2002; Leuven et al., 2009) this might have been also a possible dispersal route for *C. sowerbii* for long-distance transport. The modification of river systems for human purposes leads to huge, connected water systems and numerous artificial ponds and impoundments (Figure 3).

These artificial habitats provide easy stepping stones over long distances and are easily accessible environments for aquatic organisms.

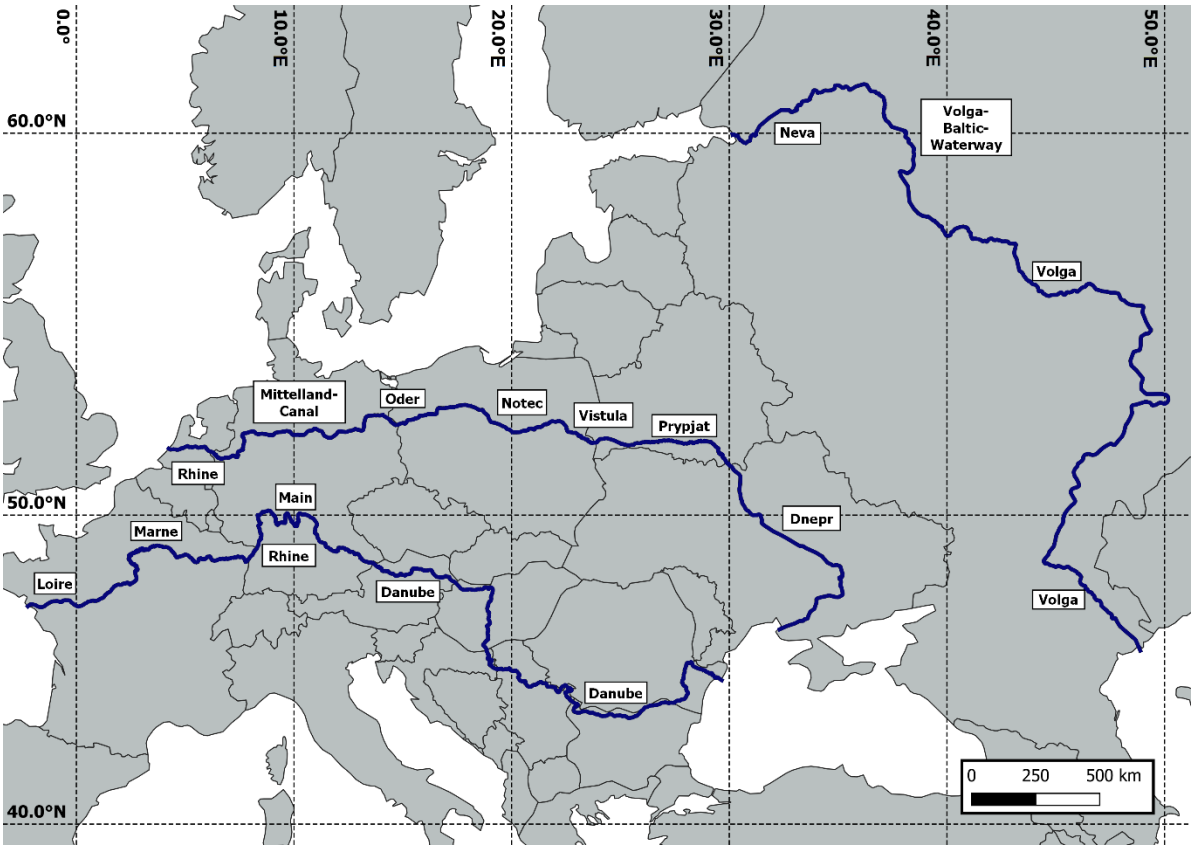


Figure 3: Three important, long distance, connected waterways known to facilitate species invasion all over Europe (after Bij de Vaate et al. (2002)).

Such emerging habitats are also often key to an early "successive stage" of community assembly and therefore potentially allow for easier invasion (Havel et al., 2005). The question of how and to what extent biotic and abiotic vectors contribute to the dispersal of *C. sowerbii* is still an open topic.

One of the problems faced when investigating the distribution of *C. sowerbii* is the difficulty of detecting them. In contrast to the small and inconspicuous polyps, the medusa stage can be found easily in a waterbody. However, at least in Europe, polyps are the only reproductive stage, and therefore give a much better indication of the actual distribution, since not all polyp populations produce medusae (Duggan & Eastwood, 2012). In literature, the more easily detectable medusae stage is used as the

only indicator for the presence of *C. sowerbii* in most cases (Fantham & Porter, 1938; Deacon & Haskell, 1967; Arbačiauskas & Lesutienė, 2005; Figueroa & De Los Ríos, 2010; Minchin et al., 2016; Marchessaux et al., 2021b). With a simpler way of detecting the medusa stage comes the enormous advantage of being able to monitor large geographical areas with very low risk of misidentification. In contrast, searching for the polyp and polyp-related benthic stages requires much more effort, time and professional experience, since polyps can easily be overlooked or misidentified.

As described in 1.1 the establishment of *C. sowerbii* in a new environment is dependent on numerous factors. Obvious and logical factors in this context are abiotic environmental factors, for example water chemistry, toxic substances and/or water temperature. Some abiotic factors can be easily approximated from geographical factors; geographical factors can therefore be used as proxies to determine abiotic factors limiting distribution. One example for such a link between geographical and environmental factors is the approximation of temperature depending on the altitude of a certain location (Klanderud & Birks, 2003; Hoiss et al., 2012). *C. sowerbii* has been reported in many kinds of freshwater (Potts, 1897; Fantham & Porter, 1938; Dexter et al., 1949; Beckett & Turanchik, 1980; Lundberg et al., 2005). However, investigations into inhabitation patterns or favourable environmental factors are still rare.

1.4. Potential agricultural factors influencing the distribution of *Craspedacusta sp.*

1.4.1. Nitrate

The current global loading of nitrogen exceeds the optimal boundaries the planet can handle to maintain similar environmental conditions to those we know today (Steffen et al., 2015). Due to human activity, the amount of globally available active nitrogen compounds has multiplied vastly in the last century. Most nitrogen (78%) can be found in the atmosphere as molecular nitrogen (Galloway & Cowling, 2002). This molecular form of nitrogen (N_2) is not biological available. Plants and algae need the so-called reactive nitrogen forms such as ammonium (NH_4) or nitrate (NO_3) for uptake. Only certain bacteria and a few plants, in symbiosis with bacteria, have the ability to fix airborne N_2 by splitting the

molecule and transforming it into a reactive form. For about 100 years humans have also been able to split airborne N_2 via the so-called Haber Bosch process. Since then, reactive nitrogen forms have been increasing globally. All of these compounds synthesised by humans end up in the environment at some point (Galloway & Cowling, 2002). As most plants and algae evolved under nitrogen-limited conditions in the past, recent increased reactive nitrogen levels have had a large effect on community dynamics (Vitousek et al., 1997). In particular, the use of nitrogen fertilisers in modern agriculture is a major source of nitrogen, mostly in the form of nitrate in surface waters (Galloway & Cowling, 2002; Rockström et al., 2009). While nitrogen is an essential resource for primary producers, for most animals nitrate is a moderately toxic substance (Camargo & Ward, 1992). At higher concentrations or with longer exposure times the toxic effect becomes more severe (Camargo et al., 2005). For example, it is known that nitrate reduces neonate production in *Ceriodaphnia dubia,* reduces growth rates in amphibians, and increases mortality in fish larvae (Scott & Crunkilton, 2000). The amount of nitrate in a river can be roughly predicted from the surrounding landscape. Watersheds with high proportions of agricultural land are indicators for elevated concentrations of nitrate and pesticides in the water (Allan, 2004). High human population densities within the catchment area also indicate higher amounts of nitrate in rivers and lakes within a region (Peierls et al., 1991).

Water chemistry plays an important role for the local community of organisms in a river or lake (Dodson et al., 2005). For any chemical substance transported from terrestrial to aquatic habitats, there are two possible ways it can be supplied. Firstly, the substance can be supplied in a permanent way, i.e. the concentration of the substance is roughly stable over a longer period of time. This is called a “chronic condition”. Secondly, the concentration of a substance can be supplied as a pulse and can rise suddenly to high levels and decrease or vanish completely after a short time. This is called an “acute condition”. Both conditions can have severe impacts on organisms and can therefore result in community shifts. These shifts might provide opportunities for the invasion of alien species. The ability of the invading species to survive and reproduce in sufficient numbers in the new habitat is crucial for this outcome to arise. Hence, successful invasive species are often considered to be generalists capable

of tolerating a broad range of environmental factors, and as such able to invade habitats which are less suitable for less tolerant species (Karatayev et al., 2009; Keller et al., 2011).

1.4.2. Pesticides

Agricultural pesticides are another important group of substances that can potentially restrict invasion of newly arriving alien species in aquatic ecosystems. The improvement of production yields is an important task in agriculture. With increased productivity of agricultural areas, more potential pollutants reach nearby waterbodies (Allan, 2004; Buck et al., 2004). A major reason for this pollution is the use of pesticides to prevent crop failure. Additionally, global warming has resulted in an increase in heavy rainfall events (Witze, 2018). The combination of more pesticides and heavier rainfall results in increased amounts of pesticides reaching aquatic ecosystems. These pesticides will act on aquatic organisms and can, depending on the substance itself, result in severe ecosystem changes, since they are often also toxic for non-target species. Such disturbances can be seen as pulse disturbances, with sudden peaks of pesticide concentrations (acute exposure) after heavy rainfall events, but also as more permanent disturbances if such events happen regularly over the season (chronic exposure).

In Germany, some of the most common pesticides used in agriculture are the herbicide Terbuthylazine (TBA), the fungicide Pirimicarb, and the insecticide Tebuconazole. Experiments with *Daphnia magna* showed no direct toxic effect of the TBA herbicide itself, but enhancing effects in pesticide mixtures (Marchini et al., 1988; Pereira et al., 2017; Silva et al., 2018). For the zebra fish *Danio rerio,* experiments showed that TBA has significant influence on the swimming behaviour of the fish larvae (Pérez et al., 2013). The Federal Research Center for Cultivated Plants reported that TBA is the most widely used herbicide in intensive corn production areas in Germany (Roßberg, 2016). The toxicity of the fungicide Tebuconazole was tested on *D. magna,* and resulted in a significant decrease of energy resources and a reduction in feeding activity (Sancho et al., 2009). The insecticide Pirimicarb can drastically reduce the reproductive success of *D. magna,* even after a short pulse exposure (Andersen et al., 2006). Accordingly, the EU Pesticides Database lists all three substances as “Very toxic to aquatic life” and “Very toxic to aquatic life with long lasting effects”. The presence of these substances in water changes the

local environmental conditions—and as a consequence also communities—and as such acts as a chemical disturbance. This disturbance is a potential threat to local communities, but also to successful invasion. It seems likely that environmental factors shape the invasion success and thereby the distribution of the polyp stage of *C. sowerbii.* However, exactly which factors hinder the successful establishment of polyps is unknown. Initial experiments investigating the role of agricultural fertilisers (nitrate) and pesticides for the growth of polyps are described in my thesis. The chosen concentrations were related to those potentially occurring in rivers after heavy run-off events, where the peak concentrations can exceed the average concentrations severalfold.

1.5. Technical aspects of the handling of the polyp stage of *Craspedacusta sp.*

Over the last decade the research interest in *C. sowerbii* has risen constantly. In particular, subjects concerning the medusa stage were investigated. Both laboratory and field experiments considered the circumstances of appearance, distribution, and effects on the environment. However, given the complex life cycle of *C. sowerbii* and its many different life stages, research focused almost solely on the medusa stage gives an inaccurate representation of this species. The polyp stage is the most abundant, and seems to represent the main distribution stage, yet ecological investigations of polyps are rare. Successful research of this life stage requires some basic but scarce knowledge of their handling. Effective ways of collecting polyps in the field, as well as fundamental knowledge about the effects of experimental day-to-day handling, will increase the possibility and success of studying them.

1.5.1. Facilitating the detection of the polyp stage of *Craspedacusta sp.*

The collection of the polyp stage from natural environments proved to be quite challenging. At only a few millimetres in size, almost transparent in colour, and often overgrown by algae or covered in detritus, their detection is highly challenging (Folino-Rorem et al., 2016). A possible way of simplifying the work with the polyp stage would be to improve its visibility—increasing the contrast between the polyp and its surroundings would facilitate its detection. One very important aspect in this context is

that the method used must be completely harmless to the polyp, as further culturing in the laboratory must remain possible.

In plankton research, several staining methods are used for different purposes, but only rarely to stain living animals for easier detection in the field. The discrimination between live and dead individuals in samples is more often the main focus (Elliott & Tang, 2009; Zetsche & Meysman, 2012). As the samples are fixed immediately after staining the consequences for the organism are not relevant.

An example of a substance used in vital staining is neutral red, a lipophilic, pH-value sensitive dye. It changes state from uncharged to cationic if the pH-value falls below 7.5. This change from uncharged to charged status also induces a change in colour from yellow to red. In organisms it diffuses into lysosomes where it remains trapped, as the pH-value within lysosomes is usually below 5 (Nemes et al., 1979). As such, a bright red colour stain remains in the animal for between a few hours and several weeks (Simon, 1974; Gray et al., 1983; Anstensrud, 1989). Following the standards of the "Ordinance on facilities for handling substances that are hazardous to water (German designation) (AwSV)" neutral red is classified as slightly hazardous to water organisms. For this reason, its suitability for the detection of polyps of *C. sowerbii* still needs to be investigated.

1.5.2. Influence of medium changes on the growth of the polyp stage of *Craspedacusta sp.*

Ecological experiments are often concerned with the effects of single environmental factors on organisms. To get informative results about such environmental factors influencing the dynamics of organisms, all other variables and growth conditions must be kept in a known, stable state. In aquatic laboratory experiments this stability can be achieved by refreshing the culture growth medium regularly. This helps reduce the accumulation of waste products (which can change the chemical environment), the level of oxygen due to respiration, or a shift in pH-values. Depending on the experiment design the refreshing process with new growth medium can occur at different intervals.

However, the process of refreshing the growth medium can itself be considered a kind of disturbance that could potentially influence the organism. For the standard refreshment process, the container

containing the polyps is emptied and refilled with fresh medium. This entails disturbance in several ways. Firstly, there is the short amount of time where no medium is in the container; and secondly, the incoming medium can disturb the organism physically, depending on the refilling method. If experiments require different refresh intervals and/or quantities, the growth medium experimental results can in theory be influenced as a consequence. To analyse whether this factor has a crucial impact on experimental outcomes, the degree to which refreshing growth media has an impact on the results should be quantified beforehand.

1.5.3. Regeneration capability of the polyp stage of *Craspedacusta sp.*

The stem of cnidarians is generally known to have good regenerative abilities (Holstein et al., 2003). The abilities of *Hydra*—the nearest freshwater relative to *C. sowerbii*—go beyond this generally good potential. It can regenerate an entire head within 48-72 hours (Bode, 2003), and can even regenerate from cell suspensions within 5-6 days (Gierer et al., 1972). This trait gives *Hydra* great capabilities for persisting in all habitats, and facilitates dispersal into new ones. In the laboratory, good regeneration abilities can also be a useful trait for successful experimental research. They facilitate handling and shorten waiting times until the specimens are ready for use in experiments after being collected from natural environments or laboratory cultures. The polyp state of *C. sowerbii* is, as mentioned above, tiny in size and barely visible. Injuries during the handling process in the culture are inevitable for such a delicate species. To get an impression of how fast polyps regenerate after injury, more data on these regeneration traits are essential.

1.6. Research Topics

I addressed four different research topics in my thesis:

1.6.1. Analysis and assessment of the distribution of *Craspedacusta sp.* in Germany

In this part of my thesis I want to answer following questions:

- What is the current distribution of the easily detectable medusa stage of *C. sowerbii* in Germany?

► To answer this question, I used a so called “Citizen Science“ approach, combined with a literature review.

- How well does the medusae population reflect the actual distribution of *C. sowerbii*?

  ► To answer this question, I sampled lakes in Bavaria for the polyp and medusa stages and compared the proportion of lakes inhabiting polyps and polyps and medusae.

- What are the possible geographical and physical limitations for the distribution of polyps and medusae?

  ► To answer this question, I collected several basic parameters of all sampled lakes and compared them to the polyp and medusa distributions.

1.6.2. Impact of agricultural chemicals on the distribution of *Craspedacusta sp.*

The invasion success, and therefore the distribution of *C. sowerbii,* may be hindered by harmful environmental factors. Well-known factors that can negatively influence the growth, survival and reproduction of organisms in freshwater include agricultural runoff (ARO) by rainfall, which often shows high concentrations of fertilisers (nitrate) and pesticides.

I therefore investigated the following questions:

- Does nitrate negatively affect the growth dynamics of polyps of *C. sowerbii* at chronic or acute exposures?

  ► To answer this question, I performed several laboratory experiments exposing *C. sowerbii* to nitrate.

- Is it possible to find support for laboratory results in field investigations?

  ► To answer this question, I monitored two rivers in Upper Bavaria with contrasting nitrate concentrations.

- Do the most common pesticides used in Germany affect the survival, growth and reproduction of polyps at low and environmentally relevant concentrations?

    ► To answer this question, I performed several controlled laboratory experiments exposing *C. sowerbii* polyps to three common pesticides.

1.6.3. Polyp handling

The polyp stage is the most abundant life stage of *C. sowerbii*. Due to its unremarkable appearance and small size it is still mostly overlooked and seldom used for experiments in the laboratory. Hence, knowledge and experience of detection and handling is still scarce.

Within this part of my thesis I investigated the following questions:

- Is it possible to facilitate the detection of the polyp stage in the field by staining without harming the polyps and hindering further investigations?

    ► To answer this question, I tested neutral red on polyps of *C. sowerbii* to see how it affects the polyp stage of *C. sowerbii.*

- How do polyps react to different frequencies of growth medium renewal?

    ► To answer this question, I monitored differences in the growth of polyps of *C. sowerbii* with different medium exchange intervals.

- How fast do polyps recover from potential injuries resulting from the sampling and handling process?

    ► To answer this question, I decapitated polyps of *C. sowerbii* and observed the regrowth process.

2. Material and Methods

2.1. General methods

2.1.1. Taking the polyp stage of *Craspedacusta sp.* into culture

For my main polyp cultures, I sampled rivers and lakes in upper Bavaria from July to September 2018. As the polyp stage of *C. sowerbii* is only visible under a microscope I collected stones in the field and took them to the laboratory in 1l buckets filled with water from the sampling spot to prevent the stones from drying. These buckets were kept in climate chambers at 18°C until further use. Each stone was then examined under a stereo microscope at 16-fold magnification. Polyp colonies were then removed from the surface with a needle and transferred into suspension culture plates (Greiner bio-one, Kremsmünster, Austria). As medium a Hydra Medium was used ($CaCl_2 \cdot 2H_2O$: 1 M,$NaHCO_3$: 0.5 M, $MgCl_2 \cdot 6H_2O$: 0.1 M, $MgSO_4 \cdot 7\ H_2O$: 0.08 M, KNO_3: 0.03 M (Folino-Rorem et al., 2016)). After their first introduction into the new wells the polyps needed an adaption period of 10-14 days. During this time the removed polyps transformed into the frustule stage, then back to the polyp stage. To ensure undisturbed transformations and to avoid losses due to handling, no feeding or medium changes were made during the adaption period. The most common polyp colony type, comprising two individuals, transforms into one frustule and back to a colony of two polyps. The time needed for transformation differs between colonies. Colonies with faster transformations may even be able to produce frustules in the same time other colonies need to complete one transformation. Due to the small size of the well and refraction effects obstructing a clear view at the lower edges of the well, freshly grown polyps or frustules are easily overlooked. Therefore, all wells need to be checked closely for additional polyps or frustules before an experiment starts.

2.1.2. Laboratory culture of the polyp stage of *Craspedacusta sp.*

Polyps in culture were kept at 18°C. As the polyps can not be cleaned completely after taking them from the field, algae are always present in the wells. To avoid uncontrolled growth of these algae it is useful to store the cultures in darkness. As a food source I used the freshwater rotifer *Brachionus*

calyciflorus. Since these rotifers also occur in the polyps' natural habitat, nutrition close to a natural diet was achievable. However, a broad variety of other freshwater species are also viable prey source, as polyps are widely generalistic about prey species. In my laboratory the cultures were fed with *B. calyciflorus* ad libitum three times a week. Once a week the medium in each well was changed completely, to clean the wells of algae, bacteria and detritus.

2.1.3. Evaluation of the experiments

All statistical analyses for this thesis were performed with the R software version 3.4.0 (R Core Team, 2018)**.** Geographical analyses were performed in QGIS (version 3.4.15 - Madeira (QGIS Development Team, 2018)) with an ESRI base map.

2.2. Analyses of distribution patterns, pathways and potential limitations

2.2.1. Data collection

Reports of medusae sightings in freshwater habitats in Europe arrived at my desk following several appeals on the internet, and in reports on the radio and in television. For my citizen science approach, I selected only reports from Germany. As *C. sowerbii* is the only freshwater jellyfish in Germany, misidentifications are highly unlikely. The reports were collected from 2015 to 2019 but a few reports (3) were from previous sightings. Additionally, I collected available literature data about published medusae sightings in Germany for comparative analysis (Reisinger, 1934; Beck & Krach, 1984; Kronfelder, 1984; Jankowski, 2000; Tappenbeck, 2008). These data consist of sightings from 1931-2019.

For further analyses of both medusae and polyp distribution patterns I chose 75 lakes within a radius of 120 km from the Seeon Limnological Station and the laboratory in Munich (Figure 4). The chosen lakes included several deep and large pre-alpine lakes; small, shallow kettle lakes; and artificial lakes of young age. A few additional samples taken randomly across Bavaria completed my data set.

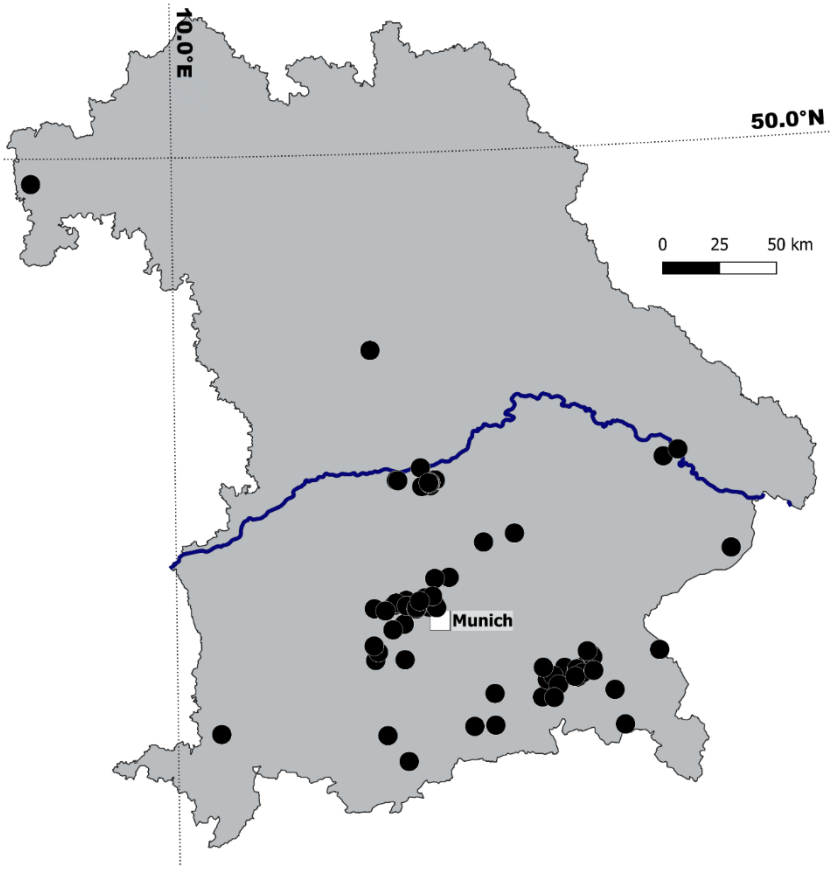


Figure 4*:* Map of the lakes in Bavaria sampled for this project (black dots).

2.2.2. Sampling of the medusa and polyp stages of *Craspedacusta sp.*

Polyps were sampled as described in 1.1. In each lake stones were collected at a depth of 50 cm along two 10 m transects, with about 1 m intervals between collected stones.

For sampling the pelagic medusa stage of the jellyfish, the water surface of each lake was screened from a boat and by snorkeling. For the analyses reported here medusa data were converted into an absence/presence matrix.

2.2.3. Geographical analyses

All lakes were analysed in regard to their nearest distance to one of the four rivers with the largest river basin districts in Germany (Danube, Elbe, Rhein and Weser). Together the river basin districts of these rivers cover about 90% of the total area of Germany. The river Danube rises in the southwest of

Germany and flows through the southern part of Germany, over the eastern border into Austria. The river Rhine crosses western Germany from south to north, in an almost straight line. The river Elbe enters Germany from Poland in the east and flows in an almost straight line in a north-westerly direction until it enters the North Sea. The last river Weser begins at the conjunction of two rivers in central Germany and flows northwards to the North Sea. Frequency distributions of the number of lakes inhabiting medusae within a certain distance from the four rivers were plotted for 1) my citizen science data of jellyfish sightings and 2) the published historical data about medusa sightings. For comparison I also plotted a frequency distribution of the nearest distance of the 730 largest lakes in Germany to one of the four chosen rivers. For geographical analyses of the lakes sampled for polyps and medusae I used data from the Bavarian Environmental Agency (Grimminger, 1982). I extracted surface area and altitude above sea level and grouped lakes by natural or artificial origin.

2.2.4. Statistical analyses

For the analysis of the shortest distances between the medusae lakes and the four German rivers I grouped the lake distances in intervals of 10 km. The resulting groups were displayed in a histogram, and an exponential regression line was fitted to the data. I performed this analysis with 1) my reported medusae lakes, 2) the medusae lakes from the literature and 3) reference lakes. Additionally, I further combined the two data sets (1, 2) including medusa sightings into one analysis.

My sampling of polyps and medusae in lakes near to Seeon Limnological Station resulted in three categories of lakes: lakes where I found no stages of *C. sowerbii;* lakes where I found *C. sowerbii,* regardless of stage; and lakes where I found the medusa stage. I calculated the share of each of the three categories for the total number of monitored lakes.

I compared the average altitude above sea level of each group with a one-way ANOVA. If the results indicated a significant difference, a post-hoc Tukey test was performed. For a visual display of the altitudes of the lakes' locations, all lakes were displayed in a boxplot with altitude on the y-axis. The same was done to the average surface area.

For the comparison of the lakes' origins I grouped them into artificial and natural lakes and used a Fisher's exact test to analyse whether the proportion of both types is significantly different for lakes without *C. sowerbii*, lakes with *C. sowerbii* regardless of stage, or lakes with both polyps and medusa stages.

2.3. Analyses of the effects of nitrate on the polyp stage of *Craspedacusta sp.*

2.3.1. Laboratory experiments

The small and highly variable shape of the polyps of *C. sowerbii* makes it difficult to evaluate the condition of this life stage. I therefore performed experiments based on the reproduction of polyps. This method is common in research on the effects of toxic substances on aquatic specimen. For my experiments with nitrate, I decided to test the effect of both chronic and acute exposure.

As preparation for all experiments, polyp colonies were separated into new wells, and a further adaption period was given, similar to the one used when the colonies were first taken into the laboratory. After the standard check for additional polyp colonies or frustules, the medium was changed once and the polyps were fed with *B. calyciflorus*, to ensure good physical condition. During the experiment, the feeding and well cleaning was performed in the same way as in the common laboratory culture.

Nitrate in the form of KNO_3 was dissolved in regular Hydra Medium. For the acute experiment, concentrations of 200, 400, 600 and 800 mg/l were used. For the chronic experiments, concentrations of 5, 10, 20, 40, 50, 100, 150 and 200 mg/l were used. For the acute experiment, nitrate was added to the wells for 24h before standard Hydra Medium was reintroduced. In the chronic experiments the nitrate exposure lasted the full duration of the experiment. Besides the different medium, the polyps were treated exactly the same in both cases. This also allowed for a consistent nitrate concentration in the chronic experiments. The tests at each concentration—plus the control tests (without nitrate) for both experiments—were replicated 12 times. The duration of the experiments was 29 days, during which the number of polyps per well was counted three times a week.

2.3.2. Polyp and water samplings in the rivers Amper and Maisach

For the field experiment checking for potential nitrate limitation on the distribution of polyps, two rivers in upper Bavaria were chosen. These two rivers are located next to each other in the west of Munich, however one flows mainly through agricultural areas (Maisach), while the other flows mainly through protected areas (Amper).

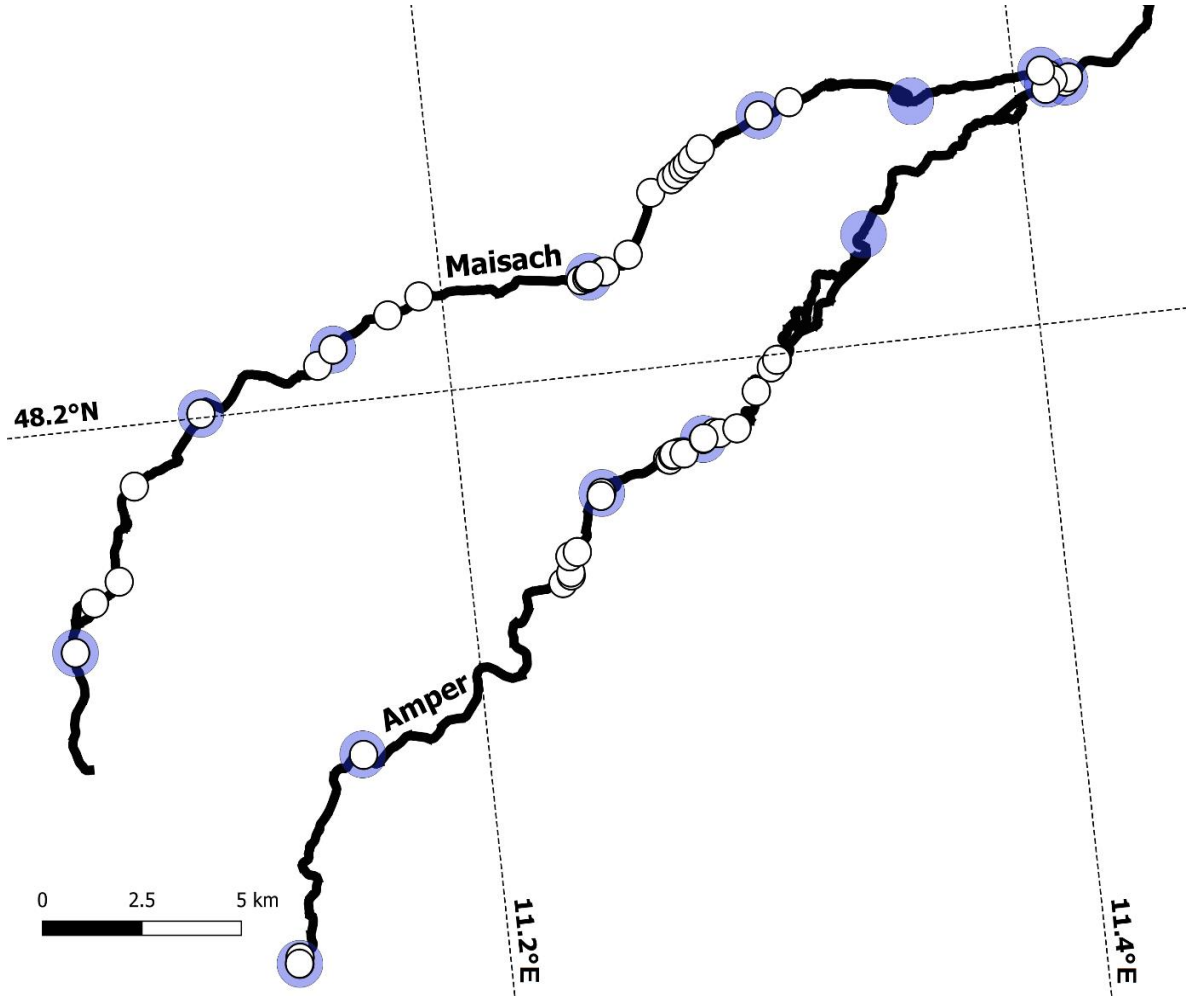


Figure 5: Routes of the two sampled rivers Maisach (upper) and Amper (lower). White circles display polyp sampling sites and blue circles display water sampling sites.

Both rivers were screened for the presence of polyps of *C. sowerbii*. To get an overview of the distribution of polyps in both rivers, 270 stones from 27 sites were sampled in each river. For the shorter

river Maisach, almost the entire length was covered during sampling. The longer Amper was sampled from its origin to the point where the Maisach runs into it. This part of the Amper has the same length as the Maisach, about 35 km (Figure 5). I tried to distribute the sampling sites as evenly as possible over the entire lengths of the rivers, but preservation areas or inaccessible riverbanks made a completely even distribution impossible. The sampling itself and the screening for polyps were carried out as described in 2.1.1. The results of these samplings were recorded in a presence/absence matrix. Additionally, water samples were taken at 6 sites in every river. These samples were transported to the laboratory in a cooling box. The analyses of nitrate concentration in both rivers was performed with a Dionex (DIONEX ICS 1100, Thermo Scientific) and displayed on a point graph.

2.3.3. Comparison of the basic information on the rivers Amper and Maisach

For the comparison of the two rivers, I used data provided by the German Federal Institute of Hydrology. From this source I used data on drainage areas and average runoff. Afterwards a geographical analysis was performed with QGIS, from which I derived information about the size of the rivers, the size and frequency of riparian areas on the banks, and the maximum distance between both rivers.

2.3.4. Statistical analyses of the laboratory experiments

For the statistical analyses of the laboratory experiments, the number of individuals per well was first converted to biomass C by the factor 0.21 µg C (Schachtl, 2019) and later averaged for each treatment and observation day. These data points were plotted on a point graph displaying the population development over time, accompanied by an error bar showing the standard error. Additionally, the mean biomass per replicate over the final three days was calculated and plotted on a point graph. A linear regression line was fitted through these data points, along with a 95% confidence interval. Lastly, an effect size was calculated for every concentration with the formula for the log-transformed response ratio: ln(mean(treatment)/mean(control)) (Hedges et al., 1999; Osenberg et al., 1999). The effect sizes were then displayed on a bar graph for each treatment.

2.4. Laboratory analyses of effects of ARO on the polyp stage of *Craspedacusta sp.*

In this part of my work, I chose three pesticides commonly occurring in ARO in Germany and tested their effect on the population increase of the polyp stage of *C. sowerbii*. The chosen pesticides—Terbuthylazine, Tebuconazole and Pirimicarb—were used from stock solutions for which the chemicals were diluted in dimethyl sulfoxide (DMSO). The media used for the polyps in these experiments was standard Hydra Medium mixed with each of the pesticide stock solutions. In the acute experiments the polyps were kept in the Hydra Medium with the pesticide for 24 hours before being changed back to normal Hydra Medium. In the chronic experiments the Hydra Medium with a pesticide was used over the entire duration of the experiment and renewed once a week as per standard procedure. All experiments ran over 31 or 32 days, with 12 replicates per treatment. The counting of individuals in each well took place three times a week, as in the nitrate experiments. To exclude the effects of DMSO, an additional DMSO control was performed. This treatment showed no effect on polyp reproduction. A complete overview of the different experimental set-ups is shown in Table 1.

Table 1: Overview for the laboratory experiments on the effect of three agricultural pesticides on the reproduction of polyps of *C. sowerbii*.

pesticide	exposure	stock solution	concentrations [µg/l]	replicates	control treatment
Terbuthylazine	acute	Terbuthylazine + DMSO	2.5, 5, 10	12	Hydra Medium DMSO
Terbuthylazine	chronic	Terbuthylazine + DMSO	0.1, 0.5, 1	12	Hydra Medium DMSO
Pirimicarb	acute	Pirimicarb + DMSO	1, 2, 4	12	Hydra Medium DMSO
Tebuconazole	acute	Tebuconazole + DMSO	5, 7.5, 10	12	Hydra Medium DMSO

To evaluate the outcome of the experiments the same statistical methods were used as for the nitrate experiments (2.3.4.).

2.5. Analyses of different handling aspects

2.5.1. Staining with neutral red

The staining method used in this experiment was based on the method from Elliott and Tang (2009). In their work they used a concentration of 0.015 g/l to stain the copepods in their samples. In my experiment I additionally used concentrations of 0.007 g/l and 0.022 g/l to see whether colour intensity and/or reproduction were affected by those differences. Differing from the experiment of Elliott and Tang, the polyps in my experiment were stained with each of the three concentrations for 30 minutes. The three treatments were replicated three times each. Additionally, three control treatments with unstained polyps were used. After staining the colour intensities were evaluated by eye for differences. Over the following 27 days the reproduction of the stained polyps was observed and analysed as in the nitrate experiment (2.3.4.).

To discover whether staining also works for field samples with polyps on stones with typical "Aufwuchs", I also stained five stones from field samples, using the same method as described above. The polyps on the stones were counted twice, once before staining and once after, so as to be able to compare the visibility of both. The counting of polyps was done by two researchers, one with several years of experience in detecting polyps, and another who had only worked with polyps for a few weeks. To see whether the improvement in detection of polyps on the stones is different between the experienced and the inexperienced researcher, a two-sided t-test was performed.

2.5.2. Changing the medium

In this experiment the effects of three different intensities of disturbance on the polyps' reproduction were examined. The disturbances were created by changing the culture medium at different intervals for each treatment. For treatment one, the medium was changed once a week; for treatment two, twice a week; and for treatment three, three times a week, over a period of 31 days. During the exchange of the medium, all six wells on one plate were first emptied one after the other and afterwards refilled with new medium in the same order. Using this method, each well was empty for several

seconds before the new medium was put in. Each treatment was replicated twelve times. During the experiment the number of polyps in each well was counted three times a week. Subsequent analyses of the results were performed as in the nitrate experiment (2.3.4.)

2.5.3. Regeneration of the head structure

To evaluate the regenerative capabilities of the polyp stage of *C. sowerbii* I examined the amount of time needed for polyps to regenerate a complete head structure. In this experiment I first removed the heads of twelve polyp colonies. To identify the colonies over the duration of the experiment I marked the outside of the respective transparent bottom of the wells with coloured spots. I could then discern whether all heads of a colony were regenerated, or whether different head counts occurred. To prevent bacterial overgrowth of polyps the medium was refreshed every twelve hours. Once a day the condition of all colonies was documented. The different conditions were: complete head (evaluated with a stereomicroscope); stump (polyp without visible head structure with nematocysts); podocyst; and nothing (colony not visible). The observations were documented for the whole colony. The experiment was ended after 90% of the experimental species had regenerated. For the evaluation I calculated the frequencies of all observed conditions for each day. The frequencies were displayed in bar graphs for each day of the experiment.

3. Results

3.1. Distribution analysis

3.1.1. Distance of lakes to rivers in Germany

The analysis of 149 lakes with recorded sightings from the citizen science approach and the literature research (data set 1 and data set 2, 1931-2019) of *C. sowerbii* medusae in Germany shows that accumulations of medusae sightings are found near the rivers Danube, Rhine, Elbe and Weser (Figure 6).

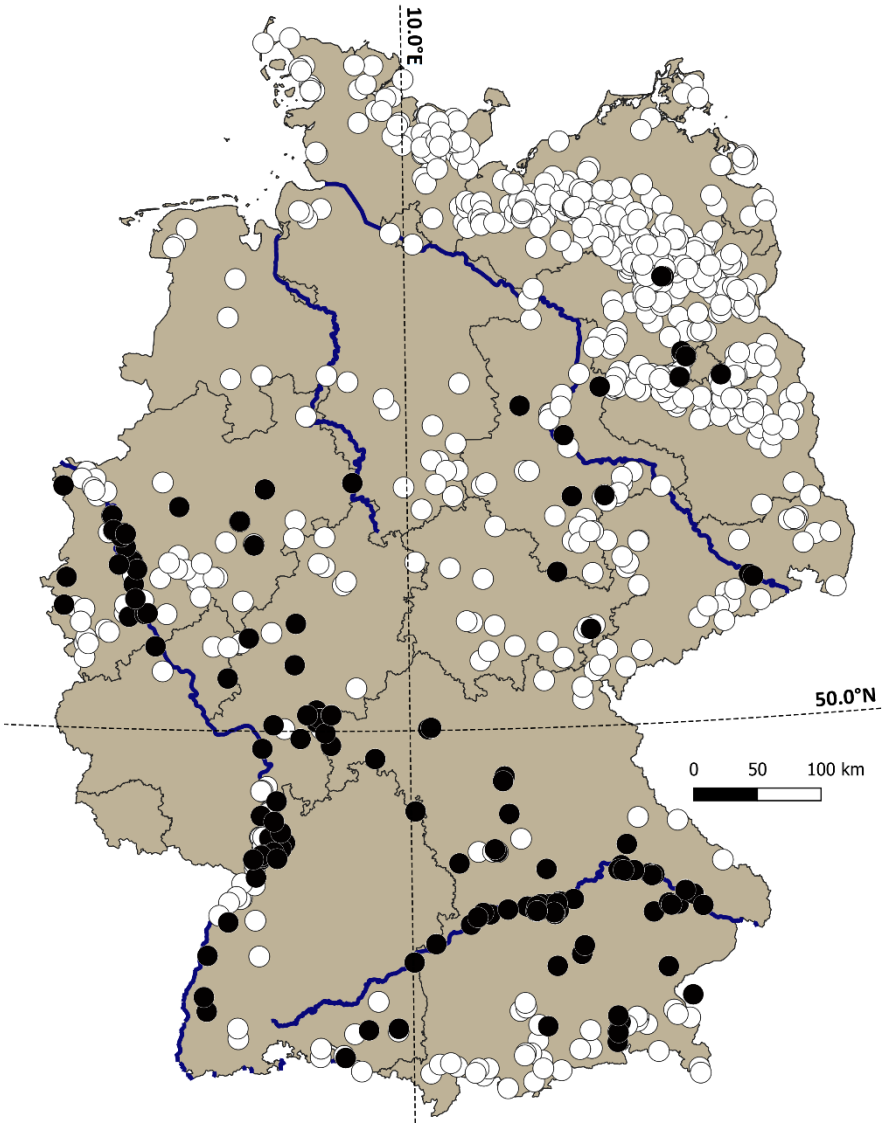


Figure 6: Map of the 149 received medusae sightings from the citizen science approach and the literature research in Germany (black points). White points display the 730 largest lakes in Germany. The basic map is taken from DIVA GIS.

The joint analysis (data sets 1 and 2) revealed that 51 % (N=76) of the lakes with medusae records (N=149, 1931-2019, Figure 7a) are located within a distance of zero to ten kilometers from one of the four described rivers (distance interval 1). Regression analysis revealed a significant exponential decay in the proportional distribution of lakes with medusae records and their distance from one of the four chosen German rivers ($y = 0.15\ e^{-0.18x}$, $r^2 = 0.63$, $F = 22.92$, $p < 0.001$; Figure 7a). Analysing lakes with medusae sightings from my citizen science approach (data set 1) separately revealed a very similar significant relationship between the distribution of medusa sightings and the distance to the German rivers ($y = 0.131\ e^{-0.15x}$, $r^2 = 0.40$, $F = 9.13$, $p = 0.012$; Figure 7b, data set 1). The relationship of the distribution of lakes with medusae sightings and the distance to the German rivers from published literature (data set 2) separately resulted in a very similar exponential decay relationship as seen in the citizen science data. However, the regression was not significant at the 5% level but showed a strong trend ($y = 0.107\ e^{-0.12x}$, $r^2 = 0.23$, $F = 4.34$, $p = 0.064$; Figure 7c, data set 2). 50 % (N=32) of the lakes with medusa sightings from my citizen science approach (data set 1, N=64) and 52 % (N=44) of the lakes with medusa sightings from published literature (data set 2, N=85) are located within a distance of zero to ten kilometers to one of the four mentioned rivers (Figure 7b, Figure 7c). For comparison, we also plotted the distances of the 730 German reference lakes to the nearest of the four German rivers in question. In contrast to lakes with medusa sightings—where the largest proportion of lakes inhabiting medusa were found close to a river (less than 10 km, see Figure 7a)—the data set with 730 lakes showed the highest percentage (13 %, N=96) of lakes occurring at an intermediate distance from the rivers (80-90 km) (Figure 7d).

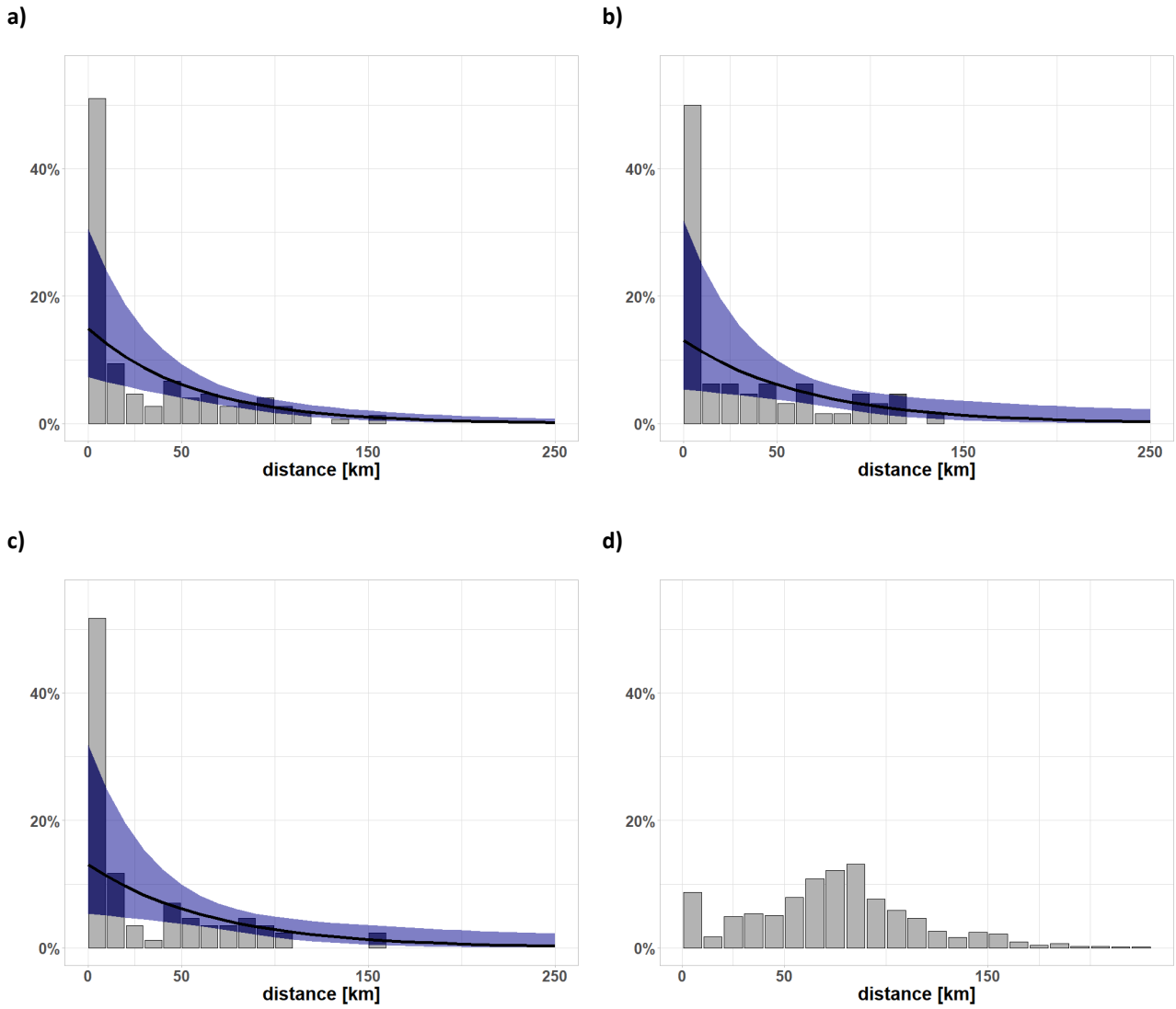


Figure 7: a) Distance x colonization relationship based on pooled long-term and current data from Germany. Percentage of the 149 jellyfish lakes (data set 1 and data set2) according to their shortest distance to one of the four considered German rivers. The regression line displays an exponential relationship between the percentage of the jellyfish lakes and the distance to the rivers by the function: $y = 0.15\ e^{-0.18x}$, ($r^2 = 0.63$, $F = 22.92$, $p < 0.001$) with a 95% confidence interval. b) Distance x colonization relationship based on current data from Germany. Percentage of the 64 jellyfish lakes from the citizen science approach (data set 1) according to their shortest distance to one of the four German rivers. The regression line displays an exponential relationship between the share of the jellyfish lakes and the distance to the rivers by the function: $y= 0.131e^{-0.15x}$, ($r^2 = 0.40$, $F=9.13$, $p=0.012$) with a 95% confidence interval. c) Distance x colonization relationship based on long-term data from Germany (data set 2). Percentage of the 85 jellyfish lakes from literature according to their shortest distance to one of the four German rivers. The regression line displays an exponential relationship between the proportion of the jellyfish lakes and the distance to the rivers by the function: $y = 0.107\ e^{-0.12x}$, ($r^2=0.23$, $F=4.34$, $p=0.064$) with a 95% confidence interval. d) Distribution of the shortest distances of 730 largest German lakes from the nearest of the four German rivers independent of medusae occurrence.

3.1.2. Analysis of the French data

The analysis of the French data set 3 (N = 123, see Supplementary Table 4) revealed a similar pattern for French lakes inhabiting medusae as for German ones (Figure 8).

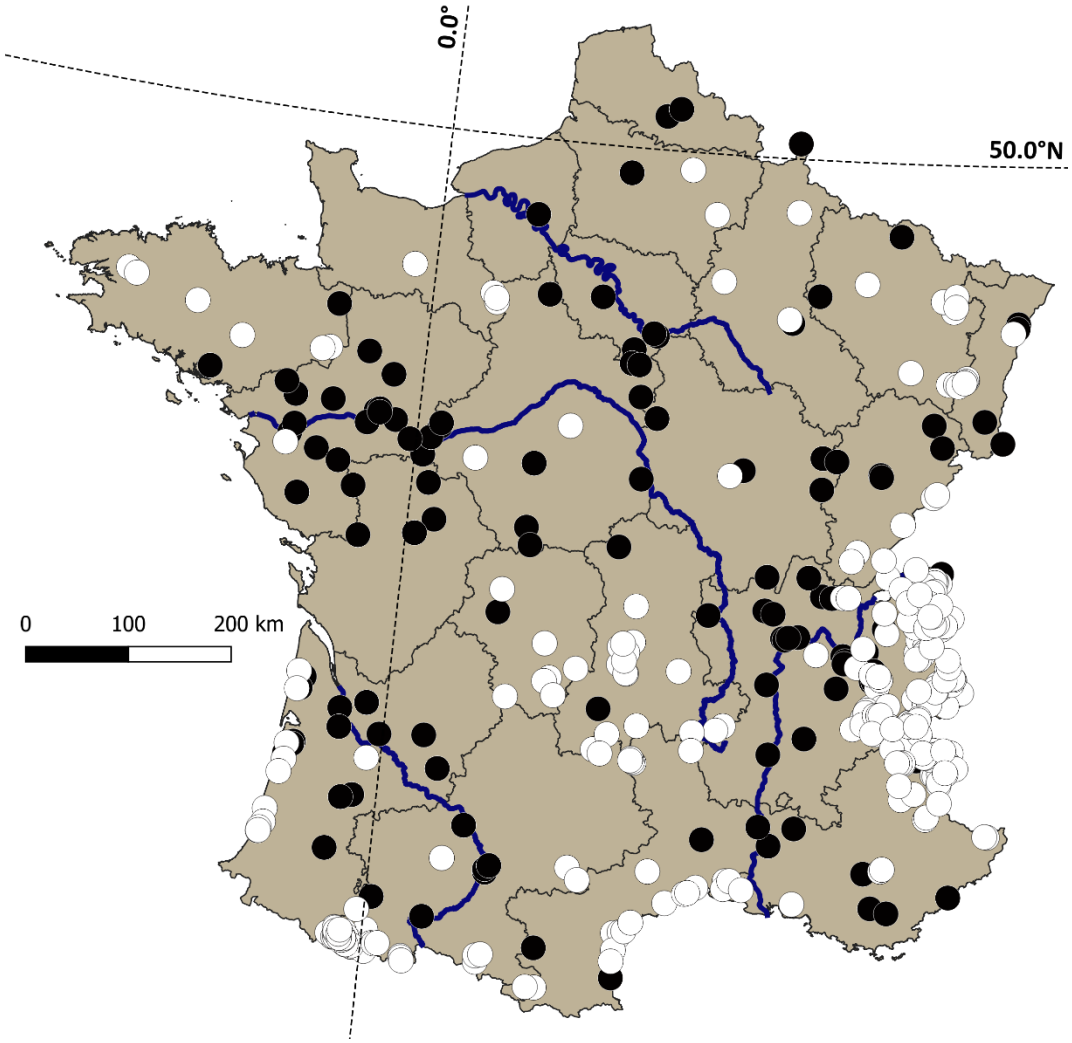


Figure 8: Map of received medusae sightings in France (black points) (Marchessaux et al., 2019; Marchessaux et al., 2021a). White points display 348 reference lakes in France, black points 123 colonized lakes. The basic map is taken from DIVA GIS. The whole generation of the map was made with the software QGIS.

Of these lakes, most (29 %, N = 35) were in the shortest distance category of 0–10 km to the nearest of the four considered French rivers (Figure 9a). As with German lakes, an increase in distance to the French rivers corresponds to an exponential decrease in the number of lakes with medusae ($y = 0.098e^{-0.11x}$, $r^2 = 0.50$, $F = 20.14$, $p < 0.001$, Figure 9a). The analysis of the French reference lakes (independent

of medusa occurrence) also shows a completely different distribution pattern of lakes within respective distance intervals from large rivers than observed for lakes inhabiting medusae (Figure 9b). Only one clear peak was found in the interval of 60-70 km (16 %, N=53).

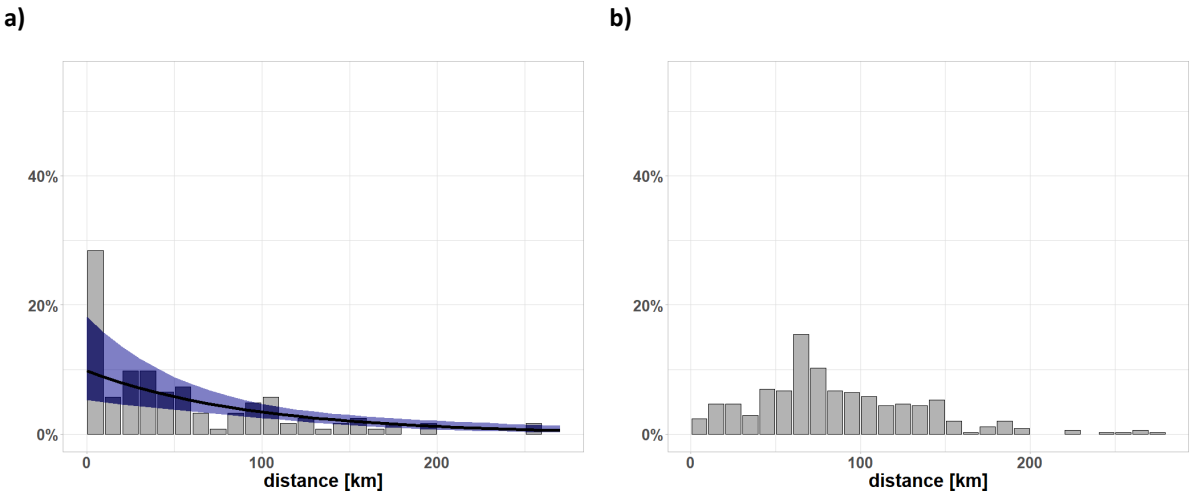


Figure 9: Distance x colonization relationship based on long-term data from France (data set 3). (a) Percentage of the 123 lakes inhabiting from (Marchessaux et al., 2019; Marchessaux et al., 2021a) as a function of their shortest distance to one of the four largest French rivers. The regression line displays an exponential decay between the proportion of the jellyfish lakes and the distance to the rivers by the function: $y = 0.098\ e^{-0.11x}$, (r^2=0.50, F=20.14, p<0.001) with a 95% confidence interval. (b) Distribution of reference lakes (independent of medusa occurrence) based on shortest distances to one of the four considered French rivers. Shown is the percentage of 348 reference lakes according to their shortest distance to one of the four rivers in France.

3.1.3. Distribution of polyps and medusae

During my monitoring program in Bavaria, I did not detect polyps or medusae in 43 % (N=32, Figure 10) of all investigated lakes (N=75, see Supplementary Table 1). However, more than half of the lakes (57 %, N=43) contained life stages of *C. sowerbii.* Polyps alone were found in 26 % (N=20, Figure 10) of all investigated lakes, and polyps and medusae combined in 31 % (N=23, Figure 10). In 54% of the lakes containing any stage of *C. sowerbii* (categories II and III) medusae were identified. In 46 % of the lakes with *C. sowerbii* (categories II and III) only polyps could be detected.

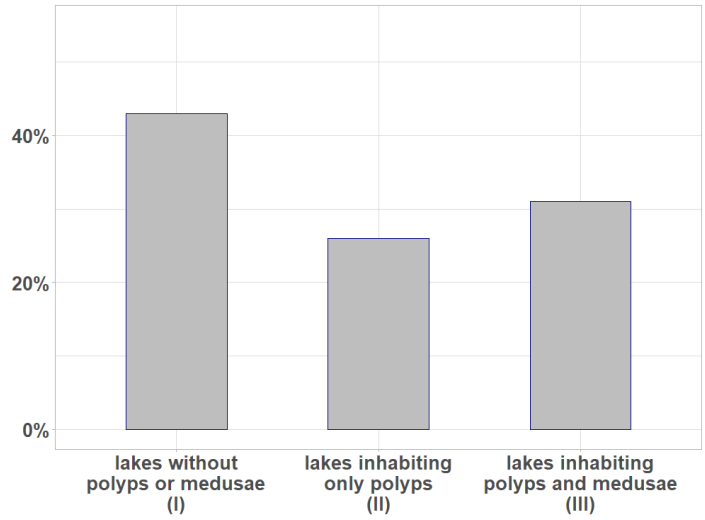


Figure 10: Percentage of lakes containing no *C. sowerbii*, only polyps or polyps and medusa.

3.1.4.Lake characteristics influencing polyp and medusae abundance

The altitudes of the monitored lakes are shown in Figure 11. The average altitude of all lakes was 484 m above sea level (m a.s.l.).

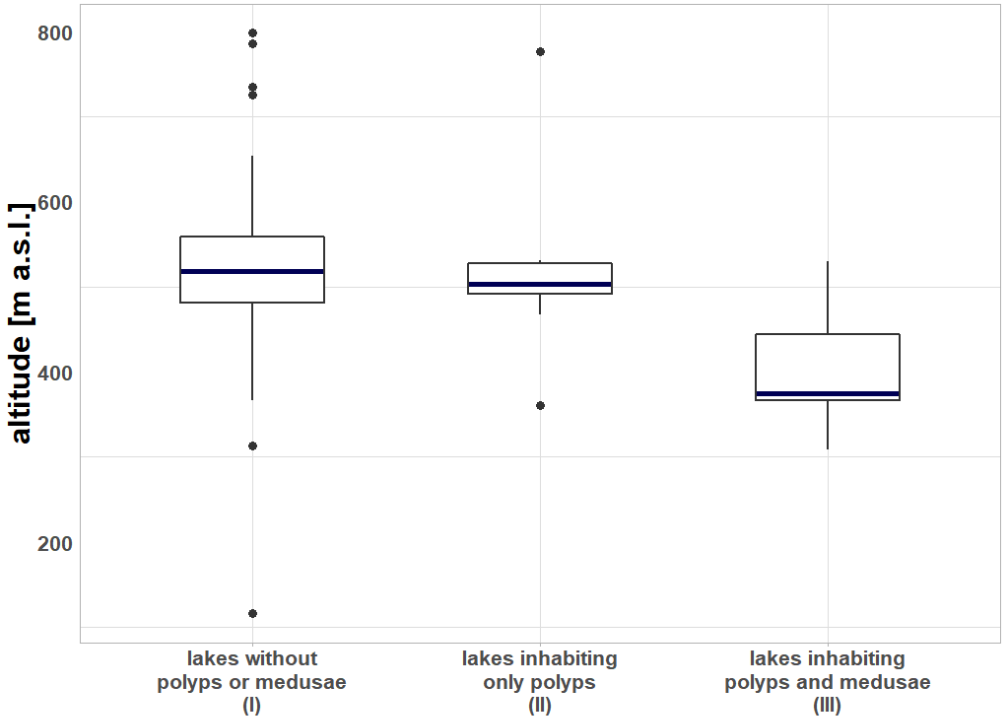


Figure 11: Boxplots of the altitudes of the lakes in the three lake type groups (lakes without *C. sowerbii*, lakes only with polyps, lakes with polyps and medusae).

The altitude of lakes without *C. sowerbii* (I) life forms (523 m a.s.l. ± 135 m a.s.l.) were on average almost similar to the average altitude of lakes inhabited by polyps only (II) (513 m a.s.l. ± 75 m a.s.l.). Lakes also inhabited by medusae (III) showed a much lower (about 100 m) average altitude (406 m a.s.l. ± 9 m a.s.l.). A significant difference emerged in mean altitudes among the three groups of lakes (ANOVA, $F=19.62$, $p<0.001$; Figure 11). While a post hoc Tukey test revealed no difference in mean altitude between lakes without *C. sowerbii* (I) and lakes inhabited by *C. sowerbii* polyps only (II), lakes containing medusae (III) had a significantly lower mean altitude ($p < 0.001$).

The log transformed surface areas of the monitored lakes are shown in Figure 12. The average areas (mean ± SE) of the three groups are 12.52 m^2 ± 0.41 m^2 for lakes without *C. sowerbii* (I), 11.93 m^2 ± 0.36 m^2 for lakes with only polyps (II), and 11.75 m^2 ± 0.54 m^2 for lakes with medusae (III). No significant difference emerged after applying a one-way ANOVA.

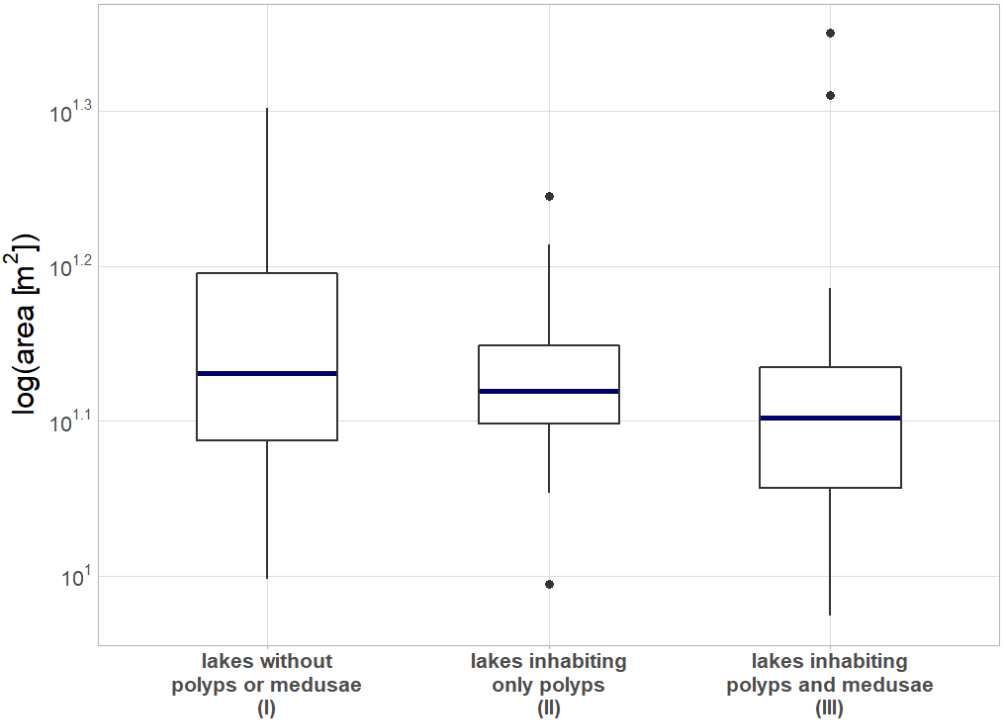


Figure 12: Boxplots of the areas of the lakes in the three lake type groups (lakes without *C. sowerbii*, lakes only with polyps, lakes with polyps and medusae). Y-axis is log scaled.

I had more artificial lakes (N=50) than natural lakes (N=25) among my monitored lakes (Table 2). The percentages within each group of lakes without polyps or medusae (I), and lakes with only polyps (II), are almost identical (41% in group I and 40% in group II; Table 2). In the group of lakes with polyps and medusae (III), the percentage is only 17%. Fisher's exact test indicates no significant difference between the three groups.

Table 2: Results of a Fisher's exact analyses of the shares of lakes with natural origin between lakes without *C. sowerbii*, lakes with only polyps and lakes with medusae. The last column shows the share of lakes of natural origin in each group. n.s. indicates no significant difference.

	lakes without polyps and medusae (I)	**lakes with polyps only (II)**	**lakes of natural origin [%]**
lakes without polyps and medusae (I)	-		0.41
lakes with polyps only (II)	P = 0.975 n.s.	-	0.40
lakes with polyps and medusae (III)	P = 0.08 n.s.	P = 0.17 n.s.	0.17

3.2. Effects of nitrate

3.2.1.Laboratory experiments

From the nitrate experiments I can see an influence of nitrate on the polyp stage of *C. sowerbii* in both the acute and chronic treatments. In both experiments the growth in all treated populations is lower than in the control treatment (1.650 µg C/well ± 0.16 µg C/well; Table 3, Figure 13a).

Table 3: Results of the nitrate experiments. Averaged final weights for each concentration in both treatments and the control treatment (± s.e.). Contrast analysis comparing the final weight of each treatment with the control treatment and the resulting effect size. * indicates a significant difference, n.s. indicates no significant difference.

concentration	treatment	Ø final weight [µg C/well] (± s.e.)	contrast analysis (p – value)	effect size
0 mg/l	control	1.650 (0.16)		
5 mg/l	chronic	1.540 (0.25)	0.630 n.s.	-0.07
10 mg/l	chronic	1.435 (0.21)	0.361 n.s.	-0.14
20 mg/l	chronic	1.208 (0.27)	0.066 n.s.	-0.31
40 mg/l	chronic	1.103 (0.15)	0.024 *	-0.40
50 mg/l	chronic	0.770 (0.09)	<0.001 *	-0.76
100 mg/l	chronic	0.595 (0.10)	<0.001 *	-1.15
150 mg/l	chronic	0.315 (0.17)	<0.001 *	-1.66
200 mg/l	chronic	0.070 (0.05)	<0.001 *	-3.16
200 mg/l	acute	1.164 (0.16)	0.047 *	-0.36
400 mg/l	acute	0.963 (0.14)	0.001 *	-0.72
600 mg/l	acute	0.455 (0.10)	<0.001 *	-1.55
800 mg/l	acute	0.193 (0.09)	<0.001 *	-3.85

In the acute experiment the average final biomass of each treatment differs significantly from the control treatment (200 mg/l: p=0.047, 400 mg/l: p=0.001, 600 mg/l: p<0.001, 800 mg/l: p<0.001; Table 3, Figure 13a)

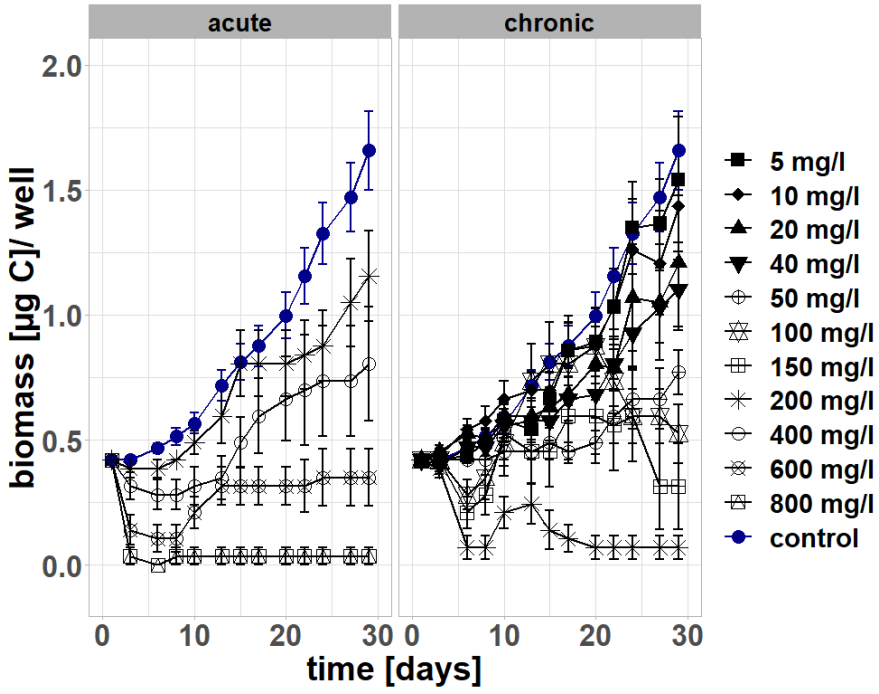
This difference increases with increasing nitrate concentration (200 mg/l: 1.164 µg C/well ± 0.16 µg C/well, 400 mg/l: 0.963 µg C/well ± 0.14 µg C/well, 600 mg/l: 0.455 µg C/well ± 0.10 µg C/well, 800

mg/l: 0.193 µg C/well ± 0.09 µg C/well; Table 3, Figure 13a). The negative effect size also increases similarly (200 mg/l: -0.36, 400 mg/l: -0.72, 600 mg/l: -1.55, 800 mg/l: -3.85; Figure 14).

In the chronic experiment the results show a similar pattern (5 mg/l: 1.540 µg C/well ± 0.25 µg C/well, 10 mg/l: 1.435 µg C/well ± 0.21 µg C/well, 20 mg/l: 1.208 µg C/well ± 0.27 µg C/well, 40 mg/l: 1.103 µg C/well ± 0.15 µg C/well, 50 mg/l: 0.770 µg C/well ± 0.09 µg C/well, 100 mg/l: 0.595 µg C/well ± 0.10 µg C/well, 150 mg/l: 0.315 µg C/well ± 0.17 µg C/well, 200 mg/l: 0.070 µg C/well ± 0.05 µg C/well; Table 3, Figure 13a). However, for the three lowest concentrations used in this experiment the contrast analysis of the final weights shows no significant difference from the control treatment (5 mg/l: p=0.63, 10 mg/l: p=0.361, 20 mg/l: p=0.066; Table 3).

For all treatments with higher concentrations the contrast analysis revealed significant differences (40 mg/l: p=0.024, 50 mg/l: p<0.001, 100 mg/l: p<0.001, 150 mg/l: p<0.001, 200 mg/l: p<0.001, Table 3). The effect sizes increase with increasing nitrate concentration (5 mg/l: -0.07, 10 mg/l: -0.14, 20 mg/l: -0.31, 40 mg/l: -0.40, 50 mg/l: - 0.76, 100 mg/l: -1.15, 150 mg/l: -1.66, 200 mg/l: -3.16; Table 3, Figure 14). The comparison of the mean biomass of the last three experimental days across the different treatments is displayed in Figure 13b.

a)



b)

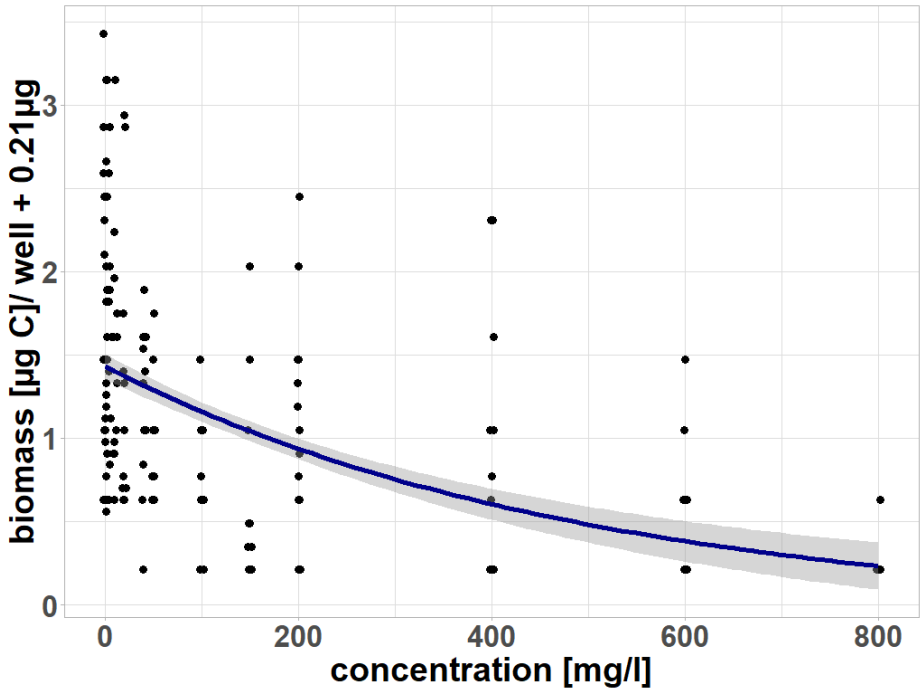


Figure 13: Growth under nitrate exposure. a) Average biomass per well for each treatment over the experiments duration. The error bars are displaying standard errors. The acute experiment is displayed on the left, the chronic experiment on the right. Each treatment was replicated twelve times. b) Mean biomass of the last three days for all treatments. The regression line with the 95% confidence interval is described by the function: $y = 1.32\text{-}0.004x + 0.0000003x^2$, ($r^2 = 0.247$, $F = 106.1$, $p < 2.2x10^{-16}$). Circles are representing acute treatments; triangles are representing chronic treatments.

It shows that the mean biomass decreases with increasing nitrate concentration. A quadratic regression line was fitted to the plotted data points ($y = 1.32\text{-}0.004x + 0.0000003x^2$, $r^2 = 0.247$, $F = 106.1$, $p < 2.2x10^{-16}$; Figure 13b). Effect sizes of the different concentrations (Figure 14) show that the effect of nitrate increases with an increased concentration, for both, chronic an acute exposure. Of note is that chronic exposure at 200 mg/l shows an effect size of -3.16, while the effect size of acute exposure with the same concentration is about ten times lower (-0.36; Table 3).

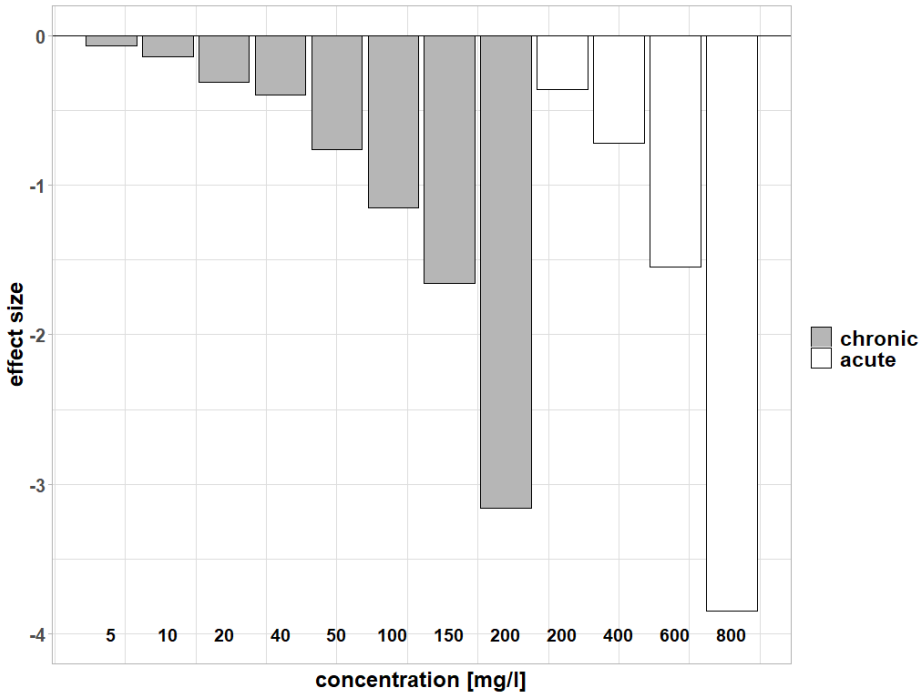


Figure 14: Display of the effect sizes of the different nitrate concentrations. Grey columns display chronic concentrations, white columns display acute concentrations.

3.2.2. Analysis of field data

The investigation of the two rivers in the west of Munich shows different patterns of polyp abundances. In the smaller river Maisach there is only one sample site out of the 27 sampled sites where polyps could be found (3.7%), while along the larger river Amper polyps were found at nine out of 27 sites (33.3%). The highest observed polyp density at a spot was almost the same in both rivers (Amper: 8, Maisach: 9; Table 4). A comparison of the general parameters of both rivers shows that the mean width, drainage area and mean run-off is greater for the river Amper than for the Maisach (Table 4).

Table 4: Comparison between the two sampled rivers. Considered were width, drainage area, mean runoff, maximum distance between the rivers, quantity of sampled sites with polyps and the maximum number of polyps per sampling site.

Factor	**Amper**	**Maisach**
River width [m]	24.22 ± 1.27	6.46 ± 0.38
Drainage area [km^2]	3100	208
Mean Runoff [m^3/s]	44.9	1.98
Maximum distance between the rivers	9.3 km	

quantity of sites with polyps	9 (33.3%)	1 (3.7%)
Maximum number of polyps per site	8	9

Analysis of the banks of both rivers shows that along the total length of the river Amper 32 kilometres are forested. For the river Maisach only eight kilometres of its banks are forested (Figure 15).

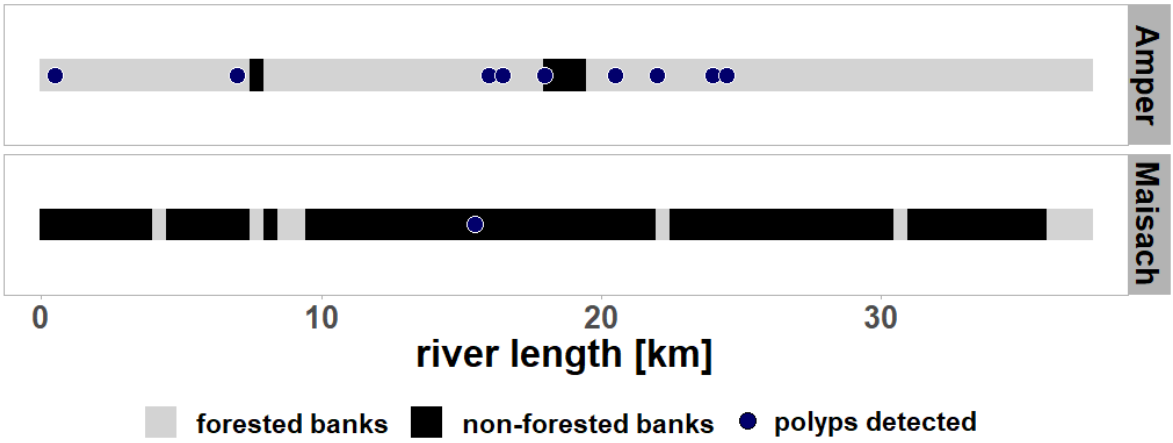


Figure 15: Forested areas besides the two rivers Amper (upper) and Maisach (lower). Grey parts indicate forested banks while black parts indicate non-forested banks. The dots are displaying if polyps were found in this part of the river.

The analysis of water samples shows that in the river Maisach the nitrate concentration fluctuates between 29 mg/l and 22 mg/l. In the river Amper the concentration increases from 3 mg/l to 5 mg/l along the investigated river length (Figure 16).

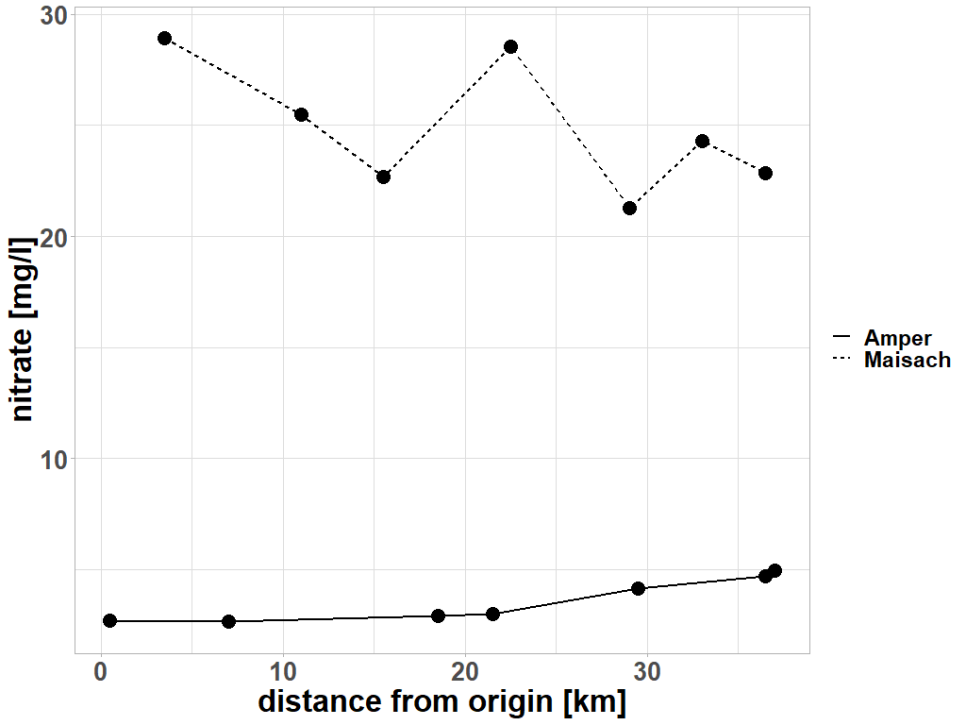


Figure 16: Nitrate concentrations of the rivers Maisach (dashed line) and Amper (line) over the investigated length of the rivers.

3.3. Effects of pesticides from ARO

For reasons unknown, single polyps sometimes disappear from the wells. This happens in general culturing as well as in experiments, and up until now no concrete reason has been found. In experiments on the influence of ARO on the growth of polyps of *C. sowerbii,* I also had few cases of initially lost polyps. All of them vanished before reproducing for the first time, so the wells remained empty. This initial loss cannot securely be linked to the pesticide treatments, as this also happens in non-experimental contexts. As these were too few to affect the results, I excluded them from the statistics.

3.3.1. Effects of Tebuconazole

In the Tebuconazole experiment the average final biomass of the control treatment was highest (2.86 µg C/well ± 0.28 µg C/well; Table 5, Figure 17a), followed by the 10µg/l Tebuconazole treatment (2.10 µg C/well ± 0.31 µg C/well; Table 5, Figure 17a), and lastly by the 7.5µg/l treatment (1.47 µg C/well ± 0.29 µg C/well; Table 5, Figure 17a).

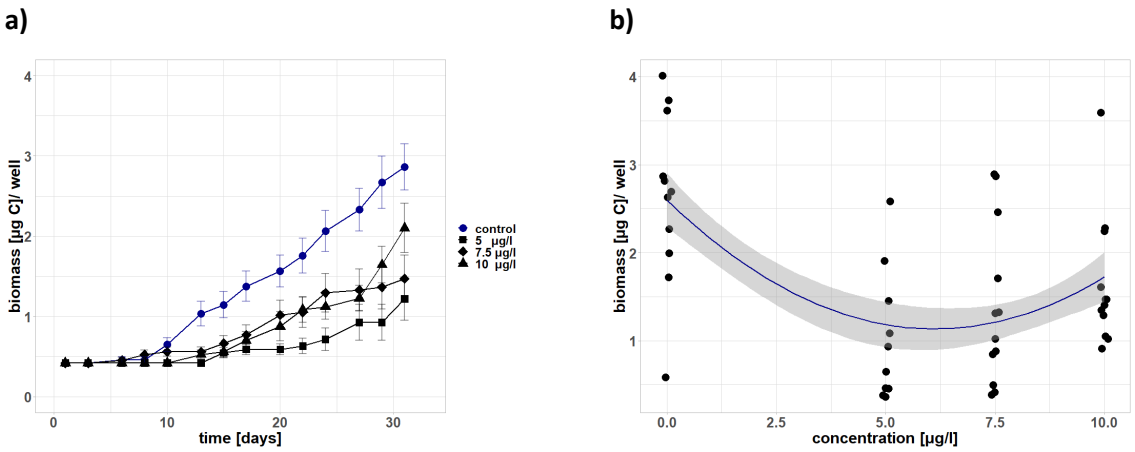


Figure 17: Growth with Tebuconazole: a) Average biomass per well for each treatment over the experiments duration. Each treatment was replicated twelve times. The error bars are displaying standard errors. b) Average number of individuals per treatment of the last three experimental days against the concentrations of Tebuconazole. The dashed line represents a quadratic regression model with the function $y=2.37-0.47x+0.04x^2$ accompanied by a 95% confidence interval.

In the 5 µg/l treatment the average final biomass was the lowest (1.22 µg C/well ± 0.27 µg C/well; Table 5, Figure 17a). A quadratic regression of biomass versus Tebuconazole concentration was significant ($y=2.60-0.48x+0.04x^2$; $r^2 = 0.24$; $F = 22.08$; $p < 0.001$; Figure 17b). The contrast analysis showed significant differences between two of the three Tebuconazole treatments and the control treatment. Significant differences were seen between the control and 5µg/l treatments (p=0.0005), and the control and 7.5µg/l treatments (p=0.01).

Table 5: Results of the acute experiment with Tebuconazole. * indicates a significant difference, n.s. indicates no significant difference.

concentration	**Ø final weight [µg C/well] (± s.e.)**	**contrast analysis (p – value)**	**effect size**
0 µg/l	2.86 (0.28)		
5 µg/l	1.22 (0.27)	0.0005 *	-0.85
7.5 µg/l	1.47 (0.29)	0.01 *	-0.66
10 µg/l	2.10 (0.31)	0.23 n.s.	-0.31

Between the 10µg/l treatment and the control I could not detect a significant difference. Within the Tebuconazole treatments the effect sizes became less negative with increasing Tebuconazole concentrations (5 µg/l: -0.85, 7.5 µg/l: -0.66, 10 µg/l: -0.31; Table 5, Figure 20).

3.3.2. Effects of Pirimicarb

In the experiment with Pirimicarb, the average final biomass showed no clear correlation to Pirimicarb exposition. The final biomass in the treatment with 4 µg/l Pirimicarb was the highest (3.41 µg C/well ± 0.45 µg C/well), followed by the 2 µg/l treatment (3.28 µg C/well ± 0.48 µg C/well), the control treatment (2.86 µg C/well ± 0.29 µg C/well), and the 1 µg/l treatment (2.91 µg C/well ± 0.58 µg C/well) (Figure 18a). The regression analysis for the averaged biomasses of the last three observation days showed no significant correlation between biomass and insecticide concentration (Figure 18b). The ANOVA analysis for the averaged biomass at the last day showed no significant difference between the treatments either.

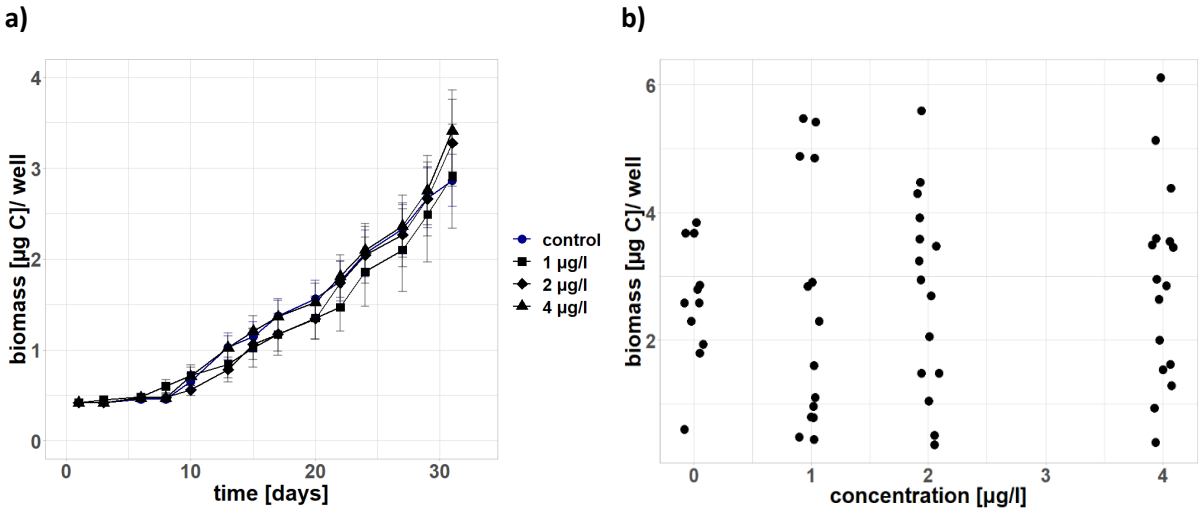


Figure 18: Growth with Pirimicarb: a) Average biomass per well for each treatment over the experiments duration. Each treatment was replicated twelve times. The error bars are displaying standard errors. b) Average number of individuals per treatment of the last three experimental days against the concentrations of Pirimicarb.

The effect sizes of Pirimicarb on growth still increased with increasing pirimicarb concentration (1 µg/l: 0.02, 2 µg/l: 0.14, 4 µg/l: 0.18; Table7, Figure 20).

Table 6: Results of the acute experiment with Pirimicarb.

concentration	Ø final weight [µg C/well] (± s.e.)]	effect size
0 µg/l	2.86 (0.29)	
1 µg/l	2.91 (0.58)	0.02
2 µg/l	3.28 (0.48)	0.14
4 µg/l	3.41 (0.45)	0.18

3.3.3. Effects of TBA

In the TBA experiments the biomasses of all treatments showed a positive increase (Figure 19a). Of the chronic treatments the final biomass in the 0.5 µg/l treatment was the highest (1.75 µg C/well ± 0.28 µg C/well; Figure 19a), followed by the 0.1 µg/l treatment (1.22 µg C/well ± 0.18 µg C/well; Figure 19a) and the 1 µg/l treatment (0.88 µg C/well ± 0.21 µg C/well; Figure 19a). Highly similar to the latter

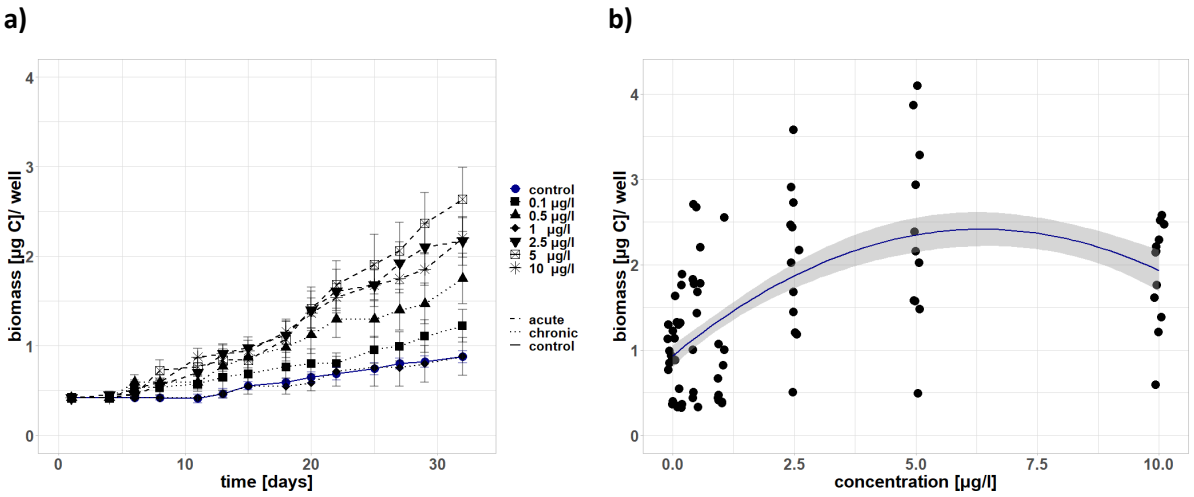


Figure 19: Growth with TBA: a) Average biomass per well for each treatment over the experiments duration. Each treatment was replicated twelve times. The error bars are displaying standard errors. Solid line is displaying the development of the control treatment, dashed lines are displaying the acute treatments and dotted lines are displaying the chronic treatments b) Average number of individuals per treatment of the last three experimental days against the concentrations of TBA. The dashed line represents a quadratic regression model with the function $y=0.92-0.49x+0.04x^2$ accompanied by a 95% confidence interval.

was the final biomass of the control treatment (0.84 µg C/well ± 0.1 µg C/well; Figure 19a). The three acute treatments with TBA developed in a similar way. The final biomasses of these treatments were almost identical (2.5 µg/l: 2.17 µg C/well ± 0.27 µg C/well, 5 µg/l: 2.64 µg C/well ± 0.36 µg C/well, 10 µg/l: 2.21 µg C/well ± 0.22 µg C/well; Figure 19a, Table 7). A significant correlation between average biomasses for the last three observation days against TBA concentrations can be displayed with a quadratic regression ($y=0.92+0.49x-0.04x^2$; $r^2 = 0.33$; $F = 64.8$; $p < 0.001$, Figure 19b).

To analyse the difference between the treatments and control, an ANOVA with a post hoc contrast analysis was performed. This analysis showed a significant difference between the chronic 0.5 µg/l TBA treatment (p=0.003, Table 7) and the control treatment, as well as between all acute TBA treatments and the control treatment (2.5 µg/l: p=0.001, 5 µg/l: p<0.001, 10 µg/l: p<0.001; Table 7). The 0.1 µg/l treatment and the 1 µg/l treatment of the chronic exposures showed no significant difference to the control treatments (0.1 µg/l: p=0.2, 1 µg/l: p=0.89, Table 7).

Table 7: Results of the experiments with TBA. * indicates a significant difference, n.s. indicates no significant difference.

concentration	**treatment**	**Ø final weight [µg C/well] (± s.e.)**	**contrast analysis (p – value)**	**effect size**
0 µg/l	control	0.84 (0.1)		
0.1 µg/l	chronic	1.22 (0.18)	0.20 n.s.	0.37
0.5 µg/l	chronic	1.75 (0.28)	0.003 *	0.73
1 µg/l	chronic	0.88 (0.21)	0.89 n.s.	0.05
2.5 µg/l	acute	2.17 (0.27)	0.001 *	0.87
5 µg/l	acute	2.64 (0.36)	0.00003 *	1.06
10 µg/l	acute	2.21 (0.22)	0.0009 *	0.89

On the whole TBA showed positive effect sizes on growth for all treatments. Within the chronic treatments the effects increased from 0.1 µg/l (0.37) to 0.5 µg/l (0.73) but decreased at the highest concentration (1 µg/l: 0.05). For the acute Treatments TBA showed positive effects on growth with almost similar effect sizes for all treatments (2.5 µg/l: 0.87, 5 µg/l: 1.06, 10 µg/l: 0.89, Table 7, Figure 20).

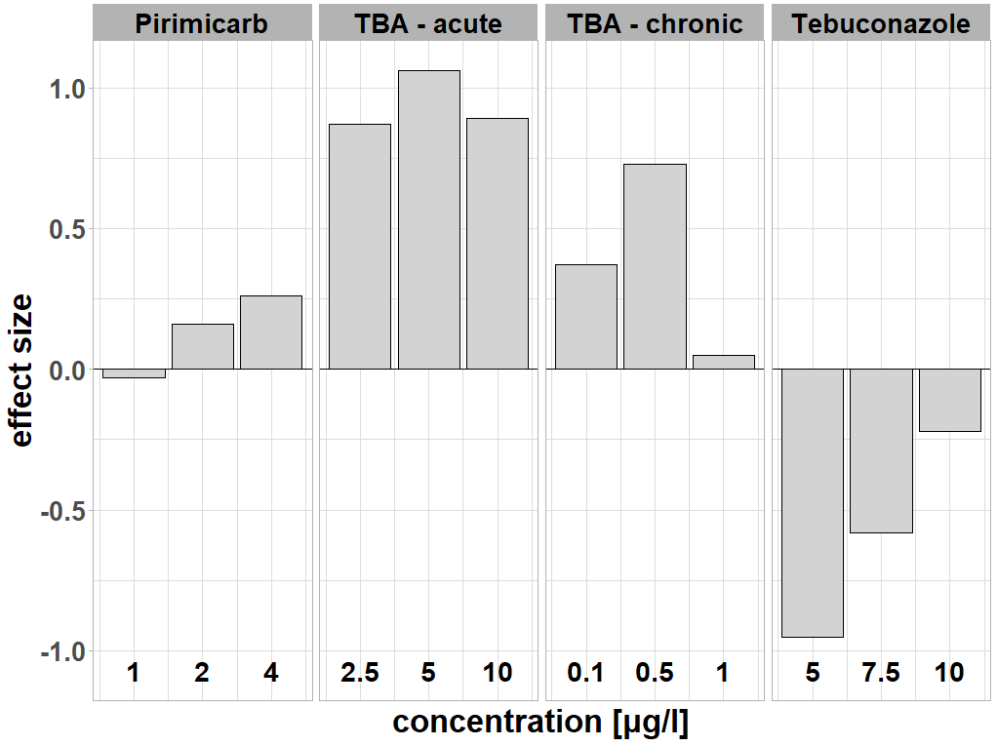


Figure 20: Display of the effect sizes of the different pesticide concentrations (Pirimicarb, TBA – acute, TBA – chronic, Tebuconazole).

3.4. Effects of handling aspects

3.3.4. Detection improvement of polyps with neutral red

3.3.4.1. General staining

Staining with neutral red was possible with the polyps. All staining concentrations caused a similar red colouring in the polyp stage, as judged by eye (Figure 21).

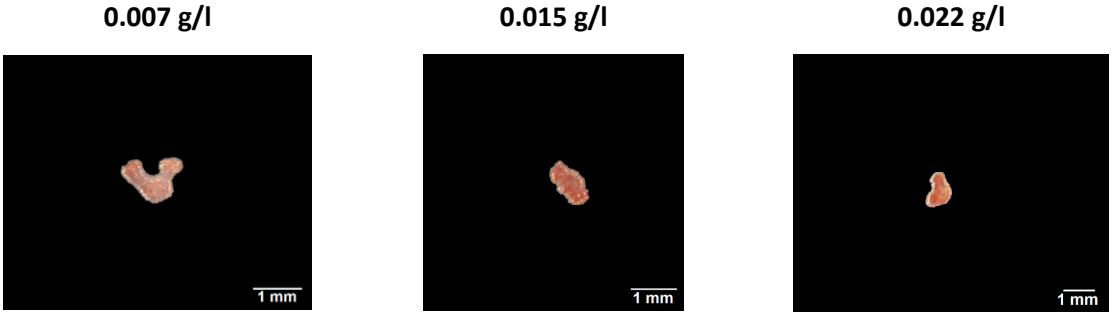


Figure 21: The polyp stage of *C. sowerbii* after staining with different concentrations of neutral red.

3.3.4.2. Development of stained polyp cultures

The following growth of the three treatments and the control showed different results. In the treatment with the lowest concentration of neutral red the biomass increased the most, up to 1.26 µg C/ well ± 0.24 µg C/ well (Table 8, Figure 22a). The intermediate concentration caused a growth similar to the control (control: 0.70 µg C/ well ± 0.28 µg C/ well, 0.015 g/l: 0.77 µg C/ well ± 0.19 µg C/ well; Table 8, Figure 22a). The highest concentration of neutral red (0.022 g/l) caused a decrease in biomass (0.28 µg C/ well ± 0.07 µg C/ well; Table 8, Figure 22a).

Table 8: Results of the experiment with neutral red.

concentration	**Ø final weight [µg C/well] (± s.e.)**	**effect size**
0 g/l	0.70 (0.28)	
0.007 g/l	1.26 (0.24)	0.59
0.015 g/l	0.77 (0.19)	0.1
0.022 g/l	0.28 (0.07)	-0.92

The one-way ANOVA revealed a barely non-significant difference between the three treatments and the control ($p=0.06$).

Regarding the average biomass of the last three days of the experiment there is a significant correlation between the neutral red concentration and the decrease in biomass, described by an exponential regression ($y=1.68\ e^{-37.21\ x}$, $r^2= 0.26$, $F=11.73$, $p=0.002$; Figure 22b). The effect sizes decrease from the lowest concentration (0.007 g/l), which has a positive effect on growth (0.59, Figure 22c), through the intermediate concentration (0.015 g/l), which has a slightly positive effect (0.1; Figure 22c), to the highest concentration (0.022 g/l), which has a negative effect on growth (-0.92; Figure 22c).

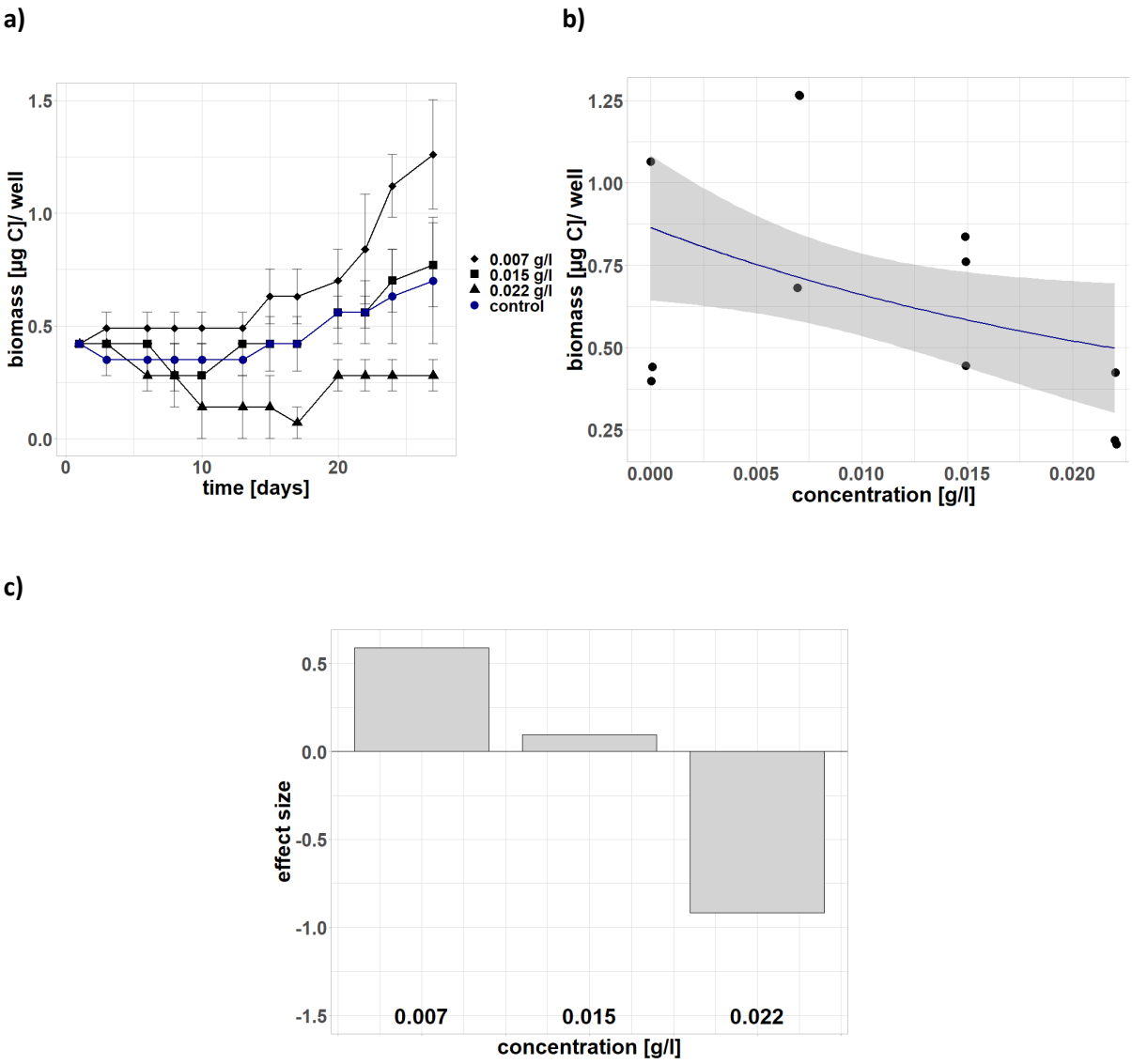


Figure 22: Growth with neutral red: a) Average biomass per well for each treatment over the experiments duration. Each treatment was replicated three times. The error bars are displaying standard errors. b) Average number of individuals per treatment of the last three experimental days against the concentrations of neutral red. The dashed line represents a quadratic regression model with the function y=1.68 * exp(-37.21*x) accompanied by a 95% confidence interval. c) Display of the effect sizes of the different neutral red concentrations.

3.3.4.3. Usability of staining in field samples

By staining field samples, I figured out that, besides the polyps, only few of the surrounding algae, benthic species and abiotic materials took on the stain. As such, the polyps become more visible using this method (Figure 23).

a) b)

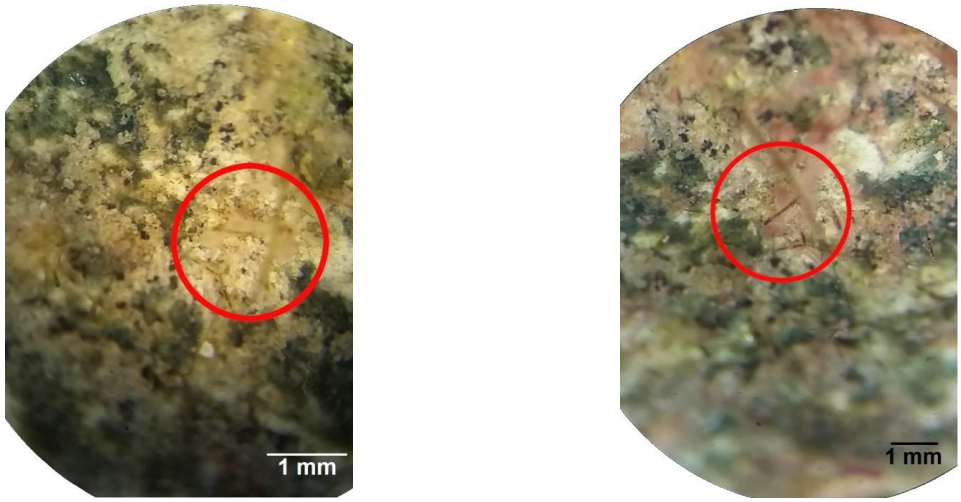


Figure 23: Polyp colony on a stone before staining with neutral red (a) and after staining with neutral red (b)

The results of the polyp counts show that the inexperienced researcher detected more polyps while counting stained polyps on stones 1, 3, 4 and 5, and the same amount on stone 2. The difference between the unstained and the stained polyps for this researcher was between 45 % and 100 % (Table 9). The experienced researcher detected more polyps on stones 3, 4 and 5, the same amount on stone 1, and fewer polyps on stone 2. The increase in detection here was between 24 % and 66%, and the decrease in the number of detected polyps was 25 % (Table 9).

Table 9: Results of the counting of polyp colonies on five stones and the appeared differences before and after the staining with neutral red. The countings were done by an experienced and an inexperienced researcher.

	inexperienced researcher			**experienced researcher**		
	before	**after**	**difference**	**before**	**after**	**difference**
stone 1	0	4	100%	0	0	0
stone 2	0	0	0	4	3	-25 %
stone 3	11	20	45 %	16	21	24 %
stone 4	2	6	66 %	4	10	60 %
stone 5	2	6	66 %	2	6	66 %
average difference			**69 %**	**average difference**		**44 %**

The average increase in detected polyps was 69 % for the inexperienced researcher and 44 % for the experienced researcher (Table 9). The t-test for differences in the increase showed that there was no significant difference between the increases for the experienced and inexperienced researchers (t=1.27, df=8, p=0.24).

3.3.5. Effects of medium change intervals on polyp growth

The growth in the treatments with different medium change intervals was similar in all three treatments (Figure 24a).

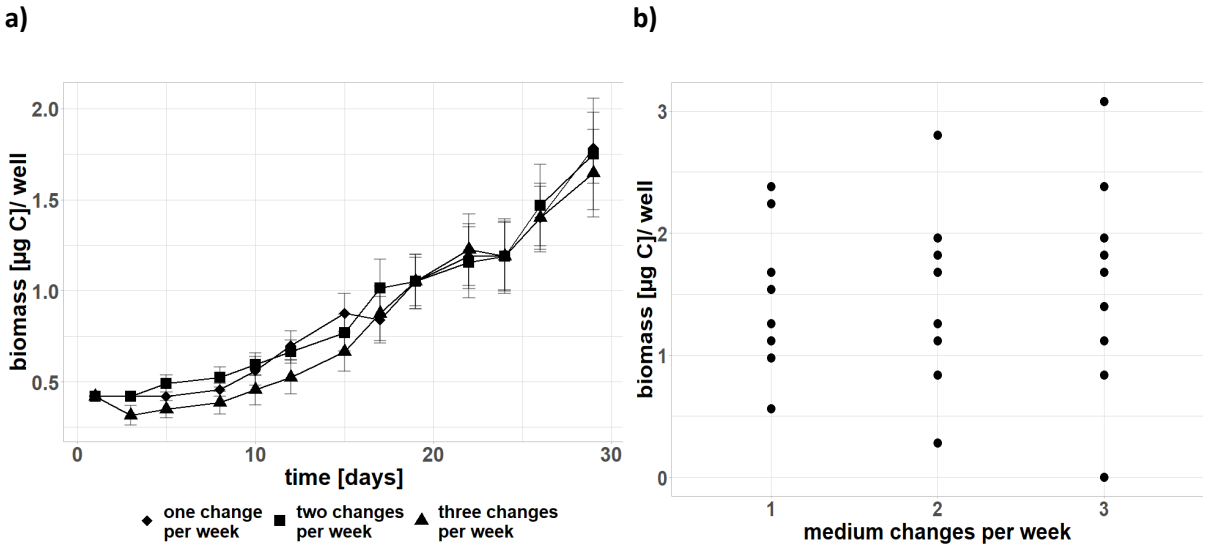


Figure 24: Growth with different intervals of medium refreshing. a) Average biomass per well for each treatment over the experiments duration. Each treatment was replicated twelve times. The error bars are displaying standard errors. b) Average number of individuals per treatment of the last three experimental days against the concentrations of neutral red.

The final biomasses were 1.79 µg C/well ± 0.19 µg C/well for the treatment with one change per week, 1.65 µg C/well ± 0.24 µg C/well for the treatment with two 2 changes per week, and 1.75 µg C/well ± 0.31 µg C/well for the treatment with three changes per week. The ANOVA revealed no significant differences between the three treatments (p=0.92). The analysis of the averaged final biomasses for the last three days in each well shows no correlation between the number of medium changes and measured biomass per well (Figure 24b).

3.3.6. Regeneration times for complete head structures

The results of the regeneration experiment are displayed in Figure 25. In the regeneration experiment a total of 24 heads were removed from polyps. These 24 polyps were grouped in twelve colonies, ten of which consisted of two polyps. The remaining four polyps were arranged in one three polyp colony, and one single polyp. After 24 hours no heads had regenerated and the total number of colonies had decreased to ten colonies. These colonies consisted of six two-polyp colonies, three one-polyp colonies, and one three-polyp colony. Over the next 48 hours the total number of colonies remained at ten colonies, while the total number of regenerated heads increased to 6 after 48 h and to 17 after 72 h.

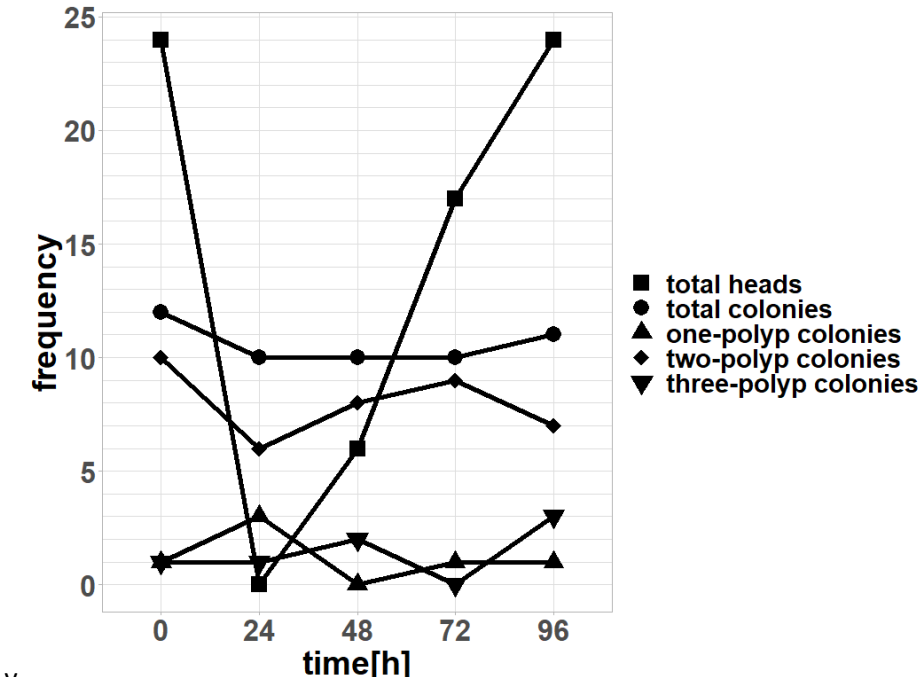


Figure 25: Development of polyp colonies after removing the head structures of all individuals over 96 hours.

The number of two-polyp colonies also increased to eight after 48 h and to nine after 72 h. The one-polyp colonies vanished in the period from 24 h to 48 h. In the following counts (72 h) there was again one one-polyp colony. The number of three-polyp colonies increased by one between the 24 h count (1 colony) and the 48 h count (two colonies), and had vanished by the 72 h count. In the final count, the total number of regenerated heads had reached the initial number of 24 heads. The total number of colonies remained at eleven, so over the course of the whole regeneration experiment only one

colony was lost. Compared to the initial numbers, the number of two-polyp colonies dropped to seven, the number of one-polyp colonies remained the same, and the number of three-polyp colonies increased to three (Figure 25).

4. Discussion

4.1. Distribution patterns of *Craspedacusta sp.*

4.1.1. Influence of rivers on the distribution of *Craspedacusta sp.*

The aim of my project was to estimate distribution patterns of the invasive freshwater jellyfish *C. sowerbii*. With data sets from different approaches (current citizen science data from Germany, long-term published data from Germany, published data from France, and data from my own monitoring), combined with a meta-analysis of basic geographical factors, I was able to explore common distribution patterns and overcome certain weaknesses of using a single approach alone. The comparison of my regional data from Germany with a French data set supports my findings that rivers are important freshwater distribution vectors, not just in Germany but across Europe and probably globally.

4.1.1.1. Comparison of colonisation patterns based on long-term and current jellyfish observation reports from Germany

The literature research data (data set 2) of 85 lakes inhabited by medusae is based on jellyfish sighting reports across Germany between 1931 and 2019 (Supplementary Table 3), while the data sets of reported medusa sightings from my citizen science approach (data set 1) are based on data obtained between 2015 and 2019 (Supplementary Table 2). Notably, although both data sets overlapped for only four lakes, a striking similarity emerged in the distribution pattern of colonised lakes. Taking each data set individually, I found that the proportion of jellyfish lakes declined exponentially with increasing distance from a river. This finding was supported by the joint analysis of the combined German data sets (Figure 7a). Importantly, this is not in line with the general distribution of lakes in Germany, which does not show an exponential decline with increasing distance to a river (Figure 7d). The extremely similar relationship between distance and colonisation in the recent data set from the citizen science approach and published data since 1930 (without substantial overlap of lakes, see above) indicates a general and stable pattern, pointing clearly towards the importance of large rivers for the distribution of this invasive species. The shape of the distribution function (exponential decline) is

typical of colonisation processes along the distance from a source pool of organisms to a new habitat (Simberloff, 1974). I assume that rivers may therefore act as one possible source pool for dispersal into lakes. Whereas rivers seem not to be a preferred habitat for medusae of *C. sowerbii* (except those with very low water current, e.g. dead arms) (Marchessaux et al., 2019) the asexually reproducing polyps can establish populations in running waters (Dejdar, 1934)(own observations). Flooding events, water birds, or other vectors could then transport polyps or other benthic life stages from rivers into nearby lakes, resulting in the observed exponential "colonisation" relationship with distance. Regarding the similarity of the results over about 100 years in Germany, it seems that distribution from rivers occurs at a relatively slow pace. Otherwise, the number of lakes with medusae at greater distances from rivers should converge with the numbers nearer rivers. As a distribution with biotic vectors like humans or other animals would occur regularly, a slight shift towards a saturation should at least be visible in the current data. As this saturation effect is heretofore lacking, a higher impact of irregularly occurring events like floods seems to be more important for the small-scale distribution of *C. sowerbii*.

4.1.1.2. Common distribution patterns of jellyfish in Europe

In all likelihood, rivers are not the only significant means of distribution of *C. sowerbii* in Germany. Natural rivers connected by canals—to improve their economic value—form a vast network of waterways. In Europe, about 28,000 km of waterways connect the North and Baltic Seas with the Mediterranean, and the Atlantic Ocean with the Caspian Sea (Galil et al., 2008). Given this context the rapid distribution of *C. sowerbii* all over Europe is understandable. It is not surprising, therefore, that the data available from France (Marchessaux et al., 2019) also points towards a highly similar pattern between the proportion of jellyfish sightings and the distance of colonised lakes from large rivers, such as seen in Germany. The first observations of the jellyfish stage were both made at the beginning of the 20th century (1901: France (Vaney & Conte, 1901), 1905: Germany (Boecker, 1905)), which implies a similar time of initial arrival in both countries. The rivers considered for my analyses are in both cases the four rivers with largest river basin districts. In both countries, the distribution pattern shows very similar functions of distance to large rivers. Based on these analogical circumstances for the

distribution of *C. sowerbii,* the similar patterns in both countries can be seen as an indicator of similar mechanisms in operation, driving the distribution pattern of this invasive species.

4.1.1.3. Inclusion of Citizen data and biases

Citizen science projects are a valuable source of data on the distribution of invasive species, especially for ecological purposes (Silvertown, 2009). With such an approach I was able to obtain distribution data that were otherwise difficult to detect, since these lakes are not necessarily monitored by scientists. There is only a slight chance of misidentification by non-experts of jellyfish in European freshwater, due to their unique occurrence and shape, and as such the reported sightings are highly reliable. A possible weakness of the analysis could be the non-random geographical data coverage across Germany. The Faculty of Biology of the Ludwig-Maximilians-Universität is located in Munich in southern Germany. Even though the appeals were broadcast across Germany, local publishers like radio stations and newspapers spread the requests more frequently. My data will therefore definitely show a bias towards higher resolution in southern Germany. To overcome such a potential bias, I also included distribution patterns from existing published reports of jellyfish sightings all over Germany.

4.1.2. Comparison of polyp and medusae distribution ranges

From my studies I was able to draw several conclusions. Primarily, *C. sowerbii* inhabits lakes at least twice as frequently as the observation of the medusa stage alone would indicate. Basic geographic parameters indicate that altitude and/or associated parameters such as temperature were an important factor in the occurrence of medusa, while the distribution of the polyp stage seems to be independent of this factor.

4.1.2.1. Role of life stages for detection, dispersal and colonisation

C. sowerbii has a complex life cycle, including pelagic and benthic life stages. For population dynamics outside its native range, it seems that sexual reproduction by pelagic medusae is highly limited (Acker & Muscat, 1976; Lundberg et al., 2005). Therefore only asexual reproduction by benthic polyps is important for building up new populations, thereby determining the local invasion success and observed

distribution patterns. Sightings of the more easily visible medusae of *C. sowerbii* usually also indicate the presence of polyps producing the medusae. However, it is unclear how many lakes have already been invaded by polyps but do not show substantial numbers of medusae, as not all polyps necessarily produce medusae (Fritz et al., 2007). Regarding existing studies on factors triggering the medusa budding in polyps, it seems that this is most probably a multi-causal process. Several factors like temperature, food, pH-value and light are believed to play an important role in this process, but the exact conditions needed to induce budding are as yet unknown (McClary, 1959; Acker & Muscat, 1976; Folino-Rorem et al., 2016).

One important question still remaining after establishing the distribution of lakes inhabited by medusae is how well the distribution of the medusa stage reflects the overall distribution of *C. sowerbii*. Polyps are too inconspicuous and too small to be detected by routine citizen science projects or monitoring. As the initial introduction of polyps in a lake happens most probably unnoticed and possibly by waterfowl or by human vectors like boats or angling gear (Burton, 2021), no indicators for the presence of *C. sowerbii* exist until either medusae occur or the polyp stage is detected. However, most scientific publications reporting *C. sowerbii* occurrences refer to medusae sightings, and not polyps (Karaouzas et al., 2015; Fraire-Pacheco et al., 2017; Jaksic et al., 2017; Kozuharov et al., 2017; Riyas & Kumar, 2017; Marchessaux et al., 2021b). I therefore installed an institutional monitoring program for *C. sowerbii*. In this program I search for the medusa stage as well as for the polyp stage. The latter task requires bringing stone samples to a laboratory for screening under a microscope. I therefore restricted my monitoring program to lakes close to the laboratories in Munich and the Limnological Station Seeon of LMU in Southern Bavaria.

My monitoring data shows that *C. sowerbii* is found in many more lakes than estimated from medusae sightings alone. This finding is similar to results from lakes in New Zealand (Duggan & Eastwood, 2012). In this screening the authors screened 26 lakes, and found medusae in 8 and polyps in 18 (medusae 31% of screened lakes, polyps 69% of screened lakes). These results match my own results (medusae found in 31% of screened lakes, polyps found in 57% of screened lakes), which indicates that the polyp

stage can be found in about twice the number of lakes as compared to the medusa stage. One should take into account that I did not screen very large areas of lake shorelines for polyps. My data indicate that with a straightforward and manageable effort, polyps can be already found in more than half of the investigated lakes (57%). A very detailed screening, including much larger areas of shoreline, would have probably further increased the number of polyp observations. One could even speculate whether polyps of *C. sowerbii* are already "regular" members of benthic food webs in lakes in general.

4.1.2.2. Impact of lake characteristics on establishing jellyfish populations

Beside the logistical constraints restricting sampling to lakes near the field station and laboratory, my sampling still included 75 lakes. The geographical situation in upper Bavaria enabled us to sample different types of lakes. Located on the northern foothills of the Alps, this region provides major lake types found in Germany including deep peri-alpine lakes, shallow glacial kettle lakes, and artificial lakes, mostly remaining from gravel mining or construction work. Despite my regional restriction, including all major types of lakes still allows for a certain amount of generalisation.

For all monitored lakes, data about their size (area), origin (natural versus artificial) and altitude were available (Grimminger, 1982). It seems that the presence of polyps was independent of lake size (area), origin of the lake (natural/artificial), or its altitude above sea-level. The distribution pattern of lakes inhabited by polyps does not differ significantly from the pattern found for lakes without any life stage of *C. sowerbii*. Polyps therefore show an enormous niche width in terms of abiotic parameters, probably much larger than that of the medusa stage. Additionally, polyps can form large and stable populations without the presence of medusae, meaning that no specific niche requirements important for medusae must be fulfilled for asexually reproducing polyp populations.

In contrast, basic geographical parameters showed some influence on medusae occurrence. A clear effect of altitude was visible; lakes inhabited by medusae were located at a significantly lower altitude compared to lakes without the presence of medusae. On average, lakes inhabited by medusae were located at an altitude about 100m lower than lakes without medusae. This accumulation of lakes with

medusae at lower altitude is also visible from the study of Marchessaux et al. (2021b). In this work higher numbers of lakes with medusae below about 500 m a.s.l. were proven for all continents.

Temperature is considered to be one of the most important factors influencing the medusa budding in polyps. In some studies, water temperatures of at least about 28°C are described to be necessary for medusae production, explaining the occurrence of medusae mainly in summer in temperate regions (Stefani et al., 2010; Minchin et al., 2016; Marchessaux et al., 2019). This matches my results, since at lower altitudes the average temperature is higher. The so-called adiabatic lapse rate states the mean air temperature decrease is about 0.6° C per 100 m higher altitude (MacArthur, 1984). The decrease in air temperature is also apparent in lake temperatures, as the surface temperature of lakes at least during summer is about 5° C above air temperature (Livingstone et al., 1999). Altitude is therefore a well-established proxy for temperature in numerous ecological studies (Klanderud & Birks, 2003; Hoiss et al., 2012). The other factors I examined for relevance in the medusa budding process were lake area and origin. I assumed lakes with smaller surface area and lakes of artificial origin to be warmer than lakes with larger surface area and of natural origin. However, my investigation shows some trends but no mathematical significant differences between lakes with different surface areas, nor artificial and natural ones. This reinforces the assumption that temperature is not the only factor influencing the budding process.

However, altitude is not only a proxy for temperature. A broad variety of other factors also change with altitude (Müller et al., 1998). With increasing altitude, the amount of agrochemicals and nutrients decreases, as the catchment of each lake is smaller. This has a cascading effect on the organic matter in the lake; with less nutrients the primary production gets depleted, with knock-on effects on the whole food chain. Furthermore, with lower amounts of organic matter in the lake, the amount of light reaching the water column increases. Additionally, CO_2 concentration in the lakes decreases with increasing altitude, since at higher altitudes the partial pressure of CO_2 in the atmosphere decreases. This decrease in CO_2 also affects the pH-value of the water as these values are also closely linked. All of these effects (chemical composition, organic matter, light, pH and CO_2) of changing altitude are

reckoned to affect the budding process of polyps of *C. sowerbii* (Acker & Muscat, 1976). Since all developments at higher altitudes (fewer organic matter, higher light intensities, lower CO_2 concentrations, higher pH (Müller et al., 1998)) are factors hindering the development of medusae, less medusae are to be expected there.

As mentioned above, my monitoring included a large range of temperate lake types. I assume that my findings are therefore not restricted to Bavaria, but to temperate regions in general. *C. sowerbii* are much more common in lakes than one would estimate from medusae sightings alone, and it seems that environmental parameters such as temperature may determine which of the lakes already containing polyps will also be inhabited by medusae.

4.2. Effects of nitrate on the growth of *Craspedacusta sp.*

My experiments on the effects of nitrate on the polyp stage of *C. sowerbii* show that nitrate causes negative effects on the growth of polyps. These negative effects occur at both chronic and acute exposure. The effects increase constantly with increasing amounts of nitrate in the water, and even low concentrations have a negative effect on the growth of polyps.

In the European Union the maximum legal value for nitrate in water for human consumption is 50 mg/L NO_3^- (European Commission, 1998). The effects on human health assumed to be linked to elevated nitrate concentrations in drinking water are several kinds of cancer, thyroid diseases, and birth defects in the central nervous systems. Studies on freshwater species show serious effects on growth and reproduction of invertebrates, fish and amphibians (Camargo et al., 2005).

One common species for aquatic invertebrate toxicology experiments, *Hydra sp.*, is among the species affected by nitrate in the water. However, comparing concentrations affecting *Hydra attenuata* reveal that the polyp stage of *C. sowerbii* is even more sensitive. In a study from 1990 the threshold for a toxic effect of nitrate on *H. attenuata* ranged between 150 mg/L NO_3^- and 250 mg/L NO_3^- (Tesh et al., 1990).

In my experiment this threshold was between 20 mg/L NO_3^- and 50 mg/L NO_3^- for *C. sowerbii* at chronic exposure.

One major source of nitrate in surface waters is the food and energy production. Only a small part of the nitrogen produced to fertilize crops actually reaches plants; the rest is emitted into the atmosphere or into ground or surface waters (Galloway & Cowling, 2002; Rockström et al., 2009). As a result, the greater extent to which agricultural land surrounds a waterbody, the more nitrate reaches and negatively affects the quality of this water, its habitats and associated biological assemblages (Allan, 2004). Riparian areas located between agricultural areas and rivers act as filters for chemicals and sediments for run-off from the anthropogenically used land (Gilliam, 1994). Even narrow zones with stable, dense vegetation are effective in the reduction of chemical run-off. Nevertheless, the larger the riparian zones are, the greater positive effect they have on rivers (Daniels & Gilliam, 1996).

My field study to support my results from the laboratory experiment was therefore performed with one river flowing through a landscape with high agricultural use, and one flowing through a forested and protected landscape. The results of the analysis of nitrate concentrations in both rivers followed my expectations. The river Maisach, with only short ranges (eight kilometres) of forested banks, had 4 – 6 times higher nitrate concentrations than the river Amper. Despite this lack of forested banks, the concentrations are still within the European Commission's limits for surface waters. The river Amper flows mainly through protected areas and has 32 km of forested banks in the stretch examined. The nitrate concentration in this river is quite low, but increases along the length of the river, implying a constant, low inflow of nitrate. The polyp abundances in both rivers followed predictions from my laboratory experiments. In the river Amper (low nitrate concentration) the number of sites where polyps were found was nine times higher than in the river Maisach (high nitrate concentration).

From these data I can conclude that nitrate is a potentially limiting factor in the distribution of *C. sowerbii*. As the concentrations found in these particular rivers are not lethal to the specimens, the distribution is not likely to fail due to lethally toxic effects of nitrate, but could be slowed due to lower

growth and reproduction rates. As fewer colonies are present in rivers with elevated nitrate concentrations the potential of these rivers as sources for new invasions is low, and fewer downstream rivers and lakes can be reached from there. As these results base on a rather small sample size and timespan, further investigations using repeated samplings and additional rivers with varying nitrate concentrations would support these first results from the field.

4.3. Effects of pesticides occurring in ARO on the polyp stage of *Craspedacusta sp.*

4.3.1. Effects of Tebuconazole

In my experiments, the fungicide Tebuconazole was shown to have a negative effect on the growth of the polyp stage of *C. sowerbii*. In all treatments, the growth was lower than in the control treatment. A noteworthy aspect of the results was that the highest concentration (10 µg/l) had the lowest effect of the three treatments, while the lowest concentration had the highest effect. From these results, an effect based solely on the toxicity of the chemical can be excluded.

An obvious outcome in terms of the growth in all treatments with Tebuconazole was that until day 13, all treatments stayed at an equally low level; at this point in the control treatments the amount of biomass had already doubled. After day 13 the biomass increased in all treatments. However, as mentioned above, the average biomass in the fungicide treatments increased with increasing fungicide concentration.

The low or non-existent growth in the first days of these treatments suggests a stress reaction and initial inhibition of the polyps due to new environmental circumstances. One effect of stress induced by, for example, environmental conditions, is an elevated risk of infection by pathogenic fungi (Wong et al., 1998). Such an infection can potentially affect the reproductive success of the polyp stage of *C. sowerbii*.

Tebuconazole is also known to affect non-target fungi, as it inhibits the synthesis of a basic component of fungi membranes (Dijksterhuis et al., 2011). With Tebuconazole potentially reducing the fungi present in the medium, and since no polyp can be cleaned completely from any unwanted

microorganisms, the possibility for polyps to become infected is reduced. Increasing concentrations of the fungicide will decrease the likelihood of a fungi infection in the polyps, as the amount of fungi in the medium is reduced.

At higher concentrations the direct toxic effect of the Tebuconazole is potentially likely to affect the polyp itself, but at the concentrations I used the positive effect from reducing the risk of fungi infections prevails.

4.3.2. Effects of Pirimicarb

In my experiments, the insecticide Pirimicarb showed no effect on the growth of the polyp stage of *C. sowerbii*.

In previous experiments on the effect of Pirimicarb on daphnids, several effects were visible, such as higher mortality, decreased body mass, and decreased offspring numbers (Kusk, 1996; Andersen et al., 2006). Pirimicarb concentrations and exposure times were somewhat different in my experiments and both Daphnia experiments. The highest Pirimicarb concentrations were 5 to 25 times higher in the Daphnia experiments compared to my experiments with the polyp stage. Also, the exposure times in the Daphnia experiments ranged from 0.5 to 72 hours, while in my experiments the exposure time was always 24 hours.

The differences in concentration in particular could explain the lack of visible effects of Pirimicarb on the polyp stage of *C. sowerbii.* But considering the targeting point of Pirimicarb on the animal organism another possible explanation occurs. Pirimicarb works as an inhibitor of Acetylcholinesterase (AChE) which works as a neurotransmitter in bilateral species.

In cnidarians AChE was found to have a probable non-neural function. In *Hydra sp.* AChE was detected in endodermal epithelial cells. In this study it was hypothesised that AChE could influence the cell differentiation in regeneration processes in *Hydra* (Takahashi & Hamaue, 2010). As *Hydra and C. sowerbii* are closely related, similar functions of AChE in *C. sowerbii* are plausible.

Pirimicarb may indeed have an effect on the polyp stage of *C. sowerbii,* but not on growth, and only at higher concentrations.

4.3.3. Effects of Terbuthylazine

The herbicide Terbuthylazine has an overall positive effect on the growth of the polyp stage of *C. sowerbii*. At both chronic and acute exposure, a higher growth than the control treatments is visible.

In experiments with zebrafish (*Danio rerio*, HAMILTON 1822) it was shown that TBA affects their endocrine functions. Endocrine disruptions possibly inhibit development, sexual differentiation, and all reproduction processes (Hostovsky et al., 2014).

In invertebrates the endocrine systems are less well documented than in vertebrates, but chemical signaling pathways are present in all known invertebrate taxa (Oetken et al., 2004). Some chemical compounds are able to disrupt such signaling pathways by mimicking hormones, working as antagonists or by interfering in the regulation of hormone synthesis processes (Oetken et al., 2004).

In cnidarians, in which circulation of internal body liquids result from diffusion through the whole organism, chemicals are able to reach and, as a consequence, affect every cell. It therefore follows that the potential attack points for disruption in signaling pathways are numerous. While the exact pathways are widely unexplored (Tarrant, 2007) some examples of hormones affecting, for example, the spawning of corals, were documented (Atkinson & Atkinson, 1992; Twan et al., 2003).

Since TBA affects endocrine processes, and therefore possibly reproduction of the polyps of *C. sowerbii,* the elevated growth with increasing exposure to TBA seems reasonable.

4.3.4. General influence of pesticides

My results show that different pesticides have varying effects on the polyp stage of *C. sowerbii*. However, my experiments mainly aimed to evaluate the ecological impact of the substances on the growth dynamics of polyps. I can therefore conclude that chemicals from agricultural run-off affect the polyp stage, and have a provable ecological impact, since the reproduction of the polyps is influenced. The

dispersal, establishment and distribution of the species are also therefore affected. To get more detailed information about possible consequences of chemicals on this species, further eco-toxicological experiments are needed. As chemicals are only seldom in the water exclusively (Altenburger et al., 2013), other agrochemicals and combinations of such chemicals should also be tested on *C. sowerbii*. Detailed dose-response analyses, a broader knowledge about molecular impact, and target organs of chemicals are required to assess the impact of whole compositions of agrochemicals on this species. Another question still to be answered is how other life stages, and the transitions between them, are influenced by these chemicals.

4.4. Technical aspects of polyp handling in experimental and monitoring work

Crucial for successful research on *C. sowerbii* in the laboratory is a stable and manageable culture of the polyp stage. Due to its small size and low visibility, the search for this stage in the field to even start a culture, as well as its handling in culture plates in the laboratory, is a difficult task. Despite a good and sturdy connectivity to the substrate, the body of the polyp stage is fragile and easily damaged during handling. A simplification of the search and handling processes was therefore a major task in my work with this species.

4.4.1. Improvement of detection possibilities in the field

The polyp stage is the prominent life stage of *C. sowerbii* in freshwater, building up the main population throughout the year. All other life stages occur only sporadically, and change back to the polyp stage or die off after a short time. Despite this importance in the life cycle of *C. sowerbii* the polyp stage plays only a minor role in scientific investigations. In general, *C. sowerbii* attracts increasing scientific interest because the cosmopolitan freshwater jellyfish occurs in a rising number of lakes all over the world. Most probably due to its small size, inconspicuous appearance and hidden lifestyle at the bottom of lakes and other freshwater habitats, the polyp stage attracts only low interest. The difficulties in finding this stage in the benthic areas of freshwater probably discourages greater scientific attraction.

My staining experiments in the laboratory indicate that the possibility of staining polyps in order to facilitate their detection in the field can be achieved. The bright red colour resulting from the staining solves the problem of locating the polyps in field samples. The method I used was based on the work of Elliott and Tang 2009. In their work they tried to distinguish between live and dead copepods in marine samples (Elliott & Tang, 2009). With neutral red only living tissues can be stained, as only then the dye can diffuse into the cell. This is also reasonable for experimental purposes, as dead polyps would not be of any use. In contrast to my aim of taking the stained, live polyps into laboratory culture, in the work of Elliott and Tang (2009) the copepod samples became fixed after the staining. As a result, any long-term influence of the staining did not matter for their use of copepods. My results show that growth of polyps was influenced slightly, only on a non-lethal base, by staining with neutral red. As the concentration used by Elliott and Tang (2009) of 0.015 g/l showed the least effect on the growth of the polyp population I highly recommend this concentration for staining polyps.

The counting of polyps in field samples shows that staining facilitates the detection to a remarkable degree. As both experienced and inexperienced investigators were able to find more polyps after the staining, this method seems highly efficient in the search for the polyp stage of *C. sowerbii*. However, as some organisms on stones can also be stained by neutral red they could accidentally be misidentified as polyps. Besides possible misidentifications, the method of staining field samples to facilitate the detection of polyps on stones appears to be a clear improvement in searching for polyps.

Potential misidentifications while counting on stones by researchers with less experience in the identification of the polyp stage might be a negligible disadvantage for this method, as further brief investigation of questionable organisms can eliminate these misidentifications easily. Furthermore, in my experience, the misidentification of unstained polyps is much more likely than the misidentification of stained ones.

4.4.2. Effects of medium change intervals on the growth of the polyp stage of *Craspedacusta sp.*

Medium changes during experiments ensure stable conditions in experimental design. Chemical concentrations and unfavourable side-effects can be controlled and adjusted to meet the necessary environmental conditions for the experiment. However, the regular change of growth medium can potentially influence the results of the experiment itself. As the removing and refilling of the container in which the experiment takes place can cause stress in the test animal, the process has to be taken in consideration for experimental designs.

Different substances with unique stabilities in test solutions need different renewal intervals (OECD, 2012). To ensure comparability between substances, the effects of different renewing intervals must be excluded.

From my experiments one can see that the reproduction of the polyp stage of *C. sowerbii* is not influenced by medium-renewal intervals. The growth for all three intervals (once a week, twice a week and three times a week) was almost identical. As such, the process of changing the test medium can be excluded as an influencing factor affecting the growth of polyps.

4.4.3. Regeneration capabilities

Due to its small size and fragile body, the polyp stage is prone to injuries occurring during daily laboratory handling. Cleaning the containers, as well as removing other polyps or whole colonies, can cause injuries in single polyps. The whole phylum of cnidarians is known for its excellent regeneration capabilities (Holstein et al., 2003). In *C. sowerbiis'* next freshwater relative, *Hydra sp.,* the high regeneration potential has been investigated in great detail. This species is able to regenerate from single cells which first reaggregate in a cell suspension and afterwards regenerate to whole animals again (Gierer et al., 1972).

My results of the regeneration process in *C. sowerbii* show that the first regenerated polyp heads were present 48 hours after the initial removal. Since the duration between each count of the heads in my

experiment was 24 hours, the regeneration of these heads was complete any time between the 24 and 48 hour observation points. This duration for head regeneration is similar to the results of McClary (1964) who examined the regeneration of *C. sowerbiis* polyps with a focus on histology. In his experiments, the first regenerated heads were found after 33.5 hours (McClary, 1964), matching my results closely.

Regarding the regeneration of heads over the entire course of my observation, the results show that the initial number of heads was reached after 96 hours. As regards the number of polyp colonies, they show that one colony vanished during the experiment. This means that the other colonies produced additional polyps during the experiment. As the fluctuation in the numbers of the different types of colonies (one-, two- and three-polyp colonies) indicates, the number of polyps per colony is constantly changing. This increase and decrease is also visible in the results of another study of McClary, in which he investigated the growth increase of polyps per colony at different temperatures (McClary, 1959). Another reason for the loss of the colony could be due to further injuries in the colony. Due to their small size, the removal of the head structures of single polyps is a challenging task. Further injuries, or even squashing the entire colony, is possible, and may cause the polyps of the colony to transform into the frustule stage and move to another spot.

4.4.4. Future working possibilities on *Craspedacusta sp.*

Concluding from the experiments described in 4.1.1., 4.1.2. and 4.1.3. it becomes evident that the polyp stage of *C. sowerbii* is an important life form to be used in further laboratory experiments. With the staining method described within 4.1.1. the search for polyps in the field is simplified and can be performed more efficiently than without staining.

The results drawn from the experiments described in 4.1.2. and 4.1.3. show that the polyp stage carries traits that make them very useful for such experimentation. While the medusae stage of *C. sowerbii* is quite delicate, during handling the polyp stage shows a high tolerance to injury and experimental procedures such as medium exchange. This facilitates the experimental work in the laboratory with this

life stage of the species. Together with a generalistic prey spectrum (Morpurgo & Alber, 2015) and high reproduction rates (McClary, 1959), the polyp stage is easy to cultivate under laboratory conditions. As research on *C. sowerbii* has until now been mostly based on the medusa stage, further research on the polyp stage should be promoted in the future. Since the polyp stage is the most important life stage of the species in terms of reproduction and therefore also distribution, a deeper knowledge of the ecology of the polyp and the environmental conditions promoting and limiting its abundances is needed.

5. Outlook

C. sowerbii is a highly invasive freshwater species which has increasingly drawn the attention of scientists over recent decades. In the beginning of the 20th century there were several promising attempts to investigate the entire life cycle of this species in all its complexity (Dejdar, 1934; Reisinger, 1934; Dunham, 1941). Now, at the beginning of the 21st century, science almost exclusively focuses on the medusa stage of this species (Fuentes et al., 2019; Oualid et al., 2019; Schifani et al., 2019). This is not surprising, as it is the most easily detected life stage in the whole life cycle. However, as it displays only a small fraction of the complex life cycle of this species it is impossible to get a complete picture of the dynamics of the species by analysing medusa-based data alone. Further investigations on the polyp stage, which is likely to represent the majority of the global *C. sowerbii* populations, are therefore inevitable.

From my experiments it becomes clear that the dispersal and distribution of this species clearly benefits from lotic water systems. The further distribution from lotic to lentic waters still remains as yet unclear. There are conceivably multiple dispersal possibilities at this small scale. It is well known from other freshwater species that passive transportation can occur via several vectors such as animals, humans and human-related activities (Havel & Medley, 2006; Coughlan et al., 2017). Mammals, for example, could transport polyps or frustules in their fur, as long as they do not dry up between origin and destination. For the podocyst stage, even drying up would not be a problem. Internal or external

transportation by birds or waterfowl could also be possible for *C. sowerbii*; such transport has already been observed for several aquatic invertebrates (Frisch et al., 2007; Anastácio et al., 2014). Human activities like the transportation of boats from one lake to another or the trade of aquatic plants could possibly enable the spread of the species from isolated freshwater systems (Waterkeyn et al., 2010; Banha et al., 2016).

Predicted distribution limitations for polyps seem to be rather scarce from my experiments. Physical parameters presumably play a minor role in such a distribution limitation, as I found that the polyp stage is not affected by any of the environmental parameters I tested. Limitation by chemical parameters is nonetheless possible, particularly chemicals from agriculture, which enter surface waters by leaching farmland and are major sources for such pollution (Pericherla et al., 2020). Such chemicals influence the reproductive success of the polyp stage, as I was able to show in my results. Political attempts and environmental regulations currently aim to restrict the entry of agrochemicals into rivers and lakes (Chave, 2001). In the European Union, the Water Framework Directive tries to improve the chemical condition of water by 2027 (Völker & Mohaupt, 2016). If these restrictions succeed in the future, they may possibly even facilitate or accelerate the distribution of *C. sowerbii*. Regions that were hitherto unsuitable for the species could transform to provide the chemical conditions to allow for permanent establishment. Given the already high number of rivers and lakes hosting the polyp stage of *C. sowerbii,* the establishment of polyps as a common inhabitant of the benthic regions of freshwater is highly possible. Until now the exact impact of the polyp stage on the benthic food web was not investigated. However, considering the high densities of polyps in certain spots in freshwater, a strong effect on benthic food webs should be expected.

The impact of the medusa stage on the pelagic food web of freshwater has already been proven (Jankowski & Ratte, 2001; Jankowski, 2004; Jankowski et al., 2005). The high numbers of medusa specimen sometimes occurring simultaneously in lakes are clearly diminishing the number of zooplankton in lakes. As such they compete with other predators of similar trophic positions, like fish, and can cause cascading effects within the whole food web (Schachtl, 2019).

Under the influence of climate change an increase in medusa abundances is also highly possible. Several factors influenced by climate change are, directly or indirectly, influencing the life cycle of *C. sowerbii* (Nazari-Sharabian et al., 2018). Elevated temperatures are, as described above, considered to have a direct influence on the medusa production of the polyp stage of *C. sowerbii* (McClary, 1959, 1961; Folino-Rorem et al., 2016). Predicted changes in precipitation patterns and intensities (Nazari-Sharabian et al., 2018) can also affect *C. sowerbii* populations, irrespective of the direction of these changes. If precipitation decreases, the temperature of lakes will increase due to lower water levels, enhancing the production of medusae by polyps. If precipitation increases in the form of flood events, the dispersal of *C. sowerbii* might be enhanced, as formerly isolated bodies of freshwater could become temporarily connected.

Besides these direct effects of climate change on *C. sowerbii,* indirect ones are possible. Most changes to freshwater caused by climate change result in an eutrophication of lakes (Nazari-Sharabian et al., 2018). The main aspect of eutrophic lakes is an increase in nutrient concentrations (Qin et al., 2013). While this nutrient enrichment most likely has no impact on the jellyfish itself, many of the consequences resulting from eutrophication may eventually have an influence on the medusa stage. Typical consequences include increases in algal and dissolved organic matter (DOM) concentrations in lakes. Both consequences of eutrophication result in a reduction of light availability and thereby visibility (Qin et al., 2013). In marine environments it has been shown that with decreasing visibility there is a shift from fish being the top predator in the food web to jellyfish becoming the top predator (Eiane et al., 1999). As an exclusively tactile hunter, jellyfish are independent of visibility to catch their prey and can therefore outcompete optically orientated hunters, including most fish. If this is also true for freshwater systems, a shift from fish to jellyfish as top predators in lakes could be possible, with subsequent significant effects on lake food webs.

From all that is known about the ecology of *C. sowerbii* until now, the species is an obvious benefactor of future environmental shifts related to climate change. It is probable that this species will further increase its distribution range and, with ongoing climate change and improvement of (chemical) water

quality, could become a common inhabitant of global freshwater. Of course, all effects of an invading species on new environments can never be fully predicted, but the first available data indicate that negative effects of *C. sowerbii* on freshwater ecosystems are possible. Until now *C. sowerbii* has not shown to be harmful to humans and human activities related to lakes, but with potentially increasing numbers, distribution ranges and occurrence periods, such threats are conceivable. One possible threat is to commercial freshwater fishing, as medusae could compete with fish for prey. In the case of jellyfish blooms, a significant decrease in prey specimen accessible to fish is possible, and could affect fish recruitment. However, more subtle effects on ecosystem dynamics are also possible, such as changes in the benthic – pelagic coupling of the fluxes of energy and matter, changes in phytoplankton concentrations by trophic cascades, and shifts in zooplankton community composition by selective predation. To quantify such possible changes, a closer, concerted and directed monitoring of lakes inhabited by freshwater jellyfish is therefore necessary and important.

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Supplementary Tables

Supplementary Table 1: Bavarian lakes screened for polyps and medusae of C. sowerbii. Geographical information (coordinates, area, altitude and origin) was taken from (Grimminger, 1982).

name	latitude	longitude	origin	altitude [m a.s.l.]	area [ha]	polyps	medusae
Allacher Lohe	48.20029	11.491991	Artificial	499	6.3224	0	0
Ammersee	47.99578	11.16782	Natural	531	4724.60	0	0
Ampersee	48.22978	11.35831	Artificial	494	8.1769	0	0
Auwaldsee	48.75218	11.467458	Artificial	366	14.9687	0	0
Baarer Weiher	48.68023	11.49099	Artificial	374	15.3254	x	x
Bodensee	47.59393	9.44068	Natural	392	47257.8	x	x
Brandsee	48.6802	11.5235	Artificial	367	9.6163	x	x
Brunnensee	47.98445	12.436212	Natural	528	5.3866	x	0
Chiemsee	47.92545	12.448562	Natural	515	8108.80	x	x
Danglweiher	48.75979	12.92087	Artificial	314	29.0932	x	x
Ebenhausener See	48.67818	11.47157	Artificial	371	3.6335	0	0
Echinger Weiher	48.31569	11.61668	Artificial	461	1.9127	0	0
Eichinger Weiher	48.22286	11.45988	Artificial	491	1.7072	0	0
Eschenauer See	47.94569	12.397769	Natural	524	16.2942	0	0
Fasaneriesee	48.2068	11.53087	Artificial	493	14.2262	x	0
Feldmochinger See	48.21384	11.5143	Artificial	491	14.9396	x	0
Förchensee	47.70807	12.62023	Natural	735	2.9499	0	0
Fridolfinger See	47.99575	12.841625	Artificial	383	5	x	0
Geisenfelder See A	48.7047	11.5551	Artificial	361	8.1979	x	0
Geisenfelder See B	48.7017	11.5566	Artificial	362	1.9446	x	x
Geisenfelder See C	48.7019	11.553	Artificial	366	1.9697	x	x
Germeringer See	48.13563	11.34224	Artificial	532	2.501	0	0
Gernlindener See	48.21143	11.283624	Artificial	510	0.6699	x	0
Gernlindener Waldsee	48.22014	11.298131	Artificial	511	0.4434	x	x
Gilchinger See	48.11466	11.274854	Artificial	554	6.7716	0	0
Griessee	47.98598	12.442315	Natural	531	7.9291	x	0
Haager Badeweiher	48.4503	11.8283	Artificial	420	3.2334	x	x
Halfinger Badesee	47.94348	12.27751	Artificial	498	3.1514	x	0

name	latitude	longitude	origin	altitude [m a.s.l.]	area [ha]	polyps	medusae
Halfinger Biotop	47.94245	12.27834	Artificial	504	0.7162	0	0
Happinger Ausee	47.82899	12.14117	Artificial	449	15.8484	x	x
Hartsee	47.92765	12.367087	Natural	525	86.6348	x	x
Haselfurther Weiher	48.48118	12.0139	Artificial	408	5.5567	x	x
Heiglweier	48.31271	11.5315	Artificial	461	2.4257	x	x
Herrenwieser Weiher	47.71609	10.25347	Artificial	785	6.7527	0	0
Hofstätter See	47.89765	12.1729	Natural	481	55.3514	0	0
Karlsfelder See	48.23716	11.468322	Artificial	482	24.7053	x	0
Kesselsee	47.91599	12.35259	Natural	530	2.7263	x	x
Kleiner Leitner Wei-	48.70557	11.320778	Artificial	373	9.992	x	x
Klostersee	47.97918	12.44663	Natural	528	44.2648	x	0
Langenbürgner See	47.90238	12.352028	Natural	531	100.975	x	0
Langwieder See	48.19462	11.416357	Artificial	502	16.2734	x	0
Lerchenauer See	48.19733	11.53685	Artificial	499	7.9918	x	0
Luberweiher	48.78411	13.010377	Artificial	309	22.6245	x	x
Lußsee	48.19939	11.418292	Artificial	504	17.6867	0	0
Mammendorfer See	48.20046	11.168347	Artificial	531	4.8041	0	0
Niedernberger See	49.89941	9.13839	Artificial	116	22.887	0	0
Obinger See	48.00354	12.415538	Artificial	553	30.5028	0	0
Olchinger See	48.20799	11.359949	Artificial	503	14.3285	x	0
Pelhamer See	47.93443	12.350095	Natural	527	71.7562	x	0
Pilsensee	48.02758	11.1853	Natural	542	195.091	0	0
Pockinger Badesee	48.38536	13.295731	Artificial	313	22.2993	0	0
Pucher Meer	48.19044	11.233617	Artificial	513	5.7064	0	0
Regattaparksee	48.24337	11.5149	Artificial	479	12.9208	0	0
Reichertshofen A	48.6911	11.5237	Artificial	366	4.9916	x	x
Reichertshofen B	48.6931	11.5244	Artificial	366	2.4325	x	x
Reichertshofen C	48.6911	11.5193	Artificial	365	11.1707	x	x
Reichertshofen D	48.6887	11.5176	Artificial	368	7.8419	x	x
Riegsee	47.69692	11.227023	Natural	654	186.936	0	0
Rinssee	47.91152	12.20965	Natural	481	41.7663	0	0
Rothsee	49.22271	11.186037	Artificial	372	161.353	0	0

name	latitude	longitude	origin	altitude [m a.s.l.]	area [ha]	polyps	medusae
Schliersee	47.72453	11.86113	Natural	776	216.702	x	0
Seehamer See	47.85015	11.86327	Artificial	651	107.245	0	0
Simssee	47.87529	12.23877	Natural	468	647.586	x	0
Starnberger See	47.9952	11.3411	Natural	583	5831.09	0	0
Straß	47.91753	12.38199	Artificial	528	2.4069	x	0
Tegernsee	47.72246	11.737564	Natural	725	895.056	0	0
Thaler See	47.90476	12.337871	Natural	533	4.141	0	0
Tinninger See	47.82515	12.21003	Natural	486	25.3666	0	0
Tüttensee	47.84653	12.568325	Natural	522	10.1549	x	0
Walchensee	47.59228	11.34693	Natural	798	1627.82	0	0
Waldschwaigsee	48.22534	11.43773	Artificial	467	10.9315	x	0
Waldsee Manching	48.69259	11.51337	Artificial	375	3.5963	x	x
Waldsee Schechen	47.946	12.153	Artificial	439	1.9301	x	x
Weicheringer See	48.70431	11.329899	Artificial	376	33.7304	x	x
Wörthsee	48.0528	11.16075	Natural	571	460.317	0	0

Supplementary Table 2: Lakes with the medusa stage of C. sowerbii from the citizen science approach.

name	date	latitude	longitude
Almer Weiher	2019	48.96551	12.2933
Alter Steinbruch Gumping	2017	49.15604	12.32067
Badesee Hochmuehl	2016	48.27943	12.7084
Baggersee bei Muellenbronn	2015	47.88312	9.52783
Baggersee Diez-Limburg	2015	50.3704	7.99187
Baggersee Johanneswiese	2019	49.09101	8.29301
Bahnweiher	2017	49.39992	8.37231
Baiersdorfer Badesee	2019	49.66464	11.02339
Bodensee	2015	47.68815	9.28094
Brandsee	2017	48.68079	11.52404
Braunweiher	2019	48.71075	11.49456
Brombachsee	2017	49.13249	10.92157
Dortmund-Ems-Kanal	2019	51.57745	7.41472
Eireiner See	2019	48.68955	11.52108
Feilensee	2017	48.70405	11.56233
Fischweiher	2017	47.88815	11.41249
Gondelheimer Seenplatte	2019	51.75667	9.38041
Guggenberger See	2017	48.97854	12.22071
Haager Weiher	2016	48.45149	11.82816
Hafenbecken Main-Donaukanal	2019	49.39821	11.06329
Happinger Ausee	2019	47.83048	12.13971
Heiglweiher	2019	48.31269	11.53233
Heratinger Badesee	2019	48.07133	12.95154
Hoeuslweiher	2017	48.81493	12.92874
Hohwiesensee	2019	49.35936	8.51336
Igelsbachsee	2015	49.14993	10.89902
Juteteich	2019	50.68715	12.02826
Kessel 1	2015	51.62974	11.87387
Kiessee	2017	48.73927	11.22969
Kleiner Leitner Weiher	2019	48.70504	11.32045
Klostersee	2019	49.80302	9.61477
Kratzmuehlsee	2019	49.00102	11.44731
Lenasee	2019	48.6802	11.5235
Luberweiher	2015	48.78356	13.01098
Mainparksee	2019	49.9861	9.0715
Mauerner Badesee	2017	48.78227	11.72892

name	date	latitude	longitude
Mooswaldsee	2019	48.48769	10.24989
Mossandl Weiher	2016	48.67055	12.57919
Muenstersee	2019	49.42942	10.04843
Neubeuerner See	2019	47.75967	12.13512
Nimburger Baggersee	2019	48.11234	7.78487
Oberndorfer Weiher	2018	49.63167	11.00512
Ostwaldsee	2019	48.69149	11.51911
Pfatter Au	2019	48.96931	12.38699
Riedlinger Baggersee	2019	48.70254	10.75793
Roither See	2019	48.97326	12.27303
Sattlinger Weiher	2019	48.69929	13.10878
Schafirrsee	2015	48.74094	11.33361
See ohne Namen	2012	47.89239	9.84054
See ohne Namen	2017	48.62055	10.62387
See ohne Namen	2017	51.00047	13.86715
Singerhofweiher	2017	48.81148	12.92614
Stechlinsee	2019	53.15221	13.02778
Sportsee Burgheim	2013	48.72181	11.02263
Tapfheimer Badesee	2019	48.67119	10.69717
Teich 1	2015	50.11604	9.13646
Teich 2	2015	50.11495	9.13649
Teich 3	2015	50.11386	9.13712
Thansauer Badesee	2019	47.82587	12.14875
Tillyweiher	2017	48.75628	11.55002
Waldsee	2017	47.94582	12.15289
Waldsee	2017	48.69253	11.51307
Wallersdorfer Kiessee	2019	48.72768	12.74516
Wallersdorfer Moos	2019	48.71278	12.76817
Weicheringer See	2014	48.70615	11.33242

Supplementary Table 3: Lakes with the medusa stage of *C. sowerbii* from the literature research.

name	date	latitude	longitude	source
Achernsee	2005	48.64414	8.03279	Fritz et al. (2007)
Adolfo-See	2002	51.05976	6.16862	Fritz et al. (2007)
Aje-See	2002	49.94648	8.7983	Fritz et al. (2007)
Alsdorfer Weiher	1985	50.86278	6.15217	Fritz et al. (2007)
Althäuser See	2016	49.21017	8.63418	https://www.dietaucher.com/ althaeuser-baggersee-kronau.html (access 13.11.2020)
Attinger See	2004	48.94182	12.55237	Fritz et al. (2007)
Auwaldsee	1983	48.75115	11.46868	Fritz et al. (2007)
Baggersee	1981	48.93294	12.60279	Fritz et al. (2007)
Baggersee	2005	50.65612	8.22091	Fritz et al. (2007)
Baggersee Fuchs	2016	49.0892	8.37232	https://taucher.net/forum- suesswasser-quallen_2016-ioz82674 (access 13.11.2020)
Baggersee Spöck	2005	49.12595	8.50859	Fritz et al. (2007)
Belicker See	2002	52.39589	12.24435	Fritz et al. (2007)
Berggeistweiher	2002	50.79345	6.8812	Fritz et al. (2007)
Canyon	2002	52.28233	11.31276	Fritz et al. (2007)
Dagowsee	2006	53.15165	13.05327	Fritz et al. (2007)
Dungkopfsee	2002	50.58845	7.18491	Fritz et al. (2007)
Einlaufweiher	2002	48.76893	11.57715	Fritz et al. (2007)
Elbsee	2005	51.18645	6.90297	Fritz et al. (2007)
Epplesee	2002	48.9649	8.32371	Fritz et al. (2007)
Erlensee	2002	50.14818	8.97418	Fritz et al. (2007)
Feilenmoos Seen	1982	48.70376	11.55802	Fritz et al. (2007)
Fischteich	2002	48.7177	12.84624	Fritz et al. (2007)
Flückigersee	2005	48.01067	7.81769	Fritz et al. (2007)
Foerstergrube	2002	51.62802	12.24442	Fritz et al. (2007)
Forsterweiher	1983	48.75349	11.37622	Fritz et al. (2007)
Freigerichtsee (West)	1947	50.084	8.99643	Fritz et al. (2007)
Freyersee	2005	49.24353	8.46475	Fritz et al. (2007)
Friedenhainsee	1979	48.92799	12.57499	Fritz et al. (2007)
Fühlinger See	2005	51.02287	6.92386	https://www.ksta.de/koeln/chorweiler/hitze-phaenomen-in-koeln-zigtausende-quallen-schwimmen-im-fuehlinger-see--31091248 (access 13.11.2020)
Gambacher Baggersee	1982	50.46835	8.73129	Fritz et al. (2007)

name	date	latitude	longitude	source
Gerolfinger Badeweiher	1983	49.05574	10.51287	Fritz et al. (2007)
Gloeckle-See	2002	50.00949	10.19969	Fritz et al. (2007)
GochNess	2005	51.70527	6.09388	Fritz et al. (2007)
Grüner See	2003	51.28494	6.82135	Fritz et al. (2007)
Grüner Waldsee	2002	52.06387	11.80725	Fritz et al. (2007)
Gurrensee	2005	48.35988	10.01602	Fritz et al. (2007)
Hamlarer Baggersee	2017	48.69082	10.8327	https://www.br.de/nachrichten/bayern/quallen-im-baggersee;QRn4oQy (access 13.11.2020)
Haubachsee	2003	51.37508	6.80407	Fritz et al. (2007)
Heidesee	2002	49.16678	8.59389	Fritz et al. (2007)
Henne	2003	51.31402	8.2677	Fritz et al. (2007)
Hofstätter See	1974	47.90088	12.17392	Fritz et al. (2007)
Hohwiesensee	2005	49.3592	8.51332	Fritz et al. (2007)
Kiesgrube	2002	51.0993	11.67643	Fritz et al. (2007)
Kiesgrube Mittelgrund	2016	49.1082	8.39211	https://taucher.net/forum-suesswasser-quallen_2016-ioz82674 (access 13.11.2020)
Kiessee	1983	48.70896	11.35526	Fritz et al. (2007)
Kiessee	2002	51.01655	13.82096	Fritz et al. (2007)
Kiessee	2005	50.81529	7.0564	Fritz et al. (2007)
Krotzenburger Badesee	2002	50.06628	8.97395	Fritz et al. (2007)
Langenfelder See	2002	51.12935	6.95716	Fritz et al. (2007)
Lindensee	1979	50.09004	9.01328	Fritz et al. (2007)
Loebejun	2002	51.62978	11.87414	Fritz et al. (2007)
Loheider See	2003	51.50542	6.6602	Fritz et al. (2007)
Lohheidesee	2005	51.5053	6.65939	Fritz et al. (2007)
Luberweiher	1979	48.78374	13.01102	Fritz et al. (2007)
Marzlinger Weiher	2005	48.39204	11.79563	Fritz et al. (2007)
Matschelsee	2002	48.40582	7.81639	Fritz et al. (2007)
Mönchswaldsee	2005	50.0426	8.50152	Fritz et al. (2007)
Moehne	2003	51.48385	8.10389	Fritz et al. (2007)
Müggelsee	2019	52.43703	13.64938	https://www.maz-online.de/Brandenburg/Havel-Mueggelsee-und-Tegeler-See-betroffen-Tausende-Quallen-in-Berlin-und-Brandenburg (access 13.11.2020)
Niederneundorfer See	2001	52.6151	13.21271	Fritz et al. (2007)

name	date	latitude	longitude	source
Niederweimar See	2003	50.76137	8.7433	Fritz et al. (2007)
Oberndorfer Weiher	2002	49.63165	11.00512	Fritz et al. (2007)
Rahmersee	2001	51.35564	6.7671	Fritz et al. (2007)
Rohrkoepfle	2002	49.12547	8.38737	Fritz et al. (2007)
Roither See	2017	48.97344	12.27331	https://altoetting.bund-naturschutz.de/natur-und-umweltthemen/biotop-und-artenschutz/artensteckbriefe/andere-tiere/suesswasserquallen (access 13.11.2020)
Sandhofsee	2005	51.15954	6.75178	Fritz et al. (2007)
Sarchinger Weiher	2002	49.00764	12.25122	Fritz et al. (2007)
Schweinfurter Bag-	1940	50.01886	10.23191	Fritz et al. (2007)
Sieben-Erlen-See	2002	49.14379	8.51378	Fritz et al. (2007)
Silbersee	2016	49.89799	9.13205	https://taucher.net/forum-suesswasser-quallen_2016-ioz82674 (access 13.11.2020)
St. Leoner See	2002	49.28217	8.58862	Fritz et al. (2007)
Stockemer See	2002	50.82121	7.08721	Fritz et al. (2007)
Streitkoepfle	2002	49.11973	8.37988	Fritz et al. (2007)
Tegeler See	2019	52.5789	13.25875	https://www.maz-online.de/Brandenburg/Havel-Mueggelsee-und-Tegeler-See-betroffen-Tausende-Quallen-in-Berlin-und-Brandenburg (access 13.11.2020)
Töppersee	2003	51.40318	6.67773	Fritz et al. (2007)
Untergrombachsee	2005	49.09762	8.55044	https://taucher.net/forum-diveinside_-_suesswasserqualle-ioz77615 (access 13.11.2020)
Vogelsberger See	2002	50.11445	8.87227	Fritz et al. (2007)
Vogelsee	2005	49.87522	8.38114	Fritz et al. (2007)
Vogelstangsee	2016	49.50399	8.54048	https://taucher.net/forum-suesswasser-quallen_2016-ioz82674 (access 13.11.2020)
Volksgartenweiher	1931	50.92183	6.94682	Fritz et al. (2007)
Wambacher See	2003	51.377	6.81711	Fritz et al. (2007)
Wannsee	2006	52.43511	13.17195	Fritz et al. (2007)
Weicheringer See	2002	48.70227	11.32674	Fritz et al. (2007)
Zachariassee	2002	51.71066	8.38492	Fritz et al. (2007)

Supplementary Table 4: French lakes with medusae sightings from Marchessaux et al. (2019) and Marchessaux et al. (2021a)

location	region	date	latitude	longitude
Abbaretz	Pays-de-la-Loire	2005	47.522853	-1.614279
Agen	Nouvelle-Aquitaine	-	43.935701	1.0952798
Aiguillon	Nouvelle-Aquitaine	-	46.312072	4.6167384
Aix-les-Bains	Auvergne-Rhône-Alpes	2005	45.6886	5.91503
Angers	Pays-de-la-Loire	2010	47.468698	-0.558814
Annecy	Auvergne-Rhône-Alpes	1962	45.900376	6.126959
Arceau	Bourgogne-Franche-Comté	2016	47.3805	5.25293
Argenton sur creuse	Centre-Val-de-Loire	2012	46.592592	1.518308
Arras	Hauts-de-France	2009	50.296881	2.896433
Aubusson	Auvergne-Rhône-Alpes	2007	45.057237	2.613386
Avignon	Provence-Alpes-Côte d'Azur	1985	43.948714	4.805927
Avrillé-les-Ponceaux	Centre-Val-de-Loire	2013	47.3944	0.286297
Bagneaux-sur-Loing	Ile-de-France	1990	48.229548	2.703951
Bâle	Suisse	1932	47.559614	7.580610
Barraux	Auvergne-Rhône-Alpes	-	45.4352014	5.987269
Beffes	Centre-Val-de-Loire	2005	47.799986	-0.386890
Bernin	Auvergne-Rhône-Alpes	2008	47.158303	1.538631
Biscarrosse	Nouvelle-Aquitaine	1977	44.395701	-1.166796
Bischheim	Grand-Est	2019	48.615284	7.761172
Bordeaux	Nouvelle-Aquitaine	-	44.845478	-0.561161
Bouguenais	Pays-de-la-Loire	1986	47.1792	-1.62472
Bourgogne	Bourgogne-Franche-Comté	1950	47.1045	5.25745
Brégnier Cordon	Auvergne-Rhône-Alpes	2003	45.648316	5.619733
Briare	Centre-Val-de-Loire	-	47.818825	2.841041
Brossac	Nouvelle-Aquitaine	2008	42.918187	2.062285
Cannes	Provence-Alpes-Côte d'Azur	1970	43.550902	7.010536
Cannes-Ecluse	Ile-de-France	1962	48.364105	2.992034
Captieux	Nouvelle-Aquitaine	-	44.101	-0.29474
Cerizay	Nouvelle-Aquitaine	-	46.7933	-0.734089
Chalonnes-sur-Loire	Pays-de-la-Loire	1986	47.3569	-0.655985
Champagney	Bourgogne-Franche-Comté	2013	47.7057	6.68209
Château Landon	Ile-de-France	-	48.1087	2.69127
Codolet	Auvergne-Rhône-Alpes	2008	44.110861	4.669422
Couziers	Centre-Val-de-Loire	1981	47.134205	0.103440
Culoz	Auvergne-Rhône-Alpes	2009	46.164031	5.328654
Dagueys	Nouvelle-Aquitaine	2006	44.917970	-0.242223
Der	Grand-Est	2010	48.554159	4.767066
Deux Sèvres	Nouvelle-Aquitaine	1949	46.578744	0.338475
Dordive	Centre-Val-de-Loire	1996	48.100096	2.790840
Douai	Hauts-de-France	2010	50.368510	3.077974
Dropt	Nouvelle-Aquitaine	1929	43.5426	1.40122
Eguzon Chatôme	Centre-Val-de-Loire	2004	46.440176	1.584713
Esparron-de-Verdon	Provence-Alpes-Côte d'Azur	2017	43.7379	5.9699
Fougères	Bretagne	1923	48.353760	-1.209096
Gétigné	Pays-de-la-Loire	2008	47.077209	-1.259932
Gizeux	Centre-Val-de-Loire	2010	47.292748	0.182120
Gour de Tazenat	Auvergne-Rhône-Alpes	1987	45.981128	2.986521
Hagetmau	Nouvelle-Aquitaine	-	43.613654	-0.552948
Hommes	Centre-Val-de-Loire	-	47.425724	0.296790
Hourtin	Nouvelle-Aquitaine	-	45.078710	-1.043316
Jazeneuil	Nouvelle-Aquitaine	2019	46.442686	0.111173
La Brède	Nouvelle-Aquitaine	2015	44.6801	-0.545866

location	region	date	latitude	longitude
La Fresnaies	Bretagne	2016	47.521520	-1.127035
La Roche Sur Yon	Pays-de-la-Loire	2015	46.669954	-1.431964
Lacanau	Nouvelle-Aquitaine	-	44.977846	-1.075465
Laffrey	Auvergne-Rhône-Alpes	2016	44.906200	5.181100
Lavau-sur-Loire	Pays-de-la-Loire	1990	47.407605	-0.295101
Le Pouzin	Auvergne-Rhône-Alpes	2016	44.751060	4.745484
Le Têt	Occitanie	-	42.7057	3.00721
Le Thouet	Pays-de-la-Loire	1949	46.995954	-0.965331
Léman	Suisse	1962	46.4015	6.83058
Lencouacq	Nouvelle-Aquitaine	-	44.071560	-0.428264
Les Gourgs lake	Occitanie	2012	44.750487	6.579871
Libourne	Nouvelle-Aquitaine	1953	44.917970	-0.242223
Limoge	Nouvelle-Aquitaine	2014	45.828521	1.261746
Loire River	Pays-de-la-Loire	1991	47.255469	-0.083404
Lormay	Centre-Val-de-Loire	1995	48.647985	1.532765
Louroux-Hodement	Auvergne-Rhône-Alpes	2016	46.490371	2.712142
Lyon	Auvergne-Rhône-Alpes	1891	45.778478	4.853746
Malafretaz	Auvergne-Rhône-Alpes	2019	46.324	5.14667
Marsac-sur-Don	Pays-de-la-Loire	2013	47.6244	-1.75133
Mayenne	Pays-de-la-Loire	1933	47.976215	-0.738654
Mervent	Pays-de-la-Loire	2010	46.367110	-0.596354
Mine du Carnier	Centre-Val-de-Loire	2014	43.435	6.07069
Miribel	Auvergne-Rhône-Alpes	1981	45.766	4.87951
Miribel-Jonage	Auvergne-Rhône-Alpes	2012	45.7964	5.0453
Mondragon	Provence-Alpes-Côte d'Azur	2007	45.605665	5.648822
Monségur	Nouvelle-Aquitaine	1958	44.689026	0.500159
Montbéliard	Bourgogne-Franche-Comté	1960	47.5096	6.79822
Montereau-Fault-Yonne	Ile-de-France	1962	47.253763	6.009912
Montereau-Fault-Yonne	Ile-de-France	2002	48.383	2.94793
Montmelas-Saint-Sorlin	Auvergne-Rhône-Alpes	1962	46.0154	4.60979
Moreuil	Hauts-de-France	2010	49.776337	2.476018
Moselle	Grand-Est	2004	49.357840	6.169297
Mulhouse	Grand-Est	2018	47.749528	7.339750
Mussey	Grand-Est	2003	48.802610	5.110626
Nantua	Auvergne-Rhône-Alpes	2013	46.158643	5.602338
Nozay	Pays-de-la-Loire	2009	48.668842	2.236333
Pas du Braou	Nouvelle-Aquitaine	2013	44.5047	-1.07846
Paty	Provence-Alpes-Côte d'Azur	2012	44.1112	5.10767
Pélussin	Auvergne-Rhône-Alpes	2008	45.365476	4.683093
Peyriat	Auvergne-Rhône-Alpes	2015	46.1527	5.45113
Pincevent	Ile-de-France	1969	46.893635	0.216650
Poitier	Nouvelle-Aquitaine	1951	44.654598	-0.046727
Pontailler-sur-Saône	Bourgogne-Franche-Comté	1990	47.35636	5.43678
Pontcharra	Auvergne-Rhône-Alpes	2019	45.433	6.01536
Pont-Saint-Pierre	Normandie	2017	49.3352	1.2749
Précy	Bourgogne-Franche-Comté	1944	47.099797	2.928622
Puivert	Occitanie	2017	43.395172	6.268959
Questember	Bretagne	2017	47.264821	-1.579614
Reynerie	Occitanie	2009	43.570761	1.397759
Romagnieu	Auvergne-Rhône-Alpes	-	45.5813	5.63845
Saint Cassien	Auvergne-Rhône-Alpes	1987	45.3601	5.55336
Saint-Barthélémy-d'Anjou	Pays-de-la-Loire	-	47.485436	-0.511653
Saint-Fargeau en Puisaye	Bourgogne-Franche-Comté	-	47.6409	3.0713
Saint-Gaudens	Occitanie	2016	43.104472	0.698168

location	region	date	latitude	longitude
Saint-Jean-de-Crieulon	Occitanie	-	43.9723	3.99211
Saint-Martin-de-la-Mer	Bourgogne-Franche-Comté	1997	47.2362	4.23404
Saint-Vit	Bourgogne-Franche-Comté	2019	47.236150	6.021635
Sanguinet	Nouvelle-Aquitaine	1982	44.481343	-1.093161
Seignosse	Nouvelle-Aquitaine	-	43.689695	-1.374304
Strasbourg	Grand-Est	2005	48.573181	7.753988
Tarbes	Occitanie	2010	43.232661	0.073950
Toulouse	Occitanie	1945	43.604462	1.444246
Tour	Centre-Val-de-Loire	2013	45.945316	3.902662
Trélazé	Pays-de-la-Loire	-	47.456364	-0.510038
Vannes	Bretagne	2015	47.658653	-2.760942
Vaulx en Velin	Auvergne-Rhône-Alpes	1982	45.7869	4.92512
Villefranche-sur-Saône	Auvergne-Rhône-Alpes	2016	45.989863	4.718663
Villeneuve-sur-Lot	Nouvelle-Aquitaine	2015	44.408282	0.707263
Viviers	Auvergne-Rhône-Alpes	-	45.527573	6.012725
Vodelée	Hauts-de-France	-	50.135591	4.745047
Vouglans	Bourgogne-Franche-Comté	2010	46.485012	5.680826

Declarations

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

München, den 07.03.2022 Ramona Klotz
(Unterschrift)

Erklärung

Hiermit erkläre ich, *

☒ dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist.

☒ dass ich mich anderweitig einer Doktorprüfung ohne Erfolg **nicht** unterzogen habe.

☐ dass ich mich mit Erfolg der Doktorprüfung im Hauptfach ..... und in den Nebenfächern ..... bei der Fakultät für ..... der ..... (Hochschule/Universität) unterzogen habe.

☐ dass ich ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen.

München, den 07.03.2022 Ramona Klotz
(Unterschrift)

*) Nichtzutreffendes streichen