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Oil Response Agents In The Arctic

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Oil Response Agents in the Arctic

Questioning the feasibility of Corexit9500 and EcoTech Oil Foam as response agents for offshore oil spills in Arctic conditions

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Front cover image: The Russian Prirazlomnaya platform in the Arctic ocean from Gazprom. Retrieved at 5-7-2014 from http://www.gazprom.com/f/posts/63/407714/avd49654_1.jpg

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Abstract

With the increase of offshore activities in the Arctic, the question arises if currently existing oil spill response plans involve effective methods for removing oil slicks in Arctic conditions. The dispersant Corexit9500 is commonly applied as response agent to disperse oil slicks. It has been applied at many oil spill accidents. EcoTech Oil Foam is a newly developed absorbent. Little is known concerning the toxicity of EcoTech and Corexit9500 in low water temperature (4°C). Aim of the research is to provide knowledge on the feasibility of oil response agents in the Arctic. Research question of this project: *Can Corexit9500 and EcoTech Oil Foam be recommended as response agents for combating offshore oil spills in low water temperatures?* Plunge tests were conducted to test the dispersion behaviour of Helder oil and Mississippi Canyon Block 252 oil with and without using Corexit9500 at different water temperatures. Acute toxicity tests have been conducted by exposing *Corophium volutator* to Mississippi Canyon Block 252 oil. Growth inhibition tests have been conducted on the phytoplankton species *Phaeodactylum tricornutum*. Tests were based on the Water Accommodated Fracture preparation. At 4°C, oil with and without Corexit9500 are less dispersed and produce larger droplets compared to dispersion at 18°C. Oil slicks treated with Corexit9500 proved more toxic compared to oil treated with EcoTech or solely oil at 18°C. At 4°C Corexit9500 proved less toxic compared to untreated oil, however it is questionable if these data are correct. EcoTech proved difficult to apply in tests, it was not possible to conduct 4°C experiments. Corexit9500 proved less toxic at 4°C compared to 18°C (0.3949 vs 0.02104 ml oil/L). The growth inhibition test resulted in no significant difference between treatments. It is assumed that the experimental set up was inefficient for this type of research. For future research, it is recommended to conduct experiments with a variation of test organisms. And to create a toxic concentration based on a dilution range of one water accommodated fracture volume.

Keywords: Corexit9500; EcoTech Oil Foam; Plunge test; WAF; dispersion behaviour; Corophium volutator; Acute toxicity test; Phaeodactylum tricornutum; Growth inhibition; Arctic; Oil spill response; Residual toxicity; LC_{50}

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1. Introduction

The Arctic is a cold and harsh environment in which it is extremely difficult to conduct human activities. At least, that is what the Arctic used to be (Allen et al., 2004, Huntford, 1987). Modern research predicts that most single year ice will disappear completely during summer in several decades (Serreze, Holland, & Stroeve, 2007). These changes create opportunities for human activities such as oil exploitation. This does not come without a threat for the environment. Disasters, such as the Deep water Horizon oil spill in the Gulf of Mexico and the Great Barrier Reef oil spill in 2010 are examples, showing the ecological risk when human activities go wrong. In today's society Oil Spill Response Plans (OSRP's) are imposed by governments in order to minimize the environmental impact of an oil spill. Currently existing OSRP's for Arctic oil exploitation need to be tested and adapted in order to minimalize the environmental impact of an oil spill in this new and challenging area.

The Arctic is defined as all of the area within 66° 33"N latitude line (Smithson et al., 2008). It consists of an ocean that is surrounded by continents, which makes it the opposite from the Antarctic region (i.e. Antarctica is a continent surrounded by oceans). The 66°N latitude line is defined as Arctic Circle due to the fact that the sun does not appear above the horizon during winter and does not disappear during summer (midnight sun). Other definitions of Arctic area exist. However, these are not applied as much as the Arctic circle is (Hassol et al., 2004). The Arctic is not a vast ice covered ocean alone. It includes large areas of land as well, such as: the northern parts of Norway, Sweden, Finland and the vast plains and forests of Siberia (USR), Canada and Alaska (USA). For this research the area of research is focused on the Arctic Ocean, the Norwegian and Chukchi Sea.

1.1 A Changing Arctic

On the 1st of May 2014, the headlines of newspapers all around the world titled: "*Greenpeace blocking Russian tanker carrying oil from Arctic from mooring in Rotterdam*" and "*Dutch police storm Greenpeace ship trying to block Arctic oil delivery*". These are two examples evoking the first delivery of oil, derived from the Arctic (Bink, 2014; Vidal, 2014; Kooren, 2014). The possibility of extracting oil from the Arctic was considered to be impossible for a long time due to the freezing temperature and harsh climate conditions. The current delivery of oil from the Russian tanker is not the result of one very soft winter or a suddenly ice free ocean. It is the effect of an ongoing rise in temperature (climate change), and the result of this is already visible (Hinzman et al., 2005).
The increase in temperature has many influences on the Arctic ecosystem, (e.g. ocean acidification, gas hydrate destabilization or shift of indigenous species) (Biastoch et al., 2011; Ware et al., 2014). The most visible effect of global warming is the decline of sea ice. The summer of 2012 showed an all-time low in sea ice coverage (see figure 1). Models that normally predict the growth and decay of ice sheets have been proven insufficient. One particular study concluded that current summer sea ice minimal in surface area are ahead of the models by 30 years (Stroeve, 2007). Comparing climate models, proved that over 40% of these models predict an ice free Arctic ocean during summer at the end of the 21st century (Serreze et al., 2007). Other researchers claim an already ice-free Arctic ocean within 30 years (Wang & Overland, 2009). Predictions differ by date, however all studies forecast an ice free Arctic Ocean during summer, in at the latest the end of this century.

1.2 Human activity in the Arctic

The overall decrease of sea ice raises the discussion if international shipping via the Arctic would be possible in the future, connecting the Pacific and Atlantic ocean via a Northern route (see figure 1). Therefore, ignoring conventional routes and passages such as the Panama and Suez Canal (Symon, 2011). The concept of shipping via Northern routes has several advantages. Overall it has a shorter distance, saving on fuel and human resources (e.g. from London to Yokohama via the Suez canal would cover 21.200km, via a North East Passage would cover 13.841km) (Lasserre & Pelletier, 2011). An Arctic sea route would be especially profitable for oversized vessels that are not able to travel passages such as the Panama canal (Schøyen & Bråthen, 2011). It is expected that the use of Arctic sea routes will increase by the end of this century. When the physical conditions become more suitable (i.e. lesser sea ice and better navigable waters) in such a rate that even vessels with a low ice resistance are able to voyage the Arctic (Østreng et al., 2013).

Another activity that arises in response to the decline of sea ice are offshore activities. The Arctic is expected to contain approximately 13% of the worlds undiscovered oil resources. It is estimated that 84% of this amount is located offshore, in the Arctic Ocean (Harsem et al., 2011; Schiermeier, 2012). The development of offshore activities in the Arctic is already taking fast leaps. Since the mid-sixties oil is exploited at lower Arctic regions such as the Baffin bay area and the shoreline of Alaska (Collett et al., 2011). It is expected that, triggered by the latest activities from Shell and Gazprom, Arctic offshore oil exploration will provide 18% of the global oil exploration by mid-century (Lindholt & Glomsrød, 2012). Between 1980 and 2000 already one fifth of the entire oil production of the United States came from Alaska (Schmidt, 2012).

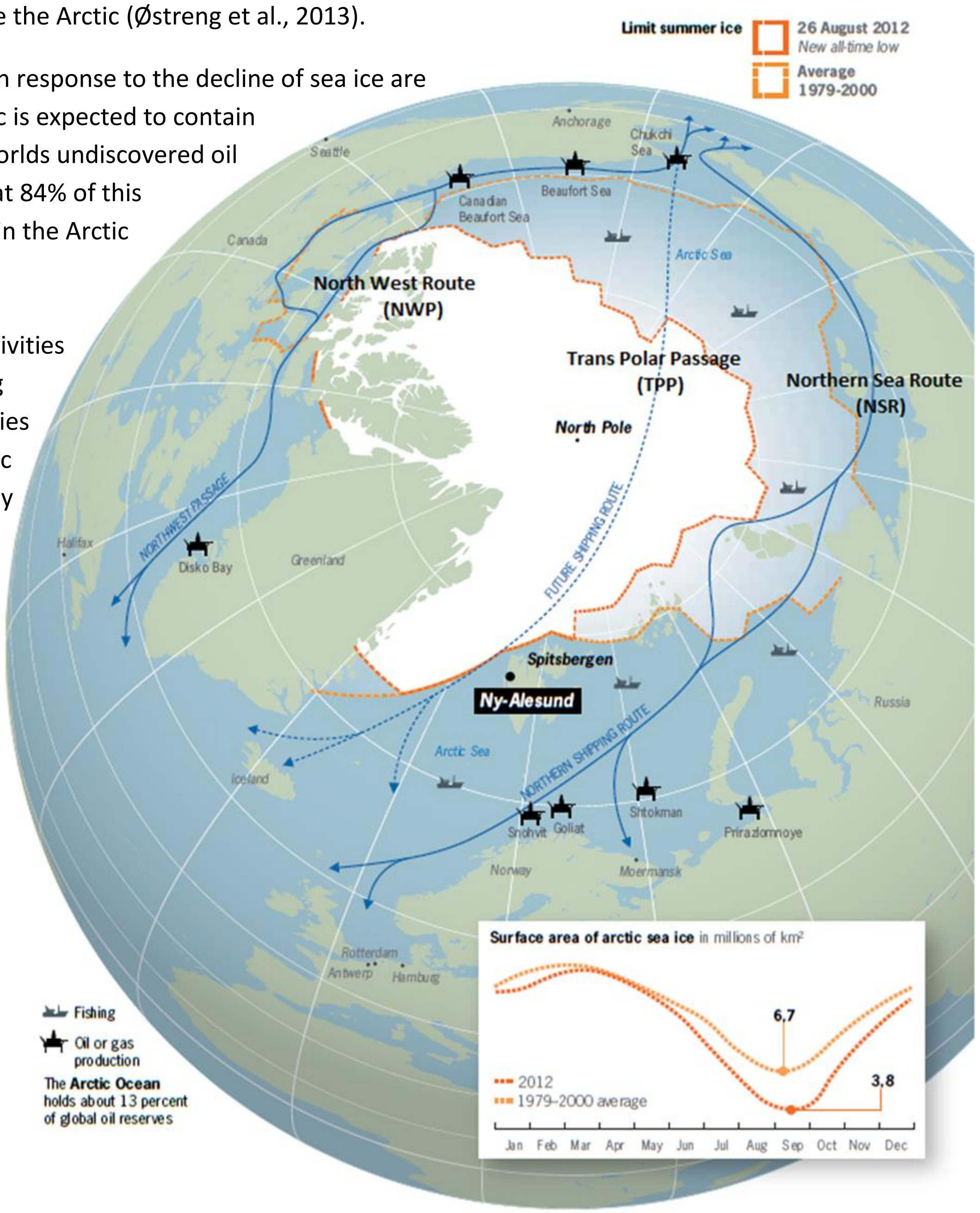


Figure 1. The Arctic with proposed routes, NWP left, TPP centre and NSR right. Several proposed oil fields indicated by the oil-rig icon. Image from Wageningen World magazine (Bothe et al., 2014).

Arctic Oil exploration in the past decades has only been land based. Offshore installations, which are completely different from land based exploration, are a feature from the last few years only. The proposed oil fields are located in the Arctic Ocean, more remote compared to the already producing oil fields in Alaska. Offshore drilling is not a new concept; the very first offshore locations were explored in the early 20th century. Offshore drilling in the Arctic however is different from other locations such as in the Gulf of Mexico or the North Sea region.

Due to the everlasting quest for fossil fuels, oil companies are drilling at larger depths to find undiscovered oil fields. The Deepwater Horizon oil rig from British Petrol (BP) was specifically designed to drill deeper than any other oil rig. In 2009 it successfully drilled the deepest well on earth, at a depth of 10.000metres (Bozeman, 2011). The Arctic Ocean is on average 1000m deep with an ocean floor consisting of submerged continental plates (Pinet, 2009). The harsh and totally dark winter season combined with the formation of ice, lack of infrastructure and low temperatures make the Arctic region a harsh environment to conduct activities.

There are several reasons why it is more difficult to conduct these types of activities in the Arctic region compared to other regions on earth (e.g. Gulf of Mexico or the North Sea). For example, the proposed Arctic sea routes are barely charted or sufficiently mapped (Østreng et al., 2013). The Northern Sea Route (NSR) has not been used frequently since the Soviet era. Many areas are still uncharted and only used by fishing vessels and small sized cargo ships. In 1969 the American oil tanker *Manhattan* was the first vessel of its size to cross the North West Route. From that date until the 1980’s, around 30 complete transits were made, making it a moderately used ship route. The Trans Polar Passage (TPP) is indicated on the map (figure 1), still it remains a future concept. The heavy and thick multiyear ice layers (≥5 metres) currently make it impossible for even the heaviest icebreakers to navigate (Østreng et al., 2013). The low amount of traffic that has used the Arctic ship lanes in the past, indicates that many parts of the routes are still unknown territory. Much of the treacherous parts are undiscovered yet, creating a possible hazard for large cargo ships.

The great difference in seasonality is another burden to activities in the Arctic. During wintertime, the sun does not appear above the horizon. Five months of total darkness is combined with temperatures as low as -30°C. The formation of ice sheets (mostly single year ice) can be problematic if they are too thick to allow navigation for the proposed oversized vessels.

Another issue involved with Arctic shipping is the lack of infrastructure and possibilities to supply. The Arctic region (e.g. along the NSR) does not inhabit a sufficiently large harbour to accommodate vessels. This means that there is a lack of response methods in the area near the shipping lanes (Harsem et al., 2011). Same threats are relevant for the drilling of oil and the use of oil rigs, in history it is proven that accidents involving oil exploration require fast response (e.g. The Exxon Valdez disaster in 1989). Oil rigs are mostly static objects that are kept in place by large sinkers or anchors attached to the seafloor. In recent years oil companies have developed ice resistant rigs that should withstand the pressing force of the pack ice when it builds up. Nevertheless an accident can always occur, especially under extreme conditions. In the Arctic it would mean that there is no direct assistance in case of emergency.

To minimize the threat of an oil spill or blowout, oil spill response plans are created. These plans include the methods on how to react during a spill, what preparations should be taken and how the clean-up onshore should be conducted. The plan also describes how oil is removed offshore at the location itself. The following chapter discusses which methods can be applied for offshore oil spill removal.

1.3 Oil Spill Response Plan (ORSP)

To minimize or even avoid a disaster, oil companies are obligated to produce an oil spill response plan (OSRP) to ensure adequate handling in case of emergency. The most commonly applied methods to remove oil slicks offshore are listed below.

Skimmers and oil booms that mechanically collect the oil in order to remove it from the water. This mostly works for thick oil slicks. The downside of these methods is the limited size of the skimmers. Skimmers are never able to fully contain an oil spill, and the recovered oil has turned into a thick mass which does not resemble the initial composition anymore. Different types of skimmers exist and the development for “Arctic-proof” devices is an ongoing process, it is claimed that skimmers suitable for the Arctic are present (Shell, 2011).

Controlled ***In-situ* burning**. When weather conditions prevent mechanical recovery, non-mechanical methods such as *In-situ* burning can be applied (Shell, 2011). This means that the oil slick is gathered and enclosed by booms before it is set on fire. Under optimal conditions the oil slick can be removed by 85 to 95% (Schmidt, 2012)(see figure 2). The burning of oil should start quickly after the spill. Fresh oil is a prerequisite for successful burning and could be a sufficient method when mechanical recovery is blocked by dense sea ice (Berkman & Vylegzhanin, 2010). Other research claims that *In-situ* burning proves insufficient in case of sea conditions with high waves (1.5m) or an ice coverage of above 80%, due to the difficulty of reaching the oil slicks (Fritt-Rasmussen & Brandvik, 2011; Schmidt, 2012). Research by Schmidt (2011) claims that *In-situ* burning has been tested only under highly controlled experimental conditions, not in real situations (Schmidt, 2011). *In situ* burning involves environmental consequences such as the decrease of ice mass due to the emission of black carbon. The deposition of black carbon negatively influences the ability of the ice to reflect sunlight, resulting in a subsequent ice melt (Jacobson, 2010).

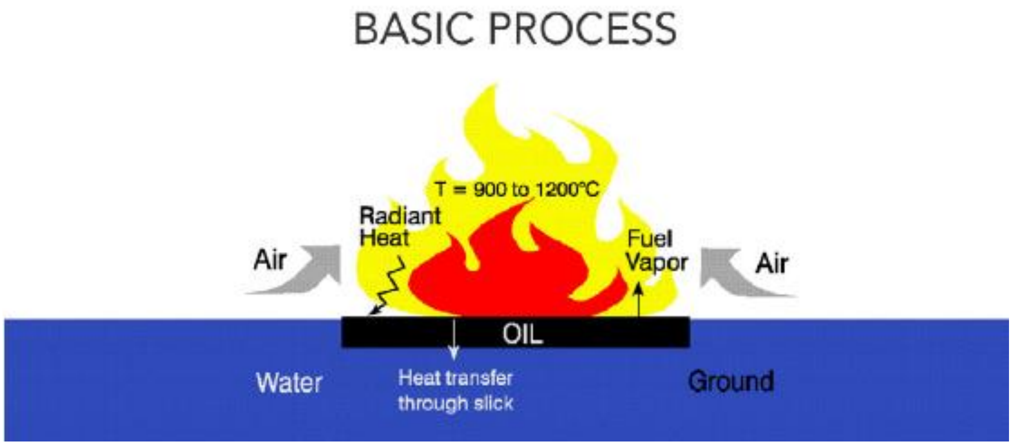


Figure 2. The basic process of *In-situ* burning of an oil slick. Keep in mind the figure does not contain information concerning possible toxic effects (Bothe et al., 2014)**.**

The third solution is the use of **dispersants** (Shell, 2011). These are chemical solutions that break up an oil layer into finer oil droplets (Wang et al., 2003) (see figure 3). Wave action should further disperse the oil into the water column from where it disappears off the surface. During the Deepwater Horizon oil spill approximately 7 million litres of dispersant (mainly Corexit9500) were added to disperse the oil slick (Campo, Venosa, & Suidan, 2013; Finch et al., 2012). Shell claims the use of dispersant has been proven "highly effective" in case of an oil spill in the Arctic, securing a responsible OSRP (Shell, 2011). However, a multitude of research shows that dispersants are less beneficial than assumed (Berkman & Vylegzhanin, 2010; Moles, Holland, & Short, 2002). Corexit9500 is a type III dispersant (the latest version) which acquirers an 1:20 dispersant:oil ratio for efficient dispersion. This dispersant has been applied in great amounts during the Deepwater Horizon oil spill and the after effects have become visible in a radius of approximately 11km around the oil well. One of the effects is the accumulation of marine snow or detritus (large amounts of dead phytoplankton). Under normal conditions the production of marine snow is a natural occurrence. After the Gulf of Mexico spill, marine snow was found present in much larger amounts (Passow et al., 2012).

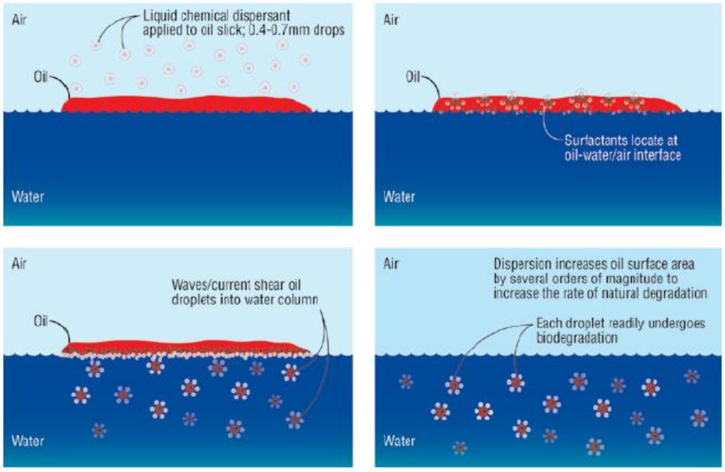


Figure 3. The schematic working of a dispersant on an oil slick (Potter, Buist, Trudel, Dickins, & Owens, 2012).

An alternative option of removing an oil slick after a blow-out is to apply **absorbents** to the oil, instead of dispersing it into the aquatic environment. This means that the oil is contained and collected. The absorption of oil does sound appealing, and methods to do so are still in development. One absorbent which has been developed in recent years is EcoTech Oil Foam (hereafter EcoTech). As the name describes: It consists of foam which is highly porous (>90%), it can absorb large quantities of oil and is water repellent. It is expected that the foam is non-toxic and environmentally friendly since it does not absorb any species of plankton (Murk, 2014). Oil companies are currently relying on dispersants since absorbents are still under development.

When looking at OSRP for the Arctic, Shell can be taken as an example, as this company is already conducting activities in the Arctic for future exploration. In their OSRP for the Arctic region the methods to remove oil as described above make up for the largest part of the response plan. This does not differ much from other protocols such as applied during the Deepwater Horizon oil spill by BP. Oil companies claim to be fully prepared for accidents in the remote Arctic region and promote Corexit9500 as a highly effective agent to disperse the oil (Shell, 2011)

Oil released in the Arctic is exposed to sea water of temperatures between -1.8°C and 4°C. The viscosity of oil (a fluids resistance to flow) increases at lower temperatures, creating a layer of highly viscous oil. As temperature drops and viscosity rises, the effectiveness of dispersants decreases (Lewis & Daling, 2007). When dispersant is added to a layer of oil and the time of penetration takes too long, the dispersant is washed off the oil slick by wave action, leaving an intact oil layer and a chemical solution floating around which is harmful to marine life (Brandvik & Faksness, 2009; Moles et al., 2002). The formation of ice sheets could change the effect of dispersants on oil dramatically. When present at a loose and regular amount, ice sheets could alter the dispersion of oil in a natural way. When ice coverage is more than 80%, it is possible that the oil is contained instead of being dispersed. This way it can be stored for several seasons in the ice layer which prevents a quick clean up or even the use of dispersants (Brandvik & Faksness, 2009)

Corexit9500 proved to effectively disperse the oil slick from the surface during the Deepwater Horizon oil spill in 2010. However, research suggests that planktonic organisms are highly sensitive to the residual toxicity of dispersants and dispersed oil and dispersants are responsible for the increased formation of marine snow (Passow et al., 2012). Marine snow is formed by bacteria, phytoplankton, detritus and minerals. It is a regular component in the water column and is responsible for most of the downward transport of material in the ocean. In the months after the oil spill in the Gulf of Mexico, marine snow was found, spread over the ocean floor in increased volumes. It was especially abundant near the spill site and in the direct vicinity of the dispersed oil plumes, containing remnant toxicants from the oil and Corexit9500. Marine snow functions as a food source for planktonic species and fish. When containing remnant of toxicants, derived from oil and dispersant, the feeding on toxic marine snow could lead to a spread of toxicants to higher trophic levels (Passow et al., 2012; Steinberg, 1995).

The formation of marine snow is a regular occurrence in oceans. In the Arctic Ocean, the short summer period triggers a fast and powerful bloom of Arctic planktonic species. These form the main element of the diet of benthic organisms, but also large marine mammals such as the bowhead whale (*Balaena mysticetus*) (Søreide et al., 2013). In wintertime, open spots in the water (Polynya's), formed by wind and water currents provide refuge for many organisms, including plankton. Plankton forms the main food supply year round, not only in summer (Saunders et al., 2003). This means that use of dispersants for combatting oil spills in the Arctic may result in an increased formation of marine snow which may include toxic compounds. This will result in a decline of food production for higher trophic levels or even the spread of remnant contaminants by consumption of marine snow by higher trophic levels (i.e. fish, molluscs etc.) (Passow et al., 2012; Søreide et al., 2013).

BP was taken by surprise considering the size of the spill at the Gulf of Mexico and applied rigorous methods to end the spill, for example by injecting dispersant at the well head just above the ocean floor. British petrol had the permission of the United States government to apply this method in addition to its original OSRP. It depends on the legislation of the hosting nation how an oil company can operate. In the Arctic this is another case. The Arctic is shared by Arctic nations which claim territorial borders that are not clearly defined yet, making the Arctic a topic for international discussion. The Arctic region is not listed as an internationally protected nature reserve such as the Antarctic is (ATS, 1959). The Arctic is therefore largely openly accessible for exploitation and usage. Currently, Arctic regulations and agreements are established by the Arctic Council. To fully understand the geopolitical situation in the Arctic it is necessary to take a look at the Arctic legal framework first.

1.4 Arctic Legal Framework

The Arctic Council (AC) was officially erected in 1996 and exists of eight member states (Norway, Sweden, Finland, Iceland, Russia, USA, Canada and Denmark). Together, these nations act as an international, high level forum; promoting cooperation, coordination and interaction amongst the Arctic nations for the benefit of the region. The international forum was established formally in 1996. Besides the Arctic member states, the AC exists of the following eight permanent participants which represent the Arctic tribes: The representatives of the Arctic Athabaskan Council, Aleut International Association, Gwich`in Council, Russian Association of Indigenous Peoples of the North and the Saami Council. The permanent participants safeguard the values of the indigenous tribes that live within the Arctic region (including land areas). Besides the member states and permanent participants, the Arctic Council is supported by working groups that are dedicated to monitoring, research and preservation of the Arctic flora and fauna, and stimulation of sustainable development. The Arctic Council does not have the mandate to act as police force, it depends on the willingness for cooperation from the member states to achieve goals such as regulations and sound oil spill response plans. Currently, the international willingness to cooperate in the Arctic Council is present and member states do work together.

Figure 4. Svalbard Treaty territory. Marked areas are National Park (Green) and Nature Reserve (Red). Map from Norwegian Polar Institute.

The Spitsbergen treaty was signed in 1920 by 42 countries in Paris, the treaty was erected after centuries of international whaling and poaching activities. Until that time no country was officially the guardian of the archipelago. After the discovery of large amounts of coal the situation changed, expeditionary settlements were erected to house miners from several nations and land was claimed by companies for exploration. A form of legislation and governance was needed to accommodate and legalize the activities undertaken. Norway was appointed as caretaker of the Arctic Archipelago at the Versailles negotiations. Norwegian law was introduced including the police system as the archipelago became part of the Kingdom of Norway. Due to the great amount of fossil fuels found, the Spitsbergen treaty was erected in such a way that all countries (including Norway) had the same chance to benefit (Sysselmannen, 2013). In short, the Spitsbergen treaty consists of the following principles:

Non-discrimination- equality to conduct activities on Spitsbergen by every company and civilian on earth if not interfering the Norwegian law or treaty.

Taxation- Norway is not allowed to raise taxes or earn money from activities undertaken at Spitsbergen. Profit gained on the Archipelago is meant to be reinvested or stay on the Archipelago.

Military Restrictions- No military activities can be undertaken at Spitsbergen or its territorial waters from any country, Norway is allowed to ensure this remains.

Environment conservation- Norway is obliged to protect Spitsbergen's flora and fauna ("Svalbard Treaty," 1920)

The treaty is currently still existing, at the moment over 65% of the entire archipelago is listed as nature reserve (figure 3); meaning that no human influences or pressures can be executed in that particular area. Areas that are not designated as reserve are still protected by legislation according to the treaty (Stange, 2012).

1.4.1 United Nations Convention on the Laws of the Sea

The United Nations Convention on the Law of the Seas (UNCLOS) applies to the whole Arctic Basin and is in force for all Arctic member States except the United States. It was erected firstly in 1956 to replace the law of *Mare liberum* from Hugo Grotius. The law dated from the medieval era and was not applicable for modern society anymore. After the first UNCLOS convention, Two more followed. UNCLOS III was signed and rectified by 165 countries and became active in 1994. UNCLOS defined rules and regulations concerning the use of the world's oceans in order to safeguard the environment and international cooperation. According to the UNCLOS convention a nation has the right to protect and exploit its territorial waters, an extension of 200 nautical miles or Economic Exclusive Zone (EEZ) is allowed, which provides the host nation the right to exploit, manage and conserve the area. An EEZ can be claimed within 10 years after signing the treatment. An EEZ in the Arctic results in the benefit of the ruling nation since ship lanes and natural resources are completely owned by the host (UNCLOS, 1970). The rush for territory has created a maze of claims across the Arctic Ocean, resulting in international disputes. Figure 5 provides a clear overview of the current situation formed by claims of Arctic member states (Durham University, 2008).

The potential borders are reason for debate; few examples are Hans Island and the Russian Lomonosov Ridge expedition. In 1984 the Danish minister of Greenlandic affairs travels to Hans Island by helicopter to plant the Danish flag. From then on several other Danish flags have been planted, subsequent by Canadian ones. The dispute between Canada and Denmark has led to political commotion and in 2005 an agreement has been made. Hans Island itself is a small rocky island, inhabited by a seal colony, in the middle of the Kennedy channel (in between Greenland and Canada). While the island itself is not of great value, the presumed oil reserves underneath the island and strategic location in the ship lane are (Jarashow, Runnels, & Svenson, 2006). Another example of ongoing Arctic exploitation occurred in 2007; an expedition led by the prime minister of the Russian Doema (Russian parliament) planted a Russian flag on the edge of the Lomonosov Ridge, some 4000 metres below sea-level. This publicity stunt supported the Russian claim they proposed in 2001, stating that the entire area on the Russian continental shelf rightfully belonged to the Russian federation (figure 4, the light green area) (Chivers, 2007). These actions were criticized by the international community, especially the other Arctic nations. The criticism reflects the actual fear of the worldwide community: if the claim is rightful it would mean that all fossil fuels located within that region belong to a single nation. It would also mean that future sea routes such as the NSR and TPP would travel over one countries territory, making it able to claim taxes and wages, something other countries are trying to avoid.

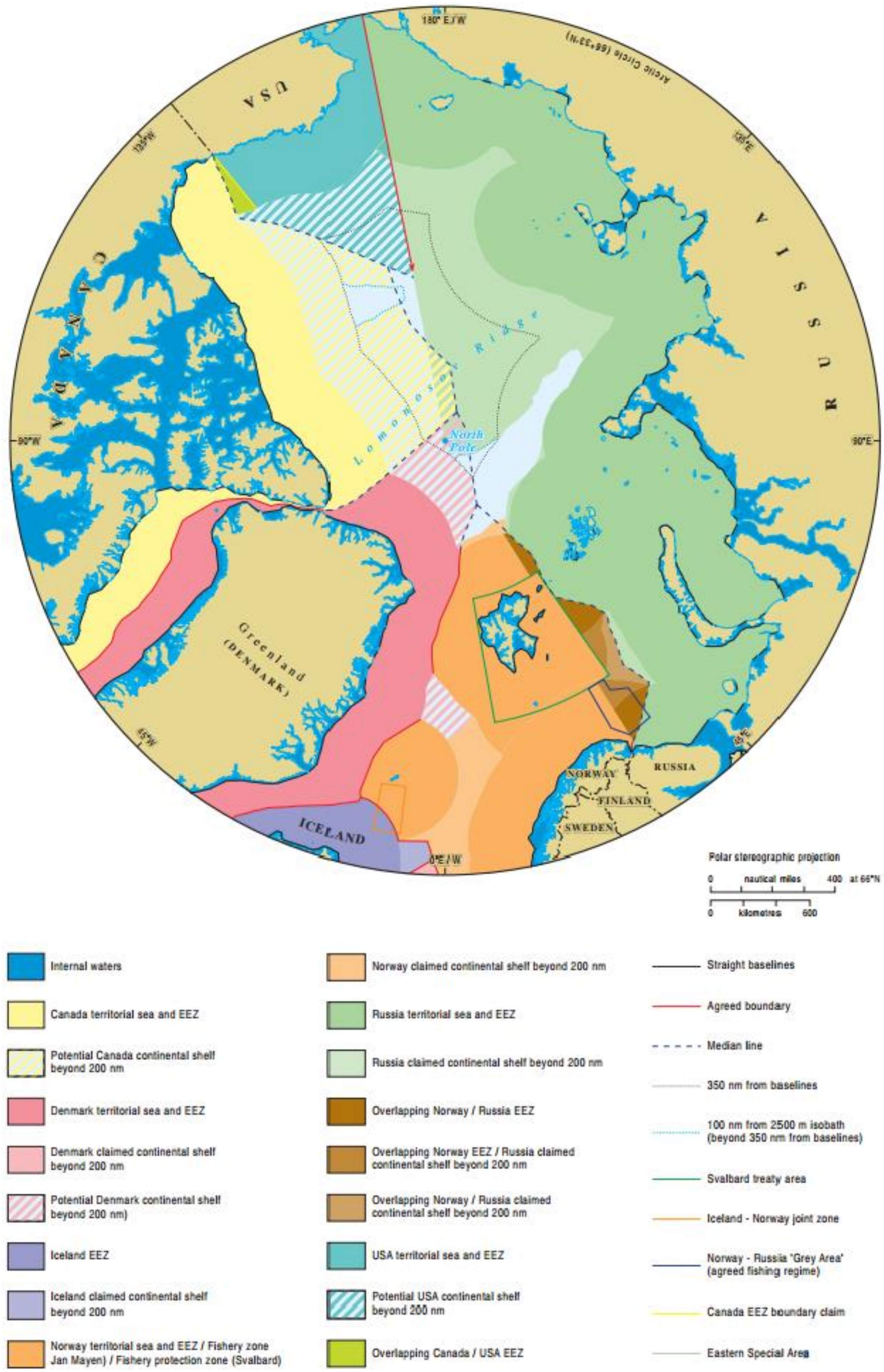


Figure 5. The Arctic subdivided with geopolitical borders (*Durham University*, 2008).

1.4.2 Artic Strategic Plans

All Arctic nations have produced an Artic strategy plan, which states their future activities in the Arctic. Listed below are the strategic plans of all Arctic nations, concerning their future involvement in the Arctic.

Canada - In its strategy plan produced in 2009 Canada has listed priorities for the Arctic region that are similar to other nations, its priorities are: exercising sovereignty, promoting economic and social development, protecting the Arctic environment and the last: Improving and developing governance. Canada is involved in a struggle with especially the US concerning the ownership of the Northwest route, the US wishes to keep the route international, Canada is regarding the route located in its territorial waters, and renamed it into the “Canadian Northwest passage” (Canadian-parliament, 2009).

Denmark - Shortly summarized Denmark`s priorities are aimed at the enhancement of maritime safety and to enhance its sovereignty. Second aim is to promote renewable energy sources, also to exploit mineral resources, whilst maintaining to lead a role in Arctic research and to promote cooperation on human health. Third aim is to conduct more research concerning Arctic climate change. Final priority is to enlarge the global cooperation and enhancement in the Arctic Council (Danish-government, 2011).

Finland - In the Finnish strategic report of 2010 it is explained that due to the Northern geographic location Finland already is bounded with the Arctic. Its objectives for the future are to promote Arctic research and stimulate nuclear safety (mostly on own territory). Concerning the economy, Finland wishes to strengthen its role as international expert in Arctic knowhow, to use the Finnish experience of winter shopping and Arctic technology. Finland expects to mostly to increase its mining activities and infrastructure to promote transport(Finnish-Parliament, 2010).

Norway - In the summary of the Arctic strategy report of the Norwegian governments the following aspects are stated as main political priority: The Norwegians will exercise their authority in the High North in a credible, consistent and predictable way and will take active place in the frontline of international efforts to develop knowledge in and about the High North. Norway will further develop a framework for petroleum activities in the Barents Sea. Norway will intend the High North policy to play a role in safeguarding the livelihoods, traditions and cultures of indigenous peoples in the High North. The statute mentions the importance of maintaining close bilateral relations with Russia in order to overcome challenges such as environmental security and resource management. Another aspect of priority which is closely related to this is the fact that Norway will intend to be the best steward for environment and natural resources in the High North. Thus Norway intents to remain and improve its involvement in the Arctic with emphasis on safeguarding nature’s wellbeing (Norwegian-parliament, 2006).

Russia - The main objective for Russia is to secure and prepare the Arctic region to become the nation’s largest supplier of natural resources (e.g. fossil fuels and important metals and minerals) by 2020. One of the main goals is to increase extraction of natural recourses and to secure and become the leading Arctic power. The document, named: “*The fundamentals of state policy of the Russian Federation in the Arctic in the period up to 2020 and beyond*” specially outlines the importance of securing a “necessary combat potential”, clear definitions of this meaning remains vague in the document, it is clear that the Russian

Federation is preparing special military Arctic units to secure its territory. To conclude, it is clear that the Arctic will be exploited as main source of fossil fuels by 2020 (Russian-parliament, 2009).

Sweden - Sweden wants to reduce the emission of greenhouse gasses and to make sure that Arctic climate change is highlighted in international climate negotiations. Sweden is willing to invest in scientific research for climatic and environmental purposes. On the other hand Sweden wishes to pursue business (e.g. mining and forestry) in the free trade parts of the Arctic. The wellbeing of native Arctic tribes such as the Sámi who life in Northern Sweden shall be ensured(Swedish-parliament, 2014).

The EU - On itself not a member of the Arctic Council, some of its member states are (i.e. Sweden and Finland). The EU does have introduced a suggestion in the form of an Arctic policy, the document itself confirms the strategic plans of its member states and does not differ much from it. The EU does aim to increase its involvement in the Arctic by for example becoming a permanent observer in the Arctic Council and the call for intensification of dialogues between Arctic states (EU-Council, 2014).

United States - The US published its Arctic policy (named: Arctic region policy) in 2009 and is very similar to the policies of other nations. The US enhances the need for homeland security and defence. Besides that the US is focussed on operating independently in the Arctic, on the other hand the need for international cooperation is requested. The Arctic Council is regarded as a positive system, but its mandate should not further exceed (Presidential-directive, 2009).

1.5 Summary of the Introduction

While sea ice decreases with fast leaps, the value of the Arctic increases even faster. Boundary lines are drawn and territories are marked in order to secure natural resources for the benefit of one's nation. The Arctic nations have detailed strategy plans for the next decade in which they all aim to increase the involvement in the area, especially with the focus on oil exploitation. Agreements and treaties are achieved via the Arctic Council, which consists of all Arctic nations and representatives of the Arctic indigenous tribes. The Arctic Council aims to protect the Arctic region and all involved nations have agreed on the need for conservation. Still the focus of most countries is emphasized on the extraction of the previously mentioned fossil fuels. The offshore activities are already taking place and the future foresight predicts these activities will even further increase. This results in an increasing chance of accidents and possible oil spillage (i.e. blowout on an oil rig or oil tanker wreckage).

The existence of a proper working oil spill response method is highly relevant for an intensively used Arctic region. The members of the Arctic Council signed the "Agreement on Cooperation on Marine Oil Pollution, Preparedness and Response in the Arctic". Which This shows that authorities are already aware of the risk of an oil spill in Arctic areas and the needed cooperation among the different countries within this topic is already known by authorities (ArcticCouncil, 2013). Until now there are no common requirements to grant permission for oil exploitation between nations. The mandatory oil response plans are based on oil exploitation at lower latitudes. Oil companies claim to possess a sufficient OSRP (Shell, 2011). Other research claims that the proposed methods applied are only tested under highly controlled conditions and that the proposed methods may not be efficient in Arctic conditions (Berkman & Vylegzhanin, 2010).

1.6 Problem description

Predictions made by climate models of an ice free Arctic Ocean during summer within 30 years boost offshore activities. At the same time, the need for fossil fuels creates a large interest for the Arctic region since an estimated 13% of the world's oil reserves are located in this area. Oil spill response methods are currently heavily relying on the use of oil dispersants as response method in case of an oil spill (e.g. Deepwater Horizon blowout). Dispersants have not been tested specifically for the Arctic region. Increased viscosity of oil, lesser penetration and decreased dispersion could be less efficient when applied at Arctic temperatures. Other Oil spill recovery methods such as absorbents are still under development. Oil spill response plans are still heavily relying on the use of dispersants. These OSRP's are accepted by the member states of the Arctic Council in order to conduct offshore activities in the Arctic and to safeguard the Arctic ecosystem while little is known regarding the use of dispersants in the Arctic. On behalf of IMARES new data will be collected concerning the applicability of the dispersant Corexit9500 under Arctic temperatures. In addition, the absorbent EcoTech Oil Foam will be tested for application at Arctic temperatures.

1.7 Aim of research

Aim of the research is to provide knowledge on the feasibility of oil response agents in the Arctic.

1.8 Main question

Can Corexit9500 and EcoTech Oil Foam be recommended as response agents for combating offshore oil spills in low water temperatures?

1.9 Sub question

1. How does the dispersion behaviour of oil and oil with Corexit9500 in temperate water (18°C) differ from the dispersion behaviour of oil in Arctic water (4°C)?
2. How does the LC_{50} of *Corophium volutator* change after treating oil slicks with Corexit9500 and EcoTech Oil Foam in temperate water temperature (18°C) compared to Arctic water temperature (4°C)?
3. How does the growth ratio of *Phaeodactylum tricornutum* change after treating oil slicks with Corexit9500 and EcoTech Oil Foam in a temperate water temperature (18°C) compared to Arctic water temperature (4°C)?
4. What issues are involved concerning the practical applicability of the deployment of Corexit9500 and EcoTech Oil Foam in the Arctic?

2. Material & Methods

This chapter will describe the methods applied during this research and the apparatus needed. It is divided in several paragraphs which are dedicated to the different types of experiments conducted during this research.

2.1 The dispersion behaviour of oil

This chapter describes the material and methods conducted during the plunge-test experiments. A more detailed description of the material and methods of the plunge-test are described in Appendix I. The objective was, to provide data concerning the efficiency of the dispersant Corexit9500 using an oil-dispersant ratio of 1:100 to disperse at different temperatures (4°C & 18°C). The data was collected by so called plunge-tests. During these tests, an amount of water was plunged into an aquarium that contained a 4mm layer of crude oil. The wave activity created by the plunge disturbed the oil layer. Droplets of oil circulating through the water column (by the plunge) were recorded by two high speed cameras and stored in Visionlab software systems. By using a specific computer program, the oil volume in the water column was calculated by using the amount of pixels showing oil droplets on the pictures. The procedure of the calculations is outside of the scope of this research and is not mentioned in the report.

An aluminium structure kept the 9 Litre glass aquarium in place. Two high speed cameras were fixed to the aluminium frame, aiming at the centre of the aquarium. A background LED-plate illuminated the aquarium for optimal light conditions for the high speed cameras. The experimental setup can be seen in figure 6. In advance of the test, the artificial seawater was prepared as described in Appendix I. An oil-layer of 400μm, mixed with or without Corexit9500, was applied to the water surface 10 minutes before the plunge. The types of oil applied in test are 'Mississippi Canyon Block 252 oil (MC252)' representing oil with low viscosity and Helder oil representing an oil type with higher viscosity. 300ml of artificial sea water was placed at the glass container (plunge container) that would tip over at a fixed height of 15cm, resulting in a plunge. After the plunge the high speed cameras automatically shoot images triggered by the sensor placed below the plunge container. This experiment was conducted at 18°C and 4°C resembling an oil spill at temperate and Arctic water temperatures.

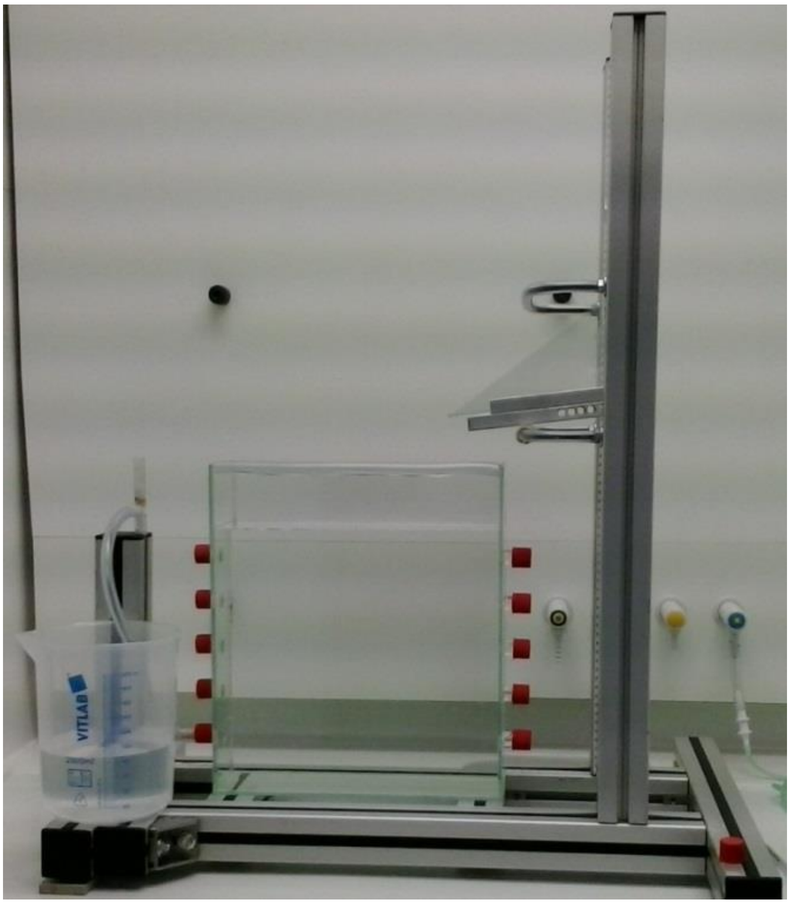


Figure 6. experimental setup of the plunge-test.

2.2 Water Accommodated Fracture for toxicity tests

Acute toxicity tests were conducted to determine the residual toxicity of oil and oil treated with different techniques. The residual toxicity was tested with non-treated oil, oil treated with a dispersant (Corexit9500) and oil treated with an absorbent (EcoTech Oil Foam) at 18°C and 4°C water temperature. *In vivo* tests were conducted on the test organism *Corophium volutator*, from which the Lethal Concentration of 50% of the test population (LC50 in ml oil/L) was calculated in order to answer sub question 2. The test population consists of 10 *C. volutator.* The standard test with the algae *Phaeodactylum tricornutum* was used for a growth inhibition test, monitoring the growth rate while exposed to different concentrations in order to answer sub question 3. All tests were based on the Water Accommodated Fraction Preparation Procedure (WAF). Standards within the acute toxicity test have been applied. Every series consisted of a control group without oil (blanc test) and a reference group to be able to compare results between experiments. The used oil in this experiment was MC252.

Figure 7. Schott Duran double-bottles used during research. 1) Surface layer of the first bottle contained the layer of oil or oil-dispersant. 2) This side of the structure was placed above a stir plate, a stir bean on the bottom of the flask provided a circulating. 3) The glass tubes enabled a circulation of water from the oil carrying flask through the second flask. 4) The second flask contained a mesh cylinder in which *C. volutator* were kept.

For the WAF preparation Schott Duran double-bottles were filled with 2.2Litres of filtered Oosterschelde Estuary seawater. One bottle (with a narrow opening) was used for the adding the oil or treated oil to the system (figure 7). This bottle was located on a stir plate. The second bottle with a wider opening contained a mesh cylinder, which would later on contain the organisms for the *in* vivo test. A vortex of approximately 1cm was created by a propelling glass covered stir bean. Glass was the preferred material for the stir bone instead of regular Teflon beans, since Teflon could absorb oil. The vortex created a water circulation through both bottles via the glass tubes. During the WAF preparation, the entire setup was kept in total darkness for 24 hours according to the protocol in Appendix II. The bottles were closed airtight with plastic lids covered in tin-foil on the inside, to avoid attachment of volatile oil compounds to the plastic fabric. After 24 hours, a 200ml sample for chemical analysis was taken by pipetting the volume with a 50ml glass pipettes from the second bottle with the wider opening. The chemical analysis could not be conducted during the research period. Another 90ml WAF extract was removed from the second bottle and put into a 150ml Erlenmeyer flask for the *P. tricornutum* growth inhibition tests, as described later on. The remaining WAF in the double-flasks was used for the *C. volutator* based toxicity test, which is described in the following paragraph. The first test set was conducted in an 18°C climate room to represent the water temperature in a temperate region (between the Arctic Circle and tropic zone) during summer. The second test temperature was at 4°C, which represents the general Arctic water temperature. For one week, one treatment was tested. Tests were conducted in triplicates and at 6 different oil loadings (See table 1).

	MC252 (ml oil/L)						**MC252 with Corexit9500 (ml oil/L)**						**MC252 with EcoTech (ml oil/L)**					
18°C	0	0.003	0.01	0.1	0.3	1	0	0.0075	0.015	0.030	0.1	0.3	0	0.01	0.03	0.1	0.3	1
4°C	0	0.01	0.03	0.1	0.3	1	0	0.01	0.03	0.1	0.3	1						

Table 1. Applied oil loading ml per litre seawater.

Oil loadings described in table 1 have been applied to achieve 0 to 100% mortality, by 6 different concentrations in the *C. volutator* toxicity test. The oil loading of the first toxicity test for non-treated oil at 18°C was taken from former research ("Personal communication Chiel Jonker," 2014). The results of this test were used as baseline for the oil loadings in all other tests. A higher mortality was expected when using dispersants and a lower mortality at lower temperatures. By doing so, it was possible to determine an LC_{50} of each treatment. In addition, one reference treatment of 30µl oil/Litre seawater was conducted at 18°C during all treatments to assess the sensitivity of the test organisms. All loadings were conducted in triplicate. The oil loadings within a replicate series were randomly distributed along the double-bottles.

For conducting the oil with Corexit9500 treatment, a set dispersant to oil ratio of 1:20 was applied. This ratio is derived from a regular OSRP (ITOPF, 2014). The Corexit9500 was sprayed on the oil layer in each bottle. For the treatment of oil with EcoTech at 18°C, permeable nylon bags containing 0.25g of the EcoTech, figure 8, were put into each bottle on top of the oil layer. The amount of EcoTech was decided by the ability to absorb the highest oil loading.

Figure 8. Permeable nylon bag containing EcoTech Oil Foam made from panties.

2.3 *Corophium volutator* toxicity test

This test is conducted in the double flasks containing the WAFs at the different temperatures, directly after the WAF extractions were taken.

The test organism: *C. volutator* or mudshrimp (see figure 9). The test organisms were retrieved from the Oesterput, which is currently part of the Natura 2000 reserve plan. The Oesterput is located at the Oosterschelde near the village of Colijnsplaat (The Netherlands) and has been a point of collection of *C. volutator* for research purposes in previous years.

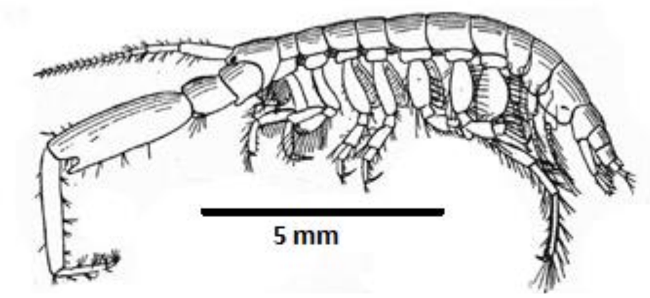


Figure 9. *Corophium volutator,* male ("*C. volutator* ," n.d.)**.**

C. volutator were collected by sieving the very fine sediment at the upper 5-10cm layer, using a 1mm mesh size the *C. volutator* would remain in the sieve and could be placed in a bucket for transport. The bucket was filled with a layer of sediment and seawater from the same location. During the collection of *C. volutator* no juvenile specimens were gathered (≤6 mm) in order to avoid the chance of change in sensitivity and response between juveniles and adult species during experiments. Both male and female species have been applied during experiments. At arrival at the laboratory in Yerseke the organisms were stored in an aquarium, containing a layer of sediment from the Oesterput and unfiltered seawater from the Oosterschelde. Continuous aeration provided oxygen in the aquarium. In order to acclimatise and avoid stress *C. volutator* were kept in the aquarium for no longer than 6 days before being put into the tests.

The choice for *C. volutator* as test organism is supported by the International Organization for Standardization (hereafter ISO), such as the standard test protocol for determination of acute toxicity of marine or estuarine sediment to amphipods (ISO 16712). Governmental permission allows IMARES to collect flora, fauna and sediment from protected or restricted areas.

C. volutator were collected from the start of April till the end of May. During this period *C. volutator* grew in size and abundance as summer temperatures were reached. It is proved that the test species prefers temperatures between 15-20°C (Mills & Fish, 1980; Taylor, Meadows, & Ruagh, 2011). The first test-temperature of 18°C lies well within the preferred lower and upper limit of *C. volutator.* In order to conduct the experiments at 4°C and 18°C and to prevent mass mortality, the organisms were acclimatised to these temperatures prior to the start of the tests. *C. volutator* were placed in a climate room overnight that was either lowered or raised in temperature every 2 hours about 2°C until 4°C (test temperature) was reached.

At day 0, the start of the test, 22 glass beakers were filled with 10 organisms of the relative same size in filtered Oosterschelde Estuary seawater. Male and female species were randomly distributed over each beaker. The same day, just after taking the WAF-extractions, the organisms were added to the double-flasks containing the WAFs with the different oil loadings. The 10 organisms were added to the fine mesh cylinder which was placed in the second bottle. By doing so, *C. volutator* was prevented to swim to the other bottle avoiding mortality by direct contact with oil slick or treated oil, and the glass covered stir beans. After addition of the test organisms, the double-bottles were closed air tight and left in the climate room at the set temperature with an 8-16 hour (light/darkness) rhythm. It was assumed no O_2 limitation occurred during the test period. After five days (120 hours) the mortality rate of *C. volutator* was monitored in each bottle. The mesh cylinders were taken out of the double-bottles. All test organisms were removed from the cylinder one by one and carefully placed in glass beaker filled with sea water in order to watch movement of the surviving organisms. An effect of exposure to oil loading can be temporary anaesthesia. Therefore, observation of the mortality should last for at least 30 seconds in order to allow *C. volutator* to recover from the narcotic effect of the tested compounds.

2.4 *Phaeodactylum tricornutum* growth inhibition test

The growth inhibition experiment was conducted on the algae species *Phaeodactylum tricornutum*. The method applied to culture this diatom species is based on the water quality protocol: "Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*" (ISO 10253:2006), see Appendix III.

The test was started with a pre-cultured algal stock with low cell density to maintain reaching exponential growth until the start of the test. Two 100ml batches of *Phaeodactylum* previously cultured in an Algae-stove, were added to a 2.5 litre Erlenmeyer flask. The Erlenmeyer flask contained 2.5l filtered and sterilized seawater (from the Oosterschelde Estuary), 2.5 ml Walné medium, 0.25ml vitamins and 10ml silicate to enable culture growth. A 60 hour pre-culturing period was regarded sufficient to obtain a sufficient density. The 2.5L flasks were kept under a continuous light regime supported by filtered aeration to enable maximum algal growth. The flasks, seawater and apparatus were sterilized before using to avoid culture contamination. After the 60 hour pre-culturing period 10ml of the culture stock was extracted and put into test.

10ml algal solution was added to a 250ml Erlenmeyer flask which was filled with 90ml of WAF extract containing the different oil loadings and a growth medium. As growth medium 0.09ml Walné medium, 0.36ml silicate and 0.009ml vitamins were used, calculated for the 90ml of WAF extract. In total 6 different oil loadings were tested in triplicates plus a reference oil loading. After addition of the 10ml algae to the Erlenmeyer flasks, the flasks were sealed with tin-foil and parafilm. The tin-foil prevents attachment of volatile oil components to parafilm. All flasks were placed under a continues light regime at 18°C, see figure 10, and were manually shaken for 10 seconds, three times per day to provide re-suspension.

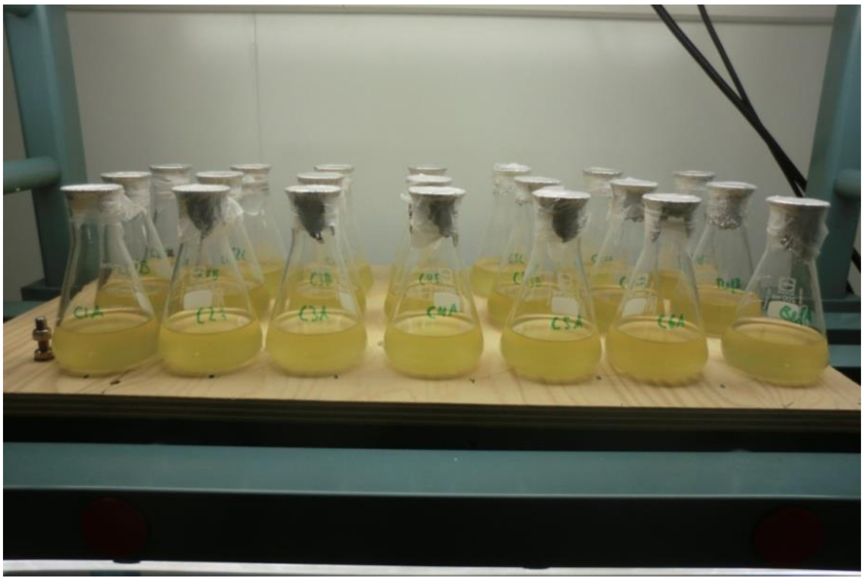


Figure 10. Growth inhibition test of *P.triconutum.*

The cell density of each replicate was monitored at day 1, 2 and 3 using a Bürker-Türk or haemocytometer. When monitoring, a 1ml sample was taken from each Erlenmeyer and stained with 50µl Lugol to secure the sample. After staining, a set amount of the sample was placed on the Bürker-Türk counting rafter. This thick glass counting rafter contains two chambers on the upper surface that can be covered with a glass coverslip, creating a volume of $0.1mm^3$. In total all the algae cells located within 25 rafters per chamber were counted. This provides the mean amount of algal cells per 1/10 of a microliter. To know the amount of cells per millilitre the average counted algae cells of both chambers were multiplied by 10,000.

The growth ratio (µ in day-1) of each replicate was calculated by using the following formula:

$$\mu = (\ln X_t - \ln X_0) / t$$

Where X_0 is the initial cell density and X_t is the cell density after t-days, calculation according to R.R.L Guillard (Stein, 1973).

The percentage of growth inhibition of each replicate was calculated according to the formula of the ISO standard protocol:

$$I_{\mu i} = \frac{\bar{\mu}_c - \mu_i}{\bar{\mu}_c} * 100$$

Where $I_{\mu i}$ is the percentage inhibition, $\bar{\mu}_c$ is the mean growth ratio of the control and μ_i is the growth ratio of the replicate.

2.5 Applicability and deployment of oil response agents in the Arctic

The experiments conducted in this research were focused on the environmental side of the topic. To answer whether Corexit9500 and EcoTech Oil Foam are recommendable as response agents for combating offshore oil spills in the Arctic region, different factors (e.g. logistic and infrastructural applicability) are of importance as well. In order to answer the 4^{th} sub question "What issues are involved concerning the practical applicability of the deployment of Corexit9500 and EcoTech Oil Foam in the Arctic?" a desk study was conducted. Additionally, experiences concerning the applicability of the agents gathered during the experimental part of this study were taken into consideration as well as expert opinions.

2.6 Data Analysis

This chapter describes the methods of statistical and visual analyses conducted during this research.

2.6.1 The dispersion behaviour of oil

Statistical analysis has been conducted by data derived from the plunge-test pictures. Images showing the plunge-test 5 seconds after impact have been statistically analysed. Images from other timeframes proved insufficient: 2.5 seconds contains many large droplets of oil and air, making reliable calculation difficult if not impossible. The timeframe of 30 seconds showed that most droplets had surfaced again and was regarded not valuable.

To analyse the difference of natural dispersion behaviour based on temperature, an independent sample t-test for both Helder oil and MC252 has been conducted after verifying the applied assumptions. The layer thickness of the oil volume is the applied variable, groups were defined by 4°C and 18°C water temperature. Dispersion values for each temperature of Helder oil were normally distributed, as assessed by Shapiro-Wilk test (18°C: p= 0.266, 4°C: p.=0.393). Homogeneity of variances was violated, as assessed by Levene`s Test for Equality of Variances(p=0.382).Dispersion values for each temperature of MC255 were normally distributed, as assessed by Shapiro- Wilk test (18°C:p.= 0.290, 4°C:p.=0. 367). Homogeneity of variances was violated, as assessed by the Levene`s Test for Equality of Variances (p.=0.108).

In addition to the statistical analysis, the images from the plunge-test experiments have been assessed in a visual analysis. The behaviour of Helder and MC252 oil and the addition of dispersant have been compared at 18°C and 4°C.

2.6.2 *Cororphium volutator* based toxicty test

Prior the actual analysis, the dataset for the *C. volutator* toxicity tests were checked for reliability of data: Mean mortality of the blanc concentrations of all tests were not allowed to exceed 10%, otherwise the experiment would be excluded from further analysis. Mortality above 10%at the blanc treatment would mean that the test organisms were affected by other factors (i.e. stress, old age or injuries), resulting in unreliable data.

After proving the reliability of the data, each experiment was analysed separately to calculate the LC50 or lethal concentration of 50% of the population, using Graphpad Prism 6. This is conducted via a non-linear regression analysis with log(agonist) versus normalized response test with a variable slope.

The analysis of this test is based on Pearson Chi- Square Tests. For using the Pearson Chi- Square Test following assumptions were ratified for all conducted tests: at most 20% have expected count less than 5 and the minimum expected count is 1 or higher. The SPSS outputs of all Pearson Chi- Square Tests can be found in Appendix IV.

First, the references of each test were analysed by a Pearson Chi- Square Test using mortality as weight. Only the experiments with no significant difference in the references are able to compare. All 18°C experiments do not differ from each other:

18°C MC252 and 18°C MC252 with Corexit9500: Pearson Chi- Square Test, df=2, p= 0.952,

18°C MC252 and 18°C MC525 with EcoTech: Pearson Chi- Square Test, df=2, p= 0.726, 18°C MC252 with Corexit and 18°C MC252 with EcoTech: Pearson Chi- Square Test, df=2, p= 0.574.

The experiment references of MC 252 at 18°C and at 4° differ from each other and are not able to compare(Pearson Chi- Square Test, df=2, p= 0.000).

The experiment references of MC252 with Corexit9500 at 18°C and 4°C do not differ from each other(Pearson Chi- Square Test, df=2, p= 0.098).

Next, the differences between each experiment (able to compare according to the reference analysis) were analysed by a Pearson Chi- Square Test using mortality as weight. After initial analysis, outlying or interrupted data has been separated. These data points have been further analysed by a Pearson Chi-Square Test.

2.6.3 *Phaeodactylum tricornutum* growth inhibition test

The validity criteria written in the ISO standard protocol were analyzed by using Excel. The test is valid if the control increases by a specific growth rate of 0.9 d^{-1}.

The mean growth inhibition and the standard error of each oil loading are calculated with Excel.

Only descriptive statistical analysis has been conducted since high standard errors and the test does not meet the validity criteria.

3. Results

This chapter describes the results of the conducted experiments. Per sub-question a separate paragraph was made.

3.1 The dispersion behaviour of oil

In this paragraph, the results of the plunge test are represented to answer sub question 1: How does the dispersion behaviour of oil and oil with Corexit9500 in temperate water (18°C) differ from the dispersion behaviour of oil in Arctic water (4°C)?

3.1.1 Natural dispersion of Helder oil

During the plunge tests, after 5 seconds the natural dispersion of Helder oil did not differ between 4°C and 18°C (independent sample t-test, df=6, t=1.501, p=0.184). Average oil volume in the water column was 0.374±0.167ml (average± standard error) for the 4°C treatment and 0.628±0.088ml for the 18°C treatment, see figure 11.

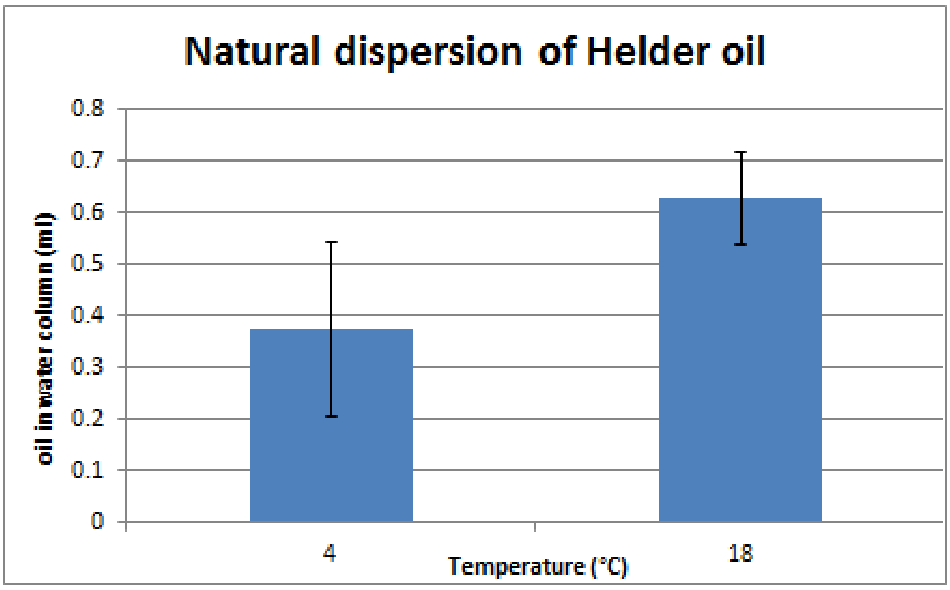


Figure 11. Average natural dispersion with standard error of Helder oil at different temperatures (4°C: n=3, 18°C: n=5).

In figure 12, a selection of images of two tests per temperature is presented of Helder oil which were taken at different timeframes (2.5, 5, 30 and 60sec.) after the plunge. Images of all tests can be found in the Appendix V**.** The natural dispersion behaviour is shown of a 0.4mm thick oil layer at 18°C and 4°C water temperature.

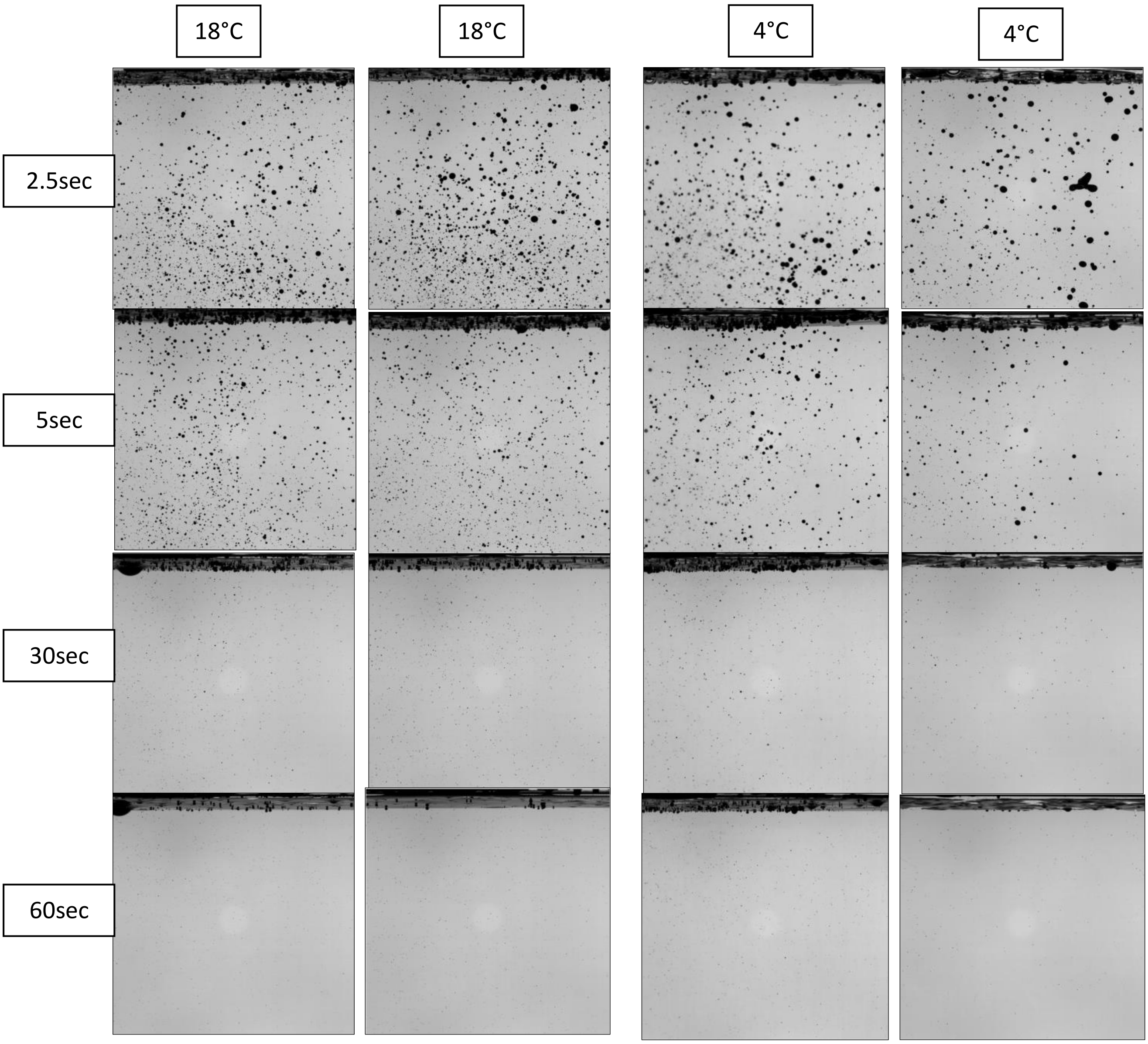


Figure 12. Natural dispersion of Helder oil

Due to the plunge activity, the oil layer was dispersed into oil droplets of different sizes in the water column. 2.5 Seconds after impact, oil droplets of various sizes together with air bubbles were distributed through the water column in figure 12. After 5 seconds the images reveal a different situation: the air bubbles have already ascended towards the surface and the largest oil droplets have gathered near the water surface. The ascending of large oil droplets was visible at the 18°C experiments. The lower water column was mostly filled with the small oil droplets. After 30 seconds only the smallest oil droplets were residing in the water column. Comparing the pictures of different temperatures it could be seen that the particle size of the oil droplets in the water column are bigger at 4°C water temperature than in 18°C water temperature. It seemed the amount of particles is less at 4°C than at 18°C. Whereas the particle size at 4°C was28 larger, by comparing the images (at 5 sec.) of both 4 and 18°C water temperatures.

3.1.2 Natural dispersion behaviour of MC252

In the plunge tests the natural dispersion of MC252 oil did not differ between 4°C and 18°C (independent sample t-test, df=5, t=1.122, p=0.108). Average oil volume in the water column was 0.771±0.235ml for the 4°C treatment and 0.518±0.094ml for the 18°C treatment.

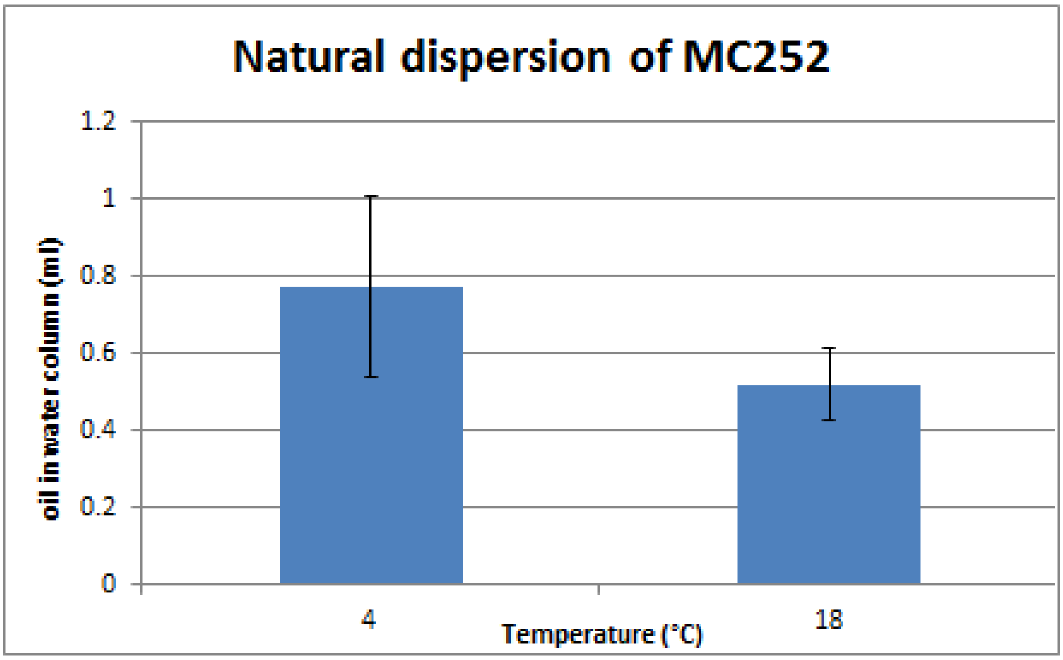


Figure 13. Average natural dispersion with standard error of MC252 at different temperatures (4°C: n=3, 18°C: n=3).

In figure 14 a selection of images of two tests per temperature is presented which were taken at different timeframes (2.5sec., 5sec., 30sec. and 60sec.) after the plunge using MC252. The pictures of all tests can be found in Appendix V**.** Figure 13 shows the natural dispersion behaviour of a 0.4mm thick oil layer of MC525 at 18°C and 4°C water temperature. The same change of dispersion behaviour comparing the two different temperatures as in the chapter of natural dispersion of Helder oil was seen. But the droplets of dispersed MC252 were in general smaller than the droplets of dispersed Helder oil.

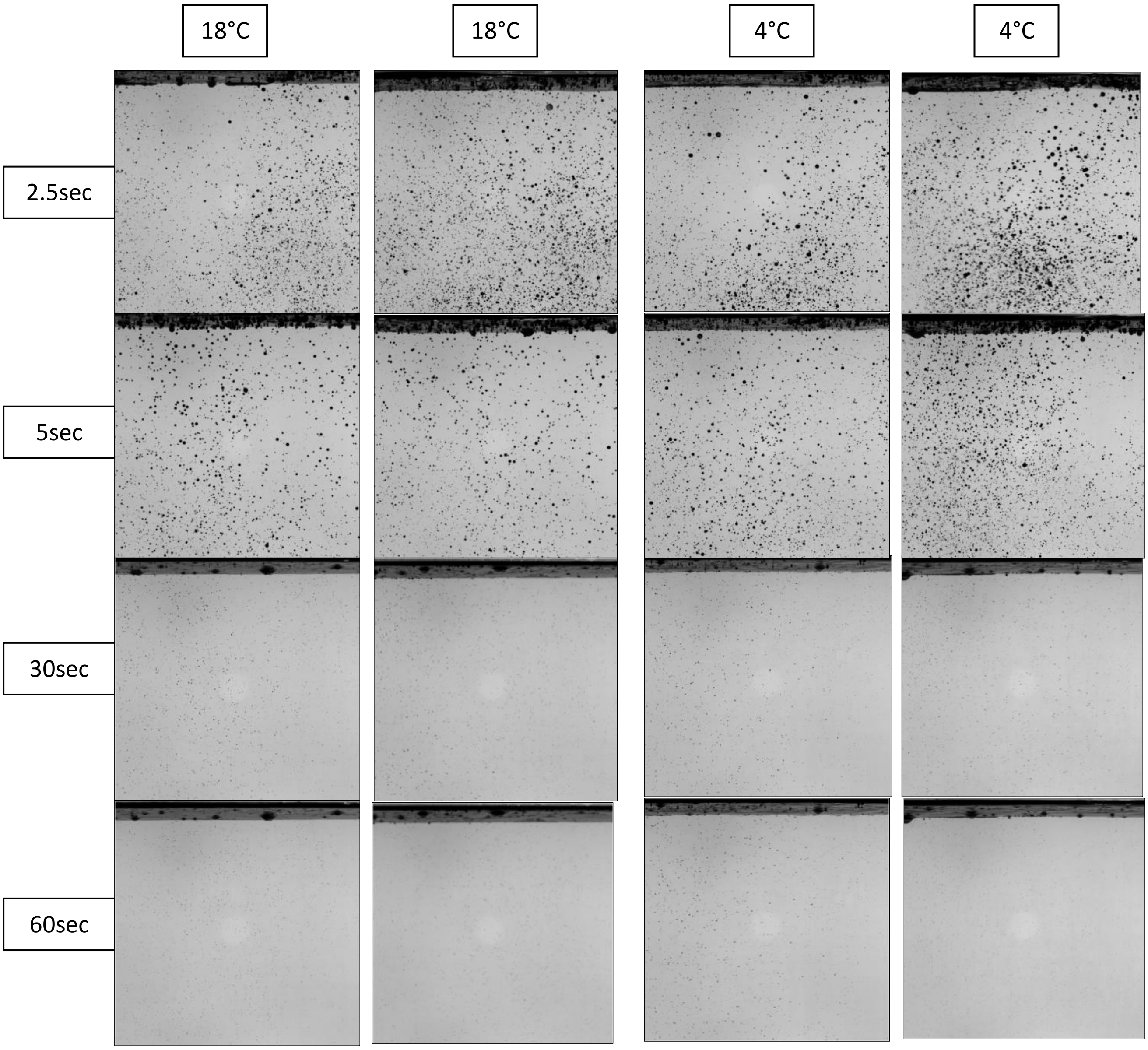


Figure 14. Natural dispersion of MC252 at 18°C and 4°C.

3.1.3 Chemical dispersion

Looking at figure 15 of the dispersion behaviour of oil treated with Corexit9500 (1:100 dispersant-oil ratio) at 4°C and 18°C, similar to the natural dispersion differences in particle sizes were seen. The particle sizes of the dispersed oil were much smaller at 18°C than at 4°C. Also, the amount of small particles was different. On the pictures taken after 60 seconds where just the smaller particle remaining in the water column more particles could be seen at 18°C. These results were comparable for both oil types, Helder oil and MC525. The addition of Corexit9500 on a MC252 oil slick at 18°C resulted in a dense layer of very fine droplets which are dispersed throughout the water column, where most of the fine particles remained even after a long period (≥60 sec). The situation is similar at 4°C, however here the dispersed oil droplets were larger in size and the quantity of droplets remaining dispersed was lower compared to 18°C. After the plunge, Helder oil and Corexit9500 resulted in an increased amount of small sized droplets at 18°C. These droplets were larger compared to MC252 and dispersant. At 4°C the droplets were much larger in size, looked more like oily strings circulating which quickly ascend to the surface (≤5sec). In all tests the particle size of dispersed Helder oil (in all time shots) was bigger than particle size of dispersed MC252.

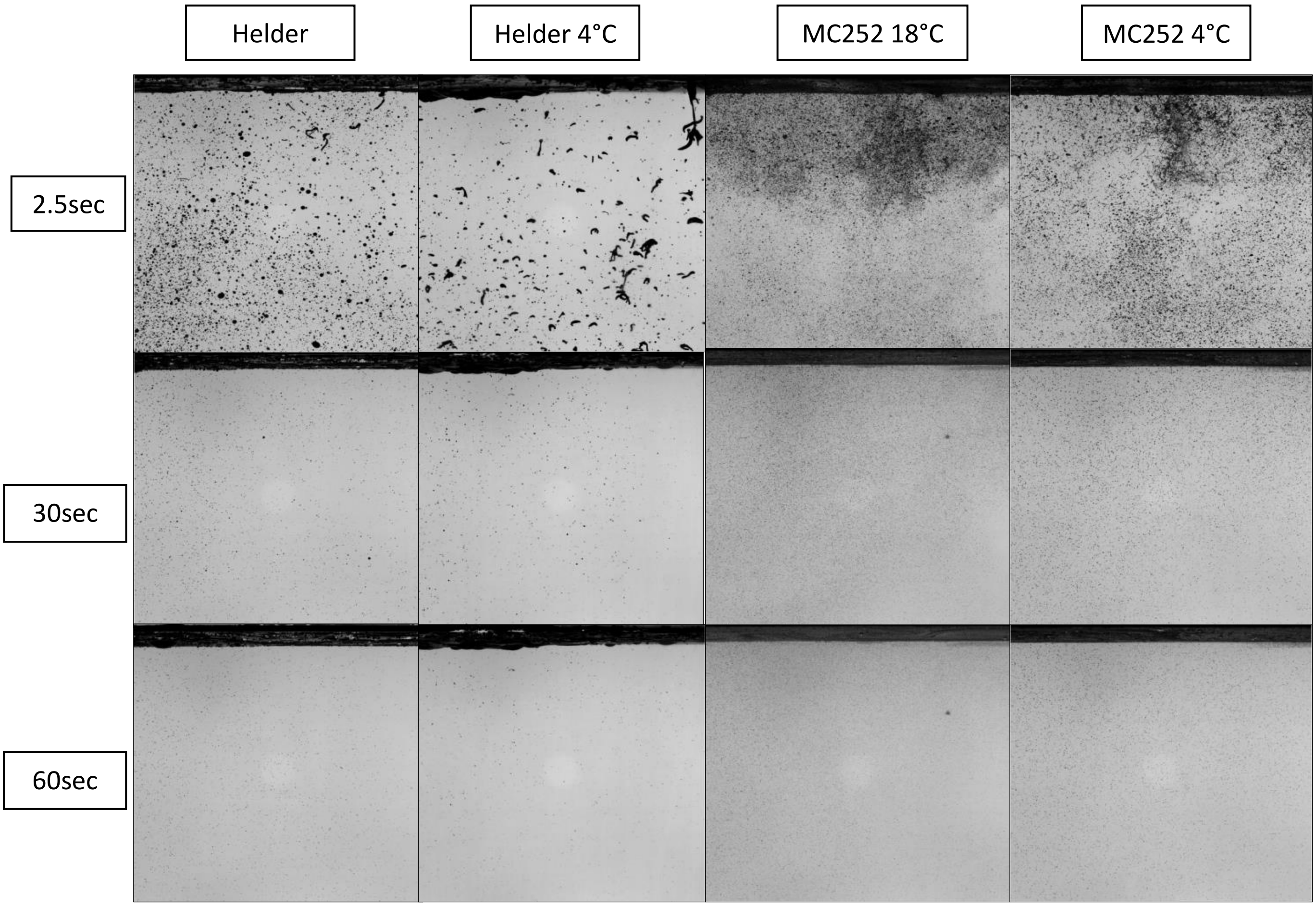


Figure 15. Chemical dispersion of MC252 and Helder oil with Corexit9500 at 18°C and 4°C.

Closer analysis of the images derived from the plunge-test experiments reveals the following: Oil layers consisting of solely MC252 resulted in small sized oil droplets at 18°C. At 4°C the MC252 droplets er slightly larger compared to the droplets occurring at 18°C. When exposed to the plunge, the more viscous Helder oil resulted into droplets of a larger volume compared to MC252. Similarly, Helder oil produced larger droplets at 4°C compared to the 18°C experiment. Oil Slicks (of both types) exposed to wave activity at low water temperatures (4°C) resulted in larger sized oil droplets circulating in the water column, compared to temperate water temperatures (18°C).

3.2 *Corophium volutator* toxicity test

In the following the results of the *C. volutator* toxicity test are presented.

Figure 16 shows **t**he dose response curve of *C. volutator,* exposed to MC252 at 18°C on which the LC_{50} value was calculated. The LC_{50} value of this treatment was 0.02126ml oil/L. The curve shows that the mortality of *C. volutator* slightly increased at the oil loading of 0.003ml/L with a mean of 3.33% and a standard deviation, hereafter SD, of 5.77% to the oil loading at 0.01ml oil/L with a mean mortality of 13.33% (SD=5.77). The strongest increase of mortality was seen between the oil loading of 0.01(Mean=13.33%, SD=5.77) and 0.03ml oil/L (Mean=70%, SD=17.32). From there the mortality increased up to almost 100% (Mean=96.6, SD=5.77) at the oil loading of 0.1ml oil/L. At the oil loading of 0.3ml oil/L the mortality of *C. volutator* reached 100%.

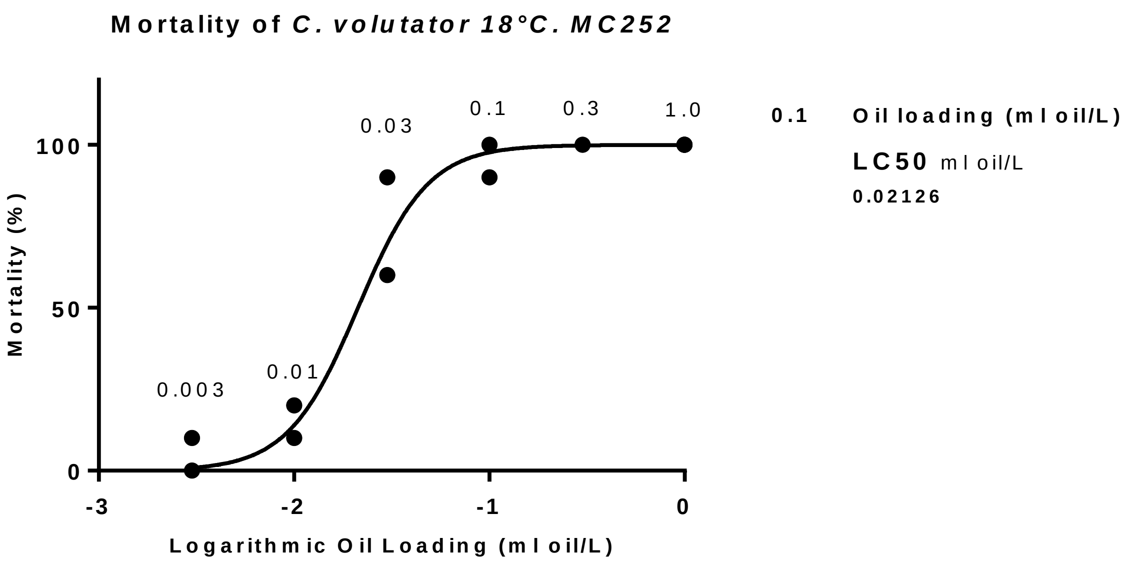


Figure 16. Dose response curve *C. volutator* exposed to MC252 at 18°C.

Figure 17 shows the dose response curve of *C. volutator* exposed to MC252 with Corexit9500 at 18°C on which the LC_{50} value was calculated. The LC_{50} value of this treatment was 0.02104 ml oil/L. At an oil loading of 0.0075 ml oil/L the mean mortality ratio was 26.67(SD=23.09). At the oil loading of 0.01ml oil/L the mortality increased to a mean of 43.33% (SD= 20.82). From there the mortality increased to a mean value of 56.67% (SD= 5.77) at the oil loading of 0.015ml oil/L. Within these 3 loadings the mortality increased the strongest relative to the logarithm scale. At the oil loading of 0.03ml oil/L the mean mortality reached 80% (SD=10). The 100% mortality was reached in this treatment with an oil loading of 0.1ml oil/L.

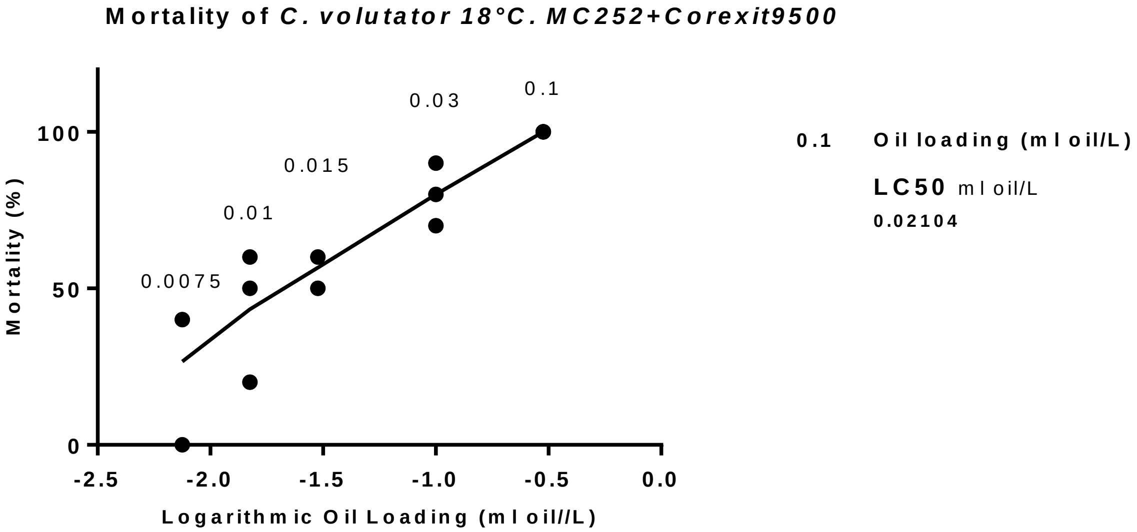


Figure 17.Dose response curve *C. volutator* exposed to MC252 with Corexit9500.

Figure 18 shows the dose response curve of *C. volutator* exposed to MC252 with EcoTec Oil Foam at 18°C on which the LC_{50} value was calculated. The LC_{50} value of this treatment was 0.05436ml oil/L. At the start oil loading of 0.01ml oil/L the mean mortality ratio was 20% (SD=10). From there to next oil loading of 0.03ml oil/L the mean mortality decreased to 10%(SD=10). Between the oil loading of 0.03ml oil/L and 0.1ml oil/L the mortality increased from 10% to 90%(SD=17.32). 100% mortality was reached at an oil loading of 0.3ml oil/L.

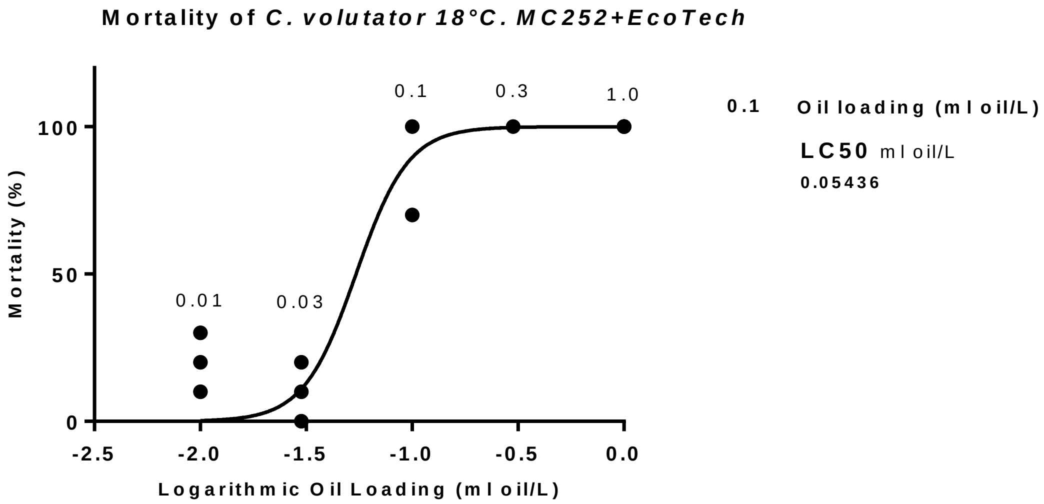


Figure 18.Dose response curve *C. volutator* exposed to MC252 with EcoTech Oil Foam.

Figure 19 shows all dose response curves of the experiments conducted at 18°C.Between mortality of the oil experiment and the mortality of oil treated with Corexit9500 experiment at 18°C, a significant difference was seen (Pearson Chi-Square Test, df=2, p=0.002). A significant difference was seen between the oil and the EcoTech treatment experiment, conducted at 18°C (Pearson Chi-Square Test, df=2, p=0.000). Comparing oil response methods, the mortality of the oil with Corexit9500 experiment differed from the mortality of the oil with EcoTech experiment. (Pearson Chi-Square Test, df=2, p=0.000).

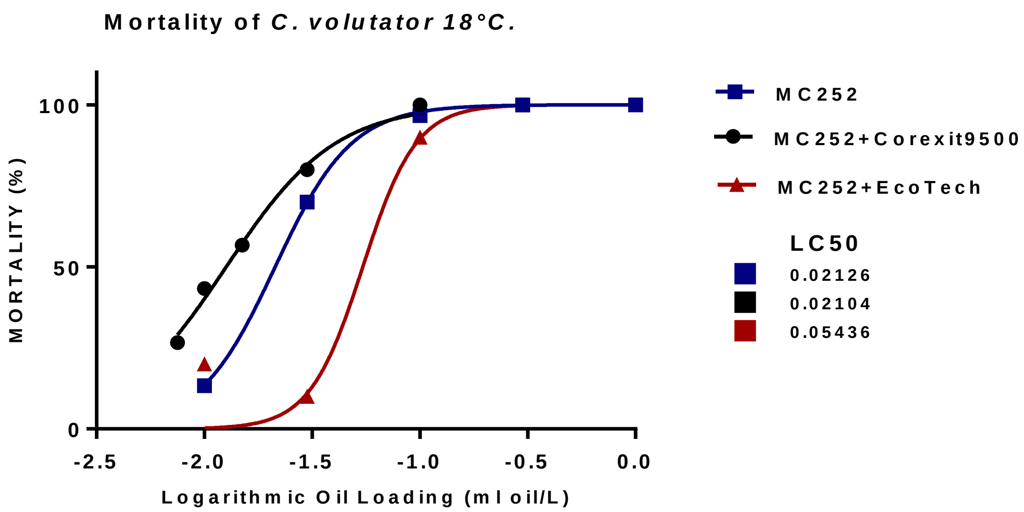


Figure 19. Dose response curve of *C. volutator* of all experiments conducted at 18°C.

Figure 20 shows the dose response curve of *C. volutator*, exposed to MC252 at 4°C on which the LC_{50} value was calculated. The LC_{50} (ml oil/L) of this treatment was 0.01351ml oil/L. Within this test the used oil loadings started at 0.01ml oil/L. At this loading no mortality was seen. At 0.03ml oil/L the mortality changed to 16.67% (SD=5.77). At an oil loading of 0.1ml oil/L, the highest mortality was observed. The highest mean mortality reached in this test is 53.33% (SD= 15.28). Towards higher oil loadings the mean mortality decreased to 13.33% (SD=15.28) at 0.3ml/L and 3.33% (SD= 5.77) mean mortality at the highest oil loading of 1.0ml oil/L.

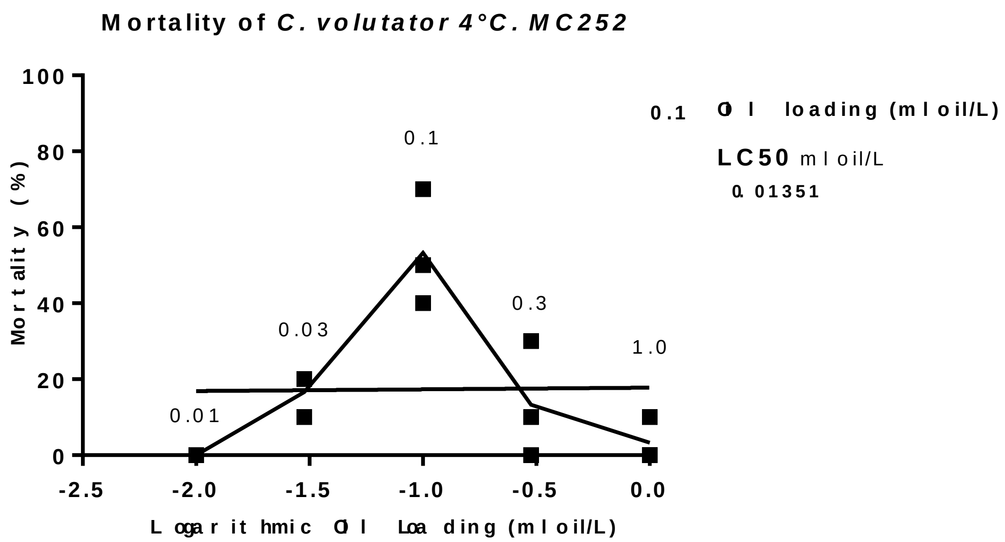


Figure 20. Dose response curves of *C. volutator* exposed to MC252 at 4°C.

Figure 21 shows the dose response curve of *C. volutator* exposed to MC252 with Corexit9500 at 4°C on which the LC_{50} value was calculated. The LC_{50} value of this treatment was 0.3949ml oil/L. The start oil loading within this test is 0.01ml oil/L. There the mean mortality was 3.33% (SD=5.77**).** At the following oil loading of 0.03ml oil/L the mortality raised towards 6.66% (SD=11.55). From the oil loading of 0.1ml oil/L the mean mortality increased from 20% (SD=0) towards a mean mortality of 26.67% (SD=25.17). The oil loading of 0.1ml oil/L resulted in a 100% mean mortality.

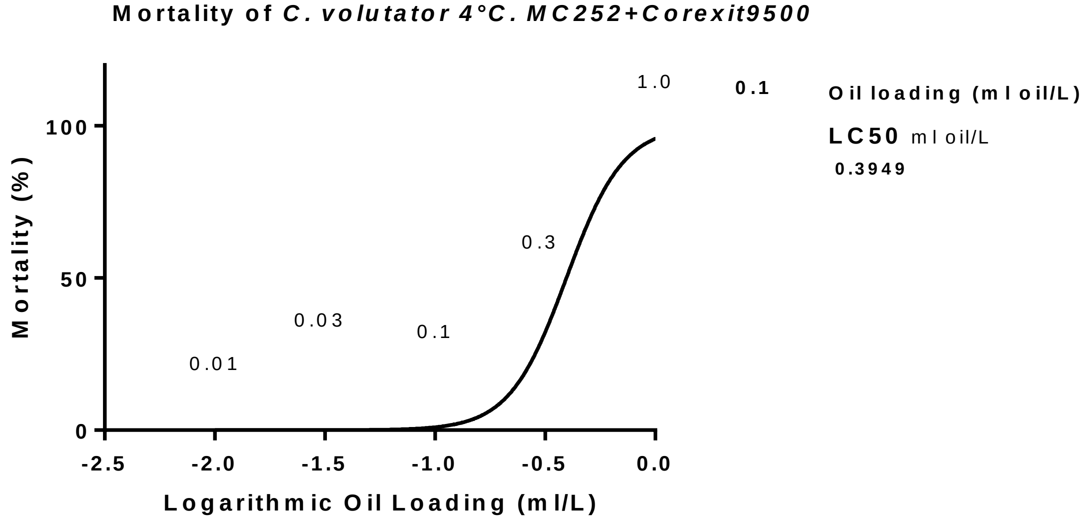


Figure 21. Dose response curves of *C. volutator* exposed to MC252 with Corexit9500 at 4°C.

Figure 22 shows the dose response curves of the experiments applying Corexit9500 at different temperatures. Looking at the different temperatures the mortality of MC252 with Corexit9500 at 4°C and 18°C differs (Pearson Chi-Square Test, df=2, p=0.000). The figure shows that the dose response curve of MC252 with Corexit9500 at 18°C is higher that the mortality curve of the 4°C experiment.

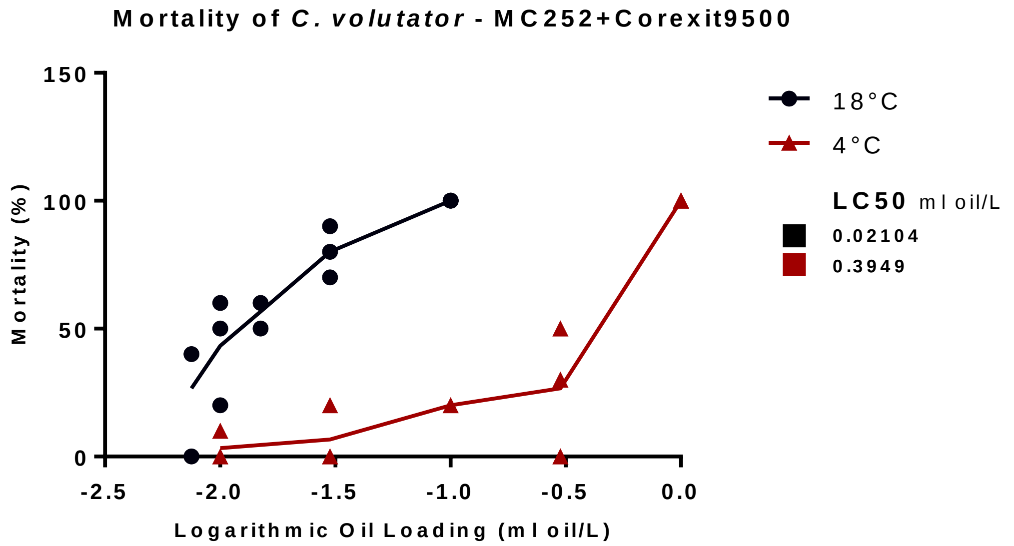


Figure 22. Dose response curve of *C. volutator* exposed toMC252 with Corexit9500 at different temperatures.

In table 2 all LC_{50} values are presented.

	MC252 (ml oil/L)	MC252+Corexit9500 (ml oil /L)	MC252+EcoTech (ml oil/L)
18°C	0.02126	0.02104	0.05436
4°C	0.13510	0.39490	

Table 2. LC_{50} values of all experiments.

The LC_{50} values of the 18°C experiment showed that a lower oil loading causes 50% mortality of the test population by using Corexit9500 compared to applying EcoTech. The LC_{50} value at 18°C was the highest at oil with EcoTech. The value was higher than the calculated LC_{50} value of the experiment using oil only.

3.3 *Phaeodactylum tricornutum* based growth inhibition test

Described in this paragraph are the results of the *P. tricornutum* based growth inhibition experiments. All experiments did not meet the validity criteria of the test. A linear logarithm line would indicate exponential growth. The exponential growth in the control group in the 18°C oil test never took place. Between day one and day two, see figure 22, the growth was higher than between day two and day three. The average growth ratio between day one and two of the control was 0.85 in this test. The results of analysing the validity criteria for all other experiments can be found in Appendix VI.

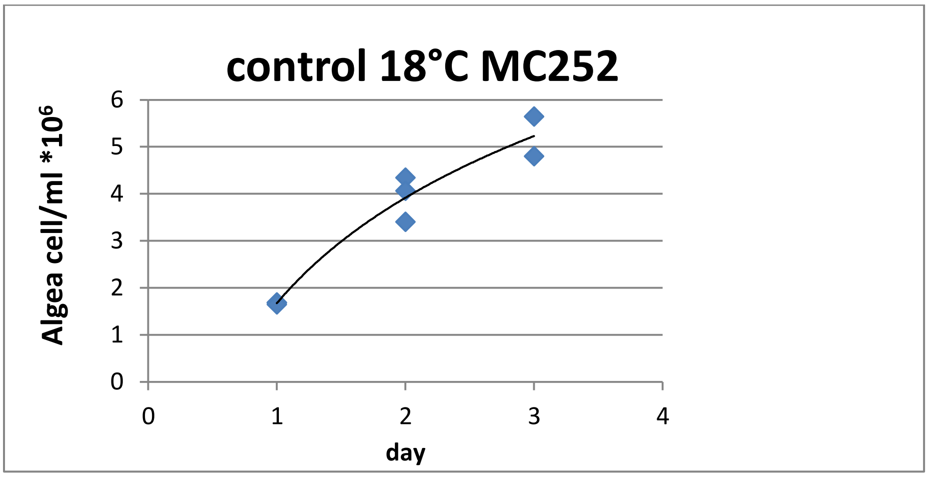


Figure 22. Growth curve of the controls with logarithmic trend line of the 18°C MC252 test.

In the following, the calculated growth inhibition ratios of all tests, based on day one and two are presented.

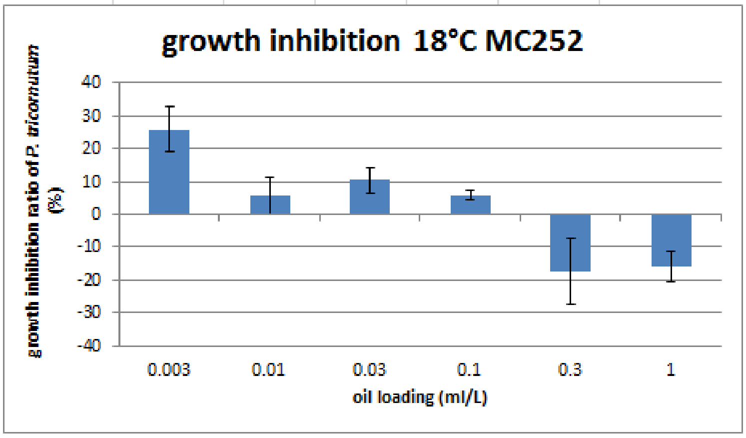
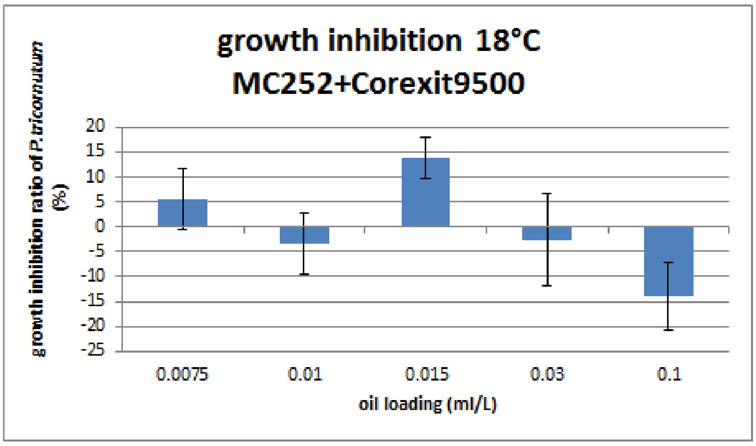


Figure 23. Average growth inhibition ratios of *P.tricornutum* with standard error of the 18°C MC525 experiment.

Figure 23 shows the calculated growth inhibition results of the experiment using MC252. Until the oil loading of 0.1ml oil/L a growth inhibition took place. At the two highest oil loading the growth ratio increased. Table 3 shows the growth inhibition values of *P. tricornutum* and the standard error of each oil loading.

oil loading (ml\L)	0.003	0.01	0.03	0.1	0.3	1
growth inhibition (%)	25.93	5.39	10.30	5.69	-17.39	-15.77
standard error (%)	7.00	5.63	4.08	1.43	10.18	4.68

Table 3. Average growth inhibition ratios of *P.tricornutum* with standard error of the 18°C MC525 experiment



In the test of using MC252 with Corexit9500, see figure 24, the inhibition ratio values vary from positive to negatives values among all different oil concentrations. The standard error is high in this experiment. Table 4 shows the growth inhibition values of *P. tricornutum* and the standard error of each oil loading.

Figure 24. Average growth inhibition ratios of *P. triconutum* with standard error of the 18°C MC252 with Corexit9500 experiment.

oil loading (ml\L)	0.0075	0.01	0.015	0.03	0.1
growth inhibition (%)	5.48	-3.42	13.77	-2.67	-13.93
standard error (%)	6.06	6.23	4.04	9.25	6.79

Table 4. Average growth inhibition ratios of *P. triconutum* with standard error of the 18°C MC252 with Corexit9500 experiment.

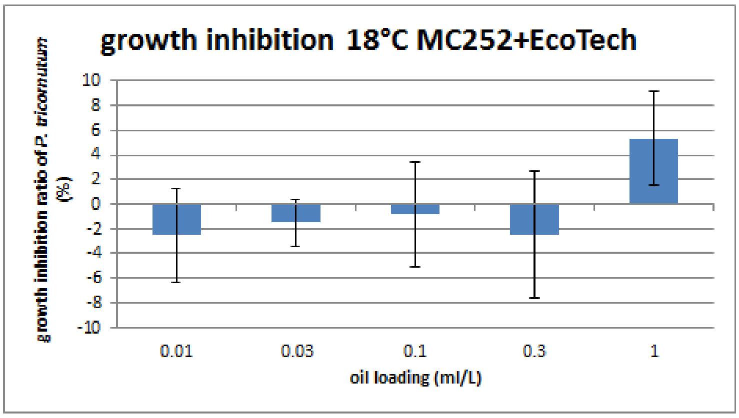


Figure 25 shows the results of using MC252 with EcoTech at 18°C. As to see on the negative growth inhibition values within this experiment, except from the highest oil loading, the algae growth ratio increased compared to the control. Table 5 shows the growth inhibition values of *P. tricornutum* and the standard error of each oil loading.

Figure 25. Average growth inhibition ratios of *P. tricornutum* with standard error of the 18°C MC252 with EcoTech experiment.

oil loading (ml\L)	0.01	0.03	0.1	0.3	1
growth inhibition (%)	-2.25	-1.53	-0.82	-2.49	5.32
standard error (%)	3.38	1.9	4.22	5.19	3.82

Table 5. Average growth inhibition ratios of *P. tricornutum* with standard error of the 18°C MC252 with EcoTech experiment.

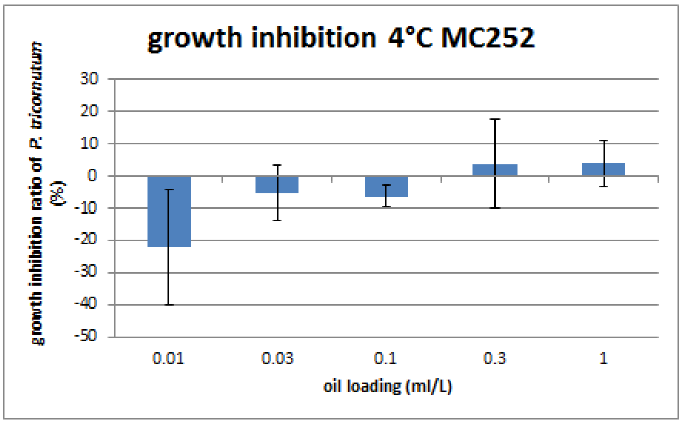


Figure 26. Average growth inhibition ratios of *P.tricornutum* with standard error of the 4°C MC252 experiment.

At the MC252 experiment at 4°C (figure 26) an average increase of growth ratio compared to the control took place using an oil loading of 0.01ml/L, 0.03ml/L and 0.1ml/L. The two highest oil loadings resulted in a growth inhibition compared to the control group. Table 6 shows the growth inhibition values of *P. tricornutum* and the standard error of each oil loading.

oil loading (ml\L)	0.01	0.03	0.1	0.3	1
growth inhibition (%)	-22.19	-5.25	-6.25	3.74	3.91
standard error (%)	18.12	8.5	3.49	13.92	7.30

Table 6. Average growth inhibition ratios of *P.tricornutum* with standard error of the 4°C MC252 experiment.

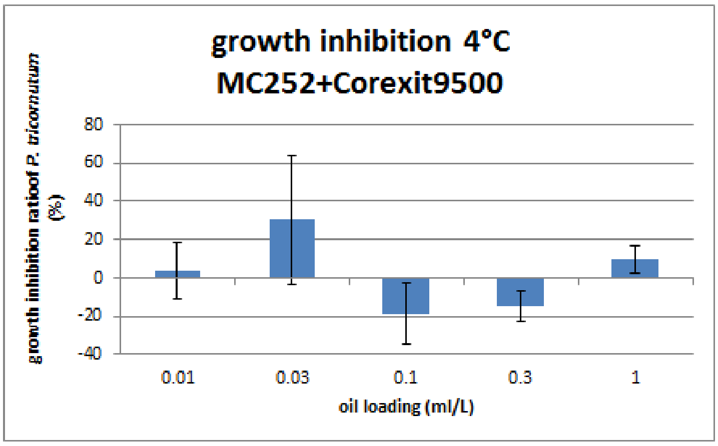


Figure 27. Average growth inhibition ratios with standard error of the 4°C MC252 with Corexit9500 experiment.

The growth inhibition test of the experiment of MC252 with Corexit9500 (figure 27) resulted in a fluctuation of positive and negative growth inhibition values along the oil loadings. Table 7 shows the growth inhibition values of *P. tricornutum* and the standard error of each oil loading.

oil loading (ml\L)	0.01	0.03	0.1	0.3	1
growth inhibition (%)	3.63	30.23	-18.77	-14.75	9.29
standard error (%)	15.02	33.83	16.01	7.80	7.08

Table 7. Average growth inhibition ratios with standard error of the 4°C MC252 with Corexit9500 experiment.

3.4 Issues involved concerning the practical applicability of the deployment of Corexit9500 and EcoTech Oil Foam in the Arctic

In this chapter the practical issues of applying Corexit9500 and Ecotech Oil Foam in the Arctic are described. Corexit9500 is applied globally as a response agent for oil spills.

Regarding the availability large quantities of Corexit9500 are already produced in stock as a precautionary measure, in case an oil spill occurs. EcoTech Oil Foam, however, is still in a test phase, therefore there are no large amounts available at the moment ("Personal communication Tinka Murk,2014").Further production would need time and financial investment. Another practical issue is the storage of the agents. Corexit9500 is a concentrated solution which can easily be stored in canister("Personal communication Wierd Koops,2014")s. EcoTech Oil Foam, on the other hand, is made of a highly porous structure and can be compared with a sponge. Thus, the storage of EcoTech would take up large amounts of space, which are not available at the moment. If EcoTech should be applied in the Arctic new storage room needed to be build("Personal communication Wierd Koops,2014"). This would come along with further expenditures. The method of application differs between the agents. Corexit9500 can be applied via a sprinkler system by a vessel or airplane and does not need any retrieval since it disperses together with the oil slick into the water column. Application by airplane allows a fast response to oil spills (National Research Council,2005). With regard to EcoTech, this advantage totally depends on the method of application("Personal communication Wierd Koops,2014"). If EcoTech is applied directly to an oil slick (e.g. contained in a permeable bag) it will absorb the present oil. In contrast to Corexit9500, EcoTech will have to be removed and disposed after absorbing the oil. Again, the storage capacity of vessels is a limiting factor. While clean EcoTech will mostly just consume space, EcoTech saturated with oil also comes with additional weight. When left floating in the ocean awaiting its transport to onshore disposal sites, EcoTech can be subject to degradation and could be releasing oil compounds and breaking up in smaller particles (in case of rough seas). Marine wildlife (e.g. seabirds, zooplankton, seals, fish) could mistake the filled absorbent for food which could have lethal results("Personal communication Wierd Koops,2014"). Applying the foam as filter within a skimmer system will avoid the previously described issues with EcoTech floating on the water. But it will lose its ability to become a fast response option, as the vessel operating the skimmer will have to navigate for a certain period before arrival on site. When the EcoTech in the skimmer is saturated it has to be stored on board which is again limited by the storage capacity of the vessel. The practical application of oil spill response agents is also regulated by legislation. As mentioned above, Corexit9500 is currently accepted by many nations as an effective response agent. Reviewing EcoTech as a possible new response method and reaching agreements between governments on its application would be a time-consuming process. This could cause the implementation of new OSRPs Arctic to take even longer than it does nowadays.

4. Discussion

In this chapter the results of this research and the applicability of the treatments in practice are discussed.

4.1 The dispersion behaviour of oil

The effect of temperature on chemical dispersion is researched in a lot of studies with different results. The research of Moles et al. (2002) stated that the chemical dispersion is dependent on temperature. The same is concluded in the research of Fingas et al. (2002)(Fingas, Bobra, & Velicogna, 1987). In contrast in the research of Ross (1997) temperature is not seen as a major factor influencing chemical dispersion behaviour. In the study of Mackay et al. (1980) a lower effectiveness of dispersant in lower temperature is based on the increase of oil viscosity. It has to be considered that all this studies were conducted with different oil types (Mackay & McAuliffe, 1988). In this research no significant difference in the behaviour of dispersed oil without dispersant at different temperatures was estimated. This can be based on the limitation of replicates. During analysis it has to be considered that a 2 dimensional model is used. That means droplets are taken into account which are seen from the camera side. The applied oil values are therefore not the absolute value of volume oil within the entire water column. Oil droplets of smaller volume blocked behind larger droplets are not measured. In addition, the software is not able to analyse droplets smaller than one pixel. Looking at the images a difference can be seen: Dispersion of bigger oil droplets at 4°C and dispersion of bigger oil droplets by using Helder oil compared to using MC252 oil. Measuring the viscosity of the oil, the results of the study confirm the findings of Mackay et al. (1997). The viscosity of MC252 changed from 15mPa·s (milipascal per second) at 18°C to 50mPa·s at 4°C. The viscosity of Helder oil changed from 250mPa·s at 18°C to 2500mPa·s at 4°C (*Personal communication Marieke Zeinstra, 2014*). The smaller change in viscosity of MC252 may explain why the mean values of the oil volume in the water column at different temperatures do not differ as much for this oil type as it does for the Helder oil test. The amount of different researches and results shows the complexity of dispersion behaviour of oil. Factors such wave energy, salinity, weathering process are factors influencing the dispersion behaviour next to the temperature (Chandrasekar, Sorial, & Weaver, 2006; Fingas et al., 1987; Moles et al., 2002; Wang, Zheng, & Lee, 2013).

4.2 *Corophium volutator* toxicity test

Acute toxicity experiments on *Corophium volutator* at 18°C indicate that a treatment of oil with the dispersant Corexit9500 is the most toxic. These results were expected since the dispersion of oil by Corexit9500 does increase toxic effect, as is shown in other research as well (Gulec, Leonard, & Holdway, 1996; Rico-Martínez, Snell, & Shearer, 2013;Hansen et al., 2014). Literature describing the residual toxicity of crude oil and oil treated with the methods applied in this research are scarce. Most research has a different set up, conducting experiments with contaminated sediments or different species of crustaceans. Comparing the values for oil treated with dispersants at 18°C of this research with other literature, it becomes clear that there are no toxicity experiments involving WAF based exposure to *C. volutator*. There is some literature related to this specific research (Chase et al., 2013; Finch et al., 2012; Roddie, Ashby-crane & Crane, 1994). Plenty of experiments have been conducted regarding the toxicity of MC252 to a wide variety of aquatic organisms. Gulec et al. (1996) described a study on the toxicity of an amphipod species *Allorchestes compressa*, exposed to dispersed oil with Corexit9500 (1:10 dispersant/oil ratio) using a 96 hour acute toxicity tests at 17°C. WAF of non-treated oil resulted in an LC50 of 0.311 ml/L. In this study, the WAF of non-treated oil had an LC_{50} of 0.02126 ml/L using *C. volutator*. Differences in LC_{50} can be caused by e.g. the type of oil applied in the tests and the specific sensitivity of the test organisms. WAF with Corexit9500 resulted in an LC_{50} of 0.0148 ml/L (Gulec, Leonard & Holdway, 1996), whereas this study with *C. volutator* exposed to chemically dispersed MC252 oil shows an LC_{50} of 0.02104 ml/l. Both studies indicate increased toxicity of oil after addition of dispersants at temperate temperatures (17-18°C). Similar research has been conducted concerning the toxicity of dispersants such as Corexit9500. Hansen et al. (2014), without the use of oil. Several species were exposed to different types of dispersant, amongst other Corexit9500. *Corophium volutator* was exposed to spiked sediment (only Corexit9500) at 15°C for 10 days resulting in LC_{50} (1.006 ml/l). In the same research, *Acartia tonsa* was exposed to WAF of Corexit9500 for 48 hours at 20°C, revealing a much lower LC_{50} value of 0.0065ml/L (Hansen et al., 2014). During the research of Hansen et al. (2014), the toxicity test of *C. volutator* was conducted with treated sediment. In the entire research it was proven that *A. tonsa* was the most sensitive species of the test organisms (Hansen et al., 2014). Difference in sensitivity can be caused by e.g. size dependency (*tonsa* 1mm vs *Corophium* ≥5mm) and exposure route (sediment versus water exposure). During the experiment, *Corophium volutator* proved most sensitive to the dispersant; Gamlen OD4000 (LC_{50}: 0.140ml/L), Corexit9500 was therefore not the most toxic dispersant for *C. volutator*. As no oil was used in the experiments of Hansen et al (2014) and exposure was conducted via sediment, we cannot directly compare our results to this study. None of these types of research are dedicated to the effects of residual toxicity in low temperature water (4°C) or the exposure of *C. volutator* in water based toxicity tests with oil. There is a lack of knowledge concerning the toxicity of oil and dispersant at low water temperatures. It proved difficult to compare results of this research with relevant literature. A study of Campo et al., (2013) describes the biodegradability of oil and dispersant at 5°C and 25°C. He concludes that oil treated with dispersant at 25°C biodegrades more successfully compared to chemical dispersion at 5°C. In short: Toxic oil components biodegrade more slowly at low temperatures compared to high temperatures. Meaning that the addition of dispersant at low temperatures does not enhance the biodegrading of oil compounds (Campo et al., 2013). In our research, organisms were exposed for 6 days. It might be possible that a longer exposure period (longer than 6 days) could result in a higher toxicity. Compounds are released more slowly and dissolve more slowly. This could explain why dispersed oil proved less toxic at 4°C than at 18°C. However, more research is required to support this assumption. To conclude, it is difficult to compare the results of this research with other literature. LC_{50} values of non-treated oil in water exposure studies are difficult to compare to our LC_{50} value of exposure of *C. volutator* to non-treated oil at 18C. Literature supports our finding of an increasing residual toxicity of oil after application of dispersants. No studies on the toxicity of oil and treated at low temperatures were found in literature.

4.3 *Phaeodactylum tricornutum* growth inhibition test

The response of *Phaeodactylum tricornutum* to petroleum contamination has been investigated in several studies. In some reports *P. tricornutum* was found to be relatively unsensitive to soluble petroleum compounds and naphthalene (Kusk, 1981; ØStgaard, Eide, & Jensen, 1982). In contrast, in other reports the species was found to be sensitive to the studied fuel oil (Bate & Crafford, 1985; Siron et al., 1991). Whether the species is sensitive for oil loadings within this study is questionable due to high standard error values and the fluctuation in growth increase and growth decrease among the oil loadings using MC252 with Corexit9500 at different temperatures. Growth inhibition at higher oil loadings and stimulation of growth at low oil loadings is common in former studies, not only based on this specific species(Lewis & Pryor, 2013; Siron et al., 1991). The experiments of this research with MC252 with EcoTech and the MC252 at 4°C confirm these findings. However, results of the oil experiments at 18°C show the opposite, with an increase in algal growth at higher oil loadings. Comparing the experiments of treated MC252 with Corexit9500 with the other experiments it seems that using Corexit9500 influenced the growth rate. A decrease in growth rate can be seen in the tests with Corexit9500. Acute toxicity of crude oil is mainly based on the volatile water-soluble fractions. By preparing a WAF these compounds shall be largely dissolved in water. While using dispersants small oil droplets occur in the water column as well. It can be assumed that, next to the water soluble fraction, medium-weight and heavy-weight compounds, also present in the water column, increase the toxicity. Oil droplets in the water column can also influence the algal growth by direct contact or by limiting the light availability. The fluctuation among the different oil loading can be explained by variations in the presence of oil droplets in the water during WAF preparation. No significant difference in growth rate at different oil loadings compared to the control could be observed. This may be due to the fact that the exponential growth phase was not reached. The growth inhibition results depend on the growth phase (M. Lewis & Pryor, 2013; Siron et al., 1991) although Siron et al. (2013) shows that growth inhibition can also be observed without exponential growth. It is also possible that the effect concentration was not reached yet and that higher oil loading may be necessary to establish a significant effect in the *P. tricornutum* test. The results of the *P. tricornutum* growth inhibition test do not show a specific trend according to the oil loadings, treatment or temperature. This is might be due to the chosen WAF preparation. Instead of using a dilution range based on one WAF, each extract was taken from a new WAF. The components of the WAF extract may not be identical between WAFs. Analysing the components of the WAF extract would give a further insight into this.

4.4 Experimental limitations of toxicity tests

The double flask set-up as applied during this study comes along with some advantages and disadvantages compared to other studies. For each replicate a new WAF was prepared instead of using one WAF and creating different oil loadings by dilution of this WAF as used in the most studies (Couillard et al., 2005; Jiang et al., 2012). Variation of the initial oil loading can occur due to an error in pipetting. The use of different stir plates and fluctuations in vortex also increase the possibility of toxicity variation among the bottles. The relation of toxic compounds in the water column and the oil loadings is influenced by the variation of vortex (Couillard et al., 2005). In literature WAF based experiments always allowed settlement for several hours of the WAF after mixing(Couillard et al., 2005; Hansen et al., 2012; Jiang et al., 2012; Scarlett et al., 2007). This way, direct exposure of oil to test organisms is avoided as non-dispersed oil droplets can cause unintended mortality due to suffocation. The WAF extract for the *P. tricornutum* growth inhibition test were taken while circulation of the water flow continued inside the double flasks. Not only water soluble components may have been sampled. In some cases an oil layer occurred in the mesh flask and WAF extracts had to be taken through this layer. In the *P. tricornutum* growth inhibition test the test organisms were exposed to the toxic compounds which were present in the water column at the moment of extraction. The probability of variance among the flasks increased. To overcome evaporation of volatile toxic compounds the flasks were kept airtight. But that means that a CO_2 mass transfer from air to water was limited (Hailing-Sørensen, Nyhohn, & Baun, 1996). The algae could only consume the amount of CO_2 provided by the air within the test flask. Therefore it is most likely that CO_2 was a limiting factor for optimal growth in this test. A limitation of CO_2 would also result in a shift of the pH value. However, this assumption cannot be confirmed or rejected since pH values were not measured. *C. volutator* was put into the mesh avoiding direct oil contact as much as possible. However, water circulation took place during the whole test period and an oil layer in the mesh flask could not always be prevented. The mortality due to oil contact cannot be excluded in this case. The same can be stated for the mortality due to O_2 limitation since measurements of O_2 were only conducted at the control group. No experiments were conducted with EcoTech at 4°C. At the 18°C test circulating EcoTech cushions for 6 days did create several uncertainties. It is not known if toxic components were released from the EcoTech or nylon material itself, nor is it known if oil is releasing toxicants once trapped in the EcoTech. In pre-tests it was concluded that oil passed through the nylon successfully, it is however unknown to what rate oil was absorbed by the nylon itself.

4.5 Issues involved concerning the practical applicability of the deployment of Corexit9500 and EcoTech Oil Foam in the Arctic

Little research is available concerning the infrastructure present in the Arctic. It is however clear that the Arctic is remote and difficult to access. Regarding the current issues for ship movement and activities in the region relevant literature is known. The available information on oil spill response agents, however, is much more limited. Thus information had to be gathered from various sources, including interviews with individuals involved in the subject. After this desk study, a better image of the situation could be acquired regarding the practical applicability of the two oil response agents in the Arctic. It is in the benefit of this research that Corexit9500 is a widely accepted oil spill response agent worldwide. Thus, some information is available on its working, deployment and storage. The major issue is related to the use of EcoTech Oil Foam, as this new agent is mostly unknown in the sector as a possible response method and therefore only contributes to the rising amount of uncertainties concerning this agent. It always has to be considered that the most issues concerning EcoTech were studied due to own experience during the conducted toxicity experiments and further discussions with experts and are not scientifically proven.

5. Conclusion

This chapter answers the sub-questions, which combined form the answer to the main question of this research: *Can Corexit9500 and EcoTech Oil Foam be recommended as response agents for combating offshore oil spills in low water temperatures?*

Dispersion behaviour of oil

Naturally dispersed Helder oil at 18°C disperses less successfully compared to MC252. Dispersion with a 1:100 dispersant/oil ratio of Corexit9500 results in smaller particles at both types of oil, MC252 is dispersed most successful. At 4°C, the natural dispersion is less successful at both oil types. Chemical dispersion with Corexit9500 at 4°C results in a better dispersion compared to natural dispersion at 4°C. The dispersed oil droplets tend to form flocks and accumulate at the surface, especially with Helder oil. More Corexit9500 is required for the same dispersion as it was at 18°C. Thus, natural and chemical dispersion are less effective at 4°C compared to 18°C.

The difference of LC_{50} of C*orophium volutator* after treating oil slicks with Corexit9500 and EcoTech Oil Foam in temperate water temperature (18°C) and Arctic water temperature (4°C)

At 18°C, the LC_{50} of oil treated with Corexit9500 decreased, resulting in an increased toxicity. The LC_{50} of oil treated with EcoTech Oil Foam is high compared to dispersant, indicating a lower toxicity. Solely oil has an intermediate LC_{50} the toxic range is in between Corexit9500 and EcoTech. At 4°C, Corexit9500 shows an increased LC_{50} and lower toxicity. EcoTech was not measured at 4°C, oil alone provided ambiguous data. It is assumed the lesser rate of natural dispersion of oil due to EcoTech results in a higher LC_{50}.

The change in growth ratio of *Phaeodactylum tricornutum* after treating oil slicks with Corexit9500 and EcoTech Oil Foam in a temperate water temperature (18°C) and Arctic water temperature (4°C)

The *Phaeodactylum tricornutum* based growth inhibition experiments provided ambiguous data. It seemed the diatom species was not sensitive to the exposed WAF concentrations. It is possible that WAF concentrations were too low to induce significant effects on the growing diatom cultures. It is impossible to state any conclusions concerning the change in growth ratio of *P. tricornutum* after treating oil slicks with Corexit9500 and EcoTech Oil Foam from this research. It is questionable if the WAF extract was applied in an appropriate method.

Issues involved concerning the practical applicability of the deployment of Corexit9500 and EcoTech Oil Foam in the Arctic

Since the best method of application of EcoTech is not yet determined, it is very hard to predict the practical applicability of EcoTech in the Arctic. Currently, too many uncertainties exist to clearly define whether or not EcoTech is a suitable solution. At the moment it seems that EcoTech needs further development to be an effective system or device. Corexit9500 on the other hand is already widely known worldwide. The method to apply the agent is relatively quick (by plane) and does not involve any retrieval as it is the case with EcoTech. Therefore, Corexit9500 is a more preferable method since it requires minor effort (only the deployment is needed). To conclude, it is clear that EcoTech Oil Foam is effective in the absorption of oil. On the other hand, the deployment and methods to do so are still undefined and are expected to involve more logistic difficulties compared to Corexit9500. From a logistical point of view, Corexit9500 is currently recommended as oil spill response method in the Arctic. This recommendation could however change, in case the method to deploy EcoTech Oil Foam is further developed.

The feasibility of Corexit9500 and EcoTech Oil Foam as response agents for combating offshore oil spills in low water temperatures

EcoTech Oil Foam can be a recommendable oil spill response agent if the method of application is determined. If applied effectively, EcoTech has the capability to reduce the toxicity of an oil slick by absorbing the oil and removing it from the marine environment. Until the exact method of usage is determined it will be difficult to state whether or not EcoTech is an efficient oil spill response agent. From the environmental point of view, EcoTech Oil Foam is the more effective option. It removes the oil by absorbing it, instead of spreading it further in the water column as dispersant does, adding toxic concentrations to the environment. From the logistical point of view, Corexit9500 is the more recommendable oil spill response agent at the moment.

The storage, transportation and applicability are more suitable with the currently existing equipment available. From the ecological perspective, Corexit9500 would be the least recommendable option. The residual toxicity decreases with lower water temperature, a higher oil:dispersant ratio needed in colder water would compensate the temperature based decrease of toxicity. At the moment Corexit9500 is a more recommendable method, regarding the logistical and technical applicability. EcoTech Oil Foam, despite being less practical, is a more environmentally friendly option. If the practical applicability of EcoTech is enhanced, it would be the better option.

6. Recommendations

The dispersing behaviour of naturally and chemically dispersed oil slicks are recorded at temperate and low water temperatures. For further research it would be useful to make use of software that is capable of monitoring the actually dispersed droplet size and using a 3 dimensional model. This would avoid the chance of predicting the behaviour of dispersed oil by solely visual monitoring. Furthermore, several procedures for the production of WAF exist. During this research adaptions had to be made since the double flasks made it impossible to follow the protocols exactly. The double flasks did enable exposure of *C. volutator* to a direct flow of toxicants derived from the oil slick in the adjacent flask. On the other hand, it did prove difficult to extract WAF for the growth inhibition experiment since oil reached the second flask, contaminating the extract directly. To conclude whether extracts were contaminated with crude oil a chemical analysis should be conducted. The direct contact of oil could also affect results for the acute toxicity experiments. However the effect of this direct contamination has not been observed yet. It proved impossible to contain the oil loadings with dispersant into the first bottle, dispersant literally dispersed oil into the entire water column, blurring both bottle with very fine oil droplets. For following research a pre-mixed WAF could be made in advance of exposure in the acute toxicity experiment. By doing so the possible direct contact could be avoided. In most literature, *C. volutator* is exposed to spiked or treated sediment. The organisms remain in the sediment and after the test period the survival is being monitored. This way the natural conditions of *C. volutator* are closely mimicked. During this research, *C. volutator* was placed in a steel mesh cylinder enabling exposure to the water flow. The test organisms are not able to cover themselves in sediment and are fully exposed. Future research could investigate if the test organisms provide different results when applied in sediment based toxicity experiment. Otherwise it might be possible to conduct experiments with a similar setup, only with different crustacean species instead of only one. During this research test organisms for the acute toxicity experiments were derived from a mudflat and selected by size and activity. The chance exists of variation of sensitivity between organisms. Organisms could be winter survivors or young brood that hatched in early spring. Culturing own test organisms under controlled conditions could delineate any variance in age and development of the test organisms. Difference in test temperature could affect test organisms even without effect of the toxicant. To avoid cold or heat shock 24 hours of temperature adaption were applied. It remains well possible that organisms are not used to a sudden change in temperature, possibly affecting the test results. Again the culturing of test species at a set temperature would be a suggestion. To test the toxicity of oil response methods in the Arctic, it is suggested to apply indigenous Arctic organisms. *C. volutator* is a widespread crustacean and is found in north Norway as well. In this case, it remains a low Arctic species; more northern species could be suitable for this research. The growth inhibition experiment provided knowledge concerning the effectiveness of the test setup and use of *P. tricornutum* as test species. For future research it might be suitable to produce a WAF extract with higher oil concentrations since the WAF applied in this research proved to have no impact on the rate of growth. Other species which are generally more sensitive could be applied as well. Other variables could have an impact on test results too, such as, light availability, growth medium and circulation. Remarkable is the fact that none of the control indicated exponential growth during the test period; this indicates that there is a lack of nutrients or abiotic factors. Since growth medium was added to every concentration it is unlikely that nutrients were the limiting factor in the experiments. It is for example more likely that a lack of Co2 occurred, limiting the ability for *P. tricornutum* to grow exponentially. The occurrence of possible limitations needs to be kept in mind for future research. Abiotic factors were not measured during this research.

This is due to the threat of oil contamination of equipment. It is stated in literature and previous research that factors such as: temperature, salinity, PH-value and oxygen level always need to be documented. The importance of this knowledge exceeds the threat of oil contamination to measuring equipment. Furthermore, it might be beneficial to conduct the growth inhibition and acute toxicity experiments with one standard WAF. Making a concentration range based on the initial WAF would limit variation between the different exposure mediums. This is a much applied method in previous research, and proved successfully for the types of experiments similar to this research.

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Appendix I Plunge test

In this part of the research the efficiency of the dispersant Corexit9500is determined. Especially the difference of efficiency in arctic water in comparison to the efficiency in temperate water is researched. Water temperatures of 4°C and 18°C are used. In addition two types of oil are used to determine the difference of efficiency by occurring oil with high viscosity and low viscosity. The oil with high viscosity in this study is represented by Helder oil which is from the North Sea. The oil with less viscosity is Mississippi Canyon Block 252 oil (MC 252) which is from the Gulf of Mexico. The oil layer thickness in this experiment is 400 µm. This part of the research is done in cooperation with the PhD student Marieke Zeinstra in the laboratory of the Water Application Centre in Leeuwarden.

Experimental setup and structure

The experimental setup, called plunging jet system (see fig.2) is created by Marieke Zeinstra. It consists of aluminium rig, a rectangle glass tank(30cm*10cm wide and 35cm high) with extraction points, a small triangular glass plunge container (4cm wide and a capacity of 300ml), 2 speed cameras and computer system called Visionlab, a background illumination device and an overflow system. For the 4°C tests additionally a thermometer and thermal packs are used.

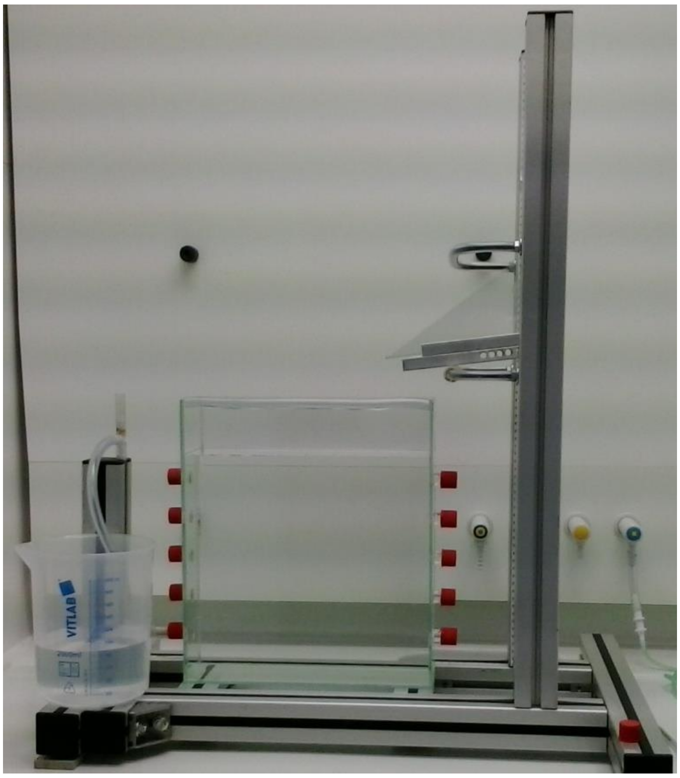


Figure 1. Plunging jet system

The glass tank is filled with filtered artificial seawater. The overflow system assures that the level of water remains constant at 30cm high while the outflowing water is collected outside the tank. A plunge container, which is able to flip over, is fixed at 15cm above the glass tank. This plunge container is filled with overflowed seawater from the tank. When filled with water, the container can tilt towards the glass tank and pour the water into the tank, simulating a plunging breaking wave. In front of the tank two cameras are installed. These cameras are connected to the plunge container by a switch, triggering the photographic sequence as the plunge container falls. To make the contrast in the picture higher between water and oil a lamp is placed behind the glass tank.

variables	Helder oil	MC 252
18°C		
4°C		

Table 1. variables

Standard procedure – Plunging test with oil at 18°C

1) Artificial Seawater with conductivity of 41-42 MS/cm at 25°C is made by adding 130mg salt to 4l demi water while rotating in a plastic measuring pitcher on a magnetic stirrer. In total 12L artificial seawater is prepared and stored in a 25l plastic can. Afterwards the salinity is measured with a conductivity measuring device and if necessary the salinity is adjusted by adding demi water or salt directly to the can according to the following correction formula of Marieke Zeinstra:

+ 0.5mS/cm: add salt 0.38 g/l artificial seawater

- 0.5mS/cm: add demi water 12.68 ml/l artificial seawater.

The prepared seawater is aerated overnight .

2)On the test day the conductivity of the artificial seawater is tested and if necessary adjusted as described before. The glass tank is placed on the installation. The overflow is fixed at the installation and a 500ml Erlenmeyer is placed under the overflow end. The cold seawater is filtered through a 50µm mesh sized sieve and filled into the glass tank until the overflow mechanism starts. A thermometer is placed and fixed in a corner of the glass tank. The water level and the plunge hight is measured. Air bubbles seen at the glass are removed by moving a ruler along the glass. 15ml oil is stored in 25ml Erlenmeyer and closed with a glass cap. For chemical dispersion, a mixture 150µl Corexit9500is stored in a 15ml plastic tube using reverse wise a pipette and a tip of 2-200µl volume range. In total 15ml (5*3ml) oil is added to the plastic tube using reverse wise a pipette and a pipette tip of 1000-5000µl volume range. The mixture is shaken by hand. The lamp is turned on.

3)Oil is added to the water surface in the tank by using a pipette reverse wise with a pipette tip of 1000-5000µl volume range. For a layer thickness of 400µm 4*3ml oil is added drop wise and widespread on the water surface. Waiting for 10 minutes for homogenisation of the oil layer. The plunge container is lifted and filled with 100ml filtered of seawater which was measured in a 100ml measuring cylinder. After the 10 minutes a photo of the oil layer is taken from above. The plunge container is released. Photos are taken automatically. The tank is cleaned by sweeping the entire water surface with an oil only absorbent sheet which has the width of the tank. With a new sheet the tank sides are cleaned. Re-wiping of the water surface is done until no new oil can be seen on the sheet. Afterwards the experiment is repeated from Paragraph 3 by adding the oil dispersant mixture which has been shaken before until the mixture is homogeny distributed.

Standard procedure – Plunging test with oil at 4°C

Two 5l plastic cans are stored with the artificial seawater and shaken shortly. 15ml oil is poured in 25ml Erlenmeyer bulb and closed with a glass cap. For the oil dispersion mixture 150µl Corexit9500 is filled in a 15ml plastic tube using reverse wise a pipette and a tip of 2-200µl volume range. In total 15ml (5*3ml) oil is added to the plastic tube using reverse wise a pipette and a pipette tip of 1000-5000µl volume range. The mixture is shaken by hand. The two 5l plastic cans with artificial seawater, the Erlenmeyer bulb with oil, the oil dispersant mixture and the glass tank including extraction installation is stored overnight in a fridge at 1.5°C. Additionally five thermal packs are stored in a freezer.

2)On the test day the conductivity of the artificial seawater is tested and if necessary adjusted as described before. The glass tank is placed on the installation. The overflow is fixed at the installation and a 500ml Erlenmeyer bulb is placed under the overflow end. The cold seawater is filtered through a 50µm mesh sized sieve and filled into the glass tank until the overflow mechanism starts. A thermometer is placed and fixed in a corner of the glass tank. The water level and the plunge high is measured. Air bubbles seen at the glass are removed by moving a ruler along the glass. Thermal packs are settle down as much as possible in front of the glass tank.

3)Oil is added to the water surface in the tank by using a pipette reverse wise with a pipette tip of 1000-5000µl volume range. For a layer thickness of 400µm 4*3ml oil is added drop wise and widespread on the water surface. Waiting for 10 minutes for homogenisation of the oil layer. The plunge container is tilt up and filled with 100ml filtered cold seawater which was measured in a 100ml measuring cylinder. After the 10 minutes thermal packs are removed and the sides of the tanks are dried with a towel. The lamp is turned on and a photo of the oil layer is taken from above. The plunge container is released. Photos are taken automatically. The thermal pack are settled down again. The tank is cleaned by sweeping the entire water surface with an oil only absorbent sheet which has the width of the tank. With a new sheet the tank sides are cleaned. Re-wiping of the water surface is done until no new oil can be seen on the sheet. Afterwards the experiment is repeated from Paragraph 3 by adding the cold oil dispersant mixture which has been shaken before until the mixture is homogeny distributed.

Appendix II WAF protocol

Protocol of Water Accommodated Fraction preparation and Liquid-Liquid Extraction

Materials

Aqua Holland artificial salt
Weigh balance
Schott Duran glass bottle 1 liter
Magnetic stirrer and stir bars
Salinity meter
1 L or 500 mL glass aspirator flask with sidearm at bottom
125mL serum bottles
Viton stoppers and crimp caps
Glass pasteur pipettes
Cotton
1.5 mL screw top GC vials
Micro-vials
Screw top Micro-vials

Chemicals
Hexane
Diethyl ether
Na_2SO_4
Demi Water
Nitrogen gas
DMSO

Note when working with oil:

- **Avoid using plastics.**
- **Use glass containers and glass pipettes.**
- **Avoid sunlight as much as possible, store everything in the dark (covered with for example aluminum foil).**
- **Work in a fume hood.**

Methods

1. *Artificial sea water (ASW) preparation*

1.1 Add 1 liter demi water to a clean and dry Schott Duran glass bottle.

1.2 Weigh 32g Aqua Holland artificial salt (synthetic sea salt) and add to Schott Duran glass bottle.

1.3 Stir the solution by using magnetic stirrer to make a homogenous solution.

1.4 Check the salinity, this should be 31±1 ‰ or 49 ±2 mS/cm.

1.5 Store the prepared ASW in the refrigerator.

2. *Water Accommodated Fraction (WAF) preparation*

2.1 Prepare 1 L or 500 mL glass aspirator flask, connect the sidearm of flask with a glass tube and close off with rotary switch (Fig 1).

2.2 Add crude oil and artificial sea water into the flask at a ratio of 1:9 oil:water.

2.3 Stir mixture by using magnetic stir plate with zero vortex for 24 hours at ambient room temperature. The flask needs to be covered with aluminum foil during the stirring period to minimize photooxidation of the solutions.

2.4 WAF solution can be drawn from the bottom of the container through rotary switch.

2.5 WAF solution can be stored with aluminum foil cover in refrigeration within one week.

3. Liquid-liquid extraction(LLE)

3.1 Dry the Na_2SO_4 powder in an oven at 500 degrees for three hours before beginning.

3.2 Blend 5% Diethyl ether (DEE) and 95% Hexane.

3.3 Add 100 ml WAF solution in 125ml serum bottle.

3.4 Add 10 ml Hx:DEE mixture to the serum bottle and close with Viton stopper and crimp cap. Manually shake the bottle rigorously for 10 minutes (Fig 2).

3.5 Allow the bottom phase to settle and collect top liquid phase with glass pipette (Fig 3).

3.6 Repeat 3.4 and 3.5 three times and totally collect 30 ml mixture solution.

3.7 Fill a glass pasteur pipette with cotton and dried Na_2SO_4 (from 3.1) (Fig 4). 30 ml mixture needs about 5 or 6 glass pipettes columns.

3.8 Final dried solution can be measured in GC-FID for Total Petroleum Hydrocarbons or transferred to DMSO for bioassays (see 4). The extract can be stored in the refrigerator covered with aluminum foil, use within one week.

4. Concentrate Extracts

4.1 If TPH concentration of the extract is not high enough compared to expectation then the extract should be concentrated.

4.2 Using nitrogen gas, very gently evaporate extract solution in graduated cylinder (easier to know evaporation volume) until ~ 1 ml is left (Fig 5).

4.3 Use a glass pipette to transfer the last 1 ml of the extract into a micro-vial (Fig 6) or other smaller glass container and continue to evaporate the extract until it is nearly dry, then add 100-500 ul DMSO (depends on preferred final concentration). Then, evaporate the remaining hexane.

4.4 The DMSO stocks should be stored in the dark at room temperature (Don’t put this stock in refrigerator, otherwise it will freeze).

Figure 1 glass aspirator flask connected with glass tube and closed off with rotary switch

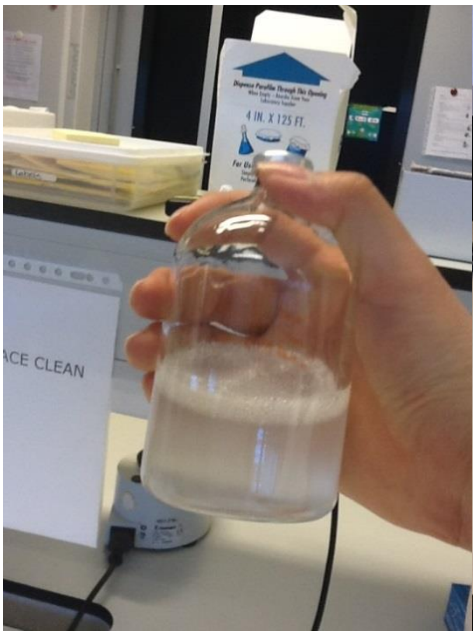


Figure 2 manually shake serum bottle Figure 3 settle the bottom phase

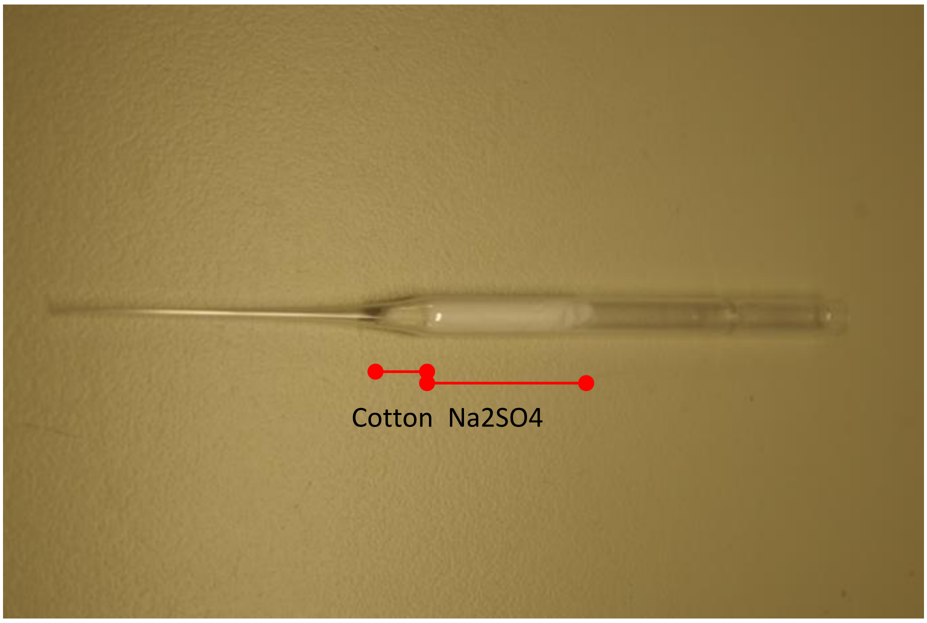


Figure 4 glass pasteur pipette with cotton and dried Na_2SO_4

Figure 5 Nitrogen gas for evaporation   Figure 6 micro-vial

Appendix III ISO Standard procedure

Appendix IV SPSS outputs *C. volutator* toxicity test

Cross tabulation and Pearsons Chi-Square test between references

Zusammenfassung der Fallverarbeitung

	Fälle					
	Gültig		Fehlend		Gesamtsumme	
	H	Prozent	H	Prozent	H	Prozent
method * replicate	390	100,0%	0	0,0%	390	100,0%

Kreuztabelle method*replicate

Anzahl

		replicate			Gesamtsumme
		1,00	2,00	3,00	
method	18disp	50	50	80	180
	18oil	60	60	90	210
Gesamtsumme		110	110	170	390

Chi-Quadrat-Tests

	Wert	df	Asymp. Sig. (zweiseitig)
Pearson-Chi-Quadrat	,099[a]	2	,952
Likelihood-Quotient	,099	2	,952
Anzahl der gültigen Fälle	390		

a. 0 Zellen (0,0%) haben die erwartete Anzahl von weniger als 5. Die erwartete Mindestanzahl ist 50,77.

Zusammenfassung der Fallverarbeitung

	Fälle					
	Gültig		Fehlend		Gesamtsumme	
	H	Prozent	H	Prozent	H	Prozent
method * replicate	340	100,0%	0	0,0%	340	100,0%

Kreuztabelle method*replicate

Anzahl

		replicate			Gesamtsumme
		1,00	2,00	3,00	
method	18Ecotec	40	40	50	130
	18oil	60	60	90	210
Gesamtsumme		100	100	140	340

Chi-Quadrat-Tests

	Wert	df	Asymp. Sig. (zweiseitig)
Pearson-Chi-Quadrat	,641[a]	2	,726
Likelihood-Quotient	,642	2	,725
Anzahl der gültigen Fälle	340		

a. 0 Zellen (0,0%) haben die erwartete Anzahl von weniger als 5. Die erwartete Mindestanzahl ist 38,24.

Zusammenfassung der Fallverarbeitung

	Fälle					
	Gültig		Fehlend		Gesamtsumme	
	H	Prozent	H	Prozent	H	Prozent
method * replicate	310	100,0%	0	0,0%	310	100,0%

Kreuztabelle method*replicate

Anzahl

		replicate			Gesamtsumme
		1,00	2,00	3,00	
method	18disp	50	50	80	180
	18Ecotec	40	40	50	130
Gesamtsumme		90	90	130	310

Chi-Quadrat-Tests

	Wert	df	Asymp. Sig. (zweiseitig)
Pearson-Chi-Quadrat	1,110[a]	2	,574
Likelihood-Quotient	1,113	2	,573
Anzahl der gültigen Fälle	310		

a. 0 Zellen (0,0%) haben die erwartete Anzahl von weniger als 5. Die erwartete Mindestanzahl ist 37,74.

Zusammenfassung der Fallverarbeitung

	Fälle					
	Gültig		Fehlend		Gesamtsumme	
	H	Prozent	H	Prozent	H	Prozent
method * replicate	380	100,0%	0	0,0%	380	100,0%

Kreuztabelle method*replicate

Anzahl

		replicate			Gesamtsumme
		1,00	2,00	3,00	
method	18oil	60	60	90	210
	4oil	60	70	40	170
Gesamtsumme		120	130	130	380

Chi-Quadrat-Tests

	Wert	df	Asymp. Sig. (zweiseitig)
Pearson-Chi-Quadrat	15,966[a]	2	,000
Likelihood-Quotient	16,287	2	,000
Anzahl der gültigen Fälle	380		

a. 0 Zellen (0,0%) haben die erwartete Anzahl von weniger als 5. Die erwartete Mindestanzahl ist 53,68.

Zusammenfassung der Fallverarbeitung

	Fälle					
	Gültig		Fehlend		Gesamtsumme	
	H	Prozent	H	Prozent	H	Prozent
method * replicate	390	100,0%	0	0,0%	390	100,0%

Kreuztabelle method*replicate

Anzahl

		replicate			Gesamtsumme
		1,00	2,00	3,00	
method	18disp	50	50	80	180
	4disp	80	50	80	210
Gesamtsumme		130	100	160	390

Chi-Quadrat-Tests

	Wert	df	Asymp. Sig. (zweiseitig)
Pearson-Chi-Quadrat	4,643[a]	2	,098
Likelihood-Quotient	4,676	2	,097
Anzahl der gültigen Fälle	390		

a. 0 Zellen (0,0%) haben die erwartete Anzahl von weniger als 5. Die erwartete Mindestanzahl ist 46,15.

Cross tabulation and Pearsons Chi-Square test between experiments at 18°C

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Treatment * conc	300[a]	100,0%	0	0,0%	299,990	100,0%

a. Number of valid cases is different from the total count in the crosstabulation table because the cell counts have been rounded.

Treatment * conc Crosstabulation

			conc			Total
			,0100	,0300	,1000	
Treatment	EcoTech	Count	20	10	90	120
		Expected Count	13,2	32,0	74,8	120,0
		% within conc	60,6%	12,5%	48,1%	40,0%
	Oil	Count	13	70	97	180
		Expected Count	19,8	48,0	112,2	180,0
		% within conc	39,4%	87,5%	51,9%	60,0%
Total		Count	33	80	187	300
		Expected Count	33,0	80,0	187,0	300,0
		% within conc	100,0%	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	36,195[a]	2	,000
Likelihood Ratio	40,297	2	,000
N of Valid Cases	300		

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 13,20.

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Treatment * conc	343[a]	99,9%	,333	0,1%	343,333	100,0%

a. Number of valid cases is different from the total count in the crosstabulation table because the cell counts have been rounded.

Treatment * conc Crosstabulation

			conc			Total
			,0100	,0300	,1000	
Treatment	Corexit	Count	43	80	100	223
		Expected Count	41,0	58,5	123,5	223,0
		% within conc	68,3%	88,9%	52,6%	65,0%
	EcoTech	Count	20	10	90	120
		Expected Count	22,0	31,5	66,5	120,0
		% within conc	31,7%	11,1%	47,4%	35,0%
Total		Count	63	90	190	343
		Expected Count	63,0	90,0	190,0	343,0
		% within conc	100,0%	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	35,653[a]	2	,000
Likelihood Ratio	39,685	2	,000
N of Valid Cases	343		

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 22,04.

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Treatment * conc	403[a]	99,9%	,320	0,1%	403,320	100,0%

a. Number of valid cases is different from the total count in the crosstabulation table because the cell counts have been rounded.

Treatment * conc Crosstabulation

			conc			Total
			,0100	,0300	,1000	
Treatment	Corexit	Count	43	80	100	223
		Expected Count	31,0	83,0	109,0	223,0
		% within conc	76,8%	53,3%	50,8%	55,3%
	Oil	Count	13	70	97	180
		Expected Count	25,0	67,0	88,0	180,0
		% within conc	23,2%	46,7%	49,2%	44,7%
Total		Count	56	150	197	403
		Expected Count	56,0	150,0	197,0	403,0
		% within conc	100,0%	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12,336[a]	2	,002
Likelihood Ratio	13,061	2	,001
N of Valid Cases	403		

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 25,01.

Cross tabulation and Pearsons Chi-Square test between experiments MC252 with Corexit9500 at 18°C and 4°C

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
concentratie1 * Temperatur1	760	100,0%	0	0,0%	760	100,0%

concentratie1 * Temperatur1 Crosstabulation

			Temperatur1		Total
			4	18	
concentratie1	,010000	Count	10	130	140
		Expected Count	16,6	123,4	140,0
		% within Temperatur1	11,1%	19,4%	18,4%
	,030000	Count	20	240	260
		Expected Count	30,8	229,2	260,0
		% within Temperatur1	22,2%	35,8%	34,2%
	,100000	Count	60	300	360
		Expected Count	42,6	317,4	360,0
		% within Temperatur1	66,7%	44,8%	47,4%
Total		Count	90	670	760
		Expected Count	90,0	670,0	760,0
		% within Temperatur1	100,0%	100,0%	100,0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15,277[a]	2	,000
Likelihood Ratio	15,454	2	,000
Linear-by-Linear Association	14,974	1	,000
N of Valid Cases	760		

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 16,58.

Cross tabulation and Pearson Chi-Square tests-concentrations peak at 4°C MC252

Zusammenfassung der Fallverarbeitung

	Fälle					
	Gültig		Fehlend		Gesamtsumme	
	H	Prozent	H	Prozent	H	Prozent
loading * replicate	210	100,0%	0	0,0%	210	100,0%

Kreuztabelle loading*replicate

Anzahl

		replicate			Gesamtsumme
		1,00	2,00	3,00	
loading	0,03	20	20	10	50
	0,1	40	50	70	160
Gesamtsumme		60	70	80	210

Chi-Quadrat-Tests

	Wert	df	Asymp. Sig. (zweiseitig)
Pearson-Chi-Quadrat	9,516[a]	2	,009
Likelihood-Quotient	10,105	2	,006
Anzahl der gültigen Fälle	210		

a. 0 Zellen (0,0%) haben die erwartete Anzahl von weniger als 5. Die erwartete Mindestanzahl ist 14,29.

Zusammenfassung der Fallverarbeitung

	Fälle					
	Gültig		Fehlend		Gesamtsumme	
	H	Prozent	H	Prozent	H	Prozent
loading * replicate	200	100,0%	0	0,0%	200	100,0%

Kreuztabelle loading*replicate

Anzahl

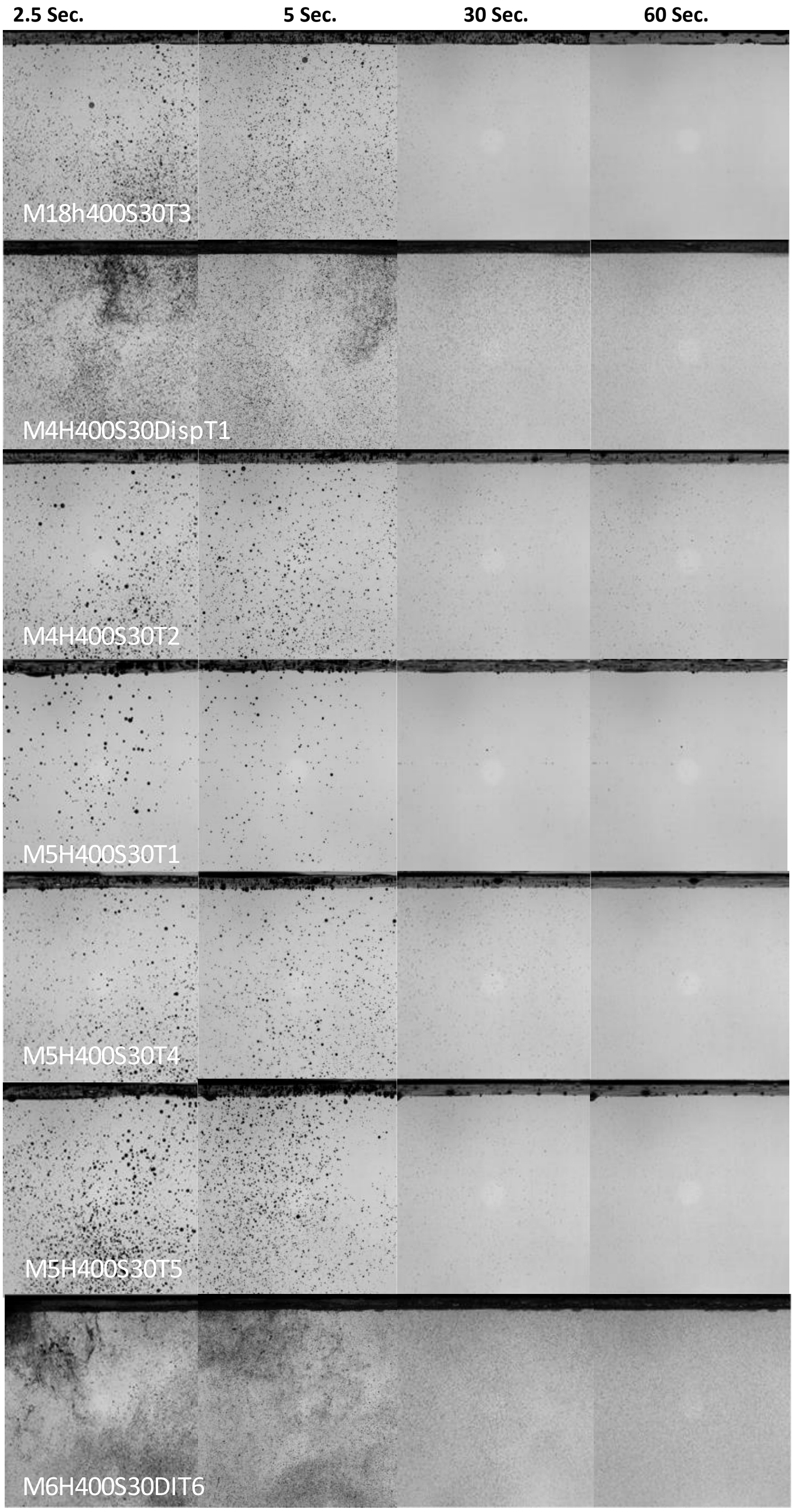
		replicate			Gesamtsumme
		1,00	2,00	3,00	
loading	0,1	40	50	70	160
	0,3	10	0	30	40
Gesamtsumme		50	50	100	200

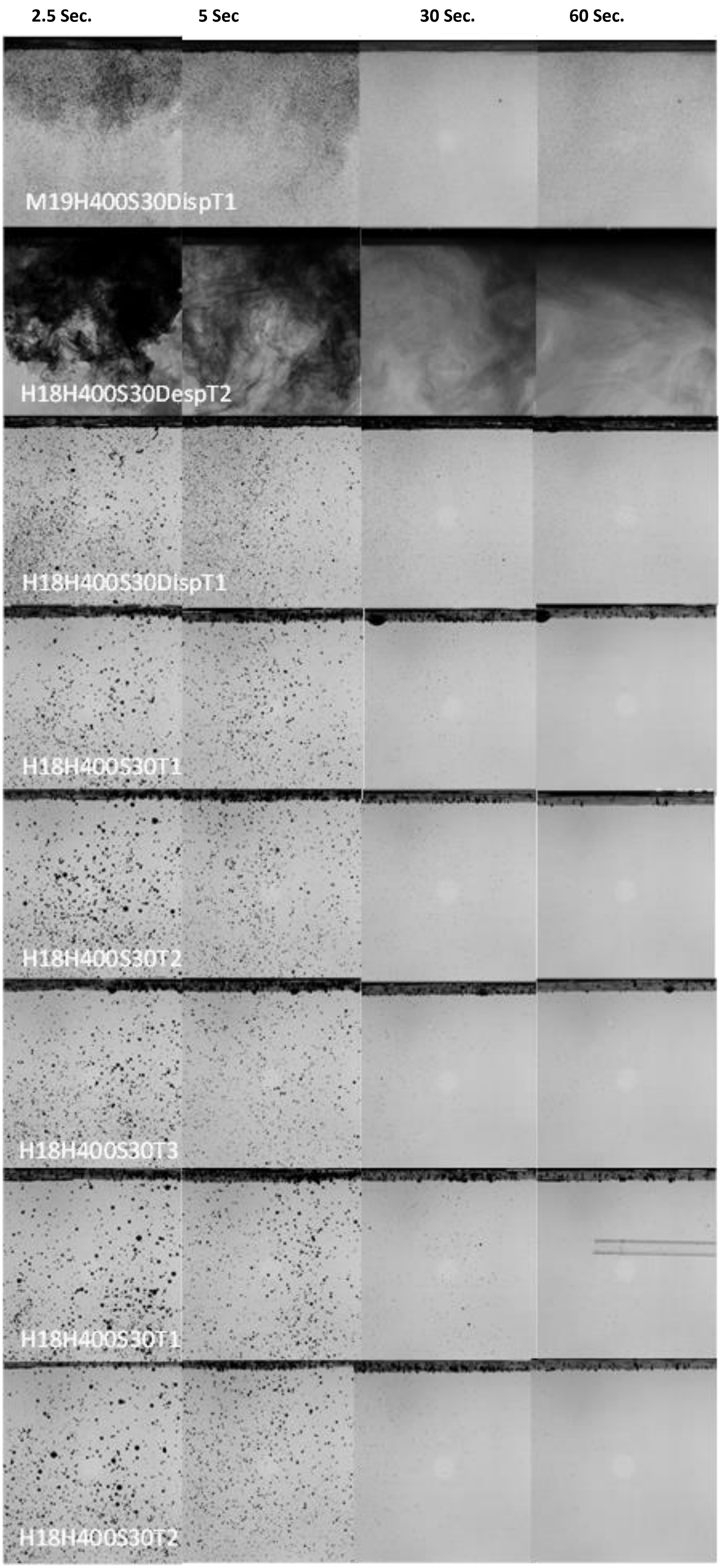
Chi-Quadrat-Tests

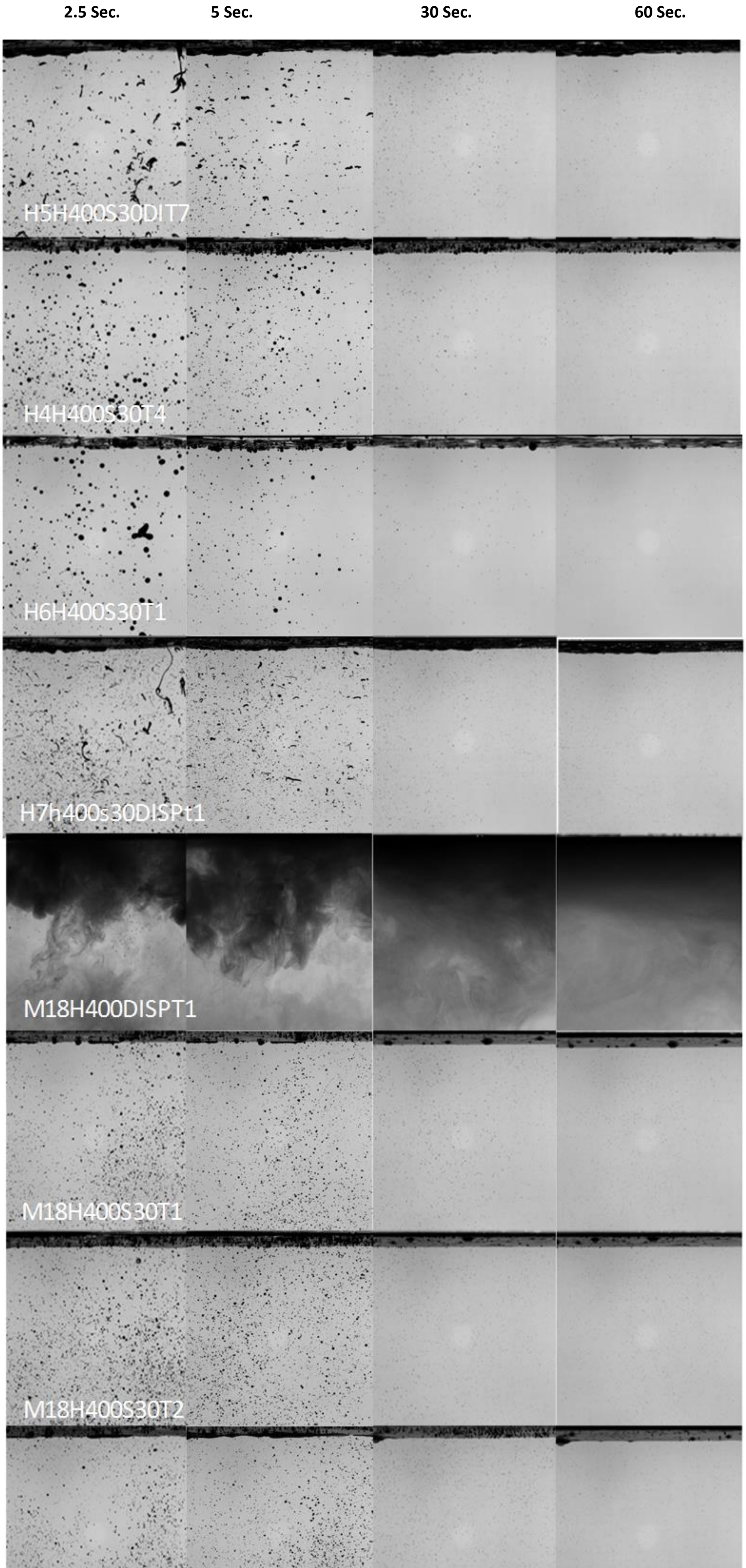
	Wert	df	Asymp. Sig. (zweiseitig)
Pearson-Chi-Quadrat	18,750[a]	2	,000
Likelihood-Quotient	27,948	2	,000
Anzahl der gültigen Fälle	200		

a. 0 Zellen (0,0%) haben die erwartete Anzahl von weniger als 5. Die erwartete Mindestanzahl ist 10,00.

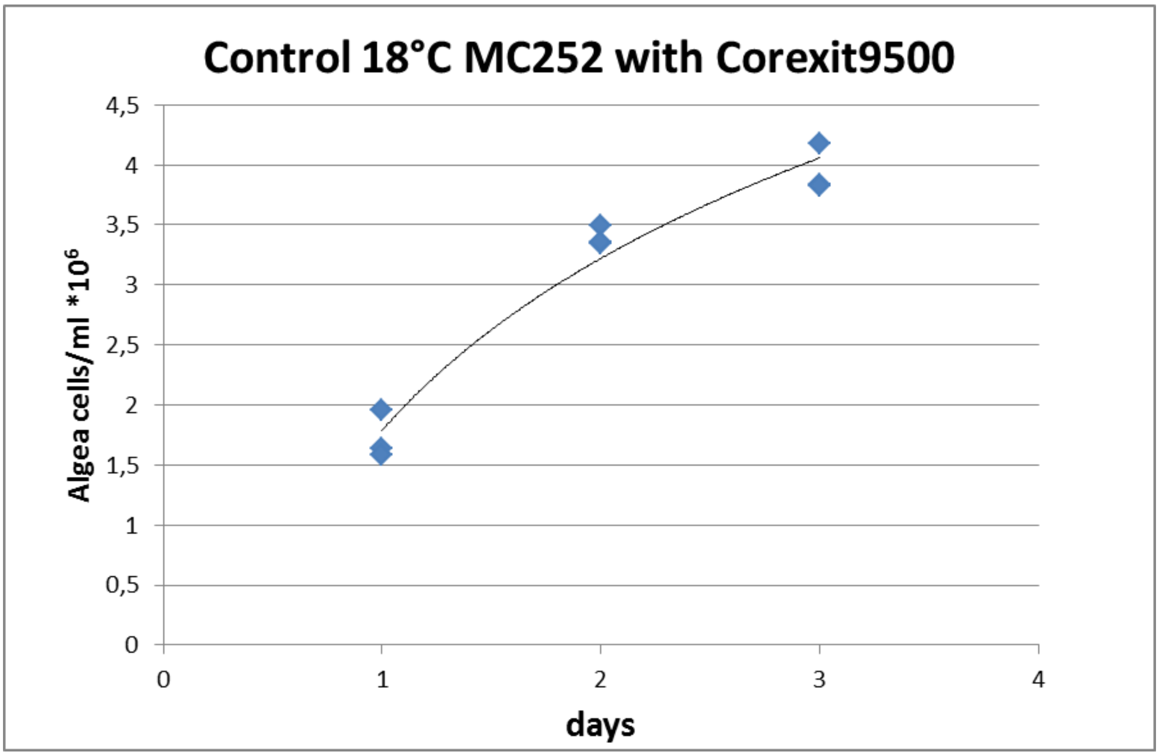
Appendix V Pictures from the plunge test experiments



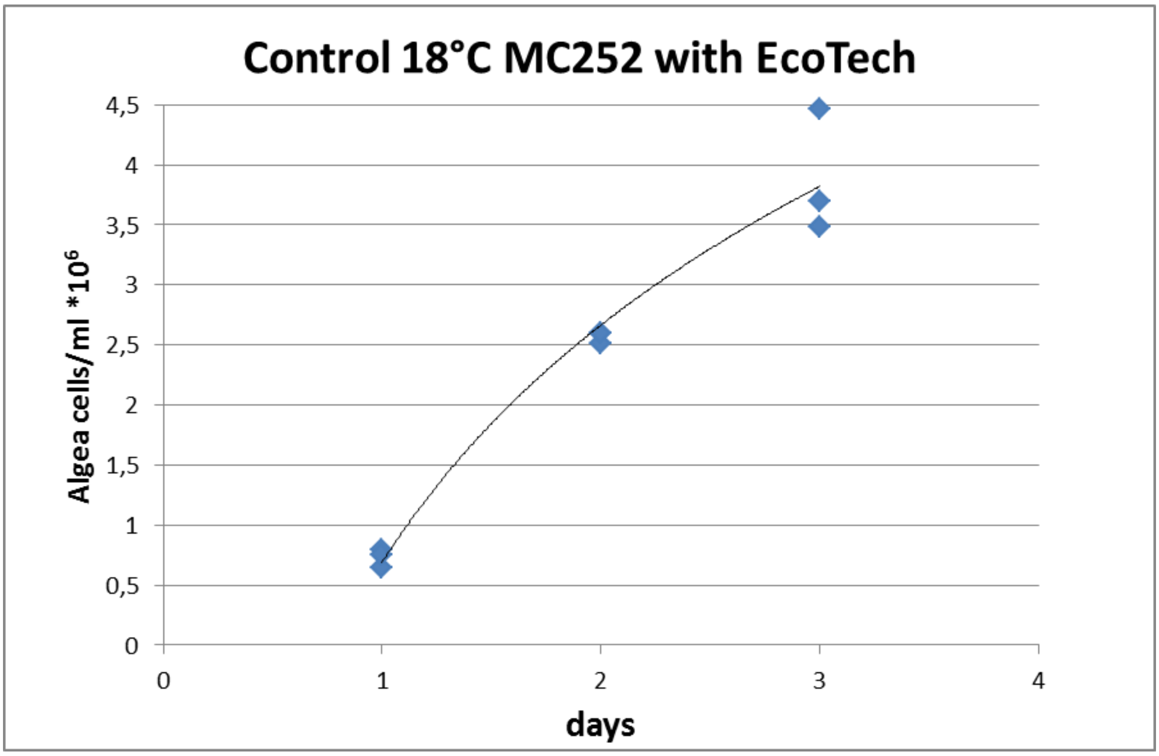

2.5 Sec.
5 Sec
30 Sec.
60 Sec.
M19H400S30DispT1
H18H400S30DespT2
H18H400S30DispT1
H18H400S30T1
H18H400S30T2
H18H400S30T3
H18H400S30T1
H18H400S30T2


2.5 Sec.
5 Sec.
30 Sec.
60 Sec.
H5H400S30DIT7
H4H400S30T4
H6H400S30T1
H7h400s30DISPt1
M18H400DISPT1
M18H400S30T1
M18H400S30T2

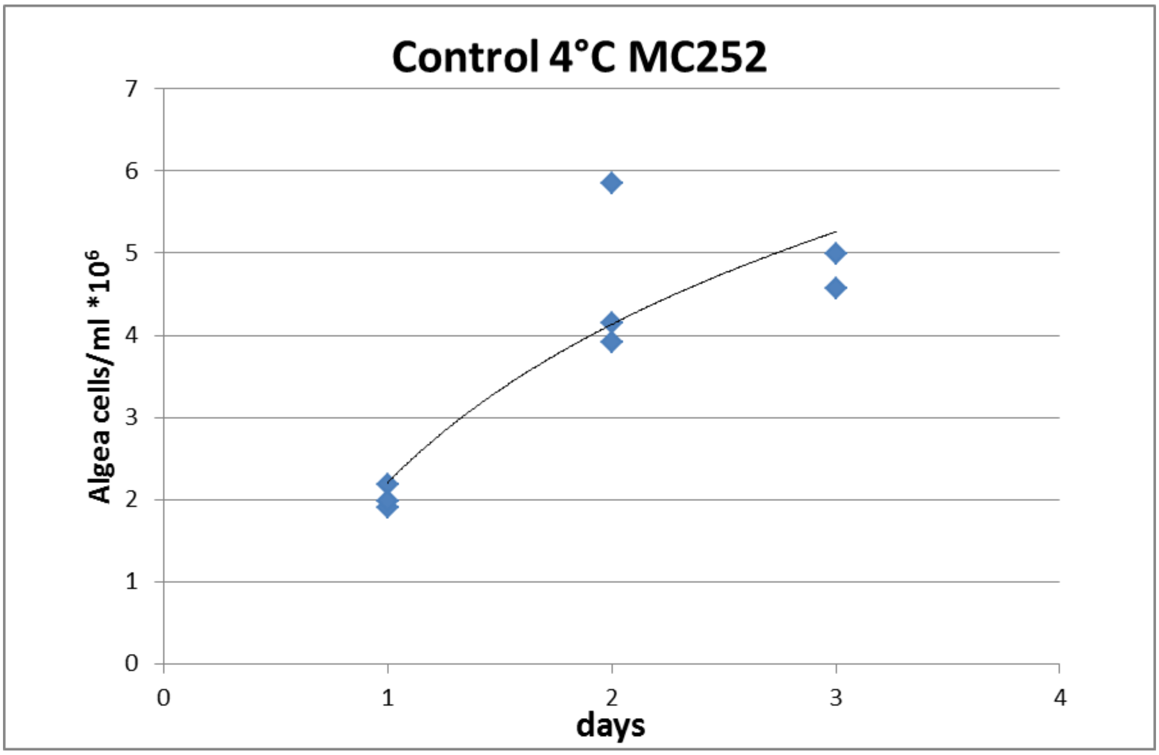
Appendix VI validity criteria of the *Phaeodactylum tricornutum* growth inhibition test.



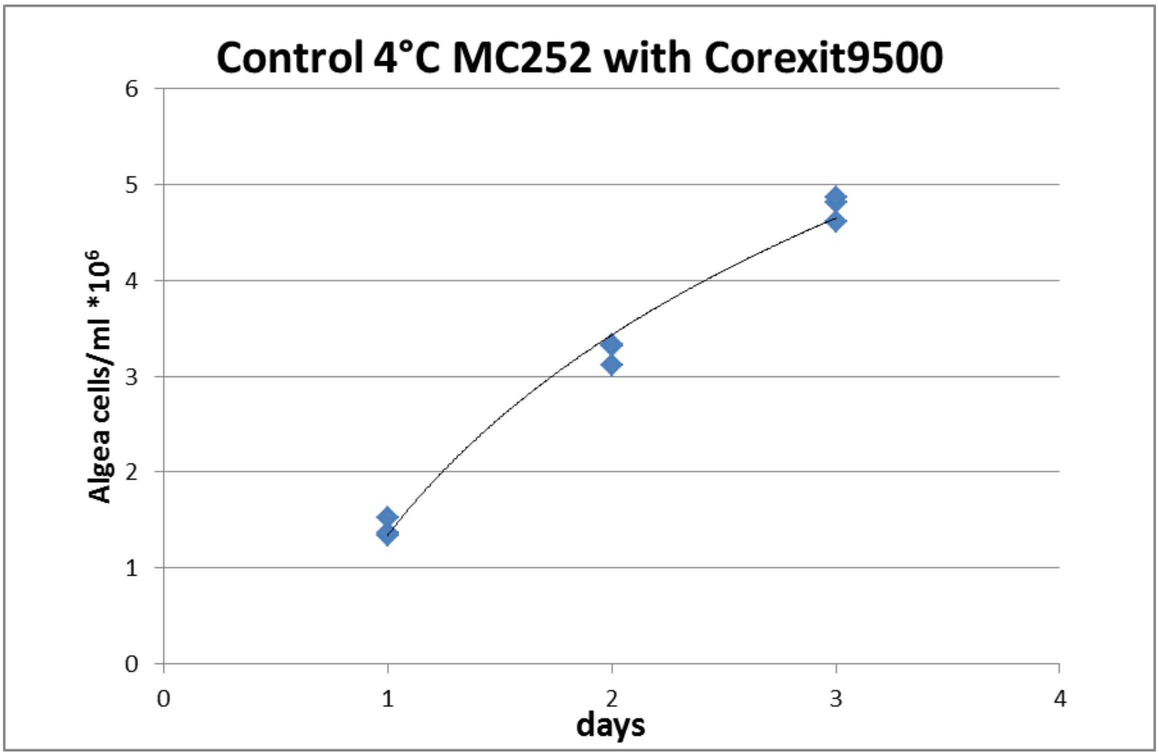
Mean growth rate between day one and day two: 0.68



Mean growth rate between day one and day two: 1.26



Mean growth rate between day one and day two: 0.82



Mean growth rate between day one and day two: 0.84