Investigating the prey spectrum of two co-occurring *Themisto* amphipods in the Fram Strait (Atlantic-Arctic gateway) using DNA metabarcoding

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Zusammenfassung

Pelagische Amphipoden sind Schlüsselorganismen in den Polarregionen. Im Arktischen Ozean, dominieren die zwei hyperiiden Arten *Themisto libellula* und *Themisto abyssorum*. Diese Amphipoden sind nicht nur eine wichtige Nahrungsquelle für höhere trophische Ebenen, sondern auch wichtige Räuber, die dazu fähig sind in manchen arktischen Regionen den Zooplanktonbestand zu kontrollieren. In den vergangenen Jahren, wurden einige Studien durchgeführt, um die Nahrung von *T. libellula* und *T. abyssorum* zu untersuchen. Diese Studien nutzten Stereomikroskopie und Biomarker und zeigten, dass die Nahrung hauptsächlich aus den abundantesten Zooplanktonarten besteht, inklusive Copepoden, Euphausiaceen und Chaetognathen. Zusätzliche wurde herausgefunden, dass die beiden Amphipoden unterschiedliche Nischen im arktischen Ökosystem besetzen, wobei *T. libellula* stark vom Eisalgen Pfad abhängig zu sein scheint. Dies führt zu der Vermutung, dass die beiden unterschiedlich durch die Folgen der Atlantifizierung der Arktis beeinflusst werden. In dieser Studie wird DNA metabarcoding verwendet, um das gesamte Beute Spektrum dieser Räuber zu entschlüsseln und bisher übersehenes gelatinöses Zooplankton in der Nahrung zu identifizieren. Es wurden signifikante Unterschiede in der Beutezusammensetzung der beiden Prädatoren gefunden, was bedeutet, dass die beiden nicht um Nahrung konkurrieren. Zusätzlich scheinen calanoide Copepoden nicht so wichtig als Nahrungsgrundlage für *Themisto* Amphipoden zu sein wie zuvor vermutet. Die RRA (relative read abundance) für andere Zooplanktonarten waren höher als die RRA für die calanoiden Copepoden. Es wurde auch gezeigt, dass die Beutezusammensetzungen innerhalb einer Räuberart zwischen den verschiedenen Stationen unterschiedlich sind. An kalten, arktischen Stationen wurden vermehrt eisassoziierte Beutearten in den Mägen von *T. libellula* gefunden, dazu gehörten unter anderem *Calanus glacialis* und *Boreogadus saida*. An warmen, atlantischen Stationen hingegen wurden verschiedene Copepoden- und Krillarten gefunden, die weniger von Seeeisbedeckung abhängig sind. Diese Ergebnisse führen zu der Vermutung, dass eine Ernährung basierend auf eisassoziierten Arten, eher von den vorherrschenden Umwelteinflüssen als von einer Beutepräferenz abhängt. Die Nahrung von *T. abyssorum* wurde hauptsächlich von dem Chaetognathen *Eukrohnia hamata* dominiert, zeigte aber dennoch Unterschiede zwischen den einzelnen Stationen. An Stationen mit atlantischem Einfluss wurden höhere RRAs für *Calanus finmarchicus* gefunden, als an Stationen

mit arktischem Einfluss. In einigen Proben beider Prädatoren wurden Sequenzen von Hydrozoenarten, wie *Nanomia cara* und *Aglantha digitale*, gefunden. Diese Funde waren nicht mit bestimmten Stationen verknüpft und es scheint, dass diese nur einen kleinen Teil der Nahrung dieser Prädatoren ausmacht. Allerdings zeigen diese Ergebnisse, dass *Themisto* Amphipoden im Arktischen Ozean gelatinöses Zooplankton fressen können. In dieser Studie wurde ein breites Beutespektrum für *T. libellula* nachgewiesen. Dies kann bedeuten, dass dieser Prädator in der Lage ist, sich an eine verändernde Zooplanktonzusammensetzung anzupassen, verursacht durch Klimaerwärmung und Seeeisverlust. Zudem wurde gezeigt, dass die beiden *Themisto* Amphipoden verschiedene Organismen fressen und daher nicht um Beute konkurrieren. Um das gesamte Fressverhalten der beiden Amphipoden zu erfassen, sind weitere Untersuchungen notwendig, idealerweise eine Kombination von DNA metabarcoding und Biomarkern, um sowohl eine kurzfristig als auch eine langfristige Ernährung zu bestimmen.

Abstract

Pelagic amphipods are a key zooplankton group in polar regions. In the Arctic Ocean, the two hyperiid amphipod species *Themisto libellula* and *Themisto abyssorum* are dominating the pelagic community. They are not only an important food source for higher trophic levels, but also important predators able to control the zooplankton standing stock in some Arctic regions. In recent years, several studies using stereomicroscopy and biomarkers were conducted to study the diet of *T. libellula* and *T. abyssorum*. These studies suggested a diet mainly consisting of the most abundant zooplankton species including copepods, euphausiids and chaetognaths. It was also found that the two amphipods are covering different niches in the Arctic ecosystem, with *T. libellula* being more dependent on the ice-algal pathway. This leads to the assumption that the two amphipods are differently impacted by the ongoing Atlantification and sea ice retreat. In this study DNA metabarcoding was used to assess the prey spectrum at high taxonomic resolution and potentially detect so far overlooked gelatinous zooplankton in the diet of these predators. The results indicate that the two predators are feeding on different zooplankton and ichthyoplankton species and hence, not to compete for food. Additionally, calanoid copepods, do not seem to be as important as assumed in the diet in the summer months. The diet within one predator species differed between the different sampling localities. *T. libellula*'s diet consisted of ice-associated species like *Calanus glacialis* and *Boreogadus saida* in regions with cold, Arctic waters, while these prey species were not found at stations with Atlantic waters. This leads to the assumption that a sympagic fueled diet for this species is rather linked to the location than to a preferred prey type. The diet of *T. abyssorum* was dominated by the chaetognath *Eukrohnia hamata*, but high variability was observed between the stations. At locations with Atlantic influence RRA (relative read abundances) for *Calanus finmarchicus* were higher than at stations with Arctic impact. In some samples of both predators, sequences belonging to several hydrozoan species, e.g., *Nanomia cara* and *Aglantha digitale*, were detected. Those findings were not linked to certain locations, but they show that the amphipods are able to feed on gelatinous zooplankton, although they do not make up a major part of its diet. The broad prey spectrum found for *T. libellula* shows that this flexible species may be able to adapt its diet to changes in the zooplankton community caused by climate change and sea ice retreat. To fully understand *Themisto*'s feeding behavior more sampling is needed, ideally combining DNA metabarcoding and biomarkers, to assess then both short-term and long-term diet.

1. Introduction

1.1 Atlantification of the Arctic Ocean: The role of Fram Strait

1.1.1 Hydrography of the Fram Strait

The Fram Strait is the only deep connection between the Arctic and Atlantic Ocean and is handling 90% of the heat and 75% of the mass exchange between the Arctic and other oceans (Wadhams 1983; Hop et al. 2006). The region is impacted by two opposing currents, the West Spitsbergen current (WSC), that brings warm Atlantic water northwards along Svalbard and the East Greenland current (EGC), which transports cold Arctic water southwards along the east coast of Greenland (Hop et al. 2006). The high quantities of heat brought within the WSC influence the climate in the whole region (Hop et al. 2006). The northward transport of warm Atlantic waters through the WSC is assumed to be 9.5Sv, while the southward transport of cold polar waters and ice through the EGC is assumed to be 13.7Sv, leaving a southward net transport and loss of cold Arctic water and ice of 4.2Sv (Fahrbach et al. 2001; Maslowski et al. 2004). It is known that the inflow of Atlantic water and the outflow of polar waters and ice is linked to the Arctic Oscillation (AO) and the North Atlantic Oscillation (NAO) (Rigor 2002; Zhang, Ikeda, and Walsh 2003; Dickson et al. 2000). These pressure systems are mainly influenced by the atmospheric heat balance, which means that climate changes can alter the strength of the large-scale ocean circulation in the Fram Strait (Hop et al. 2006). The inflow of Atlantic waters through the WSC is increasing during a strong, positive NAO (Dickson et al. 2000; Schlichtholz and Goszczko 2006). This might also have led to an intensification of the EGC with according increased southward ice transport (Hop et al. 2006).

1.1.2 Sea ice decline and its causes

Several studies dealt with the decline of sea ice extent and sea ice thickness during the last decades. Carmack et al. (2015) found a decline of sea ice extent from 1980 to 2010 of 3.8±0.3% per decade and for the sea ice thickness of nearly 1.7m. Historically, in total 40% of the Arctic Ocean's ice cover consists of first year ice (FYI, melting completely during spring and summer each year) and 60% of multi-year ice (MYI, having already endured at least one melting season) (Hop et al. 2006). Due to the warming trend and resulting thinning of the sea ice, by the end of 2010 only 15% of the Arctic ice consisted of MYI (Polyakov, Walsh, and Kwok 2012). The Arctic Ocean has lost 42% of its MYI between 2005 and 2008 (Polyakov, Walsh, and Kwok

2012). A large fraction of the lost MYI is exported, mainly wind-driven, through the Fram Strait (Polyakov, Walsh, and Kwok 2012). The thicker MYI normally consists of more ice-associated (or "sympagic") biomass like ice-algae or ice-associated zooplankton than thinner FYI and allows overwintering of the sympagic communities (Barry et al. 1993; Lønne and Gulliksen 1991a, 1991b). Models predict the Arctic may become sea-ice free in summer within a decade or two (Overland and Wang 2013) which will disrupt the sympagic productivity. This loss of sympagic biomass and biodiversity could have large consequences for the Arctic ecosystem as it is an important food source for the Arctic fauna (Hop et al. 2006). Polyakov et al. (2017) assume an increasing role of incoming warm Atlantic water (AW) through the Fram Strait causing sea ice decline in the eastern Eurasian Basin. In recent years the stratification of the eastern Eurasian Basin was strong enough to provide an insulation layer and to avoid ventilation of the water column during winter (Polyakov et al. 2017). In contrast to this, the stratification in the western Nansen Basin was not as strong and enabled a ventilation caused by the cooling and haline convection during ice formation (Ivanov et al. 2016). This leads in the western Nansen Basin to a reduction in sea ice thickness along the slope off Svalbard (Onarheim et al. 2014; Ivanov et al. 2016). In most other regions in the Arctic Ocean this strong vertical mixing, leading to the entrainment of AW to the upper ocean layers, is prevented by a cold and high saline layer, the cold halocline layer (CHL) which mainly stops the haline convection, so that mixing only happens in the upper 40m (Ivanov et al. 2016). In the winters 2013/14 and 2014/15 a warming and shoaling of the Atlantic Water layer in the eastern Eurasian Basin together with a weakening of the CHL could be observed (Polyakov et al. 2017). This phenomenon is also called the Atlantification of the eastern Eurasian Basin (Polyakov et al. 2017). This Atlantification has enabled the vertical mixing of the upper 130m, which led to the disappearance of the CHL, which can furthermore lead to a fundamental change in the structure of the water column (Polyakov et al. 2017). As a consequence, the eastern Eurasian Basin was already nearly ice free by the end of summer since 2011 (Polyakov et al. 2017). In addition, a recent study of Wang et al. (2020) shows that the decline in sea ice can have an altering effect on the inflow of Atlantic water through the Fram Strait. The potential reason for this altering effect is an increased salinity in the Greenland Sea due to the reduction of transported sea ice in this region. This increased salinity can lead to a stronger circulation in the cyclonic gyre in the Nordic Seas (i.e., Iceland, Norwegian and Greenland seas) (Wang et al. 2020). The altered strength of the cyclonic gyre in the Nordic Seas can strengthen the Atlantic Water Boundary Currents which leads to a higher

inflow of Atlantic water into the Arctic Ocean (Wang et al. 2020). This stronger inflow can result in a feedback loop in the Arctic Ocean: the heat carried within the Atlantic water and the increased vertical mixing will intensify the ongoing sea-ice decline (Polyakov et al. 2017; Wang et al. 2020).

1.2 Consequences of the warming and ongoing Atlantification on the ecosystem

1.2.1 Effects of warming on primary production and higher trophic levels

Due to the sea ice decline in the Arctic Ocean, less sea ice is reaching the Fram Strait (Krumpen et al. 2019). Sea ice is an important habitat for the sympagic community including not only sympagic microalgae, heterotrophic protists and metazoans such as copepods, rotifers and turbellarians (utilizing brine channels within the ice), but also under-ice meiofauna (Gradinger, Friedrich, and Spindler 1999; David et al. 2015; Bluhm et al. 2018). Ice-algae found in MYI can make up 50% of the primary production in the Arctic Ocean (Gosselin et al. 1997; Fernández-Méndez et al. 2015). In contrast, productivity of FYI is lower, since more algal biomass is found in MYI (Werner, Ikävalko, and Schünemann 2007; Fernández-Méndez et al. 2015). Ice-algae are also an important food source for primary consumers (Søreide et al. 2006; Falk-Petersen et al. 2009; Søreide et al. 2013; Kohlbach et al. 2016) and those grazers represent an important link between sympagic production and higher trophic levels (Ehrlich et al. 2020). The calanoid copepods *Calanus glacialis* and *C. hyperboreus* are depending strongly on the ice algae as a food source during their life cycle (Søreide et al. 2010; Kohlbach et al. 2016). The later phytoplankton bloom is crucial for the offspring of *C. glacialis*, an earlier ice break resulting in a shorter ice-algal bloom and an earlier second phytoplankton bloom could result in a mismatch for *C. glacialis* and in a poor survival of the species (Søreide et al. 2010; Haug et al. 2017). The lack of *C. glacialis* as a food source can have consequences for the whole Arctic marine food web (Leu et al. 2011; Søreide et al. 2010). The boreal, smaller and less lipid-rich *C. finmarchicus* is less dependent on sea ice and is therefore assumed to replace the more lipid-rich *C. glacialis* and *C. hyperboreus* in a more atlantified Arctic (Hirche and Kosobokova 2007), with unknown consequences for the entire Arctic food web. Further increase in *C. finmarchicus* abundance may be caused by an increased advection via the WSC through the Fram Strait (Basedow et al. 2018; Polyakov et al. 2020).

Sea-ice decline can also have a direct impact on the benthic secondary production, which is higher in ice-covered areas than in ice-free areas (Degen et al. 2016). This could result in a decrease in the benthic secondary production due to a further sea-ice decline in the Arctic (Haug et al. 2017). The benthic organisms living on the continental shelves of the Arctic are important for the energy flow (Piepenburg et al. 1995; Piepenburg and Schmid 1996), as they make organic carbon usable for other organisms by bioturbation, redistribution and remineralization (Piepenburg et al. 1995; Bluhm et al. 2009; Blicher and Sejr 2011). This may be translated up the food chain with community changes from benthivorous to pelagic-feeding fish (Frainer et al. 2017)

The sea ice decline in the Arctic Ocean not only impacts the smallest organisms, but also top consumers such as marine mammals. For example, ringed seals (*Pulsa hispida*) use sea ice for breeding and as haul out platform (Haug et al. 2017). Hence, they seemed to have followed the ice edge further north, which might have resulted in increased energetic costs in finding food (Hamilton et al. 2015; Haug et al. 2017). Ringed seals are a keystone species in the Arctic Ocean and a further decline in sea ice will probably finally lead to a population decrease (Hamilton et al. 2015). Another seal species, the harp seal (*Pagophilus groenlandicus*) is also suffering from the sea ice decline as it prefers to be close to the sea ice throughout the year (Haug et al. 2017). Their blubber thickness has decreased in recent years, which resulted in a poor pup production (Øigård et al. 2013; Haug et al. 2017). This decrease in body condition might not only be a result of the sea ice decline, but also of the competition for food with the large fish stocks including capelin, polar cod and Atlantic cod in the region (Øigård et al. 2013; Bogstad et al. 2015). It was found that in September and mid-October harp seals are mainly preying on *Themisto* amphipods in the Barent Sea (Nilssen et al. 1995), a reduction of one of these important prey species in autumn could lead to a further decline of the body condition of adult harp seals.

1.2.2 Boreal species in the Arctic as indicators for the ongoing Atlantification.

The Atlantic inflow through the Fram Strait is not only bringing heat to the Arctic Ocean, it also transports boreal species into the sub-Arctic and Arctic Seas. As mentioned before, a lot of zooplankton like *C. finmarchicus* are advected into the Arctic Ocean via the Fram Strait, but it also favors dispersal of other organisms like fish and bivalve species (larval transport). Atlantic cod (*Gadus morhua*) and capelin (*Mallotus villotus*), for example, have undergone a recent poleward range expansion into the Barents Sea to the northern Svalbard shelf (Haug et al. 2017).

Over the last decade, other boreal species like haddock (*Melanogrammus aeglefinus*), Greenland halibut (*Reinhardtius hippoglossoides*), redfish (*Sebastes* spp.) and prawn (*Pandulus borealis*) were also newly found at the northern Svalbard shelf break (Haug et al. 2017). Hollowed, Planque, and Loeng (2013) have calculated the potential for several boreal fish species to move in the Arctic Ocean and to establish there. They calculated a very high potential for the beaked redfish (*Sebastes mentella*) as soon as temperature and feeding conditions are appropriate (Hollowed, Planque, and Loeng 2013). They also found a high expansion potential for the Greenland shark (*Somniosus microcephalus*), which is currently found in the Barents Sea and around Spitsbergen (Hollowed, Planque, and Loeng 2013). For Greenland halibut, herring (*Clupea harengus*) and capelin a moderate potential for a distribution range expansion is estimated (Hollowed, Planque, and Loeng 2013). A successful establishment of these species is mainly depending on appropriate prey conditions and suitable drifting conditions for larval transport (Hollowed, Planque, and Loeng 2013). For some other Atlantic species like the Atlantic cod a low potential is estimated, as the seasonal sea ice cover in the Arctic Ocean is thought to act like a barrier to spawning (Hollowed, Planque, and Loeng 2013). The topography of the deep Arctic may not be suitable for the demersal Atlantic cod (Hollowed, Planque, and Loeng 2013). The same accounts for some Pacific fish species like the Walleye pollock (*Theragra chalcogramma*), Alaska plaice (*Pleuronectes quadrituberculatus*) and the Bering flounder (*Hippoglossoides robustus*) which were found to have a moderate or low potential to migrate into the Arctic Ocean (Hollowed, Planque, and Loeng 2013). Haug et al. (2017) assume that if the warming of the Arctic will lead to higher plankton production further north, this might result in a poleward migration of pelagic fish species like *Clupea harengus*, *Scomber scombrus* and *Micromesistius poutassou* in the case that these fish stocks become food limited in their original feeding grounds. The introduction of boreal species to the Arctic Ocean can cause an increase in the predation pressure on the Arctic fish community including polar cod, an important resource for Arctic top predators such as ringed seals, beluga whales and polar bears (Hamilton et al. 2015; Steiner et al. 2019).

1.2.3 Atlantification and its potential effect on jellyfish

The Atlantification of the Arctic Ocean may not only favor the survival and establishment of boreal fish species in the region, but also an increase in jellyfish biomass. So far it is not known if gelatinous zooplankton like ctenophores, scyphomedusae and hydromedusae can take advantage

of the sea ice retreat and the newly available habitat and food (Purcell 2005; Purcell et al. 2010). From many other regions of the world an increase of gelatinous zooplankton was already observed (Graham 2001; Mills 2001; J. Purcell, Graham, and Dumont 2001; Link and Ford 2006; Kawahara et al. 2006; Lynam et al. 2006), but it is so far not known if this phenomenon will be seen in the Arctic Ocean as well. Brodeur et al. (2008) pointed out that the increasing ocean temperatures will not necessarily lead to an increase in jellyfish biomass in all regions in the world. There are however some factors that seem to favor the occurrence of high jellyfish abundances, such as climate change, overfishing, eutrophication and the introduction of invasive species (Shiganova 1998; Arai 2001; Parsons and Lalli 2002; Purcell 2005, 2007; Attrill, Wright, and Edwards 2007). The Arctic Ocean and surrounding (sub-)Arctic seas are heavily impacted by climate change, and rising levels of pollution and invasive or range-expanding species (Halpern et al. 2008). (Sub-)Arctic seas such as the Bering and Barents Sea also undergo a heavy fishing pressure (Christiansen, Mecklenburg, and Karamushko 2014). Hence, some jellyfish species might get the chance to increase their biomass in the Arctic Ocean.

The potential increase of gelatinous zooplankton could have a negative impact on the productivity of higher trophic levels, as some jellyfish species are also known to prey on juvenile fish and large amounts of zooplankton (Purcell et al. 2010; Haug et al. 2017). Since the 1990s a dramatic increase of jellyfish on the eastern Bering Sea shelf was observed, and until 2000 the jellyfish biomass was nearly 4 times higher than back in the summers between 1975 and 1990 (Brodeur et al. 2008). However, jellyfish biomass has decreased again after 2001 despite the sea surface temperatures being warmer than the long-term average in the region (Brodeur et al. 2008). After the jellyfish peak in 2002, the zooplankton biomass has decreased leading probably to poor feeding conditions for the large jellyfish (Brodeur et al. 2008). An increase in jellyfish biomass was also noted in the Barents Sea since 2013 (Eriksen 2014). Warmer temperatures in the Barents Sea may have favored the proliferation of jellyfish, since during these times more zooplankton and fish eggs and larvae are brought to the Barents Sea (Ottersen 2000) leading to better food conditions (Loeng and Gjøsæter 1990; Ottersen 2000). Like many other organisms also jellyfish are reaching the Arctic through the WSC within the Atlantic water masses (Eriksen et al. 2012). That is why Knutsen et al. (2018) pointed out that especially boreal gelatinous species have the chance to enter the Arctic Ocean and extent their distribution range further north. With further sea ice retreat, the primary production in the Arctic

Ocean will no longer be light-limited and benefit from a longer growth season (Arrigo and Dijken 2015). Thus, an increasing primary production will lead to higher secondary production and will provide increasingly favorable food conditions for gelatinous predators (Purcell et al. 2010). Through these changes, the Arctic Ocean might become a more suitable habitat for some gelatinous plankter (Purcell et al. 2010).

Since gelatinous zooplankton is able to reach large abundances in short time and can exploit the zooplankton standing stock very fast, a potential increase in jellyfish biomass can lead to dramatic consequences for the ecosystem (Brodeur, Sugisaki, and Jr 2002). The exact predation impact of gelatinous predators was not yet estimated properly but is considered to be very high (Purcell et al. 2010). A study of Siferd and Conover (1992) concerning the predation impact of *Mertensia ovum* in the Resolute Passage showed that this ctenophore is able to consume 9% of the population of larger *Calanus* copepods per day. These copepods are very vulnerable against high predation pressure due to their long life cycle (Madsen, Nielsen, and Hansen 2001; Purcell et al. 2010), and as mentioned before, represent important lipid-rich prey for a variety of other predators, including macrozooplankton, fish and seabirds. In another study by Majaneva et al. (2013) even higher consumption rates for *Mertensia ovum* were found, suggesting that it might be able to consume up to 33% per day of the *Calanus* population in the upper 20m and 1.4% per day throughout the whole water column. It is likely that other jellyfish will have similar consumption rates and will be competitors for food for other planktivorous organisms in the pelagic ecosystem (J. E. Purcell and Arai 2001). However, the scarcity of datasets with a sufficient spatial and temporal coverage does not allow us to validate this assumption of an increase in jellyfish biomass in the Arctic Ocean (Aubert et al. 2018).

1.3 The role of the key pelagic amphipods *Themisto libellula* and *T. abyssorum* in the Arctic food web

1.3.1 Ecology of the two hyperiid amphipods

Pelagic amphipods form one of the major zooplankton groups in polar regions (Longhurst 1985; Bowman 1960). In the Arctic Ocean, two hyperiid amphipod species are dominating the amphipod communities, the amphipods *T. libellula* and *T. abyssorum* (Dalpadado, Borkner, and Skjodal 1994) (Fig. 1). These two species and *T. libellula* in particular, are known to be an important food source for many higher trophic levels like some fish species (Dempson, Shears,

and Bloom 2002), seabirds like the little auk (Pedersen and Falk 2001), harp seals (Haug et al. 2020) and some other seals (Nilssen et al. 1995; Hop et al. 2006) and even some whales (Lowry and Frost 1984). On the other hand, these hyperiid amphipods are also important predators in the Arctic pelagic food web, preying mainly on calanoid copepods (Auel et al. 2002; Hop et al. 2006). For example, in some regions, like the Bering Sea shelf, *T. libellula* is able to control the zooplankton standing stock and thereby affects the recruitment success of many pelagic fish species (Pinchuk et al. 2013). *T. libellula* is mainly associated with Arctic waters and is nearly absent in Atlantic waters, which is vice versa for *T. abyssorum* (Dalpadado, Borkner, and Skjodal 1994). But it was observed that *T. libellula* can increase its temperature tolerance up to 13-15°C in some regions like an Alaskan fjord (Percy 1993). *T. abyssorum* seems to have a broad temperature tolerance too, since it can be found in colder waters as well (Havermans et al. 2019). Both species are long lived species with life spans from 2-3 years (Koszteyn et al. 1995). It has already been observed that *T. abyssorum* became more abundant in the Barents Sea and Fram Strait, while the population of *T. libellula* has decreased in these regions (CAFF 2017). A possible explanation for this distribution shift could be the ongoing Atlantification in the region (Polyakov et al. 2017), as *T. abyssorum* is mainly associated with waters of Atlantic origin (Dalpadado, Borkner, and Skjodal 1994). The change in the abundance and distribution of *T. libellula* and *T. abyssorum* can have consequences for the pelagic food web in the Arctic Ocean, since higher trophic levels preferably feed on *T. libellula* (max. 31mm) due to its larger size and higher lipid-content and may not be able to feed on the smaller *T. abyssorum* (max. 18mm) (Lønne and Gabrielsen 1992; Koszteyn et al. 1995).

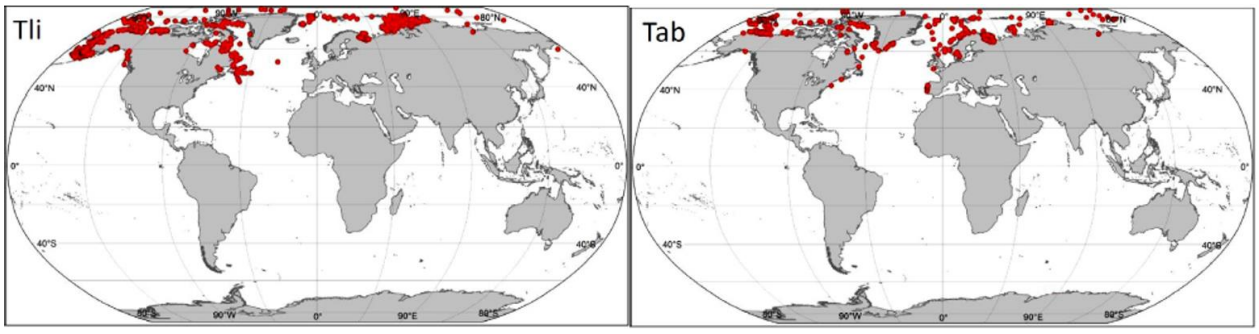

Fig. 1: Occurrence map of Themisto libellula (Tli) and T. abyssorum (Tab) in the Arctic and Atlantic Ocean (Havermans et al. 2019).

1.3.2 So far known diet of *T. libellula* and *T. abyssorum*

In recent years, a number of studies have been conducted to investigate the diet of these Hyperiidae. Several studies found that *Themisto* amphipods are mainly feeding on the most abundant zooplankton species including copepods, euphausiids and chaetognaths (Pakhomov and Perissinotto 1996; Auel et al. 2002). These studies were conducted using stereomicroscopy for prey identification. So far, the diet of *T. libellula* and *T. abyssorum* has also been investigated using biomarker analyses based on stable isotopes and fatty acids (Olsen et al. 2013; Kohlbach et al. 2016; Auel et al. 2002). These studies revealed that the two *Themisto* species occupy different niches in the Arctic ecosystem (Auel et al. 2002). The authors found that *T. libellula* is more ice-dependent in its diet, while *T. abyssorum* seems to be less dependent on the sea-ice algal pathway and seems to even cover a slightly higher trophic position (Auel et al. 2002; Kohlbach et al. 2016). These observations lead to the assumption that *T. libellula* may be more impacted by the consequences of climate change and sea ice loss, as one of its major food sources (calaniod copepods) is depending on sympagic primary production (Auel et al. 2002; Kohlbach et al. 2016). Based on their affinities for distinct water masses and differing feeding habits it is assumed that climate change will lead to a range expansion of *T. abyssorum*, while *T. libellula* may undergo a poleward range contraction (Havermans et al. 2019).

1.4 Recently used methods for dietary studies on *Themisto*

The so far applied methods for dietary analyses of *T. libellula* and *T. abyssorum*, which include biomarker and morphological analyses are limited in resolution and may overlook certain prey items. The characterization of fatty acids as biomarkers is only specific for determining the source of primary production and primary consumers, while the complete prey spectrum of the predator cannot be estimated with this method (Auel et al. 2002). Microscopic analyses are problematic as soft-bodied and highly digested prey cannot be clearly (or not at all) identified with this method (e.g., Olsen et al. 2013). A study by Olsen et al. (2013) using a denaturing high performance liquid chromatography with optimized primers to target the 18S rDNA gene, showed the presence of cniderians in the guts of some *T. abyssorum*. Anyhow, the most abundant sequences belonged to the amphipod itself and copepods, showing that the diet of both amphipods in the Arctic mainly consists of copepod species (Olsen et al. 2013). As this study was

conducted for *T. abyssorum* sampled at Arctic hydrothermal and cold vents (Olsen et al. 2013), it might not be representative for other locations in the Arctic Ocean.

Since the beginning of the 2000s great developments in next-generation sequencing (NGS), were achieved. The first sequencing methods were developed by Sanger and Coulson and another method by Maxam and Gilbert in 1976 (Sanger, Nicklen, and Coulson 1977; Maxam and Gilbert 1977). Those methods used both size measuring of fragments by using polyacrylamide gel electrophoresis (Maniatis, Jeffrey, and deSande 1975). In 1987 the first automated, fluorescence-based Sanger sequencing device was developed and could generate around 1000 bases per day (Smith et al. 1986; Connell et al. 1987). Before this methodology it could take up to one month to determine one base of a sequence (Gilbert and Maxam 1973). Between 1980 and 1990 several research groups looked for an alternative to the use of electrophoretic sequencing, and finally in 2003, the first approach of NGS using sequencing-by-synthesis was developed (Shendure et al. 2017). In 2005 the first NGS instrument was released by 454, and since 2012 Illumina sequencing platforms are dominating the market (Shendure et al. 2017). It was a long way from sequencing one base per month to achieving over a billion reads within two days (Shendure et al. 2017).

In this study, NGS in form of DNA metabarcoding is used to determine the stomach content of both *Themisto* species. DNA metabarcoding is a recent application of high-throughput sequencing on different substratum, like organismal bulk or environmental samples including soil, water, feces and dietary samples to identify the community or prey composition (Taberlet et al. 2012). With this method a region of DNA, called barcode or amplicon is targeted. This amplicon region should have enough variability to distinguish different taxonomic groups (Pompanon et al. 2012). The advantage of using metabarcoding is that the prey can be identified to the species level as long as a comprehensive DNA reference database is available, which makes it possible to assess the whole prey spectrum of the two important species (Weigand et al. 2019). In contrast to other biomarker methods, metabarcoding (like microscopy) only represents a temporary snapshot of the diet, whereas biomarker studies reveal the dietary signal integrated over a longer time (Pompanon et al. 2012). This method is only semi-quantitative, as the number of reads detected for each prey item is influenced by a lot of factors besides the ingestion (Deagle et al. 2013, 2018). Even universal primers appear to have taxon-

specific biases (binding more easily to DNA of one prey item than the other), and the DNA copy number can vary between taxa as well (Deagle et al. 2013; Nakahara et al. 2015).

1.5 Research goal

Since *T. libellula* and *T. abyssorum* are known to be key organisms in the Arctic, pelagic food web (Auel et al. 2002; Marion et al. 2008; Havermans et al. 2019), it becomes crucial to gain more knowledge on their diet composition. This is even more urgent in the light of the rapid changes that the Arctic is undergoing, by which *T. abyssorum* and *T. libellula* are considered to be differently impacted (Havermans et al. 2019). This study will therefore focus on the prey spectrum of the two *Themisto* species by using DNA metabarcoding of the gut content of individuals from different locations in the Fram Strait. By doing so, the prey spectra between and within the species will be compared and their regional variation will be assessed. Finally, the role of maybe so far overlooked gelatinous zooplankton in the prey of *T. libellula* and *T. abyssorum* will be investigated. The outcome of this study will ultimately allow us to make better predictions on how both species will be impacted by climate change and its consequences for the Arctic ecosystem.

1.6 Hypothesis

This study aims to test the following hypothesis, while applying DNA metabarcoding to assess the prey spectrum of two *Themisto* amphipods in the Arctic Ocean.

Hyp1: The prey spectrum between the two predators will differ significantly, even at the same location.

Hyp2: The prey spectrum of each predator will differ between the tested localities and the diet of *T. libellula* will contain more genuine Arctic and ice-associated species like *C. glacialis*, *C. hyperboreus* or *B. saida*, while *T. abyssorum* will be more opportunistic feeder with high variation between localities.

Hyp3: Both *Themisto* amphipods are feeding occasionally on gelatinous zooplankton.

2. Materials and Methods

2.1 Sample collection

Tab.1: Overview of all stations from cruises PS107, PS100 and TUNUVII. BO = bongo net, PT = pelagic trawl, MN = multi net, BT = bottom trawl. (size classes: 1: <10mm; 2: 10-20mm; 3: >20mm).

Station	Predator	Size class	Latitude	Longitude	Gear	Depth	Date	Time
PS107_007/5	*T. libellula*	2-3	79.058	3.752	BO	450	29.07.2017	03:16
TUNU_1376	*T. libellula*	2	79.251	-7.308	PT	NA	24.09.2017	11:29
TUNU_1278	*T. libellula*	2	77.371	2.154	PT	NA	15.09.2017	12:09
TUNU_1300	*T. libellula*	3	75.974	-21.717	BT	NA	18.09.2017	06:04
PS107_002/18	*T. libellula*	2	78.556	5.063	BO	60	26.07.2017	18:20
PS107_38/5	*T. libellula*	2	79.020	4.446	BO	60	11.08.2017	12:29
PS107_007/5	*T. abyssorum*	1	79.058	3.752	BO	450	29.07.2017	03:16
PS107_28/9	*T. abyssorum*	1	78.928	-4.583	BO	450	05.08.2017	15:00
PS107_002/7	*T. abyssorum*	1	78.608	5.057	MN	NA	26.07.2017	07:27
PS107_30/4	*T. abyssorum*	1-2	79.315	-2.010	BO	450	06.08.2017	21:30
PS107_45/10	*T. abyssorum*	1	79.009	8.227	BO	450	14.08.2017	07:00
PS100_002/4	*T. abyssorum*	1	75.113	8.541	BO	NA	20.07.2016	10:57

Themisto samples were collected during two Polarstern cruises in 2016 (PS100) and 2017 (PS107), and the Norwegian TUNUVII cruise with R/V Helmer Hansen (2017). Samples were either collected with bongo or multinets (Polarstern) and bottom or pelagic trawls (Helmer Hansen). They were stored in 96-100% ethanol after identification. The sampling stations for both species are shown in Fig. 2 and Tab.1.

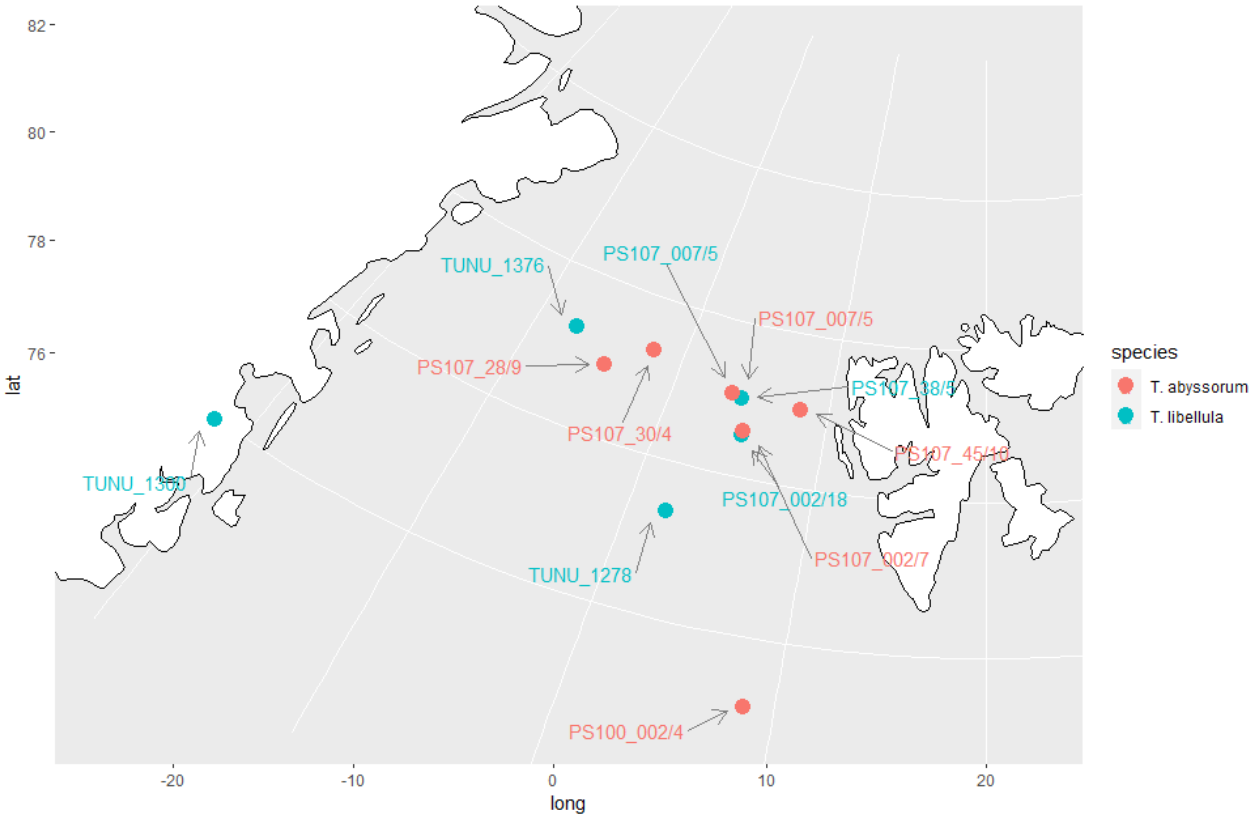

Fig. 2: Sampling sites of Themisto libellula and T. abyssorum in the Fram Strait.

2.2 DNA extractions and sequencing

Stomach contents of *T. libellula* and *T. abyssorum* were isolated. For the slightly larger *T. libellula*, 6-10 stomachs of individuals were pooled and for the smaller *T. abyssorum* 10-25 stomachs (following Siegenthaler et al. 2018; Ray et al. 2016). For each station, three replicates of these pooled stomach contents were taken. After taking out the stomach content, it was dried on a sterile wipe, since ethanol left in the tissue could inhabit the extraction reactions. DNA extractions were performed using the DNeasy PowerSoil Kit Handbook (Qiagen 2017). The collected tissues off 6-25 individuals were then transferred into the PowerBead Tubes provided in the kit. Those tubes already contained a buffer to protect nucleic acids from degradation, when lysing cell membranes (Qiagen 2017). Between each individual, the dissection instruments were cleaned using 70% ethanol and flamed off after. Before adding the tissue, the so-called solution C1 was added to the PowerBead Tube. This solution contains SDS for the complete cell lysis and breaking down fatty acids and lipids (Qiagen 2017). The PowerBead tubes were weighed before and after adding the stomachs. After adding the stomachs, the tubes were vortexed horizontally

using the Vortex-Genie2 (Scientific Industries, serial-no: 2-131313) with an adapter for 24 tubes at maximum speed for 10 minutes (Qiagen 2017). After the vortexing step the samples were centrifuged for 30s at 10,000 x g using the Eppendorf Centrifuge 5425 (serial-no: 5405II911013) (Qiagen 2017). After transferring 510µl of supernatant to a new 2ml collection tube provided in the kit, 250µl of the patented Inhibitor Removal Technology (IRT) within the so-called solution C2 was added (Qiagen 2017). This solution was used to precipitate cell debris and proteins. The sample was then vortexed for 5s using PV-1 (Grant-bio, serial-no: 01020319101906) and then incubated at 8-10°C for 5min in a Mixer HC (StarLab, serial-no: 9201N800986) (Qiagen 2017). After this incubation, the samples were centrifuged for 1min at 10,000 x g. Next, 600µl of the supernatant were transferred to a new 2ml collection tube and 200µl of another IRT solution (C3) were added to the sample (Qiagen 2017). This mixture was also vortexed briefly and again incubated for 5min at 8-10°C (Qiagen 2017). In the next step the samples were first centrifuged for 1min at 10,000 x g, before 700µl of the supernatant were transferred into a new 2ml collection tube (Qiagen 2017). Then, 1200µl of solution C4 were carefully added and the whole solution was vortexed briefly (Qiagen 2017). Solution C4 is a highly concentrated salt-solution, that should ensure that the DNA will bind to the MB Spin Columns. The samples were briefly centrifuged at low speed, to avoid sample droplets on the lid of the collection tube. Then, the sample was loaded onto the MB Spin Columns in three steps using each 675µl of the sample (Qiagen 2017). After loading the sample onto the MB Spin Column, the columns were centrifuged for 1min at 10,000 x g (Qiagen 2017). The flow-through was then discarded and a new round of sample was loaded (Qiagen 2017). When the entire sample was processed through the column, 500µl of solution C5 was added to the MB Spin Column and centrifuged for 30s at 10,000 x g (Qiagen 2017). Solution C5 washes the column and removes residual salt and other contaminants, while the DNA stays bound to the column (Qiagen 2017). After centrifuging, the flow-through was discarded and the columns were centrifuged again for 1min at 10,000 x g to ensure a wash out of all C5 (Qiagen 2017). Afterwards, the column was placed in a clean 2ml collection tube and 75µl of C6 was added to the column (Qiagen 2017). C6 is a sterile elution buffer (10mM Tris), that washes the DNA from the column (Qiagen 2017). Then the samples were incubated for 5min at room temperature, before centrifuging at 10,000 x g for 30s (Qiagen 2017). After the centrifuge step the column was discarded and the flow-through was kept, as this now contained the DNA (Qiagen 2017).

After the extraction, the DNA quantity of each sample was measured using the NanoDrop ND-1000 (PeqLab Biotechnologie GmbH, serial-no:6189), before freezing the samples at -20°C.

2.2 Library preparation

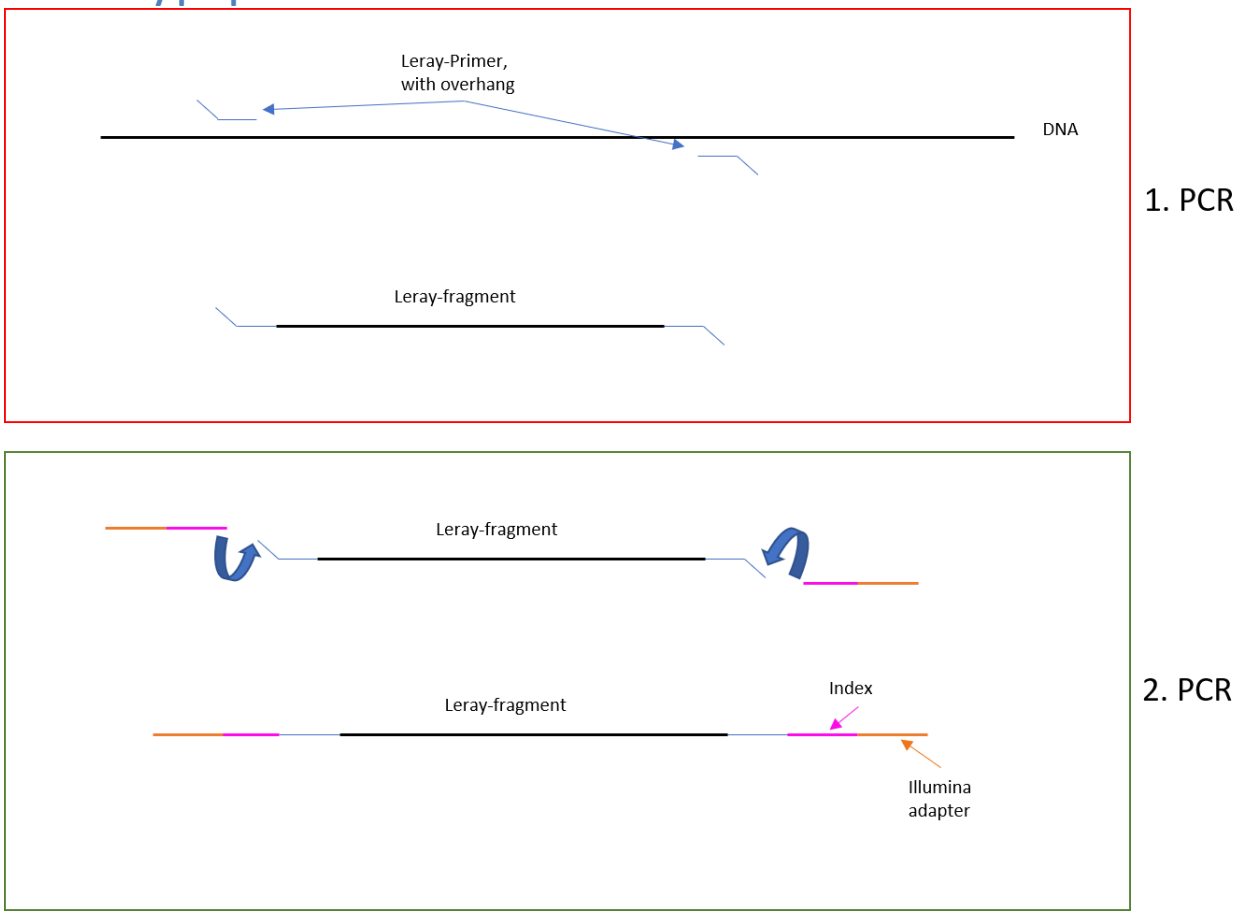

Fig. 3: Simplified presentation of the two PCR steps performed during the library preparation.

DNA extracts were sent to AllGenetics & Biology SL in Spain (www.allgenetics.eu), where the library preparation and sequencing was performed. For the library preparation, a two PCR approach was used (Fig. 3). According to AllGenetics the following described protocol was used for the library preparation and sequencing. In the first PCR step, the target DNA fragment was amplified. In this case a 313bp long fragment of COI was used, called Leray fragment (Leray et al. 2013). This fragment was already used in previous studies concerning the stomach content of coral reef fish and brown shrimp (Leray et al. 2013; Siegenthaler et al. 2018), as well as of *Themisto gaudichaudii* from the Southern Ocean (Havermans, unpublished results). It appeared to have a high resolution for a very broad spectrum of eukaryotes, including gelatinous zooplankton. For the first PCR a mlCOIintF-XT primer was used as forward primer

(5’ GGWACWRGWTGRACWITITAYCCYCC-3’) (Wangensteen et al. 2018) and jgHCO2198 was used as reverse primer (5’-TAIACYTCIGGRTGICCRAARAAYCA-3’) (Geller et al. 2013). Both are referred to as Leray-primers in the continuing explanation. To the 5’ end of the Leray-primers Illumina sequencing primers were attached as the overhang on the Leray-primer in PCR 1 (Fig. 3). For each sample, three replicates were created during the first PCR in order to increase the probability to also amplify rare DNA fragments (Mata et al. 2019). Those three replicates were also separately processed for the library preparation. The first PCR was carried out in a total volume of 25µl, containing 2.5µl of DNA, 0.5µM of the Leray-primers, 12.5µl of Supreme NZYTaq 2x Green Master Mix (NZYTech), and ultrapure water. Within the Green Master Mix, the polymerase and dNTPs were included. The temperature profile for the first PCR is shown in Fig. 4.

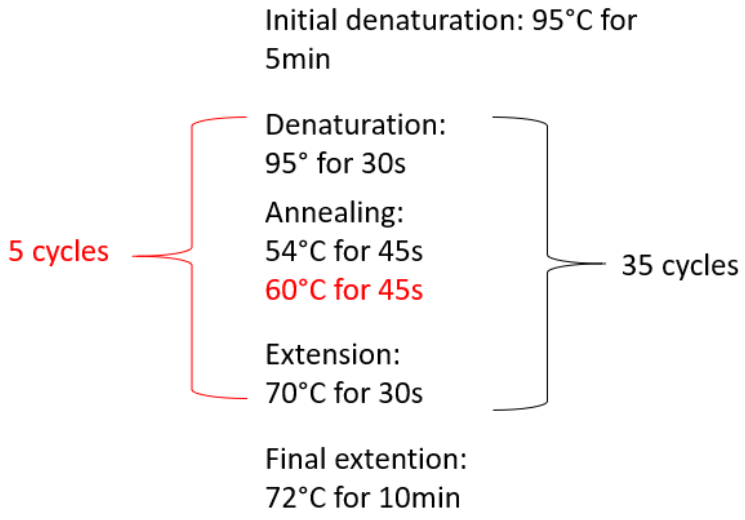

Fig. 4: PCR profile of the first and second PCR (changes for the second PCR marked in red).

In the second PCR oligonucleotides, were used as indices to enable multiplexing of different libraries. They are attached to the overhang of the Leray-primers, together with the Illumina adapters. The second PCR was carried out in the same volume as the first PCR (25µl). It contained 2.5µl of the first PCR product, 0.5µM of the indexed primers, 12.5µl of Supreme NZYTaq 2x Green Master Mix (NzYTech), which is a mix containing a taq-polymerase and dNTPs, and ultrapure water. The temperature profile for the second PCR is also shown in Fig. 4. During the whole library preparation process a negative control with no DNA was added to check for contamination. The second PCR was performed on all PCR replicates obtained from the first PCR, leading to a total number of 108 libraries.

To check the library size, 3µl of each library was run on a 2% agarose gel, which was stained with GreenSafe (NZYTech) and imaged under UV light. Again, a negative control containing no DNA was included to check for contaminants during the library preparation.

After verifying the library size, the libraries were purified using the Mag-Bind RXNPure Plus magnetic beads (Omega Biotek) following the instruction of the manufacturer. The libraries were then quantified using the Quibit High-Sensitivity dsDNA Assay kit (Thermo Fisher Scientific) and then equimolarly pooled. Subsequently, the pool was sequenced in 1/2 of a MiSeq PE300 run (Illumina).

2.3 Bioinformatics

The sequences were pre-processed by the sequencing facility: they were demultiplexed and sequencing primers and adapters had been removed. The quality of the sequences within each sample was checked using FastQC (version: 0.11.9) (Brown, Pirrung, and McCue 2017) for forward and reverse reads, separately. After that, forward and reverse reads were assembled for each sample using vsearch (version: v2.15.0_linux_x86_64) (Rognes et al. 2016), allowing 5-10 differences in the overlapping region of both reads with no Ns, reaching for at least 80% of assembled sequences. The minimum overlap was set to be 200bp. After the assembly, the Leray-primers were removed from each sample using cutadapt (version: 2.10. with python 3.8.5) (Martin 2011) with linked primers anchored at the 5' end. In the next step, the parts with low quality (quality score less than 30), were cut off using vsearch and then the sequences shorter than 200bp were sorted out again using vsearch. Finally, the sequences were dereplicated and clustered using vsearch by allowing 97% of identity within one cluster. The generated MOTUs (Molecular Operational Taxonomic Unit) were then assigned taxonomically using a local database created from NCBI GenBank data (Bethesda 2008) and BOLD (Barcode of Life Data System; Ratnasingham and Herbert 2007) data, using the blastn algorithm from NCBI (Camacho et al. 2009) and applying an identification threshold of >95%. Every MOTU that was assigned with less than 100% to the NCBI database, it was checked again using the BOLD database and was only considered correct if both assignments led to the same result. For further analysis only samples with at least 50 sequences belonging to prey organisms were considered (Jarman et al. 2013; McInnes et al. 2017). All taxa except algae and dinoflagellates, terrestrial mammals, insects and the predator itself were considered for further analysis. For the remaining

taxa identified as prey organisms, with at least 50 sequences per sample a threshold of 0.05% of the total dietary (non-predator) sequences for each sample was applied to determine an occurrence of a certain prey (Jarman et al. 2013; McInnes et al. 2017; Deagle et al. 2018).

2.4 Statistical analysis

All statistical analysis as well as data wrangling and presentation of all data were performed in RStudio (R version 4.0.2 (2020-06-22)). For data wrangling and presentation, the R package `tidyverse` (Wickham et al. 2019) was used and, tables were created using `flextable` (Gohel 2020). For the presentation of the data, relative read abundances (RRA) (Deagle et al. 2018), were calculated at the station and predator level, following eq. 1. This data was not used for the statistical analysis, since the abundance of reads in a sample is strongly biased by factors like binding success of primers, the digestion of the predator, duration in the stomach of the predator etc. (Deagle et al. 2018). For the statistical analysis, the MOTU-table (not shown) was transformed into a data set with presence/absence data, using the 0.05% threshold as definition for an occurrence of a certain prey item.

$$RRA = \frac{p}{T} * 100$$

Eq. 1: Equation for calculating RRA for either a station or a predator, with: p = reads for one prey item at either a station or for one predator, T = total food reads at either a station or for one predator

To identify whether the reads accomplished in the samples were sufficient to access the whole stomach content of the amphipods, rarefaction curves were created using the `vegan` package in RStudio (Oksanen et al. 2020).

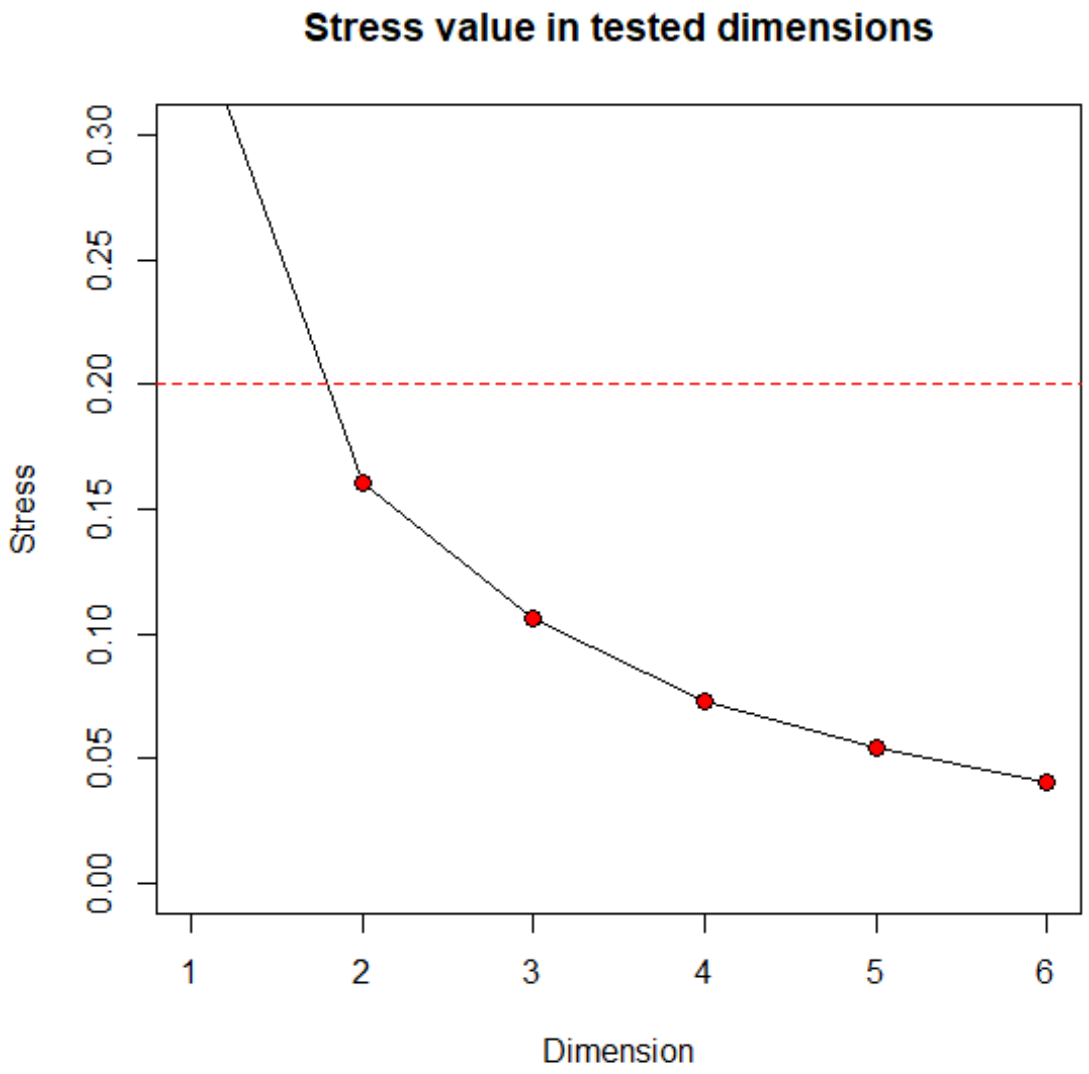

Fig. 5: Screeplot of both T. libellula and T. abyssorum, plotting stress over dimensions. The red line indicates the threshold of 0.2.

To access the differences between the two predators and within each predator species non-metric multidimensional scaling (NMDS) and a PERMANOVA were used. At first the best fit for the dimensions (k) was determined. Therefore, stress calculated within the NMDS model was plotted over the dimensions. A threshold of 0.2 stress was used for finding k, according to Clarke (1993) a stress of <0.2 can still lead to an interpretable picture. The screeplot and the NMDS were created using RStudio and the `vegan` package and were modified using the package `goeveg` (Oksanen et al. 2020; Goral and Schellenberg 2018). The screeplot for the whole dataset, including presence/absence data for both predators is shown in Fig. 5. This plot helped to find the

best fit for the dimensions (k), for the NMDS model for both predators. In this case k=2 was used for the model. For each single predator a similar screeplot was created, which can be found in the appendix. For *T. libellula*, two dimensions were the first k-value, with a stress below 0.2. On the other hand, for *T. abyssorum* one dimension lay also just below the threshold, but for the comparability the same k-value as for the model for *T. libellula* was used. Shephards diagrams were used to check for the goodness of fit of the NMDS model, these are shown in the appendix (Fig. 23, Fig. 25, Fig. 27). These were produced with the `vegan` package and the function `stressplot()`. The screeplots for each NMDS can be found in the appendix (Fig. 24, Fig. 26).

The influence of either the predator species or the location (sampling station) on the prey spectrum was analyzed by using a PERMANOVA. The PERMANOVA was calculated using `adonis` from the package `vegan` in RStudio (Oksanen et al. 2020) with the Jaccard's similarity and 1,000 permutations according to the suggestion in Siegenthaler et al. (2018). A PERMANOVA follows the assumption that there is homogeneity of the multivariate dispersion in the sample, therefore an ANOVA was calculated using the distance matrix using Jaccard's similarity and either the predator species or the location (station) as explanatory variable. The assumptions for the PERMANOVA are met if the ANOVA shows a p-value larger than 0.05.

3. Results

3.1 Evaluation of sequencing success

During the cleaning of the data, all samples with less than 50 sequences representing potential prey organisms were discarded. In general, a lot of predator and contaminant reads were sequenced in the samples (>95% in average). This means that for some stations less than three replicates were available. Accordingly, for *T. libellula* for PS107_007/5 and PS107_38/5, only two samples were left and for TUNU_1300 and PS107_002/18, only one sample was left. For the same reason, not all three PCR replicates could be used for certain samples. This was the case for TUNU_1300, for which only one of the PCR replicates contained more than 50 prey sequences. For *T. abyssorum*, less samples had to be discarded due to the threshold of 50 prey sequences. Only a couple of the PCR replicates contained less sequences this, was the case for samples from stations PS107_28/9 and PS107_30/4. For the former station, from two samples, one PCR replicate could not be used. For the latter, two of the three PCR replicates from one sample had to be removed.

The negative control, used during the sequencing procedure by AllGenetics, contained sequences for *T. libellula*, *T. abyssorum* and flies.

Tab.2: Reads achieved for all samples. Reads for contaminants like insects, phytoplankton, human or other terrestrial mammals are not listed.

Sample	Reads total	Reads predator	Reads prey	MOTUs prey	% predator reads
TliZ001-1	40366	40266	0	0	99.75227
TliZ001-2	45232	45111	7	3	99.73249
TliZ001-3	47938	47824	1	1	99.76219
TliZ003-1	33625	33001	514	8	98.14424
TliZ003-2	39590	38952	529	9	98.38848
TliZ003-3	33958	33456	435	9	98.52170
TliZ004-1	29706	28475	1063	18	95.85606
TliZ004-2	53072	50954	1861	16	96.00920
TliZ004-3	37844	36434	1230	13	96.27418
TliZ005-1	45146	33168	6187	17	73.46830
TliZ005-2	39539	30415	4702	22	76.92405
TliZ005-3	47422	36392	5903	18	76.74075
TliZ006-1	34941	31991	177	4	91.55720
TliZ006-2	53769	50012	192	5	93.01270
TliZ006-3	39005	36210	181	7	92.83425
TliZ007-1	42141	34106	2018	14	80.93306
TliZ007-2	37396	30563	1795	14	81.72799
TliZ007-3	40341	33311	1769	14	82.57356
TliZ008-1	53170	46619	1501	5	87.67914
TliZ008-2	40881	35818	1163	9	87.61527
TliZ008-3	43564	37887	1384	9	86.96860
TliZ009-1	41345	37091	2388	9	89.71097
TliZ009-2	44270	39422	2659	7	89.04902
TliZ009-3	62326	55729	3779	7	89.41533
TliZ010-1	41420	39262	1039	11	94.78996
TliZ010-2	44192	42002	1203	11	95.04435
TliZ010-3	51092	48826	1128	5	95.56486
TliZ011-1	38188	38156	14	8	99.91620
TliZ011-2	35891	35861	20	9	99.91641
TliZ011-3	41787	41762	19	4	99.94017
TliZ012-1	42699	42657	26	7	99.90164
TliZ012-2	37430	37369	33	10	99.83703
TliZ012-3	44462	44428	17	2	99.92353
TliZ013-1	45005	44962	4	3	99.90446

Sample	Reads total	Reads predator	Reads prey	MOTUs prey	% predator reads
TliZ013-2	39565	39537	4	2	99.92923
TliZ013-3	50100	50079	2	2	99.95808
TliZ014-1	43448	42799	19	2	98.50626
TliZ014-2	56871	56066	37	4	98.58452
TliZ014-3	54033	53384	24	5	98.79888
TliZ015-1	37760	36739	534	10	97.29608
TliZ015-2	50580	49248	678	11	97.36655
TliZ015-3	47386	45439	564	9	95.89119
TliZ016-1	34911	34332	299	8	98.34150
TliZ016-2	33430	32968	198	4	98.61801
TliZ016-3	51819	51149	275	4	98.70704
TliZ017-1	38772	38627	103	2	99.62602
TliZ017-2	47841	47698	106	2	99.70109
TliZ017-3	72168	71926	189	3	99.66467
TliZ018-1	48016	47814	36	6	99.57931
TliZ018-2	39714	39560	20	3	99.61223
TliZ018-3	62169	61801	166	16	99.40807
TliZ019-1	41312	41265	2	2	99.88623
TliZ019-2	44735	44646	4	2	99.80105
TliZ019-3	66241	66183	5	1	99.91244
TabZ002-1	25619	24967	495	6	97.45501
TabZ002-2	29401	28647	582	7	97.43546
TabZ002-3	30643	29705	524	11	96.93894
TabZ021-1	33459	31395	1735	9	93.83126
TabZ021-2	38119	35672	1930	10	93.58063
TabZ021-3	32777	30699	1694	9	93.66019
TabZ022-1	30348	24407	4903	15	80.42375
TabZ022-2	24616	19848	4111	14	80.63048
TabZ022-3	24404	19530	4246	21	80.02786
TabZ023-1	30102	29691	67	6	98.63464
TabZ023-2	37950	37426	143	7	98.61924
TabZ023-3	44569	44071	149	7	98.88263
TabZ024-1	31787	31429	43	4	98.87375
TabZ024-2	33125	32699	75	4	98.71396
TabZ024-3	33293	32997	53	6	99.11092
TabZ025-1	42355	41891	53	7	98.90450
TabZ025-2	33168	32849	63	6	99.03823
TabZ025-3	30349	30054	31	8	99.02797
TabZ026-1	37383	16710	18412	18	44.69946
TabZ026-2	29531	13245	14791	15	44.85117

Sample	Reads total	Reads predator	Reads prey	MOTUs prey	% predator reads
TabZ026-3	36563	16787	17920	21	45.91253
TabZ027-1	38319	9513	27901	34	24.82580
TabZ027-2	30838	7412	22575	23	24.03528
TabZ027-3	38575	9136	27876	20	23.68373
TabZ028-1	24875	9828	12133	16	39.50955
TabZ028-2	36011	13217	18198	26	36.70267
TabZ028-3	32723	12367	6997	26	37.79299
TabZ029-1	34398	34020	95	2	98.90110
TabZ029-2	31801	31438	72	3	98.85853
TabZ029-3	33461	33150	89	2	99.07056
TabZ030-1	24467	24046	128	6	98.27931
TabZ030-2	34710	34088	163	5	98.20801
TabZ030-3	31586	31155	162	7	98.63547
TabZ031-1	35578	35470	10	4	99.69644
TabZ031-2	38830	38662	63	7	99.56734
TabZ031-3	27672	27603	5	2	99.75065
TabZ032-1	33690	24157	6288	13	71.70377
TabZ032-2	33686	24031	6344	13	71.33824
TabZ032-3	40229	29324	7298	15	72.89269
TabZ033-1	23500	17477	3767	14	74.37021
TabZ033-2	24888	18204	4049	13	73.14368
TabZ033-3	27665	20342	4556	15	73.52973
TabZ034-1	33681	28875	4264	16	85.73083
TabZ034-2	35499	30886	4153	17	87.00527
TabZ034-3	31884	24356	3811	12	76.38941
TabZ036-1	39525	39158	190	5	99.07147
TabZ036-2	32439	32180	96	2	99.20158
TabZ036-3	32305	31964	141	4	98.94444
TabZ037-1	27148	26830	182	3	98.82864
TabZ037-2	26038	25747	184	5	98.88240
TabZ037-3	42178	41766	246	4	99.02319
TabZ038-1	37917	37331	63	3	98.45452
TabZ038-2	35611	35136	116	3	98.66614
TabZ038-3	34163	33635	79	3	98.45447

In all sequences variations in the achieved reads could be observed (Tab. 2). For *T. libellula* at minimum 0 prey reads were achieved and at maximum 5903. For the further analysis only samples with at least 50 prey reads were considered. The numbers of MOTUs recovered in the

samples were also differing between 0 and 22. For *T. abyssorum* the reads achieved varied as well, here at minimum 5 prey reads were recovered and at maximum 27901. Here also different numbers of MOTUs were defined varying between 2 and 34.

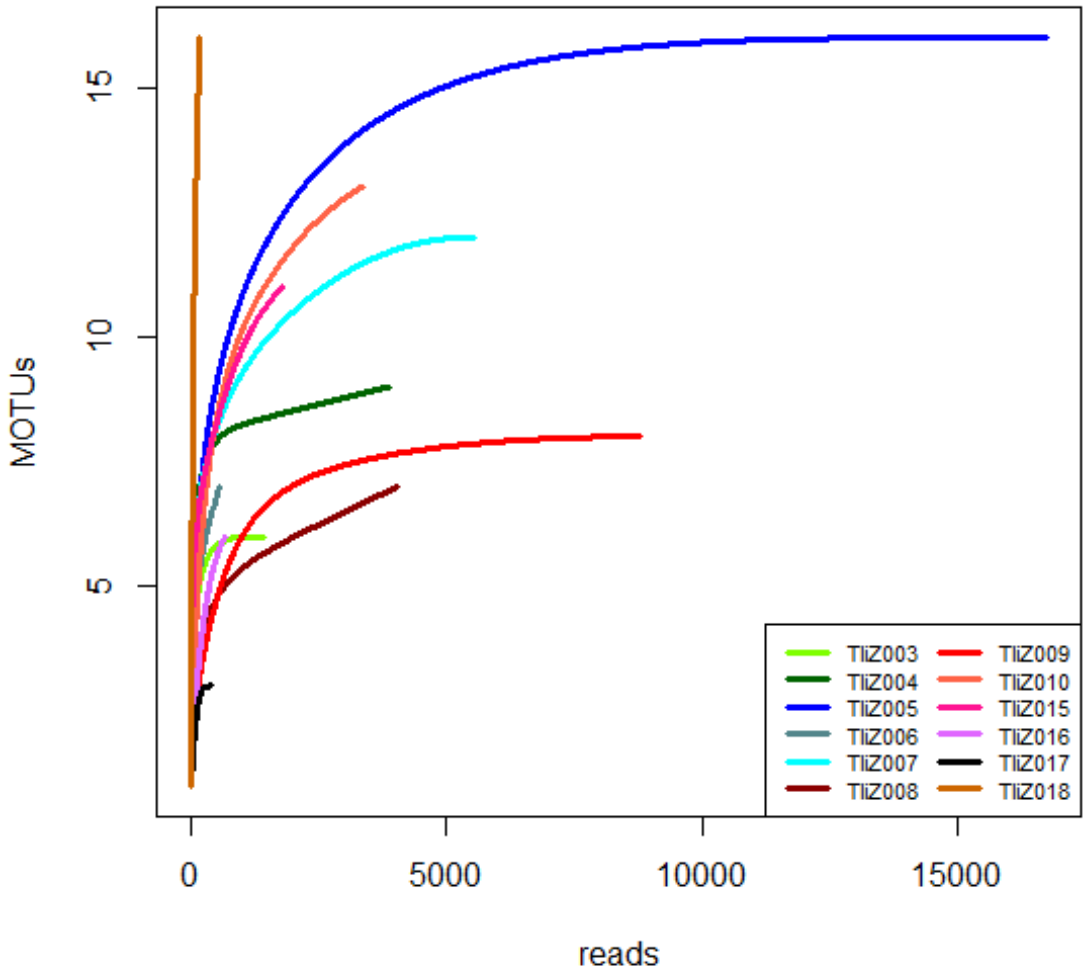

Fig. 11: Rarefaction curves of T. libellula for each sample (n=12). Plotting number of MOTUs over number of reads.

In Fig. 11, rarefaction curves for the samples of *T. libellula* are shown, plotting the number of Molecular Operational Taxonomic Unit (MOTUs) over the amount of reads in the samples. The number of MOTU increased exponentially for each sample with some reaching a plateau. For some samples, the plateau was not reached, for example for TliZ006, TliZ015 and TliZ016. This was particularly so for the rarefaction curve of TliZ018, which did not reach a plateau and increased steeply. This was caused by low numbers of achieved reads but a high number of MOTUs detected in the sample.

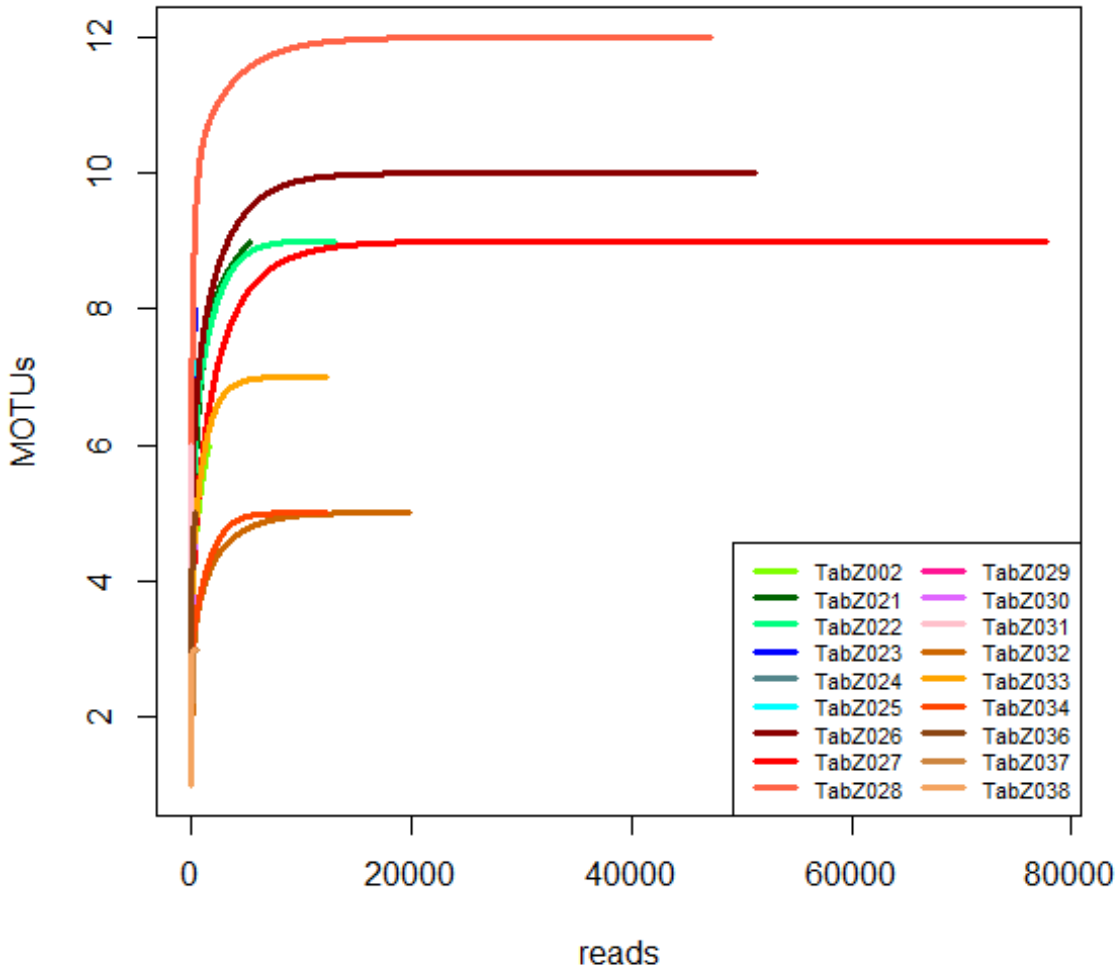

Fig. 12: Rarefaction curves of T. abyssorum for each sample (n=18). Plotting number of MOTUs over number of reads.

In Fig. 12, the rarefaction curves for all samples of *T. abyssorum* are shown. All curves showed an exponential increase before reaching a plateau. An exception is sample TabZ021, where the plateau was not reached. The amount of MOTUs found in the samples was strongly differing between the samples. For example, in the samples TabZ032 and TabZ034 about five MOTUs were found, while in sample TabZ028, 12 different MOTUs were found (Fig. 12).

3.2 Differences in the diet composition between *T. libellula* and *T. abyssorum*

The relative read abundance (RRA) of different prey groups was compared between the two predators, *T. libellula* and *T. abyssorum* (Fig. 13). For *T. libellula*, reads of copepods such as *Oithona similis* or *Pseudocalanus minutus* and reads of bony fish such as *Boreogadus saida* and *Liparis fabricii* were most abundant among all samples. For *T. abyssorum*, reads of chaetognaths

(*Eukrohnia hamata*) and krill (*Thysanoessa longicaudata*) were most present. In both species a broad range of food items was found. The proportion of reads of calanoid copepods, i.e., *Calanus glacialis*, *C. hyperboreus* and *C. finmarchicus*, was low in both predators. Additionally, prey organisms that were abundant in the stomachs of the one predator were rare or absent in the stomachs of the other species. For example, chaetognaths were very abundant in the stomachs of *T. abyssorum*, but were rare for *T. libellula*, while reads of bony fish were dominating the diet in terms of reads for *T. libellula*, but rare for *T. abyssorum*.

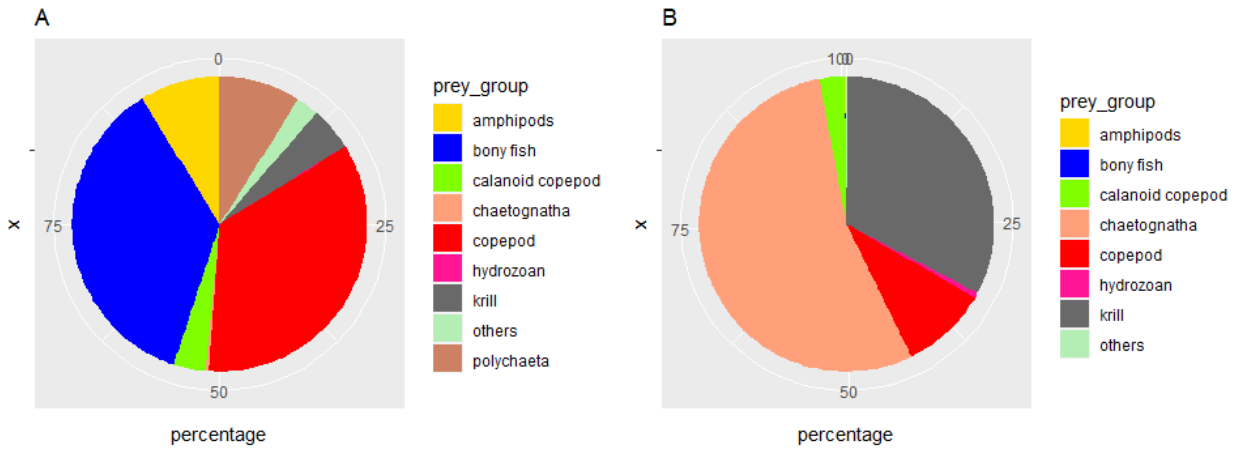

Fig. 13: RRA of different prey groups in the stomachs of the two predators (A: T. libellula; B: T. abyssorum).

In Fig. 14 the plot of the NMDS model for the two predators is shown. The two groups defined by the predators are clearly separated from each other. The PERMANOVA showed that the predator species has a significant influence ($p<0.05$) on the prey composition. The ANOVA showed that the assumptions for a PERMANOVA were met (ANOVA: $p>0.05$).

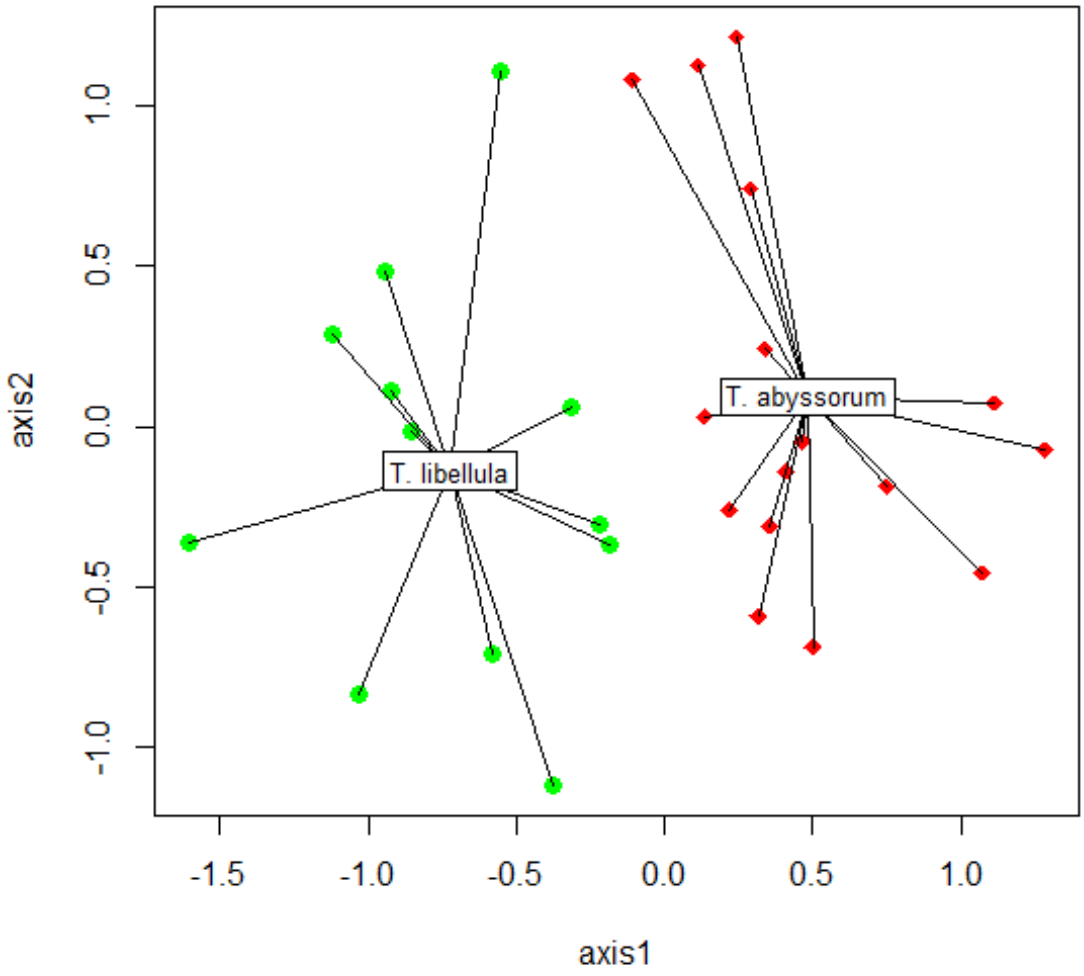

Fig. 14: NMDS for both predators using k=2 and stress=0.1602471

3.3 Spatial variation in the prey spectrum of *T. libellula* in the Fram Strait

In the next step, the prey spectrum of *T. libellula* and its link to the different sampling locations in the Fram Strait was investigated. The prey spectrum for each location is shown, after merging the results of the PCR replicates for the different samples. Additionally, the results of the PCR replicates are shown separately. In Tab. 3 the different prey organisms found in *T. libellula* (all samples combined) are shown. The most diverse prey groups were copepods and bony fish (Tab. 2). For other groups such as pteropods or chaetognaths, only one species was found in the samples. At some stations cnidarians including *Aglantha digitale*, *Obelia longissima* and *Nanomia cara*, were detected.

Tab. 3: Prey taxa found in T. libellula stomachs at different stations in Fram Strait (size classes: 1: <10mm; 2: 10-20mm; 3: >20mm).

Station	Size class	Phylum	Species
PS107_007/5	2-3	Copepoda	*Pseudocalanus minutus*
	2-3		*Calanus finmarchicus*
	2-3		*Calanus hyperboreus*
	2-3		*Metridia longa*
	2-3		*Calanus glacialis*
	2-3	Chaetognatha	*Eukrohnia hamata*
	2-3	Amphipoda	*Themisto abyssorum*
	2-3	Arthropoda	*Thysanoessa longicaudata*
	2-3	Pteropoda	*Clione limacina*
	2-3	Aves	*Alle alle*
TUNU_1376	2	Copepoda	*Calanus hyperboreus*
	2		*Pseudocalanus minutus*
	2		*Paraeuchaeta norvegica*
	2		*Calanus glacialis*
	2		*Calanus finmarchicus*
	2		*Oithona similis*
	2	Chaetognatha	*Eukrohnia hamata*
	2	Amphipoda	*Themisto abyssorum*
	2		*Gammarus wilkitzkii*
	2	Bony fish	*Boreogadus saida*
	2		*Liparis fabricii*
	2		*Sebastes mentella*
	2		*Anarhichas minor*
	2		*Triglops nybelini*
	2		*Lycodes pallidus*
	2		*Arctogadus glacialis*
	2	Arthropoda	*Thysanoessa longicaudata*
	2	Annelida	*Phyllodoce groenlandica*
	2	Cephalopoda	*Gonatus steenstrupi*
	2	Echinodermata	*Lophaster furcilliger*
	2	Cnidaria	*Catablema vesicarium*
TUNU_1278	2	Copepoda	*Oithona similis*
	2		*Pseudocalanus minutus*
	2		*Calanus finmarchicus*

Station	Size class	Phylum	Species
	2		*Metridia longa*
	2	Amphipoda	*Themisto abyssorum*
	2	Arthropoda	*Thysanoessa longicaudata*
	2	Bony fish	*Sebastes mentella*
	2		*Liparis fabricii*
	2		*Melanogrammus aeglefinus*
	2		*Amblyraja radiata*
	2	Cephalopoda	*Gonatus steenstrupi*
	2	Pteropoda	*Clione limacina*
	2	Annelida	*Phyllodoce groenlandica*
	2	Cnidaria	*Aglantha digitale*
	2		*Obelia longissima*
Ps107_38/5	2	Copepoda	*Calanus glacialis*
	2		*Paraeuchaeta norvegica*
	2		*Pseudocalanus minutus*
	2		*Calanus hyperboreus*
	2		*Calanus finmarchicus*
	2		*Oithona similis*
	2	Chaetognatha	*Eukrohnia hamata*
	2	Amphipoda	*Themisto abyssorum*
	2	Mollusca	*Hiatella sp.*
	2	Pteropoda	*Clione limacina*
	2	Arthropoda	*Thysanoessa longicaudata*
	2		*Thysanoessa inermis*
	2	Bony fish	*Boreogadus saida*
	2	Cnidaria	*Nanomia cara*
PS107_002/18	2	Copepoda	*Calanus finmarchicus*
	2	Amphipoda	*Themisto abyssorum*
	2	Pteropoda	*Clione limacina*
TUNU_1300	3	Copepoda	*Boroecia maxima*
	3		*Microcalanus pusillus*
	3		*Oithona similis*
	3		*Metridia longa*
	3		*Gaetanus tenuispinus*
	3	Chaetognatha	*Eukrohnia hamata*
	3	Amphipoda	*Themisto abyssorum*

Station	Size class	Phylum	Species
	3	Arthropoda	*Discoconchoecia elegans*
	3		*Thysanoessa longicaudata*
	3	Cephalopoda	*Rossia palpebrosa*
	3	Bony fish	*Boreogadus saida*
	3		*Salmo salar*
	3		*Arctogadus glacialis*
	3		*Lycodes pallidus*
	3		*Leptoclinus maculatus*
	3	Echinodermata	*Ophiopleura borealis*
	3		*Strongylocentrotus pallidus*
	3	Cnidaria	*Nanomia cara*

CTD data from PS100, PS107 were used to create temperature and salinity profiles (Fig. 15). Those profiles were used to show if a station was more under influence of Arctic vs. Atlantic water masses. For TUNUVII no CTD data was available for this study. Therefore, the dominating water mass can only be assumed based on the location of the stations. TUNU_1300 was located in the Bessel Fjord in Greenland. Since the EGC is transporting cold Arctic water southwards along the Greenland shelf (Khan et al. 2014; Sejr et al. 2017; Frederiksen et al. 2020), it can be assumed that this station as well as TUNU_1376 can be considered as Arctic stations. TUNU_1278 was located in the middle of the Fram Strait and it is difficult to distinguish whether it was an Arctic or Atlantic station. The PS107 stations were showing similar temperature and salinity profiles, with a warm water layer in the upper 500m. These stations were showing strong Atlantic influence and were therefore considered to be Atlantic station (Fig. 15).

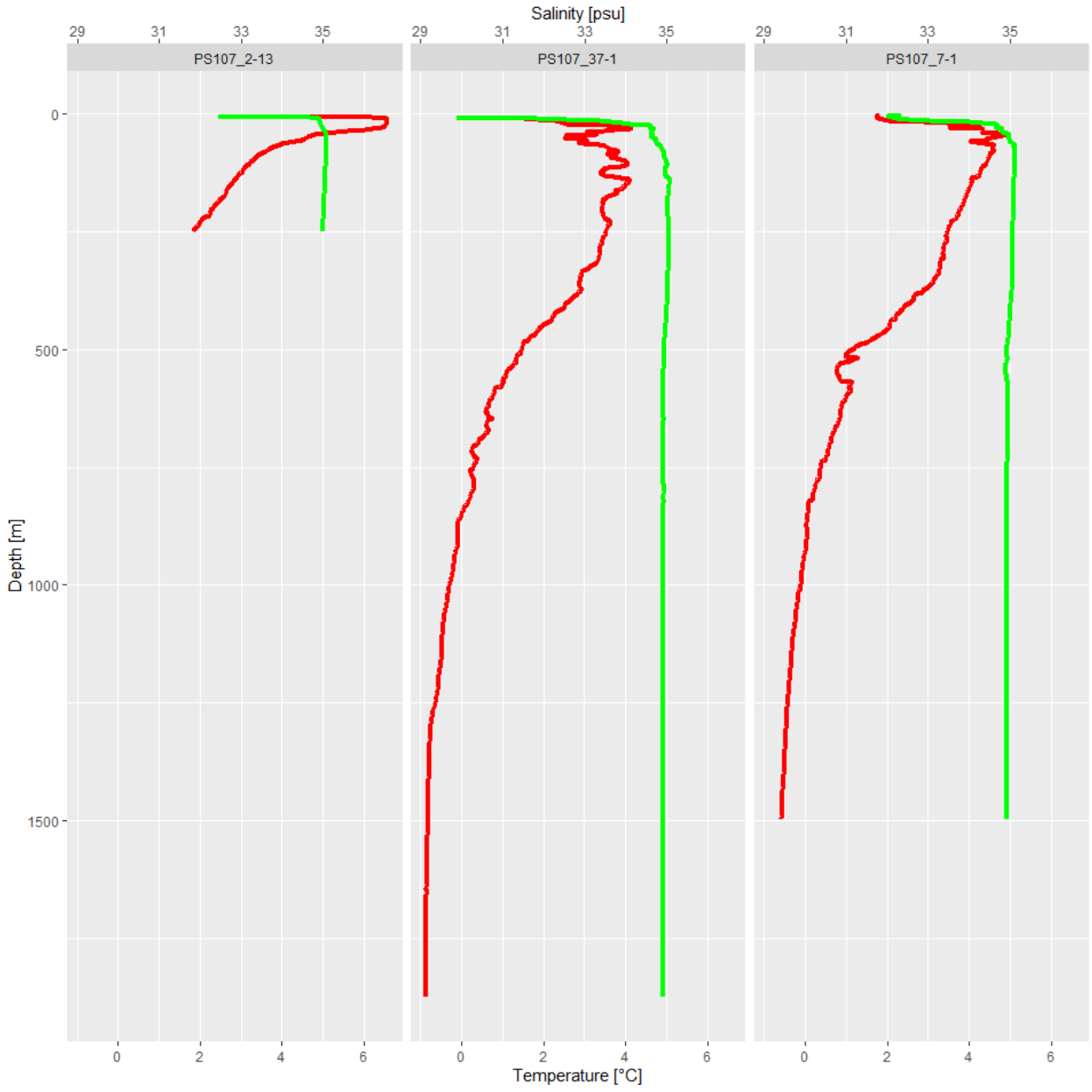

Fig. 15: Temperature (red) and salinity (green) profile of the stations for T. libellula, using potential temperature provided by CTD data.

The diet composition of each sample is shown in Fig. 16. Not all replicates of one station were showing the same prey composition. In all but one station, the dominating prey species was varying. Only for TUNU_1278, all replicates showed the same dominant species. At station PS107_002/18, the most abundant reads were belonging to *Calanus finmarchicus* (assigned to 99.68%), which was not the case for any of the other stations. At station TUNU_1278 and in the second sample of PS107_38/5, sequences belonging to *Oithona similis* (assigned to 100.00%) were the most abundant relative to the amount of all prey reads in the specific sample. At station

PS107_007/5 sample 1 was mainly dominated by reads of *Thysanoessa longicaudata* (assigned to 99.36%) and sample 2 is dominated by *Themisto abyssorum* (assigned to 99.36%). At station TUNU_1376, most reads in sample 1 belonged to *Boreogadus saida* (assigned to 100.00%) and in sample 2 and 3 to *Phyllodoce groenlandica* (assigned to 99.68%). *B. saida* was not found in the samples closer to the Svalbard shelf and was found in higher RRA at the station closer to Greenland. At station TUNU_1300, located in the Bessel fjord in Greenland, reads of *B. saida* (assigned to 100.00%) and other fish species as well as *Eukrohnia hamata* (assigned to 100.00%) and several copepod species were found. The samples from stations close to Svalbard showed a high variation in prey items, with the prey compositions between the samples differing strongly from each other. TUNU_1278, where the stomach content was dominated by *O. similis* (assigned to 100.00%) is located in the middle of the Fram Strait.

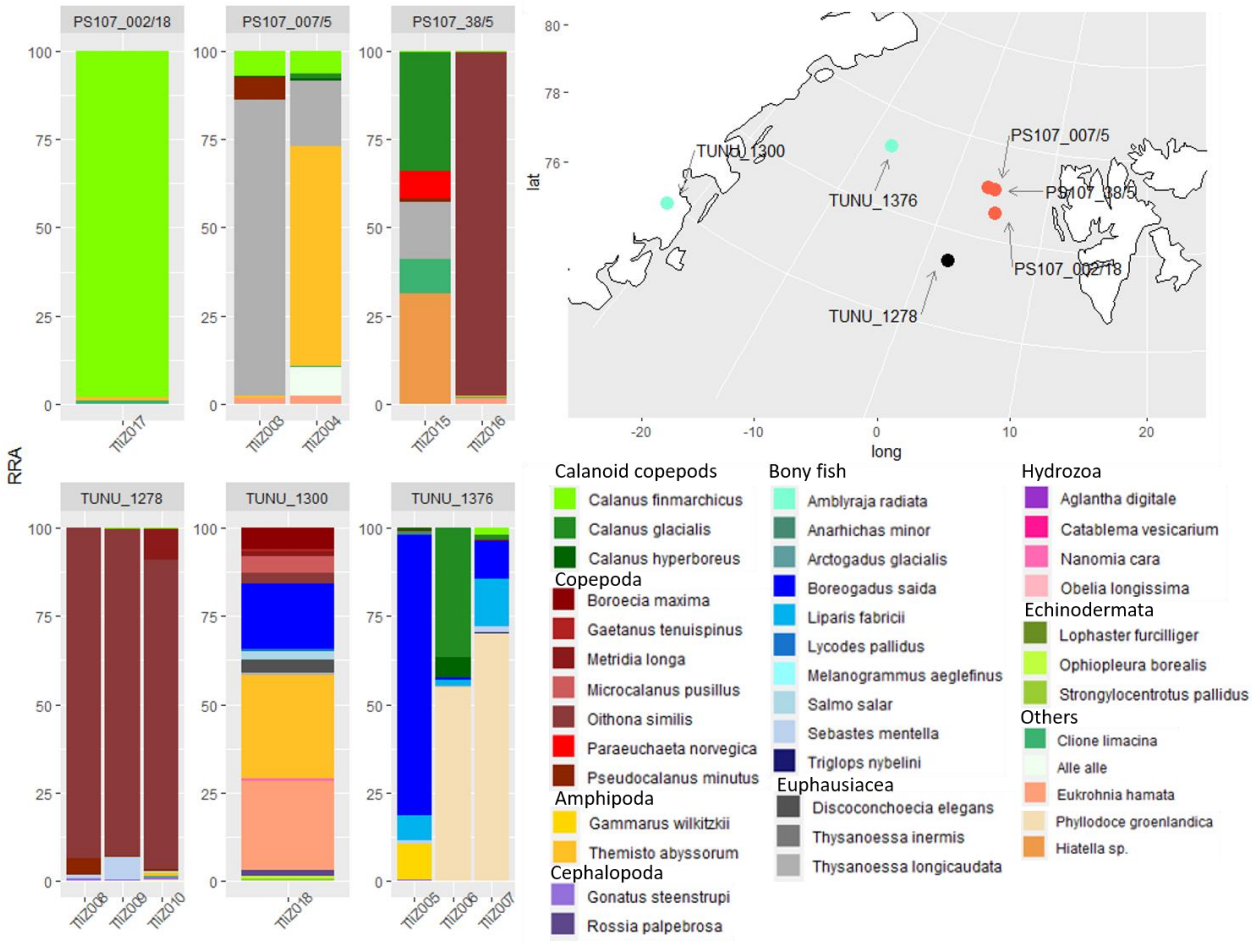

Fig. 16: Relative read abundance of prey organisms found in each sample of T. libellula. Atlantic stations in the map are marked red and Arctic stations blue.

PCR replicates for all samples of *T. libellula* are shown in Fig. 17. For each sample, three PCR replicates were performed to ascertain the amplifications of also the rarest prey sequences. The results showed that all PCR replicates per sample have similar species compositions, but in some samples (e.g., TliZ006 and TliZ017) additional species like *Liparis fabricii* (assigned to 100.00%) or *Clione limacina* (assigned to 99.68) were found, which were not present in the other replicates of the same sample. For nearly all samples, all three PCR replicates could be used, only for TliZ018 only one PCR replicate had sufficient prey reads.

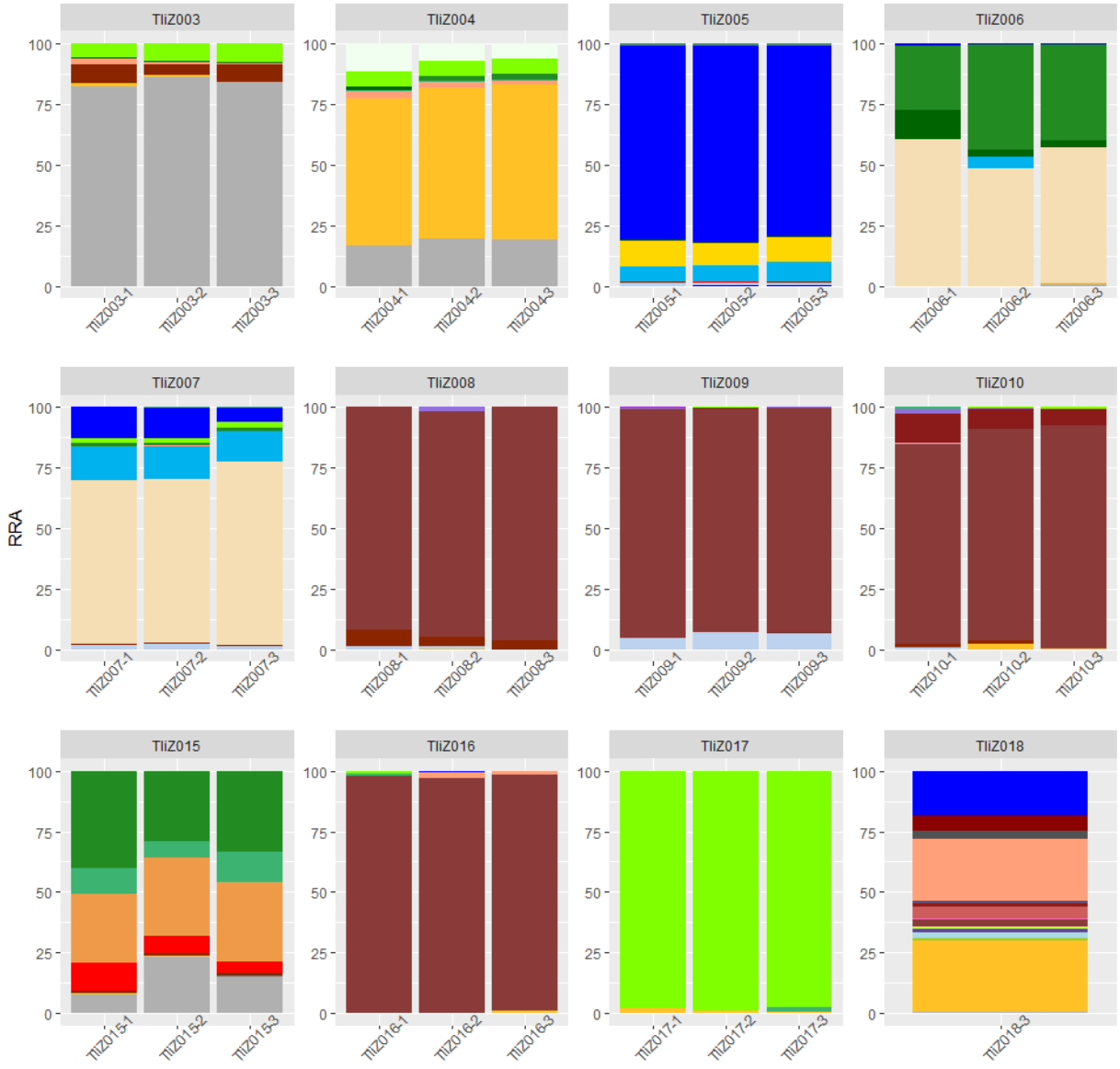

Fig. 17: RRA for each of the PCR replicates of the different T. libellula samples. Color coded as in fig. 14.

In order to show the difference in the prey spectrum between the different sampling localities, a NMDS model was produced (Fig. 18). From the NMDS model and the further statistical analysis the stations TUNU_1300 and PS107_002/18 were excluded, since for those stations only one sample was left after excluding all samples with less than 50 prey sequences. It was observed that the samples belonging to station TUNU_1278 were clustered together and were separated from the other samples. Station TUNU_1376 showed a higher variance between the samples and the signals for the different samples was widely spread. The blue and green dots, indicating station PS107_007/5 and PS107_38/5 respectively, were closer to each other and could be also clustered together.

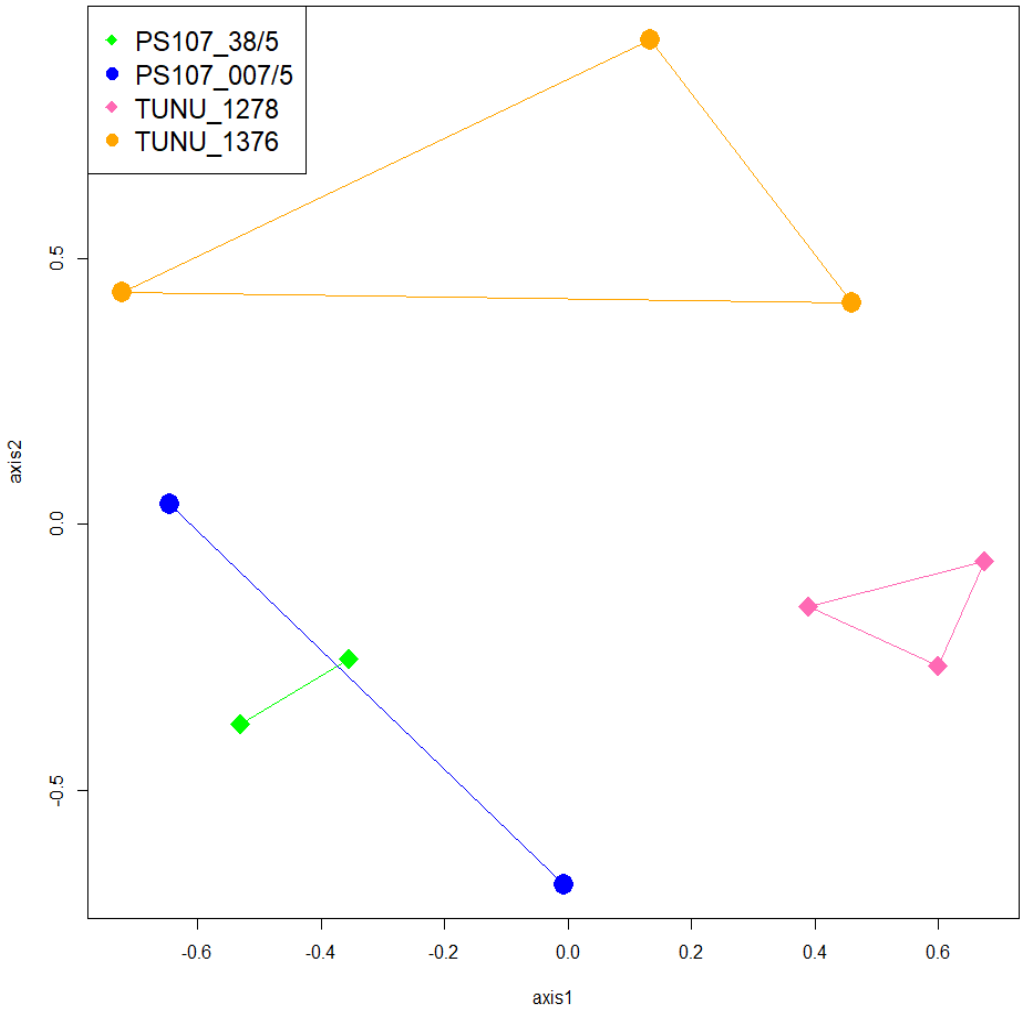

Fig. 18: NMDS plot for T. libellula samples. Colours are linked to the different locations, with k=2 and stress=0.09232075.

The PERMANOVA showed that the station, which was linked to a geographic location in the Fram Strait, had an influence on the prey composition ($p<0.05$). This p-value showed that the diet composition differed significantly between the different locations. The assumptions for the PERMANOVA were met after removing station TUNU_1300 and PS107_002/18 (ANOVA: $p>0.05$).

3.4 Spatial variation in the prey spectrum of *T. abyssorum* in the Fram Strait

In this section the prey composition of *T. abyssorum* linked to the different locations in Fram Strait is investigated. First, the results of the PCR replicates merged together for each sample are presented and are shown separately in a second step. The diet composition of the predator was also linked to the environmental conditions at the different stations, taking temperature and salinity into account to determine the dominant water masses (warmer and more saline Atlantic vs. colder and fresher Arctic waters) at the different stations.

Tab.4 lists the taxonomic assignments of the DNA reads found in stomachs of *T. abyssorum* at different stations. The individuals of *T. abyssorum* that were used for analysis were in general much smaller than *T. libellula*. The chaetognath *Eukrohnia hamata* (assigned to 99.36-100.00%) was found at all stations.

Tab. 4: Prey taxa found in T. abyssorum at different stations in Fram Strait (size classes: 1: <10mm; 2: 10-20mm; 3: >20mm).

Station	Size class	Phylum	Species
PS107_007/5	1	Copepoda	*Calanus finmarchicus*
	1		*Calanus hyperboreus*
	1		*Paraeuchaeta norvegica*
	1		*Oithona similis*
	1		*Calanus glacialis*
	1		*Metridia longa*
	1	Chaetognatha	*Eukrohnia hamata*
	1	Amphipoda	*Themisto libellula*
	1	Arthropoda	*Discoconchoecia elegans*
	1		*Thysanoessa longicaudata*
	1	Pteropoda	*Clione limacina*
	1	Aves	*Alle alle*
PS107_28/9	1	Copepoda	*Calanus glacialis*

Station	Size class	Phylum	Species
	1		*Calanus hyperboreus*
	1		*Calanus finmarchicus*
	1		*Pseudocalanus minutus*
	1		*Boroecia maxima*
	1		*Scolecithricella minor*
	1	Chaetognatha	*Eukrohnia hamata*
	1	Annelida	*Nereimyra aphroditoides*
	1	Amphipoda	*Themisto libellula*
	1	Echinodermata	*Ophiocten sericeum*
	1	Pteropoda	*Limacina helicina*
	1		*Clione limacina*
PS107_002/7	1	Copepoda	*Oithona similis*
	1		*Microcalanus pusillus*
	1		*Microcalanus pygmaeus*
	1		*Metridia longa*
	1		*Paraeuchaeta glacialis*
	1		*Scolecithricella minor*
	1		*Boroecia maxima*
	1		*Calanus finmarchicus*
	1		*Gaetanus tenuispinus*
	1	Chaetognatha	*Eukrohnia hamata*
	1	Arthropoda	*Discoconchoecia elegans*
	1		*Thysanoessa longicaudata*
	1	Amphipoda	*Themisto libellula*
	1	Pteropoda	*Clione limacina*
	1	Cnidaria	*Aglantha digitale*
	1		*Nanomia cara*
PS107_30/4	1-2	Copepoda	*Calanus hyperboreus*
	1-2		*Microcalanus pusillus*
	1-2		*Microcalanus pygmaeus*
	1-2		*Metridia longa*
	1-2		*Oithona similis*
	1-2	Chaetognatha	*Eukrohnia hamata*
	1-2	Amphipoda	*Themisto libellula*
	1-2	Arthropoda	*Thysanoessa inermis*
	1-2	Aves	*Alle alle*

Station	Size class	Phylum	Species
	1-2	Cnidaria	*Aglantha digitale*
PS107_45/10	1	Copepoda	*Calanus finmarchicus*
	1		*Metridia longa*
	1		*Oithona similis*
	1	Chaetognatha	*Eukrohnia hamata*
	1	Arthropoda	*Thysanoessa longicaudata*
	1		*Meganyctiphanes norvegica*
	1	Amphipoda	*Themisto libellula*
	1	Hydrozoan	*Physophora*
	1	Pteropoda	*Clione limacina*
PS100_002/4	1	Copepoda	*Calanus finmarchicus*
	1	Chaetognatha	*Eukrohnia hamata*
	1	Amphipoda	*Themisto libellula*
	1	Arthropoda	*Thysanoessa longicaudata*
	1	Bony fish	*Boreogadus saida*

CTD data from PS107 and PS100 were used to create temperature and salinity profiles to highlight the different water temperatures at the different locations (Fig. 19). For the stations PS107_002, PS107_45 and PS107_007 the temperature profile showed a similar pattern, with a layer of warm water in the upper 500m. Thus, these stations are impacted strong from Atlantic water. At the stations PS107_28 and PS107_30 a different pattern was found. Here, the upper layers were cooler, and a warm layer was found only at 250m. These stations can be considered as Arctic stations. For station PS100_002 no CTD data was available. Based on the location, further in the south of Fram Strait, it can be assumed that this is an Atlantic station.

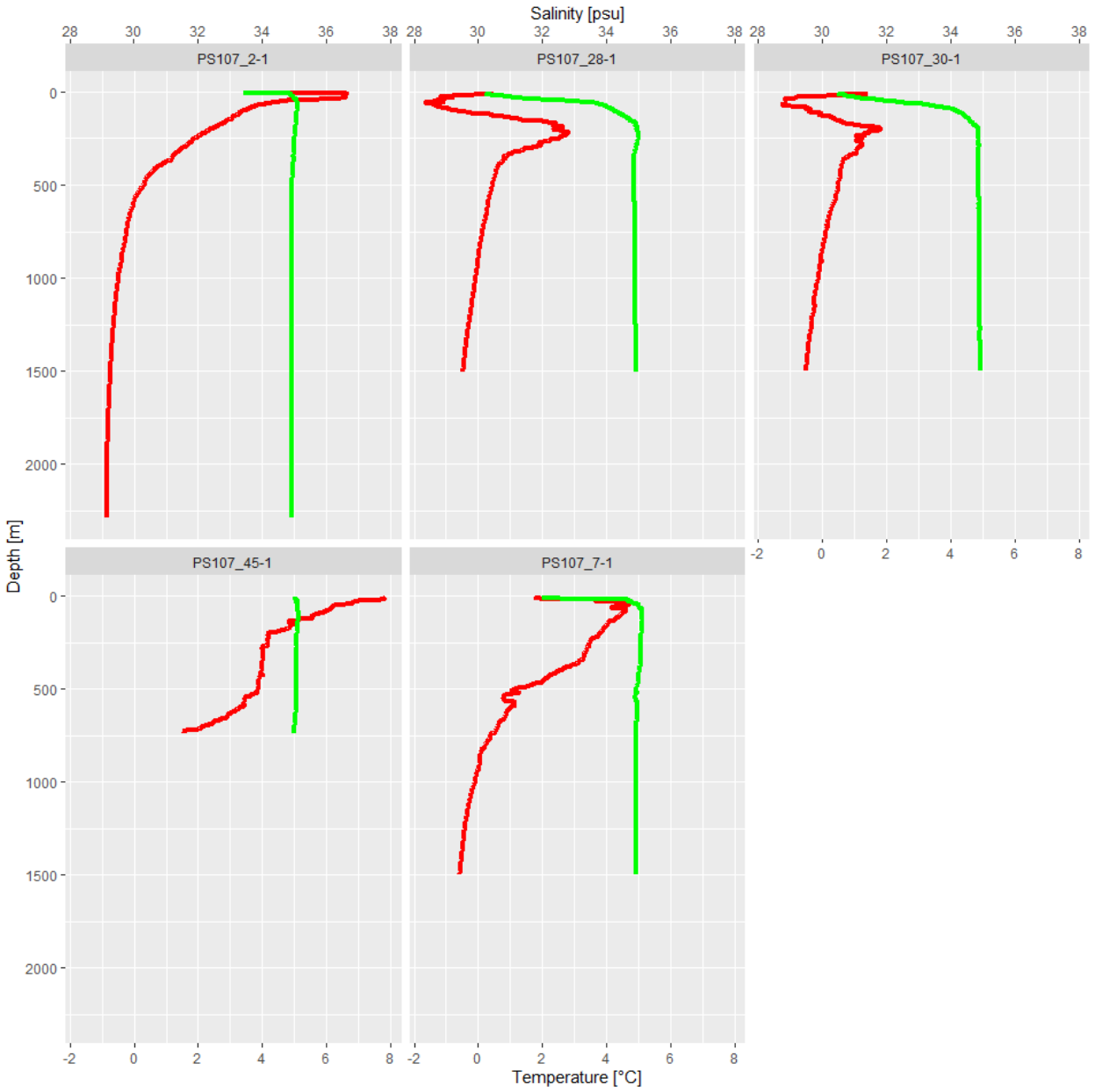

Fig. 19: Temperature (red) and salinity (green) profile of the stations for T. abyssorum, using potential temperature provided by CTD data.

In Fig. 20 the relative read abundances for the different prey organisms for each sample are shown. Diet composition for all three samples was similar for the stations PS100_002/4, PS107_007/5 and PS107_45/10. *Eukrohnia hamata* was dominating in all these samples. The second most abundant prey species at these stations was either *Calanus finmarchicus* (assigned to 99.681%) or *Thysanoessa longicaudata* (assigned to 99.36-99.68%). At the stations PS107_002/7, PS107_28/9 and PS107_30/4, not all samples showed the same prey species composition. For PS107_002/7, the sample 1 and 3 were nearly similar, while in sample 2 the

most relative abundant reads belonged to *T. longicaudata* (assigned to 99.36%). For station PS107_28/9 the samples 1 and 2 are similar, while in sample 3 the highest amount of reads among all prey reads belonged to *Clione limacina* (assigned to 100.00%). The three samples of PS107_30/4 differed completely from each other. In sample 1 of this station *Thysanoessa inermis* (assigned to 99.68%) showed the highest abundant reads. In sample 2 *Calanus hyperboreus* (assigned to 100.00%), *Themisto libellula* (assigned to 100.00%) and *Thysanoessa inermis* (assigned to 99.68%) represented similar proportions of the total abundances. In sample 3, the chaetognath *E. hamata* dominated the prey composition. Although station PS100_002 was located further south than the other stations, the prey composition did not differ from stations further north. The proportion of reads belonging to *C. finmarchicus* was in general higher at stations closer to Svalbard compared to stations offshore and closer to the Greenland shelf.

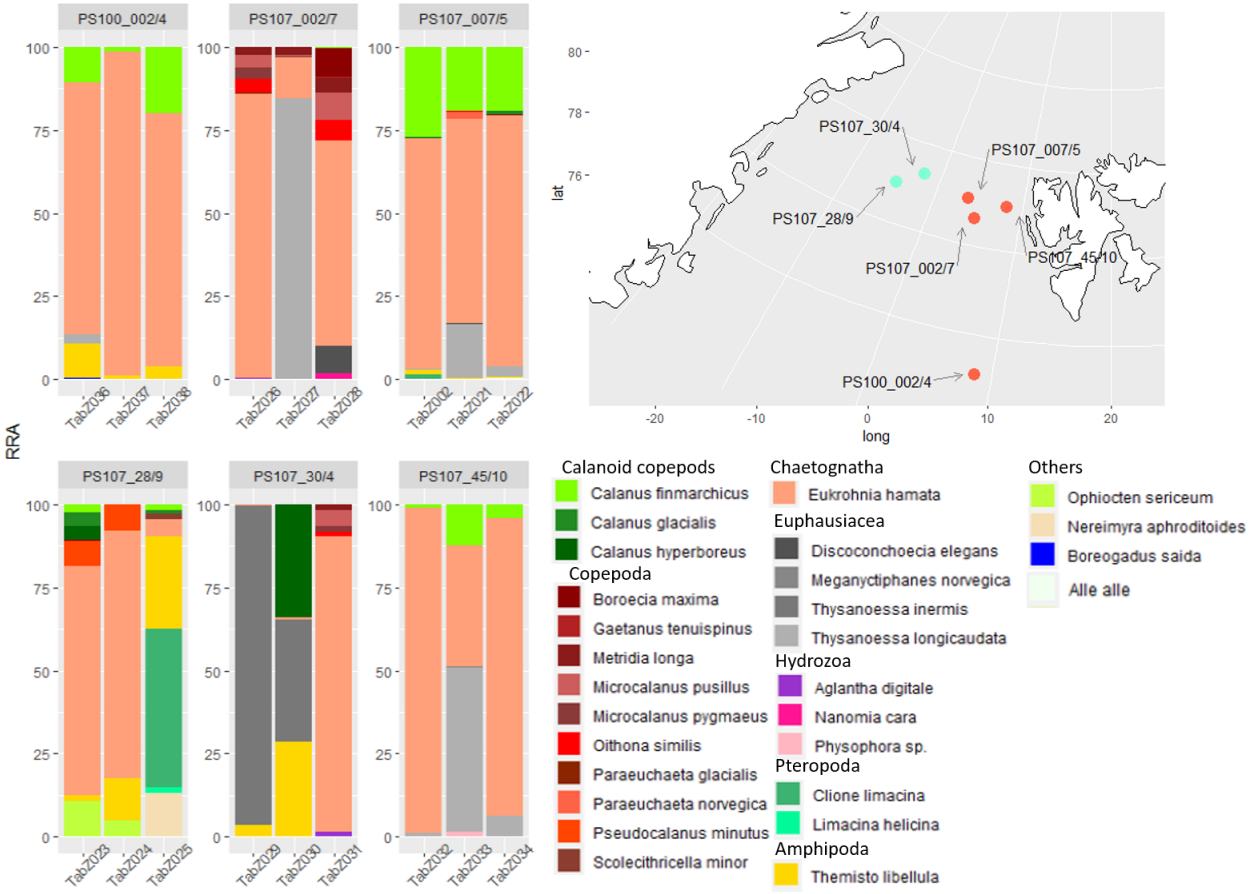

Fig. 20: Relative read abundance of prey organisms found in each sample of T. abyssorum. Atlantic stations in the map are marked red and Arctic stations blue.

The relative read abundances for the PCR replicates are shown in Fig. 21. In general, most of the replicates were similar in terms of species composition, which was dominated by *E. hamata*. Some replicates were dominated by krill species like *T. longicaudata* and *T. inermis*.

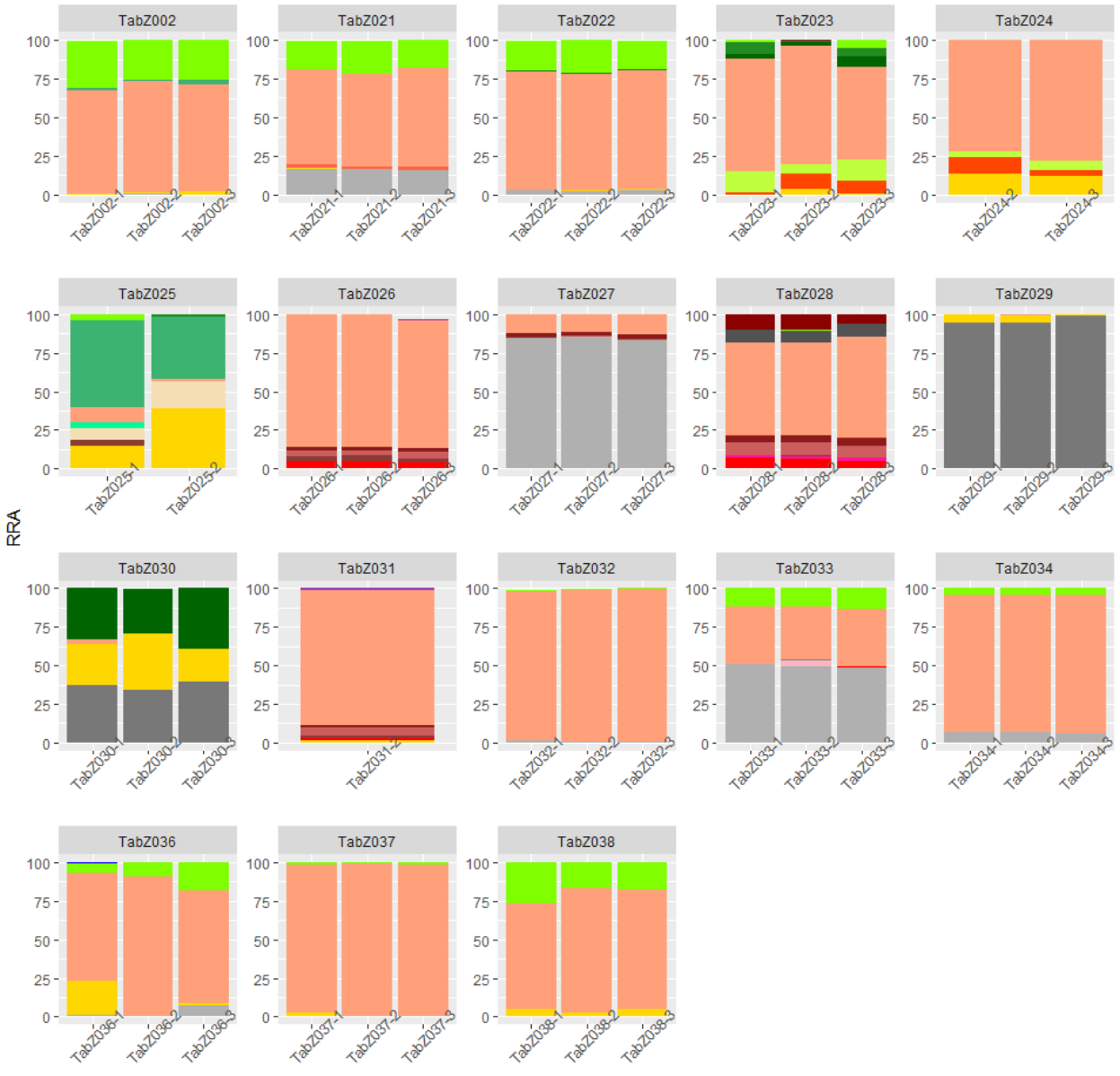

Fig. 21: RRA for all PCR replicates for all samples of T. abyssorum. Color coded as in Fig. 18.

Fig. 22 shows the NMDS plot for all *T. abyssorum* stations using 2 dimensions with a stress of 0.117193. All stations were distinct and clearly separated from each other. Only stations PS100_002/4, PS107_45/10 and PS107_007/5 were closer to each other, indicating that they were less different from each other compared to the other locations in the Fram Strait.

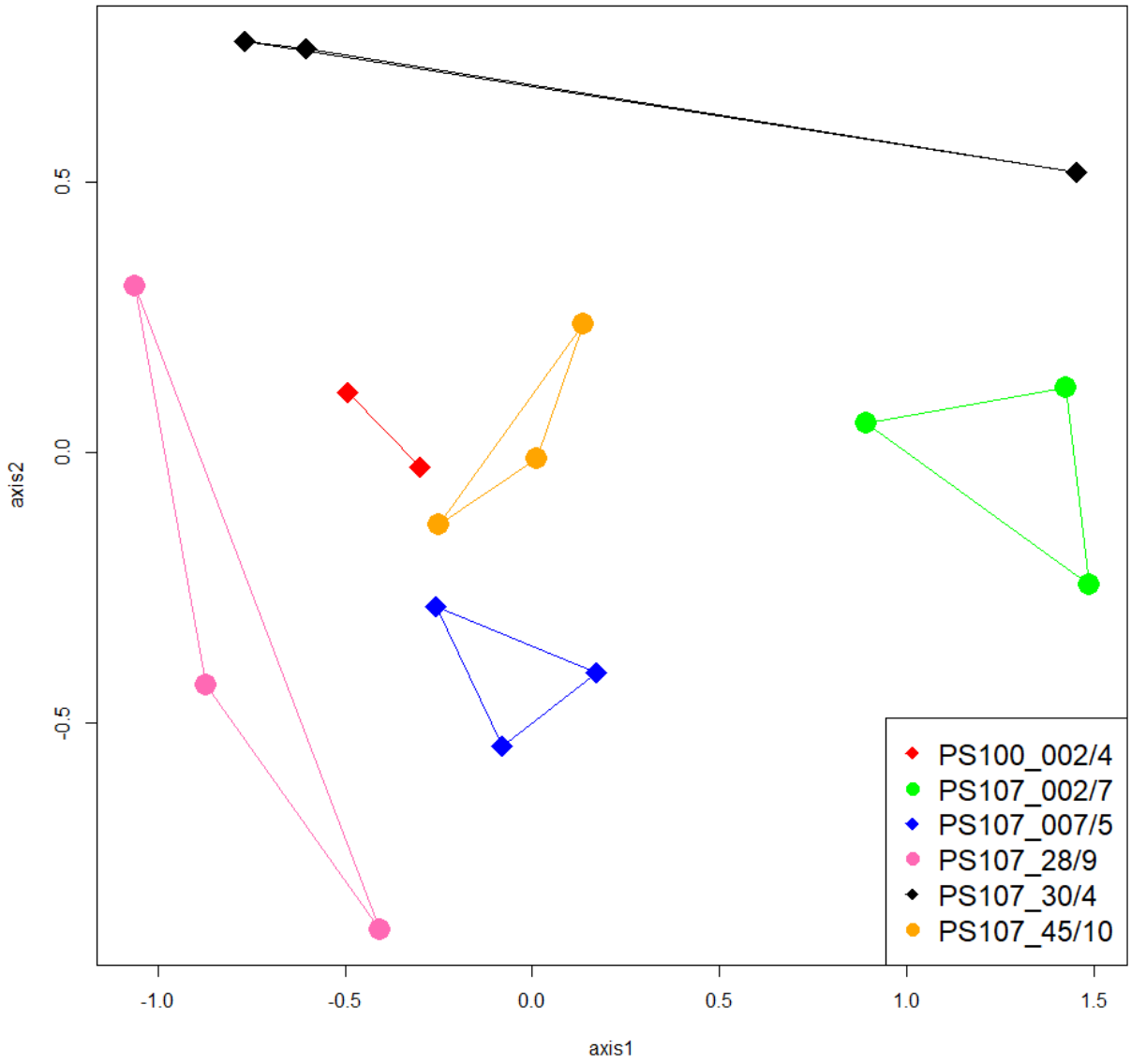

Fig. 22: NMDS for T. abyssorum, colours are linked to the different locations, with k=2 and stress=0.1284792.

The PERMANOVA calculated for these samples showed a significant difference between the prey compositions linked to the different stations ($p>0.05$). This showed, as already seen in Fig. 18, that the station or location influenced the diet composition of both *T. abyssorum* and *T. libellula*. Here, the assumptions for the PERMANOVA were also met (ANOVA: $p>0.05$).

4. Discussion

The two hyperiid amphipods *T. libellula* and *T. abyssorum* are key species in the Arctic, pelagic food web, being important predators (Auel et al. 2002; Hop et al. 2006), but also known as an important food source for higher trophic levels like fish species (Dempson, Shears, and Bloom 2002), seabirds (Pedersen and Falk 2001) and marine mammals (Nilssen et al. 1995; Hop et al. 2006; Lowry and Frost 1984). In a changing Arctic Ocean, it becomes crucial to gain more knowledge about the prey of *T. libellula* and *T. abyssorum*, since it is likely that the zooplankton community will change with warming and further Atlantification of the Arctic Ocean (e.g., Søreide et al. 2010; Hirche and Kosobokova 2007; Degen et al. 2016). Previous studies concerning the diet of *Themisto* amphipods were mainly based on microscopy or biomarkers (Pakhomov and Perissinotto 1996; Auel et al. 2002; Olsen et al. 2013; Kohlbach et al. 2016), whereas in this study DNA metabarcoding was used to assess the prey spectrum of the two predators. With this method, a more precise taxonomic assignment of prey organisms can be achieved (Auel et al. 2002). This approach can also detect highly digested and soft-bodied prey, which are often overlooked in studies using a traditional microscopic approach (Olsen et al. 2013). An advantage of biomarker analyses is the possibility to gain an insight in the long-term diet of an organism, while with metabarcoding only a snapshot of the diet can be shown (Pompanon et al. 2012). Therefore, more sampling throughout the year is necessary for a more complete picture of the diet.

4.1 Differences in the diet composition between the two predators

The prey spectra of *T. libellula* and *T. abyssorum* and their respective dominant prey taxa clearly differ (Fig. 13). These findings support the first hypothesis assuming that the prey spectrum of the two predators is differing, even at the same location, since the PERMANOVA showed a significant difference. Such a differing diet for the two predators was already shown by Auel et al. (2002), who found that the two amphipods are occupying different niches in the Arctic ecosystem.

In both predators the RRAs for calanoid copepods were low, although previous studies showed that calanoid copepods make up a major part of the diet of *T. libellula* (Auel et al. 2002; Kohlbach et al. 2016). The samples tested in this study, were collected between July and

September and are therefore only showing the prey spectrum during this period in the Fram Strait. It is possible that the prey spectrum is different for other seasons.

Besides the low proportions of calanoid copepods in the two predators, many differences could be seen in the diet of *T. libellula* and *T. abyssorum*. The diet of *T. libellula* showed in general a high proportion of bony fish and different copepod species like *Metridia longa* and *Pseudocalanus minutus*, respectively. This was not found for *T. abyssorum* for which the diet was dominated by chaetognaths and krill species. This difference can be caused by feeding preference, but also by the size difference of the two predators. Therefore, larger prey items like fish larvae, may have been too large for the small *T. abyssorum* (<10mm). Dalpadado (2002) found that *T. abyssorum* within this size range are mostly juveniles or females. If the tested *T. abyssorum* were juveniles, the differences in the prey composition can also be caused by the different life stages compared. Larger *T. abyssorum* may feed on other, larger prey species (e.g., fish larvae) than the smaller ones in this study. The individuals of *T. libellula* investigated were mainly between 10-20mm and some even larger. Therefore, larger prey items like polar cod larvae and krill might be more easily to catch and handle for those predators.

One prominent difference in the prey compositions of the two amphipods was mainly found when looking at the role of the chaetognath *E. hamata*. For *T. abyssorum*, high RRAs of this chaetognath were found in almost all samples, while this was certainly not found for *T. libellula*, even at stations where both predators were sampled (e.g., PS107_002 and PS107_007). *T. abyssorum* was mainly sampled with deep Bongo hauls with up to 450m depth, while *T. libellula* was collected at varying depths between shallow (60m) hauls and deep (450m) hauls. Depth segregation between the two predators (Dalpadado et al. 2001) could also explain the differences in the prey spectrum. Grigor, Schmid, and Fortier (2017) investigated the reproduction and growth of *E. hamata* in the Canadian Arctic and showed that young chaetognaths were found throughout the water column in the Amundsen Gulf and newborns were mainly found at 300m depth (Grigor, Schmid, and Fortier 2017). Due to the small size of the tested *T. abyssorum* it can be assumed that it fed on the larvae of *E. hamata*. Dalpadado et al. (2001) found that *T. abyssorum* prefers deeper depths, while *T. libellula* preferred shallower waters. This could mean that *E. hamata* was not available for *T. libellula* due to depth segregation of predator and potential prey. Anyhow, since Bongo nets cannot be closed

at a certain depth like a Multinet, it remains uncertain from which exact depth the sampled individuals were collected.

Due to the small overlap in the diet of the two amphipods, it can be assumed that the predators are not competing for food, at least in late summer in the Fram Strait. Hence, in terms of food availability, *T. libellula* may not be impacted by an increase in *T. abyssorum* biomass in the Arctic Ocean due to the ongoing Atlantification. To give better estimates regarding the diet of the two *Themisto* amphipods and its consequences for the ecosystem, further investigations of prey composition, as well as experiments regarding prey preference and clearance rates are needed.

4.2 Spatial variation in the prey spectrum of *T. libellula* in the Fram Strait

The PERMANOVA showed significant differences in the prey composition between the different stations for *T. libellula*. In these terms the findings of this study supported the second hypothesis. Nevertheless, it was assumed to find a diet based on genuine Arctic and ice-associated species like *Calanus glacialis*, *C. hyperboreus* or *Boreogadus saida*, since this was also suggested by previous studies using biomarkers (Auel et al. 2002). In this study a broad and variable prey spectrum was found, suggesting a less ice-dependent diet. Arctic fish species like *B. saida* and the gelatinous snailfish *Liparis fabricii* were detected in *T. libellula*'s diet and were exclusively found in specimens sampled in cooler water masses at the East coast of Greenland. At these stations, also other ice-associated prey was found in the samples of *T. libellula*: the sympagic amphipod *Gammarus wilkitzkii* and *C. glacialis* (Lønne and Gulliksen 1989; Gradinger and Bluhm 2004; Fortier et al. 2006; Søreide et al. 2010; Kohlbach et al. 2016; Johannesen et al. 2017; Węsławski, Legeżyńska, and Włodarska-Kowalczuk 2020). Since at those locations colder Arctic water were detected, the feeding on sympagic species was mainly linked to the environmental conditions.

At Atlantic-influenced stations, a prey assemblage of different copepod and krill species was detected, that seems to be less ice-dependent. A study of Stige et al. (2019) found that the biomass of krill has increased in the short term in years with warm winters and less sea ice. This leads to the assumption that *T. libellula* is able to adapt its diet to the available prey and is not limited to the sea-ice associated pathway. The amphipods collected at the warmer stations were slightly smaller than the ones close to the Greenland shelf. The size difference might be explained by the different methods used to catch the amphipods. The amphipods caught during

the TUNU cruise, where collected with pelagic and bottom trawls and are therefore more likely to collect larger amphipods. The difference in the size of *T. libellula* might also be an explanation for the differences in the prey composition. Anyhow, the size difference was not very large, so it is more likely that all species tested in this study are able to feed on the same prey species and that differences in the diet are rather explained by environmental factors. In the future it might be important to also test the lipid content of *T. libellula* at the different locations to assess the conditions of the individuals. In most samples of *T. libellula*, the warm-water *C. finmarchicus* only played a minor role in the diet, except for station PS107_002/18, where it was dominant. Since this station was closer to the Svalbard shelf and influenced by Atlantic waters, it can be assumed that *C. finmarchicus* was abundant at this location (Basedow et al. 2018; Polyakov et al. 2020), which can explain its dominance in the diet at this location. At other stations, the RRAs found for *C. finmarchicus* were rather low, so it might be that *T. libellula* feeds on this copepod, when it is very abundant. Nevertheless, for this station only one sample was analyzed, and therefore more sampling should be performed in the future. There is only station PS107_007/5, where higher RRAs of *C. finmarchicus* were found, which showed warm water temperatures at the upper 500m, likely of Atlantic origin.

It is interesting to see that at station TUNU_1278 *Oithona similis* was found to be nearly the only prey in the stomach of *T. libellula*, whereas at other stations no single prey species seemed to dominate the diet. It might be that *O. similis* was very abundant at this station and therefore *T. libellula* was able to ingest a high proportion of this small copepod.

4.3 spatial variation in the prey spectrum of *T. abyssorum* in the Fram Strait

The PERMANOVA showed that the differences between the prey found in the stomachs of *T. abyssorum* at different locations were significant. Thus, the second hypothesis was also proven for *T. abyssorum*. It was assumed before that *T. abyssorum* is a more opportunistic feeder with a high variation between the localities. Here, the diet of *T. abyssorum* was dominated by the chaetognath *E. hamata* of which the DNA was omnipresent in the samples which is not supporting an opportunistic behavior.

In the Arctic Ocean only three chaetognaths species are found, including *Parasagitta elegans*, which is a neritic species mainly found in the epipelagial, *Pseudosagitta maxima*, which is found in bathy-pelagic and surface waters and *E. hamata*, which is mainly found in meso-pelagic and

deep waters (Bieri 1959; Alvarino 1964; Terazaki and Miller 1982; Samemoto 1987). As written before, based on the size of the tested *T. abyssorum*, it is more likely to have fed on the larvae of *E. hamata*. Grigor, Schmid, and Fortier (2017) found that *E. hamata* spawns throughout the year and larvae hatch between December and July. The larval *E. hamata* are kept in the folded lateral fins of the adults for some time and hatch from brood sacs (Alvarino 1968; Grigor, Schmid, and Fortier 2017). *E. hamata* makes up a large proportion of the zooplankton biomass and is a key species in the pelagic food web, since they are thought to be very effective predators (Terazaki 2000). Although they are important predators it is not known how much they contribute to higher trophic levels (Grigor, Schmid, and Fortier 2017). When looking at the results achieved in this study, the chaetognath *E. hamata* seems to be an important prey organism for *T. abyssorum* and might therefore be also important for the pelagic food web. Since this study represents only a temporal snapshot, a more extensive sampling is needed to confirm whether this chaetognath dominates *T. abyssorum*'s diet throughout the summer.

It was found that the proportion of calanoid copepods, as well as their species composition, in the diet of *T. abyssorum* was varying between the stations. In other studies, calanoid copepods were identified as one of the major food sources for both *Themisto* amphipods (Auel et al. 2002; Kohlbach et al. 2016), which was not the case in this study. Higher RRAs for *C. finmarchicus* were found at warmer locations closer to Svalbard and associated with the warmer Atlantic water entering Fram Strait with the WSC (Fig. 20). These stations (PS107_45/10, PS107_007/5 and PS100_002/4) seemed to have a strong Atlantic influence, explaining the presence of this boreal species. At station PS100_002/4, the diet is dominated by *E. hamata* and *C. finmarchicus*. These species are found in North Atlantic waters and *E. hamata* as well in the Arctic Ocean (WoRMS Editorial Board 2020), which matches well with the distribution of *T. abyssorum*. No CTD data were available, but based on the location further south of Svalbard, it can be assumed that the water is from Atlantic origin. At this station the diet of *T. abyssorum* did not contain krill, whereas at other locations the reads for *T. inermis* and *T. longicaudata* were high. Hence, it can be assumed that these species did not occur at this southern location.

At stations linked to lower surface temperatures, or with only a thin warm surface layer like PS107_28 and PS107_2, less *C. finmarchicus* RRAs were found. Instead, Arctic species like *C. glacialis* and *C. hyperboreus* were detected (Conover 1988; Falk-Petersen et al. 2009; Visser, Grønning, and Jónasdóttir 2017). The second most abundant prey species found in *T. abyssorum*

were the krill species *T. longicaudata* and *T. inermis*. These prey species were found at warmer and cooler stations and appear not to be linked to a certain water mass or temperature. At many stations the diet contained as well several different copepod species including *Metridia longa*, *Pseudocalanus minutus*, *Microcalanus pusillus* and *Microcalanus pygmaeus*, which were found at nearly all stations except for station PS100_002/4 and PS107_007/5 where mainly *E. hamata* and *C. finmarchicus* were found. One explanation might be that *Metridia longa* and *Pseudocalanus minutus* are rather found in cold water (Frost 1989; Auel and Werner 2003; Daase et al. 2008) and are therefore not available in the warm Atlantic waters at such lower latitudes.

Specimens sampled of *T. abyssorum* were in general very small (<10mm) and Hop et al. (2006) suggests different trophic levels for small and larger *T. abyssorum*, even suggesting an herbivorous diet for juveniles. In the samples of both predators many MOTUs assigned to diatoms and dinoflagellates were found, that could be a signal for herbivory, but these reads could also be explained with secondary predation since most copepod species are feeding on phytoplankton. With stomach content analyses, it remains impossible to validate an herbivorous diet for both predators. Hence, it can be assumed that larger *T. abyssorum* might show a different prey spectrum than what was found in this study.

4.4 The role of jellyfish in the diet of *Themisto* amphipods

Jellyfish were found only in low RRAs in both predators and only in some samples. Nevertheless, these findings support the third hypothesis as well. The reads could be assigned to the hydrozoan species *Nanomia cara*, *Aglantha digitale*, *Obelia longissima*, *Catablema vesicarium* and *Physophora* sp. In *T. libellula*, cnidarians were found at all TUNU stations and at station PS107_38/5. In *T. abyssorum*, these were detected at stations PS107_30/4, PS107_45/10 and PS107_002/7, always in only one of three samples. It does not seem like the ingestion of jellyfish is linked to a certain location or water mass, as it was found both in warm and cold waters. In general, only few sequences were assigned as jellyfish species, no ctenophores were found in the stomachs, also the RRAs of the jellyfish reads were low. This can be due to several reasons: First, jellyfish might not play a major role in the diet of these to amphipods and might be a survival food. Second, due to the insufficient read depth for *T. libellula* some reads might have been overlooked. Additionally, some sequences were only assigned to 80% to ctenophore or jellyfish

species and where therefore not considered. In the future it might be important to further improve the reference databases. Last, jellyfish might be digested very fast, and therefore they might be hard to detect in the diet of the amphipods.

In this matter, more studies are needed, since jellyfish might play a major role in the changing Arctic. There are reports from *Themisto* amphipods in the Southern Ocean, showing that they are feeding mainly on the stomach of salps and could be a major predator of salps due to its anatomy (Stowasser et al. 2012; Smetacek, Assmy, and Henjes 2004).

4.5 *Themisto*'s diet in the light of environmental change

Based on the prey spectrum found for both *Themisto* amphipods in this study, a minor impact of environmental change on the diet of the two predators can be assumed. Additionally, the two predators seem not to compete for prey in this ecosystem and focus on different prey types. These findings can be used to estimate some implementations for higher trophic levels in the Arctic Ocean.

Based on the results, the two predators do not seem to compete for prey in this ecosystem and target different prey types. If both predators indeed co-exist without the possibility of one outcompeting the other, a more productive, atlantified system could promote an increase of biomass of both species, of which higher trophic levels will benefit. However, previous studies already showed a decrease in the biomass of *T. libellula* in some Arctic regions (CAFF 2017), indicating that other factors besides the prey composition have an impact on the survival of this species in the changing Arctic. A study of Percy (1993) showed that *T. libellula* is able to tolerate warmer temperatures in some regions like the Alaskan fjord, leading to the assumption that it is able to adapt to warmer conditions. To estimate consequences for the pelagic food web in the future more sampling is needed, ideally combining DNA metabarcoding of stomach content and biomarkers for a more complete insight of the diet of the two predators throughout the seasons in this region (Pompanon et al. 2012). Additionally, the tolerance of both predators towards temperature and salinity must be included in these studies.

T. libellula feeds on *C. finmarchicus*, but it seems not to make up a major part of its diet and might be linked to the abundance of this copepod in the water column. Nevertheless, it is possible that the role of *C. finmarchicus* as part of the diet of *T. libellula* will increase in a more atlantified Arctic Ocean as it is thought to replace the sympagic copepod *C. glacialis* (Hirche and

Kosobokova 2007). This could have an impact on the winter survival of *T. libellula*, since *C. finmarchicus* contains less lipids than *C. glacialis* (Hirche and Kosobokova 2007). So far it is not much known about the winter-survival strategies of *T. libellula*, but due to its high amounts of wax esters it might be possible that they reduce their metabolism during the winter months when less prey is available (Auel et al. 2002). It is not known whether the amphipods can synthesize the wax esters or if they have to ingest prey with high amounts of wax esters (Falk-Petersen, Sargent, and Tande 1987). But Falk-Petersen, Sargent, and Tande (1987) found in their study high amounts of wax esters in *C. finmarchicus*, indicating that they might be a good alternative copepod prey for *T. libellula*.

In general, it was found that *T. libellula* can prey on a broad range of organisms, while *T. abyssorum* showed a less opportunistic feeding behavior than previously assumed. From the findings on the diet of *T. libellula*, it can be assumed that an ice-associated feeding behavior for this species is rather linked to environmental conditions than to a preferred prey species.

4.6 Limitations of methods

In this study three PCR replicates were carried out, to decrease the chances that one prey item is missed during the PCR and sequencing step (Mata et al. 2019). Mata et al. (2019) suggest that technical replication (PCR replicates) has a lower impact on the efficiency than biological replicates. Other studies concerning eDNA and soil samples suggest that high numbers of PCR replicates are needed to assess the biodiversity of a samples, these studies suggest using 8-20 replicates to reach the maximum outcome (Ficetola et al. 2014; Dopheide et al. 2018). Nevertheless, Ficetola et al. (2014) pointed out that the number of PCR replicates must match the goal of a study. In this study the main goal was to detect important food items for the two amphipods to estimate the consequences of climate change and Atlantification, therefore lower numbers of PCR replicates were sufficient (Ficetola et al. 2014). With the approach used in this study, it might be possible that some species in the diet were overlooked, but it would be unlikely to miss the dominating prey items (unless caused by inefficient primer-binding). It also seemed that the PCR replicates were sufficient to reach the goal of the study, since even so far overlooked species like jellyfish and larval fish were detected in the diet.

When looking at the prey spectrum revealed in this study, it must be mentioned that relative read abundances cannot be interpreted as absolute abundances of a certain prey. This is because the

number of reads achieved for one prey taxon is biased by the binding success of the primers, the time for which a prey item had been ingested, and the size of an ingested prey. Highly digested prey will be sequenced in lower read abundances than newly ingested prey (Deagle et al. 2018). However, RRAs were used to visualize and compare the proportion of a certain prey between different samples and species since it can be assumed that the primer pair will have the same affinity to a particular sequence (taxon). With this assumption, at least the bias caused by primer binding is minimal.

In the negative control, that was created with the library preparation, reads for *T. libellula* and *T. abyssorum* were found. This means that it could not be determined whether the reads of *T. abyssorum* found in the stomachs of *T. libellula* and vice versa, are caused by predation or are resulting from contamination. Furthermore, it was found that for *T. abyssorum* in general more reads were achieved, than for *T. libellula*, although both predators were handled equally. The rarefaction curves showed that the read depth achieved for *T. libellula* was in most samples not sufficient. It can thus be that some less dominant species were overlooked in the diet of *T. libellula*. It is not very likely that dominating species were overlooked caused by the read depth. Another important aspect, when using DNA metabarcoding, is the reference database used to assign the different MOTUs to taxa (Pompanon et al. 2012). In this study the NCBI GenBank database (Bethesda 2008) and BOLD (Barcode of Life Data System; Ratnasingham and Herbert 2007) were used. Most of MOTUs could be assigned, but several could not be matched to a certain taxon. This could lead to the assumption, that some prey species might be overlooked or not assigned, since there was no reference sequence available.

For each sample 6-25 individuals were pooled together to increase the amount of stomach content. It was assumed that organisms sampled at the same location will probably feed on the same organisms as the same zooplankton assemblage is available for all amphipods. But as seen, the prey composition between the samples within one station varied strongly. Therefore, it can be assumed, that the predators feed on different organisms even at the same location. In general, this could mean that some prey items were not detected, since other newly ingested prey or larger prey items could overlay other prey items (Mata et al. 2019). A study of Mata et al. (2019) carried out on a bat species showed that pooling of pellets of bats led to poorer results. This could also be the case here.

The differences in the diet found in this study and previous studies might be explained by the different methods that were used. Biomarkers like fatty acids or stable isotopes can give insights in the long-term diet of a species, while DNA metabarcoding allows to look more precisely on a snapshot of the diet (reviewed by Sunderland, Powell, and Symondson 2005). It is possible that in general the diet of *T. libellula* as well as the diet of *T. abyssorum* is strongly dominated by calanoid copepods during other seasons. For *T. libellula* no clear pattern in the prey spectrum was found, but it seems be very flexible in its prey spectrum. This leads to the assumption that it can cope well with the possible change in the zooplankton assemblage in a warmer, ice-free Arctic Ocean. What remains unclear is whether it can also cope with the ongoing and predicted temperature increase since it is a truly Arctic species, preferring low water temperatures (Frost 1989; Auel and Werner 2003; Daase et al. 2008). Thus, it remains important to assess the possible change in the diet of *T. libellula* in the future and monitor its population size and distribution in the Arctic Ocean.

This study can be used as starting point for further investigations regarding the prey spectrum and the diet of *Themisto* amphipods in the Arctic Ocean. It will be important to have a closer look on the diet throughout the year combining several methods like biomarkers, metabarcoding and lipid content analysis, to gain a more complete picture on the ecology and future scenarios for these two amphipods (Pompanon et al. 2012).

5. Conclusion

All hypotheses were supported by the findings of this study. Significant differences in the diet of both *Themisto* amphipods were detected, indicating that they are not competing for food. These findings were also supported by previous studies using biomarkers (Auel et al. 2002). Additionally, in previous studies an ice-dependent prey was found for *T. libellula* (Auel et al. 2002; Kohlbach et al. 2016), but this study showed that this was only the case for cold, Arctic stations whereas at warmer, Atlantic stations, a broad prey spectrum was found, containing several copepod and krill species. Based on this broad prey spectrum this amphipod may cope well with the changes in the zooplankton community caused by climate change, Atlantification and sea ice retreat. For *T. abyssorum*, a less opportunistic feeding behavior was revealed, with a diet dominated by the chaetognath *E. hamata*. Additionally, it was possible to detect sequences belonging to jellyfish species in the stomachs of both predators. This means that

they are able to feed on gelatinous zooplankton, but it seems that it makes only up a minor part of the diet.

In general, it is worth mentioning that DNA metabarcoding only gives a temporal snaphot of the diet and for a better understanding of the feeding behavior more sampling is needed, ideally with a combination of metabarcoding and biomarkers, to assess both short-term and long-term diet (Pompanon et al. 2012).

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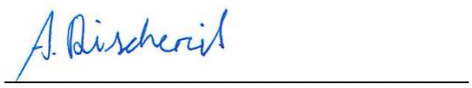
Declaration of Authorship

Hiermit bestätige ich an Eides statt, dass die vorliegende Arbeit von mir selbstständig verfasst wurde und ich keine anderen als die angegebenen Hilfsmittel - insbesondere keine im Quellenverzeichnis nicht benannten Internet-Quellen - benutzt habe und die Arbeit von mir vorher nicht einem anderen Prüfungsverfahren eingereicht wurde. Die eingereichte schriftliche Fassung entspricht der auf dem elektronischen Speichermedium. Ich bin damit einverstanden, dass die Masterarbeit veröffentlicht wird.



Annkathrin Dischereit, Brake 29th January 2021

References

Alvarino, Angeles. 1964. “Bathymetric Distribution of Chaetognaths.” *Pacific Science* 18: 64–82.

Alvarino, Angeles. 1968. “Egg Pouches and Other Reproductive Structures in Pelagic Chaetognatha.” *Pacific Science* 22: 488–92.

Arai, Mary N. 2001. “Pelagic Coelenterates and Eutrophication: A Review.” *Hydrobiologia* 451: 69–87.

Arrigo, Kevin R., and Gert L. van Dijken. 2015. “Continued Increases in Arctic Ocean Primary Production.” *Progress in Oceanography* 136 (August): 60–70. https://doi.org/10.1016/j.pocean.2015.05.002.

Attrill, Martin J., Jade Wright, and Martin Edwards. 2007. “Climate-Related Increases in Jellyfish Frequency Suggest a More Gelatinous Future for the North Sea.” *Limnology and Oceanography* 52 (1): 480–85. https://doi.org/10.4319/lo.2007.52.1.0480.

Aubert, A., E. Antajan, C. Lynam, S. Pitois, A. Pliru, S. Vaz, and D. Thibault. 2018. “No More Reason for Ignoring Gelatinous Zooplankton in Ecosystem Assessment and Marine Management: Concrete Cost-Effective Methodology During Routine Fishery Trawl Surveys.” *Marine Policy* 89 (February): 100–108. https://doi.org/10.1016/j.marpol.2017.12.010.

Auel, Holger, Miriam Harjes, Régine da Rocha, Dorothee Stübing, and Wilhelm Hagen. 2002. “Lipid Biomarkers Indicate Different Ecological Niches and Trophic Relationships of the Arctic Hyperiid Amphipods Themisto Abyssorum and T. Libellula.” *Polar Biology* 25 (5): 374–83. https://doi.org/10.1007/s00300-001-0354-7.

Auel, Holger, and Iris Werner. 2003. “Feeding, Respiration and Life History of the Hyperiid Amphipod Themisto Libellula in the Arctic Marginal Ice Zone of the Greenland Sea.” *Journal of Experimental Marine Biology and Ecology* 296 (2): 183–97. https://doi.org/10.1016/s0022-0981(03)00321-6.

Barry, R. G., M. C. Serreze, J. A. Maslanik, and R. H. Preller. 1993. “The Arctic Sea Ice-Climate System: Observations and Modeling.” *Reviews of Geophysics* 31 (4): 397. https://doi.org/10.1029/93rg01998.

Basedow, Sünnje L., Arlid Sundfjord, Wilken-Jon von Appen, Elisabeth Halvorsen, Slawomir Kwasniewski, and Marit Reigstad. 2018. “Seasonal Variation in Transport of Zooplankton into the Arctic Basin Through the Atlantic Gateway, Fram Strait.” *Frontiers in Marine Science* 194.

Bethesda, M. D. 2008. „Get NCBI Blast Databases. Available from: https://www.ncbi.nml.nih.gov/Books/Nbk53777/. Accessed 2021-01-29”. National Center for Biotechnology Information (US).

Bieri, Robert. 1959. “The Distribution of the Planktonic Chaetognatha in the Pacific and Their Relationship to the Water Masses1.” *Limnology and Oceanography* 4 (1): 1–28. https://doi.org/10.4319/lo.1959.4.1.0001.

Blicher, Martin Emil, and Mikael Kristian Sejr. 2011. “Abundance, Oxygen Consumption and Carbon Demand of Brittle Stars in Young Sound and the Ne Greenland Shelf.” *Marine Ecology Progress Series* 422: 139–44.

Bluhm, BA, K Iken, S Mincks Hardy, BI Sirenko, and BA Holladay. 2009. “Community Structure of Epibenthic Megafauna in the Chukchi Sea.” *Aquatic Biology* 7 (December): 269–93. https://doi.org/10.3354/ab00198.

Bluhm, Bodil A., Haakon Hop, Mikko Vihtakari, Rolf Gradinger, Katrin Iken, Igor A. Melnikov, and Janne E. Søreide. 2018. “Sea Ice Meiofauna Distribution on Local to Pan-Arctic Scales.” *Ecology and Evolution*, January. https://doi.org/10.1002/ece3.3797.

Bogstad, Bjarte, Harald Gjøsæter, Tore Haug, and Ulf Lindstrøm. 2015. “A Review of the Battle for Food in the Barents Sea: Cod Vs. Marine Mammals.” *Frontiers in Ecology and Evolution* 3 (March). https://doi.org/10.3389/fevo.2015.00029.

Bowman, Thomas E. 1960. “The Pelagic Amphipod Genus Parathemisto (Hyperidea: Hyperidae) in the North Pacific and Adjacent Arctic Ocean.” *Proceedings of the United States National Museum* 112 (3439): 343–92.

Brodeur, Richard D., Mary Beth Decker, Lorenzo Ciannelli, Jennifer E. Purcell, Nicholas A. Bond, Phyllis J. Stabeno, Erika Acuna, and George L. HuntJr. 2008. “Rise and Fall of Jellyfish in the Eastern Bering Sea in Relation to Climate Regime Shifts.” *Progress in Oceanography* 77: 103–11.

Brodeur, Richard D., Hiroya Sugisaki, and George L. Hunter Jr. 2002. “Increases in Jellyfish Biomass in the Bering Sea: Implications for the Ecosystem.” *Marine Ecology Progress Series* 233 (May): 89–103.

Brown, J., M. Pirrung, and L. A. McCue. 2017. “FQC Dashboard: Integrates Fastqc Results into a Web-Based, Interactive, and Extensible Fastqc Quality Control Tool.” *Bioinformatics* 33: 3137–9.

CAFF. 2017. “State of the Arctic Marine Biodiversity Report.” Akureyri, Iceland: Conservation of Arctic Flora; Fauna International Secretariat.

Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, and T. L. Madden. 2009. “BLAST+: Architecture and Applications.” *BMC Bioinformatics* 10 (421).

Carmack, E., I. Polyakov, L. Padman, I. Fer, E. Hunke, J. Hutchings, J. Jackson, et al. 2015. “Toward Quantifying the Increasing Role of Oceanic Heat in Sea Ice Loss in the New Arctic.” *Bulletin of the American Meteorological Society* 96 (12): 2079–2105. https://doi.org/10.1175/bams-d-13-00177.1.

Christiansen, Jørgen S., Catherine W. Mecklenburg, and Oleg V. Karamushko. 2014. "Arctic Marine Fishes and Their Fisheries in Light of Global Change." *Global Change Biology* 20 (2): 352–59. https://doi.org/10.1111/gcb.12395.

Clarke, K. R. 1993. "Non-Parametric Multivariate Analyses of Changes in Community Structure." *Australien Journal of Ecology* 18: 117–43.

Connell, C., S. Fung, C. Heiner, J. Bridgham, V. Chakerian, E. Heron, B. Jones, et al. 1987. "Automated Dna-Sequence Analysis." *BioTechniques* 5 (4): 342–48.

Conover, R. J. 1988. "Comparative Life Histories in the Genera Calanus and Neocalanus in High Latitudes of the Northern Hemisphere." *Hydrobiologia* 167: 127–42.

Daase, Malin, Ketil Eiane, Dag L. Aksnes, and Daniel Vogedes. 2008. "Vertical Distribution ofCalanusspp. andMetridia Longaat Four Arctic Locations." *Marine Biology Research* 4 (3): 193–207. https://doi.org/10.1080/17451000801907948.

Dalpadado, Padmini. 2002. "Inter-Specific Variations in Distribution, Abundance and Possible Life-Cycle Patterns of Themisto Spp. (Amphipoda) in the Barents Sea." *Polar Biology* 25: 656–66.

Dalpadado, Padmini, Nina Borkner, Bjarte Bogstad, and Sigbjørn Mehl. 2001. "Distribution of Themisto (Amphipoda) Spp. In the Barents Sea and Predator-Prey Interactions." *ICES Journal of Marine Science* 58: 876–95.

Dalpadado, P., N. Borkner, and H. R. Skjodal. 1994. "Distribution and Life History of Themisto (Amphipoda) Spp., North of 73°N in the Barents Sea." *Fishen Havet* 12: 1–42.

David, Carmen, Benjamin Lange, Benjamin Rabe, and Hauke Flores. 2015. "Community Structure of Under-Ice Fauna in the Eurasian Central Arctic Ocean in Relation to Environmental Properties of Sea-Ice Habitats." *Marine Ecology Progress Series* 522: 15–32. https://doi.org/10.3354/meps11156.

Deagle, Bruce E., Austen C. Thomas, Julie C. McInnes, Laurence J. Clarke, Eero J. Vesterinen, Elizabeth L. Clare, Tyler R. Kartzinel, and J. Paige Eveson. 2018. "Counting with DNA in Metabarcoding Studies: How Should We Convert Sequence Reads to Dietary Data?" *Molecular Ecology* 28 (2): 391–406. https://doi.org/10.1111/mec.14734.

Deagle, Bruce E., Austen C. Thomas, Amanda K. Shaffer, Andrew W. Trites, and Simon N. Jarman. 2013. "Quantifying Sequence Proportions in a DNA-Based Diet Study Using Ion Torrent Amplicon Sequencing: Which Counts Count?" *Molecular Ecology Resources* 13 (4): 620–33. https://doi.org/10.1111/1755-0998.12103.

Degen, R, LL Jørgensen, P Ljubin, IH Ellingsen, H Pehlke, and T Brey. 2016. "Patterns and Drivers of Megabenthic Secondary Production on the Barents Sea Shelf." *Marine Ecology Progress Series* 546 (March): 1–16. https://doi.org/10.3354/meps11662.

Dempson, J. Brian, Milton Shears, and Marc Bloom. 2002. "Spatial and Temporal Variability in the Diet of Anadromous Arctic Charr, Salvelinus Alpinus, in Northern Labrador." In

Ecology, Behaviour and Conservation of the Charrs, Genus Salvelinus, 49–62. Springer Netherlands. https://doi.org/10.1007/978-94-017-1352-8_4.

Dickson, R. R., T. J. Osborn, J. W. Hurrell, J. Meincke, J. Blindheim, B. Adlandsvik, T. Vinje, G. Alekseev, and W. Maslowski. 2000. "The Arctic Ocean Response to the North Atlantic Oscillation." *Journal of Climate*.

Dopheide, Andrew, Dong Xie, Thomas R. Buckley, Alexei J. Drummond, and Richard D. Newcomb. 2018. "Impacts of DNA Extraction and PCR on DNA Metabarcoding Estimates of Soil Biodiversity." Edited by Michael Bunce. *Methods in Ecology and Evolution* 10 (1): 120–33. https://doi.org/10.1111/2041-210x.13086.

Ehrlich, Julia, Fokje L. Schaafsma, Bodil A. Bluhm, Ilka Peeken, Giulia Castellani, Angelika Brandt, and Hauke Flores. 2020. "Sympagic Fauna in and Under Arctic Pack Ice in the Annual Sea-Ice System of the New Arctic." *Frontiers in Marine Science* 7. https://doi.org/10.3389/fmars.2020.00452.

Eriksen, Elena, ed. 2014. *Survey Report from the Joint Norwegian/Russian Ecosystem Survey in the Barents Sea and Adjacent Waters, August-October 2014*. IMR/PINRO Joint Report Series.

Eriksen, Elena, Dmitry Prozorkevich, Aleksandr Trofimov, and Daniel Howell. 2012. "Biomass of Scyphozoan Jellyfish, and Its Spatial Association with 0-Group Fish in the Barents Sea." Edited by Graeme Clive Hays. *PLoS ONE* 7 (3): e33050. https://doi.org/10.1371/journal.pone.0033050.

Fahrbach, Eberhard, Jens Meincke, Svein Østerhus, Gerd Rohardt, Ursula Schauer, Vigdis Tverberg, and Jennifer Verduin. 2001. "Direct Measurements of Volume Transports Through Fram Strait." *Polar Research* 20 (2): 217–24. https://doi.org/10.1111/j.1751-8369.2001.tb00059.x.

Falk-Petersen, Stig, Patrick Mayzaud, Gerhard Kattner, and John R. Sargent. 2009. "Lipids and Life Strategy of Arctic Calanus." *Marine Biology Research* 5 (1): 18–39. https://doi.org/10.1080/17451000802512267.

Falk-Petersen, Stig, John R. Sargent, and Kurt S. Tande. 1987. "Lipid Composition of Zooplankton in Relation to the Sub-Arctic Food Web." *Polar Biology* 8: 115–20.

Fernández-Méndez, M., C. Katlein, B. Rabe, M. Nicolaus, L. Peeken, K. Bakker, H. Flores, and A. Boetius. 2015. "Photosynthetic Production in the Central Arctic Ocean During the Record Sea-Ice Minimum in 2012." *Biogeosciences* 12: 3525–49. https://doi.org/10.5194/bg-12-3525-2015.

Ficetola, Gentile F., Johan Pansu, Aurélie Bonin, Eric Coissac, Charline Giguet-Covex, Marta De Barba, Ludovic Gielly, et al. 2014. "Replication Levels, False Presences and the Estimation of the Presence/Absence from eDNA Metabarcoding Data." *Molecular Ecology Resources* 15 (3): 543–56. https://doi.org/10.1111/1755-0998.12338.

Fortier, Louis, Pascal Sirois, Josée Michaud, and David Barber. 2006. "Survival of Arctic Cod Larvae (Boreogadus Saida) in Relation to Sea Ice and Temperature in the Northeast Water

Polynya (Greenland Sea).” *Canadian Journal of Fisheries and Aquatic Sciences* 63 (7): 1608–16. https://doi.org/10.1139/f06-064.

Frainer, André, Raul Primicerio, Susanne Kortsch, Magnus Aune, Andrey V. Dolgov, Maria Fossheim, and Michaela M. Aschan. 2017. “Climate-Driven Changes in Functional Biogeography of Arctic Marine Fish Communities.” *Proceedings of the National Academy of Sciences* 114 (46): 12202–7. https://doi.org/10.1073/pnas.1706080114.

Frederiksen, R., J. S. Christiansen, E. Bonsdorf, L.-H. Larsen, M. C. Nordström, I. Zhulay, and B. A. Bluhm. 2020. “Epibenthic Megafauna Communities in the Northeast Greenland Vary Across Coastal, Continental Shelf and Slope Habitats.” *Polar Biology* 43: 1623–42.

Frost, B. W. 1989. “A Taxonomy of the Marine Calanoid Copepod Genus Pseudocalanus.” *Canadian Journal of Zoology* 67 (3): 525–51. https://doi.org/10.1139/z89-077.

Geller, J., C. Meyer, M. Parker, and H. Hawk. 2013. “Redesign of PCR Primers for Mitochondrial Cytochromecoxidase Subunit I for Marine Invertebrates and Application in All-Taxa Biotic Surveys.” *Molecular Ecology Resources* 13 (5): 851–61. https://doi.org/10.1111/1755-0998.12138.

Gilbert, W., and A. Maxam. 1973. “The Nucleotide Sequence of the Lac Operator.” *Proceedings of the National Academy of Sciences* 70 (12): 3581–4. https://doi.org/10.1073/pnas.70.12.3581.

Gohel, David. 2020. *Flextable: Functions for Tabular Reporting*. https://CRAN.R-project.org/package=flextable.

Goral, Friedemann, and Jenny Schellenberg. 2018. *Goeveg: Functions for Community Data and Ordinations*. https://CRAN.R-project.org/package=goeveg.

Gosselin, Michel, Maurice Levasseur, Patricia A. Wheeler, Rita A. Hornes, and Beatrice C. Booth. 1997. “New Measurements of Phytoplankton and Ice Algal Production in the Arctic Ocean.” *Deep-Sea Research* 44 (8): 1623–44.

Gradinger, R., C. Friedrich, and M. Spindler. 1999. “Abundance, Biomass and Composition of the Sea Ice Biota of the Greenland Sea Pack Ice.” *Deep Sea Research Part II: Topical Studies in Oceanography* 46 (6-7): 1457–72. https://doi.org/10.1016/s0967-0645(99)00030-2.

Gradinger, Rolf R., and Bodil A. Bluhm. 2004. “In-Situ Observations on the Distribution and Behavior of Amphipods and Arctic Cod (Boreogadus Saida) Under the Sea Ice of the High Arctic Canada Basin.” *Polar Biology* 27: 595–603.

Graham, W. M. 2001. “Numerical Increases and Distributional Shifts of Chrysaora Quinquecirrha (Desor) and Aurelia Aurita (Linné) (Cnidaria: Scyphozoa) in the Northern Gulf of Mexiko.” *Hydrobiologia* 451: 97–111.

Grigor, Jordan J, Moritz S Schmid, and Louis Fortier. 2017. “Growth and Reproduction of the Chaetognaths Eukrohnia Hamata and Parasagitta Elegans in the Canadian Arctic Ocean:

Capital Breeding Versus Income Breeding." *Journal of Plankton Research* 39 (6): 910–29. https://doi.org/10.1093/plankt/fbx045.

Halpern, Benjamin S., Shaun Walbridge, Kimberly A. Selkoe, Carrie V. Kappel, Fiorenza Micheli, Caterina DAgrosa, John F. Bruno, et al. 2008. "A Global Map of Human Impact on Marine Ecosystems." *Science* 319 (5865): 948–52. https://doi.org/10.1126/science.1149345.

Hamilton, Charmain D., Christian Lydersen, Rolf A. Ims, and Kit M. Kovacs. 2015. "Predictions Replaced by Facts: A Keystone Species Behavioural Responses to Declining Arctic Sea-Ice." *Biology Letters* 11 (11): 20150803. https://doi.org/10.1098/rsbl.2015.0803.

Haug, Tore, Martin Biuw, Harald Gjøsæter, Tor Knutsen, Ulf Lindstrøm, Kirsteen M. MacKenzie, Sonnich Meier, and Kjell T. Nilssen. 2020. "Harp Seal Body Condition and Trophic Interactions with Prey in Norwegian High Arctic Waters in Early Autumn." *Progress in Oceanography*, December, 102498. https://doi.org/10.1016/j.pocean.2020.102498.

Haug, Tore, Bjarte Bogstad, Melissa Chierici, Harald Gjøsæter, Elvar H. Hallfredsson, Åge S. Høines, Alf Håkon Hoel, et al. 2017. "Future Harvest of Living Resources in the Arctic Ocean North of the Nordic and Barents Seas: A Review of Possibilities and Constraints." *Fisheries Research* 188: 38–57.

Havermans, Charlotte, Holger Auel, Wilhelm Hagen, Christoph Held, Natalie S. Ensor, and Geraint A. Tarling. 2019. "Predatory Zooplankton on the Move: Themisto Amphipods in High-Latitude Marine Pelagic Food Webs." In *Advances in Marine Biology*, 51–92. Elsevier. https://doi.org/10.1016/bs.amb.2019.02.002.

Hirche, Hans-Jürgen, and Ksenia Kosobokova. 2007. "Distribution of Calanus Finmarchicus in the Northern North Atlantic and Arctic OceanExpatriation and Potential Colonization." *Deep Sea Research Part II: Topical Studies in Oceanography* 54 (23-26): 2729–47. https://doi.org/10.1016/j.dsr2.2007.08.006.

Hollowed, Anne Babcock, Benjamin Planque, and Harald Loeng. 2013. "Potential Movement of Fish and Shellfish Stocks from the Sub-Arctic to the Arctic Ocean." *Fisheries Oceanography* 22 (5): 355–70.

Hop, Haakon, Stig Falk-Petersen, Harald Svendsen, Slawek Kwasniewski, Vladimir Pavlov, Olga Pavlova, and Janne E. Søreide. 2006. "Physical and Biological Characteristics of the Pelagic System Across Fram Strait to Kongsfjorden." *Progress in Oceanography* 71: 182–231. https://doi.org/doi:10.1016/j.pocean.2006.09.007.

Ivanov, Vladimir, Vladimir Alexeev, Nikolay V. Koldunov, Irina Repina, Anne Britt Sandø, Lars Henrik Smedsrud, and Alexander Smirnov. 2016. "Arctic Ocean Heat Impact on Regional Ice Decay: A Suggested Positive Feedback." *Journal of Physical Oceanography* 46 (5): 1437–56. https://doi.org/10.1175/jpo-d-15-0144.1.

Jarman, Simon N., Julie C. McInnes, Cassandra Faux, Andrea M. Polanowski, James Marthick, Bruce E. Deagle, Colin Southwell, and Louise Emmerson. 2013. "Adélie Penguin

Population Diet Monitoring by Analysis of Food DNA in Scats." Edited by Hans-Ulrich Peter. *PLoS ONE* 8 (12): e82227. https://doi.org/10.1371/journal.pone.0082227.

Johannesen, Edda, Herdis Langøy Mørk, Knut Korsbrekke, Rupert Wienerroither, Elena Eriksen, Maria Fossheim, Thomas de Lange Wennecke, Andrey Dolgov, Tatiana Prokhorova, and Dmitry Prozorkevich. 2017. "Arctic Fishes in the Barents Sea 2004-2015: Changes in Abundance and Distribution." *IMR/PINRO Joint Report Series*, 1–50.

Kawahara, M, SI Uye, K Ohtsu, and H Iizumi. 2006. "Unusual Population Explosion of the Giant Jellyfish Nemopilema Nomurai (Scyphozoa: Rhizostomeae) in East Asian Waters." *Marine Ecology Progress Series* 307 (January): 161–73. https://doi.org/10.3354/meps307161.

Khan, Shfaqat A., Kurt H. Kjær, Michael Bevis, Jonathan L. Bamber, John Wahr, Kristian K. Kjeldsen, Anders A. Bjørk, et al. 2014. "Sustained Mass Loss of the Northeast Greenland Ice Sheet Triggered by Regional Warming." *Nature Climate Change* 4 (4): 292–99. https://doi.org/10.1038/nclimate2161.

Knutsen, Tor, Aino Hosia, Tone Falkenhaug, Rasmus Skern-Mauritzen, Peter H. Wiebe, Roger B. Larsen, Asgeir Aglen, and Erik Berg. 2018. "Coincident Mass Occurrence of Gelatinous Zooplankton in Northern Norway." *Frontiers in Marine Science* 5 (May). https://doi.org/10.3389/fmars.2018.00158.

Kohlbach, Doreen, Martin Graeve, Benjamin A. Lange, Carmen David, Ilka Peeken, and Hauke Flores. 2016. "The Importance of Ice Algae-Produced Carbon in the Central Arctic Ocean Ecosystem: Food Web Relationships Revealed by Lipid and Stable Isotope Analyses." *Limnology and Oceanography* 61 (6): 2027–44. https://doi.org/10.1002/lno.10351.

Koszteyn, J., S. Timofeev, J. M. Weslawski, and B. Malinga. 1995. "Size Structure of Themisto Abyssorum (Boeck) and Themisto Libellula (Mandt) Populations in European Arctic Seas." *Polar Biology* 15: 85–92.

Krumpen, Thomas, H. Jakob Belter, Antje Boetius, Ellen Damm, Christian Haas, Stefan Hendricks, Marcel Nicolaus, et al. 2019. "Arctic Warming Interrupts the Transpolar Drift and Affects Long-Range Transport of Sea Ice and Ice-Rafted Matter." *Scientific Reports* 9 (1). https://doi.org/10.1038/s41598-019-41456-y.

Leray, Matthieu, Joy Y. Yang, Christopher P. Meyer, Suzanne C. Mills, Natalia Agudelo, Vincent Ranwez, Joel T. Boehm, and Ryuji J. Machida. 2013. "A New Versatile Primer Set Targeting a Short Fragment of the Mitochondrial Coi Region for Metabarcoding Metazoan Diversity: Application for Characterizing Coral Reef Fish Gut Content." *Frontiers in Zoology* 10 (34): 1–14.

Leu, E., J. E. Søreide, D. O. Hessen, S. Falk-Petersen, and J. Berge. 2011. "Consequences of Changing Sea-Ice Cover for Primary and Secondary Producers in the European Arctic Shelf Seas: Timing, Quantity, and Quality." *Progress in Oceanography* 90 (1-4): 18–32. https://doi.org/10.1016/j.pocean.2011.02.004.

Link, JS, and MD Ford. 2006. “Widespread and Persistent Increase of Ctenophora in the Continental Shelf Ecosystem Off NE USA.” *Marine Ecology Progress Series* 320 (August): 153–59. https://doi.org/10.3354/meps320153.

Loeng, H., and H. Gjøsæter. 1990. “Growth of 0-Group in Relation to Temperature Conditions in the Barents Sea During the Period 1965-1989.” *ICES CM1990/G*.

Longhurst, Alan R. 1985. “The Structure and Evolution of Plankton Communities.” *Progress in Oceanography* 15 (1): 1–35. https://doi.org/10.1016/0079-6611(85)90036-9.

Lowry, L. F., and K. J. Frost. 1984. “Foods and Feeding of Bowhead Whales in the Western and Northern Alaska.” *Scientific Reports of the Whales Research Institute* 35: 1–16.

Lynam, Christopher P., Mark J. Gibbson, Bjørn E. Axelsen, Conrad A. J. Sparks, Janet Coetzee, Benjamin G. Heywood, and Andrew S. Brierley. 2006. “Jellyfish Overtake Fish in Heavily Fished Ecosystem.” *Current Biology* 16 (13): R492–R493.

Lønne, O. J., and G. W. Gabrielsen. 1992. “Summer Diet of Seabirds Feeding in Sea-Ice-Covered Waters Near Svalbard.” *Polar Biology* 12: 685–92.

Lønne, O. J., and B. Gulliksen. 1989. “Size, Age and Diet of Polar Cod, Boreogadus Saida (Lepechin, 1773), in Ice Covered Waters.” *Polar Biology* 9: 187–91.

Lønne, O. J., and B. Gulliksen. 1991a. “On the Distribution of Sympagic Macro-Fauna in the Seasonally Ice Covered Barents Sea.” *Polar Biology*.

Lønne, O. J., and B. Gulliksen. 1991b. “Sympagic Macro-Fauna from Multiyear Sea-Ice Near Svalbard.” *Polar Biology* 11: 471–77.

Madsen, S. D., T. G. Nielsen, and B. W. Hansen. 2001. “Annual Population Development and Production by Calanus Finmarchicus, c. Glacialis and c. Hyperboreus in Disko Bay, Western Greenland.” *Marine Biology* 139: 75–93.

Majaneva, S, J Berge, PE Renaud, A Vader, E Stübner, AM Rao, Ø Sparre, and M Lehtiniemi. 2013. “Aggregations of Predators and Prey Affect Predation Impact of the Arctic Ctenophore Mertensia Ovum.” *Marine Ecology Progress Series* 476 (February): 87–100. https://doi.org/10.3354/meps10143.

Maniatis, Tom, Andrea Jeffrey, and Hans Van deSande. 1975. “Chain Length Determination of Small Double- and Single-Stranded Dna Molecules by Polyacrylamide Gel Electrophoresis.” *Biochemistry* 14 (17): 3787–94.

Marion, A, M Harvey, D Chabot, and J Brêthes. 2008. “Feeding Ecology and Predation Impact of the Recently Established Amphipod, Themisto Libellula, in the St. Lawrence Marine System, Canada.” *Marine Ecology Progress Series* 373 (December): 53–70. https://doi.org/10.3354/meps07716.

Martin, Marcel. 2011. “Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads.” *EMBnet.journal* 17 (1): 10. https://doi.org/10.14806/ej.17.1.200.

Maslowski, Wieslaw, Douglas Marble, Waldemar Walczowski, Ursula Schauer, Jaclyn L. Clement, and Albert J. Semtner. 2004. "On Climatological Mass, Heat, and Salt Transports Through the Barents Sea and Fram Strait from a Pan-Arctic Coupled Ice-Ocean Model Simulation." *Journal of Geophysical Research: Oceans* 109 (C3). https://doi.org/10.1029/2001jc001039.

Mata, Vanessa A., Hugo Rebelo, Francisco Amorim, Gary F. McCracken, Simon Jarman, and Pedro Beja. 2019. "How Much Is Enough? Effects of Technical and Biological Replication on Metabarcoding Dietary Analysis." *Molecular Ecology* 28: 165–75.

Maxam, A. M., and W. Gilbert. 1977. "A New Method for Sequencing DNA." *Proceedings of the National Academy of Sciences* 74 (2): 560–64. https://doi.org/10.1073/pnas.74.2.560.

McInnes, Julie C., Rachael Alderman, Mary-Anne Lea, Ben Raymond, Bruce E. Deagle, Richard A. Phillips, Andrew Stanworth, et al. 2017. "High Occurrence of Jellyfish Predation by Black-Browed and Campbell Albatross Identified by DNA Metabarcoding." *Molecular Ecology* 26 (18): 4831–45. https://doi.org/10.1111/mec.14245.

Mills, Claudia E. 2001. "Jellyfish Blooms: Are Populations Increasing Globally in Response to Changing Ocean Conditions?" *Hydrobiologia* 451: 55–68.

Nakahara, Fumiko, Haruko Ando, Hideyuki Ito, Asako Murakami, Naoki Morimoto, Michimasa Yamasaki, Atsushi Takayanagi, and Yuji Isagi. 2015. "The Applicability of DNA Barcoding for Dietary Analysis of Sika Deer." *DNA Barcodes* 3 (1). https://doi.org/10.1515/dna-2015-0021.

Nilssen, K. T., T. Haug, V. Potelov, and Y. K. Timoshenko. 1995. "Feeding Habits of Harp Seals (Phoca Groenlandica) During Early Summer and Autumn in the Northern Barents Sea." *Polar Biology* 15: 485–93.

Oksanen, Jari, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, et al. 2020. *Vegan: Community Ecology Package*. https://CRAN.R-project.org/package=vegan.

Olsen, Bernt Rydland, Christofer Troedsson, Kenan Hadziavdic, Rolf B. Pedersen, and Hans Tore Rapp. 2013. "A Molecular Gut Content Study ofThemisto Abyssorum(Amphipoda) from Arctic Hydrothermal Vent and Cold Seep Systems." *Molecular Ecology* 23 (15): 3877–89. https://doi.org/10.1111/mec.12511.

Onarheim, Ingrid H., Lars H. Smedsrud, Randi B. Ingvaldsen, and Frank Nilsen. 2014. "Loss of Sea Ice During Winter North of Svalbard." *Tellus A: Dynamic Meteorology and Oceanography* 66 (1): 23933. https://doi.org/10.3402/tellusa.v66.23933.

Ottersen, G. 2000. "Covariability in Early Growth and Year-Class Strength of Barents Sea Cod, Haddock, and Herring: The Environmental Link." *ICES Journal of Marine Science* 57 (2): 339–48. https://doi.org/10.1006/jmsc.1999.0529.

Overland, James E., and Muyin Wang. 2013. "When Will the Summer Arctic Be Nearly Sea Ice Free?" *Geophysical Research Letters* 40 (10): 2097–2101. https://doi.org/10.1002/grl.50316.

Pakhomov, E. A., and R. Perissinotto. 1996. “Trophodynamics of the Hyperiid Amphipod Themisto Gaudichaudii in the South Georgia Region During Late Asutral Summer.” *Marine Ecology Progress Series* 134: 91–100.

Parsons, T. R., and C. M. Lalli. 2002. “Jellyfish Population Explosions: Revisiting a Hypothesis of Possible Causes.” *La Mer* 40: 111–21.

Pedersen, Carsten Egevang, and Knud Falk. 2001. “Chick Diet of Dovekies Alle Alle in Northwest Greenland.” *Polar Biology* 24: 53–58.

Percy, J. A. 1993. “Energy Consumption and Metabolism During Starvation in the Arctic Hyperiid Amphipod Themisto Libellula Mandt.” *Polar Biology* 13: 549–55.

Piepenburg, D., T. H. Blackburn, C. F. von Dorrien, J. Gutt, P. O. J. Hall, S. Hulth, M. A. Kendall, K. W. Opalinski, E. Rachor, and M. K. Schmid. 1995. “Partioning of Benthic Community Respiration in the Arctic (Northwestern Barents Sea).” *Marine Ecology Progress Series* 118: 199–213.

Piepenburg, Dieter, and Michael K. Schmid. 1996. “Brittle Star Fauna (Echinodermata: Ophiuroidea) of the Arctic Northwestern Barents Sea: Composition, Abundance, Biomass and Spatial Distribution.” *Polar Biology* 16: 383–92.

Pinchuk, Alexei I., Kenneth O. Coyle, Edward V. Farley, and Heather M. Renner. 2013. “Emergence of the Arctic Themisto Libellula (Amphipoda: Hyperiidae) on the Southeastern Bering Sea Shelf as a Result of the Recent Cooling, and Its Potential Impact on the Pelagic Food Web.” *ICES Journal of Marine Science* 70 (6): 1244–54. https://doi.org/10.1093/icesjms/fst031.

Polyakov, Igor V., Matthew B. Alkire, Bodil A. Bluhm, Kristina A. Brown, Eddy C. Carmack, Melissa Chierici, Seth L. Danielson, et al. 2020. “Borealization of the Arctic Ocean in Response to Anomalous Advection from Sub-Arctic Seas.” *Frontiers in Marine Science* 7 (July). https://doi.org/10.3389/fmars.2020.00491.

Polyakov, Igor V., Andrey V. Pnyushkov, Matthew B. Alkire, Igor M. Ashik, Till M. Baumann, Eddy C. Carmack, Ilona Goszczko, et al. 2017. “Greater Role for Atlantic Inflows on Sea-Ice Loss in the Eurasian Basin of the Arctic Ocean.” *Science* 356 (6335): 285–91. https://doi.org/10.1126/science.aai8204.

Polyakov, Igor V., John E. Walsh, and Ronald Kwok. 2012. “Recent Changes of Arctic Multiyear Sea Ice Coverage and the Likely Causes.” *Bulletin of the American Meteorological Society* 93 (2): 145–51. https://doi.org/10.1175/bams-d-11-00070.1.

Pompanon, Francois, Bruce E. Deagle, William O. C. Symondson, David S. Brown, Simon N. Jarman, and Pierre Taberlet. 2012. “Who Is Eating What: Diet Assessment Using Next Generation Sequencing.” *Molecular Ecology* 21 (8): 1931–50. https://doi.org/10.1111/j.1365-294x.2011.05403.x.

Purcell, JE. 2007. “Environmental Effects on Asexual Reproduction Rates of the Scyphozoan Aurelia Labiata.” *Marine Ecology Progress Series* 348 (October): 183–96. https://doi.org/10.3354/meps07056.

Purcell, Jennifer E. 2005. “Climate Effects on Formation of Jellyfish and Ctenophore Blooms: A Review.” *Journal of the Marine Biological Association of the United Kingdom* 85 (3): 461–76. https://doi.org/10.1017/s0025315405011409.

Purcell, Jennifer E., and Mary N. Arai. 2001. “Interactions of Pelagic Cnidarians and Ctenophores with Fish: A Review.” *Hydrobiologia*.

Purcell, Jennifer E., Russell R. Hopcroft, Ksenia N. Kosobokova, and Terry E. Whitledge. 2010. “Distribution, Abundance, and Predation Effects of Epipelagic Ctenophores and Jellyfish in the Western Arctic Ocean.” *Deep Sea Research Part II: Topical Studies in Oceanography* 57 (1-2): 127–35. https://doi.org/10.1016/j.dsr2.2009.08.011.

Purcell, J., W. Graham, and H. Dumont. 2001. “Jellyfish Blooms: Ecological and Societal Importance.” *Developments in Hydrobiology* 155: 333 p.

Qiagen. 2017. *DNeasy Powersoil Kit Handbook - for the Isolation of Microbial Genomic Dna from All Soil Samples*.

Ratnasingham, S., and P. D. N. Herbert. 2007. “BOLD: The Barcode of Life Data System. Available from: https://www.boldsystems.org/. Accessed 2021-01-29.” *Molecular Ecology Notes* 7 (3): 355–64.

Ray, Jessica L., Julia Althammer, Katrine S. Skaar, Paolo Simonelli, Aud Larsen, Diane Stoecker, Andrey Sazhin, et al. 2016. “Metabarcoding and Metabolome Analyses of Copepod Grazing Reveal Feeding Preference and Linkage to Metabolite Classes in Dynamic Microbial Plankton Communities.” *Molecular Ecology* 25 (21): 5585–5602. https://doi.org/10.1111/mec.13844.

Rigor, Ignatus G. 2002. “Response of Sea Ice to the Arctic Oscillation.” *Journal of Climate*.

Rognes, T., T. Flouri, B. Nicholas, C. Quince, and F. Mahé. 2016. “VSEARCH: A Versatile Open Source Tool for Metagenomics.” *PeerJ* 4: e2584.

Samemoto, D. D. 1987. “Vertical Distribution and Ecological Sinificance of Chaetognaths in the Arctic Einvironment of Baffin Bay.” *Polar Biology* 7: 317–28.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. “DNA Sequencing with Chain-Terminating Inhibitors.” *Proceedings of the National Academy of Sciences* 74 (12): 5463–7. https://doi.org/10.1073/pnas.74.12.5463.

Schlichtholz, Pawel, and Ilona Goszczko. 2006. “Interannual Variability of the Atlantic Water Layer in the West Spitsbergen Current at in Summer 19912003.” *Deep Sea Research Part I: Oceanographic Research Papers* 53 (4): 608–26. https://doi.org/10.1016/j.dsr.2006.01.001.

Sejr, Mikael K., Colin A. Stedmon, Jørgen Bendtsen, Jakob Abermann, Thomas Juul-Pedersen, John Mortensen, and Søren Rysgaard. 2017. “Evidence of Local and Regional Freshening of Northeast Greenland Coastal Waters.” *Scientific Reports* 7 (1). https://doi.org/10.1038/s41598-017-10610-9.

Shendure, Jay, Shankar Balasubramanian, George M. Church, Walter Gilbert, Jane Rogers, Jeffery A. Schloss, and Robert H. Waterston. 2017. "DNA Sequencing at 40: Past, Present and Future." *Nature* 550 (7676): 345–53. https://doi.org/10.1038/nature24286.

Shiganova, T. A. 1998. "Invasion of the Black Sea by the ctenophoreMnemiopsis Leidyiand Recent Changes in Pelagic Community Structure." *Fisheries Oceanography* 7 (3-4): 305–10. https://doi.org/10.1046/j.1365-2419.1998.00080.x.

Siegenthaler, Andjin, Owen S. Wangensteen, Chiara Benvenuto, Joana Campos, and Stefano Mariani. 2018. "DNA Metabarcoding Unveils Multiscale Trophic Variation in a Widespread Coastal Opportunist." *Molecular Ecology* 28 (2): 232–49. https://doi.org/10.1111/mec.14886.

Siferd, Timothy D., and Robert J. Conover. 1992. "Natural History of Ctenophores in the Resolute Passage Area of the Canadian High Arctic with Special Reference to Mertensia Ovum." *Marine Ecology Progress Series* 86 (2): 133–44.

Smetacek, Victor, Philipp Assmy, and Joachim Henjes. 2004. "The Role of Grazing in Structuring Southern Ocean Pelagic Ecosystems and Biogeochemical Cycles." *Antarctic Science* 16 (4): 541–58. https://doi.org/10.1017/s0954102004002317.

Smith, Lloyd M., Jane Z. Sanders, Robert J. Kaiser, Peter Hughes, Chris Dodd, Charles R. Connell, Cheryl Heiner, Stephen B. H. Kent, and Leroy E. Hood. 1986. "Fluorescence Detection in Automated Dna Sequence Analysis." *Nature* 321: 674–79.

Steiner, Nadja S., William W. L. Cheung, Andres M. Cisneros-Montemayor, Helen Drost, Hakase Hayashida, Carie Hoover, Jen Lam, et al. 2019. "Impacts of the Changing Ocean-Sea Ice System on the Key Forage Fish Arctic Cod (Boreogadus Saida) and Subsistence Fisheries in the Western Canadian ArcticEvaluating Linked Climate, Ecosystem and Economic (CEE) Models." *Frontiers in Marine Science* 6 (April). https://doi.org/10.3389/fmars.2019.00179.

Stige, Leif Christian, Elena Eriksen, Padmini Dalpadado, and Kotaro Ono. 2019. "Direct and Indirect Effects of Sea Ice Cover on Major Zooplankton Groups and Planktivorous Fishes in the Barents Sea." *ICES Journal of Marine Science* 76: i24–i36.

Stowasser, G., A. Atkinson, R. A. R. McGill, R. A. Phillips, M. A. Collins, and D. W. Pond. 2012. "Food Web Dynamics in the Scotia Sea in Summer: A Stable Isotope Study." *Deep Sea Research Part II: Topical Studies in Oceanography* 59-60 (January): 208–21. https://doi.org/10.1016/j.dsr2.2011.08.004.

Sunderland, K. D., W. Powell, and W. O.C. Symondson. 2005. "Insects as Natural Enemies: A Practical Perspective." In, edited by M. A. Jervis, 299–434. Springer.

Søreide, Janne E., Michael L. Carroll, Haakon Hop, William G. Jr.Ambrose, Else N. Hegseth, and Stig Falk-Petersen. 2013. "Sympagic-Pelagic-Benthic Coupling in Arctic and Atlantic Waters Around Svalbard Revealed by Stable Isotopic and Fatty Acid Tracers." *Marine Biology Research* 9: 831–50.

Søreide, Janne E., Haakon Hop, Michael L. Carroll, Stig Falk-Petersen, and Else Nøst Hegseth. 2006. "Seasonal Food Web Structures and Sympagicpelagic Coupling in the European Arctic Revealed by Stable Isotopes and a Two-Source Food Web Model." *Progress in Oceanography* 71 (1): 59–87. https://doi.org/10.1016/j.pocean.2006.06.001.

Søreide, Janne E., Eva Leu, Jørgen Berge, Martin GraeveE, and Stig Falk-Petersen. 2010. "Timing of Blooms, Algal Food Quality and Calanus Glacialis Reproduction and Growth in a Changing Arctic." *Global Change Biology*, January, no–no. https://doi.org/10.1111/j.1365-2486.2010.02175.x.

Taberlet, Pierre, Eric Coissac, Mehrdad Hajibabaei, and Loren H. Rieseberg. 2012. "Environmental DNA." *Molecular Ecology* 21 (8): 1789–93. https://doi.org/10.1111/j.1365-294x.2012.05542.x.

Terazaki, Makoto. 2000. "Dynamics and Characterization of Marine Organic Matter." In, edited by N. Handa, E Tanoue, and T. Hama, 257–76. TERRAPUB/ Kluwer.

Terazaki, M., and C. B. Miller. 1982. "Reproduction of Meso- and Bathypelagic Chaetognaths in the Genus Eukrohnia." *Marine Biology* 71: 193–96.

Visser, Andre W., Josephine Grønning, and Sigrún Huld Jónasdóttir. 2017. "Calanus Hyperboreus and the Lipid Pump." *Limnology and Oceanography* 62 (3): 1155–65. https://doi.org/10.1002/lno.10492.

Wadhams, Peter. 1983. "Sea Ice Thickness Distribution in Fram Strait." *Nature* 305: 108–11.

Wang, Qiang, Claudia Wekerle, Xuezhu Wang, Sergey Danilov, Nikolay Koldunov, Dmitry Sein, Dmitry Sidorenko, Wilken-Jon Appen, and Thomas Jung. 2020. "Intensification of the Atlantic Water Supply to the Arctic Ocean Through Fram Strait Induced by Arctic Sea Ice Decline." *Geophysical Research Letters* 47 (3). https://doi.org/10.1029/2019gl086682.

Wangensteen, Owen S., Creu Palacín, Magdalena Guardiola, and Xavier Turon. 2018. "DNA Metabarcoding of Littoral Hard-Bottom Communities: High Diversity and Database Gaps Revealed by Two Molecular Markers." *PeerJ*.

Weigand, Hannah, Arne J. Beermann, Fedor Čiampor, Filipe O. Costa, Zoltán Csabai, Sofia Duarte, Matthias F. Geiger, et al. 2019. "DNA Barcode Reference Libraries for the Monitoring of Aquatic Biota in Europe: Gap-Analysis and Recommendations for Future Work." *Science of the Total Environment* 678 (August): 499–524. https://doi.org/10.1016/j.scitotenv.2019.04.247.

Werner, Iris, Johanna Ikävalko, and Henrike Schünemann. 2007. "Sea-Ice Algae in Arctic Pack Ice During Late Winter." *Polar Biology* 30: 1493–1504.

Węsławski, Jan M., Joanna Legeżyńska, and Maria Włodarska-Kowalczuk. 2020. "Will Shrinking Body Size and Increasing Species Diversity of Crustaceans Follow the Warming of the Arctic Littoral?" *Ecology and Evolution* 10 (19): 10305–13. https://doi.org/10.1002/ece3.6780.

Wickham, Hadley, Mara Averick, Jennifer Bryan, Winston Chang, Lucy D'Agostino McGowan, Romain François, Garrett Grolemund, et al. 2019. "Welcome to the tidyverse." *Journal of Open Source Software* 4 (43): 1686. https://doi.org/10.21105/joss.01686.

WoRMS Editorial Board. 2020. "World Register of Marine Species. Available from http://www.marinespecies.org at Vliz. Accessed 2021-01-29." VLIZ. https://doi.org/10.14284/170.

Zhang, Xiangdong, Moto Ikeda, and John E. Walsh. 2003. "Arctic Sea Ice and Freshwater Changes Driven by the Atmospheric Leading Mode in a Coupled Sea Ice-Ocean Model." *Journal of Climate*.

Øigård, TA, U Lindstrøm, T Haug, KT Nilssen, and S Smout. 2013. "Functional Relationship Between Harp Seal Body Condition and Available Prey in the Barents Sea." *Marine Ecology Progress Series* 484 (June): 287–301. https://doi.org/10.3354/meps10272.

Appendix

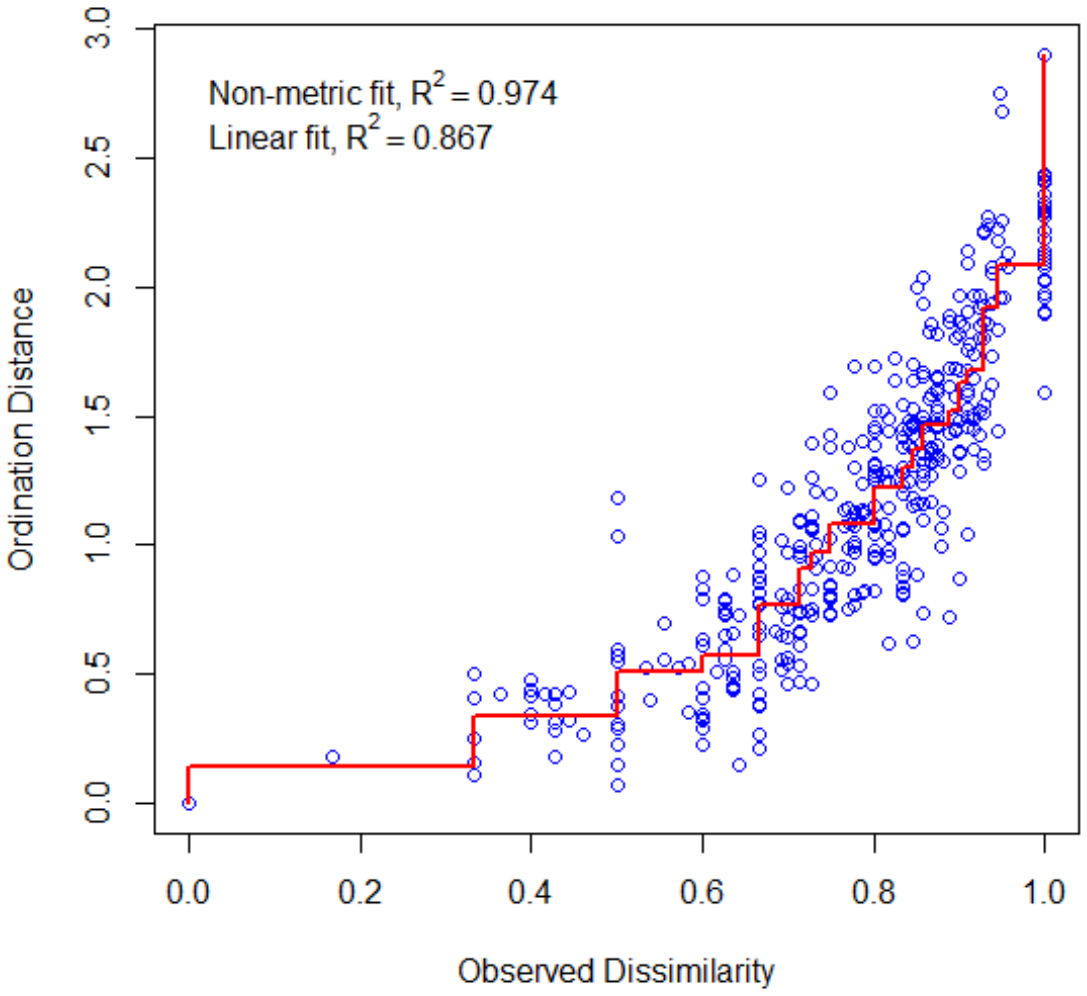


Fig. 23: Shepherds diagram for the NMDS model for both predators.

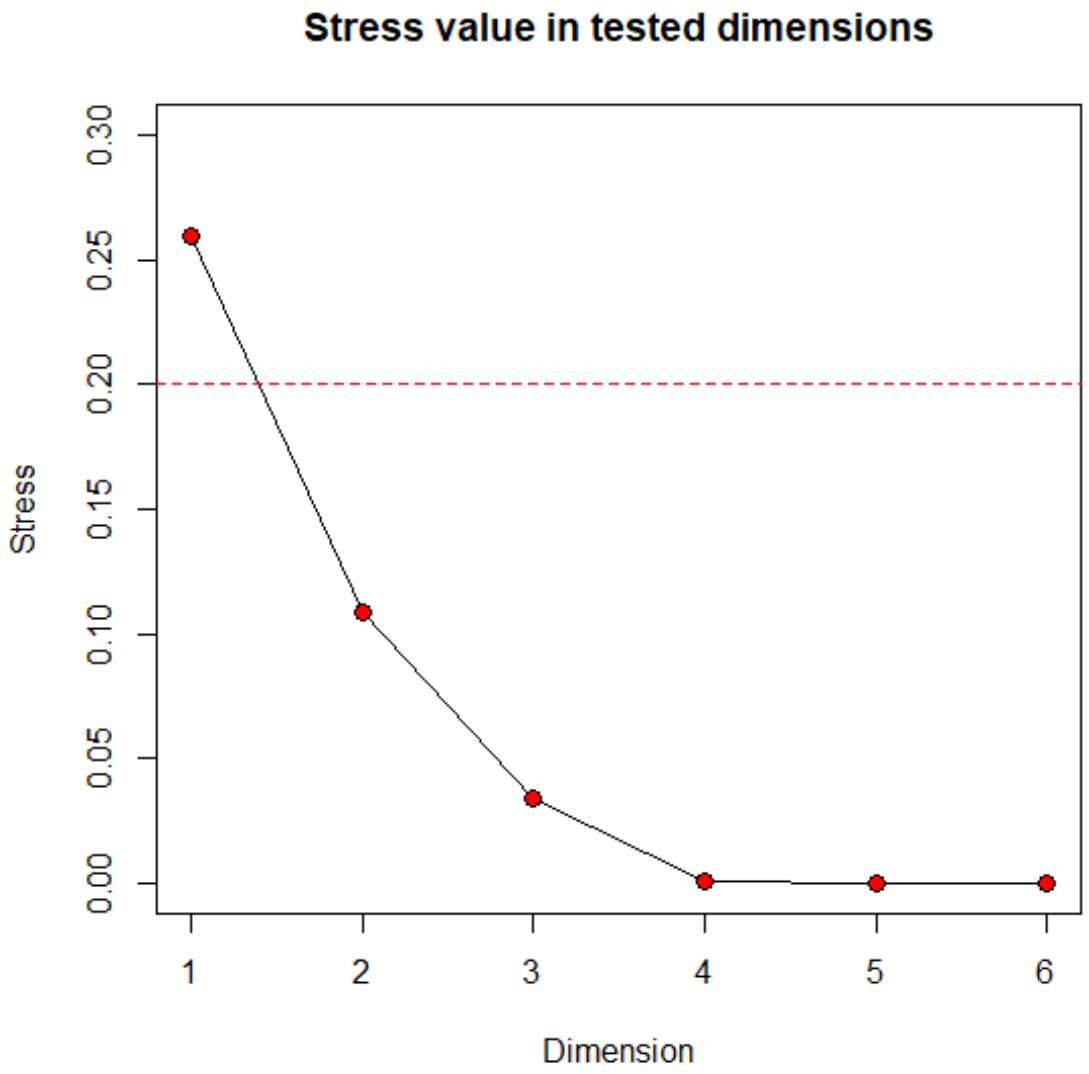


Fig. 24: Screeplot of T. libellula, plotting stress over dimensions. The red line indicates the threshold of 0.2.

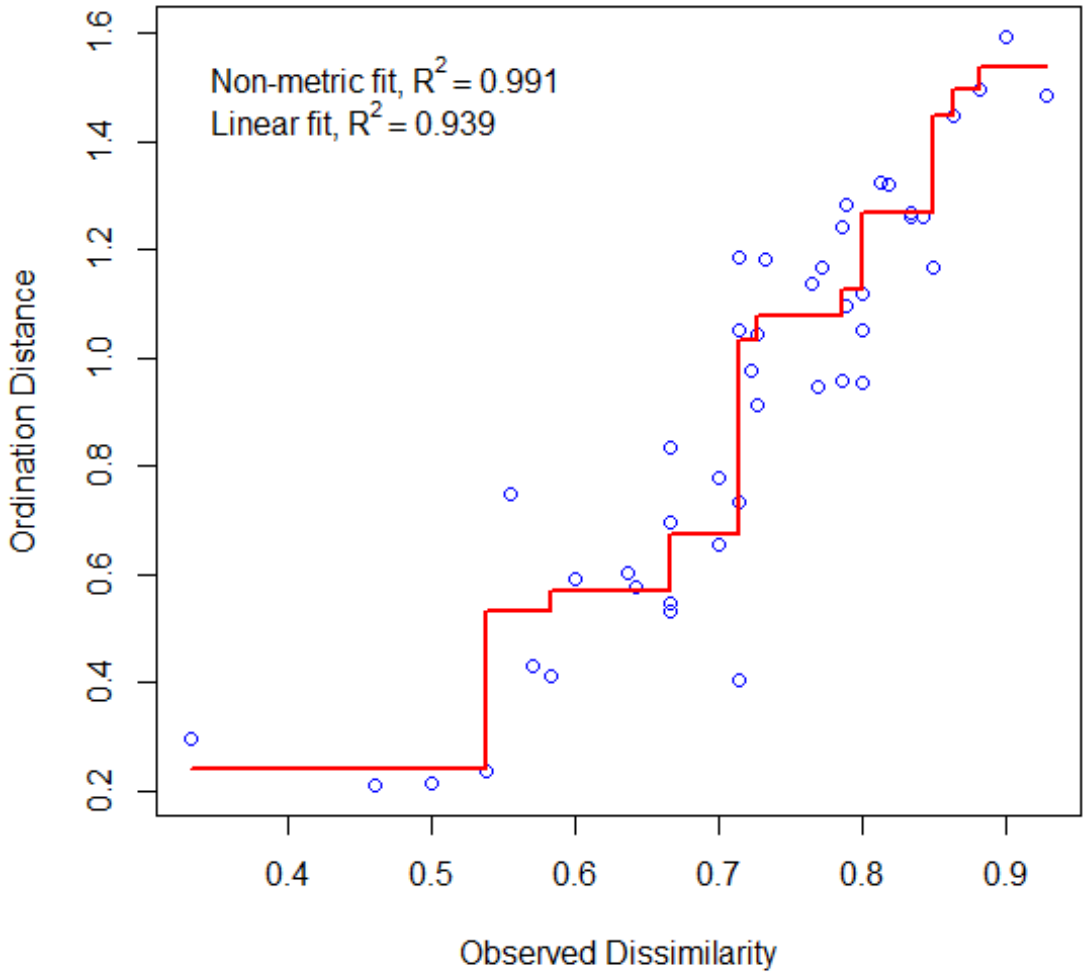


Fig. 25: Shepherds diagram of T. libellula.

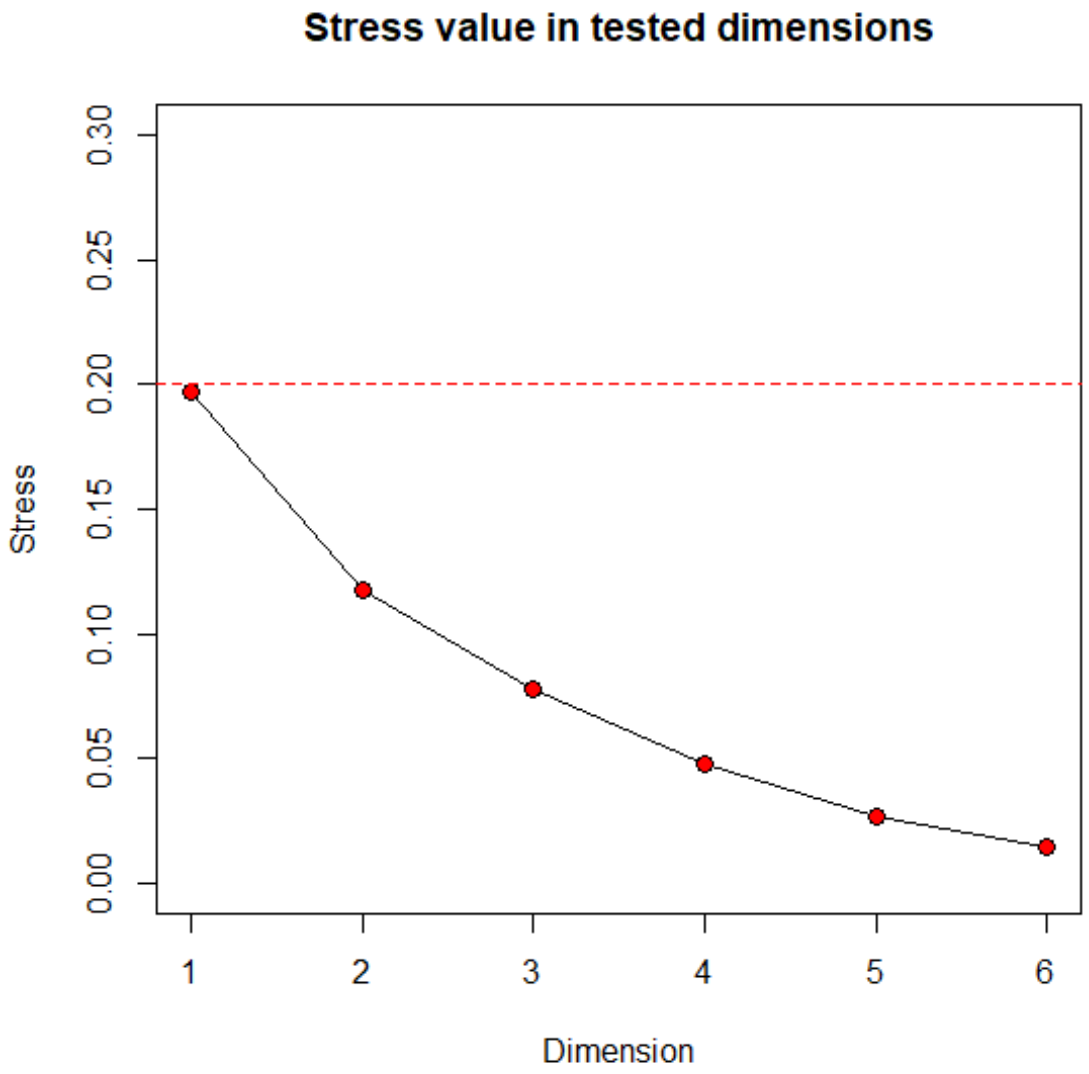


Fig. 26: Screeplot of T. abyssorum, plotting stress over dimensions. The red line indicates the threshold of 0.2.

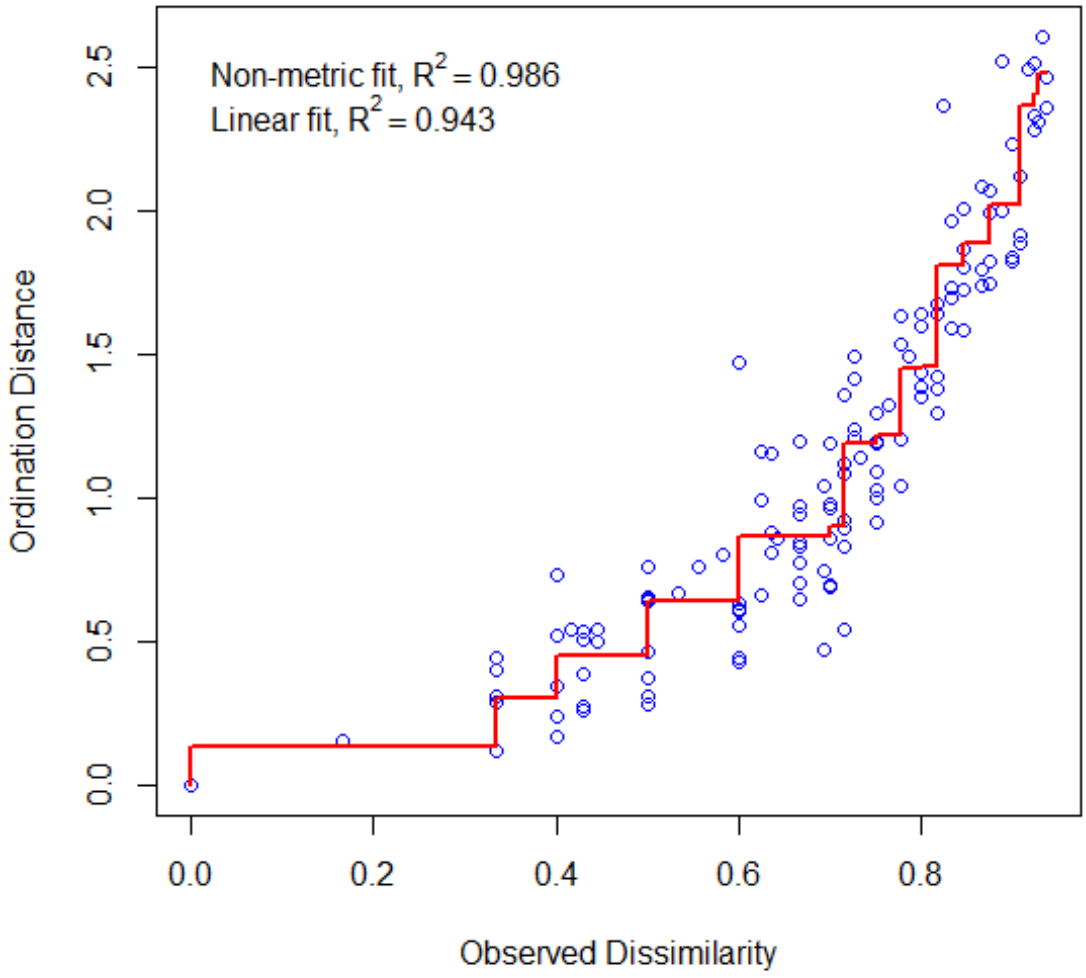


Fig. 27: Shepherds diagram of T. abyssorum.