Can we see the genetic fingerprint of fishing impact on the cold water corals of Western Greenland?

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Abstract

Deep sea trawling has heavily impacted marine environments and communities around the globe for decades, destroying habitats and reducing the biodiversity and abundance of many species. Legislation regulating the practice is often insufficient to protect vulnerable species, particularly in international waters. In order to further assess the impact on cold water coral biodiversity that shrimp trawling is having off the coast of Western Greenland the MutS mitochondrial DNA region from coral samples collected by trawlers are compared. A number of samples from Canadian waters are also included for comparison. Analysis of differing diversity in geographical regions of differing trawling intensity is made but no pattern is found, with all regions showing relatively similar levels of biodiversity independent of trawling intensity. It is therefore concluded that the trawling is not having an impact in this particular case, although further work is advised to improve reliability of results.

Introduction

Trawling history and impacts

Deep sea trawling has been one of the most contentious issues of international fishing for many decades, with studies into the damage it causes dating back as far as 1885 (Eyre and Spottiswoode, 1885). The practice most commonly consists of dragging a large weighted net along the sea bed of a deep sea environment. Two metal trawl doors sit on either side of the net and keep it open while it is dragged along the sea floor, disturbing the sediment and throwing up any organisms living there into the net (Watling and Norse 1998; Rice 2006). It is commonly used to harvest a wide variety of species, most commonly fish, but also other marine species like shrimp and squid. Overfishing of the target species is thought to have reduced the stocks of many by up to ninety percent in certain areas (Christensen, 2003; Myers & Worm, 2003), but this is far from all of the damage that trawling is responsible for.

Despite advances in trawling gear that allows improved targeting of shoals by the vessels, which has slightly reduced bycatch (Broadhurst, 2000), the practice is still hugely indelicate and damaging to the environment and still generally lacks precision

or ability to differentiate between organisms, meaning that not only the target species, but nearly all other life forms are dragged into the net. These non-target organisms that are caught in the net are known as bycatch, and once brought to the surface are usually discarded overboard by trawler crews. This is not enough to save them, as many are killed by either the changes in pressure between the sea floor and the surface or through sheer blunt trauma in the process of being caught and dragged up (Fosså et al., 2002).

Beyond the harm done by bycatch, the majority of damage done is through mortality caused through blunt trauma when organisms come into contact with the heavy machinery involved in the net (Jenkins, Beukers-Stewart & Brand, 2001). Sediment disturbance can also heavily impact some groups especially on soft substrata, as the disturbed sediment can resettle onto various organisms and smother them, also making it difficult for the larvae of some groups to settle by creating an anaerobic environment (Jones, 1992).

Trawling legislature

Before legislature effecting deep sea trawling can be discussed it is important to understand that the definition of what constitutes “deep sea” is a highly varied one, and is generally entirely relative to the region within which the term is being applied. The United Nations (UN) defines the deep sea as the region between 200 and 2000 meters (FAO, 2016), while the European Parliament defines it as the region beyond continental shelves (EPRSLibary, 2013). The definition used by individual research articles or national bodies of law can vary even further, and generally there is little agreement on the topic. For this study the UN definition of depths below 200m was used.

Deep sea trawling bans have been implemented in the national waters of a large number of countries throughout the last decade as evidence for the destructive nature of trawling has greatly increased. These include a wide range of nations across the globe with notable examples including the USA’s ban along much of their west coast in 2006 (NOAA, 2006) and New Zealand’s Kermadec Ocean Sanctuary (MFE, 2016). These and the majority of other examples consist of bans that only cover specific areas that have been identified as particularly fragile or at risk; however the major exception to this is the island of Palau, which implemented a

complete ban of trawling in 2006 and has been highly vocal on wider bans ever since (UN, 2006).

Regulation of trawling in international waters is far less common however, and little legislature protects much of the world's oceans from the damage trawling can cause. Some areas are overseen by regional fishery management organisations such as the Northwest Atlantic Fisheries Organization (NAFO) and the North-East Atlantic Fisheries Commission (NEAFC) in the North Atlantic**,** but the power these organisations wield is often limited and their effectiveness greatly varies (Molenaar, 2004)**.** Both the UN and the EU have made statements on several occasions that call for the regulation of deep sea trawling and claim urgent action is needed, encouraging regulation by individual nations (e.g UNGA, 1982; UNGA, 2004; EU, 2002; EPRS, 2013). As of yet, they themselves have done little however to regulate international waters despite being some of the few bodies capable of doing so.

The UN has created several initiatives to restock and make sustainable various global fisheries (UN, 2002) and in 2005 the UN's General Fisheries Commission for the Mediterranean (GFCM) established the first ever ban on deep sea trawling in international waters by banning the practice in all Mediterranean waters below 1000m **(GFCM, 2005).** A more ambitious attempt to regulate deep sea trawling in the UN was the push made by Tommy Remengesau, President of Palau, for a complete ban on unregulated deep sea trawling in international waters due to the damage it caused (UN, 2006). Despite receiving support from several other nations, the proposal was ultimately unsuccessful and no legislation was put into place because of it.

Trawling in European waters is currently regulated through catch limits and restrictions set by the European Parliament in 2002 (EU, 2002). In 2013 a proposal for the banning of deep sea trawling in all European waters failed to pass due pressures from the fishing industry; however, a clause was put in place to have a re-evaluation made in 2017 and another vote held based on the new findings (EU, 2013).

The EU is currently in the process of negotiating a potential depth limit to deep sea trawling for this 2017 deadline. A study from 2015 show that any trawling below 600 m is especially damaging and also of limited economic return (Clarke et al, 2015) is

thought to be influencing the decision, but lobbying has resulted in the initial proposal of 200 m slowly descending to only a ban below 800 m as the proposal has been passed through various committees (Chris Yesson, pers. comms.). The downward revision of the depth threshold has angered some conservation groups and goes against the scientific recommendations.

If deep sea trawling continues at its current rate then it may soon or already be too late to repair the damage that has been done, with recovery estimates of dozens of years for many of the effected organisms (Freese 2001, Boutilier et al. 2010). This is of course only one aspect of a much wider issue that covers the entire fishing industry, with organisms in every ocean being driven rapidly toward extinction by a wide variety of techniques which are poorly regulated and under-monitored, leading to an abundance of unknowns in terms of how near to disaster we are. One of the most telling pieces of evidence for this issue is the fact that historical fleets of relatively simplistic vessels were able to bring in hauls of fish hugely superior to modern numbers brought in by far more sophisticated vessels due largely to the fact that there are simply far fewer fish then their use to be (Thurston, Brockington & Roberts, 2010). While this article will specifically focus on deep sea trawling, it is vital to acknowledge these issues and promote further work on the international crisis facing all marine biology.

Specific area background

This paper will focus in particular on the deep sea trawling carried out by shrimp fisheries along the Western coast of Greenland with some samples from the nearby eastern coast of Canada also included. Trawling in both regions is carried out by a number of shrimp fisheries using Otter trawls along each respective coastline targeting the Northern Shrimp, *Pandalus borealis* (Lassen et al. 2013). These shrimp have become the most profitable target for fisheries in the region and now account for about 50% percent of all exports out of Greenland after stocks of various target fish species along the coast, most specifically cod, collapsed due to environmental shifts, overfishing and mismanagement. These factors had less impact on the shrimp, which were more tolerant of cold waters, and with less predation from the fish, shrimp numbers greatly expanded (Buch et al, 2003).

As series of Student Projects at ZSL have focused on the impact of the trawling along the west coast of Greenland and revealed a general decrease in abundance across all marine organisms in the region (Yesson et al, In Review)**,** as has been seen in other trawled areas (Jennings et al., 2002). Organisms living on soft substrata appear to have been most impacted, whereas hard substrata communities appear less so (Gorham, 2014). Soft substrata are generally found in northern waters, while hard or sandy habitats are generally found in the south (Gougeon, 2015). Macrobenthic community diversity was also found to have decreased on the soft substrate, but not in other areas (Simon, 2013), with some evidence for increases in community diversity on hard substrata (Chemshirova, 2013).

The Nephtheidae

The focus of this study is will be the Nephthidae, a clade of cold water corals within the Alcyonacea order of Octocorallia. Members of the *Gersemia* (Marenzeller, 1877)*, Capnella* (Gray, 1869) and *Duva* (Koren & Danielssen, 1883) genera have been found to be distributed all along the entire shelf and upper slope of West Greenland by trawling surveys (Jørgensen et al, 2014), and it is samples from these three groups that will be used in this study. However, it is notoriously difficult to distinguish between species or even higher taxonomic groupings within the Alcyonacea and specifically the Nephtheids due to the fact that they possess a gradient of features across their lineage as oppose distinct features that can distinguish groups (Kenchington et al., 2009, Figueroa & Baco, 2014).

Corals have been found to be particularly susceptible to trawling (Simpson & Watling, 2006, Prena et al., 1999) as sessile organisms that cannot move to avoid disturbances made by the nets and have been shown to be replaced in heavily trawled regions by burrowing or mobile species that are more able to escape (De Juan, Demestre & Sanchez, 2011). Corals' slow growth rates and the fact that they possess emergent structures that stick up from the sea floor also make them especially vulnerable to the negative impacts of trawling (Curtis *et al.,* 2013). Amongst the especially vulnerable stony corals a single trawl can reduce coral abundance by up to 57% in the affected area (Curtis et al, 2012).

Cold water corals are especially vulnerable to trawling, as unlike shallow-reef systems which form above 50m depths (Freiwald et al., 2004) they lack the

resistance that shallow reefs build up due to their constant exposure to wave action (Hall-Spencer et al, 2002). They also lack the structural stability that is provided by the symbiotic relationship that shallow-reef systems share with calcareous coralline algae (Chisholm, 2000).

The Neptheidae off the west coast of Greenland have been reported to have seen an overall decrease in size over recent decades, with predictions being made that this is due to selection pressure created by the trawlers, which are more likely to come into contact with larger corals and damage them. Coral abundance in the region was not found to be decreasing however (Vakarc, 2015). Those smaller Neptheidae that are left have resilience to blunt trauma from trawlers as if sufficiently small the net will pass over them which may explain some of the lack of impact. This is especially true of rockhopper gear used in Otter trawls, which include rubber discs that ease the trawl over uneven areas on hard substrata as to reduce damage to the net (Watling & Norse, 1998).

Studies focusing specifically on these corals in the region initially listed them as non-vulnerable based on an assessment made in the St Laurence estuary region (DFO, 2012); however, Gorham (2014) found them to be amongst the groups whose abundance was negatively impacted by trawling across all substrata. Murphy (2014) then analysed the impact that trawling was having on biodiversity but found no relationship between biodiversity and trawling intensity. Stock assessments of the corals in Canadian waters have been made (Wareham & Edinger, 2007) but little work has been done on assessing the impacts of trawling on biodiversity there.

Barcoding

Comparison of diversity will be made using the MutS barcoding region of mitochondrial DNA, previously called Msh1, which has been proven more successful for corals than the more commonly used COI coding region, in recent years (Pont-Kingdon, 1995). MutS also shows a far higher level of intraspecific variation than COI (McFadden et al., 2011), which is particularly important among the Nephtheidae where morphological identification is unreliable and so genetic identification is more important. This region has been frequently used before successfully in the identification and classification of octocorals (eg Sánchez et al., 2003; Brugler & France, 2008,). This will also allow use of the dataset from Murphy (2014), which

consists of data from the same Western Greenland sample set that this study is using.

Previous studies using this region, even combined with multiple other regions have still not been able to achieve 100% resolution amongst coral species and so distinctions between genera may still be difficult (Baco & Cairns, 2012). Despite these limitations, previous works using the study have still been able to identify important distribution and biodiversity patterns among the *Narella* octocoral genus in a similar effort to what is being done here.

Hypothesis

The hypothesis for this study is that biodiversity amongst Neptheidae corals will be significantly lower in regions of higher trawling intensity along the west coast of Greenland and the east coast of Canada.

Method

Sample preparation and DNA extraction

Coral fragments were collected by trawlers at stations across the entire shrimp trawl fishery region from the country's southern tip up the west coast to Upernavik, covering a range of areas with differing levels of trawling activity. Some stations were visited on multiple years while others were only visited once (Burmeister et al, 2013). At the stations the samples were pulled up in trawling nets from depths ranging between 72m and 908m as measured by the vessels acoustic sensors. Each trawl would last for a period of fifteen minutes with the vessel moving at a rate of 2.5kn for the duration. Similarly trawlers in Canadian waters collected coral fragments for the Canadian Department of Fisheries and Oceans. Data on the trawler vessels involved in collecting the Canadian samples is unavailable.

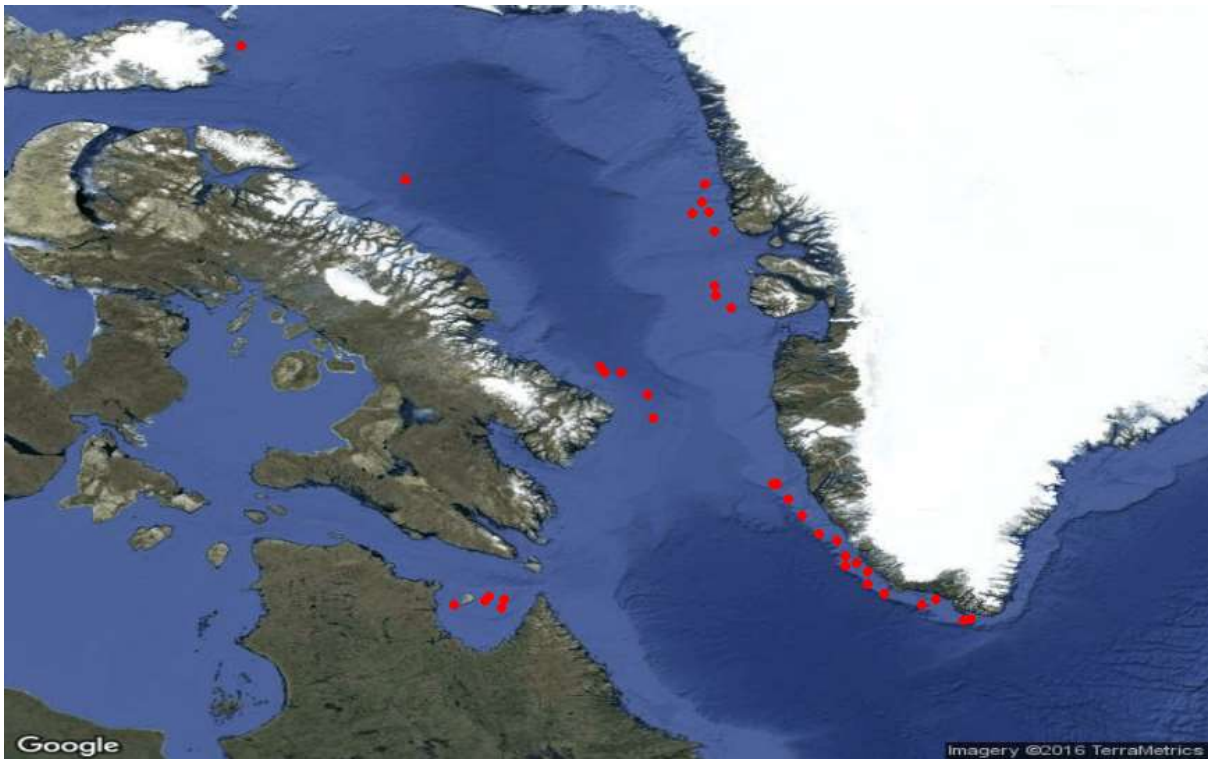

Figure 1.1 Sample collections locations for Ayre (2016) dataset.

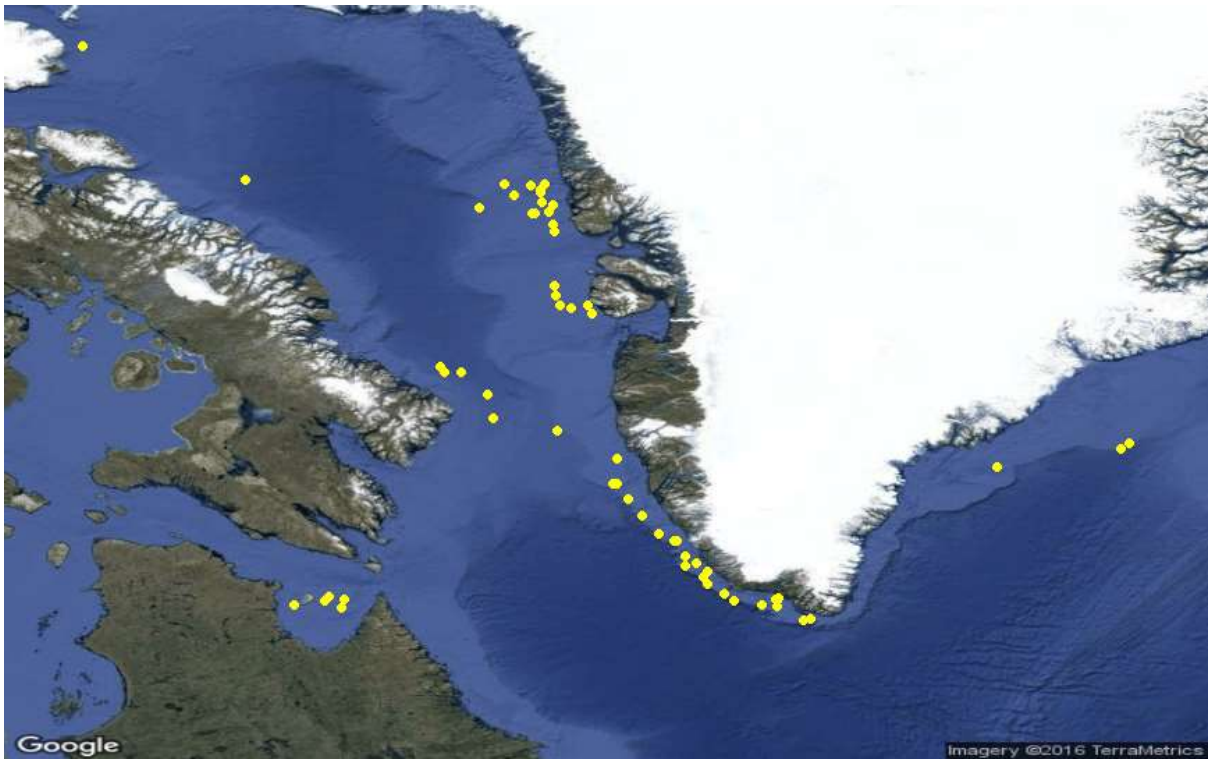

FIgure 1.2 Sample collection locations for Murphy (2014) and Ayre (2016) datasets combined.

Samples were then photographed and collected either by the trawler workers or by researchers stationed on board the trawlers and stored in either 100% ethanol or RNAlater for delivery to the UK. The samples stored in RNAlater were found to have greatly degraded by the time of this study and so all samples included in the new dataset were stored in ethanol; however, some samples included in the dataset of Murphy (2014) may have been stored in RNAlater. Collection of coral in this region was carried out with support from the Greenland Institute for Natural Resources who have been collecting samples from the bycatch since 2009.

A set of six geographical regions were chosen based on notable clusters within the sample distribution throughout the west coast of Greenland and the east coast of Canada. For the samples from Greenland, including those of Murphy (2014), the samples were assigned to three regions labelled “Greenland North”, “Greenland South” and “Greenland East”. The Canadian samples were similarly assigned one of three regions titled “Canada Central”, “Canada South” and “Canada North”.

Identification of all samples to genus level was attempted using the standard identification key of the Northwest Atlantic Fisheries Organisation (NAFO) (Kenchington et al., 2009) before lab worked commenced, but due to the known difficulty in identifying members of the Neptheidae family by morphological detail (Mortensen et al., 2008), especially limited to the details available on the fragmented samples, the identity of many of the samples could not be narrowed any further than Neptheidae. Descriptions of each genus are as follows: *Duva* - ‘soft, branching, broccoli-like with polyps in loose clusters, stem slightly rough to touch’. *Gersemia* - ‘soft but firm, branching, cauliflower-like to round with polyps in tight clusters. *Capnella* - ‘soft or firmer stem, smooth to touch, branching with polyps variable but may resemble clusters of grapes’. All samples were also photographed using a Leica IC80 HD microscope camera.

In the lab, all DNA extractions were carried out using the standard QIAGEN Genomic DNA kit and the Bioline DNA extraction protocol as detailed in appendix 1. All extractions were then tested for DNA concentration using a NanoDrop spectrophotometer and any substances found to be outside of the range 50 ng/µl-100 ng/µl were diluted with elution buffer or evaporated accordingly in order to correct

concentration. Samples with a concentration below 20 ng/µl were regarded as unsuccessful and re-extraction attempts were made.

Samples of correct concentration then underwent PCR to amplify the MutS region with the ND4L2475F forward primer 5'- TAGTTTTACTGGCCTCTAC – 3' (Brugler & France, 2008) and the MUT3458R reverse primer MutS3458R 5'-TSGAGCAAAAGCCACTCC-3' (Sánchez et al, 2003). Initially a cycle of 3 min at 94ºC for initial denaturation, followed by 35 cycles of 1 min at 94ºC, 1 min at 50ºC, 1 min at 72ºC and a final extension step of 10 min at 72ºC (van Ofwegen & Groenenberg, 2007, following methods recommended for this group by Murphy, 2014).

Due to a low success rate in our extractions, with often less than a third of samples working, a wide range of primer combinations were tested for each of the different genus of coral with other primers used consisting of forward primers ND42625F 5-TACGTG GYACAATTGCTG-3 (Lepard, 2003), and 165647F 5-ACACAGCTCGGTTTCTATCTACCA-3 and the reverse primer ND21418R 5-ACATCGGGAGCCCACATA-3 (McFadden et al, 2004). All possible forward and reverse combinations between these three and the original two primers were the used to amplify the MutS region from samples from each genus.

In a further attempt to increase the amount of successful extractions, temperature was lowered to 49ºC in phase 3 of the PCR process. All PCR products were then tested for sufficient DNA presence on agrose gel. Those that were deemed to have been a success were then sent via courier to the Macrogen lab in Amsterdam where they were sequenced as part of the Ez-Seq sequencing service, which carries out a traditional Sanger sequencing to produce a chromatogram of the results. As preparation for this service, two 5µl measurements of PCR product with concentration of at least 50ng/µl were placed in plates with 5µl of 10pmole/µl of either forward or reverse primer, in order to generate the 3' and 5' sequences respectively.

Analysis

All sequences where imported into Geneious 9.0.5 (Kearse et al, 2012), where the sequences inspected individually for quality and had areas of low quality trimmed out. Those sequences with low quality throughout were then excluded. Where both a

forward and a reverse sequence were available for a sample a contig was created from the two and then manually checked. The remaining trimmed sequences were then auto aligned using the clustal method into an array and checked manually. The array was then imported as a FASTA format file into the R software version 3.2.5 (R Core Team, 2016).

In R the googleVis package (Gesmann & Castillo, 2011) was then used to produce a map of sample locations. The ape (Paradis, Claude & Strimmer, 2004), adegenet (Jombart, 2008; Jombart & Ahmed, 2011) and pegas (Paradis, 2010) packages were then used to analyse the array, creating a list of unique haplotypes and then graphical representations of this.

A Bayesian analysis was performed to reconstruct a phylogenetic tree to describe the relationships between all samples. The DNA sequences were input into the Mr Bayes 3.2.6 (Huelsenbeck & Ronquist, 2001, Ronquist & Huelsenbeck, 2003), where 1827000 generations of trees were run, by which point stationarity had been reached as judged from a trace of log likelihoods and sufficient sample size had been achieved as judged by the effective sample size. The trace of log likelihoods and the effective sample size were monitored using the Tracer program (Rambaut et al, 2014). A consensus tree was produced using the GTR+i+G model (Huelsenbeck & Ronquist, 2001, Ronquist & Huelsenbeck, 2003) with the first 25% of trees discarded and not used in the consensus. The tree was then viewed in FigTree v1.4.2 (Rambaut, 2009).

A box plot of the log transformed trawling hours for each sample location was then produced in order to assess the trawling intensity for each region within the Greenland samples. This data was not available for the Canadian samples of the “Greenland East” samples as both sets lie outside of the West Greenland Shrimp Trawl fisheries and so they were excluded. Trawling data for each sampling location was provided by Chris Yesson, representing the total number of hours trawled by the shrimp trawl fishery in the period 1986-2013 in a 3.5km grid around the area of the sample (Yesson et al in review). The trawling intensity was then compared to the number of haplotypes found in each region in order to assess correlation between the two. For a slightly more detailed analysis the two Greenland regions were then divided by their NAFO fishing area locations and another boxplot was created. A

scatter graph showing normalized diversity per area against trawling hours was also produced.

Results

Despite attempts to improve PCR output using different primer combinations, no combination which produced significantly more successful results was found. Decreasing the temperature during the third phase of the cycle did however notably increase the number of successful samples per batch, although still to less than half of the samples per plate.

A total of 45 samples were successfully sequenced for the MutS barcode region in this study. This was added to 44 sequences deemed suitable from the dataset of Murphy (2014). For the 45 new sequences, a total of 27 haplotypes were found, although many of these were likely fragmented versions of the same haplotype due to differing lengths of many of the samples. The Murphy (2014) sequences then added an additional 28 haplotypes (see appendix 2). Sequence length across all sequences used ranged from 90 to 918, with the total alignment length being set to 918. Out of the full 89 sequences only one was the full length.

A haplotype network of just the new 45 sequences revealed two relatively distinct clusters of haplotypes, with a few haplotypes located between the two (see fig. 2.1) The haplotype network including all 89 sequences (see fig 2.2) added a third cluster

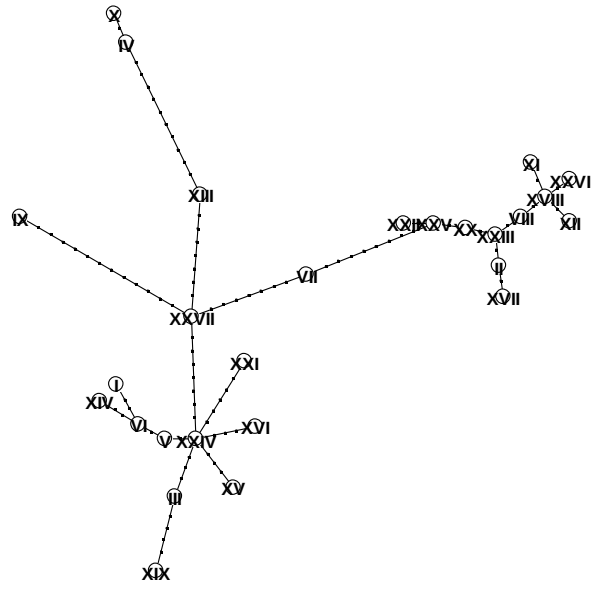

FIgure 2.1 Haplotype map for Ayre (2016) dataset

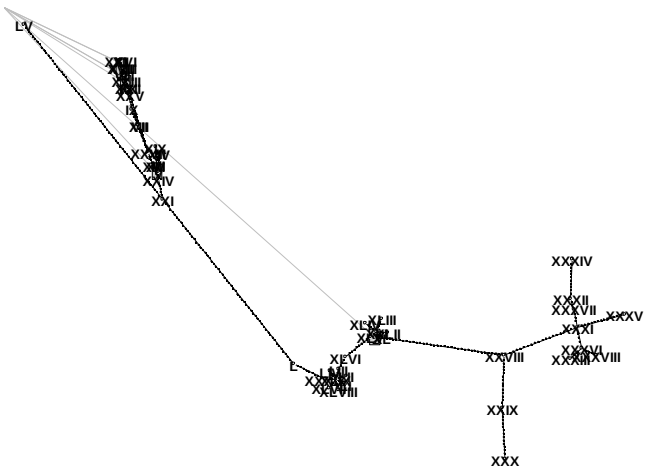

Figure 3.2 Haplotype map for Ayre (2016) and Murphy (2014) combined

that consisted entirely of the Murphy (2014) sequences; however, this was almost certainly due to the difference in length between the sequences in the two datasets, as the sequences from Murphy were only 582 base pairs long. An extreme outlier was also revealed amongst the Murphy (2014) sequences.

A plot of the haplotype network with the different haplotypes coloured for regional location then showed the two clusters were both fairly evenly split between the two Greenland regions with no obvious geographic structure. Haplotype VII, which was located between the two clusters, was revealed to contain exclusively Canadian samples from the Canada South and Canada Central regions. The outlier haplotype IV is shown to contain several samples from both Greenland North and Greenland South and can be considered its own cluster (see figure 3.1).

The third cluster representing the samples of Murphy (2014) showed a fairly clear split between two clusters. One of which was almost entirely made up of Greenland North samples and the other of which was made up of a mix of both Greenland North and South (see figure 3.2)

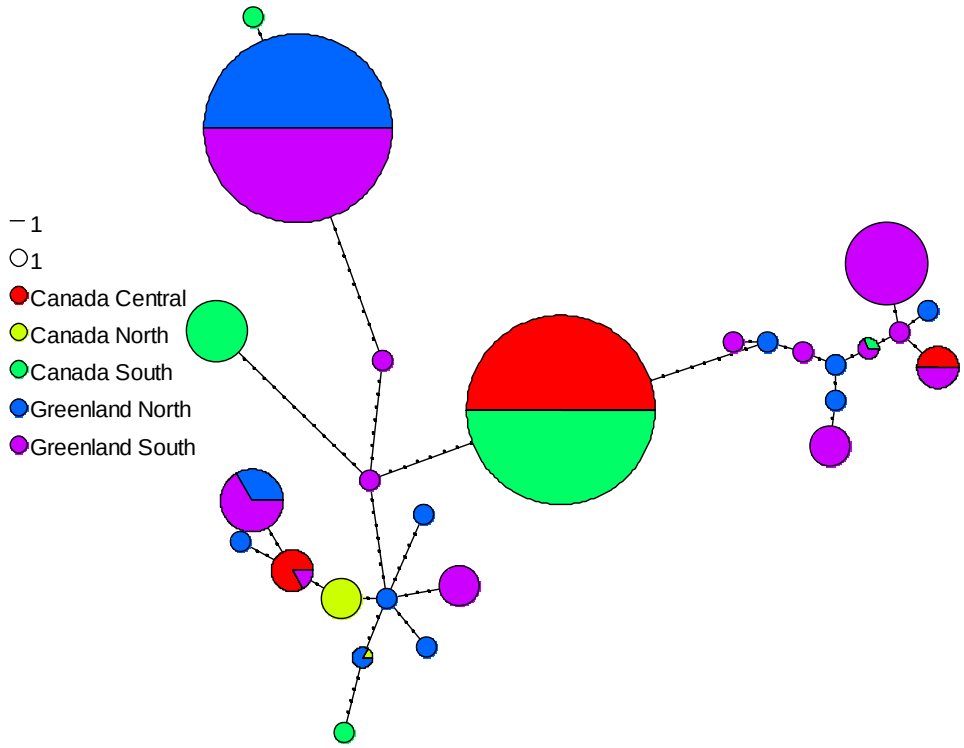

Figure 3.1 Haplotype map for Ayre (2016) dataset with graphical representation of area distribution

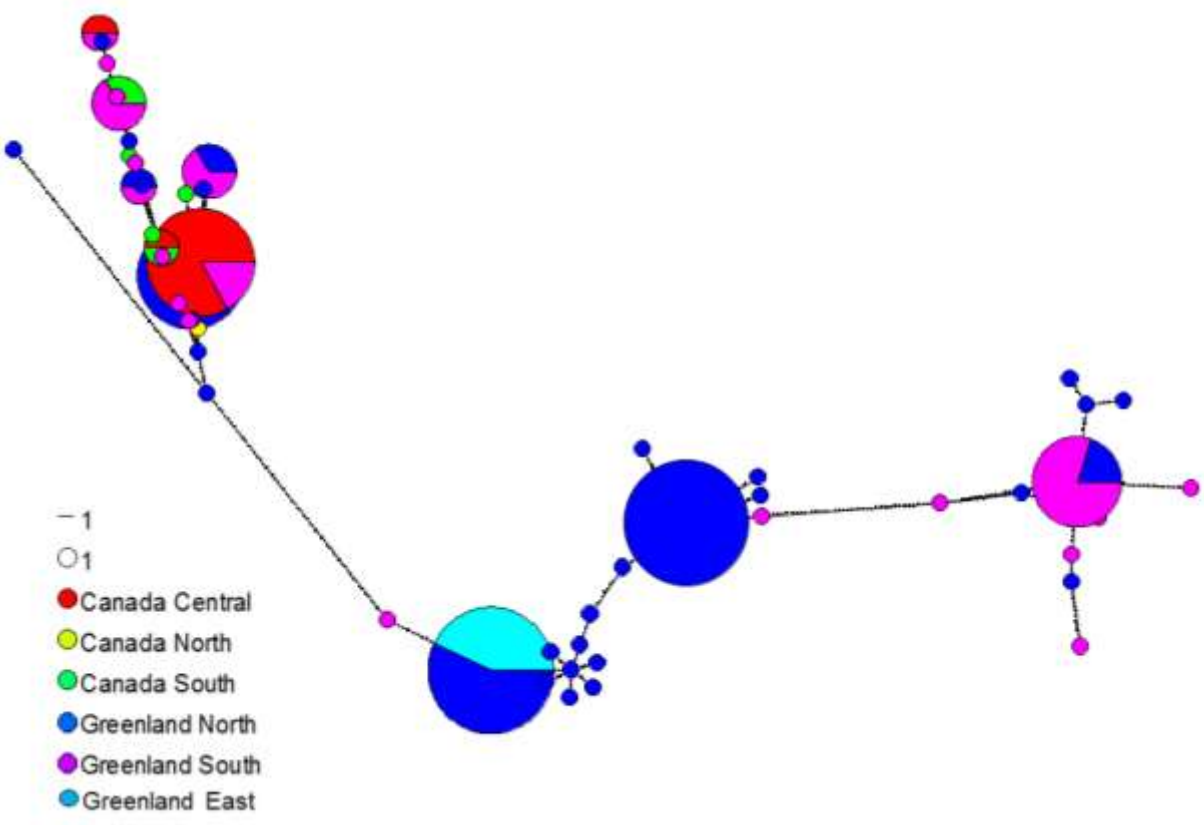

FIgure 3.2 Haplotype map for Ayre (2016) and Murphy (2014) datasets combined with graphical representation of area distribution

The consensus tree produced by phylogenetic analysis (see figure 4) showed two fairly distinct clades which seem to fairly consistently pertain to the divide between the *Capnella* and *Gersemia* genera based on the morphological identifications. The probabilities given for these clades show that the two largest *Gersemia* clades are very strongly supported, appearing in more than 90% of trees constructed, with the two clades being placed together in a clade also being strongly supported and appearing in 87.67% of trees. The remaining *Gersemia* outlier clades and the *Capnella* clades are less well supported by the consensus, with probability values all between 50 and 60%.

Gaps between the right and Greenland South region in Greenland is not notable due to the fact that previous modelling of habitat suitability for the Nephthidae in this region found that the region between the two areas was not suitable for these corals (Turner, 2014). Gaps in the distribution of the Canadian samples are due to the fact that samples collected from the DFO were limited and not representative of the entire region.

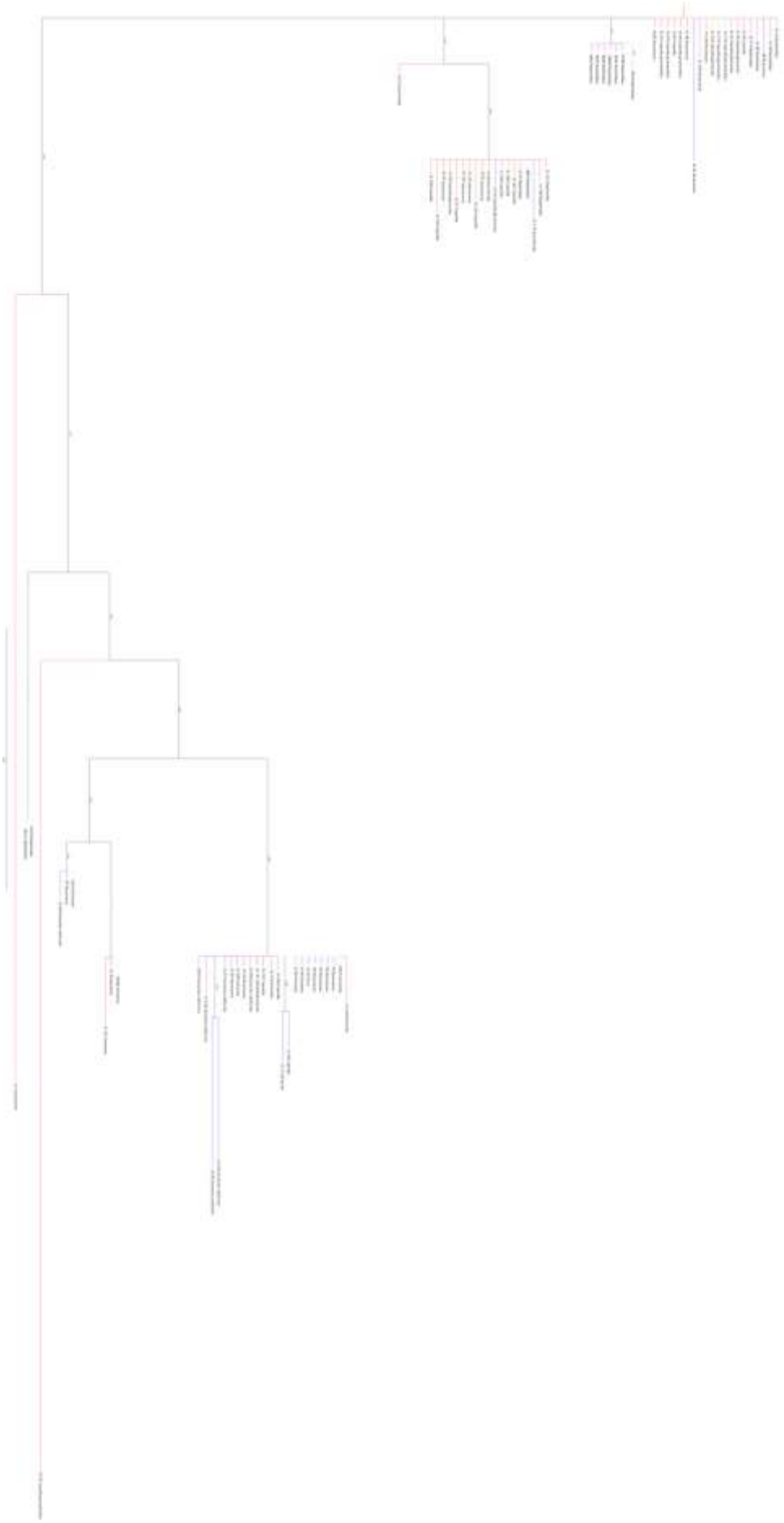

Figure 4 Consensus tree

The geographical regions seemed fairly evenly distributed between these two clades with no notable pattern of clustering. One of the only points of interest is a smaller clade within the *Capnella* clade (titled from henceforth the Canadian *Capnella* Clade) that is solely made up of samples from the “Canada Central” and “Canada North” geographical regions, both within Canadian trawled waters. It is the most strongly supported sub-clade within the *Capnella* clade, with a posterior probability score of 0.721. This clade contained seven samples and accounted for almost all of the Canadian samples from these two regions in the dataset, only omitting three, two of which were located within a separate *Capnella* subclade (Henceforth referred to as the *Capnella* Internal Clade) and one of which was located within a *Gersemia* subclade. The “Canada South” geographical region however was distributed across all clades alongside all of the samples from Greenland.

The separation of the *Capnella* Internal Clade from the other *Capnella* does not appear to follow any noted species or genus divide, and so presumably represents a fairly distinctive intra-species difference within the sequences. It mostly contains samples from the “Greenland North” region, but also includes samples from the “Canada South”, “Canada North” and “Greenland East” region.

Within the *Gersemia* clade there are several small and fairly distinct clades alongside one major subclade that contains most the *Gersemia* specimens. Only one of these shows any notable geographic pattern, which is a small clade with fair geographic distance from the other samples in the *Gersemia* Clade that contains only two samples from the “Canada South” region.

The *Duva* genus seems fairly divided between the two aforementioned clades; however, given the small number of *Duva* present in the sample, this might not represent anything more significant then several cases of misidentification and so likely requires a larger sample size to comment on.

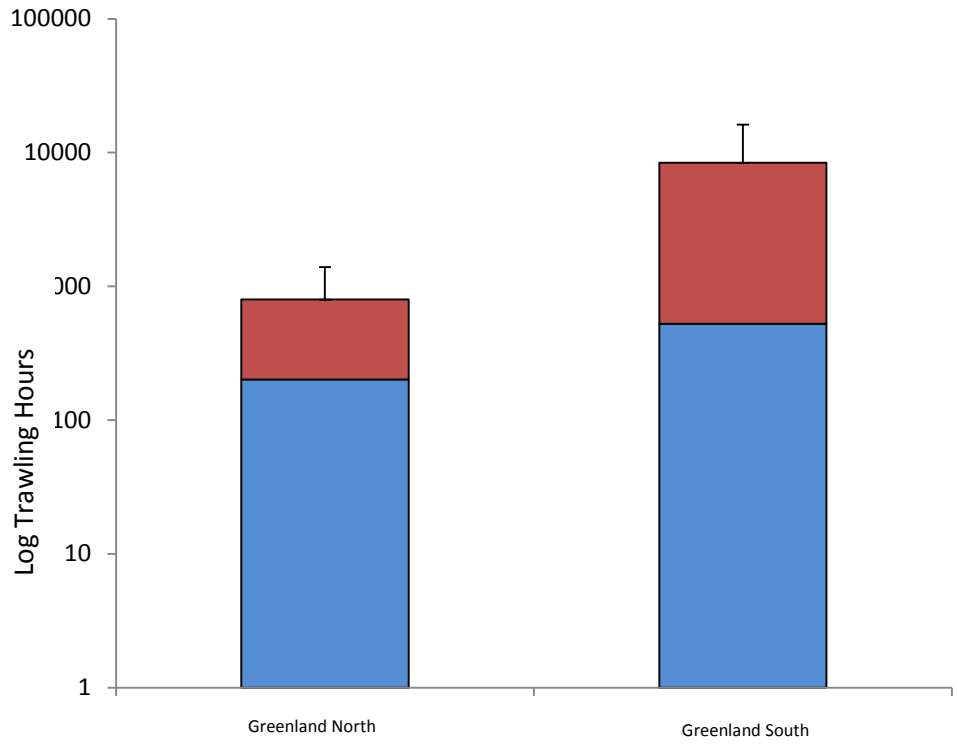

Figure 5.1 Boxplot of log trawling hours for Greenland North and Greenland South showing 2nd Quartile in Blue and 3rd Quartile in red with Median bar in between.

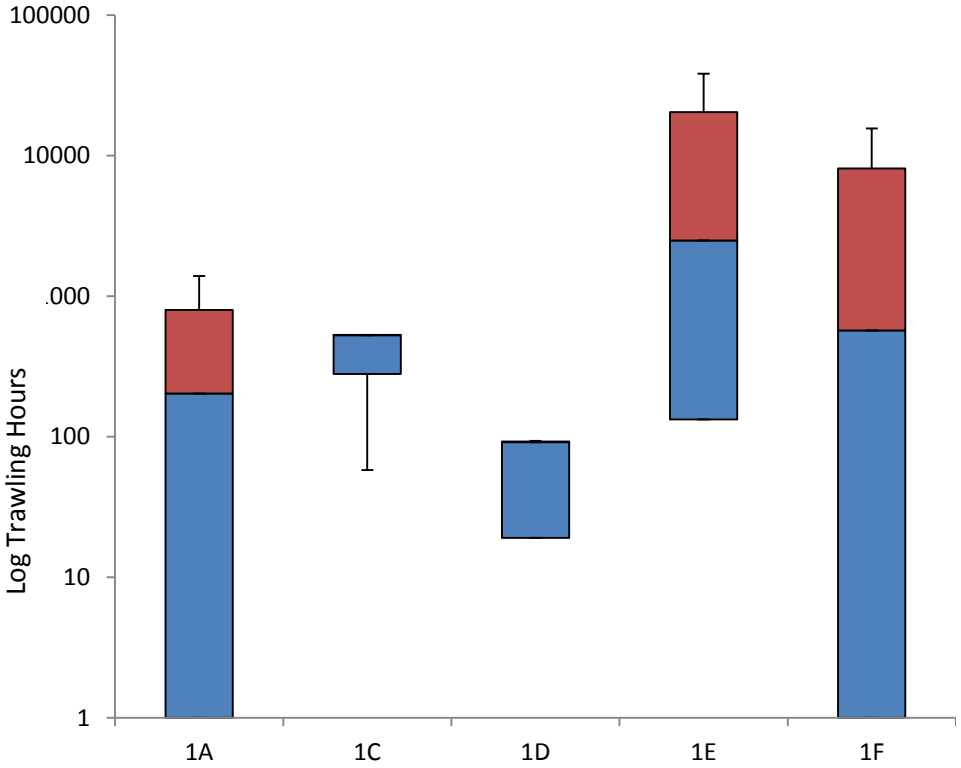

Figure 5.2 Boxplot of log trawling hours for NATO fishing areas showing 2nd Quartile in Blue and 3rd Quartile in red with Median bar in between.

A boxplot of the trawling intensity amongst the different regions revealed that the “Greenland South” region saw notably higher trawling intensity than the “Greenland North” region, with the “1F” NAFO fishing area within the “Greenland South” region having by far the highest median trawl hours of all the fishing areas observed (see figure 5), while the other areas also located in the “Greenland South” region had medians more comparable to the 1A area that made up most of “Greenland North”.

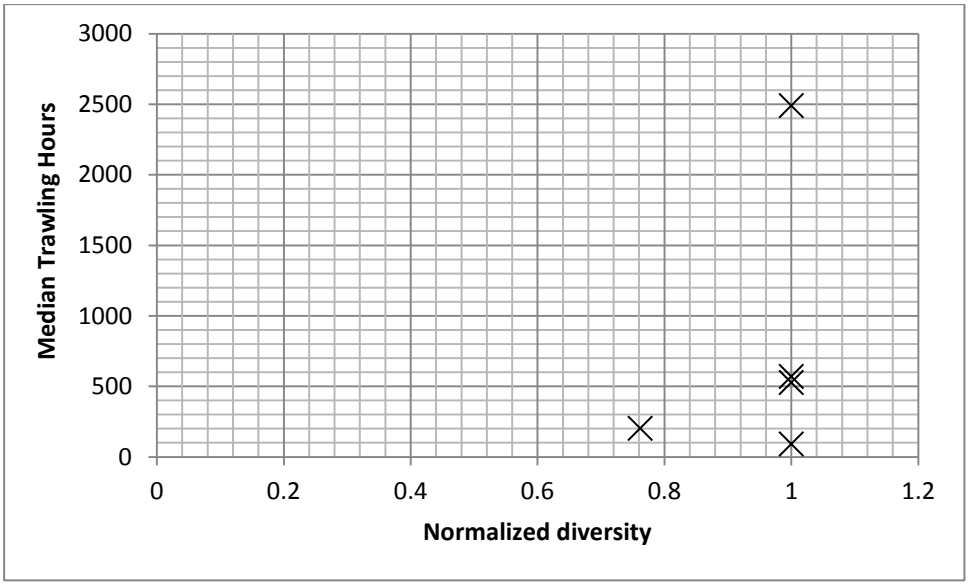

Figure 6: Plot of normalized diversity against median trawling hours for the 5 NATO fishing areas

When the median trawling intensity was then plotted against the normalized diversity of haplotypes for each region, the only region shown not to have a normalized diversity of 1 was region 1A, however this region contain 42 samples well the rest contained 11 or less (see Fig. 6).

Discussion

All of the results from this study show little in the way of genetic distinction between each of the geographic regions that samples were collected from; particularly the Greenland regions for which further analysis was carried out. This suggests that there is gene flow between the corals in all of these areas as many of the haplotypes are not limited to one region and that biodiversity is not suffering particularly in any one region, despite the differences in trawling levels within each area. The only regions that seem to be fairly isolated and show little biodiversity are the “Canada

Central” and “Canada North” regions, which almost all appear together in one subclade that contains samples from no other regions. These areas might therefore be suffering biodiversity impacts; however, due to the fact that these regions are represented by only ten samples combined it is difficult to say with any real certainty if these findings are in anyway representative of these areas as a whole.

As such though, overall the results produced with this expanded dataset seem to agree with those of Murphy (2014), in that there is no notable impact on coral biodiversity amongst the sampled genera within the sampled areas due to shrimp trawling. Those regions that were more heavily trawled provided samples that appeared across the entirety of the phylogenetic tree alongside those from the less trawled regions and so trawling intensity appears to make little difference. For a clearer answer a comparison of the number of haplotypes and species present now compared to pre-trawling numbers would need to be made, but given that genetic data from historical periods is unavailable such comparisons are not possible. Comparisons of modern and historical communities was made by Chemshirova (2014) but concluded that the time period covered by the photos was not enough to clearly show the impact trawling was having.

It is also interesting to note that the regions which might have been expected to show greater biodiversity due to the greater habitat diversity (Levin, et al, 2010), specifically the “Greenland South” region, did not appear to be any more diverse than the other regions. This might potentially suggest a reduction in biodiversity in these regions, which could make sense if changing of environmental niches by the trawling net is taken into consideration; however, as past studies have suggested diversity might actually be increasing in these areas (Chemshirova, 2013) that seems highly unlikely. Any real conclusion in this area would need a more detailed analysis of the differences in terrain between these different regions as well as a more in depth understanding of the effect that different environment types can actually have on the Nephtheidae.

Limitations

One of the reasons for the lack of data in this project is of course the limitations effecting the collection of data. All samples from the coast of Greenland used were collected by trawlers which introduce multiple issues. Firstly, trawlers have been

previously noted to be a poor tool for coral sample collection (Jorgensen et al, 2014), as many corals are crushed underneath the net instead of being caught. This means that if there are any phenotypes of coral which are able more likely to be crushed underneath instead of broken off, for example due to shorter height, greater strength or flexibility, then they will either be under-represented or entirely absent from the samples collected, as they will not be picked up by the net and so won't reach the trawlers. As previous studies have suggested that the size of coral in this region is decreasing (Vakarcs, 2015), this may increasingly represent more of an issue.

These samplings issues are of course more likely to actually reduce the amount of biodiversity seen and so could actually create an illusion of impact where there is none. As such they have likely not made much difference to the overall conclusions of this particular study, but might affect future work in this area if comparison is made between total overall biodiversity between the coast of Greenland and other regions in order to determine impact.

Another issue that would reduce the amount of seen biodiversity is that multiple specimens may actually come from the same organism. If multiple fragments from the same coral are broken off and caught in the net, then they would likely be treated as separate entities once they reached the surface, and when sampled would give the same DNA sequence and so contribute to the appearance of low biodiversity. Given that each area of trawling only produced a small number of samples, and the same organism providing samples at different sites is low, the impact this issue may have is likely very low; however, it still must be considered as there are two pairs of samples within this study where both samples share the same collection coordinates and haplotype. The issue is further reduced amongst those samples collected by researchers as oppose to trawler workers, as the researchers are able to better identify the samples and so not included duplicates.

Ideally to resolve this issue, a data collection team would be sent to collect samples from the areas using a Remotely Operated Vehicle similarly to Becheler et al (2015), that way making sure that all forms of coral on the seafloor are fairly represented and collected. A sample collection team could also collect a far greater amount of information about the environment that each sample or set of samples is collected from, creating a more complete idea of exactly what other flora and fauna might be

impacting the coral species and what environmental conditions are having an effect. This information would be vital for working out exactly what is causing any impact on biodiversity, as trawling is potentially far from the only factor that might be causing an issue, for example global warming (Harley et al, 2006) has been shown to be negatively impacting the biodiversity of marine communities as well.

Understanding what the type of seafloor that a coral is living in can take on an extra level of importance considering that studies of deep sea trawling have shown that the trawling affects not only organisms, but also the very composition of the seafloor composition it is pulled through. In almost all sediments, prolonged trawling activity "smoothens out" the seafloor and greatly reduces complexity of the surface, reducing the number of niches available (Freese et al 1999). This of course creates a more homogenous landscape which in turn reduces the opportunities for biodiversity to flourish (Puig et al, 2012).

More comparative samples should also be collected from areas off the coast of Greenland which have never been trawled, for example regions further than 74˚N in the Davis Strait, which are not trawled by the fisheries (Lassen et al, 2013). This is obviously not possible while all samples are supplied by trawlers, but could easily be collected by a separate data collection team. Given the large region of ocean floor that the corals were collected from, it is also important to remember that there is likely an effect by many other factors causing differences in biodiversity between the different regions of collection.

In the lab, it must be noted that the reduction of temperature during the third stage of PCR increased the risk of non-specific site binding (Nolan & Bustin, 2013) and may have led to some of the sequenced codes being unusable. Further issues with the number of successful samples may have been due to sample quality and not due issues in the PCR process.

Further work and conservation

Firstly, as both Murphy (2014) and this study found the set of primers currently used for PCR of the MutS region of octocoral genomes to consistently bind poorly and produce low success rates for extractions. As such for further work in this area it is highly advisable that new primers be developed with a higher success rate, or the

reason for the low success rates be tested in more detail to determine the nature of the problem as these low success rate severely reduced the sample size available for both studies and therefore the reliability of their conclusions. This will also prove incredibly valuable for further taxonomic work with these species, which is important due to the lack of clear morphological distinctions between groups.

In terms of conservation, the results of this study suggest that the corals in this region need not be prioritised for protection based on their biodiversity; although due to the lack of results for the *Duva* and the limited sample size overall further work is suggested in order to further improve the reliability of these findings. Based on previous findings, though, benthic communities as a whole in the area are at risk (Simon, 2013; Chemshirova, 2014; Yesson et al, in review) and so there are a number of options for future conservation.

The most obvious of these courses of action is the ban being placed below trawling at a certain depth. The current legislation running through the UN for a ban below 800 metres would mean that out of the 82 specimens we used in our experiment, 6 would now fall within a no-trawling zone. The fact that this number is so low shows just how ineffective such a low threshold will be and so ban thresholds at shallower depths are recommended here and indeed globally. For example if a ban below the suggested 200 metres was place, then 49 out of the 82 samples would be protected; however, as the fisheries are based entirely below 200m this would prevent them from operating at all and so be and unfeasible solution. This means some compromise between the two would need to be met in terms of a depth limit or only specific regions identified as the most vulnerable would need to be covered by the ban.

If depth bans or protected areas are introduced it is vital that they are firmly enforced as no trawling areas. It has been shown (Reed et al, 2005) that while claiming an area is protected on paper is all well and good, if all trawling vessels are not made abundantly aware of the restricted areas or depths then the areas and in truth not protected at all. One way to safeguard against this is to mark the areas with some form of buoy or other marker; however the practicality of this is questionable due to the fact that many of these areas would need to be far out at sea, where maintenance and installation would be a serious issue. Patrols of the area by the

coast guard or some other body could also work, as is practiced in terrestrial reserves. Instead of manned patrols, sonar scanners could be employed to send a warning to the coastguard whenever any vessel enters the region, allowing the coastguard to react and travel out to divert the trawler; this however introduces a time delay that will mean that some damage might already be done by the time the coast guard gets there. As currently under consideration by Palau, (Dorney, 2013) drones could also be effectively used to monitor marine spaces for trawling vessels.

Another option of enforcement is simply the application of fines to those that do trawl in the restricted areas based on identification by vessel monitoring systems (EU, 2003); however, this is a reactive measure and so is less likely to be effective than a proactive measure, as it only takes effect once the damage has already been done, whereas patrols prevent the damage happening in the first place. Fines have already been applied to other protected marine environments and while often successful in deterring trawling, they are rarely enough to entirely remove the issue (Agnew, 2000). A combination of all three methods might be the most successful, but would of course also be the most costly and difficult to instate, and so is unlikely to successfully be accepted.

Other solutions include looking into designs for trawling nets which are less damaging to coral and other organisms, such as currently proposed “floating” trawl doors that would reduce contact with the seafloor and the associated damage; however, tests of such devices have currently met with little success (C. Yesson, pers. comms.). Further advances in the existing improvements to trawling are potentially a more viable option (Broadhurst, 2000). Overfishing of target species is still of course and issue, but this would certainly be a positive step toward curbing the impacts of trawling.

Reactionary measures in response to the effects of trawling must also be considered. One potential course of action that has had success in the past (Kaufman, 2006) is some form of artificial repair to the areas of seafloor that have been damaged by trawling. As previously mentioned, trawling can reduce the complexity of the seafloor, which in turn can reduce the opportunities for biodiversity to flourish. If areas marked as conserved could have some of their pre-trawling environmental complexity restored, then this might do a lot to encourage and return to higher levels of

biodiversity. These repairs could take the form of reforming lost structures on the seabed which provided crevasses and hiding places for various creatures, including the corals. Of course these forms repairs would be costly, and great care would have to be taken in order to ensure that further damage was not done by the repair crews; however, depending on how much these repairs are estimated to aid biodiversity increase the investment might be worth considering.

Taking the patchwork state of deep sea trawling regulation across the globe and the limited success this approach is having, it is perhaps recommendable that a single global set of regulations be agreed upon for the practice. This could be done through additions to currently standing global fishing treaties like the LOS Convention or Fish Stocks Agreement (Molenaar, 2004) or through an entirely new agreement solely focusing on trawling. Implementation of such a treaty would allow for proper enforcement of restrictions in international waters, as all parties would need to be held accountable to it rather than only specific nations having to agree. This global treaty could also be the prompt needed for many countries to increase research into deep sea trawling which could in turn lead to better regulation on a national level and an overall better understanding of the impact it is having.

Conclusion

In conclusion, this report is in agreement with previous studies that the trawling done by shrimp fisheries off the coast of Greenland does not appear to be having any significant effect on the biodiversity of the *Gersemia* and *Capnella* coral genera in the region, although more work in the area is recommended. A conclusion on the effect on the *Duva* genus would require more samples. While this specific example might not have requirement for greater regulation of trawling, a global pattern of devastation has shown that as a whole the damaged caused by trawling to deep sea habitats needs to be curbed and brought under control so that recovery and repair can begin in earnest. Further work into discovering the extent of the damage it has already caused is vital and a while continuation of this specific project with a further increased dataset might not be a priority, it is recommended that similar work should be carried out anywhere that organisms have been exposed to deep sea trawling, as for many of the areas where trawling takes place this vital information is simply not known. Work like this will make it clear which areas are most at risk and so give easy

targets for regulation to protect, allowing resources to be focused on the areas that need them most.

Reference List

- Agnew, D.J (2000) The illegal and unregulated fishery for toothfish in the Southern Ocean, and the CCAMLR catch documentation scheme. *Marine Policy.* Vol. 24. No. 5. Pp. 361-374.
- Baco AR, Cairns SD (2012) Comparing Molecular Variation to Morphological Species Designations in the Deep-Sea Coral Narella Reveals New Insights into Seamount Coral Ranges. PLoS ONE 7(9): e45555. doi:10.1371/journal.pone.0045555
- Becheler, R., Cassone, A., Noël, P., Mouchel, O., Morrison, C.L. & Arnaud-Haond, S (2015) Low incidence of clonality in cold water corals revealed through the novel use of a standardized protocol adapted to deep sea sampling. *Deep Sea Research Part II: Topical Studies in Oceanography*
- Boutillier J, Kenchington E, Rice J (2010) A Review of the Biological Characteristics and Ecological Functions Served by Corals, Sponges and Hydrothermal Vents, in the Context of Applying an Ecosystem Approach to Fisheries. DFO Can. Sci. Advis. Sec. Res. Doc. 2010/048. iv + 36p.
- Broadhurst M.K (2000) Modifications to reduce bycatch in prawn trawls: A review and framework for development. *Fish Biology and Fisheries.* Vol. 10. No. 1. Pp. 27-60.
- Brugler, M.R. & France, S.C., (2008) The mitochondrial genome of a deep-sea bamboo coral (Cnidaria, Anthozoa, Octocorallia, Isididae): genome structure and putative origins of replication are not conserved among octocorals. Journal of molecular evolution, 67(2), pp.125–36.
- Buch, E., Pedersen, S. A., & Ribergaard, M. H. (2004). Ecosystem variability in West Greenland waters. Journal of Northwest Atlantic Fishery Science, 34, 13.
- Burmeister A, Kingsley M.C.S & Siegstad, H (2013) The West Greenland trawl survey for Pandalus borealis, 2013, with reference to earlier results. NAFO SCR Doc. 13/056
- Chemshirova, I (2013) Impact of trawling on benthic marine organisms off the Greenlandic shelf, 200 to 600 meters depth. Year in Industry Project, Imperial College London. 33pp.
- Chemshirova, I (2014) Establishing historical baselines of benthic diversity and community composition, Western Greenland. BSc Project, Imperial College London. 54pp.
- Chisholm, J.R.M., 2000. Calcification by crustose coralline algae on the northern Great Barrier Reef, Australia. Limnology and Oceanography, 45(7), pp.1476–1484.

- Christensen, V (2003) Hundred-year decline of North Atlantic predatory fishes. *Fish*.
- Clarke, J., Milligan, R.J., Bailey, D.M. & Neat, F.C (2015) A Scientific Basis for Regulating Deep-Sea Fishing by Depth. *Current Biology*. Vol. 25. No. 18. Pp. 2425-2429
- Commission Regulation (EC) No. 2244/2003 of 18 December 2003 laying down detailed provisions regarding satellite-based Vessel Monitoring Systems. *Official J. Eur. Union* **L333,**17–27 (2003); (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri = OJ:L:2003:333:0017:0027:EN:PDF.)
- Curtis, J.M.R., Poppe, K. & Wood, C.C., (2013) Indicators, impacts and recovery of temperate deepwater marine ecosystems following fishing disturbance. , Canadian Science Advisory Secretariat, Research Document 2012.125
- De Juan, S., Demestre, M. & Sánchez, P. (2011) Exploring the degree of trawling disturbance by the analysis of benthic communities ranging from a heavily exploited fishing ground to an undisturbed area in the NW Mediterranean. *Scientia Marina.* Vol. 75. No. 3. Pp. 507-516
- Dorney, S (2013) Palau ends drone patrol tests to deter illegal fishing. Australia News Network (http://www.abc.net.au/news/2013-10-04/an-palau-drone-patrol-tests-to-deter-illegal-fishing-ends/4999344)
- EPRSLibary (2013) Deep-Sea Fishing. European Parliamentary Research Service Blog. (https://epthinktank.eu/2013/02/13/deep-sea-fishing/)
- European Parliament (EU) (2002) COUNCIL REGULATION (EC) No 2347/2002 (http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02002R2347-20040501&from=EN)
- European Parliamentary Research Service (EPRS) (2013) Regulating North-East Atlantic deep-sea fisheries briefing. (http://www.europarl.europa.eu/RegData/bibliotheque/briefing/2013/130702/LDM_BRI(2013)130702_REV1_EN.pdf)
- Eyre and Spottiswoode (1885) Trawl Net and Beam Trawl Fishing: Report of the Commissioners, with Minutes of Evidence and Appendix. Great Britain. Trawl net commission. London.
- FAO (2016) Deep-sea fisheries. (http://www.fao.org/fishery/topic/4440/en)
- Figueroa, D.F. & Baco, A.R., (2014). Complete mitochondrial genomes elucidate phylogenetic relationships of the deep-sea octocoral families Coralliidae and Paragorgiidae. Deep Sea Research Part II: Topical Studies in Oceanography, 99, pp.83–91.
- Fisher, J (2015) Impacts of otter trawling on macrobenthic functional diversity in western Greenland. MSc Project, University College London. 39pp.
- Fosså, J. H., Mortensen, P. B., & Furevik, D. M. (2002). The deep-water coral Lophelia pertusa in Norwegian waters: distribution and fishery impacts. Hydrobiologia, 471(1-3), 1-12

- Freese L, Auster PJ, Heifetz J, Wing BL (1999) Effects of trawling on seafloor habitat and associated invertebrate taxa in the Gulf of Alaska. Mar.Eco.Prog.Ser, 182:119-126
- Freese L (2001) Trawl-induced damage to sponges observed from a research submersible. Marine Fisheries Review, 63(3):7-13
- Freiwald, A., Fosså, J. H., Grehan, A., Koslow, T., & Roberts, J. M. (2004). Cold-water coral reefs. UNEP-WCMC, Cambridge, UK, 84.
- Gesmann, M & de Castillo, D (2011) Using the Google Visualisation API with R. The R Journal, 3(2):40-44
- Gorham, T (2014) Community composition of seabed habitats around Greenland: What is the impact of the shrimp fishery?. MSc Project, Imperial College London. 93pp.
- Gougeon, S (2015) Mapping and classifying the seabed of West Greenland. MSc Project, Imperial College London. 36pp.
- Hall-Spencer, J., Allain, V. & Fosså, J.H., 2002. Trawling damage to Northeast Atlantic ancient coral reefs. Proceedings. Biological sciences / The Royal Society, 269(1490), pp.507–11.
- Harley, C.D.G., Hughes, A.R., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F., Tomanek, L., Williams, S.L. (2006) The impacts of climate change in coastal marine systems. *Ecology Letters*. Vol. 9. No. 2. Pp. 228-241
- Huelsenbeck, J. P. and F. Ronquist. (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754-755
- Ronquist, F. and J. P. Huelsenbeck. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.
-
- Jenkins, S., Beukers-Stewart, B. & Brand, A. (2001) Impact of scallop dredging on benthic megafauna: a comparison of damage levels in captured and non-captured organisms. Marine Ecology Progress Series. 215, 297-301.
- Jennings S, Nicholson MD, Dinmore TA, Lancaster JE (2002) Effects of chronic trawling disturbances on the production of infaunal communities. Marine Ecology Progress Series, 243:251-260.
- Jombart, T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24: 1403-1405. doi: 10.1093/bioinformatics/btn129
- Jombart T. and Ahmed I. (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics. doi: 10.1093/bioinformatics/btr521
- Jones JB (1992) Environmental impact of trawling on the seabed: a review. N.Z.Jour.Mar.Fresh.Res. 26:59-67.
- Jørgensen OA, Tendal OS, Arboe NH (2013) Preliminary mapping of the distribution of corals observed off West Greenland as inferred from bottom trawl surveys 2010-2012. NAFO. Nuuk.

- Kaufman, L (2006) If You Build It, Will They Come? Toward a Concrete Basis for Coral Reef Gardening. In: Coral Reef Restoration Handbook. CRC Press. pp.119-142
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data.Bioinformatics, 28(12), 1647-1649.
- Kenchington, E., Best M., Cogswell A., MacIsaac K., Murillo-Perez F.J, MacDonald B., Wareham V., Fuller S.D., Jørgensbye H.I.Ø., Sklyar V., Thompson A.B., (2009) Coral Identification Guide NAFO Area Sci. Council Studies, No. 42: 1–35
- Lassen, H., Powles, H., Bannister, C. & Knapman, P. (2013) Marine Stewardship Council (MSC) Final Report and Determination for the West Greenland Cold Water Prawn Trawl Fishery. Intertek Moody Marine Ltd.
- Levin, L. A., Sibuet, M., Gooday, A. J., Smith, C. R. & Vanreusel, A. (2010) The roles of habitat heterogeneity in generating and maintaining biodiversity on continental margins: an introduction. *Mar. Ecol.* **31**, 1–5
- McFadden, C.S. et al., 2011. Limitations of mitochondrial gene barcoding in Octocorallia. Molecular ecology resources, 11(1), pp.19–31.
- Ministry for the Environment (MFE) (2016) Regulatory impact statement: Establishment of a Kermadec Ocean Santuary. (http://www.mfe.govt.nz/sites/default/files/media/Legislation/RIS/RIS-for-kermadec-ocean-sanctuary-bill_0_0.pdf)
- Molenaar, EJ. (2004). Unregulated Deep Sea Fisheries: A need for a Multi-Level Approach. in Unfinished Business: Deep Sea Fisheries and the Conservation of Marine Biodiversity Beyond National Jurisdiction, Special Issue of the International Journal of Marine and Coastal Law 19(3): 209-364
- Mortensen, P.B. et al., (2008) Occurrence of deep-water corals on the Mid-Atlantic Ridge based on MAR-ECO data. Deep Sea Research Part II: Topical Studies in Oceanography, 55(1-2), pp.142– 152
- Murphy, L (2014) Can we see the genetic fingerprint of fishing impact on the cold water corals of Western Greenland? MSc Project, Imperial College London. 33pp.
- Myers R.A & Worm B (2003) Rapid worldwide depletion of predatory fish communties. *Nature.* Vol. 483 Pp. 280-283
- NOAA (2006) NOAA Designates Extensive Area Off West Coast As Essential Fish Habitat. (http://www.publicaffairs.noaa.gov/releases2006/mar06/noaa06-r104.html)
- Nolan T & Bustin S.A (2013) PCR Technology: Current Innovations, Third Edition. CRC Press. ISBN 9781439848050

- van Ofwegen, L. P., & Groenenberg, D. S. J. (2007). A centuries old problem in nephtheid taxonomy approached using DNA data (Coelenterata: Alcyonacea). Contributions to Zoology, 76(3), 153-178.
- Paradis E. 2010. pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics 26: 419-420.
- Paradis E., Claude J. & Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289-290.
- Pont-Kingdon, G., 1995. A coral mitochondrial mutS gene. Nature, 375, pp.109–111
- Prena, J., Schwinghamer, P., Rowell, T. W., Gordon Jr, D. C., Gilkinson, K. D., Vass, W. P. & McKeown, D. L. (1999) Experimental otter trawling on a sandy bottom ecosystem of the Grand Banks of Newfoundland: analysis of trawl bycatch and effects on epifauna. Marine Ecology Progress Series. 181, 107-124.
- Puig P, Canals M, Company J.B, Martin J, Amblas M, Lastras G, Palanques A & Calafat A.M (2012) Ploughing the deep sea floor. Nature. Vol. 489 Pp. 286-293
- R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
- Rambaut, A. (2009). FigTree v1. 3.1: Tree figure drawing tool. FigTree website.
- Rambaut A, Suchard MA, Xie D & Drummond AJ (2014) Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer
- Reed, J. K.; Shepard, A. N.; Koenig, C. C.; Scanlon, K. M.; Gilmore, R. G., 2005: Mapping, habitat characterization, and fish surveys of the deep-water Oculina coral reef Marine Protected Area: A review of historical and current research. In: Cold-water corals and ecosystems. A. Freiwald, J. M. Roberts (Eds.). Springer-Verlag, Berlin, pp. 443–465.
- Rice, J., Arvanitidis, C., Borja, A., Frid, C., Hiddink, J. G., Krause, J. & Norkko, A. (2012). Indicators for sea-floor integrity under the European Marine Strategy Framework Directive. Ecological indicators, 12(1), 174-184.
- Sánchez, J., Lasker, H.R. & Taylor, D.J., (2003) Phylogenetic analyses among octocorals (Cnidaria): mitochondrial and nuclear DNA sequences (lsu-rRNA, 16S and ssu-rRNA, 18S) support two convergent clades of branching gorgonians. Molecular Phylogenetics and Evolution, 29(1), pp.31– 42.
- Simon, P (2013) The effects of shrimp trawling on the macrobenthic fauna of West Greenland. MSc project, University College London. 42pp.
- Simpson, a & Watling, L., (2006) An investigation of the cumulative impacts of shrimp trawling on mud-bottom fishing grounds in the Gulf of Maine: effects on habitat and macrofaunal community structure. ICES Journal of Marine Science, 63(9), pp.1616–1630.

- Thurston, R.H., Brockington, S. & Roberts, C.M (2010) The effect of 118 years of industrial fishing on UK bottom trawl fisheries. *Nature Communications.* Vol. 1. Article. 15
- Turner, C (2014) Creating a benthic habitat map for the West Greenland shelf. MSc Project, Imperial College London. 57pp.
- United Nations addressed to the President of the General Assembly (https://documents-dds-ny.un.org/doc/UNDOC/GEN/N06/604/28/PDF/N0660428.pdf?OpenElement)
- United Nations (UN) (2002) World Summit on Sustainable Development: Plan of Implementation (http://www.johannanesburgsummit.org/html/documents/summit_docs/2309_planfinal.htm) (United Nations, New York, 2002)
- United Nations (UN) (2006) Letter dated 2006/10/31 from the Chargé d'affaires a.i. of Fiji to the
- United Nations General Assembly. (UNGA) (1982) United Nations Convention on the Law of the Sea
- United Nations General Assembly. (UNGA) (2004) United Nations General Assembly resolution 59/25
- Vakarcs, M (2015) A temporal comparison of benthic macrofaunal communities and the impact of bottom trawling, West Greenland continental shelf. MSc Project, Imperial College London. 34pp.
- Wareham V.E. & Edinger E.N (2007) Distribution of deep-sea corals in the Newfoundland and Labrador region, Northwest Atlantic Ocean. *Bulletin of Marine Science.* Vol. 81. Pp. 289-313.
- Watling, L., & Norse, E. A. (1998) Disturbance of the seabed by mobile fishing gear: a comparison to forest clearcutting. *Conservation Biology*, Vol. 12: Pp. 1180-1197.

Appendix 1: DNA Extraction Protocol

1. Pre-add 180µl lysis buffer, GL, to eppendorf tube

2. Add sample, 25mg

3. Add 25 µl proteinase K buffer

4. Incubate overnight, 57ºC, with tilt on

5. Remove from incubator and vortex samples, 5-10 seconds

6. Add 200µl G3 lysis buffer to each sample

7. Vortex, 10 seconds

8. Incubate for 10mins, 70ºC. Remove and vortex, 5-10 seconds

9. Add 210µl ethanol (100%). Vortex, 15 seconds

10. Use mini-centrifuge for spin, 5 seconds

11. Pipette all into spin-column

12. Spin in centrifuge, 11,000 rcf for 1 minute

13. Discard waste product from collection tube and add 500µl wash buffer GW1 to spin-column

14. Centrifuge, 11,000 rcf, 1 minute

15. Discard waste. Add 600µl wash buffer GW2

16. Centrifuge, 11,000 rcf, 1 minute

17. Discard waste. Centrifuge, 11,000 rcf, 1 minute

18. Put spin-column into eppendorf tube. Add 50µl elution buffer, directly to membrane

19. Incubate, 70ºC, 5 minutes

20. Centrifuge, 11,000 rcf, 1 minute

21. Add 50µl elution buffer, directly to membrane 22. Incubate, 70ºC, 5 minutes
23. Centrifuge, 11,000 rcf, 1 minute and remove spin-column. Extraction complete.

Appendix 2: Sample Table

Specimen	Longitude	Latitude	Depth	Morphological Identification	Haplotype	Location	Trawl Hours	Data Set
Gr 61	-52.4804	63.28885	181	Nephtheidae	1	Greenland South	19.04	Ayre (2016)
Gr 120	-56.8085	71.81735	183	Gersemia	2	Greenland North	0	Ayre (2016)
Gr 121	-56.5469	70.01333	157	Nephtheidae	3	Greenland North	0	Ayre (2016)
Gr 123	-56.4838	69.768	158	Gersemia	4	Greenland North	0	Ayre (2016)
9914	-71.0007	72.5634	1426	Nephtheidae	5	Canada North	N/A	Ayre (2016)
9798	-59.6827	67.06942	1087	Nephtheidae	6	Canada Central	N/A	Ayre (2016)
9836	-59.395	66.39426	761	Nephtheidae	6	Canada Central	N/A	Ayre (2016)
10133	-68.6815	60.16778	189	Gersemia	7	Canada South	N/A	Ayre (2016)
10175	-66.38	60.33696	111	Gersemia	8	Canada South	N/A	Ayre (2016)
10178	-67.2766	60.27983	114	Nephtheidae	9	Canada South	N/A	Ayre (2016)
10182	-66.5203	60.02028	210	Gersemia	10	Canada South	N/A	Ayre (2016)
10668	-61.7089	67.71713	1420	Nephtheidae	6	Canada Central	N/A	Ayre (2016)
79	-51.6891	62.68598	343	Alcyonacea	11	Greenland South	163.81	Ayre (2016)
82	-50.8693	62.45395	82	Alcyonacea	12	Greenland South	67085.24	Ayre (2016)
92	-50.46	61.54562	116	Alcyonacea	11	Greenland South	1.80	Ayre (2016)
99	-49.3954	61.35478	98	Alcyonacea	8	Greenland South	175.95	Ayre (2016)
87	-50.48	61.88222	116	Alcyonacea	13	Greenland South	245.63	Ayre (2016)
Gr 36	-57.5741	71.78098	251	Nephtheidae	14	Greenland North	0	Ayre (2016)
Gr 109	-57.028	72.4781	238	Nephtheidae	15	Greenland North	445.81	Ayre (2016)
Gr 174	-44.643	59.61667	146	Duva	16	Greenland South	0	Ayre (2016)
Gr 87	-46.8929	60.14983	355	Duva	17	Greenland South	569.91	Ayre (2016)
Gr 46	-49.9583	61.67167	N/A	Gersemia	18	Greenland South	17716.00	Ayre (2016)
Gr 68	-53.8258	64.32982	177	Gersemia	7	Canada Central	N/A	Ayre (2016)
Gr 69	-53.151	63.86282	166	Gersemia	12	Canada Central	N/A	Ayre (2016)
9805	-78.6067	75.29538	576	Nephtheidae	3	Canada North	N/A	Ayre (2016)
9830	-60.9156	67.71713	880	Nephtheidae	6	Canada Central	N/A	Ayre (2016)
9835	-60.9156	67.71713	880	Nephtheidae	6	Canada Central	N/A	Ayre (2016)
98	-49.3954	60.91993	98	Alcyonacea	1	Greenland South	0	Ayre (2016)
10121	-67.0892	60.47573	123	Nephtheidae	19	Canada South	N/A	Ayre (2016)
Gr 64	-44.9687	59.57462	169	Capnella	20	Greenland South	0	Ayre (2016)
9894	-61.9156	67.84988	1456	Nephtheidae	6	Greenland South	N/A	Ayre (2016)
Gr 56	-57.1522	72.03987	286	Gersemia	21	Greenland North	26617.34	Ayre (2016)
Gr 77	-46.2417	60.33393	131	Gersemia	22	Greenland South	41295.59	Ayre (2016)

Gr 97	-55.7931	69.4508	237	Nephtheidae	3	Greenland North	796.62	Ayre (2016)
Gr 98	-55.7931	69.4508	237	Nephtheidae	1	Greenland North	796.62	Ayre (2016)
Gr 107	-57.028	72.4781	238	Capnella	3	Greenland North	445.81	Ayre (2016)
Gr 108	-57.028	72.4781	238	Capnella	3	Greenland North	445.81	Ayre (2016)
Gr 109	-57.028	72.4781	238	Capnella	23	Greenland North	445.81	Ayre (2016)
Gr 111	-56.5348	71.37888	224	Nephtheidae	24	Greenland North	201.80	Ayre (2016)
Gr 112	-56.5348	71.37888	224	Gersemia	25	Greenland North	201.80	Ayre (2016)
Gr 118	-56.5348	71.37888	224	Gersemia	26	Greenland North	201.80	Ayre (2016)
Gr 70	-53.6417	64.35983	88	Gersemia	4	Greenland South	527.34	Ayre (2016)
Gr 116	-56.5348	71.37888	224	Capnella	3	Greenland North	201.40	Ayre (2016)
Gr 77	-46.2417	60.33393	131	Gersemia	8	Greenland South	41295.59	Ayre (2016)
Gr 80	-48.6525	60.53898	335	Gersemia	27	Greenland South	5349.33	Ayre (2016)
Gr 68	-53.8258	64.32982	177	Gersemia	28	Greenland South	17075.31	Murphy (2014)
Gr 123	-56.4838	69.768	158	Gersemia	29	Greenland North	0	Murphy (2014)
Gr 70	-53.6417	64.35983	88	Gersemia	30	Greenland South	527.34	Murphy (2014)
Gr 45	-48.2012	60.324	434	Gersemia	31	Greenland South	0	Murphy (2014)
Gr 69	-53.151	63.86282	166	Gersemia	31	Greenland South	5913.47	Murphy (2014)
21 42	-56.468	66.01458	576	Gersemia	32	Greenland North	278.78	Murphy (2014)
Gr 100	-55.7931	69.4508	237	Gersemia	33	Greenland North	796.62	Murphy (2014)
Gr 85	-46.1526	60.28517	83	Nephtheidae	31	Greenland South	2752.43	Murphy (2014)
18 38	-53.6201	65.1586	75	Nephtheidae	31	Greenland North	58.00	Murphy (2014)
Gr 47	-49.625	61.17167	N/A	Nephtheidae	31	Greenland South	4731.59	Murphy (2014)
Gr 145	-50.9616	62.4594	72	Nephtheidae	34	Greenland South	28260.47	Murphy (2014)
Gr 83	-46.1309	60.3818	327	Gersemia	35	Greenland South	10839.648	Murphy (2014)
Gr 118	-56.5348	71.37888	224	Nephtheidae	36	Greenland North	201.80	Murphy (2014)
Gr 77	-46.2417	60.33393	131	Gersemia	37	Greenland South	41295.59	Murphy (2014)
49 01	-56.4713	69.79038	159	Gersemia	38	Greenland North	32	Murphy (2014)
Gr 36	-57.5741	71.78098	251	Capnella	39	Greenland North	0	Murphy (2014)
Gr 44	-46.2003	60.06947	126	Capnella	40	Greenland South	0	Murphy (2014)
Gr 58	-56.6424	71.52985	185	Duva	41	Greenland North	0	Murphy (2014)
22 01	-58.9033	72.45288	290	Nephtheidae	41	Greenland North	0	Murphy (2014)
Gr 107	-57.028	72.4781	238	Capnella	41	Greenland North	445.81	Murphy (2014)
Gr 110	-57.028	72.4781	238	Capnella	42	Greenland North	445.81	Murphy (2014)
Gr 133	-60.0978	71.9228	709	Alcyoniina	41	Greenland North	0	Murphy (2014)
Gr 135	-58.4709	72.198	319	Alcyoniina	41	Greenland North	23062.38	Murphy (2014)
Gr 37	-57.1522	72.03987	286	Capnella	43	Greenland North	26617.34	Murphy (2014)

Gr 48	-56.6424	71.52985	185	Capnella	41	Greenland North	0	Murphy (2014)
Gr 97	-55.7931	69.4508	237	Nephtheidae	41	Greenland North	796.62	Murphy (2014)
Gr 129	-57.1828	72.32817	283	Capnella	44	Greenland North	38394.09	Murphy (2014)
Gr 128	-57.1828	72.32817	283	Capnella	45	Greenland North	38394.09	Murphy (2014)
Gr 51	-57.2451	72.26392	349	Duva	46	Greenland North	56239.13	Murphy (2014)
Gr 50	-56.6235	72.00173	230	Capnella	39	Greenland North	21762.84	Murphy (2014)
Gr 54	-58.8854	72.44883	288	Capnella	47	Greenland North	0	Murphy (2014)
Gr 171	-35.9408	64.88103	184	Capnella	39	Greenland East	N/A	Murphy (2014)
Gr 172	-30.1698	65.42887	670	Capnella	39	Greenland East	N/A	Murphy (2014)
Gr 161	-29.7197	65.61647	N/A	Capnella	39	Greenland East	N/A	Murphy (2014)
Gr 136	-57.6428	72.42917	228	Nephtheidae	48	Greenland North	148.69	Murphy (2014)
Gr 138	-57.4685	71.7821	244	Nephtheidae	49	Greenland North	0	Murphy (2014)
Gr 61	-52.4804	63.28885	181	Nephtheidae	50	Greenland South	19.04	Murphy (2014)
Gr 98	-55.7931	69.4508	237	Nephtheidae	51	Greenland North	796.62	Murphy (2014)
51 05	-55.0388	69.52473	138	Capnella	52	Greenland North	0	Murphy (2014)
52 05	-56.31	69.52268	226	Capnella	39	Greenland North	0	Murphy (2014)
51 04	-55.0388	69.52473	138	Capnella	39	Greenland North	0	Murphy (2014)
51 03	-55.0388	69.52473	138	Capnella	53	Greenland North	0	Murphy (2014)
66 81	-54.8073	69.30393	170	Nephtheidae	54	Greenland North	6148.42	Murphy (2014)
51. 01	-55.0388	69.52473	138	Capnella	55	Greenland North	0	Murphy (2014)