Population and Seascape Genomics of the Deep-sea Octocoral *Acanella arbuscula*

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Impact of COVID-19

I would like to make the reader aware of limitations to this project caused by the ongoing COVID-19 pandemic. The first lockdown started during the second year of my PhD. During this time, I was meant to be completing next generation sequencing DNA library preparation in the lab. However, due to the lockdown, I was unable to return to the lab for many months. Because my entire project is based on molecular work, I was unable to make significant progress or collect any data during this time. I was not able to return home to the US, and I was using my time to research methodology and work on my code. After extensive health and safety measures were put into place, a few of us were allowed to continue research with limited access and severely reduced numbers of people allowed in each room. We faced massive supply issues with gloves, reagents, micro pipette tips, and ethanol being directed, rightly so, toward the medical needs of the NHS. We also experienced crippling international shipping delays. In addition, we were unable to receive supplies from our departmental stores, because all products had to be quarantined for several days before we were allowed to touch them. All in all, this set back my research progress and greatly reduced my original research plan. Because of this, I was not able to complete two additional chapters I had planned on deep-sea coral reproduction and larval dispersal modelling. Thank you for your understanding.

Abstract

Deep-sea corals are under threat from anthropogenic factors such as destruction from bottom contact fishing gear, gas and oil exploration, deep-sea mining, pollution, and climate change. One unique coral, *Acanella arbuscula* (Isididae), is the only known species of branching coral that is found in large gardens stretching tens of kilometers where its shallow root-like hold fasts anchor it in soft sediment. They are incredibly fragile with fine branches only a few millimeters in diameter. These coral gardens provide habitat for commercially important fish species as well as several invertebrates that live obligately on *A. arbuscula*. Soft sediment coral gardens are heavily fished in the North Atlantic Ocean, and *A. arbuscula* are very commonly caught as by-catch. People working in the fisheries have reported gill nets on shore in Newfoundland, Canada containing upwards of one hundred dead *Acanella* colonies. Due to their importance to the deep-sea ecosystem, their unique nature, and the current threat to their survival, these octocorals are a sensible target for conservation. In order to conserve populations of deep-sea corals, it is imperative that researchers and stakeholders understand the connectivity of populations across the North Atlantic Ocean, because the recovery of areas decimated by bottom contact fishing gear will rely on larval recruitment from local populations. Recruited larvae are unlikely to survive if their source environment is largely different from where they settle, because corals are sensitive to changes in environmental conditions such as temperature, salinity, oxygenation, nutrient availability, and calcium carbonate concentration. Therefore, along with genetic connectivity, it is essential to understand the environmental seascape and what factors are contributing to local adaptation in populations. This research was carried out in three stages. First, the most beneficial methods of extracting DNA from deep-sea corals were investigated. Five different extraction methods were compared in the search for a method that produced high quality genomic DNA. The salting-out method and plant mini kit extractions were found to be the best methods for *Acanella arbuscula*. Second, Single

Nucleotide polymorphisms (SNPs) generated from ultra-conserved elements (UCE) sequencing were used to investigate the connectivity of 362 colonies from 33 sites spanning depths of 60-2,300 m across nearly the entire geographical range of *A. arbuscula* from Greenland, Canada, Scotland, Ireland, and Spain. Four genetic clusters emerged, and trans-Atlantic connectivity of these octocorals was discovered with high levels of geneflow between Canadian samples and European samples at similar depths. A barrier to geneflow was discovered in the Eastern Atlantic where samples collected shallower than 1,200m showed almost no genetic connectivity to samples collected deeper than 1,200 m depth. Additionally, distinct genetic cluster formed exclusively for samples collected in the Bay of Biscay (Spain) around 860 m was identified. It is suspected that warm highly saline Mediterranean outflow water traveling northward creates a barrier to geneflow in this region. Finally, we used the SNPs generated from this study to investigate local adaptation to environmental factors across the 33 study sites in the North Atlantic. We compared 12 environmental variables such as seafloor temperature, oxygenation, nutrient availability, salinity, and pH across all of the sites with the genotypic diversity of the corals found at those sites for each UCE SNP marker. Twelve significant environment marker associations were found, and all of them were associated with seafloor temperature. Temperature on the seafloor appears to be the main driver of local adaptation in *A. arbuscula.* Using the associations between genotype and the environmental variables measured, it was possible to model the potential genotype for the entire study area and predict potential stepping-stones to connectivity. The combination of connectivity and migration data with local adaptation data was used to point out areas that are potentially vulnerable to climate change and destruction from direct anthropogenic threats. Some broad suggestions were made for potential MPA locations to help maintain populations in the North Atlantic such as Eastern Greenland, Southern Iceland, the Celtic Sea, and Cantabrian Sea.

Chapter 1: Introduction- Deep-Sea, Population Connectivity, and *Acanella arbuscula*

The Deep-Sea

The deep-sea is the largest environment on earth with ninety percent of oceanic habitat lying below shallow waters. Below 200 meters, organisms have adapted to the absence of visible light, immense pressure, and temperatures only a few degrees above freezing (Gage & Tyler, 1991). Despite the environmental pressures experienced by deep-sea organisms, the seafloor is home to higher diversity than previously thought (Hessler & Sanders, 1967), and two thirds of all described coral species are described from the deep-sea (Roberts & Hirshfield, 2004; Cairns, 2007). Some corals are known to live thousands of years; the oldest known corals, *Gerardia* sp. (gold coral) and *Leiopathes* sp. (black coral), were aged 2,742 and 4,265 years old respectively (Roark et al., 2009).

Human Impacts in the Deep-Sea

Despite being out of sight, deep-sea coral communities have been and will continue to be impacted by human activities such as fishing, oil exploration, pollution, and climate change (Watling ,2005; Weaver et al., 2011; White et al., 2012; Clark et al., 2019). For instance, during deep-sea oil exploration, increased sedimentation and mud from drilling was found, and damage was observed from anchoring in deep-sea communities (Lissner et al., 1991;White et al., 2012).

While oil and gas exploration may damage cold water coral reefs, spills seem to have a much greater impact. Three to four months after the capping of the *Deepwater Horizon* oil spill in the Gulf of Mexico, one deep-sea coral community 11 km from the spill site was discovered to be heavy impacted by the spill (White et al., 2012). Forty-six percent of the corals at the site were highly stressed. This stress manifested as excess mucus production, tissue loss, sclerite (microscopic needle-like structures that form the shape of distal regions of the coral such as polyps) enlargement, covering of a brown flocculent material, and bleached symbiotic ophiuroid brittlestar (White et al., 2012). In addition to discoloration of the ophiuroids, they also experienced changes in behaviour evident in their posture and attachment to the corals which was different to that observed in healthy reefs (White et al., 2012).

Recently, researchers reported the ingestion of plastic microfibers by deep-sea organisms across several research cruise specimens (Taylor et al., 2016). They found

microplastics inside two sea cucumbers, a squat lobster, a zoanthid growing on a bamboo coral, a sea pen (octocoral), a hermit crab, and a zoanthid growing on a hermit crab shell. Plastic particles have also been found in deep sea sediment (author pers. obs; Van Cauwenberghe et al. 2013). Ingestion of plastics reduces coral fitness by affecting energy acquisition and allocation (Chaperon et al., 2018).

The largest human impact in terms of pollution is carbon dioxide and climate change; an effect of excessive fossil fuel use that the deep sea is not immune to. Within the next 80 years, it is expected that the deep sea will experience an increase in water temperature by over 1^0C, a decrease in oxygen concentrations of 0.03 mL/L, and an increase in acidification up to 0.37 pH units (Danovaro, 2004; Turley et al.,2007; Sweetman et al., 2017). In addition to these physical changes, deep-sea environments are expected to experience a 40-55% decrease in available organic particulate matter "snowing" from more shallow ocean waters due to the reduction of cascading events caused by differences in density and changes in terrestrial rainfall (Sweetman et al. 2017). Utilizing nematodes as a model for deep-sea organisms, researchers found that deep-sea environment biodiversity was highly impacted by small, as little as 0.4^0C, temperature variations (Danovaro 2004).While deep-sea scleractinian corals have persevered in the face of previous extinction events, their populations required millions of years to recover based on geological time-scales, and in geological history, carbon dioxide concentration increases of this rate are unprecedented (Turley et al., 2007). In a controlled experiment, deep-sea coral *Desmophyllum pertusum* showed reduced calcification when exposed to pH between 7.67 and 7.90 and increased mortality up to 100% in response to warming (up to 14° C) and deoxygenation (1.5 ml· l^{-1}) (Lunden et al., 2014).

In addition to the indirect environmental changes, organisms in the deep sea can also be affected directly by human activities such as fishing. Deep-sea corals living in soft substrates, on drop stones, and even gravel, are highly susceptible to damage from bottom trawl fishing gear. When trawls are dragged over coral fields imbedded in the soft sediment substrate, the corals are uprooted and damaged- the terrestrial equivalent to clear cutting a forest and removing all of the topsoil (Watling 2005; Weaver et al. 2011). Trawling alters sessile macro-invertebrate assemblages (Hall-Spencer et al., 2002; Clark and Rowden, 2009; Williams et al., 2010), and a recent survey of seamounts in New Zealand suggests that even fifteen years after

heavy exposure to trawls has ceased, benthic communities show little signs of recovery (Clark et al. 2019). Likewise, seamounts belonging to the Corner Rise complex in the Northwestern Atlantic along the Mid-Atlantic Ridge were observed in 2007 to still be heavily damaged by bottom trawling and long line fishing gear fishing that occurred through the 1970's to the 90's (Waller et al., 2007). Due to their vulnerability to human activity, being susceptible to damage from bottom fishing gear, for example, these habitats are of conservation concern, and have been listed, alongside all other deep-sea corals, by the UN General Assembly as 'vulnerable marine ecosystems' (Weaver et al., 2011). This habitat and ecology remain largely understudied with sampling events still being relatively rare activities.

While most short-term observations have found that heavily trawled areas show no signs of recovery, a recent study on the Northwestern Hawaiian Ridge and Emperor Seamounts by Baco et al. (2019) found evidence to suggest that areas that have been protected for 30-40 years after heavy trawling have started to show signs of recovery when compared to still trawled sites. They observed corals growing in trawl scars as well as on lost fishing gear- likely from fragmented corals left behind from trawling. Baco et al. (2019) also suggest that long term closures must be maintained in-order to see recovery and that a "crop rotation style" closure where areas are closed for a period and then opened for fishing again would not provide these coral communities with enough time to recover.

Fish Reliance on Deep-Sea Corals

Fishes use deep-sea coral as facultative habitat where they may seek shelter from strong currents, escape predation, or reproduce (Auster, 2005). While fish populations are unlikely to become extinct without the presence of coral, the presence of coral reefs increases fitness in fish populations (Auster, 2005). However, the lack of coral habitat may reduce fishes resilience to disturbances (Auster, 2005; 2007). Larvae from the commercially important redfish, lantern fish, and eelpout were discovered seeking shelter in sea pen (octocoral) tissue with as many as 36 juveniles per sea pen in the Laurentian Channel off the eastern coast of Canada (Baillon et al., 2012). In a long-line survey off the southwestern continental shelf in Norway, researchers found that redfish (*Sebastes marinus*) catches were the largest and contained the biggest fish when fishing in stony cup coral, *Desmphyllum pertusum* (Previously *Lophelia pertusa*) reefs when compared to fishing in areas without coral (Husebø et al., 2002).

In the Gulf of Alaska, Kreiger and Wing (2002) analysed remote operated vehicle footage of deep-water corals to observe fish associations. They found that 85% of the large fish in 6 six species of rockfish were associated with a coral. Additionally, they found hundreds of shrimps living on single coral colonies and a mating pair of golden king crabs mating beneath a Primnoid coral (Krieger and Wing, 2002). Ray egg cases have been found attached to deep-sea corals in the NE Atlantic (Costello et al., 2005), and cat shark eggs have been observed attached to octocorals in the Mississippi Canyon in the Gulf of Mexico (Etnoyer and Warrenchuk, 2007). Cold-water corals are also important for recreationally valuable sharks off the coast of Scotland; the blackmouth catsharks, *Gaaleus melastomus*, were found using the *Lophelia* (scleractinian) reefs as spawning ground (Henry et al., 2013). This suggests that cold water coral reefs may be an essential component in various life stages of many marine organisms. As sea pens and a few species of bamboo corals often share soft sedimentary bottom habitat (Baker et al., 2012).

Deep-Sea Connectivity

The connectivity in a marine environment can be summarised by the exchange of adults, recruits, juveniles, or larvae which link together populations within a species' range (Palumbi, 2003). While connectivity may be measured in the movement of these individuals, several other factors are going to influence connectivity. So, even though a species' larvae may travel for several kilometres, it may not find a suitable habitat at extreme ends of its range. Therefore, the distance that an individual can travel, find a suitable habitat, and reproduce successfully is the realised connectivity (Marshall et al., 2010).

Several theories have been suggested and tested throughout historical studies of deep-sea connectivity and biodiversity. The stability-time hypothesis suggests that as deep-sea environments are relatively stable through time (Hessler and Sanders, 1967; Sanders, 1968; Dayton and Hessler, 1972; Abele and Walters, 1979) this leads to lower genetic diversity, higher biodiversity, and higher niche specialization (Abele and Walters 1979; Thistle 1983; Taylor and Roterman 2017), and disturbances actually increase biodiversity in both terrestrial environments such as rain forests as well as coral reefs (Connell, 1978). In a study comparing copepod diversity in two deep-sea locations, one 'stable' and one 'unstable', Thistle (1983) found no difference in copepod diversity between the two locations. By examining deep-sea

sediment cores for ostracod fossils, Cronin and Raymo (1997) demonstrate that benthic communities change in relation to climactic events and that deep-sea communities are inherently unstable in timescales of 1,000 to 100,000 years- thus discounting the stability-time hypothesis.

The isolation by distance (IBD) theory suggests that populations are isolated due to the inability of larvae to travel long distances to a new population which reduces population connectivity; this is juxtaposed to isolation by resistance (IBR) which suggests that populations are separated by a difference in habitat characteristics that make the survivability of new recruits very low to non-existent, which also leads to reduced connectivity and gene flow (Palumbi 2003; Weersing and Toonen 2009; Selkoe and Toonen, 2011; Shinzato et al., 2015; Quattrini et al., 2015; Taylor and Roterman, 2017; Xuereb et al., 2018; Grummer et al., 2019). In a coastal Pacific sea cucumber, *Parastichopus californicus*, Xuereb et al. (2018) tested both the IBD and IBR theories using RAD sequencing markers by comparing 2719 SNPs from 717 individuals across 24 sampling locations alongside a larval distribution model; they found that IBR was more likely to cause differences in population connectivity than IBD.

It has been suggested that populations may overcome IBD by using a "stepping stone model" of larval distribution (Figure 1a), in which larvae are distributed from one relatively close population to another until the following generations of larvae have travelled considerable distance (Weiss and Kimura, 1965; Smith et al., 1989; Roberts, 1997; Palumbi, 2003). After finding fauna typically associated with deep-sea hydrothermal vents on whale falls, Smith et al. (1989) hypothesised that whale remains may act as stepping stones for vent fauna because vents are often short-lived and can be otherwise geographically isolated (Smith et al., 1989; Vrijenhoek, 2010; Rogers et al., 2012). Likewise, populations may be connected in an "island model" (Figure 1b) by which populations exchange larvae via immigration and emigration relatively equally and contribute to panmixis (Palumbi, 2003). If immigration and emigration of larvae between populations is unequal, specifically if one population receives larvae from adjacent populations but does not contribute to the gene pool of other populations, the single population acts a "sink" of genetic material while the others providing larvae act as "sources" (Figure 1c) in a "source and sink model" (Palumbi, 2003).

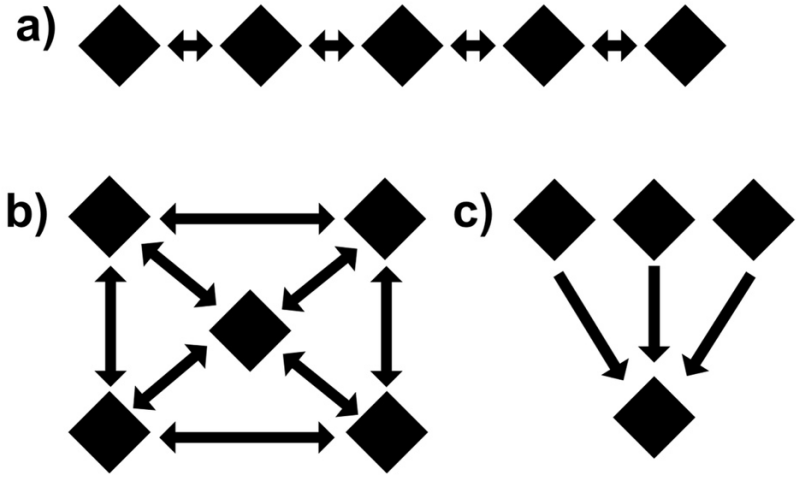

Figure 1. Models of larval distribution in populations. Diamonds represent populations while arrows represent flow of larvae. In the stepping-stone model (a) larvae may travel between close populations connecting otherwise isolated populations. When genetic material via larvae is equally distributed across close populations (b) the island model is represented. If one population acts as a sink for genetic material or larvae (c) while several other populations act as sources of genetic material without receiving any in return, a source sink model can represent this.

Recently, scientists have hypothesised that deep-sea corals show more connectivity when found at similar depths throughout their habitat range, more so than at different depths (the depth-differentiation hypothesis; Etter and Rex, 1990; Etter et al., 2005; Taylor and Roterman, 2017). In comparison to geographical distance, genetic differentiation is higher across vertical distances. Quattrini et al. (2015) used microsatellite markers to investigate connectivity between deep-sea primnoid octocoral, *Callogorgia delta,* populations in the Gulf of Mexico. Their study showed significant isolation of populations by depth (from 400 m to 900 m) as opposed to a vertical distance of 400 km, where there was high connectivity. This suggests that abiotic environmental factors found at, and influenced by, varying depth such as temperature, current, salinity, pressure, food availability, and oxygenation will drive evolutionary changes within populations (Quattrini et al. 2015).

Barriers to Marine Connectivity

Previously, it was assumed, with little evidence, that mean dispersal distance of larvae, which directly correlated to connectivity between populations, could be estimated by combining data from pelagic larval duration, rate of diffusion, and mean current speed (Selkoe and Toonen, 2011). However, aggregation and homing behaviour of larvae will increase patchiness of larval distribution (Selkoe and Toonen, 2011). Likewise, stochastic disturbances may greatly influence dispersal (Selkoe and Toonen, 2011). The environment through which larvae travel can also vary, as the deep-sea floor can be a mosaic of terrain, substrate types, and existing coral populations (Baker et al., 2012). Physical barriers to connectivity may reduce gene flow and isolate populations. Each marine habitat experiences differences in physical characteristics, some of which will form barriers to dispersal. These physical barriers may include differences in the frequency of disturbance events, different currents, temperature, hydrostatic pressure due to depth, oxygenation, nutrient availability, and sedimentation rate for example (Oleksiak, 2018).

Of the physical barriers to population connectivity, the existence of temperature clines in the water column is the most important (Oleksiak, 2018). Because physiological and chemical process rates can vary drastically (up to three-fold) for every 10º C change in temperature, and a majority of marine organisms are ectothermic, temperature becomes a crucial factor to survival (Oleksiak, 2018). Temperature will also influence metabolic rate in larvae; where lecithotrophic larvae are dependent on a limited source of nutrients, such as a yolk sac, their pelagic distribution can be shortened or extended based on temperature clines. Similarly, currents provide another physical barrier to gene flow but can also increase connectivity from otherwise isolated populations (Oleksiak, 2018).

Within the marine environment, salinity can vary and is susceptible to changes, especially near the coast where freshwater inputs from rivers and coastal flooding. Maintaining homeostasis through osmoregulation or osmoconformation is energetically taxing for the organisms exposed to these environments (Marshall et al., 2010). Likewise, in the open ocean, salinity clines can affect populations causing local adaptation that may make larvae unfit for areas with different salinity than their parental ecosystem (Marshall et al., 2010; Oleksiak, 2018).

In addition to physical barriers, biological barriers reduce realized connectivity between populations. One biological barrier to connectivity may be the presence of phenotype-

environment mismatches i.e. self-recruiting populations are more likely to be adapted to the environment in which their parental generation survived (Marshall et al., 2010; Benestan et al., 2016). While adaptation to new environments may be possible, in marine larvae, recruited larvae not adapted to an environment will perish before reaching sexual maturity, usually immediately after colonisation; predation and starvation could reduce survivability of larvae before colonization (Marshall et al., 2010). Individuals that survive an environmental mismatch but are still not suited for the habitat may reduce reproductive success of the following generation (Marshall et al., 2010; Benestan et al., 2016).

Measuring Marine Connectivity

Within marine ecosystems, observation of larval or gamete dispersal is difficult, because long term monitoring of deep-sea coral colonies is logistically not possible, and so, within deep-sea communities extremely unlikely to be observed. Likewise, mark and recapture of larvae in the deep-sea is not feasible at this time (Selkoe and Toonen, 2011). Outside of physical observation, genetic tools can help us measure and observe connectivity. Wright's Fixation Index (F_{ST}) is used to measure genetic variance relative to total variance caused by population structure (Oleksiak, 2018). The closer F_{ST} is to zero the more two populations are interbreeding without boundary and panmixis is said to be reached between the populations. When F_{ST} approaches one, the two populations do not share any genetic diversity and the existing genetic structure can be explained by population structure (Selkoe and Toonen, 2011; Oleksiak, 2018).

Reasearchers have been using a method to study connectivity called seascape genomics. This method was originally designed for terrestrial environments (landscape genomics), which uses landscape ecology alongside genetic data to understand geneflow patterns and local adaptation within populations (Manel et al., 2003). Researchers can apply the same ideas to aquatic environments by investigating the influence of water chemistry, temperature, or depth on marine populations (Benestan et al., 2016; Selmoni et al., 2020) as opposed to rainfall or other terrestrial factors. Seascape genomics will help us understand genetic adaptations to the environment in deep-sea corals and determine if there are adaptations to environmental conditions; such adaptation may be useful in our warming ocean. (Selkoe et al., 2016; Selmoni et al., 2021).

Advances in technology surrounding techniques for measuring marine connectivity have grown over the last thirty years from allozyme electrophoresis to high-throughput sequencing of millions of genetic markers. However in deep-sea research, methods have lagged behind their terrestrial and shallow water counterparts (Taylor and Roterman, 2017) with the first ever use of RAD sequencing on a deep-sea organism occurring in 2016 (Everett et al., 2016) and Ultra-conserved elements sequencing completed for the first time on deep-sea corals in 2018 (Quattrini et al., 2018). Traditionally, Sanger sequencing of DNA barcodes has been used to map connectivity in the deep sea (88 spp. in Taylor and Rotermans' review); however, traditional barcoding, for instance of Folmer's COI marker, has been unsuccessful in separating octocorals, with <1% interspecific variation reported (McFadden et al., 2011). In response to poor species delimitation in corals with traditional markers, microsatellite markers have been developed and widely used to study marine connectivity in invertebrates. In Taylor and Rotermans' review of deep-sea connectivity research up to 2017, twelve species had been studied using microsatellite data. In comparison with microsatellites, RAD-seq has been shown to yield "sharper, more consistent patterns of genetic structure" than microsatellites and were also able to detect admixture within populations (Bohling et al. 2019). Repeatedly, studies involving testing RAD-seq against microsatellite markers in fish have concurred with Bohling et al. (Bradbury et al. 2015; Jeffries et al.,2016; Thrasher et al., 2017), and Taylor and Roterman (2017) suggested that next generation sequencing will be the most useful method of studying population genetics of deep-sea organisms in the near future. However, RAD-seq requires the input of high quality whole genomic DNA for successful downstream applications- a product incredibly difficult to get from poorly preserved specimens.

Recently, phylogenomics and population genomics were investigated using the sequencing of ultra-conserved regions of DNA using tissue from aged and sometimes poorly preserved samples (Derkarabetian et al., 2019; Erickson et al., 2021) as it can be utilized on relatively small fragments of DNA, ~800bp (Faircloth et al., 2012). For example, ultra-conserved elements (UCE) sequencing was successfully used to generate SNPs in liquid preserved >30 year old octocoral museum specimens (Untiedt et al., 2021). Some of the specimens received as by-catch are poorly preserved; therefore, UCE sequencing could be the answer to generating ample SNPs for population genomics studies in deep-sea coral.

Acanella – an Unusual Genus of Deep-Sea Octocoral

Considering the success of UCE methods in various non-model species in marine environments both in the shallow and deep-sea (Erickson et al., 2021; Untiedt et al., 2021), we applied this method to a rather unique and heavily impacted deep-sea octocoral, *Acanella arbuscula* in chapter three. Unusually for a deep-sea coral, root-like shallow lobate holdfasts anchor colonies of *A. arbuscula* within the substrate; the only Isididae and non-Pennatulacea (sea pen) coral known to do so (see Figure 2). This species forms high-density fields in predominantly soft sedimentary substrate where cnidarian species richness is generally low and in relatively low abundance (Figure 2) (Baker et al., 2012). It is often found in areas with large boulders and cobble forming a habitat mosaic (Baker et al., 2012). Forming dense gardens alongside sea pens, *A. arbuscula* may help provide habitat for economically important fish, as red fish (*Sebastes* spp.) are seen in these gardens (Figure 3) (Baker et al., 2012), as well as hosting several species of brittle star (Ophiuroidae spp.), polychaete, an obligate anemone (*Amphianthus inornata*), and at least one known parasitic copepod, and one parasitic barnacle (Bronsdon et al., 1993; Grygier ,1983; Moyse, 1983; Pearson and Gage, 1984; Molodtsova et al., 2008).

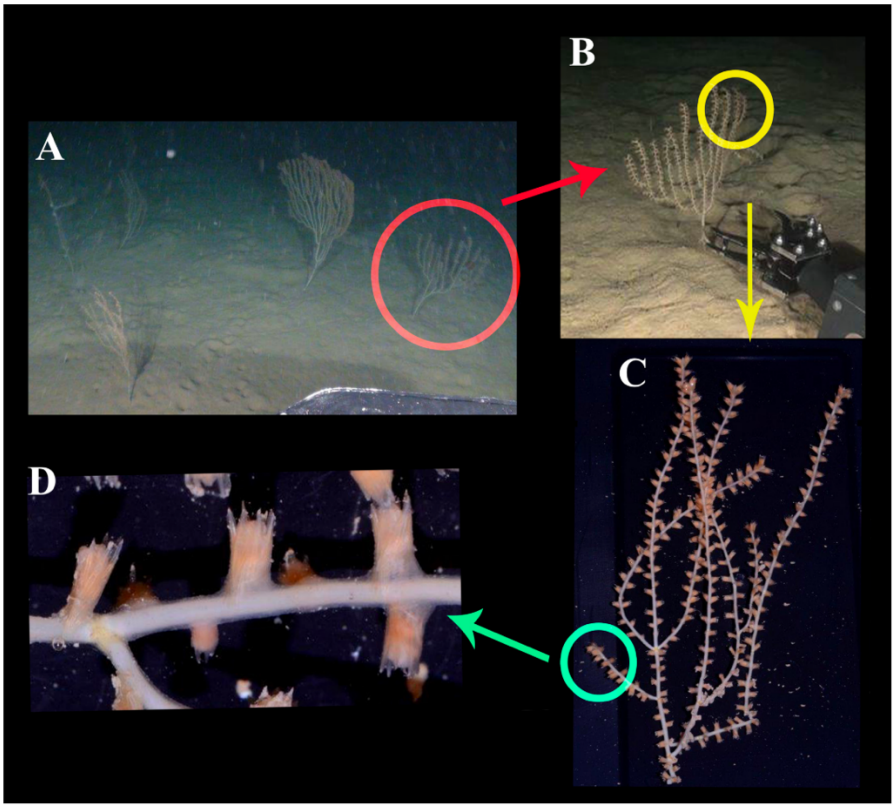

Figure 2. From Saucier et al., 2017 "*Acanella arbuscula* (M007) collected from a muddy bottom on Ausias March Seamount, Mallorca Channel, Mediterranean Sea, at 546 m depth. A) Field of colonies. Red circle indicates specimen collected. B) *In situ* image of specimen before

collection. Yellow circle indicates sampled branchlet. C) Specimen sampled with dichotomous and trichotomous branching at the node. Green circle indicates close-up of polyps on branchlet. D) Close up of sampled specimen polyps with long intertentacular sclerites. Images courtesy of Sylvia Garcia, Oceana."

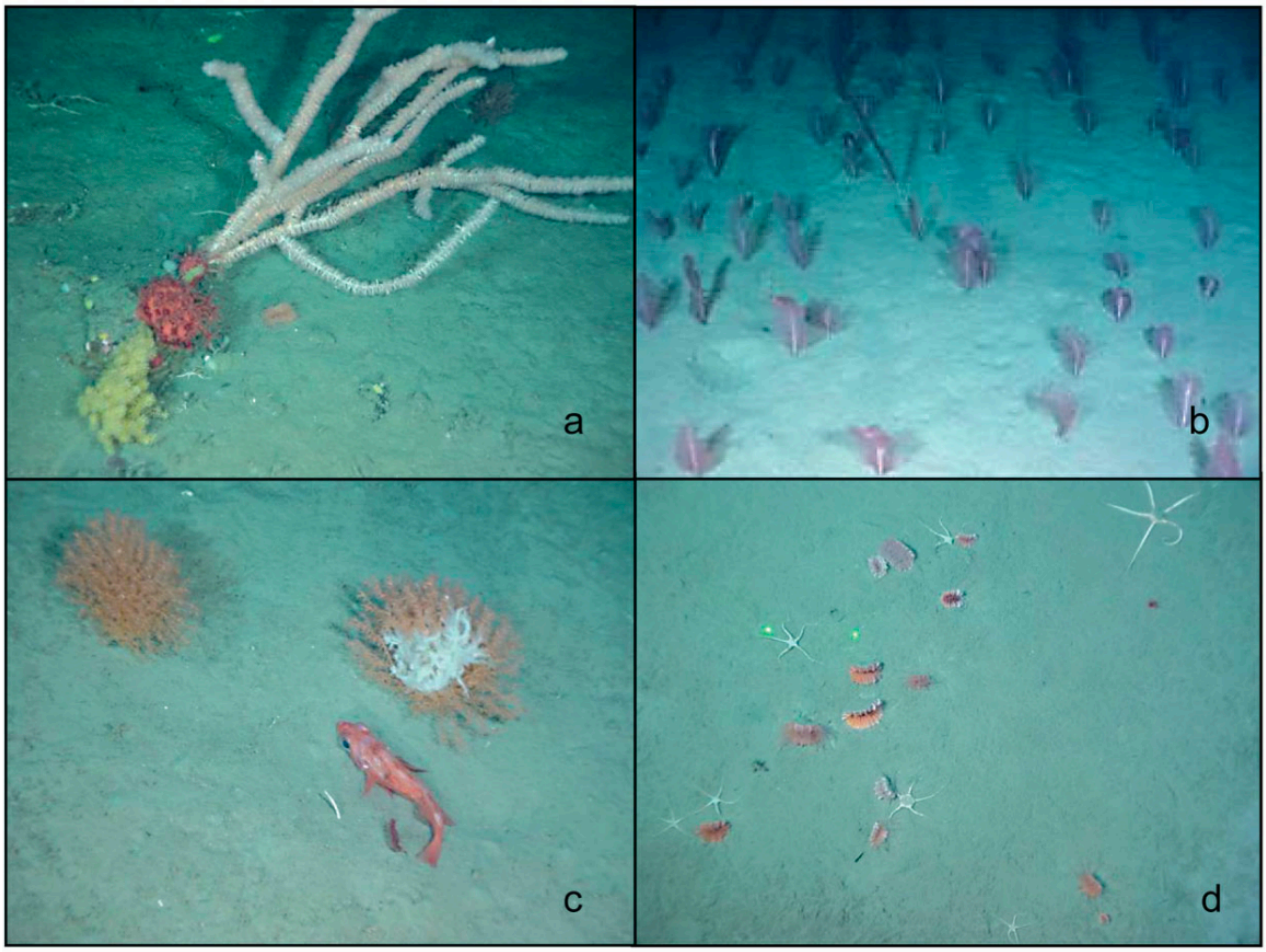

Figure 3. "Photos of coral in Remotely Operated Platform for Ocean Sciences (ROPOS) submersible transects off Newfoundland in 2007. (a) Assemblage of *Keratoisis grayi*, *Anthomastus* sp., and *Acanthogorgia armata* on a small boulder (dive R1065 at 671 m) (Flabellum sp. can also be seen), (b) sea pen field (mostly *Pennatula* spp.) in fine-sediment habitat (R1071, 835 m), (c) close up view of 2 *Acanella arbuscula* in fine sediments with *Sebastes* sp. (R1071, 594 m), (d) view of *Flabellum* spp. in fine sediments (R1065, 946 m)" From Baker et al., 2012.

Morphology and Geography

Colonies of *A. arbuscula* resemble saplings or small bushes with dichotomous or whorl-like branching at nodes. Major branches resemble bamboo with light skeletal sections being separated by disks of darker tissue formed of proteinaceous gorgonin and mucopolysaccharides. Polyps are conoidal in shape with the distal end having a larger diameter than the proximal end (Saucier et al., 2017). It is routinely found in depth ranges from 70 to 2,300 metres (Wareham 2010; Kenchington et al. 2009) with one study suggesting that colonies have been found as deep as 4,800 meters (Molodtsova et al., 2008).

Acanella arbuscula has been found throughout the Northeastern Atlantic Ocean (Figure 4) around the Mid-Atlantic Ridge to Iceland (Molodtsova, Tina N.; Sanamyan, Nadezha P.; Keller 2008) from northern Scotland through the Rockall Trough (Hughes, 2014) into the Celtic Sea (Morris et al. 2013) within the Cantabrian Sea (Sanchez, 2008) and off the coast of Morocco (Molodtsova et al. 2008). In the Northwestern Atlantic, *A. arbuscula* is prevalent from Davis Strait, Greenland, and into the Labrador Sea (Wareham 2010; Baker et al. 2012) as well as the eastern seaboard to Cape Hatteras (Watling and Auster, 2005; Buhl-Mortensen et al., 2015).

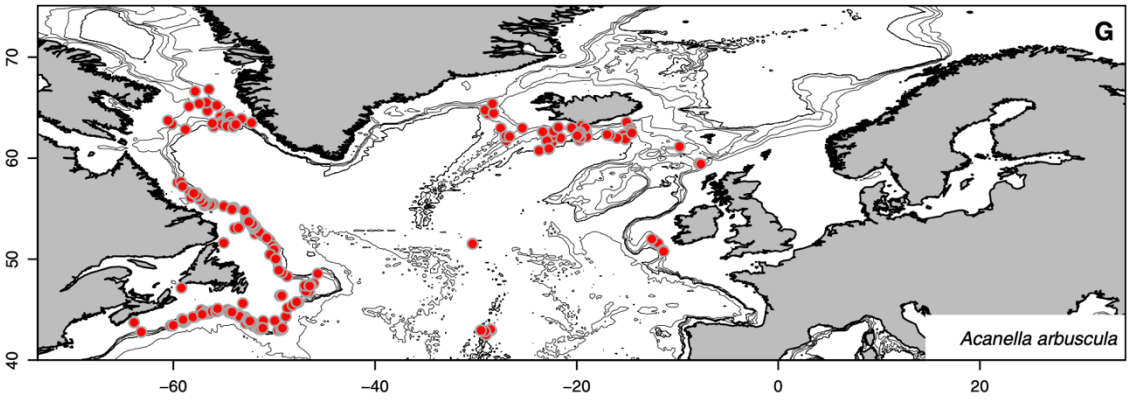

Figure 4. Previously recorded locations of *Acanella arbuscula* from (Buhl-Mortensen et al., 2015)

Acanella arbuscula Reproduction

In 2012, Beazley and Kenchington conducted a study on the reproductive biology of *A. arbuscula.* They were found to be gonochoric, with colonies being entirely male or female harbouring either sperm or eggs within the polyps. Coral colonies reached fecundity at only 3 cm with fecundity increasing relative to size. Their results suggested that *A. arbuscula* participate in broadcast spawning events; however, they were not able to observe such an event.

Because the larvae are lecithotrophic, they do not actively feed and must rely on their yolk sac for nutrients until they settle, the distance with which they can disperse is dependent upon the size and consumption rate of the yolk; scientists have however yet to determine the distance *A. arbuscula* larvae can travel (Cowen, 2009). Further evidence, potentially comparing other octocorals with similar life histories, is required to determine the potential dispersal of *A. arbuscula*.

Marine Protected Areas

Understanding of pelagic larval distribution, recruitment, and population connectivity will benefit the design of marine protected areas (MPA). Phenotype-environment mismatches may reduce recruitment to "fished" habitats from protected areas (Marshall et al., 2010). When planning a series of connected MPAs or one which will provide recruits for impacted areas, planners will have to take into account not only physical distance, but physical factors that may limit connectivity. Long duration disturbances, such as those caused by bottom trawl fishing, may remove "ecosystem engineers", which make an environment inhabitable for other species, thus creating a barrier to recruitment of species dependent on the "engineers", regardless of the rate or abundance of larvae traveling to that disturbed habitat (Marshall et al., 2010).

Utilizing marine connectivity studies, researchers can properly advise policy makers of the importance of size and placement of MPAs. When MPAs do not protect populations that act as a source of larvae, then the rest of the MPAs will not be successful in maintaining healthy populations. Designing effective MPA networks is essential to the protection of vulnerable marine ecosystems. The understanding of larval exchange between populations, which can be achieved by population genetics studies, is essential to maintaining impacted populations (Palumbi, 2003b; Oleksiak, 2018; Jenkins and Stevens, 2018).

The Canadian Department of Fisheries and Oceans (DFO) is invested in connectivity research and using *A. arbuscula* as a model for deep-sea connectivity because their habitats are unique vulnerable marine ecosystems which are heavily impacted by bottom contact fishing gear. Samples have been chosen at locations across regions of interest for the DFO (Figure 5) based on the locations of previously trawled regions that are now MPAs (areas 4, 5, 6 - Figure 5) as well as regions where fisheries observers have noted large bycatch of *A. arbuscula* (area

3 - Figure 5). Understanding of the connectivity of deep-sea coral populations in this region may directly impact the designation of new MPA networks in the future.

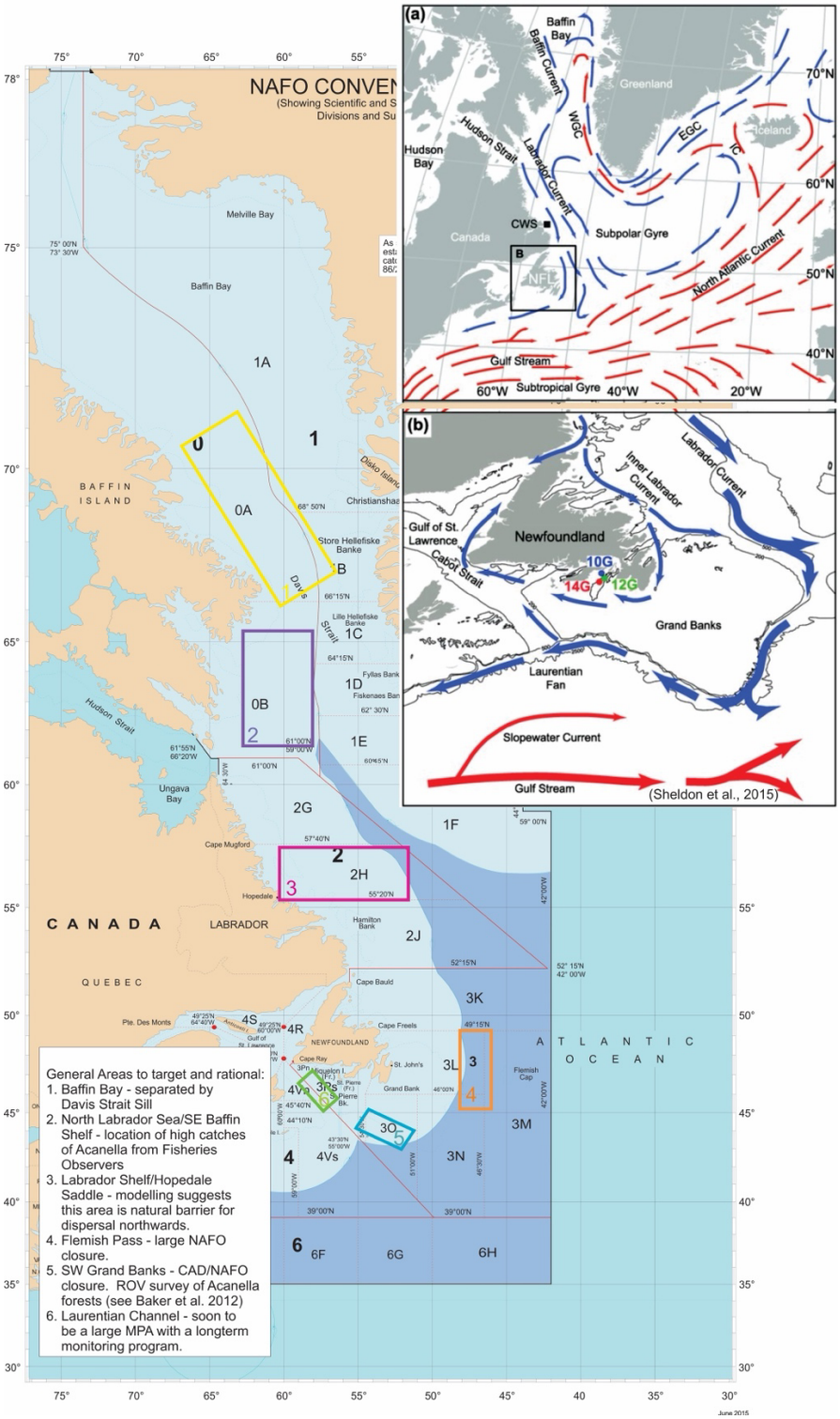

Figure 5. A DFO produced map of targeted regions surrounding Newfoundland and Labrador, Canada with models for currents which may influence connectivity of deep-sea corals. *Acanella arbuscula* colonies have been collected from parallel depth gradients within the regions labelled with colored boxes.

Project Summary

This thesis focuses on the investigation of population connectivity and genetic adaptation of *Acanella arbuscula* in the North Atlantic Ocean. First, the quantification of DNA extractions in deep-sea corals was studied in order to obtain the highest quality DNA from *A. arbuscula* for downstream genomic analysis. In that experiment, DNA was extracted from deep-sea coral tissue using five different extraction techniques, and the best method for obtaining high quality DNA was determined. The second data chapter then used this information to produce DNA extractions to prepare libraries of ultra-conserved elements (UCE) sequences. Those sequences were then analyzed to explore the population connectivity of *A. arbuscula* across the North Atlantic Ocean. The connectivity of different sample sites at similar depths was interpreted based on Fst analysis of SNPs. Geneflow, relative migration rates and directions were measured across sites. In the third and final data chapter, the environmental drivers of the connectivity patterns examined in the previous chapter were investigated. Using seascape genomics, this chapter looked at the relationship between UCE markers and environmental conditions such as temperature, salinity, and dissolved oxygen to determine if *A. arbuscula* experiences local adaptation to its environment. The combination of connectivity data and migration patterns with local adaptation data was used to point out areas that are potentially vulnerable to climate change and destruction from direct anthropogenic threats. Some broad suggestions were made for potential MPA locations to help maintain populations in the North Atlantic.

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Chapter 2: Critical analysis of DNA extraction techniques: yield, quality, time, cost, and plastic usage

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Abstract

Using non-model-organisms is becoming increasingly common in NGS. Different NGS techniques have different demands of DNA extraction; abundant DNA, "clean" HMW DNA, to be cheap, to be quick, and increasingly considered, to use minimal plastics. Here we compare five DNA extraction methods: a phenol-based extraction, salting-out protocol, and three commercially available kits from Qiagen: Blood and Tissue Kit, Plant Mini kit, and PowerSoil Pro kit. These protocols were tested across non-model organisms: deep-sea octocorals and scleractinians. We included four individuals from each of the octocorals, *Acanella arbuscula, Paragoria sp*., and *Pennatula sp*., as well as four individuals from each of the scleractinian corals, *Madrepora oculata, Lophelia pertusa*, and *Desmophyllum dianthus*. DNA yield and quality were measured and compared across methods. Phenol yielded the most DNA per mg of tissue across all corals except *Acanella* and *Madrepora*- where salting-out yielded the most DNA. The Qiagen Plant Mini kit produced the highest ratio of HMW DNA to degraded DNA in all corals tested. The purity of DNA (measured by absorbance) was equal across methods. The amount of plastic used for each protocol was also measured with kit DNA extractions using significantly more plastics than non-kit protocols. We also make recommendations on how to reduce future plastic usage. Overall, it was found that recovering HMW DNA across extraction methods was genus specific, and the method used by researchers should take into account their specific research needs.

Key words: plastic use, laboratory ethics, next generation sequencing, high molecular weight

Introduction

The use of high throughput next generation sequencing (NGS) techniques in non-model organisms has been a turning point in the study of population genetics, phylogeny, and species delimitation in many understudied taxa. However, the potential to use NGS methods on non-model organisms is often dependent on the availability of high-quality DNA for downstream applications. Recovering clean, high molecular weight DNA can be difficult in some understudied taxa yet this is required for many NGS analyses. For example, NGS techniques using long read DNA such as Oxford Nanopore and PacBio sequencing can provide scaffolding for de-novo genome assemblies (Lu et al., 2016; Jiang et al., 2019; Jeon et al., 2019; Ledoux et al., 2020). Restriction-site associated DNA (RAD) fragment single nucleotide polymorphism (SNPs) generation can lead to understanding of species delimitations (Pante et al, 2015; Herrera & Shank, 2016), to estimations of population connectivity and structure (Everett et al., 2016; Xuereb et al. 2018; Erikson et al., 2020), as well as, but not limited to, resolving phylogenomic relationships (Herrera et al., 2015; Herrera & Shank, 2016). Historically, population genetic studies require a greater number of specimens; these NGS methods require fewer specimens for population genomics studies, compared to microsatellites (Davey et al., 2011; Jenkins et al., 2019) which is particularly useful in deep-sea research where obtaining large numbers of well-preserved specimens is logistically challenging. Likewise, it is essential to recover the best quality DNA possible from these limited numbers of specimens available to researchers. Each of these NGS methods have specific requirements for DNA purity, fragment size, and quantity with protocols requiring an input of genomic DNA between 8-100 kbp in size and upwards of 1,200 ng total of DNA (data taken from various sequencing services).

When choosing a NGS technique it is crucial to understand the output of potential DNA extraction techniques. Variables such as cost, time, tissue availability, DNA yield, DNA fragment size, DNA purity, accessibility, and plastic waste generated may all be considered when choosing the DNA extraction method for a particular project. With the ever-growing use of NGS techniques in non-model organisms, it is essential to test DNA extraction protocols across taxa to determine the most appropriate isolation method.

Deep-sea coral reefs are a vulnerable marine ecosystem (Watling & Auster, 2017) potentially at risk due to destruction by fishing trawls, climate change, and ocean acidification; yet, they provide an array of services such as, providing habitat for a wide range of taxa (Watling & Auster, 2017), and are hotspots for carbon cycling (Cathalot et al., 2015). NGS methods are becoming more commonly used on deep-sea corals (Everett et al. 2016; Herrera & Shank, 2016; Xuereb et al. 2018; Jiang et al., 2019; Jeon et al., 2019; Erikson et al., 2020; Ledoux et al., 2020), and the need for optimizing DNA extractions across multiple taxonomic groups has arisen. Many extraction methods have been tested in corals ranging from phenol-based extractions to commercial kits, with varying success. While phenol is a favored extraction method due it its low cost and effective elimination of DNA contaminants, it also involves significant time spent at the bench and in the fume-hood working with toxic chemicals. Commercial kits are useful because they limit potential exposure to chemicals and have relatively easy-to-follow instructions; however, they can be expensive, may lack whole genomic DNA size selection (Schiebelhut et al., 2017), and produce significant plastic waste.

Different researchers will require specific DNA ideals for their research; for example in specific projects, finance may be a key factor in determining which DNA extraction method to use, for others the need for the highest possible amount of DNA from a specimen may be the

most important outcome, while the isolation of intact high molecular weight genomic DNA could be the most important factor to consider when attempting to complete a NGS project. In order to determine an efficient, high quality, cost effective (financially and time wise) method of DNA extraction for a wide range of deep-sea coral, this study tested five techniques on six species of coral. Given the global push to reduce plastic usage, the plastic weight of each technique was measured.

The five approaches included phenol, salting-out, a modification of the Qiagen Plant Mini Kit, Blood and Tissue Kit, and PowerSoil Pro kit. These protocols were tested across four individuals from three species of deep-sea octocoral and four from three species of deep-sea scleractinian coral. We hypothesize that optimal DNA methodologies may be taxa specific.

Methods

Collection, preservation, and preparation

Only deep-sea coral specimens determined to have ample tissue for multiple extractions were chosen. Four individuals from each the octocoral species *Acanella arbuscula*, *Paragoria* sp., and *Pennatula* sp. as well as four individuals of the scleractinian species *Madrepora oculata*, *Lophelia pertusa*, and *Desmophyllum dianthus* were used. Samples were collected from deep-sea research cruises throughout the North Atlantic. All samples were photographed with a scalebar and labeled before genetic subsamples were collected. The *A. arbuscula*, *Paragorgia* sp., and *L. pertusa* samples were stored in ≥95% ethanol at -20°C; the remaining samples were stored in ≥95% ethanol at room temperature. Tissue was weighed before downstream applications; between 8 and 270 mg of tissue was used from each specimen (Supplementary material) for each extraction. Due to the difficulty of procuring soft tissue from inside of the hard (Scleractinia) coral polyp, *M. oculata* skeletons were briefly ground with a

mortar and pestle on dry ice before being added to lysis steps. All other tissues were dissected and diced with sterile forceps and scalpel blade and excess ethanol preservative allowed to dry before being added to tissue lysis steps. Preliminary testing showed that extraction methods lacking a proteinase K digestion step failed to produce any measurable DNA from deep-sea corals, so each method was optimized and modified to include this step as described below.

Molecular Methods

Phenol Extraction

DNA was extracted from weighed, dry tissue from each individual via a CTAB and proteinase K tissue digestion followed by phenol-chloroform purification steps and ethanol DNA precipitation (Herrera et al., 2015) with the following modifications: Tissue was incubated in a micro-centrifuge tube containing 600 μL of 2% CTAB solution, 16 μL of proteinase K (Qiagen) for 12 hours in a Thermomixer (Eppendorf) set to 850 RPM at 60°C. Samples were periodically vortexed for 2 seconds. Following this lysis step 8 μL of RNase A (ThermoFisher) was added to each sample then incubated at 37°C for 30 minutes in the ThermoMixer set to 300 RPM. Following the precipitation of DNA, the DNA pellet was re-suspended in 50 μL of AE elution buffer from the Qiagen Blood and Tissue Kit and left in the refrigerator at 4°C overnight. Following gel electrophoresis, the next day, samples were stored at -20°C.

Salting-Out Extraction

Pre-weighed, dry tissue was subjected to digestion, ammonium acetate clean-up, DNA precipitation, ethanol washing, and rehydration steps as described in Jenkins (2019), modified from Li et al. (2011) as follows: Samples were vortexed and placed in the ThermoMixer for 4 hours at 65°C at 850 RPM. Following the initial digestion step, 2 μL of RNase A was placed

into each tube and incubated at 37°C for 30 minutes in the ThermoMixer at 300 RPM. DNA pellets were rehydrated with 50 μL of AE buffer (Qiagen) and left in the refrigerator overnight at 4°C. The DNA was then stored at -20°C.

DNeasy Blood & Tissue Kit (Qiagen)

Following the manufacturer's protocol with the following modifications, tissue was placed into 1.5 mL micro-centrifuge tubes with 180 μL Buffer ATL, 20 μL proteinase K. Tubes were incubated in the ThermoMixer on 600 RPM at 56°C for 2 hours. Once tissue was fully lysed, 2 μL of RNase A was added to each tube and incubated at 37°C for 30 minutes in the ThermoMixer set to 300 RPM. To increase the final DNA concentration only 50 μL of Buffer AE was added to each spin column and incubated for three minutes. They were then centrifuged at 6,000 g for one minute. The mini spin column was discarded, and DNA product visualized via gel electrophoresis and then extraction stored at -20°C.

DNeasy Plant Mini Kit (Qiagen)

The manufacturer's protocol was followed with these modifications suggested from communication with Matt Galaska in 2019 from a previous published modification (Galaska et al., 2017). This method requires the use of buffer ATL and proteinase K from the Qiagen DNeasy Blood and Tissue Kit. The tissue was first lysed in a mixture of 180 μL of Buffer ATL and 20 μL proteinase K in the ThermoMixer on 850 RPM at 60°C for 24 hours. The resulting lysate was processed following the manufacturer's suggested protocol. In the final step, only 100 μL of buffer AE was added to each spin column and incubated for 10 minutes at room temperature. The DNA was eluted by centrifuging for one minute at 6,000 g. The final product was stored at -20°C.

Modified DNeasy PowerSoil Pro Kit (Qiagen)

The manufacturer's instructions were followed with these modifications: Tissue from each specimen was added to a PowerBead Pro Tube along with 800 μL of Solution CD1 and 10 μL of proteinase K and then vortexed. Tubes were placed in the ThermoMixer for 20 hours at 60°C set to 850 RPM. Once tissue was fully lysed, 2 μL of RNase A was added to each tube and incubated in the ThermoMixer at 400 RPM for 30 minutes at 37 °C. The resulting lysate was processed following the manufacturer's published protocol. In the final step, 50 μL of Solution C6 was added to each column and incubated at room temperature for three minutes. Tubes were then centrifuged at 15,000 g for one minute. The column was discarded, and the flow-through DNA product was kept at -20°C.

DNA Extraction Product Visualization

To visualize DNA products, 2 μL of genomic DNA from DNA extraction products, were mixed with 2 μL of TriTrack DNA Loading Dye (Invitrogen) and loaded into a 1% agarose gel prepared with 5 μL of SYBR™ Safe DNA Gel Stain (Invitrogen) per 100mL of gel. Samples were run alongside a TrackIt 1Kb Plus DNA Ladder (Invitrogen) at 100 volts for thirty minutes and then visualized on the Gel Doc™ EZ System (Bio-Rad). Gel images were exported and analyzed with ImageJ open access software (Figure 1) following Mulcahy et al. (2016) quantitative assessment of genomic DNA quality. Images were inverted and their backgrounds removed allowing for more accurate histogram measurements. Using the "analyze gels" feature on ImageJ (Schnieder et al., 2012), boxes were drawn to encompass the 1Kb ladder and each sample lane. Histograms were plotted for each lane. Using the guideline from the ladder, a boundary line for DNA products greater than 8,000 base-pairs was drawn to separate histogram curves into high molecular weight (HMW), left of the boundary line, and low molecular weight (LMW), right of the boundary line. The area under the curve for each

HMW and LMW region of each lane was recorded, and the percentage of HMW vs. LMW was calculated by dividing the HMW area under the curve by the total area (Mulcahy et al., 2016).

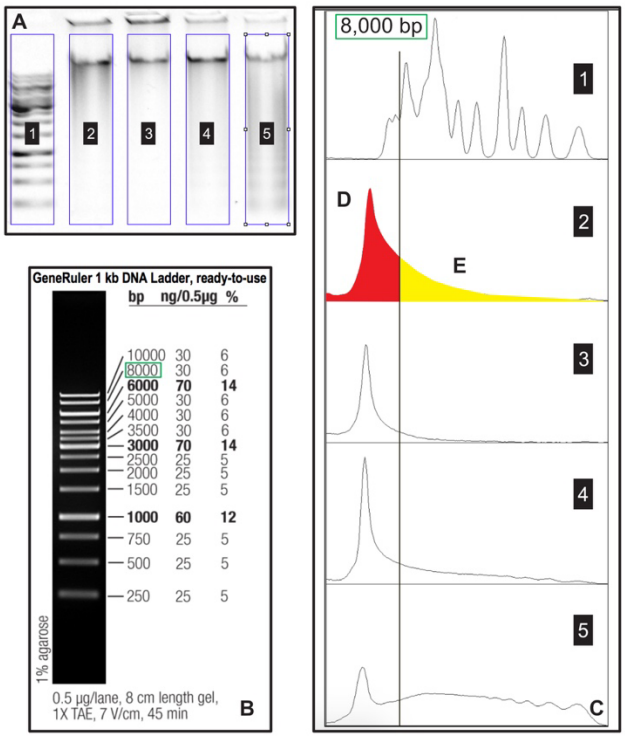

Figure 1 Gel lanes were analyzed by selecting the lane areas with blue boxes (A) and graphing the histograms of the selections (C). The histogram for the ladder (C,1) and manufacturer's guide (B) were used to identify segments of DNA greater than 8,000 bp (green boxes). A line separating DNA segments above and below 8,000 bp was drawn through histograms 1-5 (C) which correspond to lane 1-5 (A). The area under the curve for lane 2 representing genomic DNA greater than 8,000 bp was measured (D; red). Likewise, the area under the curve representing DNA less than 8,000 bp in size was measured (E; yellow). The proportion of HMW DNA was calculated by dividing the area of D by D+E. The histogram for lane 3 shows an ideal ratio of HMW to total DNA while the histogram for lane 5 shows a low ratio of HMW.

DNA Quantification

The concentration of resulting DNA products were measured three times each with a NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific), alongside the 260/280 ratios of each sample. The mean of the concentration and purity scores for each sample and each clade were calculated for each extraction method. Total DNA yield was calculated by multiplying the volume of the final product by the concentration and then dividing by the amount of tissue (in milligrams) used in each extraction. After extractions were completed, the remaining coral skeleton fragments were recovered from the initial digestion steps, washed with ethanol, dried, and weighed; skeletal weights were then subtracted from the initial total tissue weight to determine the soft tissue weight in milligrams that went into each extraction. We tested if DNA yield varied across extraction methods using a two-way ANOVA followed by a Tukey's HSD test with all statistical analysis completed in R.

Plastic Use

To estimate the amount of single-use plastics consumed for each extraction protocol, the weight of each clean pipette tip and tube -including individually packaged mini spin columns- used in each extraction was measured. The weight of each item consumed was then multiplied by the number of disposable plastics required for the extraction of twenty-four samples. The weight of pipette tip boxes, plastic wrap, and additional outer packaging were not measured.

Time/Cost Analysis

The cost of commercial kits and reagents including VAT as of January 2020 were recorded (Supplementary material). The time spent at the lab bench was calculated into an hourly wage based on the average stipend of PhD students at the University of Essex at the

time of this study (£ 9.22/hr for a 37-hour work week). Bench time spent handling specimens was included; however, lysis-based incubation periods were not.

Results

Taking all factors into account, no one method out-performs all of the others in every measured category (Figure 2). For instance, the phenol extraction method was the most efficient for the majority of the specimens tested as it yielded the most DNA per milligram of tissue used in each extraction. This was true for all specimens except in *Acanella arbuscula* where the Blood and Tissue kit produced the most DNA per milligram of tissue. Despite yielding the most DNA in most samples, the phenol extractions did not produce the highest molecular weight genomic DNA; the Plant Mini kit and salting-out protocols produced the highest ratios of HMW DNA to LMW DNA. The salting-out extraction was found to be the most cost effective, with the Power Soil Pro kit the most expensive. The commercial silica membrane-based kits produced more plastic waste than the salting-out and phenol-based protocols. All tested methods preformed similarly in producing pure uncontaminated DNA based on the 260/280 nm readings from the NanoDrop.

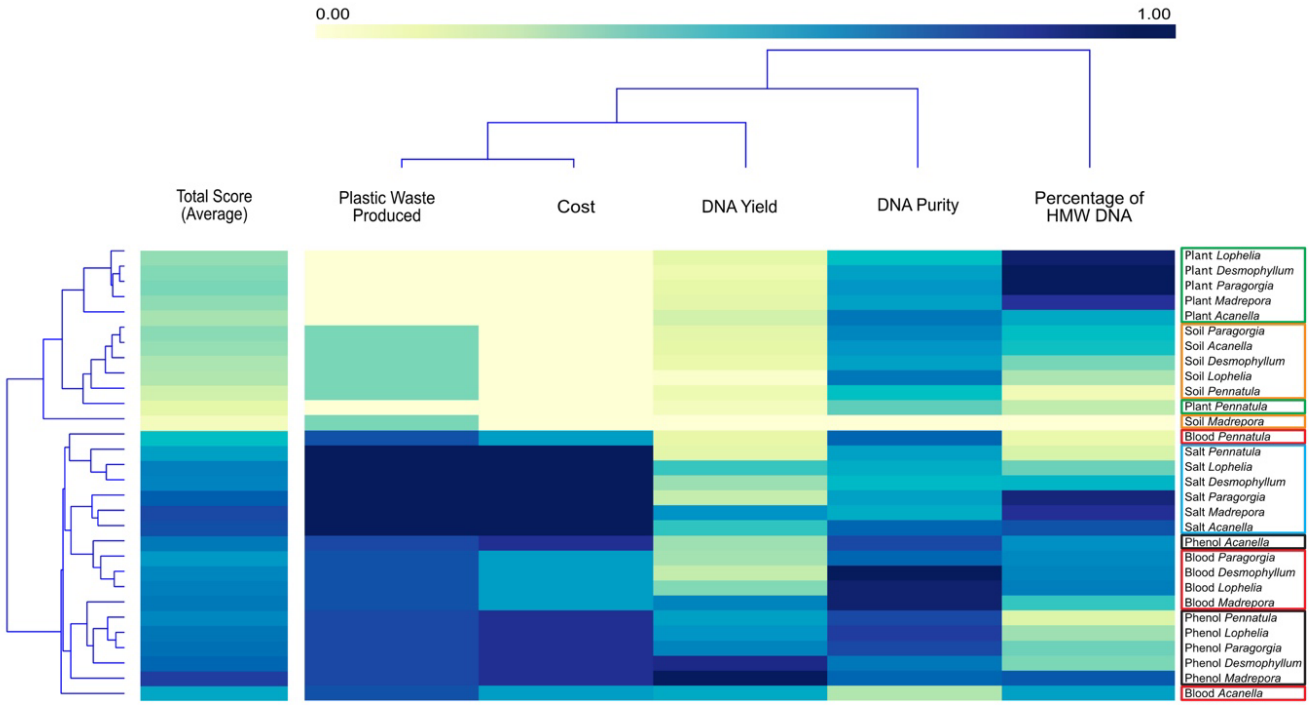

Figure 2 Measured values for each category were standardized and scaled to one with one (dark blue) being the most desirable. The values for cost and the plastic waste produced were scaled to one and then subtracted from one to indicate that the lower cost and plastic waste produced were more desirable. Rows are ordered by similarity (left tree). Columns ordered by similarity of factors measured (above tree). With all factors weighted equally, the total score on the left indicates the average values across the row for the specific genus/method combination. The grouping of methods is indicated with coloured boxes (green for plant, orange for soil, red for blood, sky blue for salting-out, and black for phenol).

Recovery of Genomic DNA

From gel lane photo analysis, the plant kit was found to yield the highest percentage of HMW DNA (greater than 8kbp) across all specimens (62.40%) followed by the salting-out protocol (49.28%), the Blood and Tissue kit (41.96%), phenol extraction (34.81%), and lastly, the soil kit produced the least HMW DNA (22.41%) (Table 1). While the plant kit extractions preformed the best overall, in *Acanella arbuscula*, the salting-out protocol produced the purest

HMW DNA (63.03% HMW DNA) vs. the plant kit (44.52%) and phenol extraction (51.01%). In *Madrepora oculata* specimens, the salting out protocol and plant kit were equally successful with 71.18% and 71.10% HMW DNA respectively. Regardless of the extraction method, *Pennatula* sp. specimens failed to produce HMW DNA; only low molecular weight smears of DNA were observed.

Table 1 Average percentage of high molecular weight DNA produced from each extraction method by specimen. Colour scales indicate the highest percentage of high molecular weight DNA (green) to lowest (red).

Specimen	Phenol	Salt	Plant	Blood	Soil
Acanella arbuscula	51.01	63.03	44.52	46.66	37.07
Paragorgia sp.	29.81	75.67	80.41	52.44	38.35
Lophelia pertusa	24.23	30.00	77.76	54.26	22.78
Pennatula sp.	13.69	14.55	20.02	9.72	7.17
Madrepora oculata	61.95	71.18	71.10	35.47	0.50
Desmophyllum dianthus	28.16	41.28	80.56	53.23	28.57
Extraction Method Average	34.81	49.28	62.40	41.96	22.41

Genomic DNA products were run on a 1% agarose gel in the same order so that lanes were directly comparable across each method to show the size of DNA recovered as well as degradation level. The majority of phenol extraction DNA products (black boxes, Figure 3) appeared as HMW DNA with smears of LMW DNA. The phenol extracted DNA also contained residual protein contamination shown as black regions in the wells of the gel. For *L. pertusa*, *Pennatula* sp., and *D. dianthus* phenol extractions there was a high concentration of

LMW DNA present (a smear) and pooling of nucleotides midway through the gel. Several of the *M. oculata* and *A. arbuscula* phenol extraction products contained clean HMW DNA; however, there was still LMW contamination. The salting-out products (blue boxes, Figure 3) produced clear HMW bands with some LMW smearing. The modified Qiagen Plant Mini kit (green boxes, Figure 3) consistently provided clean HMW DNA with strong bands and minimal contamination. For *Paragorgia* sp., *L. pertusa,* and *D. dianthus* the plant kit produced defined HMW bands. The salting-out protocol produced well-defined HMW bands in *Acanella* and, arguably, in *M. oculata* too. No method produced clean HMW DNA from *Pennatula* sp. The Blood and Tissue kit (red boxes, Figure 3) was able to produce some HMW bands for a few *A. arbuscula, Paragorgia* sp., *L. pertusa,* and *D. dianthus* corals; however, a majority of the lanes contained smears of LMW, and a few lanes had pooling of very LMW DNA fragments. The soil kit (orange boxes, Figure 3) produced a single HMW band in only one *L. pertusa* specimen with the rest containing smears of LMW DNA.

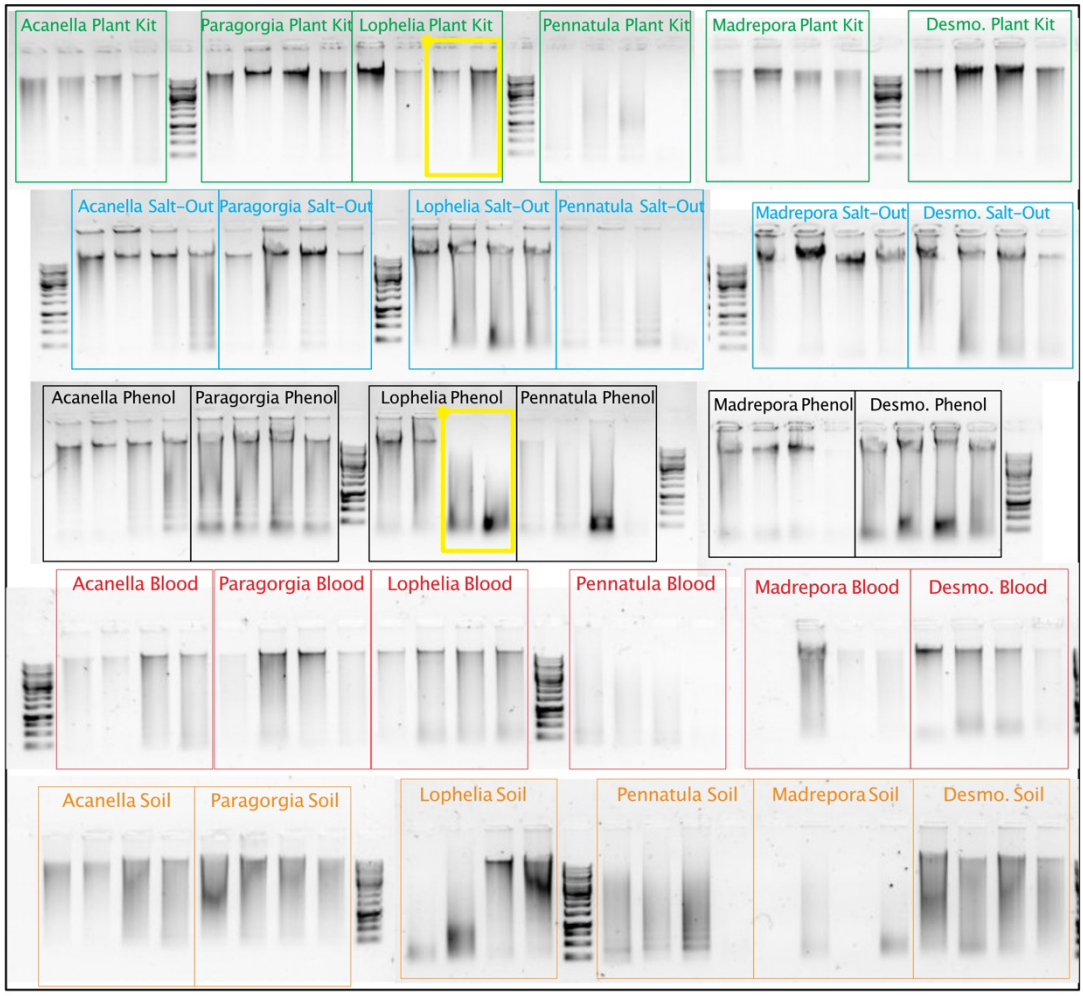

Figure 3 Agarose gel photo of DNA products for each extraction method. Samples are loaded in the same order, so that each lane within each box is directly comparable. Samples in black boxes are from phenol, blue from salting-out, green from the plant mini kit, red from blood and tissue kit, and orange from the soil kit. Yellow boxes indicate two samples that were greatly improved when extracted by the Plant Mini kit as compared to the traditional phenol method. A 1kb ladder was run alongside samples. Several gel photos have been cropped to fit this figure. **Desmophyllum* was abbreviated to fit.

DNA Quantification

Following a two-way ANOVA and TukeyHSD multiple comparisons of means, with a 95% family-wise confidence level, DNA yield was found to be significantly different across extraction methods ($p = 5.37 \times 10^{-13}$), but not across genera ($p = 0.104$). Average DNA yield across all clades was the highest from phenol extractions, except in *A. arbuscula* (Table 2). For *A. arbuscula*, the Blood and Tissue kit produced the highest yield of DNA at 1416.73 ng/mg of tissue. As per two-way ANOVA, DNA yield for each extraction method is significantly different between almost all extraction methods (Table 3).

Across all extraction methods, with few exceptions, DNA quality (260/280 ratios, Table 4) was consistently above the cut-off point for 'pure DNA' (without protein contamination) of 1.80. The PowerSoil Pro kit failed to produce pure DNA in *M. oculata* (1.68) corals, the modified Plant mini kit for *Pennatula* sp. (1.78) and the Blood and Tissue Kit for *A. arbuscula* (1.75).

Table 2 Average DNA yield (ng DNA/mg soft tissue) for each specimen in each extraction method

Genus	Phenol	Salt	Plant	Blood	Soil
Acanella	796.10	1170.29	522.49	1461.73	366.24
Paragorgia	1759.20	623.43	361.62	775.91	392.50
Lophelia	1654.00	1146.64	383.65	902.26	85.35
Pennatula	1548.28	385.48	196.54	348.56	270.66
Madrepora	2654.93	1662.11	392.25	1756.78	9.68
Desmophyllum	2431.13	810.29	290.67	640.79	316.50

Table 3 Reported p values from two-way ANOVA and TukeyHSD multiple comparison of means between each extraction method for DNA yield.

Method	Phenol	Salt	Plant	Blood	Soil
Phenol		0.000^{***}	0.000^{***}	0.000^{***}	0.000^{***}
Salt	0.000^{***}		0.013^{*}	1.000	0.002^{**}
Plant	0.000^{***}	0.013^{*}		0.011^{*}	0.965
Blood	0.000^{***}	1.000	0.011^{*}		0.001^{**}
Soil	0.000^{***}	0.002^{**}	0.965	0.001^{**}	

Table 4 Average nucleic acid purity (260/280nm) for each specimen in each extraction method

Specimen	Phenol	Salt	Plant	Blood	Soil
Acanella arbuscula	1.89	1.87	1.86	1.75	1.84
Paragorgia sp.	1.89	1.83	1.84	1.87	1.85
Lophelia pertusa	1.90	1.82	1.80	1.93	1.86
Pennatula sp.	1.89	1.83	1.78	1.87	1.80
Madrepora oculata	1.87	1.82	1.83	1.93	1.68
Desmophyllum dianthus	1.86	1.81	1.83	1.94	1.83

Time-Cost Analysis

Start-up costs for specialized equipment such as fume-hoods, centrifuges, and the ThermoMixer were not included; only the cost of reagents and specialized micro-centrifuge tubes were included in these costs. The unit cost of each reagent or commercial kit, with links to UK websites, can be found in the supplementary material. The most cost-effective method of DNA extraction was the salting-out protocol; 24 samples were processed for £20.79. The

second most cost-effective extraction method was the phenol-based extraction, £37.08 to extract 24 samples. The commercially available Qiagen kits were more expensive with the DNeasy Blood and Tissue kit being the cheapest of the kits tested which cost £82.08 to extract 24 samples. The DNeasy Plant Mini kit cost £116.64 to process 24 samples, and the PowerSoil Pro kit is the least cost-effective totaling to £171.36 for 24 extractions.

Excluding tissue digestion time, the phenol extraction method took the longest with 6 hours of bench time for 24 samples. The salting-out protocol was the second longest with 5 hours of bench time followed by the DNeasy Plant Mini kit which took 2.5 hours, the PowerSoil Pro kit with 2 hours, and lastly, the most time efficient kit was the DNeasy Blood and Tissue kit which took 1.5 hours for 24 samples. Accounting for an hourly pay of £9.22 for a 37 hour work week for a PhD student at the University of Essex, the total cost with time and materials of each method increases to £66.89 for salting-out, £92.40 for phenol, £95.91for the Blood and Tissue kit, £139.69 for the Plant Mini kit, and £189.80 for the PowerSoil Pro.

Plastic Use

It was found that the commercial kits tested produced more plastic waste than traditional methods (Table 5). The extraction method that used the least amount of plastic was the salting-out protocol (261.40g) followed by the phenol method (305.88g). Of the commercial kits, the Qiagen Blood and Tissue kit produced the least plastic waste (312.12g), followed by the Qiagen PowerSoil Pro kit (415.49g), and finally the modified Qiagen Plant Mini kit (497.59g) produced the most plastic waste of all methods tested.

Discussion

Choosing a DNA extraction method

Researchers approach NGS and their science with varying needs and constraints – financial, equipment, time, DNA yield, quality, and concentration requirements, and across the molecular field there are increasing ethical considerations around reducing plastic usage and waste. We approached this taking into account DNA yield, quality score, ratio of genomic to degraded DNA, time and cost, and plastic waste produced from each tested extraction method, no single extraction method outweighs all others in every category (Figure 2). As suspected, some methods may have genus specific successes for total DNA yield or genomic DNA concentration. Such is the case with specimens of *Acanella arbuscula*; this coral produced a higher yield of DNA in nanograms of DNA per milligram of tissue extracted using Blood and Tissue kit compared to the other corals that received the greatest yield with the phenol extraction method. Also, some methods produce a higher ratio of HMW genomic DNA to degraded LMW DNA in relation to genus. In all corals except *A. arbuscula,* the DNeasy Plant Mini kit produced the highest ratio of HMW DNA to LMW DNA. The salting-out protocol produced the highest ratio of HMW DNA to LMW DNA in *A. arbuscula,* and *M. oculata* produced an equal ratio when processed through either the salting-out or Plant Mini kit extractions. In two individuals (yellow boxes, Figure 2), the phenol extraction failed to produce HMW DNA in *Lophelia pertusa*; however, the Plant Mini kit succeeded in recovering HMW from these two samples; therefore, it may be possible to obtain HMW DNA from samples previously thought to be unsuitable for downstream NGS applications by using the Plant Mini kit.

While the phenol extraction method has long been favored due to its low cost (Schiebelhut et al., 2017), the processing of samples was more time consuming than any other

method tested here. Additionally, the risk of exposure to harmful chemicals is high, work must be conducted in a fume hood, and waste phenol must be disposed of properly with hazardous waste. This method also produces more plastic waste (creating additional phenol-contaminated hazardous waste) than the salting-out method. Several steps in the phenol protocol require a centrifuge capable of centrifuging specimens at 16,000 g which could increase start-up costs if equipment is not readily available. The salting-out protocol was the cheapest method tested here, produced high quality DNA at relatively low cost, and used the least plastic waste of all methods tested. Of these two methods, the salting-out protocol is the better choice for downstream NGS applications.

Of the tested commercial silica membrane-based kits, the Plant Mini kit out preformed the Blood and Tissue kit as well as the PowerSoil Pro kit in respect to DNA yield and HMW:LMW ratios. However, it produced the most plastic waste of all of the methods (using two silica membrane-based columns per specimen). The ratio of HMW: LMW DNA was found to be poor in the DNeasy Blood and Tissue kit despite being the most time efficient; not ideal for downstream NGS applications. The PowerSoil Pro kit performed poorly across all tested categories -except for DNA purity- and is not recommended for use in deep-sea coral DNA extractions.

In many NGS methods, such as RAD-seq, degraded DNA extracts produce fewer reads, leading to less SNP recovery (Graham et al., 2015). A project aiming to use NGS methods which require a high ratio of genomic DNA, with as little LMW as possible, may want to consider the Plant Mini kit or the salting-out method (Table 5). However, a project requiring high DNA abundance, with little regard for the ratio of HMW DNA, may benefit from using the phenol-based extraction, salting-out method, or Blood and Tissue kit.

It is important to note that regardless of method, *Pennatula* sp. samples failed to produce much DNA or any DNA of quality. It is believed that the *Pennatula* sp. samples used for this experiment were originally poorly preserved- a common issue. If quality genomic extractions are required in the future, we recommend as much effort as possible is made to preserve specimens destined for genomic research as quickly as possible after death by either flash freezing, preserving in DNAzol, or 95% ethanol (Mulcahy et al., 2016).

Table 5 Suggested DNA extraction methods for the most common next generation sequencing methods and their requirements.

NGS Method	Size (bp)	Gel Quality	Concentration (ng/ul)	A 260/280	Total DNA Required (ng)	Suggested DNA Extraction Method
RAD	>12 kb	solid band	20	1.8-2.0	1,200	Plant Mini Kit
Oxford Nanopore	>100 kb	solid band	20	1.8-2.0	1,000	Plant Mini Kit
PacBio	>40 kb	solid band	>20	1.8-2.0	>20	Plant Mini Kit

Recommendations for reducing plastic use through efficient pipetting techniques

With potentially 5.5 million metric tonnes of plastic used in scientific laboratories each year (Urbina et al. 2015), and a long history of disposable plastic use, it is perhaps the scientific challenge of the coming decade to reduce single-use plastics in research environs. We hope there is national leadership on this in the future (inclusion of sustainability in the Research Excellence Framework for example); however, there are steps each of us could undertake to reduce plastic waste produced by our labs. From the protocols explored in this research for example, when adding buffers to spin columns, if the tip is held above the column and kept sterile i.e. no touching, one tip could be used for all 24 samples; a test for inexperienced

researchers perhaps but worth the effort and training. For the sake of comparison, the amount of plastic used for each method was measured using manufacturers guidance and not these alternate efficient pipetting techniques. However, as practice and understanding of the protocol increases, we encourage users to modify pipetting steps to reduce plastic waste, which also lowers overall cost. Despite these modifications, and despite the weight of pipette tip boxes and plastic wrapping accounted for here, commercial kits still produce more plastic waste (spin columns and packaging being the main culprits). While some solutions exist to reduce the amount of plastic consumed in laboratories, such as the above-mentioned modifications and recycling, more needs to be done to make research a more sustainable practice, and companies should investigate long term solutions to waste issues.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

JDG designed the initial experiment, conducted the research, analyzed the results, and wrote the manuscript. MLT provided assistance with the experimental design, access to coral specimens, analysis ideas, funding for the experiment, and supported manuscript completion.

Chapter 3: Population structure of deep-sea octocoral *Acanella arbuscula* (Isididae) across the North Atlantic using SNPS generated from UCE sequencing

Authors of paper in preparation for submission

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Abstract

Deep-sea coral gardens provide essential ecosystem services to marine organisms at various stages of their life histories; providing shelter, breeding grounds, and food. These vulnerable marine ecosystems are targets for fishing because they host economically important species. Trawling destroys cold water coral gardens, and areas subjected to repeated bottom fishing activity have experienced the terrestrial equivalent of clear cutting a forest and removing all of the topsoil, with little signs of recovery post-trawling. These octocorals are ecologically important, because *A. arbuscula* are known to host species of ophiuroid sea star, polychaete, nematode, copepod, anemones, barnacle, and crinoid feather star. They are slow-growing (1cm/yr) and require at least three years to reach sexual maturity. In order to protect these vulnerable marine ecosystems, understanding population connectivity is essential to informing conservation measures, such as marine protected area (MPA) design. To investigate population connectivity of *A. arbuscula* across the North Atlantic, specimens were collected at Newfoundland and Labrador (Canada), Greenland, Scotland, The Celtic Sea (Ireland), and The Sea of Biscay (Spain) from 60 to 2,300 m depth. Population analyses were inferred from single nucleotide polymorphisms (SNPs) generated through high throughput sequencing of ultra-conserved elements (UCEs). Furthermore, the SNPs were used to test the depth differentiation hypothesis across the North Atlantic which proposes that there is greater genetic differentiation across depth ranges as opposed to geographical distance. Trans-Atlantic connectivity of *A. arbuscula* was uncovered with sites from Newfoundland and Labrador exhibiting high levels

of connectivity to sites in Europe. We found a barrier to connectivity around 1,200 m depth in the Eastern Atlantic where samples from sites shallower of this depth showed almost no connectivity to samples from sites below 1,200 m depth. It is suspected that some environmental barriers to connectivity exist, including those associated with currents, temperature, salinity, oxygen, and nutrient availability. Furthermore, we offer suggestions for regions of conservation concern in the North Atlantic that would benefit from the establishment of marine protected areas.

Introduction

The deep-sea constitutes the largest habitat in the world, and the invertebrates who inhabit it are largely understudied and poorly understood (Rex, 2010; Taylor & Roterman, 2017; Watling & Auster, 2017). Highly diverse and complex cold water coral assemblages, seamounts, and hydrothermal vents have been called vulnerable marine ecosystems (VMEs) by the UNGA and are widely considered under threat from human impacts such as climate change, oil and gas exploration, deep-sea mining, and destructive fishing practices (Jones et al., 2018; Watling & Auster, 2017). Deep-sea corals are slow-growing and long lived (Sherwood & Edinger, 2009) which suggests they may be slow to recover from human disturbances. Investigating the population connectivity of these deep-sea corals is essential to our understanding of their resilience to disturbance and the design of effective marine protected areas (MPAs) to help preserve existing populations and aid in the recovery of impacted cold water coral ecosystems (Cowen & Sponaugle, 2009; Jenkins & Stevens, 2018; Leiva et al., 2022; Palumbi, 2003; Taboada et al., 2022).

One such coral, *Acanella arbuscula* (Isididae) Johnson 1862, is heavily impacted by bottom contact fishing gear in the North Atlantic Ocean (Gass, 2005). Large fields of *A. arbuscula* are found anchored in soft deep-sea muddy sediments via their shallow root-like holdfasts (Figure 1) and occasionally attached to drop stones or rocky outcroppings (Buhl-

Mortensen et al., 2015). Geographically, they are found throughout the North Atlantic Ocean in Atlantic Canada through Davis Strait in Labrador and West Greenland to the southernmost tip of Newfoundland around Flemish Cap and Grand Banks into the northern region of New England, on the Mid-Atlantic ridge, south of Iceland, in the Faroe Islands, the West of Scotland marine protected area (WSMPA), down to Whittard Canyon in the Celtic Sea (Buhl-Mortensen et al., 2015), and as far south as the Cantabrian Sea (observation from sponGES expedition).

They often inhabit areas alongside octocorals such as sea pens (*Pennatula* spp.) and *Umbellula* spp. as well as the commercially important redfish (*Sebastes* sp.), as found in Newfoundland and Labrador (Baker et al., 2012). In the Grand Banks in Newfoundland, they are found in high densities with a maximum abundance of 77 colonies in a 10m square transect; they were found to have a maximum size of 30cm and an average of 7cm (Baker et al., 2012). This species of bamboo coral are slow-growing (<1cm/year) and the few measured colonies were at least 30 years old (Sherwood & Edinger, 2009).

Acanella arbuscula are ecologically important because they provide habitat for an array of deep-sea species including: ophiuroid brittlestars (Pearson & Gage, 1984), two species of epizoic anemone (Bronsdon et al., 1993), a parasitic barnacle (Moyse, 1983), polychaetes (Hamel et al., 2015), and one recently described nematode worm (Westerman et al., 2021).

Figure 1. *Acanella arbuscula* colony showcasing the white root-like holdfasts which are small compared to the size of the whole colony. (Photo credit: Scott C. France)

Obtaining samples from the deep-sea is notoriously difficult, with expeditions costing upwards of one million pounds, sampling events are thus relatively rare. With most museum specimens being poorly preserved for genetic techniques, taking advantage of what tissue is available is essential even if this may mean any resulting DNA is highly degraded (Faircloth et al., 2012). Methods of generating single nucleotides (SNPs) such as restriction site associated DNA sequencing and microsatellites used to estimate population connectivity typically require high quality DNA or they do not produce enough data to explore deeper population structure (Faircloth et al., 2012; Quattrini et al., 2018; Stiller et al., 2021). Stiller et al. (2021), in shallow temperate waters of Australia, found deeper genetic structure and higher genetic diversity in leafy sea dragons, including use of tissue from museum specimens, using UCEs than previously found in studies using microsatellites in the same region. This suggests that UCE sequencing can be more useful for population genomics than microsatellites, perhaps unsurprising given the number of markers can increase for a few dozen to many 1000s. Recently, the sequencing of ultra-conserved regions of DNA has successfully used DNA from aged and sometimes

poorly preserved samples to investigate phylogenomics as well as population genomics (Derkarabetian et al., 2019; Erickson et al., 2021; Faircloth et al., 2012) as it can be utilized on relatively small fragments of DNA, ~800bp. For example, ultra-conserved elements (UCE) sequencing was successfully used to generate SNPs in liquid-preserved >30 year old octocoral museum specimens (Untiedt et al., 2021).

The depth differentiation hypothesis (DDH) proposes that organisms in the deep-sea are more genetically connected at similar depths across far geographical distances than they are at different depths down a slope. This is due to the changes in environmental conditions experienced at different depths that influence local adaptation in populations (Rex, 2010). Testing the depth differentiation hypothesis can be done using population genomics. Here, we use UCE sequencing to generate SNPs from *A. arbuscula* DNA to investigate the population connectivity of this deep-sea octocoral across nearly the entirety of its geographical range in the largest population connectivity study of a deep-sea coral to date. We aim to test the accuracy of the DDH across the North Atlantic Ocean using *A. arbuscula* as a model organism.

Methods

Collection and preservation

Samples used in this study (Figure 2) were collected from several research expeditions and as by-catch from fisheries. Onboard the RRS *Discovery* expedition DY081 near Nuuk, Greenland, whole colonies (n=12) of *Acanella arbuscula* were collected using the *Isis* remotely operated vehicle (ROV). Whole colonies were preserved in 100% ethanol at room temperature and genetic subsamples of branches were preserved in 100% ethanol at -20°C. In the West of Scotland MPA, *A. arbuscula* colonies (n=58) were collected using the *Isis* ROV onboard the RRS *James Cook* on expedition JC136. After genetic subsampling branches were preserved in 100% ethanol and stored at -20°C; whole colonies were preserved at room temperature in 100% ethanol. From The Cantabrian Sea, samples were collected as part of the SponGES project

(n=40) using a small agassiz trawl at depths of 862m, 1050m, and 1510m. Whole colonies were preserved in 100% ethanol and stored at -20°C. Samples from the Celtic Sea were collected onboard the R/V *Celtic Explorer* using the ROV *Holland I* during expedition CE16006. Whole colonies (n=23) were preserved in 100% ethanol at room temperature, and branches for genetic subsamples were preserved in 100% ethanol and stored at -20°C. In Newfoundland and Labrador, Canada, Department of Fisheries and Oceans (DFO) fisheries observers obtained samples from bycatch onboard fishing vessels and several DFO deep-sea research expeditions (n=253). Samples were fresh-frozen and stored at -20°C. Later they were thawed, subsampled, and preserved in 100% ethanol and stored at -20°C.

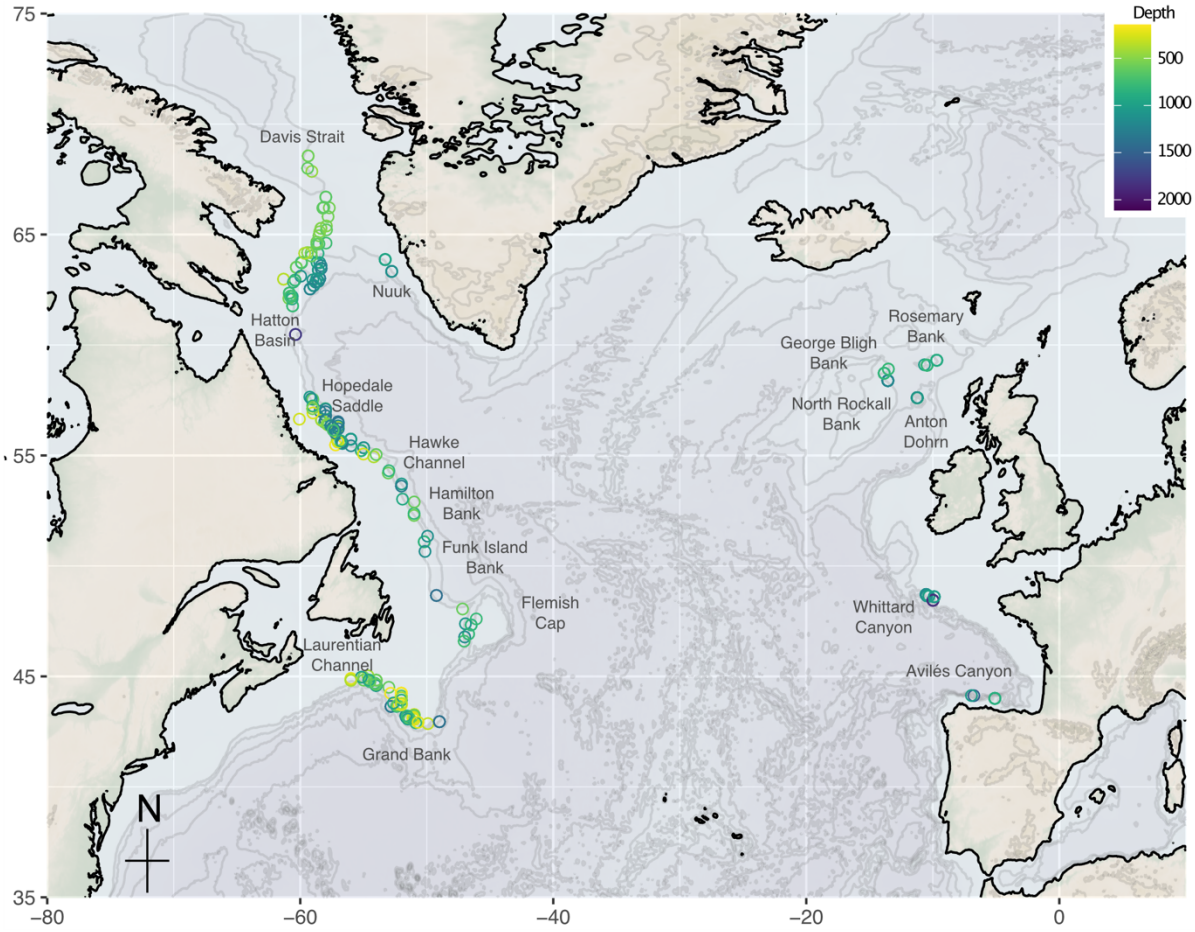


Figure 2. Samples of *Acanella arbuscula* were collected across the North Atlantic Ocean. Each circle represents a sampling location- lighter colors indicate shallower depths and darker colors indicate deeper sampling locations. Multiple individuals were collected from the same

sampling event in some instances. Bathymetric features included correlate to locations listed in Table 1.

Samples were divided into five depth categories: 0-400 m, 400-800 m, 800-1,200 m, 1,200- 1,600 m, and 1,600-2,000+ m to simplify the interpretation of the results due to the large size of the dataset. Then, samples were given a code (Table 1) based on their collection location relating to the bathymetric feature or region they were collected in. The site code is, generally, in location-depth format with the first few letters and numbers indicating the location a specimen was collected i.e. SP = Spain, and the number at the end of the site code indicates the depth category 1-5 with 1 being the shallowest and 5 being the deepest. So, AD3 is a site on Anton Dorhn where samples were collected between 800-1,200 m. Samples from similar depths and locations were aggregated into sites. Each site latitude and longitude is representative of the group of individuals and the center of all specimens collected from that area and depth.

DNA extraction

The salting-out method was chosen for DNA extractions on *Acanella arbuscula*, because it was found to be the best method for extracting high quality DNA based on the results of extraction comparisons in Chapter 2. Using this method, tissue was subjected to 1% SDS digestion, 7.5M ammonium acetate clean-up, isopropanol DNA precipitation, ethanol washing, and rehydration steps as described in Jenkins et al. (2018), modified from Li et al. (2011) as follows: samples were vortexed and placed in the ThermoMixer (Eppendorf) for 4 hours at 65°C at 850 RPM for digestion. Following the initial digestion step, 2 µL of RNase A was placed into each tube and incubated at 37°C for 30 minutes in the ThermoMixer at 300 RPM. Ammonium acetate clean-up, DNA precipitation, and ethanol washing was completed as described in Jenkins (2019). Pelleted DNA was then rehydrated with 50 µL of AE buffer (Qiagen) and left in the refrigerator overnight at 4°C. Resulting DNA concentration was

quantified using a NanoDrop spectrophotometer (Thermo-Fisher). The DNA was then stored at -20°C.

Ultra-Conserved Elements Library Preparation and Sequencing

Ultra-conserved library preparation was conducted following Quattrini et al. (2018) with a few modifications. Whole genomic DNA was fragmented enzymatically by using standardized 500 ng of DNA diluted in 26 μL 1XTE buffer (10 mM Tris pH 8.0, 1 mM EDTA) in 96 well plates with NEBNext® UltraTM II FS Module (E7810) (New England Biolabs) following manufacturer's instructions. Fragmented DNA was visualized on a high throughput precast 96 well 1% agarose SYBR Safe E-Gel (Invitrogen) with a 100 bp ladder (Invitrogen) to ensure that DNA fragment sizes were between 400-800 bp before continuing. The fragmented DNA was then cleaned using a 3X AMPure XP bead (Beckman Coulter) clean up and reconcentrated into 10mM Tris pH 8.0-8.5. The concentration of the reconstituted fragmented DNA product was measured using a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen) in a black top read 384 well opti-plate (Applied Biosystems) on a FLUOstar Omega III plate reader (BMG Lab Tech). Following the quality control step, any fragmented DNA samples within the acceptable range (10-15 ng/μL) were allowed to proceed to adapter ligation. A Kapa Hyper Prep Kit (Roche) was used to ligate iTru oligo adapters (BaDNA, University of Georgia, IDT) onto the fragmented DNA samples per manufacturer's instructions followed by a 0.8X post ligation clean up using AMPure XP beads. Ligated product underwent quality control with PicoGreen in the plate reader as before. Unique combinations of iTru i5 and iTru i7 primers (10 μM) (BaDNA, University of Georgia, IDT) were added to each ligated sample in a 96-well plate and then amplified with 2X KAPA HotStart Ready Mix following the Kapa Hyper Prep Kit instructions. Amplified libraries were then cleaned with 1X AMPure bead clean up and quality control was completed using the QuBit high sensitivity assay.

Amplified libraries were pooled in columns of 8 individuals for a total of 12 pools per plate. Pools of samples were standardized to contain 500 ng of DNA equally divided across all libraries in the pool. Library pools were then concentrated to 7 μL using a GeneVac at room temperature. The pooled DNA libraries were then hybridized in a target enrichment stage using the myBaits Hybridization Capture Kit (Arbor Biosciences) and the octocoral bait set designed by Quattrini et al. (2018). Hybridized and enriched libraries were then cleaned using the streptavidin-coated beads found in the myBAITS kit. The enriched libraries then underwent a final amplification stage using the KAPA Hyper Prep kit with illumina primers and a final 1.8X AMPure bead clean up. The concentrations of the final amplified enriched libraries were measured using a broad range QuBit assay. Pooled libraries were sent to Admera Health (NJ, USA) for HiSeq sequencing.

Bioinformatics Pipeline

Raw reads were cleaned from adapters and low-quality bases using ILLUMINAPROCESSOR v.2.0.9 (Bolger et al., 2014) with TRIMMOMATIC v0.39 (Bolger et al., 2014; Faircloth et al., 2012). The scripts implemented in PHYLUCE v 1.7.1 (Faircloth, 2016) produced a reference assembly of UCE loci for one individual with the highest number of contigs. The contigs were assembled individually using SPADES v3.12.0 (Bankevich et al., 2012). The contigs were aligned to the octocoral probe set (Quattrini et al., 2018) with minimum coverage and identity of 80, while duplicates were removed. From the contigs obtained, the longest contig for each UCE locus was selected as the reference for the SNP calling section.

Sequence reads of each sample were mapped to the reference individual using BWA mem v0.7.17 (Li & Durbin, 2009) and processed with SAMTOOLS v.1.8 (Li et al., 2009). The subsequent filtering was performed with tools implemented in GATK v 4.2.4.1(Van der

Auwera et al., 2013), such as MarkDuplicates and BuildBamIndex. Single nucleotide polymorphisms (SNPs) for each sample were called using HaplotypeCaller in GVCF mode. The COMBINEGVCFS module combined the individual GVCF files, followed by genotyping in the GenotypeGVCFs module. VariantFiltration was implemented for variant quality filtration, and only biallelic SNPs were retained. VCFTOOLS v0.1.16 (Danecek et al., 2011) was implemented to remove minor allele frequency of 0.05 and genotypes ≤ 10x depth of coverage in each individual. We identified outlier SNPs using BAYESCAN v2.1 (Foll & Gaggiotti, 2008) with 10,000 output iterations and 100 prior odds. The final putative neutral SNPs dataset was built with a missing data of 2% consisted of 5,783 SNPs in 362 individuals. Nucleotide diversity for each site was calculated using Arlequin (Excoffier, 2005).

To find the optimal number of genetic clusters, the find.clusters function from the R package adegenet (Jombart, 2008) was used alongside Bayesian information criterion (BIC). The lowest BIC was used to determine that four genetic clusters exist within this dataset. The missing no function in Poppr was used to filter out missing data at the individual and loci level (Kamvar et al., 2014). A discriminant analysis of principle components (DAPC) was completed using 12 principle components determined by the highest a-score with the dapc function in adegenet (Jombart, 2008), and a scatter plot was generated in R using the scatter function from the ggplot2 package (Wickham, 2017).

The programs VCFTOOLS and PLINK were used to generate a bed file from the vcf for input downstream into an admixture ancestry model (Chang et al., 2015; Danecek et al., 2011). A maximum value of K was tested in ADMIXTURE using the number of sampling sites (n+1= K max of 34), and the lowest cross validation error value was used to determine an optimal number of four clusters (K=4) (Alexander & Lange, 2011). The Q file with four clusters was used, and the data was checked for individuals with poorly assigned (<70%) genetic ancestry to one cluster. The Q file was used to visualize ancestry with the plotQ

function in the program POPHELPER (Francis, 2017). Directional relative migration was calculated using Jost's D (Jost, 2008) in the R-package diversity using the function divMigrate with a filter threshold of 0.05 (Keenan et al., 2013; Sundqvist et al., 2016).

Marine protected areas were mapped with the site locations from this study. Marine protected areas were mapped with the program wdpar in R (Hanson, 2022), and sampling locations and cluster assignments were added. Site colors were selected based on the assigned admixture and DAPC cluster for each site.

Results

A total of 2,570,844,736 raw sequence reads were generated. The average number of reads per individual was 6,929,500 with a median length of 151 bp. Spades contigs were assembled with a maximum length of 19,164 bp and a median of 129 bp. The average number of contigs above 1kbp per individual was 1,976 contigs, and the average number of contigs per individual was 186,477. Five samples with less than 5,000 contigs were removed from further analysis. A total of 5,783 SNPs were retained in 362 individuals after filtering which were used for downstream analysis. Nucleotide diversity (π) for each site ranged from 0.210 to 0.267 (Table 1).

Table 1. Aggregated sites region, depth range of samples, and GPS coordinates with their associated bathymetric feature and nucleotide diversity.

Site	Depth Range	Region	Bathymetric Feature	Latitude	Longitude	Diversity
SP3	800-1200	Cantabrian Sea	Avilés Canyon	44.0083	-5.1573	0.225
SP4	1200-1600	Cantabrian Sea	Avilés Canyon	43.9786	-6.483	0.251
CE3	800-1200	Ireland	Whittard Canyon	48.7063	-10.531	0.223
CE5	1600-2000+	Ireland	Whittard Canyon	48.4481	-9.9878	0.239
N0B2	400-800	Labrador	Davis Strait	62.35	-60.86	0.251
N0A2	400-800	Labrador	Davis Strait	66.6992	-57.984	0.242
N0B3	800-1200	Labrador	Davis Strait	62.8507	-58.7848	0.25
N3K3	800-1200	Labrador	Funk Island Bank	51.3483	-49.9383	0.254
N2J2	400-800	Labrador	Hamilton Bank	54.205	-53.0416	0.252
N0B1	0-400	Labrador	Hatton Basin	62.98	-61.32	0.255

N0B4	1200-1600	Labrador	Hatton Basin	62.5364	-59.2211	0.247
N2J3	800-1200	Labrador	Hawke Channel	54.3083	-52.9933	0.256
N2H1	0-400	Labrador	Hopedale Saddle	55.4766	-57.2083	0.253
N2H2	400-800	Labrador	Hopedale Saddle	57.1191	-58.0025	0.252
N2H3	800-1200	Labrador	Hopedale Saddle	55.3516	-54.99	0.255
N2H4	1200-1600	Labrador	Hopedale Saddle	56.8016	-57.9891	0.253
N2G5	1600-2000+	Labrador	Hopedale Saddle	60.4748	-60.3770	0.235
N3M2	400-800	Newfoundland	Flemish Cap	46.92	-46.6933	0.252
N3O1	0-400	Newfoundland	Grand Bank	44.2375	-52.0075	0.252
N3N1	0-400	Newfoundland	Grand Bank	43.055	-50.9783	0.253
N3O2	400-800	Newfoundland	Grand Bank	44.1133	-52.0058	0.252
N3N2	400-800	Newfoundland	Grand Bank	43.2066	-51.5433	0.252
N3O3	800-1200	Newfoundland	Grand Bank	43.145	-51.6016	0.251
N3N3	800-1200	Newfoundland	Grand Bank	47.3816	-46.9466	0.255
N3N4	1200-1600	Newfoundland	Grand Bank	42.95	-48.9975	0.25
N3P1	0-400	Newfoundland	Laurentian Channel	44.8783	-56.0083	0.253
N3P2	400-800	Newfoundland	Laurentian Channel	45.0266	-54.645	0.267
NU3	800-1200	Nuuk	Nuuk	63.8669	-53.2869	0.254
AD2	400-800	Scotland	Anton Dohrn	57.6059	-11.2342	0.212
AD3	800-1200	Scotland	Anton Dohrn	57.6121	-11.2285	0.211
GBB2	400-800	Scotland	George Bligh Bank	58.7204	-13.8402	0.21
NRB4	1200-1600	Scotland	North Rockall Bank	58.3876	-13.5517	0.255
RB3	800-1200	Scotland	Rosemary Bank	59.0818	-10.4515	0.211

Discriminant analysis of principle components and population clustering

An initial principle components analysis (PCA) was performed which showed four clusters with clusters two and four partially overlapping (supplementary material). Four major clusters emerged from the discriminant analysis of principle components (DAPC) of filtered SNPs (Figure 3) with an optimal 58 principal components and 31 discriminant functions. The proportion of conserved variance was 37.2%. Shallower samples from 862 m in The Cantabrian Sea, Spain, formed a single cluster- cluster one. While deeper samples from 1050m and 1510 m in the same geographical location formed a cluster with deeper (1690-2111m) samples from Whittard Canyon in the Celtic Sea, near the Newfoundland and Labrador cluster- cluster two. Newfoundland, Labrador, and West Greenland samples formed one large cluster which

included all depths in those locations with deeper (1205-1210m) West of Scotland MPA samples- cluster four. Shallower (984-1154m) Celtic Sea samples clustered with shallower (763-1115m) samples from the West of Scotland MPA- cluster three. A similar DAPC was run on the same dataset with depth as prior as opposed to sampling location to test the effect of depth on the clustering of sites. The results of clustering were like those found in figure 3 (supplementary figure 2).

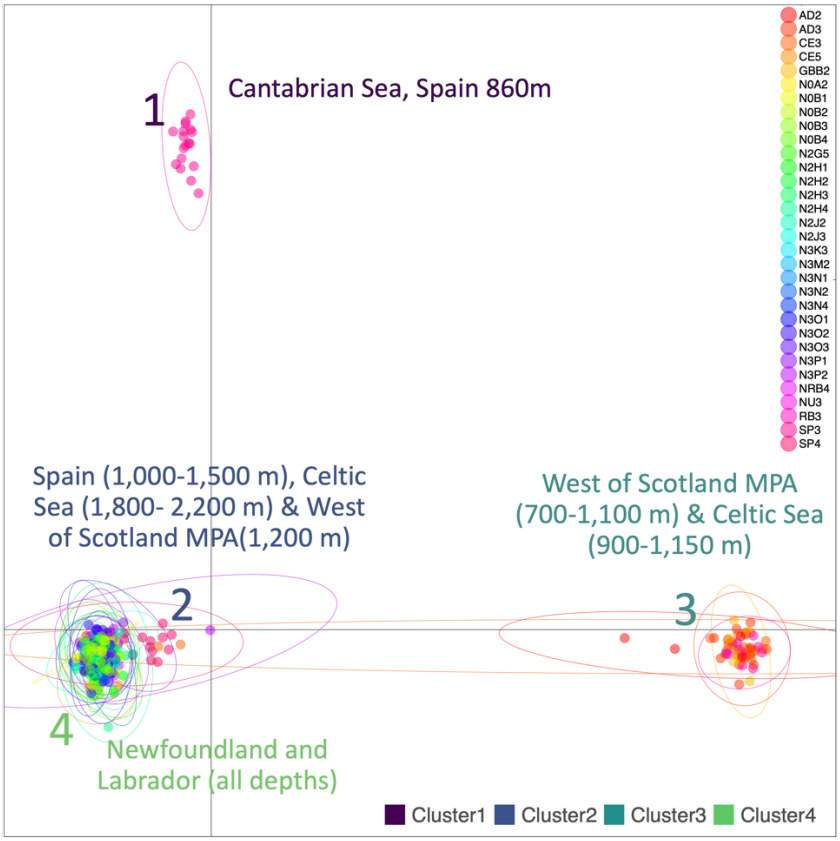


Figure 3. Plot of the discriminant analysis of principle components (K=4) for *A. arbuscula* showing four major clusters. Each colored circle indicates a sampling location. The codes for each sampling location can be found in Table 1. Cluster colors correspond to admixture plot and migration analysis throughout. Depths in cluster descriptions are generalized for ease of reading.

Population Structure

Following the population structure analysis using the program admixture, four clusters emerged across the study (Figure 4). The clustering of populations followed a similar pattern

to the results of the DAPC plot. All of Newfoundland, Labrador, and Greenland formed a distinct cluster with the deepest West of Scotland MPA from North Rockall Bank and deepest Whittard Canyon, Celtic Sea samples (Figure 4, cluster 4). There were three samples with a small amount of introgression from the shallower sites in the deep sites, indicated by small amounts of teal. Shallower Whittard Canyon, Celtic Sea samples clustered with shallower West of Scotland MPA samples from Anton Dorhn, Rosemary Bank, and George Bligh Bank (Figure 4, cluster 3). Within the shallower Celtic Sea site, one individual clustered with the Deeper Spanish site (cluster 2) and one clustered with the deeper Celtic Sea and Newfoundland sites (cluster 4). Whilst the shallower samples from the Cantabrian Sea formed an isolated population that appears to be presented in few individuals across the other sampling sites (Figure 4, cluster 1), the most notorious introgression of this cluster was identified in some specimens collected in North Rockall Bank, Scotland (1204-1208 m). The deeper Cantabrian Sea samples formed two distinct clusters despite all samples coming from the same short trawl event - one cluster appears to be distinct (Figure 4, cluster 2) while the other portion of the sampling location belongs to the same cluster as all of the Newfoundland, Labrador, and Greenland samples as well as the deepest West of Scotland MPA samples (Figure 4 lime green, cluster 4).

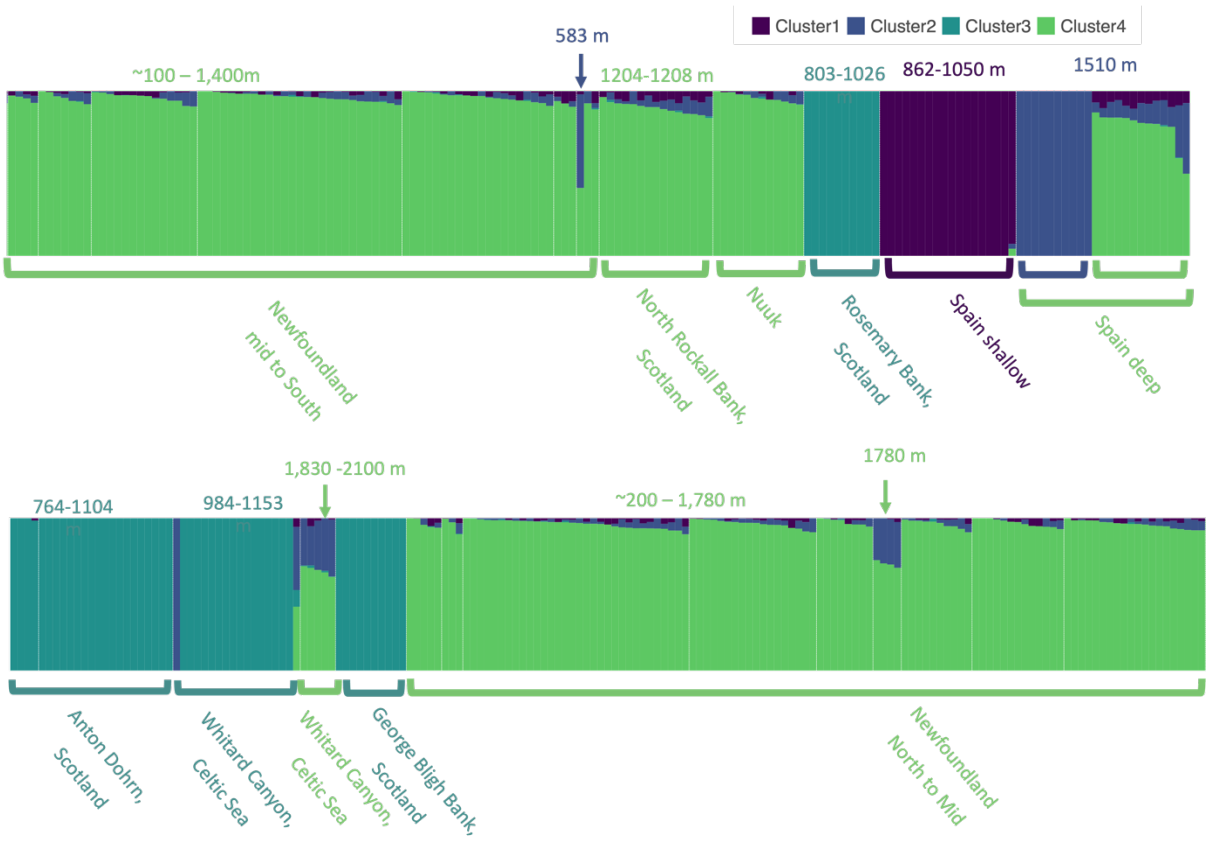


Figure 4. Admixture plot of population clusters (k=4). Each bar represents a single individual and solid white lines indicate separate collection sites. The two plots are halves of the larger plot for ease of readability and understanding. Color indicates cluster assignment.

Contemporary Migration

The results of the divMigrate analysis revealed directions of geneflow between populations in the study using a migration network (Figure 5). Bidirectional migration between the shallower WSMPA sites to the shallower Celtic Sea site was uncovered. Here, Whittard Canyon samples from 900-1,150 m are well connected to samples between 700-1,100 m in Anton Dorhn, Rosemary Bank, and George Bligh Bank while showing negligible connectivity to the rest of the study sites and even appear to be isolated from just slightly deeper sampling locations, 1,200 m in North Rockall Bank and 1,800-2,200 m in Whittard Canyon, in the same area; this suggests little genetic mixing over a relatively small range of depth, a few 100 metres.

Most sites in Newfoundland and Labrador clustered together with two sites, Hopedale Saddle Labrador samples at 1,800 m and Laurentian Channel samples from 150-250 m

appearing to be isolated away from the rest of the sites in the study, with relatively little geneflow to these populations. These two sites do show low to median levels of geneflow (D=0.28-0.39) to the Newfoundland and Labrador cluster as well as the deeper site 100-1,500 m from Cantabrian Sea, Spain. The Hopedale Saddle Site has a low level of gene flow (D=0.26) to the deepest site in the WSMPA, North Rockall Bank. This suggests that deeper populations are connected across the entire Atlantic Ocean. Further supporting this point, the deepest WSMPA site, North Rockall Bank is well connected as a source (D=0.84) and sink (D=0.63) to the large Newfoundland and Labrador cluster spanning all depth ranges in the Western Atlantic.

In sites from the Cantabrian Sea, Spain, shallower samples collected at 860 m show median geneflow (D=0.45) to the Newfoundland Labrador cluster. This shallow Spain site also has weak geneflow (D=0.34) to the deep North Rockall Bank site. Meanwhile, the deeper samples from Spain show higher levels of geneflow to (D=0.70) and from (D=0.65) the Newfoundland and Labrador cluster than they do to shallower Spanish samples (D=0.30). The deepest Spanish samples are also more well-connected to deeper samples from Whittard Canyon (D=0.38) than they are to the shallow Spanish samples.

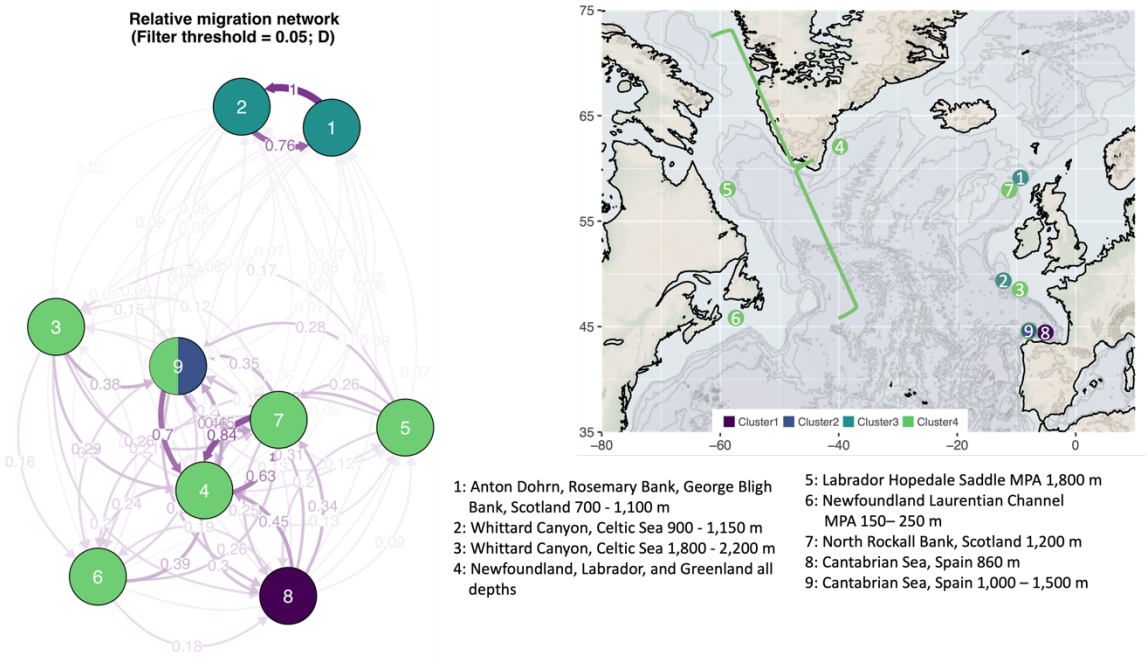


Figure 5. Relative migration network of nine aggregated sampling locations across the study area with a threshold of D=0.05 to show weak patterns of connectivity and geneflow. Purple arrows represent relative geneflow with the darker arrows representing stronger connections and lighter arrows representing weaker connections. The number on each arrow shows the relative migration rate (D). Directionality of arrows represents direction of geneflow from one site to another. Colors of sites represent the colors of the clusters in the DAPC and Admixture plot for consistency and ease of understanding.

Sites and Marine Protected Areas

The Whittard Canyon (CE3 and CE5) area and much of the slope in the Celtic Sea are not part of a MPAn (Figure 6). Other Areas that lack protection include some regions in Newfoundland (N3P1-2, N3N1-4, N3O1-3) and Labrador (N0B1-4) as well as Greenland (NU3). Although no samples of *Acanella arbuscula* were obtained from Iceland or Eastern Greenland, it is documented that they do occur here (Buhl-Mortensen et al., 2015), and these areas lack MPA zones. All corals collected from inside an MPA were done so with research collection permits. No accusations of illegal fishing in a MPA are made based on this map.

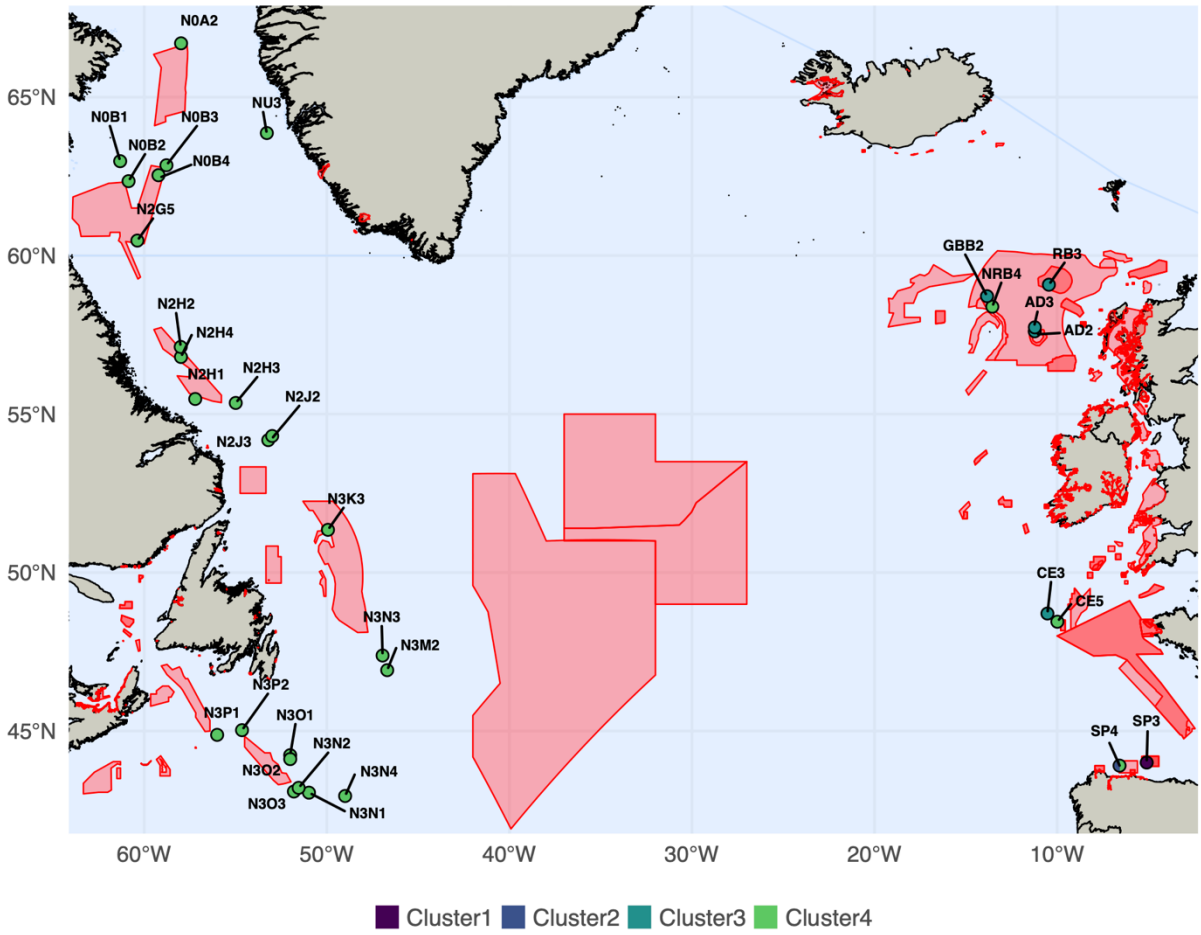


Figure 6. Map of MPAs in relation to aggregated sample site locations with site identification codes. Site color corresponds to the assigned admixture cluster. Red areas indicate known marine protected areas in the North Atlantic Ocean. Sampling site locations are representative only and are the geographical center of specimens collected at a certain depth and location. They may not represent an actual individual's exact location. No accusations of illegal fishing in a MPA are made based on this map.

Discussion

This project has again demonstrated the usefulness of UCE sequencing for population genomics and connectivity studies (Erickson et al., 2021; Petersen et al., 2022; Stiller et al., 2021). In the deep-sea, where sampling events are rare and samples are sometimes poorly preserved, making the most of every sample is essential to large geographical genetics studies. UCEs have great potential to make previously unusable samples available to researchers for

genomics projects previously thought impossible due to the protocol's utility of poor quality DNA samples. Currently only one deep-sea coral population genomics study exists using UCEs; however, it is on a much smaller scale and mostly focuses on phylogenomics (Erickson et al., 2021). Here, we were able to generate 5,783 neutral SNPs in 362 individuals across samples from a wide range of preservation methods including from fisheries observer samples that had been untangled from nets, frozen, and exposed to several freeze thaw cycles over a period of nearly 20 years. This alone is a substantial accomplishment for deep-sea genomics, and we hope that these sequencing methods and the DNA extraction techniques from the previous chapter can be utilized in future deep ecosystem species.

In addition to generating ample SNPs for genomics analysis, this study also has uncovered trans-Atlantic connectivity in *Acanella arbuscula* and complex levels of local connectivity among investigated sites. The results of our study support the depth differentiation hypothesis, because genetic breaks between populations at different depths were uncovered in several locations. On the seamounts in the WSMPA, samples collected from Anton Dorhn, Rosemary Bank, and George Bligh bank between 700-1,100 m were genetically isolated from North Rockall Bank samples at 1,200 m. This suggests that there is a barrier to geneflow between 1,100 and 1,200 m in samples collected in the East Atlantic. The addition of deeper samples from Anton Dorhn, Rosemary Bank, and George Bligh bank as well as shallower samples from North Rockall Bank could further support this evidence of a genetic break. However, no samples from those depths were available for this study. There is some evidence of introgression across sites in WSMPA which may suggest there is a small amount of geneflow between shallow and deep sites. This small amount of connectivity could classify the Whittard Canyon deep site as a refugia which provides recruits to the shallow site. Environmental conditions such as temperature or salinity may cause this genetic break between sites as New and Smythe-Wright (2001) observed a thermocline between 700-1,600 m and a halocline

between 400-1,600 m in Rockall Trough. Their observations of Labrador Sea water entering the trough may also explain why North Rockall Bank samples were so well connected with all of the Newfoundland and Labrador samples (New, 2001).

In a study conducted by Lozier et al. (2008), Mediterranean outflow water that moves northward through Rockall Trough had salinity anomalies which suggest that during high levels of North Atlantic oscillations Mediterranean outflow water is prevented from entering the subpolar gyre. When North Atlantic oscillations are low, the "salt tongue", the warmer, more saline outflow from the Mediterranean, travels past Rockall Trough and enters the subpolar gyre (Lozier, 2008). This transport of water from the Mediterranean which travels past Spain, the Celtic Sea, and into the WSMPA where it then enters the subpolar gyre (Copard et al., 2011) could help explain some of the connectivity detected between deeper sites in the east Atlantic. This "salt tongue" from the Mediterranean enters the North Atlantic between 600-1,500 m (Baringer, 1999) and was more recently discovered to have a top barrier between 800-900 m and a bottom layer between 1,100-1,200 m (Copard et al., 2011). It is characterized by being highly saline (36.9 ppt) and warm (13.22-13.76 °C) (Copard et al., 2011) which may produce a temperature and salinity barrier to geneflow between the deep (1,000-1,500 m) and shallow (850 m) Spanish samples.

Interestingly, there appeared to be very little depth differentiation in the sites sampled in Newfoundland and Labrador. Apart from the deepest population at 1,800 m in Hopedale Saddle and the shallowest population between 150-250 m in the mouth of the Laurentian Channel, Newfoundland and Labrador samples were well connected across all sites and depths sampled, along with the site from Nuuk, Greenland. Pickart (2001) found substantial depth layer mixing via deep convection in the Labrador Sea as deep as 1,500 m. This could explain why samples across such a large depth range were so well connected in Newfoundland and Labrador in this study.

The complex current system in the North Atlantic (Figure 6. from Buhl-Mortensen et al., 2015) allows distant populations to be connected across large distances. It is believed that isolated populations, such as shallow Spanish sites and shallow Celtic Sea and WSMPA sites in this study, are isolated not by distance but by resistance, because some environmental characteristics are creating barriers for geneflow such as the thermoclines and haloclines found in the Eastern Atlantic. In the next chapter, the environmental drivers of adaptation in these same *Acanella arbuscula* populations were investigated. Additionally, it is likely that there are unsampled stepping-stones to connectivity along Southern Greenland, Iceland, and the Mid Atlantic Ridge which could aid in the dispersal of *A. arbuscula* across the North Atlantic.

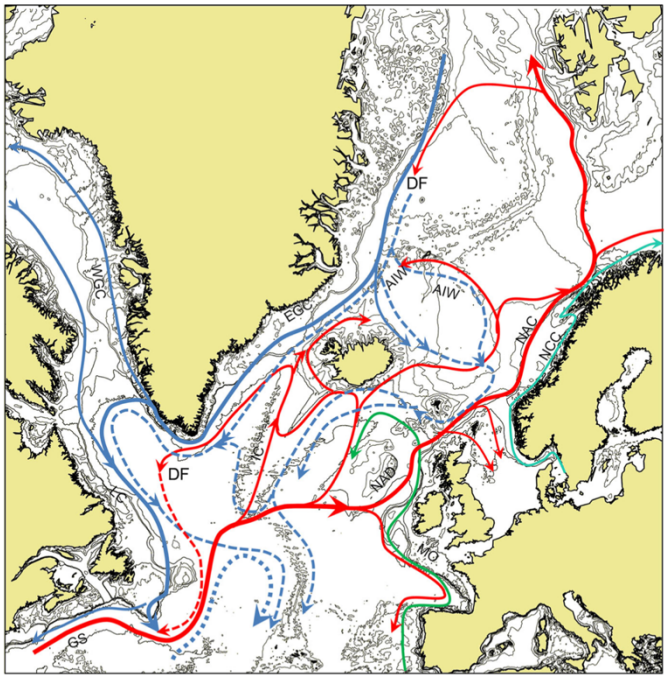


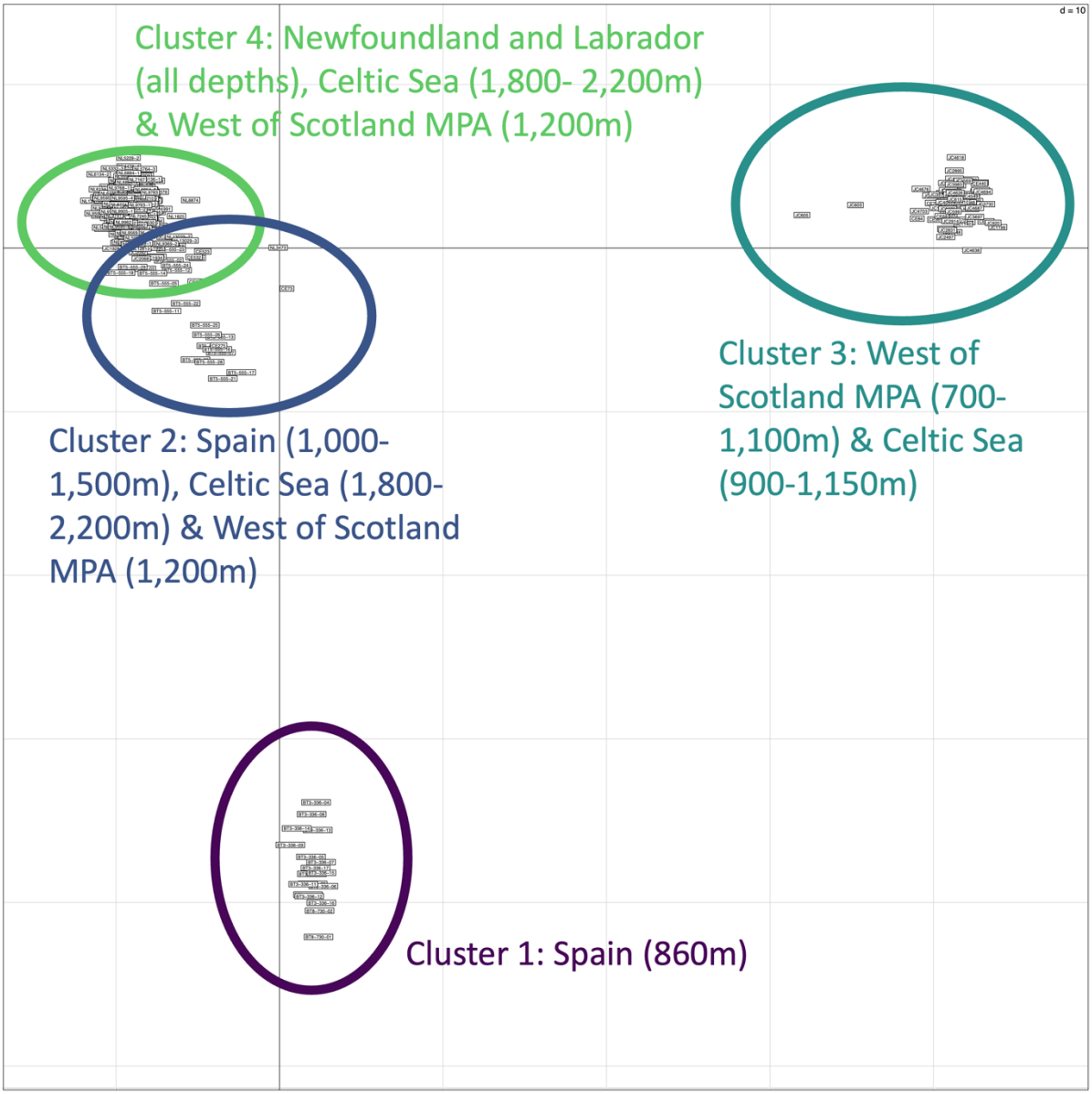
Figure 6. (Buhl-Mortensen et al., 2015) “General current patterns in the North Atlantic area relevant to this study. Red lines are warm surface currents, whereas blue lines are cold deep currents. DF deep-water formation, AIW Arctic intermediate water, NAC North Atlantic current, NAD North Atlantic drift, IC Irminger current, NCC Norwegian coastal current, EGC East Greenland current, WGC West Greenland current, LC Labrador Current, GS Gulf Stream, MO Mediterranean outflow “

One further explanation for the perceived isolation of certain populations could lie in the method of SNP generation. UCEs focus on amplifying conserved regions of the genome

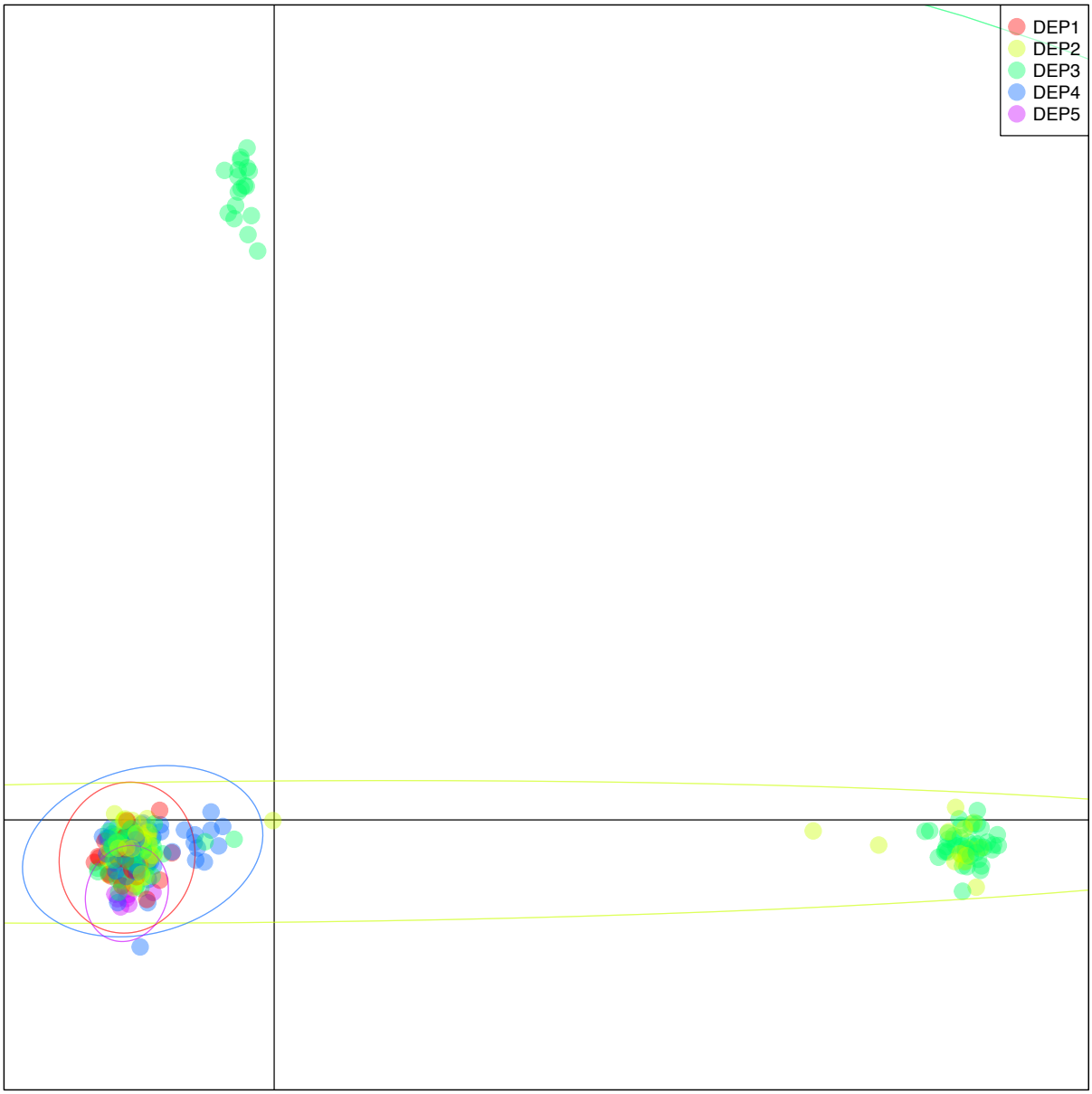
which by definition contain fewer varied regions of DNA. This may lead to an ascertainment bias which selects for regions of DNA which would not be varied enough for use in species delimitation, or population genomics. Such a bias could lead us to incorrectly assume that isolated populations are the same species – they could be cryptic species. To preemptively combat this issue, all coral samples used in this study were closely examined to ensure that they were all the same species, morphologically, however given the poor preservation of soft tissue (which is not often used in taxonomic identification of octocorals) perhaps cryptic speciation is possible as several species from the genus *Acanella* and other Isididae have similar gross morphology (Saucier et al., 2017). To further investigate the potential of cryptic species within this data set, a phylogenetic tree was created of the UCE SNPs to visualize the relationship between all samples (see supplementary figure 3). Because the major nodes separating clusters are not supported with a Bayesian posterior probability of 95 or higher (Nascimento et al., 2017), this tree is unlikely to present cryptic speciation; however, it still may exist, and our data may not be varied enough to detect cryptic species. Scanning electron microscopy will be conducted of the sclerites from several colonies from each population in the future to further support the species identification, and we are investigating using UCEs to create whole mitochondrial genomes using Mitofinder (Allio et al., 2020) of the genetic clusters to see if there are substantial differences. Time and funding were not permitting to complete this during the current study.

In previous studies assessing the connectivity of deep-sea sponges across the Northeast Atlantic, Taboada et al. (2022) found nucleotide diversities of 0.118 to 0.179. Off the Antarctic Peninsula Leiva et al. (2019) found even lower nucleotide diversities of 0.052 to 0.079 in deep-sea sponges. This study found high levels of nucleotide diversity, between 0.210 and 0.267 across all sites. This may suggest high resilience to disturbances.

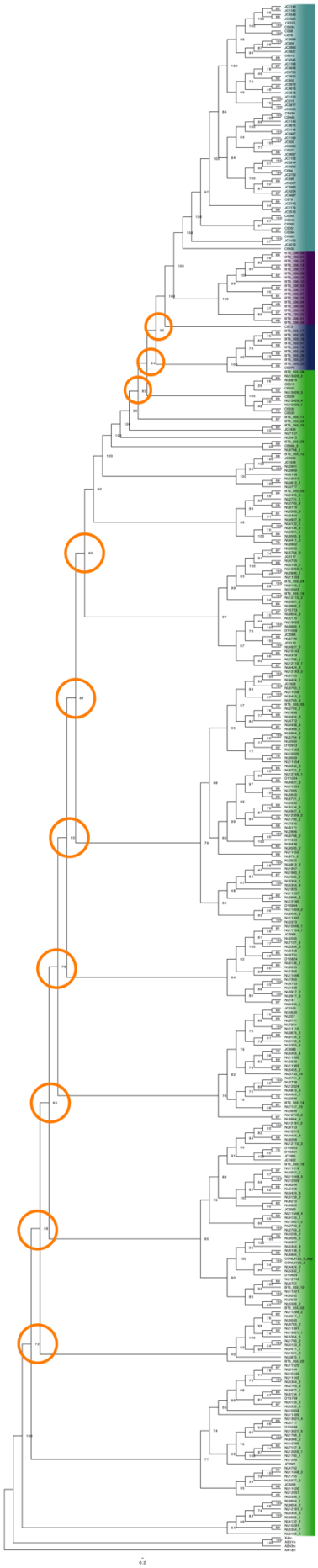
In isolated or poorly connected populations, it is recommended that these sites be monitored and protected as they may have less access to recolonizing larvae to restore damaged habitats, or may be home to novel, cryptic species of *Acanella*. They may also contain unique genetic adaptations which once destroyed would be lost from the gene pool. For example, Whittard Canyon is exposed to heavy trawling activity which disrupts the sediments *A. arbuscula* rely on for habitat (Daly et al., 2018), and this is one location where a shallower population is mostly isolated from all other parts of the study except the shallow WSMPA sites, and the shallow sites in Europe have the lowest nucleotide diversity of all the sites in this study. Some introgression and shared genetic similarity to shallower sites in the deeper Whittard Canyon site may be evidence of minimal connectivity between the shallow and deep sites; however, it is not clear whether that is enough for the deeper area to act as a refugia. Likewise, the shallow Laurentian Channel population and deep Hopedale Saddle populations are poorly connected to the rest of Newfoundland and Labrador. If these populations were damaged by fishing gear, there would be very limited larvae from the surrounding areas to help damaged areas recover. For this reason, these are areas of conservation concern that should be monitored and considered for further protection. It is hoped that this work will help conservation management teams make informed decisions on protection measures looking to incorporate genetic connectivity into MPAn in the North Atlantic region. In the future, the combination of larval distribution models and genetic connectivity studies may help conservation efforts by providing the much-needed link between water current movement and genomics to inform a connectivity network plan.



Supplementary figure 1. An initial principle components analysis (PCA) (K=4) for *A. arbuscula* showing four major clusters. Each box indicates a single individual. The first letters of an individual's unique identification indicate where they were collected- Spain (BT), Greenland (DY), Ireland (CE) Scotland (JC), Newfoundland and Labrador (NL). Cluster colors correspond to admixture plot and migration analysis throughout. Depths in cluster descriptions are generalized for ease of reading.



Supplementary figure 2. DAPC of *Acanella arbuscula* sites with depth as a prior. Samples were divided into five depth categories: 0-400 m (DEP1, red), 400-800 m (DEP2, yellow), 800-1,200 m (DEP3, green), 1,200- 1,600 m (DEP4, blue), and 1,600-2,000+ m (DEP5, purple). Four clusters emerged like the DAPC with location as a prior.



Supplementary figure 3 Consensus cladogram with Bayesian posterior probability (BPP) generated from all SNPs. Tree made of 10,000 iterations. Tip labels correspond to unique sample ID names. Tip colors indicate which genetic cluster each sample belongs to. Orange circles represent major nodes which are not supported by BPP > 95.

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Chapter 4: Potential temperature driven adaptation in deep-sea octocoral *Acanella arbuscula*

Abstract

Cold water coral reefs and gardens constitute vulnerable marine ecosystems. They are under threat from climate change, deep-sea mining, and destruction by fishing gear. Across the North Atlantic Ocean, the delicate deep-sea octocoral *Acanella arbuscula* is exposed to heavy fishing activity and potentially oceanic warming. Recovery of these damaged ecosystems is reliant on the recruitment of larvae from surrounding colonies. However, environment mismatches may hinder connectivity between populations experiencing different environmental pressures. In this study, single nucleotide polymorphisms generated from ultra-conserved elements sequencing in combination with seascape genomics are used to test for local adaptation to environmental variables in 362 colonies of *A. arbuscula* across Atlantic Canada, Greenland, Scotland, Ireland, and Spain. Twelve genomic markers were found to have significant environment allele associations. All significant associations were related to local seafloor temperature. General linear models were produced on the genotype environment associations to predict genotypic frequency across the study region for each investigated marker. We make the case for the usefulness of seascape genomic and connectivity data to be used to inform marine protected area (MPA) design to help connect existing MPAs to stepping-stones with similar environmental conditions to allow for the recruitment of larvae adapted to a similar set of conditions.

Introduction

Cold water reefs and gardens are designated as vulnerable marine ecosystems (VMEs) by the UNGA (Weaver, 2011) and are susceptible to damage from bottom contact fishing gear, oil and gas exploration, deep-sea mining, and other anthropogenic effects such as climate change (Gass, 2005; Jones et al., 2018; Watling & Auster, 2017). Deep-sea corals are slow-

growing, long-lived (Sherwood & Edinger, 2009), and likely slow to recover from disturbances, with those few studied often not recovering at all within observable decadal timescales (Althaus et al., 2009; Clark et al., 2019; Goode et al., 2020). Deep-sea corals have even been observed to ingest microplastic fibers (Taylor et al., 2016) which can reduce energy acquisition and change energy allocation, further threatening their resilience to disturbances (Chapron et al., 2018).

The deep-sea octocoral *Acanella arbuscula* (Isididae) Johnson 1862, is found in VMEs in the North Atlantic Ocean where their shallow root-like holdfasts anchor them in soft sediments and occasionally on drop stones or rocky out-croppings (Buhl-Mortensen et al., 2015). In large fields of *A. arbuscula*, other octocorals such as sea pens (*Pennatula* spp.) and *Umbellula* spp. grow in the soft sediment forming mixed gardens of corals. Many benthic invertebrates rely on *A. arbuscula* as habitat (Bronsdon et al., 1993; Hamel et al., 2015; Moyse, 1983; Pearson & Gage, 1984; Westerman et al., 2021) making them an essential part of the ecosystem. The commercially important and over-exploited redfish (*Sebastes* sp.) are often found in these coral fields throughout Newfoundland and Labrador in association with *A. arbuscula* (Baker et al., 2012), and the redfish larvae have been found taking refuge tucked in among octocoral polyps (Baillon et al., 2012).

These vulnerable coral reef ecosystems are complex habitats that are under threat from anthropogenic activities (Jones et al., 2018; Watling & Auster, 2017). Recovery of damaged reefs depends on the availability of larvae from surrounding areas. However, habitat mismatches and local adaptation may hinder the ability of recently settled larvae to thrive in previously disturbed areas. Genetic connectivity of marine populations may be fragmented by variations in environmental conditions such as salinity, currents, or temperature (Benestan et

al., 2016). Understanding gene flow and barriers to connectivity can better inform design of marine protected areas (MPAs) and specific management actions to ensure that VMEs will recover from disturbances with recruits from adjoining populations (Cowen & Sponaugle, 2009; Jenkins & Stevens, 2018; Leiva et al., 2022; Palumbi, 2003; Taboada et al., 2022).

One method that is used to understand connectivity of marine organisms is seascape genomics - derived from landscape genomics, which first introduced the idea of using genetic data alongside environmental data and landscape ecology to understand geneflow patterns and local adaptation within populations (Manel et al., 2003). However, instead of looking at terrestrial features that influence population structure such as rainfall, altitude, or air temperature, researchers can apply the same ideas to aquatic environments by investigating the influence of water chemistry, temperature, or depth on marine populations (Benestan et al., 2016; Selmoni et al., 2020). These methods will then aid our understanding of which processes lead to the development of genetic patterns and if there are adaptations to environmental conditions; such adaptation may be useful in our warming ocean. (Selkoe et al., 2016; Selmoni et al., 2021).

The potential to investigate the genetic adaptation of non-model organisms to environmental conditions has been improved by the advancement of sequencing technology (Savolainen et al., 2013; Willette et al., 2014). This chapter uses the single nucleotide polymorphisms (SNPs) generated from ultra-conserved elements (UCE) sequencing (Erickson et al., 2021; Faircloth et al., 2012; Quattrini et al., 2018), analyzed from the previous chapter, in seascape genomics to predict which *A. arbuscula* markers are associated with various environmental conditions across the North Atlantic. Does *A. arbuscula* show evidence of local adaptation?

Materials and Methods

A total of 362 *A. arbuscula* were grouped by similarity of location and depth into 33 aggregated collection sites (Table 1) with a minimum sample size of three individuals across Labrador and Newfoundland (n=253), Greenland (n=12), The West of Scotland Marine Protected Area (WSMPA) (n=58), the Celtic Sea (n=23), and the Cantabrian Sea (n=40). Samples were collected opportunistically from fisheries bycatch, from targeted expeditions using remotely operated vehicles, and from small agassiz trawl surveys. All samples were examined for morphological characteristics matching the original description and stored long term in 100% ethanol at -20°C.

Table 1. Aggregated sites region, depth range of samples, and GPS coordinates with their associated bathymetric feature

Site	Depth Range	Region	Bathymetric Feature	Latitude	Longitude
SP3	800-1200	Cantabrian Sea	Avilés Canyon	44.0083	-5.1573
SP4	1200-1600	Cantabrian Sea	Avilés Canyon	43.9786	-6.483
CE3	800-1200	Ireland	Whittard Canyon	48.7063	-10.531
CE5	1600-2000+	Ireland	Whittard Canyon	48.4481	-9.9878
N0B2	400-800	Labrador	Davis Strait	62.35	-60.86
N0A2	400-800	Labrador	Davis Strait	66.6992	-57.984
N0B3	800-1200	Labrador	Davis Strait	62.8507	-58.7848
N3K3	800-1200	Labrador	Funk Island Bank	51.3483	-49.9383
N2J2	400-800	Labrador	Hamilton Bank	54.205	-53.0416
N0B1	0-400	Labrador	Hatton Basin	62.98	-61.32
N0B4	1200-1600	Labrador	Hatton Basin	62.5364	-59.2211
N2J3	800-1200	Labrador	Hawke Channel	54.3083	-52.9933
N2H1	0-400	Labrador	Hopedale Saddle	55.4766	-57.2083
N2H2	400-800	Labrador	Hopedale Saddle	57.1191	-58.0025
N2H3	800-1200	Labrador	Hopedale Saddle	55.3516	-54.99
N2H4	1200-1600	Labrador	Hopedale Saddle	56.8016	-57.9891
N2G5	1600-2000+	Labrador	Hopedale Saddle	60.4748	-60.3770
N3M2	400-800	Newfoundland	Flemish Cap	46.92	-46.6933
N3O1	0-400	Newfoundland	Grand Banks	44.2375	-52.0075
N3N1	0-400	Newfoundland	Grand Banks	43.055	-50.9783
N3O2	400-800	Newfoundland	Grand Banks	44.1133	-52.0058
N3N2	400-800	Newfoundland	Grand Banks	43.2066	-51.5433

N3O3	800-1200	Newfoundland	Grand Banks	43.145	-51.6016
N3N3	800-1200	Newfoundland	Grand Banks	47.3816	-46.9466
N3N4	1200-1600	Newfoundland	Grand Banks	42.95	-48.9975
N3P1	0-400	Newfoundland	Laurentian Channel	44.8783	-56.0083
N3P2	400-800	Newfoundland	Laurentian Channel	45.0266	-54.645
NU3	800-1200	Nuuk	Nuuk	63.8669	-53.2869
AD2	400-800	Scotland	Anton Dohrn	57.6059	-11.2342
AD3	800-1200	Scotland	Anton Dohrn	57.6121	-11.2285
GBB2	400-800	Scotland	George Bligh Bank	58.7204	-13.8402
NRB4	1200-1600	Scotland	North Rockall Bank	58.3876	-13.5517
RB3	800-1200	Scotland	Rosemary Bank	59.0818	-10.4515

Specimen DNA was extracted using the salting-out protocol described extensively in Chapter 2. UCE libraries were then prepared with enzymatically fragmented whole genomic DNA ligated to unique iTru primer barcodes which were amplified, hybridized, and target enriched with the octocoral bait set (Quattrini et al., 2018). The pipeline for the program Phyluce was followed to de-multiplex individuals, clean raw read data, create assemblies, extract UCE loci, align and trim the data, and prepare UCE contigs (Faircloth, 2016). The individual with the most contigs was used as a reference for a Burrows- Wheeler Aligner (Li & Durbin, 2009). Then SAMtools was used to create a sequence alignment map (Li et al., 2009). The genome analysis tool kit was used to mark duplicates and build a binary alignment map (BAM) index for each individual. Then HaplotypeCaller was used to create variant call format (VCF) files from each BAM, combine the files into a single VCF, remove indels, filter variants, and keep only biallelic SNPs (McKenna et al., 2010). The program VCFtools was used to remove variants with a depth of coverage less than 10 and a minor allele frequency less than 0.05. Only the SNPs present in at least 1% of the samples were kept (Danecek et al., 2011). For further detail of this process see Chapter 3. After filtering, 354 individuals with a total of 6,422 and 12,844 alleles were used and processed into a genotype matrix accounting for each SNP being absent, occurring as a single copy or two copies for each loci.

Seven environmental variables along the seafloor were examined in this study: seafloor temperature (SFT) as a proxy for depth, salinity, mass concentration of chlorophyll, dissolved oxygen, nitrate, phosphate, and pH. Net primary productivity was excluded from analysis as the database did not have values for a majority of our study areas at depth. To represent available food sources, we have used mass concentration of chlorophyll in sea water. Environmental data was retrieved from Copernicus <http://marine.coperincus.eu/> between longitudes -65 W, 5 E and latitudes 70 N, and 35 S. The depth range for the data downloaded was -60 m to -2300 m to include all the collection sites in this study. Potential seafloor temperature data was only available from January 2020, so all data was analyzed from Jan 2020 to Jan 2022.

The data for each variable was extracted from the deepest available point across the N. Atlantic, averaged across the two years, and rasterized. Additionally, the mean and standard deviation of SFT for each month of the year was calculated. Mean and standard deviation values for the hottest (SFT_hM; SFT_hSD) and coldest (SFT_cM; SFT_cSD) month were extracted and added to the environmental variables matrix to represent the temperature extremes that these corals endure. This led to a total of eleven factors that were tested against the genotype matrix using the program SamBada to search for genetic variants potentially associated with an environmental gradient. Multiple logistic regression models were run in SamBada to compare the likelihood of each model with and without an environmental variable and test whether the model is improved with the addition of an environmental condition. This generated a G-score for each genotype and environmental association. Multiple logistic regression models were also run in SamBada to test whether the effect of the environmental variable was different from zero and whether an environmental variable was associated with a

significant change in genotype frequencies. This generated a Wald-score for each environment and marker association (Duruz et al., 2019).

A chi-square test was completed on all G-scores and Wald-scores to calculate the p-value for each. A q-value multiple test correction was performed on the p-values to test for positive false discovery rate (Storey, 2003). The q-value scores were sorted from lowest to highest and values under 0.05 were considered significant. For marker/environmental variable relationships considered significant due to q-values, a general linear model (GLM) was preformed and graphed to model the relationship between the measured environmental characteristic and allele frequency. Then, allele frequency of a given marker was plotted on a raster map of the associated significant environmental variable across the study area.

The GLM generated from significant loci and environmental characteristics were then used to model predicted allele frequencies across the study site in relation to known environmental conditions. Hundreds of thousands of points were automatically generated every 10 km across the study area, of these points a subsample of 85,424 points were chosen systematically every 10 km for only areas between 60 to 2,300 meters in depth. Each point was used to represent a potential location of *A. arbuscula*. Whether a location had appropriate substrate for the corals to live was not considered due to lack of availability of high enough resolution data for seafloor substrate across the entire study area. All points were fed into the GLMs generated for the significant environment genotype associations. Potential genotype frequency was calculated for all points based on known frequencies. Potential frequencies for each significant association were plotted on maps of the study area using color scales to represent genotypic frequency of the marker based on the known SFT.

Results

Genotype Markers and Environment Interactions

The program SamBada completed 19,137 iterations of tests across 6,379 SNPs (including SNPs under selection that were not included in the analysis in the previous chapter which used only neutral SNPs). Using SamBada allows researchers to see effects of adaptation to the environmental factors investigated rather than confounding effects of neutral genetic patterns. This program also identifies colinear environmental variables and removes them to keep only independent factors. The output uncovered twelve markers with a significant (qG & qW = $p < 0.05$) relationship to an environmental factor. All twelve of the markers were associated with seafloor temperature (SFT) during the hottest month (hM), coldest month (cM), or overall mean SFT (OM) (Table 2). To investigate what other marker environment associations may be on the cusp of significant associations, the threshold for qW was lowered while still maintaining a significant qG value and both p values until four more environment/marker associations were uncovered. These included two markers associated with phosphate concentration overall mean (PO_OM), one marker with dissolved oxygen concentration overall mean (DOX_OM), and one marker with salinity overall mean (SAL_OM) (Table 2). The GLM for each of the environmental conditions and associated allele frequencies, the plots of PO_OM, DOX_OM, and SAL_OM can all be found in the appendix. Because the relationship of each phosphate, oxygen, and salinity with genotype markers were not found to be significant, their plots were not included in further analysis of this study.

Table 2. UCEs with significant environmental associations. UCE markers labelled with (*) were used to create the maps of individual marker allele frequency against the associated environmental gradient of SFT. Bolded p and q values are significant (<0.05).

Marker	Env	pG	pW	qG	qW
uce-1112674_NL9585_639_2	SFT_hM	**4.00E-08**	**4.61E-07**	**3.73E-04**	**8.58 E-03**
uce-990291850_NL9585_674_2*	SFT_hM	**3.50E-06**	**1.50E-05**	**7.65E-03**	**3.78E-02**
uce-2232_NL9585_871_0	SFT_OM	**2.97E-07**	**6.50E-06**	**1.38E-03**	**3.93E-02**
uce-13703_NL9585_633_2*	SFT_OM	**3.06E-06**	**1.04E-05**	**6.98E-03**	**3.93E-02**
uce-16953_NL9585_551_2	SFT_OM	**3.75E-06**	**1.27E-05**	**6.98E-03**	**3.93E-02**
uce-5132_NL9585_809_0*	SFT_cM	**4.31E-10**	**1.80E-05**	**8.02E-06**	**4.78E-02**
uce-7468_NL9585_575_2	SFT_cM	**1.47E-06**	**5.95E-06**	**4.57E-03**	**4.78E-02**
uce-2241_NL9585_1140_2	SFT_cM	**4.50E-05**	**1.32E-05**	**2.70E-02**	**4.78E-02**
uce-111604_NL9585_832_2	PO_OM	**3.52E-06**	**8.62E-06**	**2.19E-02**	9.89E-02
uce-7283_NL9585_587_2	PO_OM	**2.21E-08**	**1.59E-05**	**4.11E-04**	9.89E-02
uce-113305_NL9585_758_2	DOX_OM	**5.84E-07**	**7.57E-06**	**1.09E-02**	1.40E-01
uce-2295_NL9585_578_0	SAL_OM	**2.41E-06**	**1.82E-02**	**4.47E-02**	1.0

One of each SFT category association (hottest, coldest, and overall mean) was used in a map of individual marker allele frequency against the associated SFT factor. Marker uce-13703_NL9585_633_2 (hereafter "marker 633") genotype frequency was plotted against the map of the average SFT in the North Atlantic Ocean study region (Figure 1). Genotypic frequency of marker 633 increased with increased average seafloor temperature and decreased with lower SFT. Areas of Newfoundland and Labrador had low to 50% genotype frequency of marker 633 while the eastern individuals in the WSMPA, Celtic Sea, and Cantabrian Sea displayed clear boundaries to genotype frequency in respect to SFT_OM where the warmer the SFT the more likely these samples were to have marker 633.

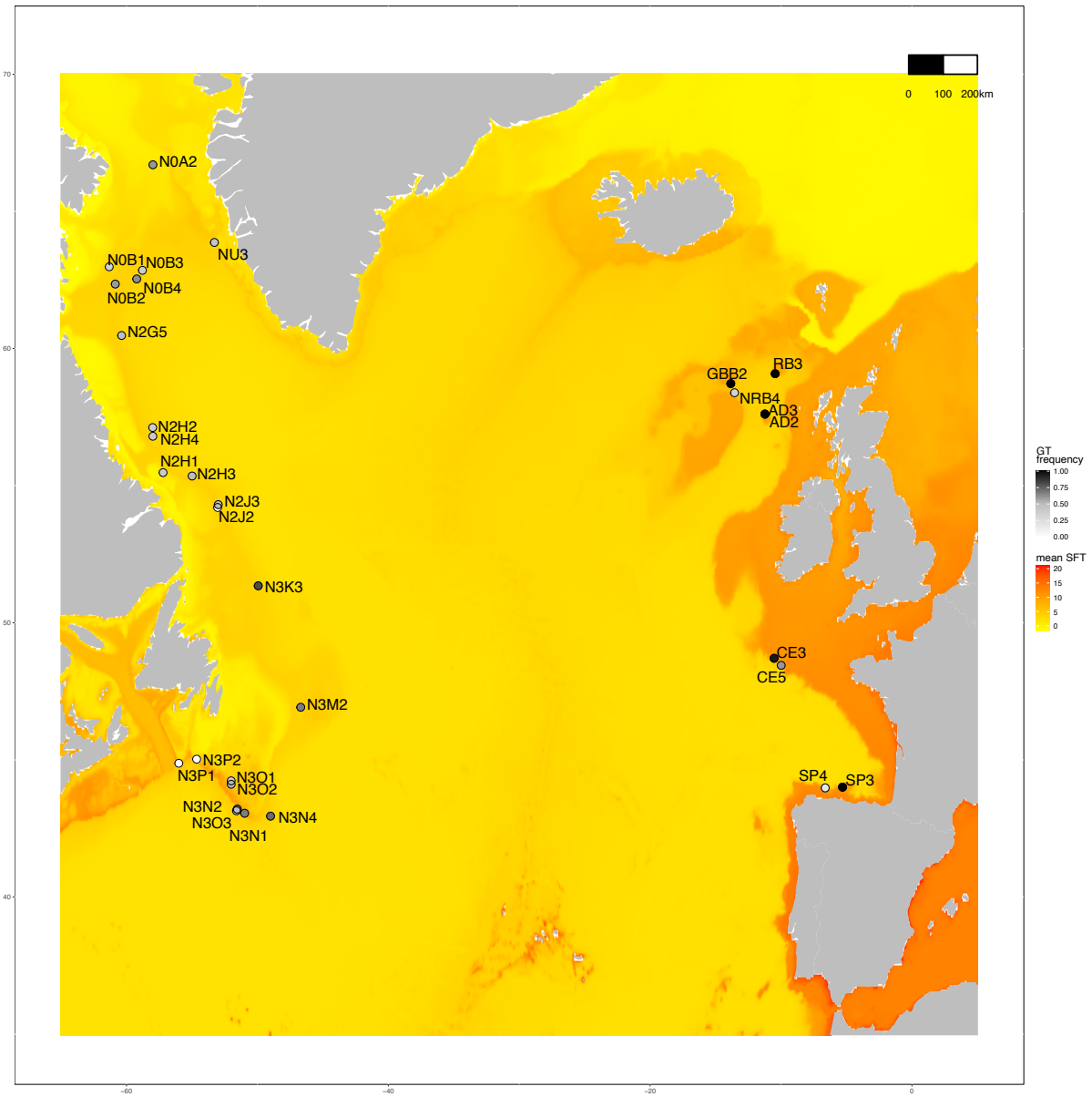

Figure 1. Marker uce-13703_NL9585_633_2 genotype frequency was plotted against mean seafloor temperature across the North Atlantic Ocean. Red areas have a higher overall average SFT and yellow areas have a lower average SFT. Lower genotype frequency of marker 633 is represented by a lighter point, and darker points represent higher genotype frequency. Information about the site codes can be found in Table 1.

Marker uce-5132_NL9585_809_0 (hereafter "marker 809") was found to have a significant relationship to mean SFT during the coldest month (cM). The raster for SFT_cM was used to create a map of the SFT during the coldest month onto which the genotype frequency of marker 809 was plotted (Figure 2). This plot shows a relationship between coral experiencing a warmer cold month and high genotypic frequency of marker 809. The colder temperatures during the coldest month appear to be correlated with an absence or low

frequency of marker 809 in *A. arbuscula.* In eastern collection sites, where the average STF_cM is higher, individuals from those sites are more likely to have a higher frequency of marker 809. In the western Atlantic, most sampled sites around Newfoundland and Labrador have low allelic frequency of UCE marker 809. The deepest site in Hopedale Saddle, Labrador, has a higher genomic frequency of 809 than the surrounding sites.

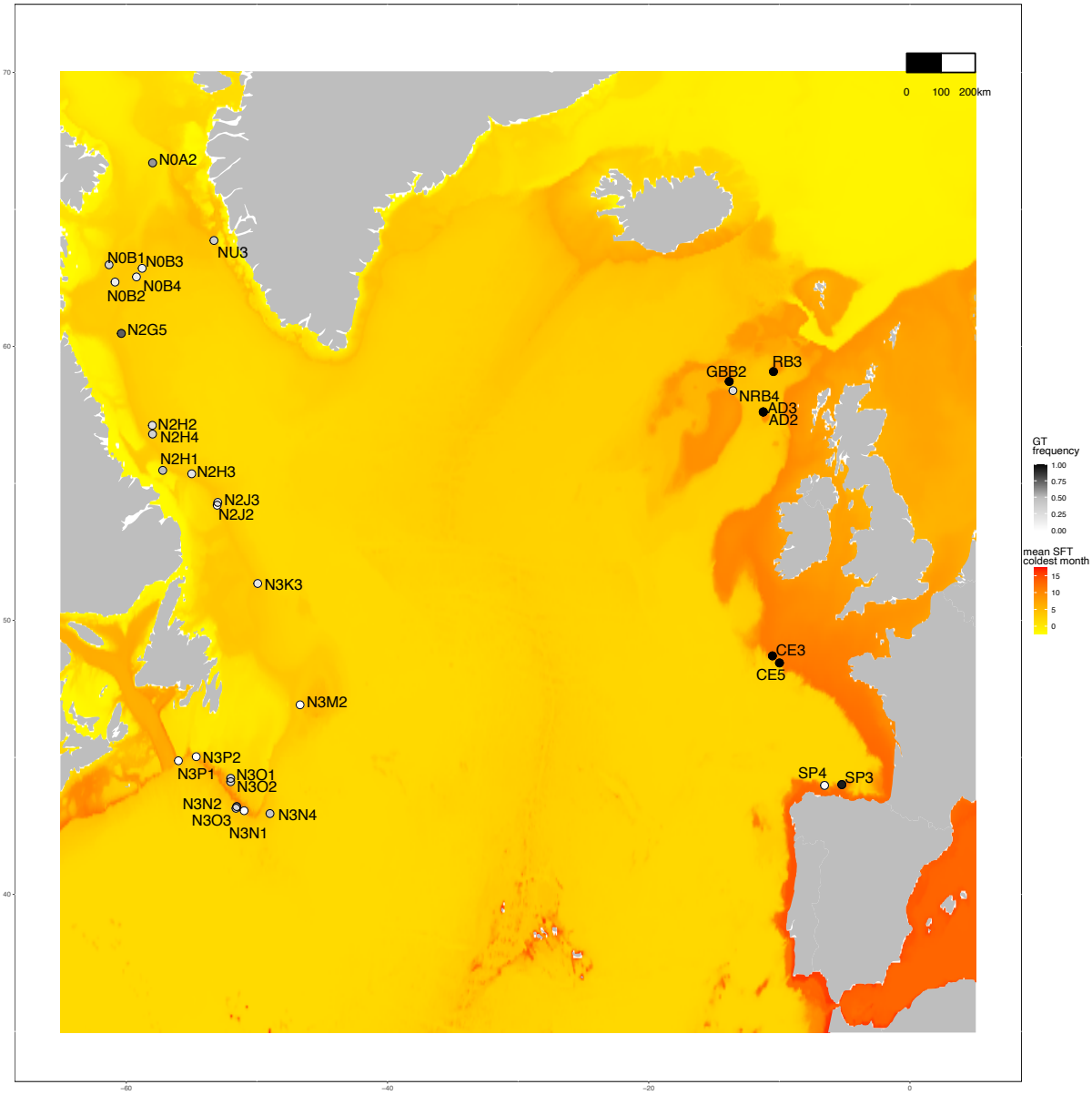

Figure 2. Marker uce-5132_NL9585_809_0 genotype frequency was plotted against mean sea floor temperature in the coldest month across the North Atlantic Ocean. Red areas have a higher overall average SFT_cM and yellow areas have a lower average SFT_cM. Lower genotype frequency of marker 809 is represented by a lighter point, and darker points represent higher genotype frequency. Information about the site codes can be found in Table 1.

The genotype frequency of marker uce-990291850_NL9585_674_2 (hereafter "marker 674") was mapped against the average seafloor temperature during the hottest measured month (SFT_hM). Here (Figure 3), the colder the SFT during the hottest month, the more likely an individual is to have a higher frequency of marker 674. In warmer areas, a low allelic frequency of marker 674 is found. So, shallower sites in Scotland, the Celtic Sea, and Cantabrian Sea have a lower frequency of marker 674 while deeper sites in these areas have increased genotypic frequency. Around Newfoundland and Labrador, a similar but not as immediately apparent trend occurs in deeper vs. shallower collection sites. Near Grand Banks in Newfoundland, some shallower sites show higher frequency of marker 674; however, deeper sites maintain the trend seen in the deeper European sampling sites.

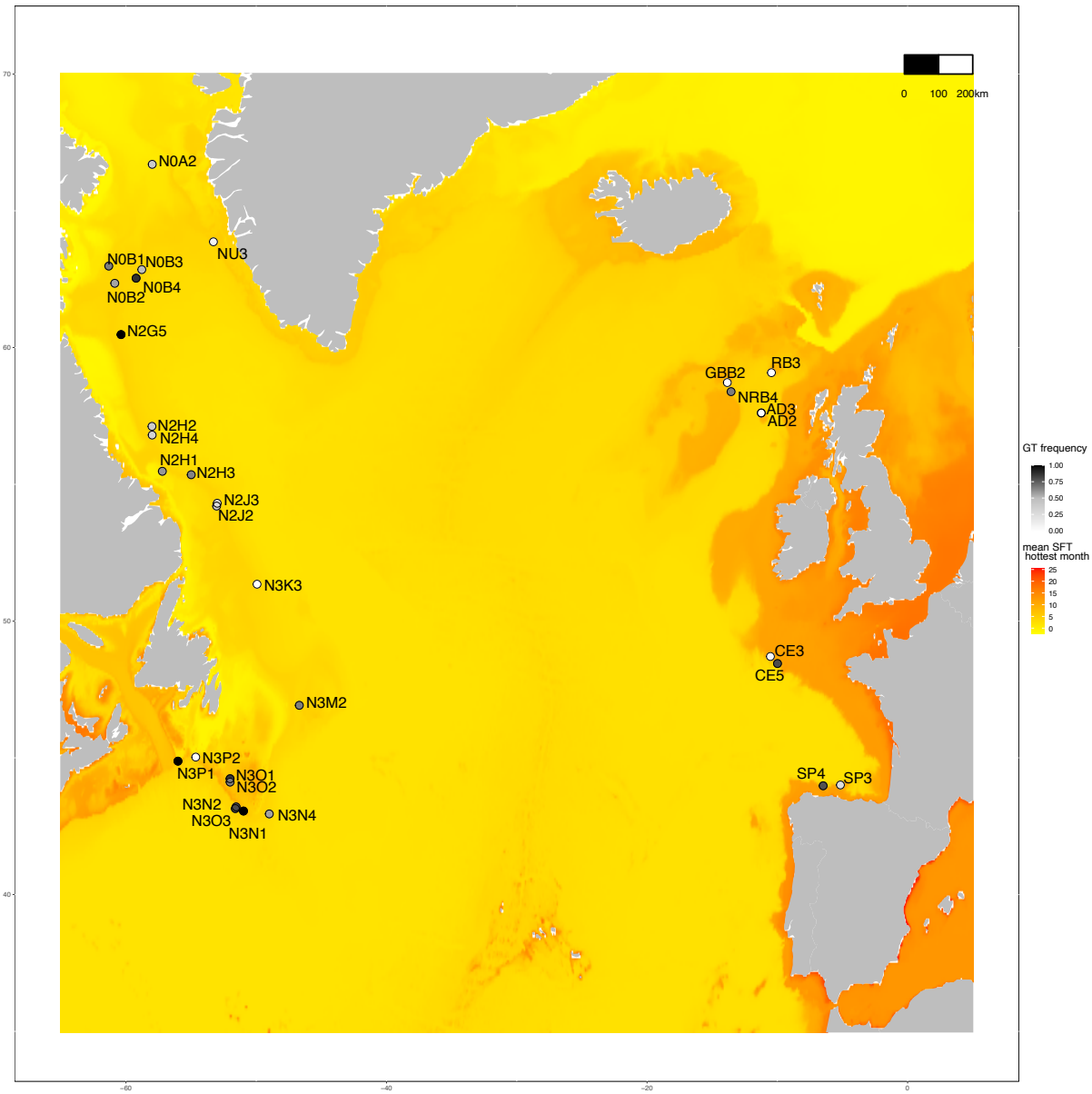

Figure 3. Marker uce-990291850_NL9585_674_2 genotype frequency was plotted against mean seafloor temperature in the hottest month across the North Atlantic Ocean. Red areas have a higher overall average SFT_hM and yellow areas have a lower average SFT_hM. Lower genotype frequency of marker 674 is represented by a lighter point, and darker points represent higher genotype frequency. Information about the site codes can be found in Table 1.

Modelling Potential Genotypic Frequency

Across the eleven measured environmental factors investigated in this study, data for each environmental variable was extracted for all 85,424 representative points. Using the GLM from markers 633, 674, and 809, it was possible to predict the allelic frequency of these markers across the entire study area based on the SFT in an area. Areas highlighted by the representative points with soft sediments could be suitable habitat for *Acanella arbuscula*. For predicted

genomic frequency of marker 633 in respect to SFT_OM (Figure 4), higher genomic frequency would be expected in the Mediterranean Sea, the Celtic Sea, areas near Scotland, SE of Iceland, and through the Laurentian Channel in Newfoundland. Lower allelic frequency would be expected in Arctic waters, on the Mid-Atlantic Ridge, and throughout Newfoundland and Labrador.

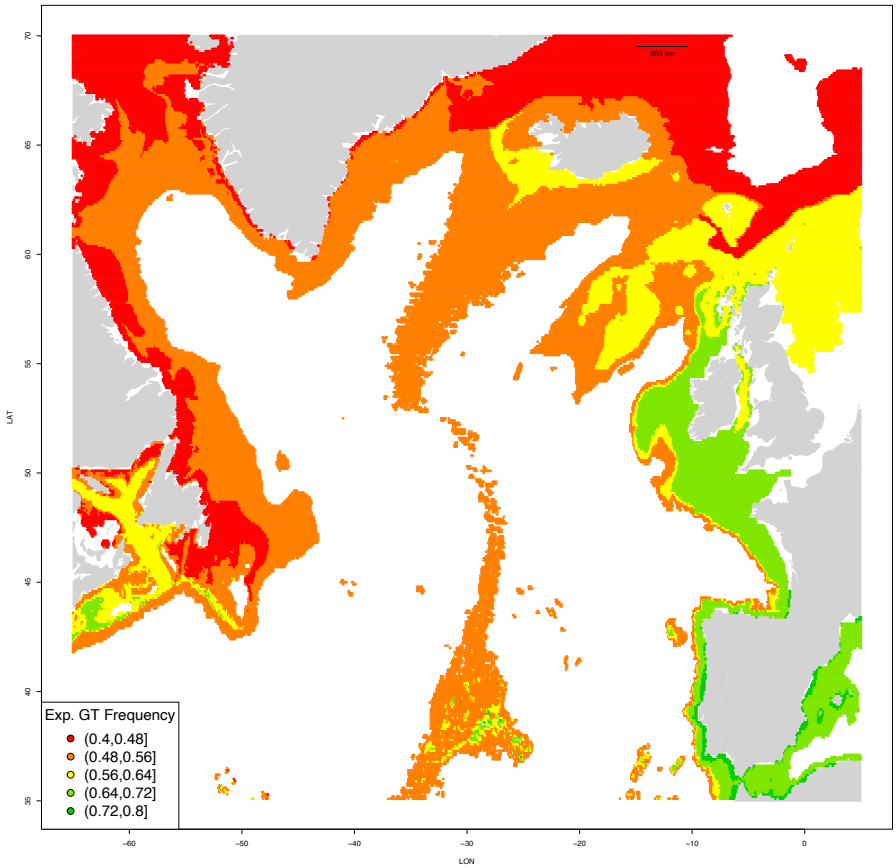

Figure 4. Predicted allelic frequency of marker 633 across the entire study site based on SFT_OM in the North Atlantic Ocean above 2,300m. Each of the 85k dots are 10 km apart. Higher expected genotypic frequency is represented by a green dot, and lower frequency by red. Areas deeper than 2,300m appear white because they were removed from the study.

Modelled potential genomic frequency of marker 674 in respect to the associated SFT_hM is nearly the opposite pattern of what was found in marker 633. In marker 674, higher genotype frequency is predicted to exist in the coldest waters in the Arctic, around Iceland, Greenland, Labrador, Newfoundland, and the Mid-Atlantic Ridge (Figure 5). Lower genotypic

frequency is predicted to exist in warmer waters of the Mediterranean Sea, the Cantabrian Sea, the Celtic Sea and around the United Kingdom.

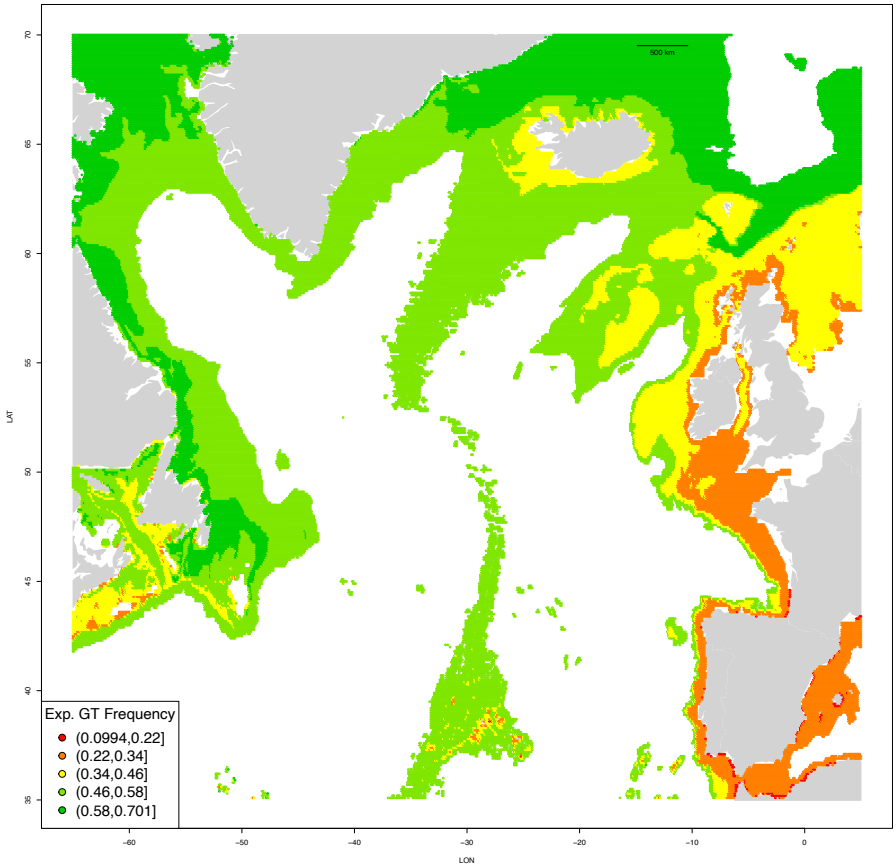

Figure 5. Predicted allelic frequency of marker 674 across the entire study site based on SFT_hM in the North Atlantic Ocean above 2,300m. Each of the 85k dots are 10 km apart. Higher expected genotypic frequency is represented by a green dot, and lower frequency by red. Areas deeper than 2,300m appear white because they were removed from the study.

The predicted genotypic frequency across the North Atlantic Ocean of marker 809 associated with SFT_cM showed similar patterns to marker 633 with a few small differences; Higher genetic frequency of marker 809 (Figure 6) is expected in the Mediterranean and outflow waters up through the Cantabrian Sea near Spain, around France, Ireland, and the United Kingdom. Small patches of higher allelic frequency were expected near Iceland, and the Laurentian Channel in Newfoundland. While mid- to low- levels of genotypic frequency of marker 809 were predicted in Greenland, Labrador, and the majority of Newfoundland.

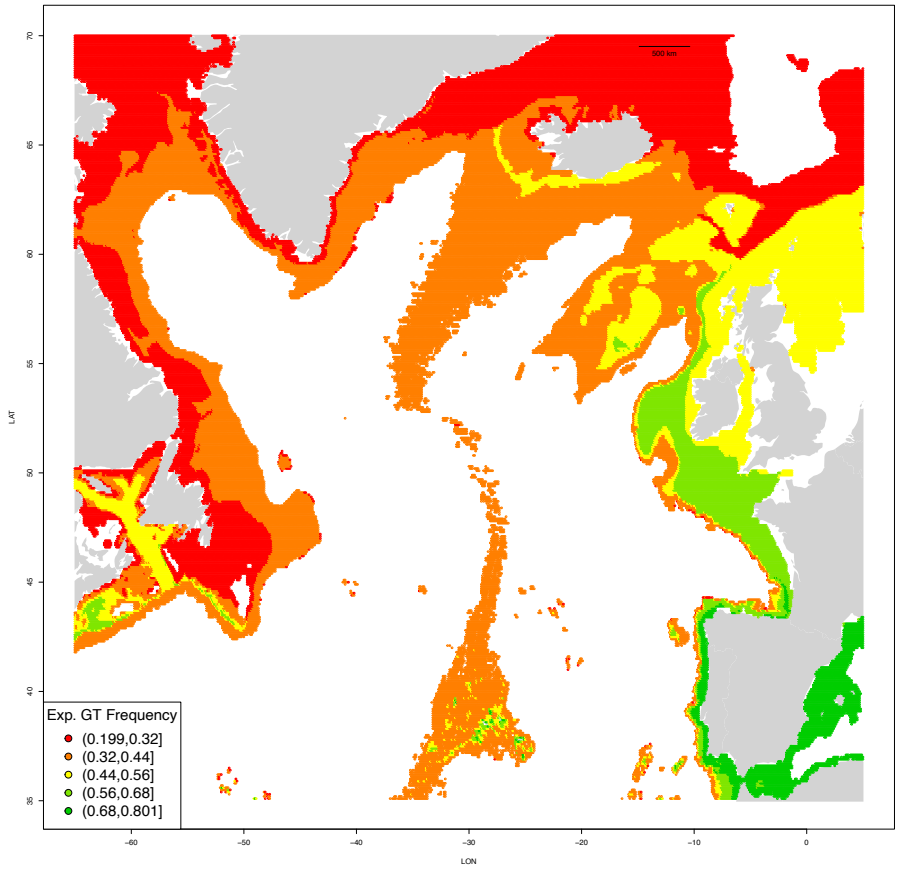

Figure 6. Predicted allelic frequency of marker 809 across the entire study site based on SFT_cM in the North Atlantic Ocean above 2,300m. Each of the 85k dots are 10 km apart. Higher expected genotypic frequency is represented by a green dot, and lower frequency by red. Areas deeper than 2,300m appear white because they were removed from the study.

Discussion

In this study adaptive loci, those that increase an organism's fitness and ability to cope with their environment (Holderegger et al., 2006; Selkoe et al., 2016), were used to test responses to environmental conditions found in the deep-sea. In previous seascape genomics studies conducted in the deep-sea, environmental selective pressures were found to be dependent on the species being studied (Selkoe et al., 2016). In one study, a deep-sea sponge, *Poecillastra laminaris*, and three deep-sea reef-building stony corals, *Goniocorella dumosa*, *Madrepora oculata*, and *Solenosmilia variabilis* were investigated using seascape genomics. All four species had specific genotype/environment associations including dissolved oxygen, dynamic topography, both sea surface temperature and net primary productivity, and tidal

current speed, respectively (Zeng et al., 2020). In the Gulf of Mexico, deep-sea octocoral, *Paramurcea biscaya*, genetic diversity was 7.37% dependent on depth, mean dissolved oxygen concentration, and mean surface primary productivity combined (Galaska et al., 2021). Solitary deep-sea coral, *Desmophyllum dianthus* (Scleractinia), in the Southwest Pacific Ocean spatial genetic variation was found to be related to topological characteristics of their habitat such as seamount occurrence and slope and to a lesser extent phosphate and particulate organic carbon flux (Holland et al., 2022). For deep-sea corals, it seems that no one environmental condition influences adaptation across all coral species.

The results from this study suggest that temperature is the largest environmental contributor to local adaptation in *Acanella arbuscula* as all significant genotype environment associations were dependent on a factor of seafloor temperature. This may have impacts on a population's ability to adapt to ocean warming. In a controlled experiment, the deep-sea coral specimens of *Lophelia pertusa* (Scleractinia) collected from the NE Atlantic and Gulf of Mexico were found to consume 30-50% more oxygen when exposed to warmer water (6.5 °C to 11 °C) and were unable to withstand dissolved oxygen concentrations 40-50% below ambient (Dodds et al., 2007). However, *L. pertusa* growing in the SE Atlantic near Angola were found thriving in warm (14°C) oxygen depleted waters (Hebbeln et al., 2020). This suggests that deep-sea corals have local adaptations to environmental conditions such as temperature and dissolved oxygen. Specimens used in this study were collected from temperatures between 1-13 °C locally; however, the difference in temperature they experience from the coldest to hottest month on average is less than a degree locally. Bathyal (200-3,000 m) seafloor temperatures are modelled to change by –0.32 to 4.41°C in the Atlantic Ocean by the year 2100 (Sweetman et al., 2017). While some corals have the ability to adapt to local environmental changes, it is unclear whether *A. arbuscula* will be able to cope with extreme

changes in temperature outside the historical range they have been exposed to. To ensure the persistence of *A. arbuscula*, it is essential that genetically diverse populations are protected so that they may provide recruits that are more resilient to anthropogenic stressors.

In addition to anthropogenic effects of sea temperature change on corals, *A. arbuscula* are exposed to heavy trawling and contact with bottom fishing gear in Newfoundland and Labrador. Local fishermen in the region have noticed the decline and eventual disappearance of corals being caught as by-catch with one area, Stone Fence in the Laurentian Channel, being described as now "a desert of what it used to be" and "flat as a table" by one fisherman compared to a period started in 1958 when he would regularly catch large corals and could fill his small boat with corals from the area (Gass, 2005). Further interviews from fishermen tell of *A. arbuscula* being more common than other coral species in Newfoundland with one notably recalling a gillnet on a wharf in a village in Newfoundland being tangled in roughly a hundred colonies (Gass, 2005). Because of their lack of attachment to hard substrate, *A. arbuscula* maybe more susceptible to becoming tangled in gillnets. In addition to the heavy trawling in NL, evidence of trawling was found in the WSMPA (Roberts et al., 2009) and a recent survey found ghost fishing gear abandoned in the area (La Beur et al., 2019) suggesting that *A. arbuscula* are under threat in multiple locations throughout their range and are of conservation concern in the North Atlantic Ocean.

The recovery of cold-water reefs disturbed by anthropogenic effects relies on the availability and successful settlement of larvae from neighboring reefs which have similar environmental conditions such as water chemistry, temperature, and substrate (Goode et al., 2020). The design of an effective marine protected area network (MPAn) to encourage connectivity of these octocorals in order to help them recover from past disturbances could

help their long-term survival in the region. Out of 148 MPA connectivity studies reviewed by Balbar and Metaxas (2019), 68 used genetics to study connectivity in MPAs. Of those studies, 16.2% informed the design of new MPAs based on the results of genetic analysis (Balbar & Metaxas, 2019). Genetic information used to study MPAs included isolation by distance, population structure, haplotype diversity, and gene flow (Balbar & Metaxas, 2019). Genetic structure data can be used in addition with ecological data by conservation planners to pinpoint populations containing unique or high levels of genetic diversity (Jenkins & Stevens, 2018). As discussed in the previous chapter, it is possible to determine genetic sources and sinks for *A. arbuscula* across its range using SNPs. The protection of source populations which provide recruits to replenish damaged populations is important and would benefit from the knowledge of local environmental adaptation or other drivers to asymmetrical connectivity (Goode et al., 2020; Jenkins & Stevens, 2018).The incorporation of the seascape genomic information from this study and the previous chapter in in the design of a MPAn could help ensure successful recolonization of *A. arbuscula* in areas that have been damaged by contact with bottom fishing gear and help with the persistence of existing populations. Spatial planning to incorporate ecological connectivity can be completed using the graphical user interface Marxan Connect which combines genetic connectivity matrices and landscape connectivity data to create a reserve design that meets multiple conservation objectives (Daigle et al., 2020).

In the future, we would like to use our seascape genomics, temperature adaptation, and projected allelic frequency information in combination with the population structure, connectivity, and geneflow data from the previous chapter with Maxan Connect to locate regions in the North Atlantic Ocean that should be protected to enhance connectivity in existing MPAs. Based on our findings, the Grand Banks near the Laurentian Channel in Newfoundland (sites N3P1 and N3P2) would be good candidates for a new MPA as they are genetically

isolated with very little geneflow to the rest of the study. One of the deepest Labrador populations, site N2G5 in Hopedale Saddle, also experiences weak gene flow to and from the rest of the study area and would be a population to investigate for protection. Across sites not represented in the study which may be stepping-stones for connectivity based on projected allele frequencies and similar environmental conditions include southern Iceland, east and southern Greenland, the slopes off of western Ireland and Portugal.

Supplementary Material

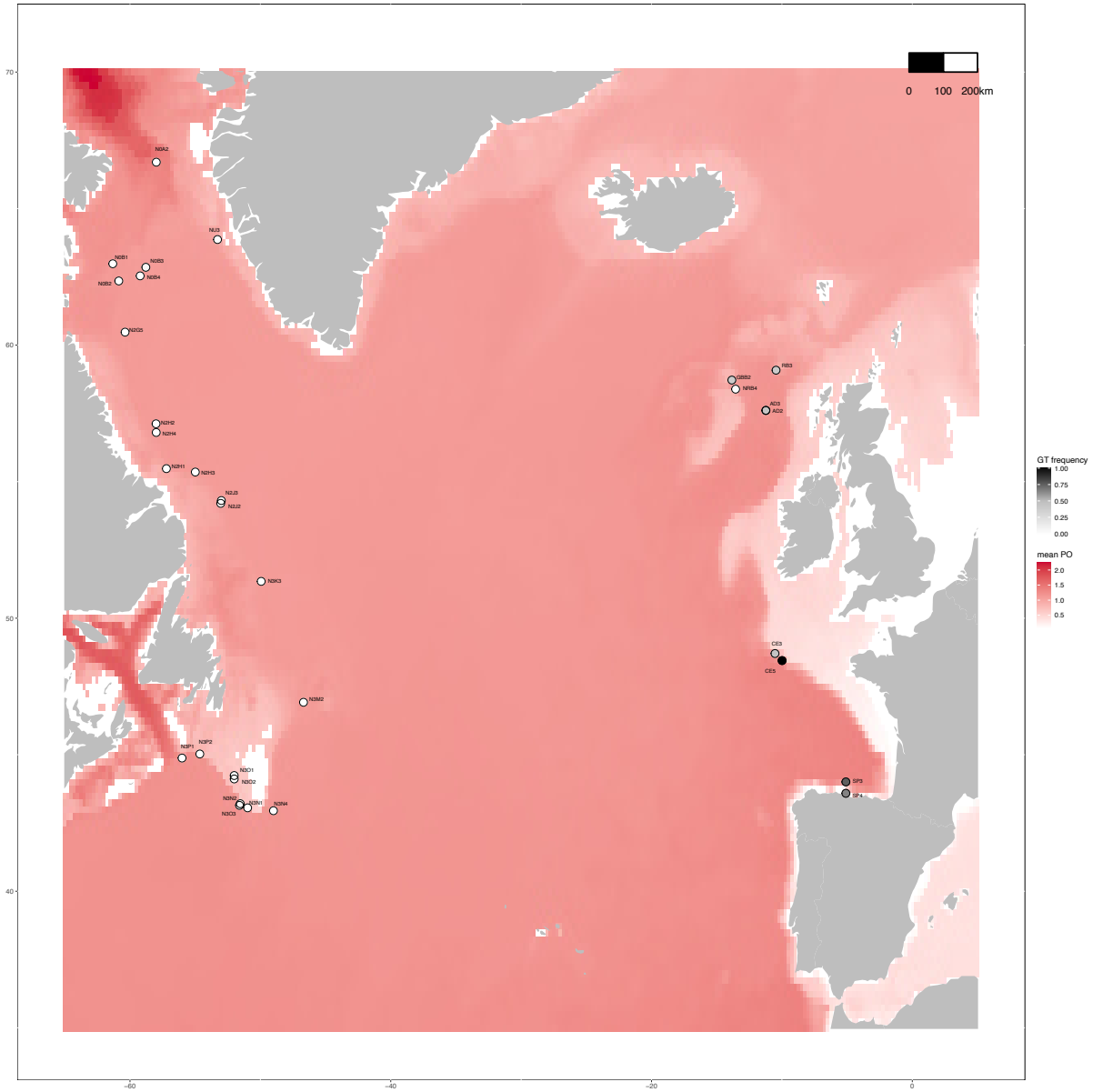

Figure S1. Marker uce-111604_NL9585_832_2 genomic frequency with yearly average phosphate concentration across 33 sample sites. Higher genomic frequency is indicated by a darker point while lower frequency is lighter. Phosphate concentration is higher in darker red areas and lower in lighter areas. Entirely white areas represent missing data from shallow regions.

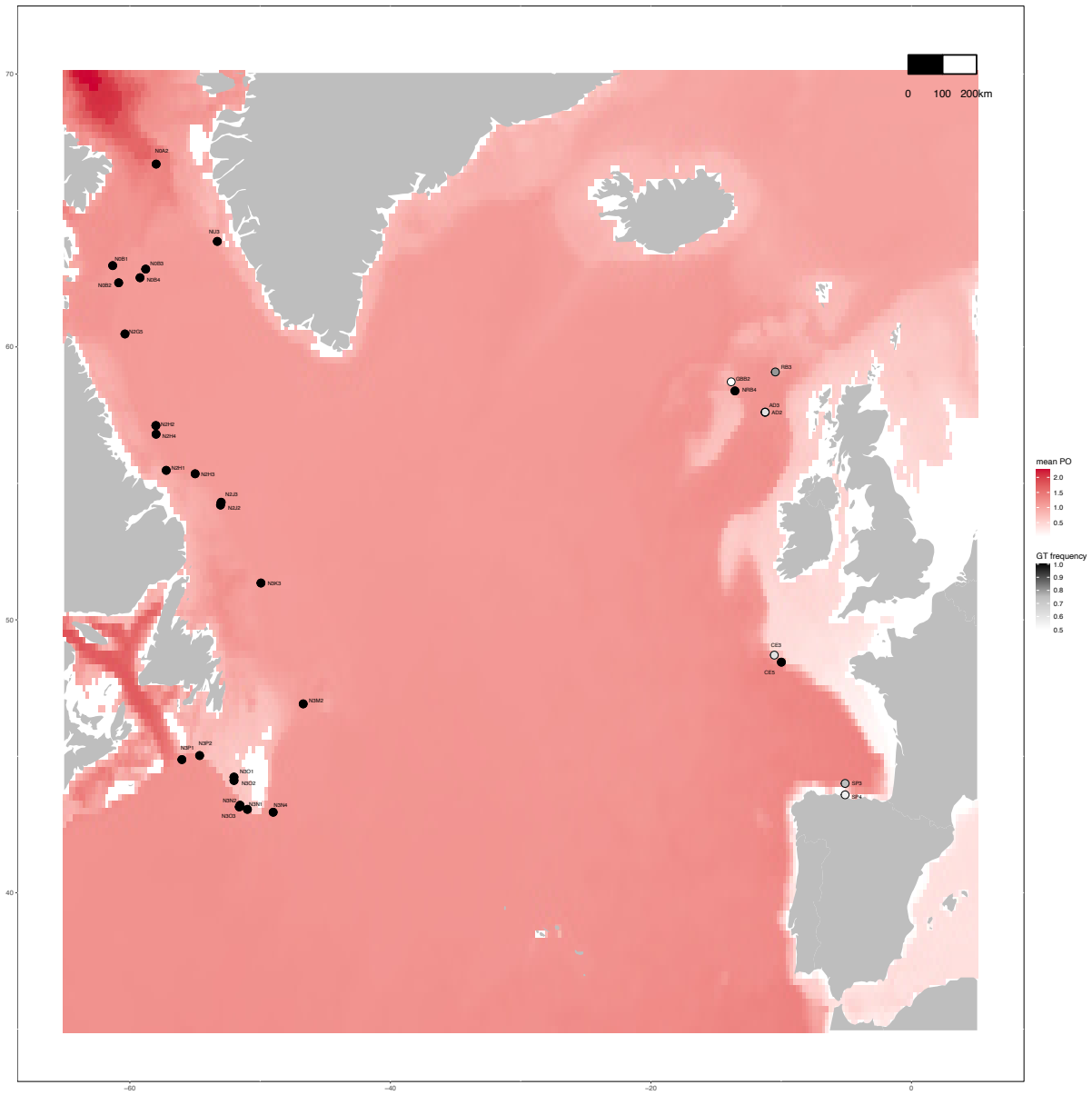

Figure S2. Marker uce-7283_NL9585_587_2 genomic frequency with yearly average phosphate concentration across 33 sample sites. Higher genomic frequency is indicated by a darker point while lower frequency is lighter. Phosphate concentration is higher in darker red areas and lower in lighter areas. Entirely white areas represent missing data from shallow regions.

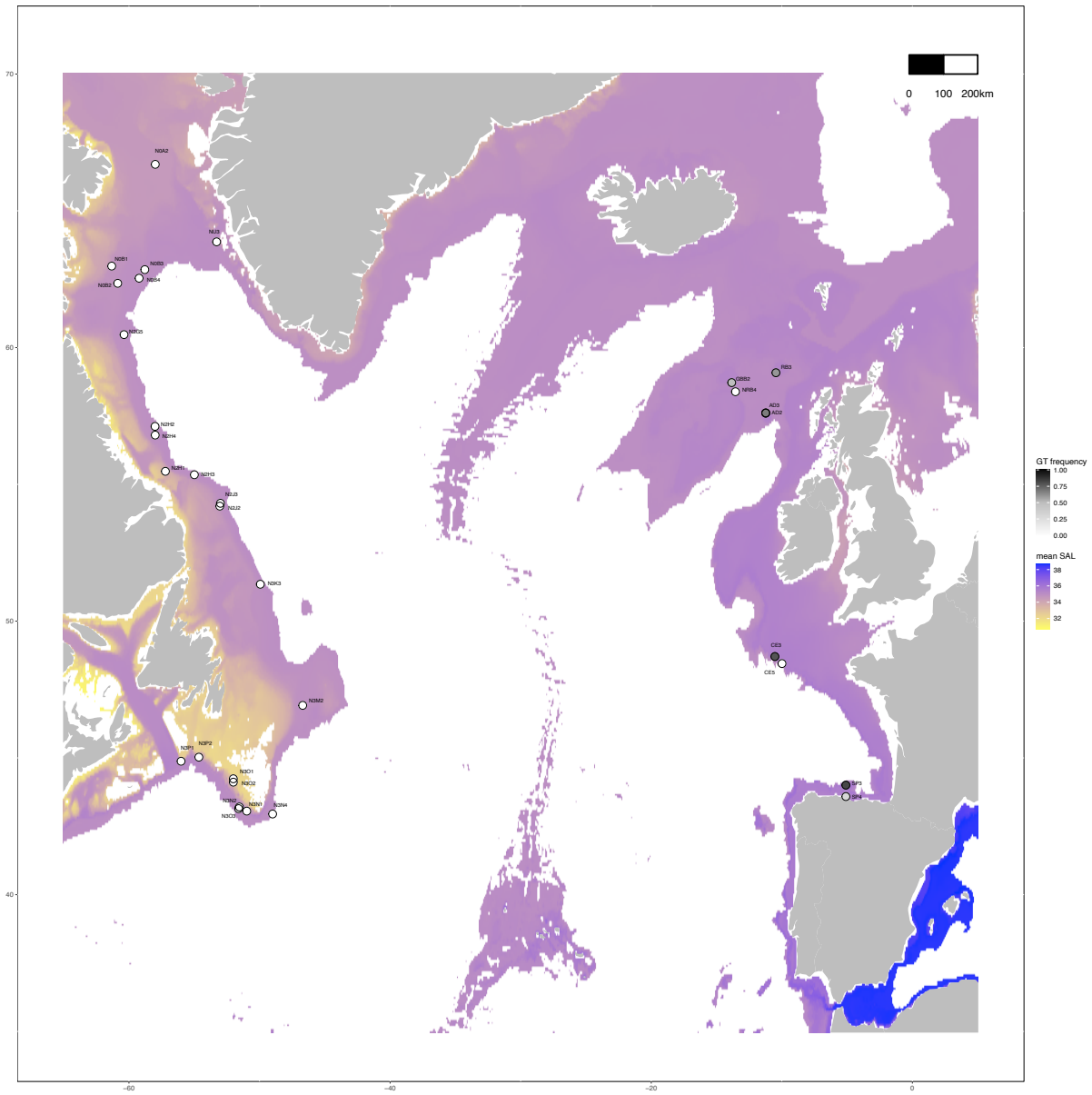

Figure S3. Marker uce-2295_NL9585_578_0 genomic frequency with yearly average salinity across 33 sample sites. Higher genomic frequency is indicated by a darker point while lower frequency is lighter. Salinity is higher in darker blue/purple areas and lower in yellow areas. Entirely white areas represent missing data from shallow regions.

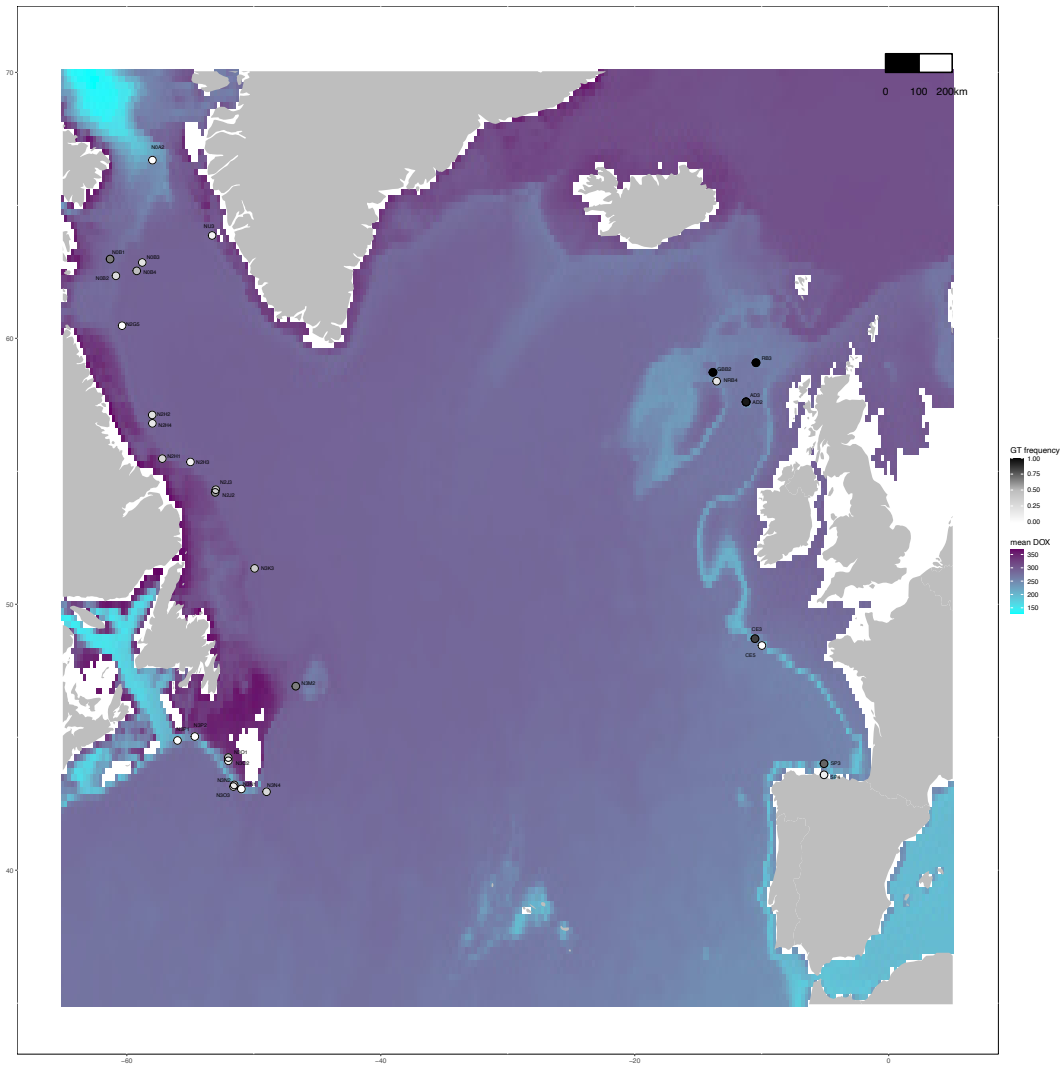

Figure S4. Marker uce-113305_NL9585_758_2 genomic frequency with yearly average dissolved oxygen concentration across 33 sample sites. Higher genomic frequency is indicated by a darker point while lower frequency is lighter. Oxygen concentration is higher in darker purple areas and lower in light blue areas. Entirely white areas represent missing data from shallow regions.

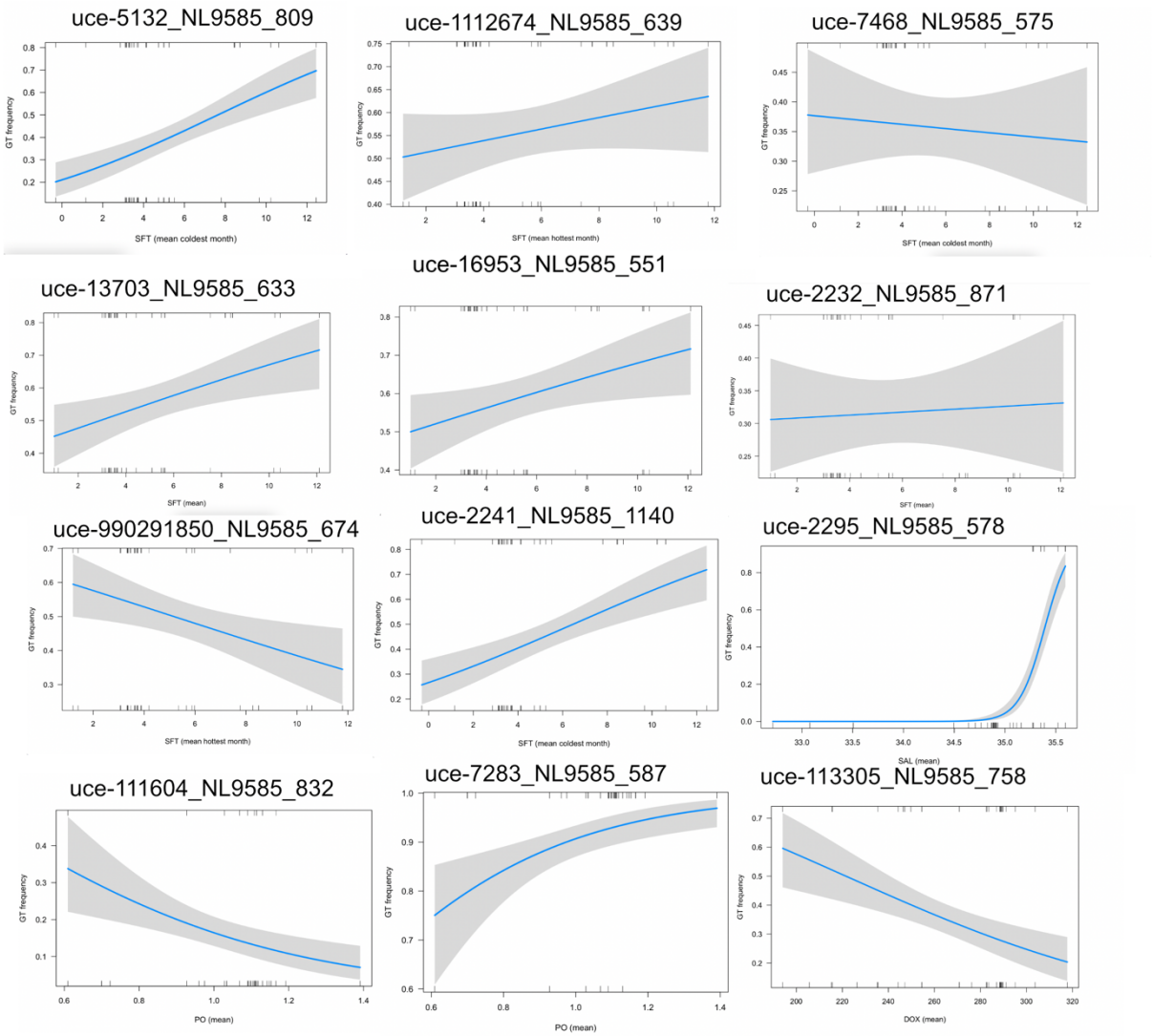

Figure S5. The results of general linear model for each of the 12 UCE markers from table 2. Genotypic frequency of each UCE marker is plotted against the environmental variable listed in the x-axis. The blue line is the best-fit.

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Chapter 5: Discussion

In DNA extraction comparisons (Chapter 2), the best techniques based on cost, DNA recovery, and plastic waste production were compared. It was found that no one technique was perfect for all organisms studied. Tissue from certain genera responded best to the salting-out technique and others responded best to a modified plant extraction kit method. The phenol extraction method yielded the most DNA per mg of tissue across all corals except *Acanella* and *Madrepora*. These two genera bore the most DNA via the salting-out method. The Qiagen Plant Mini kit produced the highest ratio of high molecular weight DNA to degraded DNA in all corals tested. The amount of plastic waste generated from each protocol was also measured with kit DNA extractions using significantly more plastics than non-kit protocols. Ways to cut down on plastic waste and improve DNA extraction quality were suggested. It is hoped that this chapter will help future researchers decide which protocols to test when conducting DNA extractions in non-model organisms. In the future, high throughput DNA sequencing may be more accessible when researchers are able to extract high quality DNA from traditionally tricky organisms. In addition to the results of how to get high quality DNA from specimen, it is hoped that the impact of plastic waste generated from molecular work is not overlooked. It is suggested in future molecular based research projects that researchers complete an ethical assessment of the anticipated volume of plastic waste that will be generated from a project and seek to reduce their plastic waste footprint wherever possible.

Using this knowledge from Chapter 2 of best DNA extraction practices to extract the highest quality DNA from the *Acanella arbuscula* a large-scale genetic connectivity analysis was commpleted. Many of these samples were poorly preserved and produced highly degraded DNA that was not suitable for downstream library preparation for most high throughput sequencing techniques. The ultra-conserved elements sequencing technique does not require

high-quality DNA (Faircloth et al., 2012; Quattrini et al., 2018), so it was perfect for these planned population genomics and seascape genomics studies. The previously published method from working on 12 samples at a time was upscaled to 96-well plates, making high-throughput library preparation of UCEs possible for 100s of samples.

The outputs of Chapter 3, whilst pushing forward ecological knowledge, also show that ultra-conserved elements sequences can be used for more than just phylogenomics (their originally designed purpose); they are useful for population genomics as well as seascape genomics analyses. With thousands of single nucleotide polymorphisms (SNPs) generated from this method, local and broadscale connectivity across our study area was investigated. Newfoundland and Labrador were surprisingly well connected across a wide depth range (60 to 2,300 m) and locations. Deeper populations in Whittard Canyon, the West of Scotland marine protected area, and the Cantabrian Sea were well connected to all of Newfoundland and Labrador as well as Nuuk, Greenland.

Populations of *Acanella arbuscula* that have low levels of geneflow in The West of Scotland MPA and Whittard Canyon in the Celtic Sea which are isolated from the rest of the study sites throughout the Cantabrian Sea, Celtic Sea, Scotland, Greenland, Newfoundland, and Labrador. Sources and sinks of genetic material within our study included the deepest site in Hopedale Saddle in Labrador which was found to be a source for genetic material to shallower sites, but it was not a sink for genetic material from other sites. The shallowest site in the mouth of the Laurentian Channel in Newfoundland was also found to be a source for genetic material, but it was not a sink from the surrounding sites.

Due to the lack of gene flow between shallow and deep sites in the WSMPA, lack of geneflow between shallow and deep sites in Whittard Canyon, and low level of geneflow between deep and shallow sites in the Cantabrian Sea, we conclude that there is a break in geneflow across depth in the Eastern Atlantic Ocean around 1,200 meters with a small amount of introgression. This genetic break is theorized to exist because of changes in salinity and temperature in a thermocline and halocline in this region originating from the warm highly saline outflow from the Mediterranean Sea. A similar barrier in geneflow across depth was not found in a majority of Canadian sample sites. However, the deepest site, Hopedale Saddle (1,800 m), and shallowest site, Laurentian Channel (150-250 m), showed lower geneflow to the rest of the sites in Newfoundland and Labrador. These results support the depth differentiation hypothesis in the deep-sea. Our sites were more genetically connected at similar depths at great distances than they were to sites geographically close but at different depths.

Ultra-conserved regions of DNA have been used to investigate phylogenomic relationships and population connectivity (Cowman et al., 2020; Erickson et al., 2021; Faircloth et al., 2012; Petersen et al., 2022; Quattrini et al., 2018; Stiller et al., 2021) and were found to be useful even in older preserved samples with degraded DNA (Derkarabetian et al., 2019). Because a majority of deep-sea corals exist in museum collections or produce highly degraded DNA, using UCEs to investigate their phylogeny and population connectivity remains the best option. However, use of UCEs presents its own unique problems. For instance, UCEs have been shown to experience stronger purifying selection than regions that code for proteins (De Silva et al., 2014) this may result in data with reduced genetic variation and bias toward genes that have experienced bottlenecks in the past as deleterious mutations would have been removed from the population. It is therefore essentially to take into account the likelihood that UCE data may present only part of the genetic “story”. We hope that the inclusion of

morphological and reproductive as well as larval dispersal modelling data with our genetic data will help resolve species boundaries and potential barriers to connectivity.

The few thousand SNPs generated from UCE sequencing were also used to investigate local adaptation to the environment. Here, the inclusion of loci under selection was essential to uncovering markers that had a significant relationship to environmental factors. Of the many environmental conditions investigated, only seafloor temperature was found to influence local adaptation. Because most of the *A. arbuscula* used in this study experience less than one degree of variation between the hottest and coldest water they are exposed to on average, understanding their local adaptation to sea temperature is important.

The above results on connectivity of *A. arbuscula* across the region as well as their local adaptation to temperature gradients to suggest regions of the North Atlantic Ocean that we believe are of conservation concern. The region in the Celtic Sea near Whittard Canyon is currently not under any protection, and it is subjected to frequent trawling. This could be detrimental to the corals and other animals that inhabit this area. Likewise, a very small portion of the waters around Iceland and Greenland are protected. We think these areas are of conservation concern.

Future Work

To expand upon the understanding of *Acanella arbuscula* and genetic adaptation in the future, I would like to research the UCE sequences that were under adaptive selection within our study. To do that, an annotated genome is needed, thus I can investigate whether these SNPs are associated with genes that have a phenotypic effect on survivorship in populations where they are abundant. I think this would be informative for conservation if the UCEs

uncovered as being significant in relation to SFT coded for some protein that helped coral survivorship in changing sea temperatures.

If I were to design this experiment with more resources, I would try to include *Acanella* samples from the Mediterranean Sea, Iceland, more samples from more locations in Greenland, the Azores, the Mid-Atlantic Ridge, and off the coast of New England. Unfortunately, samples from these locations were not available to us. However, I think the addition of samples from these areas would help fill in the gaps in our connectivity network. Regardless the genetic connectivity data presented here likely represents the best-sampled and studied deep-sea organism currently available globally. That said, finer sampling may see a sort of island-hopping model (Jenkins & Stevens, 2018) reveal itself with the inclusion of these sites or we may uncover even more isolated populations that are of conservation concern. In addition to adding samples to the study, I would also like to complete a similar populations genomics study on any invertebrates that live on *Acanella*. Specifically, studying the connectivity of the anemones that are only found on these corals would be insightful into how important it is to conserve the corals, because their connectivity is entirely reliant on the availability of a host. It may also be interesting to study the connectivity of corals found in coral gardens alongside *Acanella*, such as *Pennatula* spp. (sea pens). Unfortunately, it is incredibly difficult to get high quality DNA from sea pens (see Chapter 2); however, even highly degraded DNA can be used in UCE sequencing.

In the future, it would be useful to test larval distribution models against the genomic patterns of connectivity and migration we have found here (Gary et al., 2020). Ground-truthing distribution models could help improve their accuracy. Also, testing different larval behavioral patterns to see when distribution matches real life could help us work backwards to understand

the potential larval behavior of bamboo corals which have never been observed *in situ*. Larval distribution is partially reliant on available nutrients from a yolk sac which provide energy for the coral larvae until it can settle and start to catch food. By analyzing the size of eggs from our coral colonies, we might be able to understand their potential larval duration in the deep-sea (Graham et al., 2013). Egg maturity timings and yolk sizes alongside understanding of current modelling could help explain the connectivity patterns we see in this study.

To further support our evidence for our populations being all the same species, I would like to use a scanning electron microscope to image the small calcium carbonate structures found inside coral polyps called sclerites. This would be undertaken for specimens from each genetic cluster. Each coral species has different sclerites that are characters found only in that species (Saucier et al., 2017). So, if sclerites from samples from each of the study sites were examined and they were all the same, it would help support the theory that all specimens used are the same species and that no cryptic speciation exists.

The combination of genomics, larval distribution modelling, sclerite analysis, symbiont population genomics, and sclerite analysis would support a management option such as new marine protected area network design. All of these pieces of the puzzle would help identify areas of highest concern. We could use the program Marxan (Daigle et al., 2020) to aid in the modelling and design of an effective MPA using all of this information. Using this research to help conserve the marine environment and effect real world change would be the ideal end goal for this body of work.

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