Master's thesis

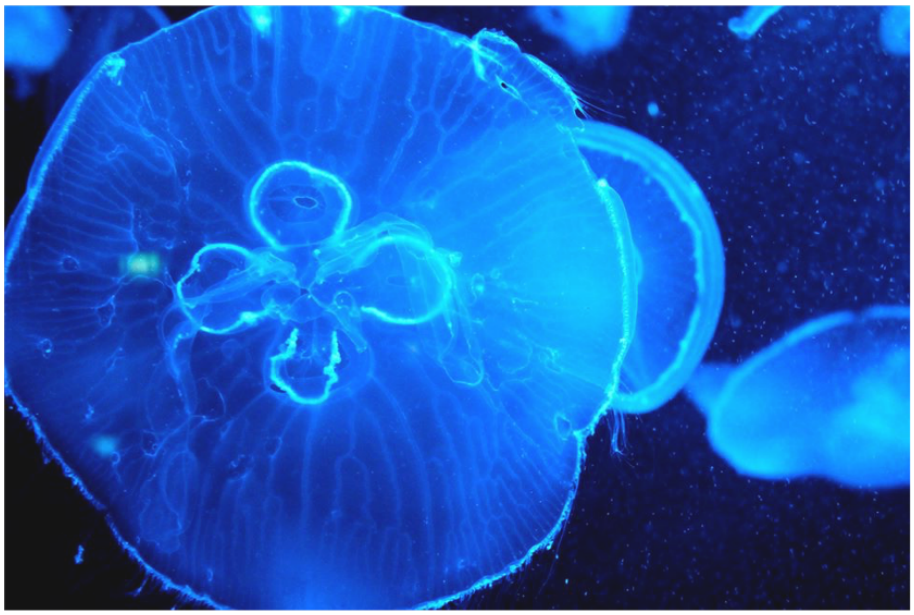
NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Aditya Wihen

Identification and population genetic analysis of jellyfish blooms in Trondheimsfjord

Role of polyps in jellyfish populations connectivity

Master's thesis in Ocean Resources
Supervisor: Nicole Aberle-Malzahn
Co-supervisor: Sanna Majaneva
May 2021



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Aditya Wihen, Trondheim, May 2021

Abstract

There is a strong need to utilize jellyfish biomass in both economic and ecological way. These two aspects rely on the capability of jellyfish to bloom in large quantities and on accurate prediction on where and when jellyfish blooms will most likely occur. However, lack of data regarding the jellyfish taxa has hindered finding the causes and dynamics of jellyfish blooms thus limiting management plans for jellyfish populations. One of the important aspects of jellyfish population dynamics is whether these blooms result from local bloom events or are rather a redispersion event of non-local jellyfish populations. In the case of local bloom events, jellyfish polyps are crucial for bloom dynamics as their asexual reproduction determines the amplitude and duration of the blooms. Nevertheless, studies on polyp populations are few in numbers and genetic connection between polyp and medusa bloom populations is not known. The main aim of this study was to find if there are population structuring in key jellyfish species in Trondheimsfjord using both medusae and polyp populations and to find connection between them. Mitochondrial cytochrome oxidase I marker revealed population differentiation between medusae populations of *Aurelia aurita* and *Cyanea capillata* in Trondheimsfjord and populations at reference locations of outer Oslofjord and Baltic Sea. While medusae populations of *A. aurita* in Trondheimsfjord were found to be significantly different compared to reference populations, analyses showed that the polyp population structure was homogenous in Trondheimsfjord and at reference locations. This suggests that this polyp populations can be considered as intermediate populations connecting the two medusae populations. In this study, no polyps of *C. capillata*. Results presented here showed a connection through the presence of intermediate polyp populations within two genetically separate medusae populations. This stresses the importance of incorporating polyp life stages in the study of jellyfish population structure and dynamics that will improve population management plans for jellyfish in the future.

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Glossary

AMOVA	Analysis of Molecular Variance
Chelex-100	Chelex I00 is a chelating resin that has a high affinity for polyvalent metal ions. Used for DNA extraction
COI	Cytochrome Oxidase subunit I. Used for DNA barcoding marker due to fast mutation rate
Contig	A contig (from contiguous) is a set of overlapping DNA segments that together represent a consensus region of DNA.
DMSO	DMSO is used in polymerase chain reaction (PCR) to inhibit secondary structures in the DNA template or the DNA primers. It is added to the PCR mix before reacting, where it interferes with the self-complementarity of the DNA, minimizing interfering reactions.
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
Ephyrae	A stage in the development of discophorous medusæ, when they first begin to swi m about after being detached from the strobila
GoJelly	Project under EU Horizon 20, source of funding for this master's thesis
Haplotype	A haplotype is a group of alleles in an organism that are inherited together from a single parent. In this thesis context, refer to one unique sequence that may be shared by more than one samples.
ITS1	Internal transcribed spacer 1, a marker
ITS2	Internal transcribed spacer 2, a marker
MAFFT	A Multiple Sequence Alignment Software. See Katoh (Katoh, 2013)
Medusae	Refer to free swimming adult jellyfish
Metagenetic	Possessing two life stages which alternate between sexual and asexual reproduction
$MgCl_2$	$MgCl_2$ is an essential cofactor that enhances the activity of Taq DNA polymerase
Microsatellite	Microsatellite are simple sequence repeats. Used for markers
Mitochondrial	Relating to mitochondria, organelle in cells which possess DNA on their own
Pairwise F_{ST}	or fixation index is a measure of population differentiation due to genetic structure
PCR	Polymerase Chain Reaction is a method widely used to rapidly make millions to billions of copies of a specific DNA sample
Planulae	Free swimming jellyfish larvae, eventually settle down on a substrate and become polyp
Primers	A primer is a short, single-stranded DNA sequence used PCR. Usually comes in pair (F and R) to flank a region of DNA to be amplified by PCR
Sessile	Fixed in one place, immobile. Here usually used to describe polyp
Strobilation	In polyp. An asexual reproduction consisting of spontaneous transverse segmentation of the body to produce ephyrae

1. Introduction

1.1 Jellyfish bloom

Jellyfish blooms refer to naturally occurring events of congregating gelatinous zooplankton within a body of water (C. H. Lucas & Dawson, 2014). Although the word 'jellyfish' itself can include a wide variety of gelatinous organisms of multiple life stages, here the term 'jellyfish bloom', is used to refer to the bloom of the medusa life stage— mature adults—belonging to Scyphozoa, Cnidaria. The documentations of occurrence and intensity of these blooms vary between species, geographical locations, size-ranges, and duration. Some notable examples of jellyfish blooms which have gained much attention in scientific literature and in global media are blooms of the giant jellyfish *Nemopilema nomurai* in east Asian marginal seas (Bohai, Yellow, East China, and Japan Seas) (Uye, 2008), blooms of *Crambionella orsini* throughout the Gulf of Oman and the Persian Gulf (Daryanabard & Dawson, 2008), blooms of *Cephea cephea* in the Red Sea (Cruz-Rivera & El-Regal, 2016), blooms of *Aurelia aurita* in Sishili Bay, Northern Yellow Sea of China (Dong et al., 2012), and blooms of *Periphylla periphylla* in the Trondheimsfjord (Tiller, Mork, Liu, Borgersen, & Richards, 2015).

Jellyfish bloom's seemingly sudden appearance in the water column can be the result of either a spike of population growth (true bloom) or a redistribution and redispersion event of jellyfish specimens through water movement (apparent bloom) or a combination of both (William M. Graham, Pagès, & Hamner, 2001). The adult stages of jellyfish are usually considered as planktonic due to their limited swimming capability. Thus, it is understandable at first to say that their dispersal is fully directed by water movement. In fact it has been observed that aggregation of larger sized medusae can frequently be found on ocean's physical discontinuities such as fronts, thermocline and halocline (William M. Graham et al., 2001), demonstrating potential for active swimming behaviour in response to environmental cues such as gravity, temperature, density, current, pressure, and other physical cues, which direct jellyfish aggregation through both active physical response and passive accumulation (William M. Graham et al., 2001). Intrinsically, local population increase (true bloom) is more related to jellyfish phenology, which may differ from species to species, and to some extent even within species through local adaptation as it is the case of globally distributed species like *A. aurita* (C. Lucas, 2001). In general, temperate species, such as *Aurelia aurita* in Norwegian waters, are known to bloom seasonally during the summer period while tropical species show

less distinct seasonality patterns on when they bloom due to their reproductive cycle happening all year round (C. Lucas, 2001).

Some studies regarded jellyfish blooms to be on the rise (Attrill, Wright, & Edwards, 2007; Brotz, Cheung, Kleisner, Pakhomov, & Pauly, 2012), which is usually speculated as a sign of marine environment degradation resulting from anthropological activities affecting marine ecosystems (Attrill et al., 2007). The impact of human activities on marine environments such as overfishing, climate change, eutrophication and coastal development are some of the observed and studied causes of jellyfish outbreaks. Overfishing, for example, has been attributed as one of the possible causes of jellyfish blooms in areas with intense human activities (Richardson, Bakun, Hays, & Gibbons, 2009; Vasas, Lancelot, Rousseau, & Jordán, 2007). Some fish are known to compete with jellyfish for zooplankton prey and some are known to be predators of jellyfish medusae and polyps (J. E. Purcell & Arai, 2001). Removal of fish from the ecosystem freed up space for jellyfish to thrive and eventually cause outbreaks, such as in the case of the Irish Sea (Lynam et al., 2011) where declines of haddock (*Melanogrammus aeglefinus*) and herring (*Clupea harengus*) population was followed by increases in jellyfish abundance. Similarly, in the northern Benguela upwelling system off the coast of Namibia where sardines (*Sardinops sagax*) population were heavily overfished, jellyfish like *Chrysaora hysoscella* took over the ecosystem (Cury & Shannon, 2004; Sparks et al., 2001). Change of ecosystem dominance by jellyfish was also observed in the context of eutrophication (J. E. Purcell et al., 2001). Nutrient-rich run-offs from farming activity are considered to cause shifts from a balanced system to a more flagellate-dominated phytoplankton community. This favours jellyfish, which are able to survive better because they feed on a wider range of prey including protists such as flagellates and are not visual predator (Colin, Costello, Graham, & Higgins Iii, 2005; Malej, Turk, Lučić, & Benović, 2007; Sullivan & Gifford, 2004). Moreover, sedimentation of large quantity of dead phytoplankton on the seafloor would increase oxygen consumption by bacteria through decomposing processes of organic material and cause localized hypoxia (Diaz & Rosenberg, 2008) where jellyfish and their polyps could survive due to their low-oxygen tolerance (J. E. Purcell et al., 2001). Jellyfish are expected to be promoted in flagellate-dominated ecosystems since an increase in sea surface temperature due to climate change could enhance water column stratification, creating low nutrient conditions in the upper surface layer. This could lead to favourable condition for flagellates compared to diatoms as they could migrate to more nutrient-rich deeper waters (Cushing, 1989). As another anthropogenic cause, coastal development increases the total area

for jellyfish polyps to attach to. Through providing more hard substrate such as concrete in areas with limited preferred substrate previously, coastal development could indirectly provide ideal conditions for polyps to thrive (W. M. Graham, 2001; Lo, Purcell, Hung, Su, & Hsu, 2008) thus causing more intensive jellyfish blooms.

However, there is still a debate whether these blooms are steadily increasing in both size and frequency, which has been heralded as leading our marine ecosystems into a more gelatinous future (Condon et al., 2012; Richardson et al., 2009). Mills (2001) reviewed not only observations on localized increases in jellyfish populations, but also some observations on the decrease of jellyfish populations such as decrease of *Aequorea victoria* in Washington State and British Columbia and the decrease of *Polyorchidae* family in the North Pacific. It is also important to note that while there is definite evidences of local increases in jellyfish populations in some places, a conclusion whether jellyfish populations are increasing or decreasing globally cannot be made in certainty. Several meta-analysis studies have published conflicting results with the view of rising jellyfish population on a global scale, with some attributing the trend to inaccurate citation practice (Sanz-Martín et al., 2016) and lack of data in terms of spatial and temporal range (Mills, 2001). But with both the push of resolving issues regarding jellyfish blooms and cautionary studies on the importance of jellyfish in the ecosystem, it is certain that there is lack of empirical data regarding these relatively ignored taxa to draw a bigger picture about their population structure and dynamics.

1.2 Jellyfish life cycle

Jellyfish life cycles usually consist of two distinct stages (C. H. Lucas, Graham, & Widmer, 2012): the free-swimming medusae stage and sessile polyp stage. Medusae reproduce sexually, producing small planktonic larvae— planulae, which settle on hard substrate like bedrock, coral, man-made structures made of wood or concrete such as aquaculture rigs, wind park installations, marinas, breakwaters (Duarte et al., 2013; Rekstad, 2019). Sexual reproduction can happen seasonally for temperate species located within shelf or coastal ecosystem with mature female and planulae present over the summer period ranging from 1 to 5 months (Brewer, 1989; Cargo & Schultz, 1966; C. Lucas, 2001). In tropical and sub-tropical ecosystems, reproduction usually happens either continuously (Dawson & Martin, 2001; W. M. Hamner, Gilmer, & Hamner, 1982) or semi-continuously (Fitt & Costley, 1998). The planulae then develop into the sessile polyp stage which can be perennial and these polyps are able to produce clones of themselves through budding, fission, and podocyst (C. H. Lucas et al., 2012). The cycle closes by performing transverse fission (strobilation) and each polyp producing several small, free-swimming jellyfish—ephyrae. The timing of strobilation shows both species- and location-specific variations. For example, *A. aurita* in Gullmar fjord, Skagerrak, Sweden, was observed to strobilate during autumn and spring period (Hernroth & Gröndahl, 1983), while in the Kiel Bight, Baltic Sea, strobilation occur over longer time-period (Möller, 1980). *Chrysaora quinquecirrha* (Calder, 1974) and *Rhopilema nomadica* (A Lotan, Fine, & Ben-Hillel, 1994) were observed to strobilate multiple times (2–5 times) per polyp per season with *R. nomadica* being able to release more than 100 ephyrae per polyp within a 2-months period (A. Lotan, Ben-Hillel, & Loya, 1992). For triggers of strobilation, there are several cues and conditions that have been observed that lead to strobilation in jellyfish, such as prey abundance (Chen, Ding, & Liu, 1985; Ishii & Watanabe, 2003), chemical compounds containing iodine (Black & Webb, 1973), and temperature (Holst, 2012a; J. E. Purcell, White, Nemazie, & Wright, 1999). But overall, there is a common trend where higher temperature and higher prey abundance lead to higher ephyrae produced per polyp both in field and laboratory settings (Hernroth & Gröndahl, 1983; J. E. Purcell et al., 1999; Willcox, Moltschaniwskyj, & Crawford, 2008). These aspects of jellyfish sexual and asexual reproduction combined with paradigm of human-induced favourable environments for jellyfish could explain the inclination of jellyfish to bloom in areas with prominent anthropological activities.

1.3 Implication of jellyfish blooms

Through an economic perspective, jellyfish blooms are commonly viewed as a nuisance. In fishing industry, mass occurrences of jellyfish could fill up fishing nets quickly as bycatch, necessitating more works and time to clean the nets (Lynam et al., 2006). There are also reports of jellyfish clogging up cooling water intake pipes of nuclear power plants, necessitating the shutdowns of the plants (J. E. Purcell, Uye, & Lo, 2007), and also blocking alluvial sediment suction in diamond mining operations (Lynam et al., 2006). Tourism can also be hit by blooms of jellyfish. Washed up jellyfish could potentially discourage beach visitors and, in some species of jellyfish, their stings could injure swimmers and the toxins can even cause death in humans, which then leads to the closure of beaches (J. E. Purcell et al., 2007). In some parts of the worlds, however, they are treated as more than just a nuisance, as jellyfish are deliberately harvested and used as a food item such as it is the case in Southeast Asia, China, and Japan (Shatz, 1998). Examples of harvested species are *Stomolophus meleagris*, *Rhopilema esculentum*, *Aurelia aurita*, and *Stomolophus nomuria*. Worldwide, harvested jellyfish biomass has been estimated to about 321 000 metric tons (Omori & Nakano, 2001).

With advancement of studies and techniques in chemistry and genetic in marine science, more knowledge is gained regarding the role of previously overlooked groups, such as jellyfish, in terms of their commercial and ecosystem service values. There are several studies regarding the roles jellyfish play within the ecosystem. In terms of carbon sequestration, jellyfish play an important role as a carbon sink through deposition of jellyfish carcasses on the ocean floor (Lebrato et al., 2012). Previously regarded as a trophic dead end within marine food webs (Verity & Smetacek, 1996), studies found that jellyfish are regularly consumed by a diverse range of marine predators such as sea turtles, birds, fish, and also invertebrates (Hays, Doyle, & Houghton, 2018). In contrast, jellyfish can also act as predators and, in large numbers, they can prey on fish larvae and eggs in such way that it can cause a trophic cascade (Jennifer E. Purcell, 1989). Jellyfish can also act as microhabitats and nursery areas such as some juvenile fish like jack mackerel that make use of jellyfish' stinging nature to protect them from predatory fish and helps them to collect prey (Masuda, Yamashita, & Matsuyama, 2008). In parallel, their commercial significance has also been noted. Other than as a food source, jellyfish can be a source of collagen for cosmetics industry (Nagai et al., 1999), or their biomass used as fertilizer (Chun, Damdinsuren, Kim, & Ezaki, 2012) or for feed in the aquaculture sector (Marques et al., 2016). There has also been an ongoing research on using substance

derived from jellyfish for nanoparticle filters, capable of filtering out tiny bits of plastic that enter the oceans from incomplete filtration of microplastic in wastewater processing plant as proposed in the *GoJelly* project under the EU Horizon (2020). These findings thus highlight the needs to further understand the dynamic of jellyfish bloom to balance ecological and economic needs.

1.4 Bloom origins and population connectivity

Understanding the blooms' origin and population structure of jellyfish is crucial for further plans on utilizing their biomass in a viable way, both economically viable and environmentally sustainable. Studying the blooms' origins, it is important to take the polyp life stage into consideration to provide a better understanding on the population size of the medusae, which is necessary to make assumption on their population dynamics (Henschke, Stock, & Sarmiento, 2018; C. H. Lucas et al., 2012). This is crucial to implement this into jellyfish population management strategy, avoid depletion of breeding stock of harvested organism (Cid, Hilker, & Liz, 2014; Raveling & Heitmeyer, 1989; Taylor, McAllister, Lawson, Carruthers, & Block, 2011) and to enable future predictions on where and how intense blooms will occur. However, studying both polyp and medusa populations poses its own challenge as jellyfish blooms can come from more than one polyp colony source (William M. Hamner & Dawson, 2009).

As previously mentioned, jellyfish blooms have been categorized into two types of blooms: either 'true' or 'apparent' blooms. While capability of detecting a currently occurring bloom in a given area has been developed (McIlwaine & Rivas Casado, 2021), tracking jellyfish blooms origin(s) requires a thorough understanding on jellyfish population structure and dynamic. A true bloom would be easier to track through the study of local polyp populations, which are responsible for local bloom events (William M. Graham et al., 2001; C. H. Lucas & Dawson, 2014). Meanwhile, predicting an apparent bloom, which population origin(s) is often unknown, would need an understanding of the population structure of a given blooming species, which might have high gene flow rate and possibly higher connectivity between populations from multiple locations under a large geographical coverage (Ben Faleh, Ben Othmen, Deli, Annabi, & Said, 2009; Dong, Liu, Liu, Liu, & Sun, 2016; Stopar, Ramšak, Trontelj, & Malej, 2010).

Investigating population connectivity of marine species can be done through combination of population genetic and environmental data (Selkoe et al., 2010). Sign of genetic partitioning

on a marine planktonic organism would mean localized dispersal through a specific current pattern, which has been demonstrated on mussel larvae in southwest England (Gilg & Hilbish, 2003). Population genetic studies on several scyphozoan species have also been performed. As examples, *Rhizostoma octopus* population in the Irish sea (Lee et al., 2013) and *A. aurita* in the southern North Sea (van Walraven et al., 2016) have both shown genetic structuring within sampled populations. Other study, however, showed contrasting results for e.g. *Pelagia noctiluca* in European seas, in which a lack of genetic structure is observed between sampled populations (Stopar et al., 2010), suggesting admixing between blooming populations in corresponding locations. It is also worth noting that most of these studies on jellyfish population structure focused on the adult stage (medusae) of jellyfish while studies on the polyp stage remain scarce with the exception of van Walraven study on *A. aurita* (van Walraven et al., 2016), which had concentrated on the polyps stage of *A. aurita* to find sign of population structuring. Seo (2021) had studied polyps of *Aurelia coerula* in the Jaran Bay, Korea, and this is another quite recent study on the polyp stage that had been published by the time of the writing of this master thesis. However, no study has been done so far on finding the population connection between the adult stage and the polyp stage.

Mitochondrial COI as genetic marker for DNA barcoding has been used to reveal population structure, or lack thereof, in many marine organism and jellyfish (Lee et al., 2013; Stopar et al., 2010) using the combination of several population structure analyses such as AMOVA and pairwise F_{ST} analysis (L. Excoffier, Smouse, & Quattro, 1992). Mitochondrial COI marker alone, however, sometimes failed to give a definitive population structuring, as in the case of *Pelagia noctiluca* across Eastern Atlantic and Mediterranean Sea (Stopar et al., 2010) where mitochondrial COI in combination with two nuclear internal transcribed spacers (ITS1 and ITS2) failed to reveal any instance of population structuring. In another study, the addition of other markers such as microsatellite markers had been performed on *P. nocticluca* on the same geographical region and managed to find a population structuring where mitochondrial COI cannot (Glynn et al., 2016). However, mitochondrial COI has been proven to be effective for discerning population structure of *A. aurita*, such as it is the case with *A. aurita* in coastal waters of the Irish Sea and Southern England sites (Dawson et al., 2015). Mitochondrial COI marker was also used to allow comparison with other studies that use of mitochondrial COI marker to find population structuring in jellyfish, which is important as this was the first study that incorporates polyp populations data to find connectivity between polyp and medusa populations. While adoption of microsatellite markers might reveal even higher resolutions of

population genetic structuring as suggested by van Walraven (2016), developing microsatellite markers for *A. aurita* would require a separate effort and time which would have extended beyond the allocated time for the completion of this master thesis, hence only mitochondrial COI was used for this study.

DNA analysis and its retrospective molecular tools such as PCR (Polymerase Chain Reaction) amplification and DNA sequencing, thus, have proven to be an effective tool in studying population connectivity in scyphozoan species. While being essential for population genetic analysis, the use of molecular tools is also important for the identification of some cryptic jellyfish species such as within the genus *Aurelia* (Dawson, 2003) and, more specifically, for species identification of scyphozoan polyp stages, which cannot be identified through morphological features alone (Holst, 2012b). Being able to identify sampled polyps is also important because as per the writing of this thesis there is no published identification of *Cyanea capillata* polyps found *in situ* (van Walraven et al., 2016).

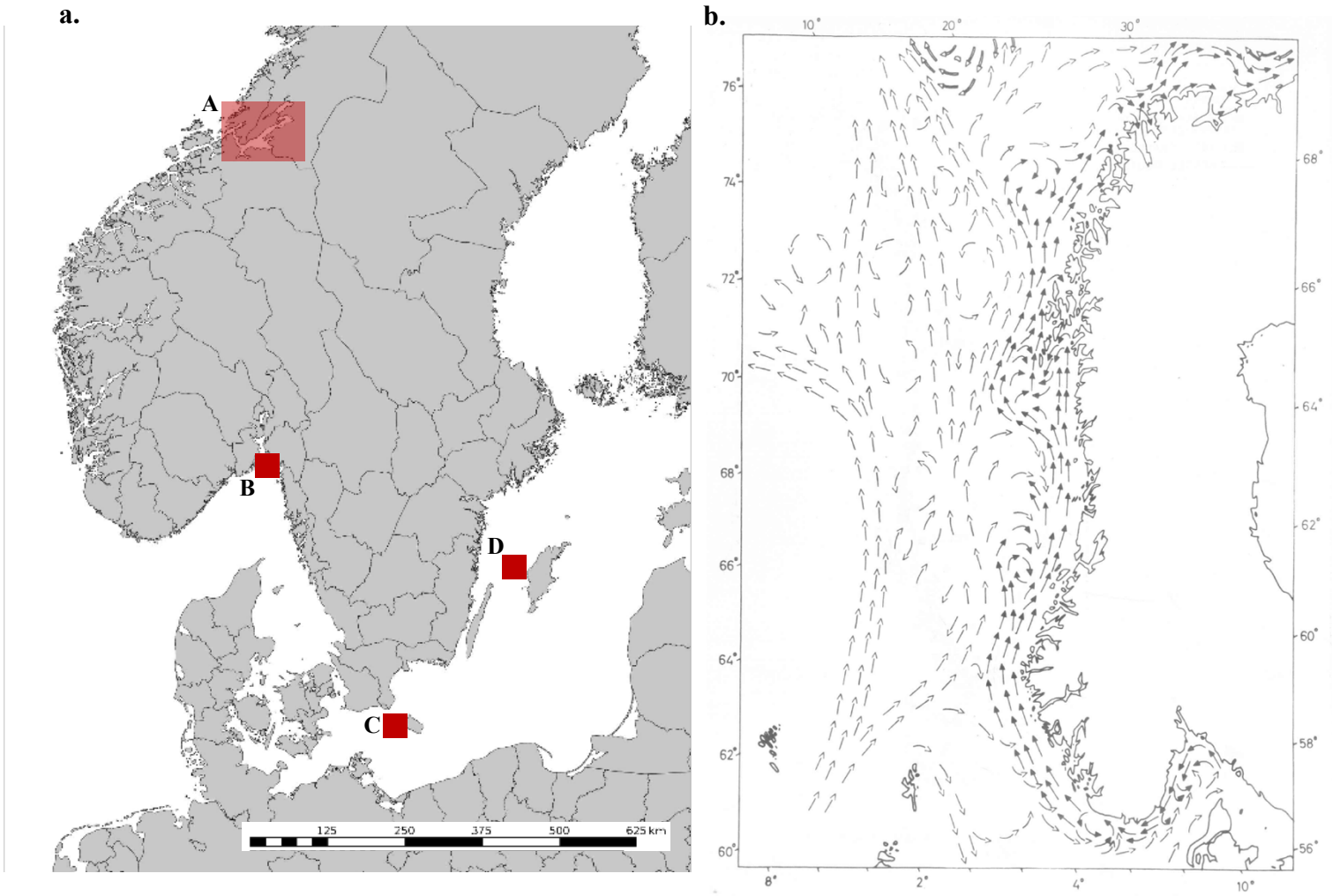
1.5 Study aims

The EU Horizon 2020 funded GoJelly project has multiple work packages with the goal of sustainable utilization of jellyfish biomass. The work at NTNU, including this thesis, fall into GoJelly's Work Package 2 '*Driving mechanisms and predictions of jellyfish blooms*' which aims on studying the drivers of the blooms of important jellyfish species by literature review, population genetics, and advancing analytical model(s) to be able to predict their blooms. This thesis focused on two scyphozoa species commonly found in Norwegian waters: *Aurelia aurita* and *Cyanea capillata*. While the GoJelly project focuses on the Baltic Sea, Norwegian Sea, Eastern Mediterranean, Adriatic Sea and the North-East Atlantic off Madeira, this thesis focuses on the connectivity of jellyfish populations found in the Trondheimsfjord and compare them with reference location from Outer Oslofjord for *A. aurita* and from Baltic Sea for *C. capillata.* In this thesis, barcoding of mitochondrial COI (Cytochrome Oxidase subunit I) gene was used to perform a population genetic analysis on the sampled jellyfish populations. With this data, the thesis aim to (1) reveal any possible intraspecific genetic structuring within the two target species in comparison to their respective reference locations, (2) investigate possible isolated polyp or medusa populations within the Trondheimsfjord system, and (3) reveal jellyfish population structure and connectivity in the Trondheimsfjord through genetic structure comparison between sampled medusae and polyps.

2. Materials and Methods

2.1 Study Area(s)

Aurelia aurita and *Cyanea capillata* specimens for this study were collected from Trondheimsfjord area. Specimens were also collected from reference sites: Outer Oslofjord area (Marius Brygge and Engø Brygge) for *A. aurita* and Baltic Sea (Bornholm Basin and Gotland Basin) for *C. capillata* (Fig. 1a). While Trondheimsfjord and Oslofjord are separated by about 500 km, the two are connected by the Norwegian Coastal Current. The Norwegian Coastal Current comprises partly of North Atlantic Drift water that mixes with the outflow from the Baltic Sea via the Skagerrak (Sætre & Ljøen, 1972). This current flows northward along the Scandinavian coast (Fig. 1b), collecting brackish water from various fjord it passes along the way (Jacobson, 1983). Jacobson (1983) showed how the depth variation of the 34 ‰ isohaline between two stations located 160 km south of the mouth of the Trondheimsfjord and within Trondheimsfjord itself correlated with each other, suggesting transference of coastal water into the fjord. With this in mind, Outer Oslofjord and Baltic Sea were selected as reference sites to see if there exist biological connectivity between the two reference sites and Trondheimsfjord. This also challenges the population structuring hypothesis for jellyfish with metagenetic life cycle, especially *A. aurita*, which has shown population structuring within geographic distance as little as 15 km (Dawson et al., 2015).


a.
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B
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c.

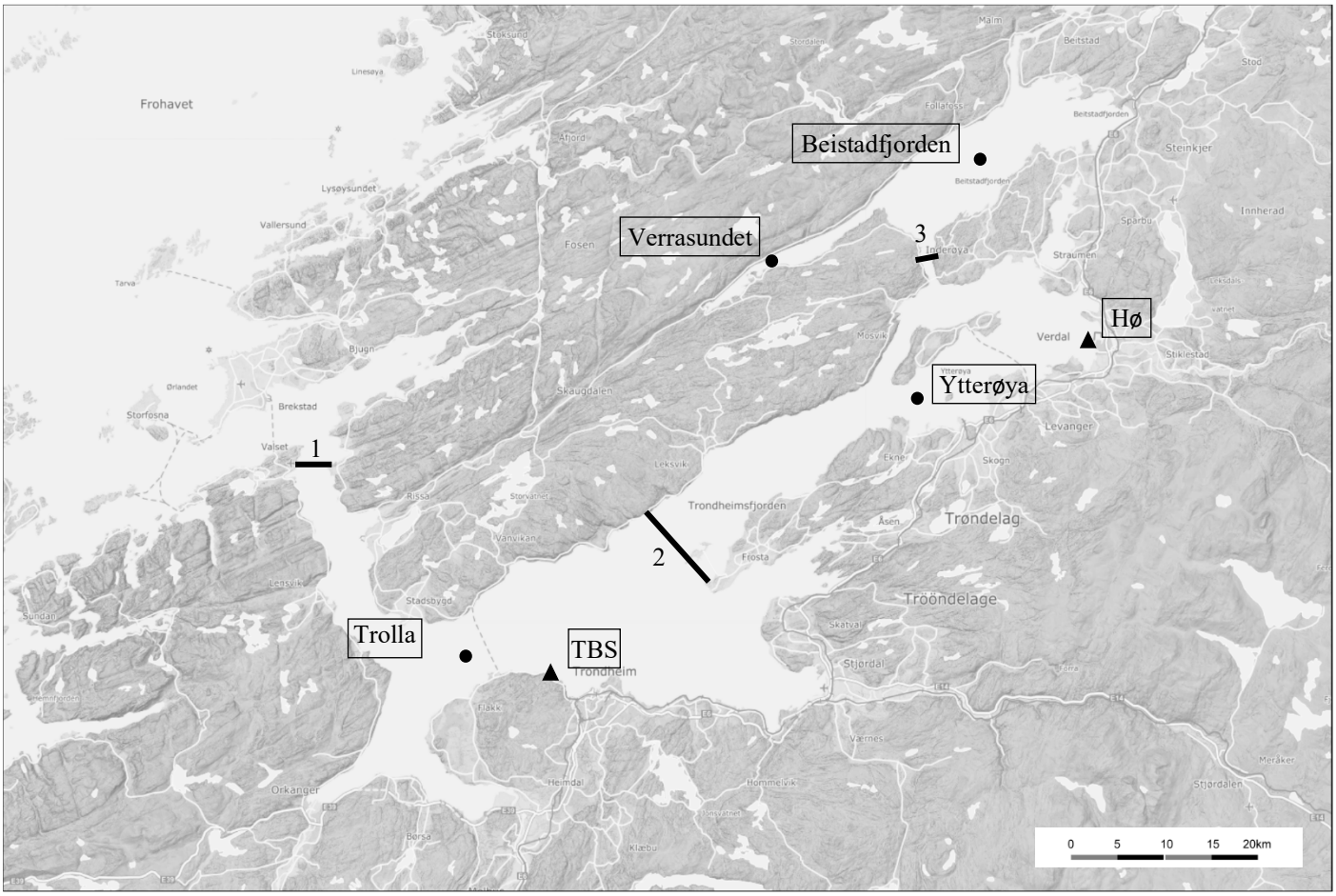


Figure 1a. Map showing approximate sampling sites denoted by red square: (A) Trondheimsfjord, (B) Outer Oslofjord, (C) Bornholm Basin, (D) Gotland Basin. B, C, and D are reference sites. (A) represents main sampling sites which are further expanded in figure 1c. Map data taken from CalTopo.com. **Figure 1b**. Map showing possible surface water movement during summertime for Norwegian Coastal Current. Bold arrow represents surface movement of Norwegian Coastal Current, while thin arrow represents surface movement of Atlantic water. Figure taken from "The Norwegian Coastal Current" (Sætre & Ljøen, 1972). **Figure 1c**. Map showing expanded Trondheimsfjord sampling areas. Black circle represent sampling sites for medusae and black triangle represent sampling sites for polyps. The locations were: Trolla, TBS (Trondheim Biological Station), Ytterøya, Hø, Verrasundet, and Beistadfjorden. Lines represent approximate location of sill within Trondheimsfjord: (1) Agdenes sill, (2) Tautra sill, (3) Skarnsund sill. Map data taken from Norgeskart.no.

Six locations were chosen as for the sampling sites within Trondheimsfjord (Fig. 1c). Sampling locations were selected based on the presence of sills in the Trondheimsfjord, as deep-water jellyfish *Periphylla periphylla* was sampled at the same time. Presence of sills had shown to restrict larval dispersal of *Ciona intestinalis* (Johannesson et al., 2018), causing population structuring between inner fjord populations and open-coast populations. There are multiple sills situated in the Trondheimsfjord (Fig. 1c). Agdenes sill (1) located at the mouth of the fjord, Tautra sill (2) and Skarnsund sill, which located just before the inner most part of Trondheimsfjord. Two sampling sites were located between Agdenes sill and Tautra sill (Trolla and TBS), two between Tautra sill and Skarnsund sill (Ytterøya and Hø), and two beyond

Skarnsund sill (Verrasundet and Beistadfjorden). However, estuarine circulation might facilitate movement of water between the sills through exchange of less saline surface water, which flows toward the open coast, and more saline water from below, which movement is caused by residual compensating current (Jacobson, 1983). This has the potential of increasing gene flow between the areas separated by the sill, thus causing panmixia among the populations found in the Trondheimsfjord.

2.2. Sample collecting methods

Medusae of *Aurelia aurita* were sampled following GoJelly cruise plan and following transect from innermost part of the Trondheimsfjord to the outermost in 26-27 August 2020, using bottom trawling (shrimp trawl, 35 mm stretched-mesh net, 11 mm inner lining mesh size in the cod-end) at various depth depending on the bottom depth of the location. Reference samples of *A. aurita* from Oslofjord were sampled opportunistically by landing net from pier-side in 05 and 18 May 2020 Tissue samples from individual jellyfish were taken using a knife by excising small part at the rim of the bell, put into a 1.5 mL Eppendorf tube, and filled up with 95% ethanol. Medusae from 2019 were sampled during similar GoJelly cruises Polyps of *A. aurita* were sampled from the coast of Trondheimsfjord within the rocky intertidal zone in Hø, Sletvik and TBS. Individual polyps were sampled from both man-made or natural substrates submerged or partially submerged within a depth range of 0.1 to 3 m. Random polyp individuals from 0.5 to 2 mm in size were collected on single substrata to prevent sampling clones of polyps. The polyps were excised and put into tubes of 95% ethanol. Medusae of *Cyanea capillata* were sampled similarly as *A. aurita* from the same cruise using trawl net. However, as no *C. capillata* specimens were detected in 2020, specimens collected in 2018 and 2019 during similar GoJelly cruises with the addition of using hand net for sampling individuals close to the coast were used for this study. Reference samples of *C. capillata* were caught from Borholm and Gotland Basin in the Baltic Sea by GoJelly partners between 2018 and 2019, with samples from 2019 collected in two different season (spring and autumn) using young fish trawl net (JFT) and Bongo net with 500 µm mesh. Samples with ethanol were then kept with or without refrigeration (-20 °C freezer room or in flammable material storage cabinet).

2.3. DNA extractions, amplifications, and purifications

Small part(s) of tissue samples, approximately 0.5 mg in total, were taken from the 95% ethanol tubes into separated new individual 1.5 mL Eppendorf tubes and left to dry overnight within a fume hood to remove the remaining ethanol. DNA extraction was performed using solution of 6% Chelex-100 resin Bio-Rad within Tris EDTA buffer at pH8 according to Granhag et al. (Granhag, Majaneva, & Møller, 2012). 0. 50 µL of the 6% Chelex-100 solution was added into the tubes and flicked gently to mix. The samples were then heated up to 98°C for 10 minutes with the tube's cap perforated to prevent pressure build-up while heating. Samples were then centrifuged at 15000 RPM for 10 minutes. The supernatant was then pipetted into a new Eppendorf tube while carefully avoiding the precipitated Chelex-100 resin in the bottom. DNA extracts were then stored at -20 °C before subsequent or immediately used for PCR amplification (Walsh, Metzger, & Higuchi, 1991). Some extractions for *C. capillata* and *A. aurita* polyps were also performed using Qiagen's DNeasy Blood & Tissue Kits following manufacturer's protocol to obtain cleaner DNA extracts for subsequent PCR amplification uses.

For *A. aurita* medusae, a fragment of mitochondrial gene cytochrome c oxidase subunit 1 (COX1) was amplified by PCR using the primers from van Walraven (2016):

Forward: ScyCOIf (5′-CTATACTTAATATTTGGTGCYTTTTC-3′)

Reverse: ScyCOIr (5′-AAATGTTGGAATARTATTGGRTCTCCT-3′).

Each 20 µL PCR reaction contained 1 µL of each forward and reverse primers, 0.6 µL solutions of 3% dimethyl sulfoxide (DMSO) (Thermo Fisher Scientific), 0.4 µL of 10 mM dNTP mix from four independent 100 mM stock solutions of dATP, dCTP, dTTP, dGTP [brand], 4 µL of 5X Phire reaction buffer (Thermo Fisher Scientific), 11.6 µL ddH_2O, and 0.4 µL Phire hot start II DNA polymerase (Thermo Fisher Scientific), and 1 µL of DNA extract. PCR was done using a thermal cycler with the setting: 5 min at 98 °C followed by 40 cycles of 8 s at 98 °C, annealing step 10 s at 60 °C, 60 s at 72 °C and a final step of 5 min at 72 °C. For *A. aurita* polyps, same primers and PCR setting were used but with added 1 µL solution of $MgCl_2$ subtracted from ddH_2O component (10.6 µL). For *C. capillata*, the same primers for *A. aurita* were used but due to limited success, it was switched to LCOjf (Lee et al., 2013) and HCO2198 (Folmer,

Black, Hoeh, Lutz, & Vrijenhoek, 1994), or CycaF and CycaR, which were designed specifically to better match the reagents used for PCR amplification.

Forward: LCOjf (5′-GGTCAACAAATCATAAAGATATTGGAAC-3′)

Reverse: HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3')

Or,

Forward: CycaF (5'-CAGCCATGATTGGTACAGC-3')

Reverse: CycaR (5'-TGCGGGGTCGAAAAAAGAG-3')

PCR setting for LCOjf and HCO2198 was the same as *A. aurita* medusae, while setting for CycaF and CycaR was 45 s at 61 °C for annealing step.

PCR products were then confirmed using 1.5% agarose gel electrophoresis made from 1X TAE buffer, with 0.01% SYBR safe DNA gel stain (Invitrogen, Thermo Fisher Scientific). 5 µL of PCR products were mixed with 1 µL of 6X gel loading dye and loaded into gel along with 1 kb DNA ladder, then were run 30 minutes at 100 Volt, 150 mAmp. The gel was then visualized using UV transilluminator. PCR products with confirmed bands were then kept in 4°C refrigerator before or immediately purified using Cytiva illustra™ GFX 96 PCR purification kit following manufacturer's protocol for purification from enzymatic reaction. Purified DNA products were then stored in 4°C refrigerator before sending to sequencing.

2.4. Sequence cleaning and genetic analysis

DNA sequences were obtained by sending amplicons to a sequencing company (Mix2Seq, Eurofins Genomics) with the samples tube mixed with 5 µL of corresponding forward primers to obtain forward sequence. DNA sequences from samples were then confirmed again by obtaining the reverse sequence by sending the purified PCR products mixed with 5 µL of corresponding reverse primers. The sequence files were then viewed using Chromas software (Chromas 2.6.6., Technelysium Pty Ltd). A manual end trimming of the sequences and manual proof-reading on the DNA sequences were performed using Chromas software. Forward and reverse sequences of each samples were aligned using contig assembly program (CAP) function of BioEdit 7.0.0 (Hall, 1999) to confirm the overlapping part of the sequence.

Discrepancies of nucleic acid(s) from forward and reverse sequences were referred back to the original raw sequence data and edited accordingly using Chromas before being put into CAP function again. Degeneracy of the nucleic base(s) was renamed according to their IUPAC nomenclatures (Biochemistry, 1986).

Phylogenetic tree were constructed using MAFFT aligned mitochondrial cytochrome oxidase I (COI) sequence data (Katoh & Standley, 2013). Maximum likelihood phylogenetic tree for genus Aurelia was constructed using MEGA X software using Tamura-Nei model (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) with 500 bootstrap replications, gamma distributed with five discrete categories. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Data set was taken from 187 cleaned and aligned COI sequences produced in this study including polyps obtained from 2020 that had been confirmed using contig assembly program, duplicates of sequences containing unresolved ambiguous nucleotide(s), and 324 COI sequences retrieved from from GenBank consisting 186 sequences of *A. aurita*, 125 *Aurelia* sp., 1 *A. limbata*, 10 *A. labiata* and 2 *Rhizostoma octopus*, and thirty *C. capillata* sequences from this study was used as an outgroup (Dawson 2005, Dawson 2015, van Walraven 2016). In this study, only sequences containing single ambiguous nucleotide were duplicated into two sequences, each representing one of the ambiguous nucleotide (e.g. sequence with ambiguous nucleotide of "Y" is duplicated into two sequences containing either "C" or "T" on the same site). Sequences containing more than one ambiguous nucleotides were removed from the alignment and subsequent analyses. This was done to avoid bias from choosing only one nucleotide without losing too many samples. Bootstrapping values are listed on branches nodes. Branches that do not belong to *A. aurita* clade were collapsed. Each red coloured text was representative of one haplotype from this study. Phylogenetic tree for *C. capillata* was constructed using the same method with data set taken from all 38 *C. capillata* sequences obtained in this study along with 23 mitochondrial COI sequences obtained from GenBank based on publication by Holst (2014), which consists of 17 *C. capillata* and 5 *C. lamarckii*, and 1 *A. aurita* as an outgroup. Branches that do not belong to *C. capillata* clade were collapsed.

Minimum spanning haplotype networks were constructed using POPART (Leigh & Bryant, 2015) under default settings. Nucleotide diversity (π) and haplotype diversity (h) were estimated using the program DnaSP 6 (Rozas et al., 2017) for both *A. aurita* and *C. capillata*. Pairwise F_{ST} analyses were performed using Arlequin suite ver. 3.5 (Laurent Excoffier &

Lischer, 2010) with p-values calculated from 20 022 permutations and AMOVA analyses using 10100 permutations for p-values. Significance levels were corrected using the Bonferroni correction method by dividing standard significant value threshold ($p = 0.05$) by the number of multiple test performed on the data set (J. Miller, 1981). There is, however, issues regarding the use of Bonferroni correction. it should be noted that Bonferroni correction can sometimes cause false negative errors due to it deemed being too conservative (Narum, 2006), and arguments against using sequential version of it in ecological studies had been presented (Moran, 2003). For this study, Bonferroni corrections were performed nevertheless to impose stricter and more accurate statistical test to better support the resulting analyses.

3. Results

3.1 *Aurelia aurita*. and *Cyanea capillata* community in Trondheimsfjord

In this study, 140 *Aurelia aurita*. were sampled in 2019 from two locations: 66 from Beistadfjorden and 74 from Trolla. In 2020, a total of 82 *Aurelia aurita*. were sampled within the same fjord system; 20 from Beistadfjorden, 10 from Trolla, 40 from Verrasundet and 12 from Ytterøya. A total of 128 polyps were sampled from Trondheimsfjord in 2020. Extra sequences of polyps—a total of 26—from 2018 were then added to supplement the data (sequence data were taken from Rekstad (2019). For *Cyanea capillata*, 23 specimens were sampled from Trondheimsfjord in 2018, 19 were sampled from 2019, but no *C. capillata* were caught during sampling campaigns in the Trondheimsfjord in 2020.

3.2 Molecular species identification

In total, 187 specimens of adult *Aurelia aurita*., 94 specimens of polyps, and 38 *Cyanea capillata* adult specimens were used for molecular species identification and further analysis which resulted 167 good-quality sequences of adult *A. aurita*, 26 sequences of *A. aurita* polyps, and 38 sequences of *C. capillata*. Among *A. aurita*, 55 sequences resulted from medusae caught in Outer Oslofjord (Marius Brygge and Engø Brygge) in 2020, 53 sequences came from medusae caught in Trondheimsfjord in 2020, and 59 came from medusae caught at Trondheimsfjord in 2019. Among *C. capillata*, 13 samples were analysed from Trondheimsfjord 2018 and seven from Trondheimsfjord 2019, while 18 samples were analysed from the Baltic Sea 2019.

PCR amplification using SCYCOI primers on adult *A. aurita*. specimens obtained in this study resulted in generally clear bands after gel electrophoresis DNA separation and gave 89.3% success rate of obtaining good-quality sequences. Polyps sampled from intertidal areas in Trondheimsfjord in 2020 had a very low amplification success rate (35.3%) from 51 samples tested using SCYCOI primer pairs. Addition of extra $MgCl_2$ into PCR reaction resulted in no immediate observable difference in band intensity. Polyp samples which gave the best amplification result using SCYCOI primers were those which were sampled from Sletvik area with 100% successful amplification rate. Further sequencing of amplicons, however, resulted in inconsistent sequencing results between pairs of forward and reverse sequences. Because of these difficulties, polyp samples from Trondheimsfjord were omitted from all analysis except

for the phylogenetic trees. Instead, supplementary data of 26 *A. aurita* polyps that were sampled from two areas in Trondheimsfjord (Hø and TBS) in 2018 were included into the population genetic analysis. *C. capillata* mitochondrial COI was amplified using SCYCOI primers initially but gave very faint to no bands which did not merit further sequencing (unpublished data). Amplification with Folmer primers (LCO1490/HCO2198) also yielded no successful results for sequencing. Highly successful amplification rate was achieved using Cyca primers pairs. Preliminary amplification test gave Cyca primer pairs a 94.4% success rate from 18 randomly selected samples of *C. capillata*.

MAFFT alignment of the compiled *Aurelia.* spp. sequences resulted in 472 nucleotides long sequences for the phylogenetic tree analysis (Fig. 2). The tree showed that all *Aurelia* spp. specimens used in this study clustered to the same monophyletic group as *A. aurita* sequences taken from GenBank, suggesting that none of the samples used in this study, neither adult nor polyp, belong to the other cryptic *Aurelia* species.

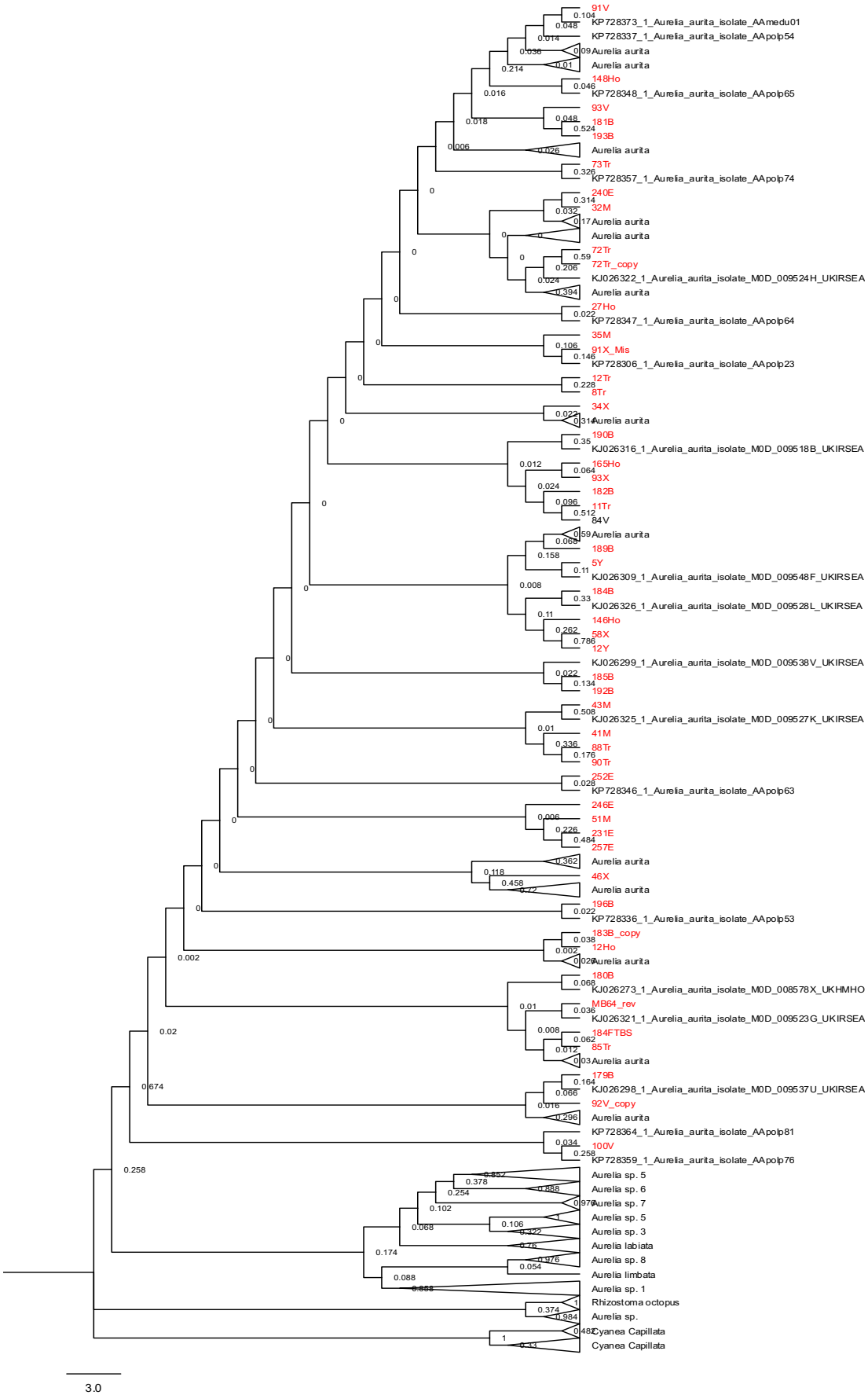


Fig. 2. Mitochondrial cytochrome oxidase I (COI) maximum likelihood phylogenetic tree for genus *Aurelia* was constructed from 187 COI sequences produced in this study including polyps obtained from 2020, duplicates of sequences containing unresolved ambiguous nucleotide(s), and 324 COI sequences retrieved from from GenBank consisting 186 sequences of *A. aurita*, 125 *Aurelia* sp., one *A. limbata*, 10 *A. labiata* and two *Rhizostoma octopus*. Thirty *C. capillata* sequences from this study are used as an outgroup. Bootstrapping values are listed on branches nodes. Branches that do not belong to *A. aurita* clade and branches that does not share nodes with samples from this study are collapsed. Each red coloured text is representative of one sampled haplotype from this study.

For *C. capillata*, MAFFT alignment resulted in 562 nucleotides long sequences for the phylogenetic tree analysis (Fig. 3). The tree showed that sequences used in this study for *C. capillata* haplotype analyses belong to the same monophyletic group as *C. capillata* sequences from publication by Holst (2014).

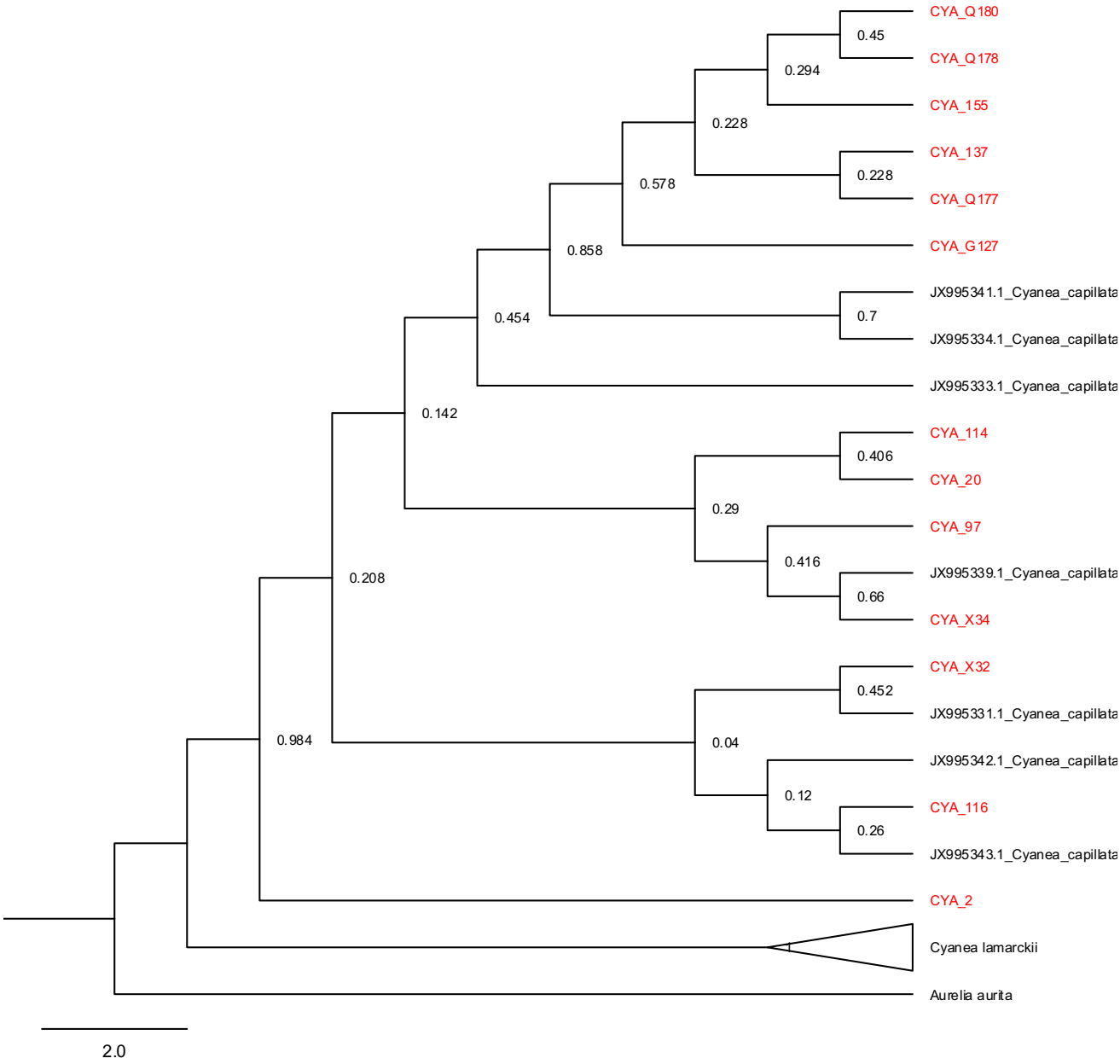


Fig. 3. COI maximum likelihood phylogenetic tree for *C. capillata* was constructed from all 38 *C. capillata* sequences obtained in this study along with 23 mitochondrial COI sequences obtained from GenBank based on publication by Holst (2014), which consists of 17 *C. capillata* and five *C. lamarckii,* and one *A. aurita* as an outgroup. Bootstrapping values are listed on branches nodes. Branches that do not belong to *C. capillata* clade are collapsed. Each red coloured text is representative of one sampled haplotype from this study.

3.2.1 Aurelia aurita

Following MAFFT alignment, analyses of 108 sequences of adult *A. aurita* (525 bp) sampled in 2020 in Trondheimsfjord and Outer Oslofjord (Engø Brygge and Marius Brygge) revealed 35 unique haplotypes (Table 1). Highest haplotype diversity was found at Beistadfjorden, Trondheimsfjord in 2020 (h ± sd = 0.949 ± 0.037, Table 1). Lowest haplotype diversity was found at Engø Brygge (h ± sd = 0.416 ± 0.112). Highest nucleotide diversity (π ± sd = 0.00717 ± 0.00115) was at Beistadfjorden, Trondheimsfjord (Table 1) while lowest haplotype diversity was observed at Engø Brygge (π ± sd = 0.00112 ± 0.00037).

Table 1. Table of haplotype and nucleotide diversity of adult and polyps of *A. aurita* from 2018, 2019 and 2020 between several areas in Trondheimsfjord (Beistadfjorden, Trolla, Verrasundet, Ytterøya) and Outer Oslofjord (Engø Brygge, and Marius Brygge) as reference. Total of each categories are in bold. Values were calculated using DnaSP. N Sample size, N_h Haplotype count, h Haplotype diversity, π Nucleotide diversity.

Area	N	N_h	h ± sd	π ± sd
Engø Brygge 2020	30	7	0.416 ± 0.112	0.00112 ± 0.00037
Marius Brygge 2020	23	10	0.688 ± 0.108	0.00342 ± 0.00091
Beistadfjorden 2020	17	12	0.949 ± 0.037	0.00717 ± 0.00115
Trolla 2020	9	4	0.806 ± 0.089	0.00233 ± 0.00036
Verrasundet 2020	18	10	0.843 ± 0.077	0.00601 ± 0.00124
Ytterøya 2020	11	6	0.873 ± 0.071	0.0036 ± 0.00097
Total 2020	**108**	**35**	**0.805 ± 0.038**	**0.00441 ± 0.00046**
Beistadfjorden 2019	13	7	0.833 ± 0.086	0.00493 ± 0.00127
Trolla 2019	33	17	0.900 ± 0.037	0.00464 ± 0.00063
Total Beistadfjorden and Trolla 2019	**46**	**22**	**0.900 ± 0.027**	**0.00470 ± 0.00058**
Beistadfjorden 2020	17	12	0.949 ± 0.037	0.00717 ± 0.00115
Trolla 2020	9	4	0.806 ± 0.089	0.00233 ± 0.00036
Total Beistadfjorden and Trolla 2020	**26**	**13**	**0.914 ± 0.033**	**0.00564 ± 0.00092**
Hø Polyps 2018	13	8	0.885 ± 0.070	0.00694 ± 0.00155
TBS Polyps 2018	13	4	0.526 ± 0.153	0.00190 ± 0.00059
Total Polyps 2018	**26**	**11**	**0.840 ± 0.053**	**0.00476 ± 0.00088**

Pairwise F_{ST} value showed significant differences between populations from Outer Oslofjord (Engø and Marius Brygge) compared to populations in Trondheimsfjord (Beistadfjorden, Trolla, Verrasundet, and Ytteroya), with Trolla (Trondheimsfjord) and Engø Brygge giving highest pairwise F_{ST} value of 0.57440 (Table 2). Populations within Trondheimsfjord showed no significant difference except for the populations at Trolla and Verrasundet (pairwise F_{ST} = 0.07480). Nested AMOVA result comparing populations from Trondheimsfjord and reference specimens from Outer Oslofjord from 2020 showed that maximum variance was obtained by grouping Marius and Engø Brygge populations as one group and Trondheimsfjord populations as another group (Table 3). This supports population differentation from pairwise F_{ST} data. The

most common haplotype was shared between all sampling locations and was shared between 46 individuals (Fig. 4).

Table 2. Pairwise F_{ST} values among samples of adult *A. aurita* in Trondheimsfjord. Engø Brygge and Marius Brygge represent samples from reference site of Outer Oslofjord. Values in bold are significant (uncorrected P-values ≤ 0.05).

	Engø Brygge	Marius Brygge	Verrasundet	Ytterøya	Beistadfjorden	Trolla
Engø Brygge	0.00000					
Marius Brygge	0.01033	0.00000				
Verrasundet	**0.24906**	**0.14933**	0.00000			
Ytterøya	**0.49929**	**0.31000**	0.05450	0.00000		
Beistadfjorden	**0.30195**	**0.19228**	-0.00541	-0.00821	0.00000	
Trolla	**0.57440**	**0.34452**	**0.07480**	0.03187	0.03022	0.00000

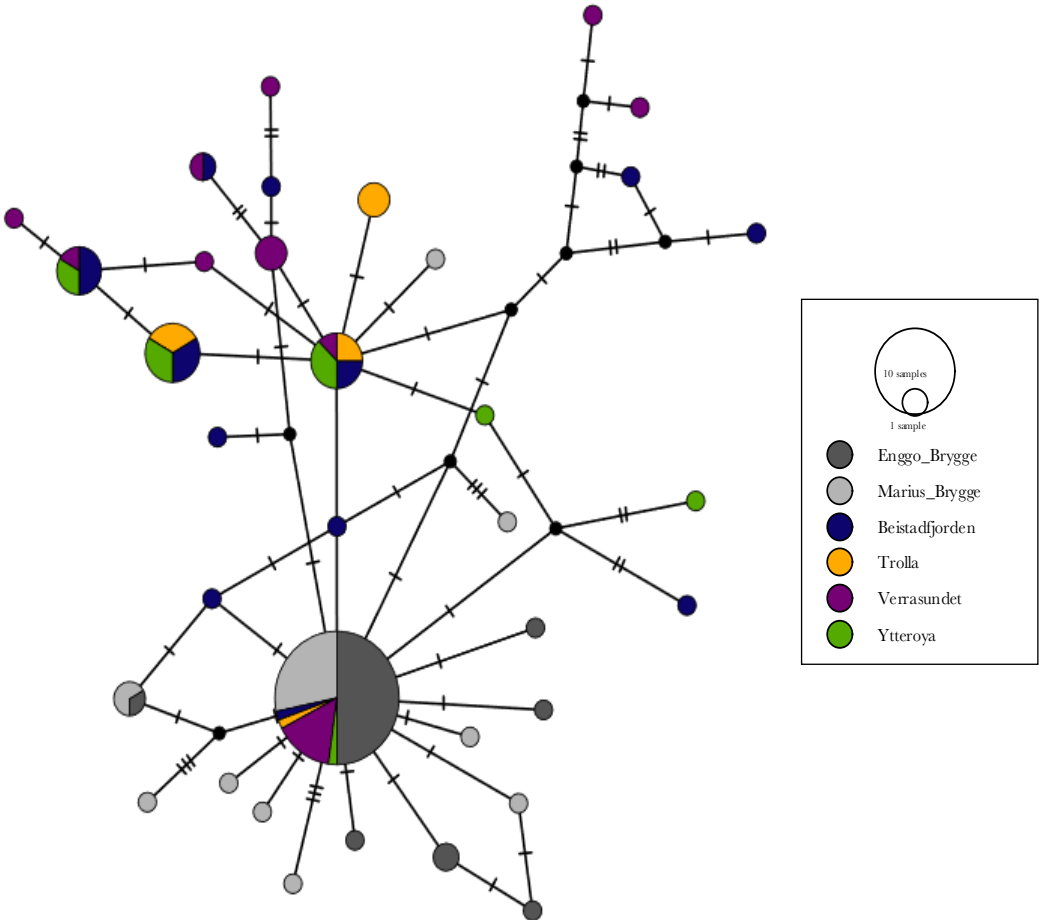


Fig. 4. Minimum spanning haplotype network of 525-bp mitochondrial COI fragments of 108 adult *A. aurita* samples from Trondheimsfjord and Outer Oslofjord as reference site calculated using PopArt. Each circle represents one haplotype with circle size proportional to quantity of sequences sharing the same haplotype. Each horizontal line on the branches represents single mutation and black dots represent hypothetical intermediate haplotypes.

Table 3. Nested AMOVA results of adult *A. aurita* from Trondheimsfjord 2020. Single grouping is enclosed within parentheses. Marius and Engø represent populations from outer Oslofjord reference site of Marius Brygge and Engø Brygge. P-values are in parentheses.

Grouping	Among groups	Among populations within groups	Within populations
	%	%	%
(Marius, Engø)(Beistadfjorden, Trolla, Verrasundet, Ytterøya)	23.18 (0.069)	2.65 (0.108)	74.17 (<0.001)
(Marius, Engø)(Beistadfjorden)(Trolla, Verrasundet, Ytterøya)	19.43 (0.063)	3.07 (0.026)	77.5 (<0.001)
(Marius, Engø)(Trolla)(Beistadfjorden, Verrasundet, Ytterøya)	22.1 (0.018)	1.82 (0.249)	76.09 (<0.001)
(Marius, Engø)(Verrasundet)(Beistadfjorden, Trolla, Ytterøya)	21.63 (0.033)	1.23 (0.234)	77.14 (<0.001)
(Marius, Engø)(Ytterøya)(Beistadfjorden, Trolla, Verrasundet)	20.86 (0.052)	2.51 (0.139)	76.63 (<0.001)

Comparison between *A. aurita* medusae from 2019 and 2020 in Trondheimsfjord showed no significant population structuring within two sampling sites (Beistadfjorden and Trolla) viewed from both haplotype network (Fig. 5) and from pairwise F_{ST} values (Table 3). Compared to 2020 data from the corresponding area, populations sampled at Beistadfjorden and Trolla in 2019 showed the same high level of haplotype diversity (respectively, $h \pm sd = 0.833 \pm 0.086$, 0.900 ± 0.037, Table 1). The two sites from 2019 had the same level of nucleotide diversity (Beistadfjorden 2019 $\pi \pm sd = 0.00493 \pm 0.00127$, Trolla 2019 $\pi \pm sd = 0.00470 \pm 0.00058$), while same sites from 2020 were observed to have different level of nucleotide diversity (Beistadfjorden 2020 $\pi \pm sd = 0.00717 \pm 0.00115$, Trolla 2020 $\pi \pm sd = 0.00233 \pm 0.00036$). Nested AMOVA results comparing populations in 2019 and 2020 of corresponding areas support these results (Table 5). Nested AMOVA was highest when all populations were put into one group.

Table 4. Pairwise F_{ST} values among samples of adult *A. aurita* in Beistadfjorden and Trolla (Trondheimfjords) in 2019 and 2020. Values in bold are significant (uncorrected P-values ≤ 0.05).

	Beistadfjorden 2020	Trolla 2020	Beistadfjorden 2019	Trolla 2019
Beistadfjorden 2020	0.00000			
Trolla 2020	0.03022	0.00000		
Beistadfjorden 2019	0.00783	0.01292	0.00000	
Trolla 2019	0.02456	0.05084	0.00793	0.00000

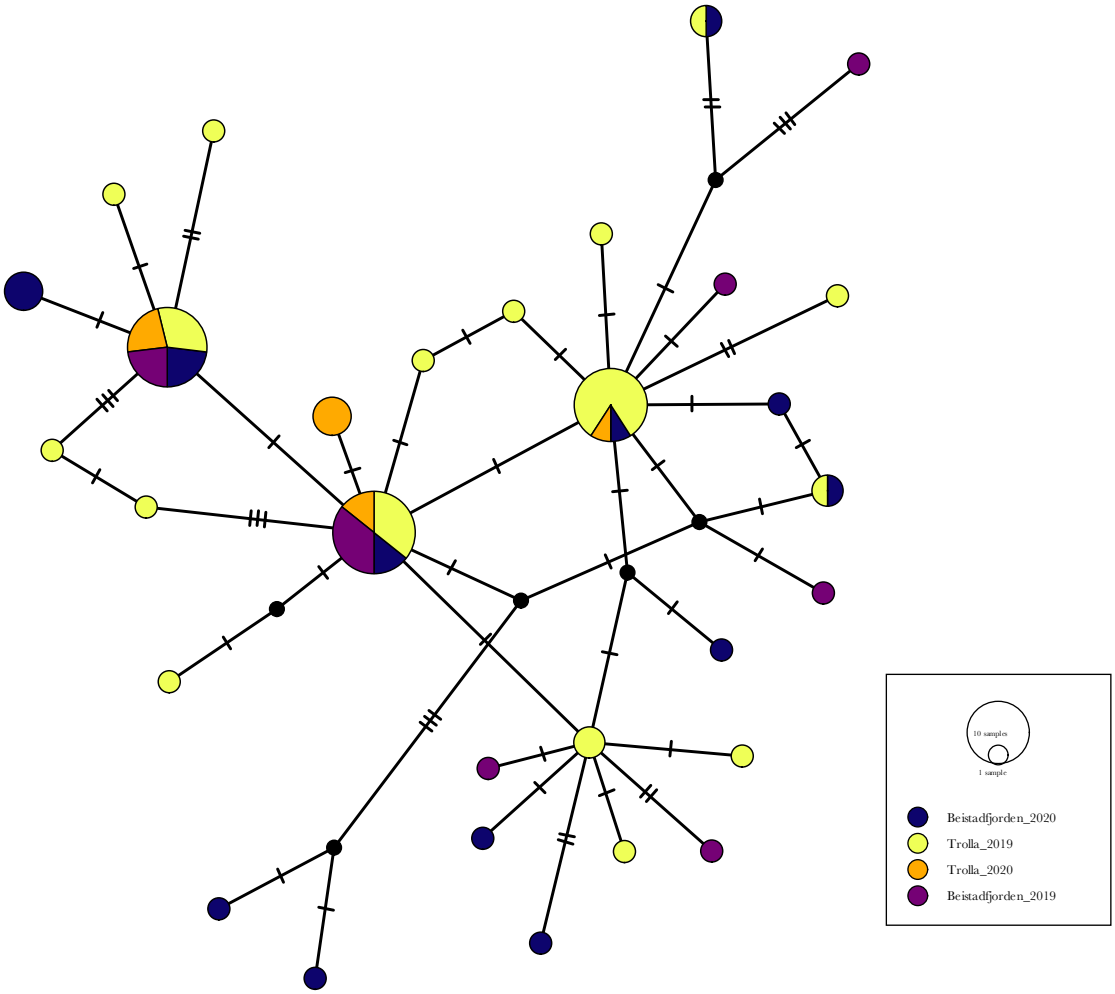


Fig. 5. Minimum spanning haplotype network of 525-bp mitochondrial COI fragments of 72 adult *Aurelia aurita* samples from Beistadfjorden and Trolla (Trondheimsfjord) in 2019 and 2020 calculated using PopArt. Each circle represents one haplotype with circle size proportional to quantity of sequences sharing the same haplotype. Each horizontal line on the branches represents single mutation and black dots represent hypothetical intermediate haplotypes.

Table 5. Nested AMOVA results of adult *Aurelia aurita* from Trondheimsfjord 2019 and Trondheimsfjord 2020 of the same sampling locations. Single grouping is enclosed in parentheses. P-values were within parentheses.

Grouping	Among groups	Among populations within groups	Within populations
	%	%	%
(Beistadfjorden 2019, Beistadfjorden 2020, Trolla 2019, Trolla 2020)	N/A	1.65 (0.133)	98.35 (N/A)
(Beistadfjorden 2019, Trolla 2019)(Beistadfjorden 2020, Trolla 2020)	0.93 (0.327)	1.02 (0.298)	98.05 (0.130)
(Beistadfjorden 2019, Beistadfjorden 2020)(Trolla 2019, Trolla 2020)	-0.37 (1.000)	1.91 (0.191)	98.45 (0.138)

Haplotype network which included data of *A. aurita* polyps from 2018 and medusae from 2020 showed roughly the same distribution with haplotype network of 2020 *A. aurelia*, and no distinct population structuring could be seen. Data of medusae *A. aurita* from 2019 was not included as no significant difference in pairwise F_{ST} was not found between 2019 and 2020 medusa populations in Trondheimsfjord. Pairwise F_{ST} values showed significant population difference between *A. aurita* from Outer Oslofjord (Engø Brygge and Marius Brygge) and *A. aurita* polyps from TBS and Hø. Comparison within Trondheimsfjord (medusae 2020 and polyps 2018), there were significant difference between TBS polyps and Ytteroya (pairwise F_{ST} = 0.16684, Table 6), Beistadfjorden (pairwise F_{ST} = 0.07130), and Trolla (pairwise F_{ST} = 0.25000), but no significant difference between Hø polyps and 2020 adult medusae in Trondheimsfjord (Table 6). Comparison between TBS and Hø polyps showed a significant difference in population structure (pairwise F_{ST} = 0.12852, Table 6). After Bonferroni correction, significant threshold became p = 0.0017. After significance correction, only values from reference outer Oslofjord compared against adult *A. aurita* from Verrasundet, Ytterøya, Beistadfjorden, and Trolla still held their significance, with the addition of polyp from Hø. Haplotype diversity analysis on polyps 2018 showed that TBS polyps had lower number of haplotype than Hø despite same number of samples from each locale (Hø N_h = 8, TBS N_h = 4). Haplotype diversity also differed, with TBS being lower in both haplotype and nucleotide diversity compared to Hø (Hø h ± sd = 0.885 ± 0.070, π ± sd = 0.00694 ± 0.00155, TBS h ± sd = 0.526 ± 0.153, π ± sd = 0.00190 ± 0.00059, Table 1). Nested AMOVA result showed highest among group percentage achieved upon grouping of TBS polyp population together with reference outer Oslofjord populations (Marius and Engø Brygge) as one group against other adult and polyp populations (Table 7). This support pairwise F_{ST} result post-Bonferroni correction on TBS comparison against Engø and Marius Brygge, which lost their significance after Bonferroni correction. Second highest among group percentage was achieved upon grouping of TBS with the other Trondheimsfjord populations.

Table 6. Pairwise F_{ST} values among samples of adult *Aurelia aurita* in Trondheimsfjord and reference outer Oslofjord in 2020 and *A. aurita* polyp populations in Hø and TBS (Trondheimsfjord) in 2018. Values in bold are significant (P-values ≤ 0.05). Values with asterisk are values that still retain their significance after Bonferroni correction. Bonferroni corrected P-values ≤ 0.0017. Above the empty diagonal cells are calculated P-values.

	Engø Brygge	Marius Brygge	Verrasundet	Ytterøya	Beistadfjorden	Trolla	TBS	Hø
Engø Brygge		0.17770+-0.0029	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00330+-0.0004**	**0.00000+-0.0000**
Marius Brygge	0.01033		**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.02182+-0.0011**	**0.00000+-0.0000**
Verrasundet	**0.24906***	**0.14933***		0.07172+-0.0018	0.50537+-0.0035	**0.04839+-0.0017**	0.06093+-0.0016	0.14164+-0.0023
Ytterøya	**0.49929***	**0.31***	0.05450		0.52015+-0.0035	0.23738+-0.0031	**0.02282+-0.0010**	0.70324+-0.0027
Beistadfjorden	**0.30195***	**0.19228***	0.00541	-0.00821		0.19308+-0.0029	**0.04030+-0.0014**	0.80428+-0.0028
Trolla	**0.5744***	**0.34452***	**0.07480**	0.03187	0.03022		**0.01029+-0.0008**	0.22504+-0.0032
TBS	**0.15679**	**0.05656**	0.05874	**0.16684**	**0.07130**	**0.25000**		**0.02153+-0.0010**
Hø	**0.40642***	**0.26662***	0.03548	-0.02355	-0.02575	0.02621	**0.12852**	

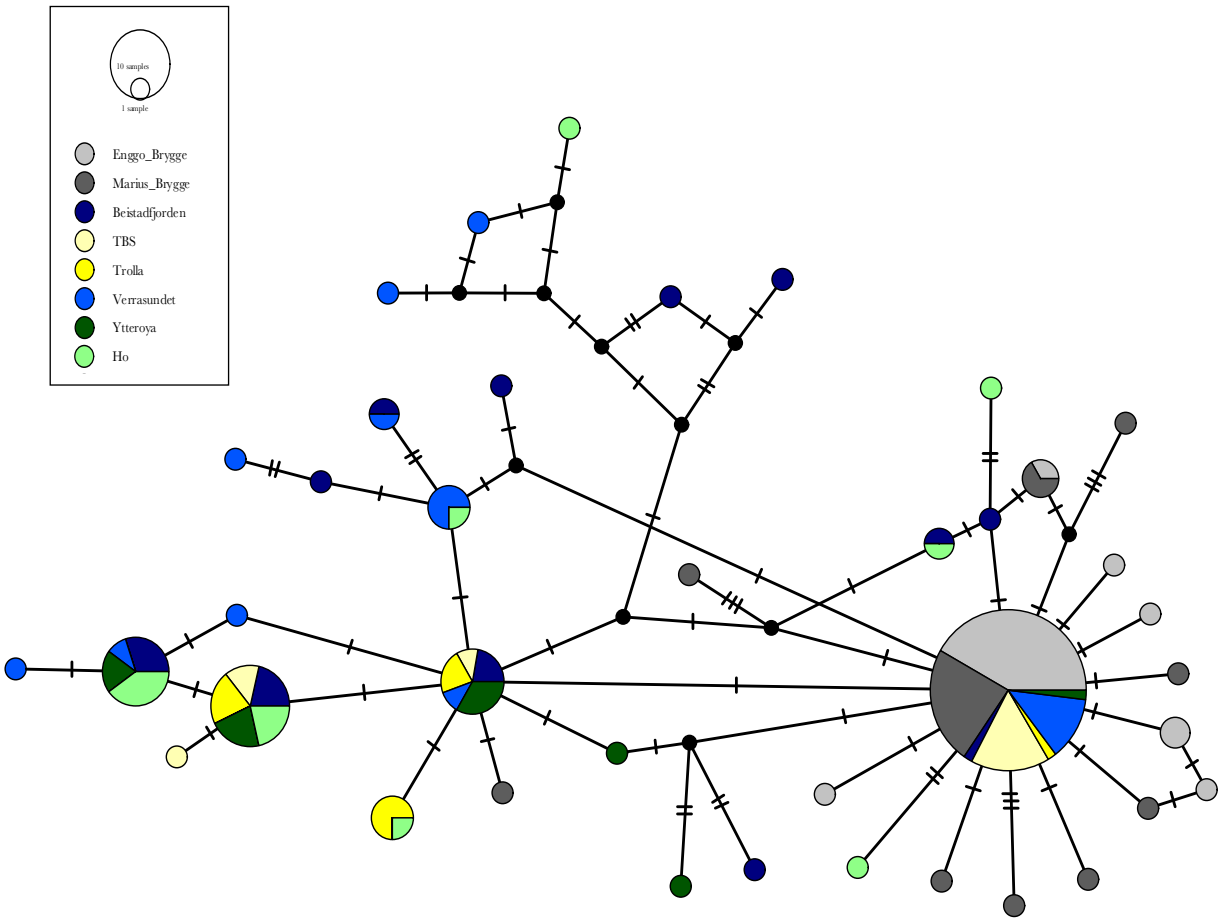


Fig. 6. Minimum spanning haplotype network of 525-bp mitochondrial COI fragments of 134 *Aurelia aurita* samples from Trondheimsfjord and reference southern fjord systems in 2020, and polyp population in Hø and TBS (Trondheimsfjord) in 2018 calculated using PopArt. Each circle represents one haplotype with circle size proportional to quantity of sequences sharing the same haplotype. Each horizontal line on the branches represents single mutation and black dots represent hypothetical intermediate haplotypes.

Table 7. Nested AMOVA results of adult *Aurelia aurita* from Trondheimsfjord 2020 and polyp from 2018. Single grouping were enclosed in parentheses. P-values were within parentheses.

Grouping	Among groups	Among populations within groups	Within populations
	%	%	%
(Marius, Engø)(Hø, TBS, Beistadfjorden, Trolla, Verrasundet, Ytterøya)	19.83 (0.034)	3.98 (0.019)	76.19 (<0.001)
(Marius, Engø)(Hø)(TBS, Beistadfjorden, Trolla, Verrasundet, Ytterøya)	17.36 (0.022)	3.88 (0.011)	78.76 (<0.001)
(Marius, Engø)(TBS)(Hø, Beistadfjorden, Trolla, Verrasundet, Ytterøya)	19.04 (0.006)	2.66 (0.171)	78.31 (<0.001)
(Marius, Engø, Hø)(TBS, Beistadfjorden, Trolla, Verrasundet, Ytterøya)	7.3 (0.213)	11.74 (<0.001)	80.96 (<0.001)
(Marius, Engø, TBS)(Hø, Beistadfjorden, Trolla, Verrasundet, Ytterøya)	21.16 (0.019)	2.7 (0.059)	76.14 (<0.001)
(Marius, Engø, TBS, Hø)(Beistadfjorden, Trolla, Verrasundet, Ytterøya)	10.73 (0.137)	9.67 (<0.001)	79.6 (<0.001)
(Marius, Engø)(Hø, TBS)(Beistadfjorden, Trolla, Verrasundet, Ytterøya)	15.2 (0.050)	4.55 (0.016)	80.26 (<0.001)
(Marius, Engø)(Beistadfjorden, Verrasundet)(Hø, TBS, Trolla, Ytterøya)	16.2 (0.039)	3.44 (0.027)	80.36 (<0.001)

3.2.2 Cyanea capillata

MAFFT alignment of *C. capillata* mitochondrial COI sequences from samples in this study yielded 562 nucleotides long sequences. Haplotype network analysis showed structured *C. capillata* populations in Trondheimsfjord (Trondheim 2018 and 2019) and the Baltic Sea (Bornholm Basin and Gotland Basin 2019) with the exception of one individual sampled from Bornholm Basin in autumn 2019 belonging to same major haplotype found in Trondheimsfjord (CYA_T21) (Fig. 7). There are six mutational difference separating the structured populations between major haplotype found in Baltic Sea (CYA_G128) and haplotype CYA_114 (Fig. 7). Pairwise F_{ST} analysis confirmed the structuring with significant pairwise F_{ST} values upon comparison of Trondheimsfjord and Baltic Sea populations (Table 8). Meanwhile, no significant difference was found in comparison of standard diversity indices within both Trondheimsfjord and Baltic Sea populations (Table 8). Haplotype diversity analysis showed relatively high haplotype diversity within all sampled *C. capillata* populations (Trondheimsfjord 2018 $h \pm sd = 0.769 \pm 0.103$, Trondheimsfjord 2019 $h \pm sd = 0.810 \pm 0.130$, Bornholm Basin Autumn 2019 $h \pm sd = 0.800 \pm 0.172$, Bornholm Basin Spring 2019 $h \pm sd = 0.714 \pm 0.181$) except for populations from Gotland Basin sampled in spring 2019, which only had single observed haplotype (Table 9). Nucleotide diversity was highest in Bornholm Basin Autumn 2019 population ($\pi \pm sd = 0.00652 \pm 0.00259$, Table 9). Nested AMOVA result obtained from comparison between *C. capillata* population sampled from Trondheimsfjord and Baltic Sea (Table 10) showed that highest variance percentage were found within among

groups, in contrast with *A. aurita*, which variance came mostly from within populations. Highest percentage of among groups variance was achieved upon grouping of Trondheimsfjord populations in one group and Baltic Sea Populations in another (Table 10).

Table 8. Pairwise F_{ST} values among samples of adult *Cyanea capillata* from Trondheimsfjord in 2018/2019 and Baltic Sea (Gotland and Bornholm Basin) in 2019. Values in bold are significant (P-values ≤ 0.05). Asterisk denote values that retain their significance after Bonferroni correction. Bonferroni corrected P-values = 0.005. Values above diagonal blank cells are calculated P-values.

	Trondheimsfjord 2018	Trondheimsfjord 2019	Gotland Basin Spring 2019	Bornholm Basin Autumn 2019	Bornholm Basin Spring 2019
Trondheimsfjord 2018		0.76442+-0.0027	**0.00015+-0.0001**	**0.00050+-0.0002**	**0.00000+-0.0000**
Trondheimsfjord 2019	-0.05021		**0.00125+-0.0002**	**0.00494+-0.0005**	**0.00070+-0.0002**
Gotland Basin Spring 2019	**0.83247***	**0.90113***		0.99995+-0.0000	0.57364+-0.0036
Bornholm Basin Autumn 2019	**0.65261***	**0.64319***	-0.03448		0.38151+-0.0029
Bornholm Basin Spring 2019	**0.78504***	**0.80606***	-0.01083	0.00839	

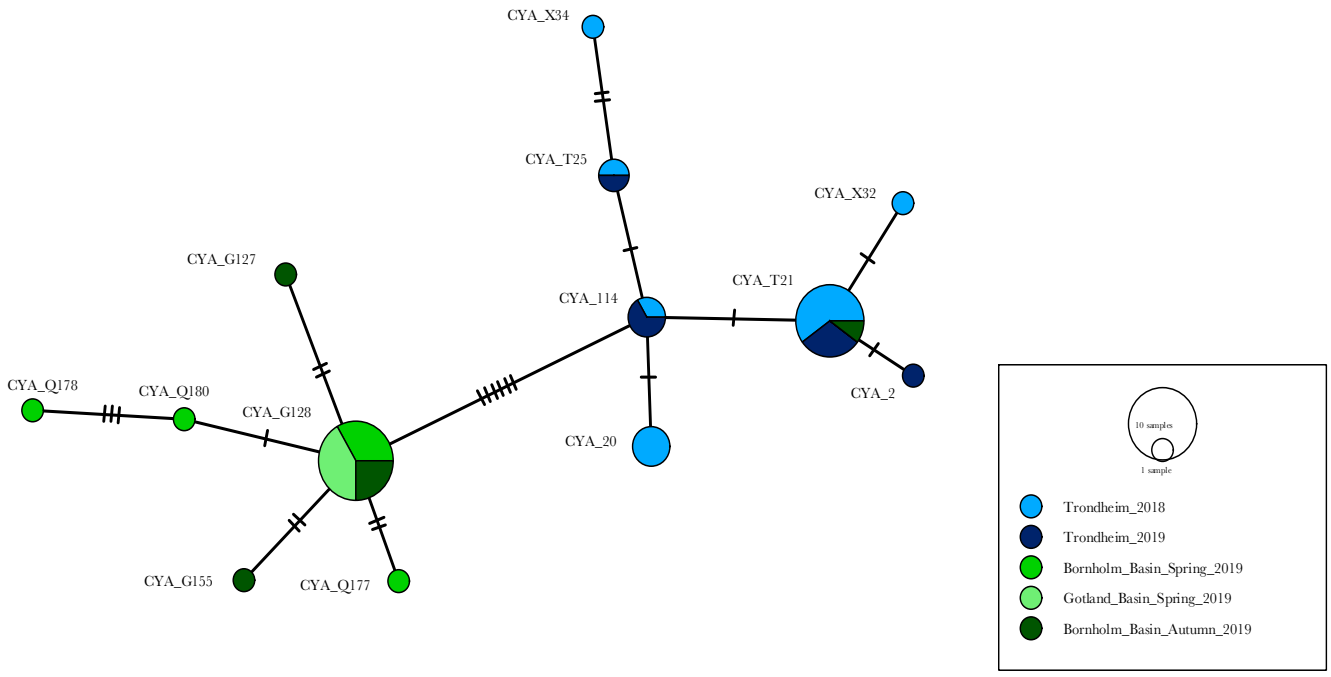


Fig. 7. Minimum spanning haplotype network of 562-bp mitochondrial COI fragments of 38 *Cyanea capillata* samples from Trondheimsfjord in 2018/2019 and reference Baltic Sea (Bornholm and Gotland Basin) in 2019 calculated using PopArt. Each circle represents one haplotype with circle size proportional to quantity of sequences sharing the same haplotype. Each horizontal line on the branches represents single mutation and black dots represent hypothetical intermediate haplotypes.

Table 9. Table of haplotype and nucleotide diversity of adult *Cyanea capillata* populations from Trondheimsfjord and Baltic Sea as reference in 2018 and 2019 (Gotland Basin Spring 2019, Bornholm Basin Autumn 2019, Bornholm Basin Spring 2019). Values were calculated using DnaSP. N Sample size, N_h Haplotype count, h haplotype diversity, π nucleotide diversity.

Area	**N**	**N_h**	**h ± sd**	**π ± sd**
Trondheimsfjord 2018	13	6	0.769 ± 0.103	0.00297 ± 0.00068
Trondheimsfjord 2019	7	4	0.810 ± 0.130	0.00203 ± 0.00052
Gotland Basin Spring 2019	5	1	0.000 ± 0.000	0.00000 ± 0.00000
Bornholm Basin Autumn 2019	6	4	0.800 ± 0.172	0.00652 ± 0.00259
Bornholm Basin Spring 2019	7	4	0.714 ± 0.181	0.00339 ± 0.00132
Total	38	13	0.832 ± 0.042	0.00814 ± 0.00055

Table 10. Nested AMOVA results of adult *Cyanea capillata* from Trondheimsfjord and reference Baltic Sea populations (Gotland spring = Gotland Basin Spring 2019, Bornholm spring = Bornholm Basin Spring 2019, Bornholm autumn = Bornholm Basin Autumn 2019). Each grouping is enclosed in parentheses on Grouping column. P-values are within parentheses.

Grouping	Among groups	Among populations within groups	Within populations
	%	%	%
(Trondheimsfjord 2018, Trondheimsfjord 2019)(Gotland spring, Bornholm spring, Bornholm autumn)	76.86 (0.097)	-0.33 (0.749)	23.47 (<0.001)
(Trondheimsfjord 2018)(Trondheimsfjord 2019)(Gotland spring, Bornholm spring, Bornholm autumn)	71.67 (0.099)	0.49 (0.583)	27.84 (<0.001)

4. Discussion

Within the framework of the GoJelly project, which has the goal of providing knowledge to support environmentally and economically responsible utilization of jellyfish biomass, the main aim of this study was to provide data and understanding of jellyfish bloom connectivity for a sustainable management of jellyfish stocks. Population genetics as a technique has contributed widely to the sustainable fishery management (Waples, Punt, & Cope, 2008; Ward, 2000). Ward (2000). In the review, it is mentioned that through genetics, harvested fish populations were able to be properly identified, especially in collection of populations that contain genera with multiple morphologically near-identical species, which is important for proper stock management plan. However, full integration of such studies for fishery stocks management plans is uncommon (Bernatchez et al., 2017). The reason is because geographic connectivity in marine vertebrates are usually poor due to high homogeneity for marine fish, which would not be useful for defining a discrete fish population (Ward, 2000). However, population genetic data on marine invertebrates has shown otherwise, with many species exhibiting local genetic differentiation and low gene flow (Thorpe, Solé-Cava, & Watts, 2000), allowing stock planning on a local scale, and thus has a great potential to be used in fishery management, such as jellyfish fisheries.

4.1. Medusae population structure

4.1.1. Aurelia aurita

Analyses of pairwise F_{ST}, haplotype network, and AMOVA analyses on mitochondrial COI of *Aurelia aurita* in this study support the idea of genetically distinct medusae populations within Trondheimsfjord compared to references from Outer Oslofjord populations. This is consistent with the findings regarding genetic structure differentiation between geographically separated population of *A. aurita*, which showed differentiation on a scale of tens to hundreds kilometres (Dawson et al., 2015; van Walraven et al., 2016). Population structure analyses showed that some level of population differentiation can be observed in *A. aurita* populations that are separated by roughly 500 km. It is also worth noting that in previous study, while separation of 15 km was observed in *A. aurita*, the localities of where the study was performed including marine lakes which are rather isolated compared to other open-coast localities (Dawson et al., 2015). This study showed population differentiation despite dispersal of populations via the Norwegian Coastal Current that connects the two fjords (Jacobson, 1983).

Presence of marine physical barriers such as sills within fjord systems have been shown to restrict dispersal of marine larvae for populations in the fjord and in more open-coastal sites (Johannesson et al., 2018). In Trondheimsfjord, the three sills at Agdenes, Tautra, and Skarnsund would then be expected to isolate populations within them, but this seems not to be the case for *A. aurita* populations in Trondheimsfjord. With a higher geographic resolution of Trondheimsfjord, significant genetic differences could only be observed for Trolla and Verrasundet populations. However, among-groups variance was highest when testing both reference Outer Oslofjord populations versus all populations in Trondheimsfjord (Table 3), showing no differentiation for population among different areas inside Trondheimsfjord. Moreover, after considering inflation of familywise error rate (FWER), which causes increase of type I error in a multiple hypothesis tests, there is chance that this borderline significant p-value would fail to confirm its significance. This type of error is prevalent when performing multiple comparisons on a data set, and it has been suggested that some form of correction would be necessary to account for such errors, thus minimizing type I error when performing hypothesis tests (Hauser, Wakeland, & Leberg, 2019). One way to account for FWER is to use the Bonferroni correction method, which controls for type I errors at the expense of increasing type II errors (J. Miller, 1981). Indeed, after applying such correction on the pairwise F_{ST} results, comparison between Trolla and Verrasundet failed to confirm its significance. These results support that there is no structured sub-population within *A. aurita* populations of Trondheimsfjord, thus suggesting a high rate of gene-flow between sampling areas in Trondheimsfjord despite presence of sills.

Also, analyses on *A. aurita* population sampled in 2019 and 2020 support the population panmixia within medusae in Trondheimsfjord with no significant difference between populations in Beistadfjorden and Trolla. It is unknown, however, if such gene flow is the result of historical geological event or cotemporary human activities. It is also worth noting that COI haplotype network of medusae *A. aurita* showed a star-shaped pattern, a typical topographical pattern as the result of founder effect by one or more dominant species or haplotypes, which is usually an indication of the introduction of non-native species or haplotypes, whose spread mediated through contemporary anthropological activities (Bayha et al., 2015; Parisod, Trippi, & Galland, 2005; Seo et al., 2021). A study by Miller (2012) had shown that the use of COI marker, along with nuclear marker of ITS1 and ITS2, allow the monitoring of change at historical scales in population structure of *Pelagia noctiluca*, and it would be interesting to see such analyses done in *A. aurita* population within Trondheimsfjord.

4.1.2. Cyanea capillata

Similarly to *A. aurita* populations in Trondheimsfjord, all analyses (haplotype network, pairwise F_{ST}, and AMOVA) suggested a structured populations between *Cyanea capillata* populations in Trondheimsfjord and reference population from the Baltic Sea. However, unlike *A. aurita*, which showed lesser degree of genetic structure differentiation, *C. capillata* seemed to be more structured when analysed using COI marker. Haplotype network analysis divided the samples into two clearly distinguishable clades (Fig. 7), one presenting population in Trondheimsfjord and another in the Baltic Sea. Two neighbouring haplotypes between the two clades were separated by six genetic polymorphisms. *C. capillata* within the same area gave non-significant values for both populations in the Trondheimsfjord and the Baltic Sea.

Despite low gene flow, however, we could see that there is one Baltic Sea haplotype of *C. capillata* present in the Trondheimsfjord haplotype (CYA_T21). There are several ways to explain this. One explanation is that there was a mistake during DNA extraction procedures, allowing a wrong sample to be amplified or cross samples DNA contamination during extraction. Sequencing error would be highly unlikely as the closest of the two haplotype groupings differed by six nucleotides. A more biological based explanation is that there were individuals that belong to the Trondheimsfjord haplotype present within the Baltic Sea population or that this particular haplotype can be found all over globe. The latest explanation deserves attention as it may skew the conclusion of this study on population structuring of *C. capillata* in Trondheimsfjord. Beforementioned scenario could mean that this particular *C. capillata* haplotype could be present globally and that a portion of population Trondheimsfjord could be part of a global population which origins is not known yet. This can be resolved by another repetition, possibly with larger sample size, to see if this is actually the case or just an isolated event or an experimental error.

4.1.3. Aurelia aurita versus Cyanea capillata populations

Aurelia aurita and *Cyanea capillata* are both metagenetic jellyfish species (see (van Walraven et al., 2016) for *A. aurita* and (Holst & Jarms, 2010) for *C. capillata*), which was proposed as one of the causes of population structuring in jellyfish due to their sessile polyps stage (Abboud, Gómez Daglio, & Dawson, 2018). Significant pairwise F_{ST} values after Bonferroni correction in *A. aurita* populations showed relatively low values (within range of 0.19–0.57, Table 2) while *C. capillata* populations showed relatively high values (within range of 0.64–

0.90, Table 8). Similarly, the highest among-population variance in *C. capillata* was obtained from nested AMOVA upon grouping of Trondheimsfjord population versus Baltic Sea populations (Table 10). A contrast to AMOVA analyses was also apparent with *A. aurita* populations having higher variance component within populations while *C. capillata* populations having higher variance among populations. These results suggest lower gene flow between *C. capillata* populations from Trondheimsfjord and reference Baltic Sea as compared to gene flow between *A. aurita* populations from Trondheimsfjord and reference Outer Oslofjord.

However, it is important to note that fewer *C. capillata* were sampled in this study compared to *A. aurita*. Thus, there is a chance that with this samples size, an incomplete estimate of genetic diversity had been presented, such as it is the case with *C. capillata* populations in Gotland Basin sampled in spring 2019 which showed only one single haplotype out of five samples. A previous study has shown the importance of adequate sample size when trying to reveal major intraspecific variation within a species (Phillips, Gillis, & Hanner, 2019). Although the same study has suggested that the typical number of adequate sample size ranges from 5 to 10 individuals for revealing intraspecific genetic variation, it further stressed that the size can also be taxon specific and no effective sample size has been proposed for *C. capillata*. Thus, it is of interest to repeat the analyses with larger sample size in order elucidate further genetic structuring of *C. capillata* in the Trondheimsfjord and the Baltic Sea.

It should also be pointed out that the contrast in genetic structuring between populations of *A. aurita* and *C. capillata* was viewed from disproportional separation of distance, with a distance between Trondheimsfjord and Outer Oslofjord of ≈500 km and Trondheimsfjord to Bornholm or Gotland Basin, Baltic Sea with a distance of ≈930 km. Longer distance and additional presence of oceanographic barriers that restrict gene flow might account for the strong genetic differentiation in *C. capillata* populations from Trondheimsfjord and the Baltic Sea. Differentiation at $F_{ST} \geq$ ~0.05 at the scale of ~15−30 km distance in the Palau marine lake system and ~30–300 km in coastal and more open ocean ecosystem is common in jellyfish with metagenetic life cycle (Abboud et al., 2018). This has also been proven to be the case for *A. aurita* in an artificial lake and adjacent localities in southern England at 15 km scale (Dawson et al., 2015), *Catostylus mosaicus* in coastal lagoons and bays of southeast Australia at 60–200 km scale (Dawson, 2005), and *Chrysaora melanaster* with more extreme spatial scale of thousands of kilometres (Dawson et al., 2015). Jellyfish with non-metagenetic life cycle

showed an even larger F_{ST} differentiation scale of thousands to tens of thousands kilometres such in the cases of *Pelagia noctiluca* and *Periphylla periphylla* (Abboud et al., 2018). Other than distance, Abboud et al. (2018) also found evidence of other geographical attributes such as onshore-offshore, depth, and latitude correlating to the structuring of jellyfish populations. These factors complicate approximation of the rate of differentiation between the two studied species as the two might have different habitat preferences (Gröndahl, 1988). Thus, in order to make more accurate comparison between species, a single reference site should be used or two species of jellyfish should be sampled from the two reference sites.

4.2. Polyp population structure and their connection to medusae populations

In jellyfish with metagenetic life cycle, the role of the asexual reproduction stage of sessile polyps has been stressed as an important part for jellyfish bloom formation (C. H. Lucas et al., 2012). Thus, to better understand the population dynamics of jellyfish with metagenetic life cycle it is crucial to not just perform studies on free-swimming medusae populations, but also on the benthic polyp life stage. While several publications have provided some knowledge regarding genetic population connectivity of the medusae stage, e.g. *A. aurita* along Mediterranean coast of Tunisia (Ben Faleh et al., 2009), *Pelagia Noctiluca* across European seas (Stopar et al., 2010), *Alatina alata* across tropical locations (Lawley et al., 2016), very few have focused on the polyp populations. As an examples, van Walraven et al. (2016) studied the population structure of *A. aurita* polyps in the southern North Sea and found evidence on population structuring in polyps on similar spatial scales. In addition, a study by Seo et al. (2021) studied the population structure of *Aurelia coerulea* polyps in the Jaran Bay and found evidence of polyp populations structuring based on mitochondrial COI and 16S rRNA markers. In the present study, data and analyses based on mitochondrial COI on polyps and medusae populations for *A. aurita* in Trondheimsfjord are presented.

The analyses of *A. aurita* polyp and medusae populations in Trondheimsfjord suggests a population structuring between Hø polyp populations and reference populations in Outer Oslofjord. Also, homogeneity between Hø polyp populations and medusae populations in inner Trondheimsfjord was suggested. This can be seen on the haplotype network analysis of *A. aurita* polyp and medusae, with lack of haplotype separation between Trondheimsfjord populations. This is expected as Hø is close to the other medusae sampling areas, especially Ytterøya and strongly suggests that Hø polyp populations recruit into local medusae

populations in Trondheimsfjord. Polyp populations sampled from TBS, however, showed contradictory results. While the initial pairwise F_{ST} analysis before Bonferroni correction suggests population differentiation at TBS when compared to Outer Oslofjord, Ytterøya, Beistadfjorden, Trolla, and Hø but not from populations at Verrasundet. Applying Bonferroni correction to these pairwise F_{ST} values caused all values to lose their significance, suggesting populations admixing between Outer Oslofjord and Trondheimsfjord. Further analysis using nested AMOVA showed highest among-group variance in grouping of the TBS polyp population to Outer Oslofjord medusae population, suggesting similarity of TBS polyp population to Outer Oslofjord population compared to other areas in Trondheimsfjord. Based on the current patterns in Trondheimsfjord (Jacobson, 1983), it can be expected that freshly strobilated ephyrae could drift from TBS to either the inner and/or outer part of Trondheimsfjord depending on whether strobilation happens during a low or high tide situation. This current pattern is likely to influence dispersal of strobilated ephyrae, increasing the rate of gene flow between inner and outer part of Trondheimsfjord. Therefore, polyp populations on TBS and possibly on the surrounding coastal area between Agdenes sill and Tautra sill could be said to be the intermediate populations that connect *A. aurita* medusae populations in Trondheimsfjord and reference Outer Oslofjord populations in term of their genetic structure. It also can be proposed that this area is the point of mixing between Trondheimsfjord populations and populations from outside the fjord that are drifting northwards with the Norwegian Coastal Current from more southerly regions e.g. the Outer Oslofjord area. Thus in term of jellyfish bloom type, this would be considered as mixture of true and apparent bloom. To create better resolutions of population connectivity, it is suggested that future sampling should cover more areas outside Trondheimsfjord. From the result, gradient of genetic connectivity along the polyp population can be made to see the rate of population differentiations of *A. aurita* polyp in Trondheimsfjord compared to reference Outer Oslofjord.

One irregularity that is worth consideration in detail is the difference between *A. aurita* polyp population at TBS and medusae population at Trolla. Interestingly, medusae sampled from Trolla, a location closest to TBS, suggest a differentiation between TBS polyp and Trolla medusa populations (based on a high pairwise F_{ST} value of 0.250, significant before applying Bonferroni correction). It has been assumed that due to the proximity of the locations, the two populations would roughly have the same genetic structure as it is the case with Hø polyp populations and neighbouring Ytterøya medusa populations. An explanation for this is that

with the high rate of water exchange of Trondheimsfjord (Jacobson, 1983), local jellyfish populations (both medusae, planulae, small ephyrae) are quickly drifted seaward before establishing themselves within the same area where strobilation happened, while jellyfish that mature from the inner part of Trondheimsfjord are able to retain themselves by producing swimming behaviour as reviewed by Graham (2001). On a site note, previously in this study, it had been shown how sills had little impact on the gene flow of *A. aurita* within Trondheimsfjord populations. This study, then, had shown population connectivity of *A. aurita* medusae through intermediate polyp populations found in rocky intertidal zones. It would be of interest to see if there are polyps of *A. aurita* can be found on the benthic zone below Trondheimsfjord sills depth and see if there are connectivity between polyps located in deeper and shallow water in the fjord. Nonetheless, aforementioned irregularity highlights difference in how physical barriers could influence the movement of non-sessile stages of jellyfish. Thus, in order to better understand jellyfish population dynamics and structure, information on the polyp populations should not be ignored.

4.3. Challenges

In this study, as stated above, several challenges were encountered. One of the major challenges was the lack of good polyp population data for population genetic analyses. In total, 128 polyp specimens were sampled from the rocky intertidal area around Trondheimsfjord. Due to time constraint and low success rate, only 51 were put through amplification process. In the end, only few were able to produce verifiable sequences, but the numbers were too few to represent populations of the area and thus omitted from population genetic analyses. From all the sampling locations, only polyps sampled from Sletvik produced clear bands on gel electroporation (unpublished data), but subsequent sequencing process produce resulted in bad quality sequences. Sequencing results showed inconsistency of quality between forward and reverse sequenced amplicon, and as good sequence data require a contig, these data were not used for population genetic analyses. Some reverse sequence data from Sletvik were put through identification and almost half matched as *Aurelia* spp. in BLAST (unpublished data). Looking back, it is suspected that the samples collected could be members of different jellyfish taxa which could not be amplified using SCYCOI primer or that the samples collected were simply other members of coelenterate like anemone or other hydrozoan polyps with similar morphology, supporting the fact that correct species identification require use of molecular tools (Holst, 2012b). Testing this speculation would require the use of universal primers such

described in several primers studies for barcoding (Leray et al., 2013; Lin, Luzon, Licuanan, Ablan-Lagman, & Chen, 2011). Other challenge relating to the use of polyps for population genetic analysis on jellyfish is the difficulty of sampling polyps compared to medusae. As polyps are part of the benthos, access to benthic habitats is necessary for sampling. In this study, polyps were collected from the intertidal zones of the Trondheimsfjord that are accessible during low tide. Polyps were not found in deeper parts of the fjord despite attempts with NTNUs remotely-operated vehicle (ROV) Minerva I (Nicole Aberle and Sanna Majaneva, personal communication). The necessity of searching for polyps also in deeper benthic habitats was also stressed by a study of van Walraven et al. (2016) where coastal sampling yielded only *A. aurita* polyps. Like in the beforementioned study, no polyps of *C. capillata* were found during the sampling campaigns of this study. So far, *C. capillata* planulae have been observed to develop into polyps in laboratory setting (Holst & Jarms, 2010), thus their polyps should also be present but haven't been observed *in situ* yet. Different explanations have been provided regarding their absence from the sampling areas. One explanation could be that *C. capillata* inhabits deeper waters (Hay, Hislop, & Shanks, 1990), and thus their polyps could be situated in deeper benthic environments which would require more intensive sampling on large spatial and temporal scales using e.g. scuba diving or remotely operated vehicles, which can be costly.

Sampling methods for medusae and polyps should also not be overlooked. In this study, reference samples from Engø and Marius Brygge were collected by hand net. This means the sampling area is relatively limited compared to the sampling campaign performed in Trondheimsfjord, where bottom trawling over larger distance was performed, thus covering larger area. Limited sampling area could mean underrepresenting haplotype from the given area. In polyps sampling, consideration should also be taken. Polyps, as previously mentioned, can undergo asexual reproduction, forming multitude of clones. Therefore, the chance that a patch of polyps on a substrate would be a colony of clones is highly likely, hence only one sample should be taken from a patch of polyp colony to avoid sampling clone. The prevalence of colonies of polyp clones also relevant for medusa sampling technique. With sampling area being close to the coastal area where *A. aurita* polyps were observed in large numbers (van Walraven et al., 2016), there is chance that a larger proportion of medusae populations in the area come from one dominant polyp colony. This could be the case with reference samples from Engø Brygge with a rather low haplotype and nucleotide diversity. To prevent this, it would be best to perform sampling all along the coastal area or perform sampling using boat

in the middle of the sampling area to make sure that local medusae population intermix with other populations within the same sampling area before being sampled.

4.4. Implication for the study

Jellyfish fishery depends on the bloom formation to harvest an economically viable quantity of biomass. While it is uncommon, a decline of local jellyfish populations due to overfishing has been documented for *Rhopilema esculentum* in China (Dong, Liu, & Keesing, 2014). Therefore, studies on the origin, timing, and amplitude of jellyfish blooms are necessary to enable a sustainable fishery. It is impossible, however, to evaluate whether these aggregations of jellyfish are the result of local blooms or the result of redispersal and redistribution events of multiple jellyfish blooms by direct observation. In this context, population genetic studies could provide valuable data where traditional stock assessments have their limitations thus improving existing stock management plans for jellyfish (Carvalho & Hauser, 1995). Some jellyfish species, such as *C. capillata* of Trondheimsfjord, show a local genetic structuring in comparison with Baltic Sea population based on genetic analyses, suggesting a separation of medusae populations. In parallel with medusae populations, Lucas et al. (2012) presented the importance of jellyfish polyps in maintaining medusae populations which subsequently cause blooms, and so a connectivity of polyp and medusa populations should also be explored to draw the whole picture of jellyfish population demography. While this study showed a differentiation in genetic structure of *A. aurita* medusae populations between Trondheimsfjord and Outer Oslofjord, some polyp populations sampled in Trondheimsfjord showed higher connectivity at some level between Trondheimsfjord and Outer Oslofjord. Mass strobilation event of polyps located within the inner part of Trondheimsfjord could point at upcoming jellyfish blooms in the same area while strobilation events of polyps located at the outer part of Trondheimsfjord might be more relevant for bloom events at other areas along the Norwegian coast. This information can be crucial when developing fishery management plans for jellyfish, as e.g. WP2 activities within GoJelly which aim for the development of a larval dispersion drift model for jellyfish to allow more reliable predictions of jellyfish blooms.

4.5. Future improvements

While this study revealed a distinct population genetic structure in *Aurelia aurita* and *Cyanea capillata*, several improvements could be included to better support the results. Sampling on polyp populations—while difficult, as discussed in previous section—should be expanded as this study has stressed the relevance of polyps as a connecting bridge between two genetically differentiated populations. Expanded sampling areas could include areas around the mouth of Trondheimsfjord and at deeper parts of the fjord to search specifically for *C. capillata* polyps. Furthermore, it would be interesting to see if this study could be expanded northward, following the Norwegian Coastal Current to see if a certain level of genetic connectivity is maintained along the coast and along the fjords passed by the current. Following the results from this study, it is hypothesized that both medusa and polyp populations would be structurally similar in terms of their population genetics along the Norwegian Coast.

In term of target species, *Periphylla periphylla* had been suggested as the third key species for this study. However, DNA extraction is a difficult task in this particular species and protocols still need to be revised and further developed (Sanna majaneva, personal communication, September 2020). Thus, *P. periphylla* was omitted from the scope of this study. However, it would have been beneficial for the current study to include *P. periphylla* into the analysis since during the sampling campaigns from 2018-2020, *P. periphylla* made up a high proportion of biomass (unpublished data). Thus, the development of reliable DNA extraction protocols for *P. periphylla* and additional analyses on its population genetics would complemented the current study .

To further improve the molecular tools used in this study, additional techniques beyond the use of mitochondrial COI to perform population genetic analyses on jellyfish species could be used. In one study, mitochondrial COI proved to be more effective in revealing genetic structure in *R. octopus* compared to nuclear gene of calmodulin (Lee et al., 2013). There were, however, some instances where mitochondrial COI failed to reveal population genetic structuring such as for the eel *Anguilla anguilla* (Avise, Helfman, Saunders, & Hales, 1986). However, the use of microsatellite markers in another study was able to reveal evidence of genetic structuring in this eel species (Wirth & Bernatchez, 2001). Thus, it is interesting to perform population genetic analysis using microsatellite markers to see if there are more distinct sub-populations that can be identified in addition to the ones obtained from this study. Moreover, it has been

demonstrated that population demographic changes like the Pleistocene climate fluctuations could give rise to contradictory signals of population structuring among markers (Larmuseau, Raeymaekers, Hellemans, Van Houdt, & Volckaert, 2010). Therefore, the addition of microsatellites along with other markers for population genetic analyses in future studies could be used to reveal if there is discrepancies in the results between different markers.

AMOVA analysis on this study can also be improved further. Here, nested AMOVA tests were performed on some grouping permutations judged to be likely to maximize among group variations, e.g. grouping by populations separated by the presence of sills in Trondheimsfjord. While the abovementioned statistical analyses are generally considered as sufficient to support population structure analyses, there is a small possibility that a non-obvious untested grouping could give a higher among-group variation than what this study has presented. In order to fully test them, spatial analysis of molecular variance or SAMOVA (Dupanloup, Schneider, & Excoffier, 2002) has been suggested. SAMOVA is a derivation of AMOVA which incorporates geographical data into the analysis, allowing detection of population grouping that maximizes the among-group variance. Other approaches can also be used such as using the program STRUCTURE (Falush, Stephens, & Pritchard, 2003) which makes use of Bayesian clustering analysis to find optimum grouping of sampled populations. Using either analysis in conjunction with pairwise F_{ST} data could provide a better understanding on the population structure presented in this study.

5. Conclusion

This study revealed population structuring in key jellyfish species between Trondheimsfjord and reference locations. Population connectivity between medusa and polyp populations for *Aurelia aurita* has also been shown. Structurally different populations of *A. aurita* medusae in Trondheimsfjord compared to reference populations sampled in Outer Oslofjord were observed. However, no significant population structure could be found among populations within Trondheimsfjord, despite the presence of sills. Polyps of *A. aurita* sampled in the area between Agdenes sill and Tautra Sill showed an intermediate population that shared genetic structure with medusae both from Trondheimsfjord and Outer Oslofjord. Using data only from medusae populations, one would assume that jellyfish blooms in Trondheimsfjord result from local polyp populations. However, when data from polyps are included, it becomes obvious that a certain proportion of medusae within the blooms in Trondheimsfjord actually derive from these intermediate polyp populations whose population dynamics are more relevant for medusae blooms at the outer part of the fjord than within the fjord. The other key jellyfish species, *Cyanea capillata,* showed a higher level of differentiation in populations between Trondheimsfjord and the Baltic Sea populations, suggesting a locally adapted population for the locations. However, existence of *C. capillata* polyps remains a mystery, and thus conclusion regarding *C. capillata* bloom originating from local polyp bloom event cannot be made without any data from the polyps. It is suggested that sampling locations for both medusae and polyps should be expanded towards other parts of the Trondheimsfjord and along the Norwegian coast to see if a gradient of population connectivity can be seen along the sampling locations. All in all, this study has highlighted a population connectivity that would not have become apparent through population genetic analyses performed on medusae populations alone. Thus, the polyp life stage should not be ignored in order to make a better population management plan for jellyfish.

References

Abboud, S. S., Gómez Daglio, L., & Dawson, M. N. (2018). A global estimate of genetic and geographic differentiation in macromedusae – implications for identifying the causes of jellyfish blooms. *Marine Ecology Progress Series, 591*, 199-216. Retrieved from https://www.int-res.com/abstracts/meps/v591/p199-216

Attrill, M. J., Wright, J., & Edwards, M. (2007). Climate-related increases in jellyfish frequency suggest a more gelatinous future for the North Sea. *Limnology and Oceanography, 52*(1), 480-485. doi:https://doi.org/10.4319/lo.2007.52.1.0480

Avise, J. C., Helfman, G. S., Saunders, N. C., & Hales, L. S. (1986). Mitochondrial DNA differentiation in North Atlantic eels: population genetic consequences of an unusual life history pattern. *Proceedings of the National Academy of Sciences, 83*(12), 4350-4354.

Bayha, K. M., Chang, M. H., Mariani, C. L., Richardson, J. L., Edwards, D. L., DeBoer, T. S., . . . Caccone, A. (2015). Worldwide phylogeography of the invasive ctenophore *Mnemiopsis leidyi* (Ctenophora) based on nuclear and mitochondrial DNA data. *Biological Invasions, 17*(3), 827-850. doi:10.1007/s10530-014-0770-6

Ben Faleh, A. R., Ben Othmen, A., Deli, T., Annabi, A., & Said, K. (2009). High genetic homogeneity of the moon jelly *Aurelia aurita* (Scyphozoa, Semaeostomeae) along the Mediterranean coast of Tunisia. *African Journal of Marine Science, 31*(1), 73-80. doi:10.2989/AJMS.2009.31.1.6.777

Bernatchez, L., Wellenreuther, M., Araneda, C., Ashton, D. T., Barth, J. M. I., Beacham, T. D., . . . Withler, R. E. (2017). Harnessing the Power of Genomics to Secure the Future of Seafood. *Trends in Ecology & Evolution, 32*(9), 665-680. doi:https://doi.org/10.1016/j.tree.2017.06.010

Biochemistry, N. C. o. t. I. U. o. (1986). Nomenclature for incompletely specified bases in nucleic acid sequences: Recommendations 1984. *Proceedings of the National Academy of Sciences of the United States of America*, 4-8.

Black, R. E., & Webb, K. L. (1973). Metabolism of 131I in relation to strobilation of *Chrysaora quinquecirrha* (Scyphozoa). *Comparative Biochemistry and Physiology -- Part A: Physiology, 45*(4), 1023-1029. doi:10.1016/0300-9629(73)90339-3

Brewer, R. H. (1989). The annual pattern of feeding, growth, and sexual reproduction in *Cyanea* (Cnidaria: Scyphozoa) in the Niantic River estuary, Connecticut. *Biological Bulletin, 176*, 272-281.

Brotz, L., Cheung, W. W. L., Kleisner, K., Pakhomov, E., & Pauly, D. (2012). Increasing jellyfish populations: trends in Large Marine Ecosystems. In J. Purcell, H. Mianzan, & J. R. Frost (Eds.), *Jellyfish Blooms IV: Interactions with humans and fisheries* (pp. 3-20). Dordrecht: Springer Netherlands.

Calder, D. R. (1974). Strobilation of the sea nettle, *Chrysaora quinquecirrha*, under field conditions. *BIOL.BULL., 146*(3), 326-334. doi:10.2307/1540408

Cargo, D. G., & Schultz, L. P. (1966). Notes on the biology of the sea nettle, *Chrysaora quinquecirrha*, in Chesapeake Bay. *Chesapeake Science, 7*(2), 95-100. doi:10.2307/1351129

Carvalho, G. R., & Hauser, L. (1995). Molecular genetics and the stock concept in fisheries. In G. R. Carvalho & T. J. Pitcher (Eds.), *Molecular Genetics in Fisheries* (pp. 55-79). Dordrecht: Springer Netherlands.

Chen, J., Ding, G., & Liu, C. (1985). Effect of nutritional conditions on the strobilation of edible medusa, *Rhopilema esculenta* Kishinouye. *Journal of Fisheries of China, 9*(4), 321-329.

Chun, K. W., Damdinsuren, E., Kim, Y. R., & Ezaki, T. (2012). Effect of Jellyfish fertilizer on Seedling Growth and Soil Properties. *日本緑化工学会誌, 38*(1), 192-195. doi:10.7211/jjsrt.38.192

Cid, B., Hilker, F. M., & Liz, E. (2014). Harvest timing and its population dynamic consequences in a discrete single-species model. *Mathematical Biosciences, 248*, 78-87. doi:https://doi.org/10.1016/j.mbs.2013.12.003

Colin, S. P., Costello, J. H., Graham, W. M., & Higgins Iii, J. (2005). Omnivory by the small cosmopolitan hydromedusa *Aglaura hemistoma*. *Limnology and Oceanography, 50*(4), 1264-1268. doi:10.4319/lo.2005.50.4.1264

Condon, R. H., Graham, W. M., Duarte, C. M., Pitt, K. A., Lucas, C. H., Haddock, S. H. D., . . . Madin, L. P. (2012). Questioning the Rise of Gelatinous Zooplankton in the World's Oceans. *BioScience, 62*(2), 160-169. doi:10.1525/bio.2012.62.2.9

Cruz-Rivera, E., & El-Regal, M. A. (2016). A bloom of an edible scyphozoan jellyfish in the Red Sea. *Marine Biodiversity, 46*(2), 515-519. doi:10.1007/s12526-015-0381-1

Cury, P., & Shannon, L. (2004). Regime shifts in upwelling ecosystems: observed changes and possible mechanisms in the northern and southern Benguela. *Progress in Oceanography, 60*(2), 223-243. doi:https://doi.org/10.1016/j.pocean.2004.02.007

Cushing, D. H. (1989). A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. *Journal of Plankton Research, 11*(1), 1-13. doi:10.1093/plankt/11.1.1

Daryanabard, R., & Dawson, M. N. (2008). Jellyfish blooms: *Crambionella orsini* (Scyphozoa: Rhizostomeae) in the Gulf of Oman, Iran, 2002-2003. *Marine Biological Association of the United Kingdom. Journal of the Marine Biological Association of the United Kingdom, 88*(3), 477-483. doi:http://dx.doi.org/10.1017/S0025315408000945

Dawson, M. N. (2003). Macro-morphological variation among cryptic species of the moon jellyfish, *Aurelia* (Cnidaria: Scyphozoa). *Marine Biology, 143*(2), 369-379. doi:10.1007/s00227-003-1070-3

Dawson, M. N. (2005). Incipient speciation of *Catostylus mosaicus* (Scyphozoa, Rhizostomeae, Catostylidae), comparative phylogeography and biogeography in south-east Australia. *Journal of Biogeography, 32*(3), 515-533. doi:https://doi.org/10.1111/j.1365-2699.2004.01193.x

Dawson, M. N., Cieciel, K., Decker, M. B., Hays, G. C., Lucas, C. H., & Pitt, K. A. (2015). Population-level perspectives on global change: genetic and demographic analyses indicate various scales, timing, and causes of scyphozoan jellyfish blooms. *Biological Invasions, 17*(3), 851-867. doi:10.1007/s10530-014-0732-z

Dawson, M. N., & Martin, L. E. (2001). Geographic variation and ecological adaptation in *Aurelia* (Scyphozoa, Semaeostomeae): Some implications from molecular phylogenetics. *Hydrobiologia, 451*, 259-273. doi:10.1023/A:1011869215330

Diaz, R. J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science, 321*(5891), 926-929. doi:10.1126/science.1156401

Dong, Z., Liu, D., & Keesing, J. K. (2014). Contrasting Trends in Populations of *Rhopilema esculentum* and Aurelia aurita in Chinese Waters. In K. A. Pitt & C. H. Lucas (Eds.), *Jellyfish Blooms* (pp. 207-218). Dordrecht: Springer Netherlands.

Dong, Z., Liu, D., Wang, Y., Di, B., Song, X., & Shi, Y. (2012). A report on a Moon Jellyfish *Aurelia aurita* bloom in Sishili Bay, Northern Yellow Sea of China in 2009. *Aquatic Ecosystem Health & Management, 15*(2), 161-167. doi:10.1080/14634988.2012.689583

Dong, Z., Liu, Z., Liu, D., Liu, Q., & Sun, T. (2016). Low genetic diversity and lack of genetic structure in the giant jellyfish *Nemopilema nomurai* in Chinese coastal waters. *Marine Biology Research, 12*(7), 769-775. doi:10.1080/17451000.2016.1196818

Duarte, C. M., Pitt, K. A., Lucas, C. H., Purcell, J. E., Uye, S.-i., Robinson, K., . . . Condon, R. H. (2013). Is global ocean sprawl a cause of jellyfish blooms? *Frontiers in Ecology and the Environment, 11*(2), 91-97. doi:https://doi.org/10.1890/110246

Dupanloup, I., Schneider, S., & Excoffier, L. (2002). A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology, 11*(12), 2571-2581. doi:https://doi.org/10.1046/j.1365-294X.2002.01650.x

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources, 10*(3), 564-567. doi:https://doi.org/10.1111/j.1755-0998.2010.02847.x

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics, 131*(2), 479. Retrieved from http://www.genetics.org/content/131/2/479.abstract

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics, 164*(4), 1567-1587. doi:10.1093/genetics/164.4.1567

Fitt, W. K., & Costley, K. (1998). The role of temperature in survival of the polyp stage of the tropical rhizostome jellyfish *Cassiopea xamachana*. *Journal of Experimental Marine Biology and Ecology, 222*(1-2), 79-91. doi:10.1016/S0022-0981(97)00139-1

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol, 3*(5), 294-299.

Gilg, M. R., & Hilbish, T. J. (2003). The geography of marine larval dispersal: coupling genetics with fine-scale physical oceanography. *Ecology, 84*(11), 2989-2998. doi:https://doi.org/10.1890/02-0498

Glynn, F., Houghton, J. D. R., Bastian, T., Doyle, T. K., Fuentes, V., Lilley, M. K. S., & Provan, J. (2016). High-resolution genetic analysis reveals extensive gene flow within the jellyfish *Pelagia noctiluca* (Scyphozoa) in the North Atlantic and Mediterranean Sea. *Biological Journal of the Linnean Society, 117*(2), 252-263. doi:10.1111/bij.12654

Graham, W. M. (2001). Numerical increases and distributional shifts of *Chrysaora quinquecirrha* (Desor) and *Aurelia aurita* (Linné) (Cnidaria: Scyphozoa) in the northern Gulf of Mexico. *Hydrobiologia, 451*, 97-111. doi:10.1023/A:1011844208119

Graham, W. M., Pagès, F., & Hamner, W. M. (2001). *A physical context for gelatinous zooplankton aggregations: a review.* Paper presented at the Jellyfish Blooms: Ecological and Societal Importance, Dordrecht.

Granhag, L., Majaneva, S., & Møller, L. (2012). First recordings of the ctenophore *Euplokamis* sp. (Ctenophora, Cydippida) in Swedish coastal waters and molecular identification of this genus. *Aquatic Invasions, 7*, 455–463. doi:10.3391/ai.2012.7.4.002

Gröndahl, F. (1988). A comparative ecological study on the scyphozoans *Aurelia aurita*, *Cyanea capillata* and *C. lamarckii* in the Gullmar Fjord, western Sweden, 1982 to 1986. *Marine Biology, 97*(4), 541-550. doi:10.1007/BF00391050

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows *95/98/NT.* Paper presented at the Nucleic acids symposium series.

Hamner, W. M., & Dawson, M. N. (2009). A review and synthesis on the systematics and evolution of jellyfish blooms: advantageous aggregations and adaptive assemblages. *Hydrobiologia, 616*(1), 161-191. doi:10.1007/s10750-008-9620-9

Hamner, W. M., Gilmer, R. W., & Hamner, P. P. (1982). The physical, chemical, and biological characteristics of a stratified, saline, sulfide lake in Palau. *Limnology and Oceanography, 27*(5), 896-909. doi:10.4319/lo.1982.27.5.0896

Hauser, S., Wakeland, K., & Leberg, P. (2019). Inconsistent use of multiple comparison corrections in studies of population genetic structure: Are some type I errors more tolerable than others? *Molecular Ecology Resources, 19*(1), 144-148. doi:https://doi.org/10.1111/1755-0998.12947

Hay, S. J., Hislop, J. R. G., & Shanks, A. M. (1990). North Sea Scyphomedusae; summer distribution, estimated biomass and significance particularly for 0-group Gadoid fish. *Netherlands Journal of Sea Research, 25*(1), 113-130. doi:https://doi.org/10.1016/0077-7579(90)90013-7

Hays, G. C., Doyle, T. K., & Houghton, J. D. R. (2018). A Paradigm Shift in the Trophic Importance of Jellyfish? *Trends in Ecology & Evolution, 33*(11), 874-884. doi:https://doi.org/10.1016/j.tree.2018.09.001

Henschke, N., Stock, C. A., & Sarmiento, J. L. (2018). Modeling population dynamics of scyphozoan jellyfish (*Aurelia* spp.) in the Gulf of Mexico. *Marine Ecology Progress Series, 591*, 167-183. Retrieved from https://www.int-res.com/abstracts/meps/v591/p167-183/

Hernroth, L., & Gröndahl, F. (1983). On the biology of aurelia aurita (L.) 1. Release and growth of *Aurelia aurita* (L.) ephyrae in the gullmar fjord, Western Sweden, 1982-83. *Ophelia, 22*(2), 189-199. doi:10.1080/00785326.1983.10426595

Holst, S. (2012a). Effects of climate warming on strobilation and ephyra production of North Sea scyphozoan jellyfish. In J. Purcell, H. Mianzan, & J. R. Frost (Eds.), *Jellyfish Blooms IV: Interactions with humans and fisheries* (pp. 127-140). Dordrecht: Springer Netherlands.

Holst, S. (2012b). Morphology and development of benthic and pelagic life stages of North Sea jellyfish (Scyphozoa, Cnidaria) with special emphasis on the identification of ephyra stages. *Marine Biology, 159*(12), 2707-2722. doi:10.1007/s00227-012-2028-0

Holst, S., & Jarms, G. (2010). Effects of low salinity on settlement and strobilation of scyphozoa (Cnidaria): Is the lion's mane *Cyanea capillata* (L.) able to reproduce in the brackish Baltic Sea? In J. E. Purcell & D. L. Angel (Eds.), *Jellyfish Blooms: New Problems and Solutions* (pp. 53-68). Dordrecht: Springer Netherlands.

Holst, S., & Laakmann, S. (2014). Morphological and molecular discrimination of two closely related jellyfish species, *Cyanea capillata* and *C. lamarckii* (Cnidaria, Scyphozoa), from the northeast Atlantic. *Journal of Plankton Research, 36*(1), 48-63. doi:10.1093/plankt/fbt093

Ishii, H., & Watanabe, T. (2003). Experimental study of growth and asexual reproduction in *Aurelia aurita* polyps. *Sessile Organisms, 20*(2), 69-73.

Jacobson, P. (1983). Physical oceanography of the Trondheimsfjord. *Geophysical & Astrophysical Fluid Dynamics, 26*(1-2), 3-26. doi:10.1080/03091928308221761

Johannesson, K., Ring, A.-K., Johannesson, K. B., Renborg, E., Jonsson, P. R., & Havenhand, J. N. (2018). Oceanographic barriers to gene flow promote genetic subdivision of the tunicate *Ciona intestinalis* in a North Sea archipelago. *Marine Biology, 165*(8), 126. doi:10.1007/s00227-018-3388-x

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution, 30*(4), 772-780. doi:10.1093/molbev/mst010

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution, 35*(6), 1547-1549. doi:10.1093/molbev/msy096

Larmuseau, M. H. D., Raeymaekers, J. A. M., Hellemans, B., Van Houdt, J. K. J., & Volckaert, F. A. M. (2010). Mito-nuclear discordance in the degree of population differentiation in a marine goby. *Heredity, 105*(6), 532-542. doi:10.1038/hdy.2010.9

Lawley, J. W., Ames, C. L., Bentlage, B., Yanagihara, A., Goodwill, R., Kayal, E., . . . Collins, A. G. (2016). Box Jellyfish *Alatina alata* Has a Circumtropical Distribution. *The Biological Bulletin, 231*(2), 152-169. doi:10.1086/690095

Lebrato, M., Pitt, K. A., Sweetman, A. K., Jones, D. O. B., Cartes, J. E., Oschlies, A., . . . Billett, D. S. M. (2012). Jelly-falls historic and recent observations: a review to drive future research directions. *Hydrobiologia, 690*(1), 227-245. doi:10.1007/s10750-012-1046-8

Lee, P. L. M., Dawson, M. N., Neill, S. P., Robins, P. E., Houghton, J. D. R., Doyle, T. K., & Hays, G. C. (2013). Identification of genetically and oceanographically distinct blooms of jellyfish. *Journal of The Royal Society Interface, 10*(80), 20120920. doi:10.1098/rsif.2012.0920

Leigh, J. W., & Bryant, D. (2015). popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution, 6*(9), 1110-1116. doi:https://doi.org/10.1111/2041-210X.12410

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., . . . Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in Zoology, 10*(1), 34. doi:10.1186/1742-9994-10-34

Lin, M.-F., Luzon, K. S., Licuanan, W. Y., Ablan-Lagman, M. C., & Chen, C. A. (2011). Seventy-four universal primers for characterizing the complete mitochondrial genomes of scleractinian corals (Cnidaria; Anthozoa). *Zool Stud, 50*(4), 513-524.

Lo, W. T., Purcell, J. E., Hung, J. J., Su, H. M., & Hsu, P. K. (2008). Enhancement of jellyfish (*Aurelia aurita*) populations by extensive aquaculture rafts in a coastal lagoon in Taiwan. *ICES Journal of Marine Science, 65*(3), 453-461. doi:10.1093/icesjms/fsm185

Lotan, A., Ben-Hillel, R., & Loya, Y. (1992). Life cycle of *Rhopilema nomadica*: a new immigrant scyphomedusan in the Mediterranean. *Marine Biology, 112*(2), 237-242. doi:10.1007/BF00702467

Lotan, A., Fine, M., & Ben-Hillel, R. (1994). Synchronization of the life cycle and dispersal pattern of the tropical invader scyphomedusan *Rhopilema nomadica* is temperature dependent. *Marine Ecology-Progress Series, 109*, 59-59.

Lucas, C. (2001). Reproduction and life history strategies of the common jellyfish, *Aurelia aurita*, in relation to its ambient environment. *Hydrobiologia, 451*, 229-246. doi:10.1023/A:1011836326717

Lucas, C. H., & Dawson, M. N. (2014). What Are Jellyfishes and *Thaliaceans* and Why Do They Bloom? In K. A. Pitt & C. H. Lucas (Eds.), *Jellyfish Blooms* (pp. 9-44). Dordrecht: Springer Netherlands.

Lucas, C. H., Graham, W. M., & Widmer, C. (2012). Chapter Three - Jellyfish Life Histories: Role of Polyps in Forming and Maintaining Scyphomedusa Populations. In M. Lesser (Ed.), *Advances in Marine Biology* (Vol. 63, pp. 133-196): Academic Press.

Lynam, C. P., Gibbons, M. J., Axelsen, B. E., Sparks, C. A., Coetzee, J., Heywood, B. G., & Brierley, A. S. (2006). Jellyfish overtake fish in a heavily fished ecosystem. *Current biology : CB, 16*(13), R492-493. doi:10.1016/j.cub.2006.06.018

Lynam, C. P., Lilley, M. K. S., Bastian, T., Doyle, T. K., Beggs, S. E., & Hays, G. C. (2011). Have jellyfish in the Irish Sea benefited from climate change and overfishing? *Global Change Biology, 17*(2), 767-782. doi:https://doi.org/10.1111/j.1365-2486.2010.02352.x

Malej, A., Turk, V., Lučić, D., & Benović, A. (2007). Direct and indirect trophic interactions of *Aurelia* sp. (Scyphozoa) in a stratified marine environment (Mljet Lakes, Adriatic Sea). *Marine Biology, 151*(3), 827-841. doi:10.1007/s00227-006-0503-1

Marques, R., Bouvier, C., Darnaude, A. M., Molinero, J.-C., Przybyla, C., Soriano, S., . . . Bonnet, D. (2016). Jellyfish as an alternative source of food for opportunistic fishes. *Journal of Experimental Marine Biology and Ecology, 485*, 1-7. doi:https://doi.org/10.1016/j.jembe.2016.08.008

Masuda, R., Yamashita, Y., & Matsuyama, M. (2008). Jack mackerel *Trachurus japonicus* juveniles use jellyfish for predator avoidance and as a prey collector. *Fisheries Science, 74*(2), 276-284. doi:10.1111/j.1444-2906.2008.01522.x

McIlwaine, B., & Rivas Casado, M. (2021). JellyNet: The convolutional neural network jellyfish bloom detector. *International Journal of Applied Earth Observation and Geoinformation, 97*, 102279. doi:https://doi.org/10.1016/j.jag.2020.102279

Miller, B. J., von der Heyden, S., & Gibbons, M. J. (2012). Significant population genetic structuring of the holoplanktic scyphozoan *Pelagia noctiluca* in the Atlantic Ocean. *African Journal of Marine Science, 34*(3), 425-430. doi:10.2989/1814232X.2012.726646

Miller, J. (1981). RG (1981): Simultaneous Statistical Inference. In: Springer-Verlag.

Mills, C. E. (2001). Jellyfish blooms: are populations increasing globally in response to changing ocean conditions? *Hydrobiologia, 451*(1), 55-68. doi:10.1023/A:1011888006302

Möller, H. (1980). Population dynamics of *Aurelia aurita* medusae in Kiel Bight, Germany (FRG). *Marine Biology, 60*(2-3), 123-128. doi:10.1007/BF00389155

Moran, M. D. (2003). Arguments for Rejecting the Sequential Bonferroni in Ecological Studies. *Oikos, 100*(2), 403-405. Retrieved from http://www.jstor.org/stable/3548200

Nagai, T., Ogawa, T., Nakamura, T., Ito, T., Nakagawa, H., Fujiki, K., . . . Yano, T. (1999). Collagen of edible jellyfish exumbrella. *Journal of the Science of Food and Agriculture, 79*(6), 855-858. doi:https://doi.org/10.1002/(SICI)1097-0010(19990501)79:6<855::AID-JSFA299>3.0.CO;2-N

Narum, S. R. (2006). Beyond Bonferroni: less conservative analyses for conservation genetics. *Conservation genetics, 7*(5), 783-787.

Omori, M., & Nakano, E. (2001). Jellyfish fisheries in southeast Asia. *Hydrobiologia, 451*(1), 19-26. doi:10.1023/A:1011879821323

Parisod, C., Trippi, C., & Galland, N. (2005). Genetic Variability and Founder Effect in the Pitcher Plant *Sarracenia purpurea* (Sarraceniaceae) in Populations Introduced into Switzerland: from Inbreeding to Invasion. *Annals of Botany, 95*(2), 277-286. doi:10.1093/aob/mci023

Phillips, J. D., Gillis, D. J., & Hanner, R. H. (2019). Incomplete estimates of genetic diversity within species: Implications for DNA barcoding. *Ecology and evolution, 9*(5), 2996-3010. doi:10.1002/ece3.4757

Purcell, J. E. (1989). Predation on fish larvae and eggs by the hydromedusa *Aequorea victoria* at a herring spawning ground in British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences, 46*(8), 1415-1427. doi:10.1139/f89-181

Purcell, J. E., & Arai, M. N. (2001). Interactions of pelagic cnidarians and ctenophores with fish: A review. *Hydrobiologia, 451*, 27-44. doi:10.1023/A:1011883905394

Purcell, J. E., Breitburg, D. L., Decker, M. B., Graham, W. M., Youngbluth, M. J., & Raskoff, K. A. (2001). Pelagic cnidarians and ctenophores in low dissolved oxygen environments: A review. *Coastal Hypoxia: Consequences for Living Resources and Ecosystems, 58*, 77-100.

Purcell, J. E., Uye, S. I., & Lo, W. T. (2007). Anthropogenic causes of jellyfish blooms and their direct consequences for humans: A review. *Marine Ecology Progress Series, 350*, 153-174. doi:10.3354/meps07093

Purcell, J. E., White, J. R., Nemazie, D. A., & Wright, D. A. (1999). Temperature, salinity and food effects on asexual reproduction and abundance of the scyphozoan *Chrysaora quinquecirrha*. *Marine Ecology Progress Series, 180*, 187-196. doi:10.3354/meps180187

Raveling, D. G., & Heitmeyer, M. E. (1989). Relationships of population size and recruitment of pintails to habitat conditions and harvest. *The Journal of Wildlife Management, 53*(4), 1088-1103. doi:10.2307/3809615

Rekstad, M. E. (2019). *Distribution, interspecific variation and habitats of scyphozoan polyp colonies in Trondheimsfjorden* [Master's thesis, Norwegian University of Science and Technology]. NTNU Open. https://ntnuopen.ntnu.no/ntnu-xmlui/handle/11250/2618089.

Richardson, A. J., Bakun, A., Hays, G. C., & Gibbons, M. J. (2009). The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Trends in Ecology & Evolution, 24*(6), 312-322. doi:https://doi.org/10.1016/j.tree.2009.01.010

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Molecular Biology and Evolution, 34*(12), 3299-3302. doi:10.1093/molbev/msx248

Sætre, R., & Ljøen, R. (1972). The Norwegian coastal current. *Proceedings of the first international conference on port and ocean engineering under Arctic conditions*, Vol. 2, Technical University of Norway, Trondheim, Norway (1971), pp. 514-535

Sanz-Martín, M., Pitt, K. A., Condon, R. H., Lucas, C. H., Novaes de Santana, C., & Duarte, C. M. (2016). Flawed citation practices facilitate the unsubstantiated perception of a global trend toward increased jellyfish blooms. *Global Ecology and Biogeography, 25*(9), 1039-1049. doi:https://doi.org/10.1111/geb.12474

Selkoe, K. A., Watson, J. R., White, C., Horin, T. B., Iacchei, M., Mitarai, S., . . . Toonen, R. J. (2010). Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. *Molecular Ecology, 19*(17), 3708-3726. doi:https://doi.org/10.1111/j.1365-294X.2010.04658.x

Seo, Y., Muhammad, B. L., Chae, J., & Ki, J.-S. (2021). Genetic Structure and Diversity of the Moon Jellyfish *Aurelia coerulea* Polyp Population in Jaran Bay, Korea, Revealed by Mitochondrial COI and 16S rRNA Genes. *Ocean Science Journal, 56*(1), 99-105. doi:10.1007/s12601-021-00008-0

Shatz, Y. (1998). Fishstat Plus (V. 2.30). *Food and Agriculture Organization of the United Nations*.

Sparks, C., Buecher, E., Brierley, A. S., Axelsen, B. E., Boyer, H., & Gibbons, M. J. (2001, 2001//). *Observations on the distribution and relative abundance of the scyphomedusan Chrysaora hysoscella (Linné, 1766) and the hydrozoan Aequorea aequorea (Forskål, 1775) in the northern Benguela ecosystem.* Paper presented at the Jellyfish Blooms: Ecological and Societal Importance, Dordrecht.

Stopar, K., Ramšak, A., Trontelj, P., & Malej, A. (2010). Lack of genetic structure in the jellyfish *Pelagia noctiluca* (Cnidaria: Scyphozoa: Semaeostomeae) across European seas. *Molecular Phylogenetics and Evolution, 57*(1), 417-428. doi:https://doi.org/10.1016/j.ympev.2010.07.004

Sullivan, L. J., & Gifford, D. J. (2004). Diet of the larval ctenophore *Mnemiopsis leidyi A. Agassiz* (Ctenophora, Lobata). *Journal of Plankton Research, 26*(4), 417-431. doi:10.1093/plankt/fbh033

Taylor, N. G., McAllister, M. K., Lawson, G. L., Carruthers, T., & Block, B. A. (2011). Atlantic Bluefin Tuna: A Novel Multistock Spatial Model for Assessing Population Biomass. *PLOS ONE, 6*(12), e27693. doi:10.1371/journal.pone.0027693

Thorpe, J. P., Solé-Cava, A. M., & Watts, P. C. (2000). Exploited marine invertebrates: Genetics and fisheries. *Hydrobiologia, 420*(1), 165-184. doi:10.1023/A:1003987117508

Tiller, R. G., Mork, J., Liu, Y., Borgersen, Å. L., & Richards, R. (2015). To Adapt or Not Adapt: Assessing the Adaptive Capacity of Artisanal Fishers in the Trondheimsfjord (Norway) to Jellyfish (*Periphylla periphylla*) Bloom and Purse Seiners. *Marine and Coastal Fisheries, 7*(1), 260-273. doi:10.1080/19425120.2015.1037873

Uye, S.-i. (2008). Blooms of the giant jellyfish *Nemopilema nomurai*: a threat to the fisheries sustainability of the East Asian Marginal Seas. *Plankton and Benthos Research, 3*(Supplement), 125-131. doi:10.3800/pbr.3.125

van Walraven, L., Driessen, F., van Bleijswijk, J., Bol, A., Luttikhuizen, P. C., Coolen, J. W. P., . . . van der Veer, H. W. (2016). Where are the polyps? Molecular identification, distribution and population differentiation of Aurelia aurita jellyfish polyps in the southern North Sea area. *Marine Biology, 163*(8), 172. doi:10.1007/s00227-016-2945-4

Vasas, V., Lancelot, C., Rousseau, V., & Jordán, F. (2007). Eutrophication and overfishing in temperate nearshore pelagic food webs: a network perspective. *Marine Ecology Progress Series, 336*, 1-14. Retrieved from https://www.int-res.com/abstracts/meps/v336/p1-14/

Verity, P. G., & Smetacek, V. (1996). Organism life cycles, predation, and the structure of marine pelagic ecosystems. *Marine Ecology Progress Series, 130*, 277-293. Retrieved from https://www.int-res.com/abstracts/meps/v130/p277-293/

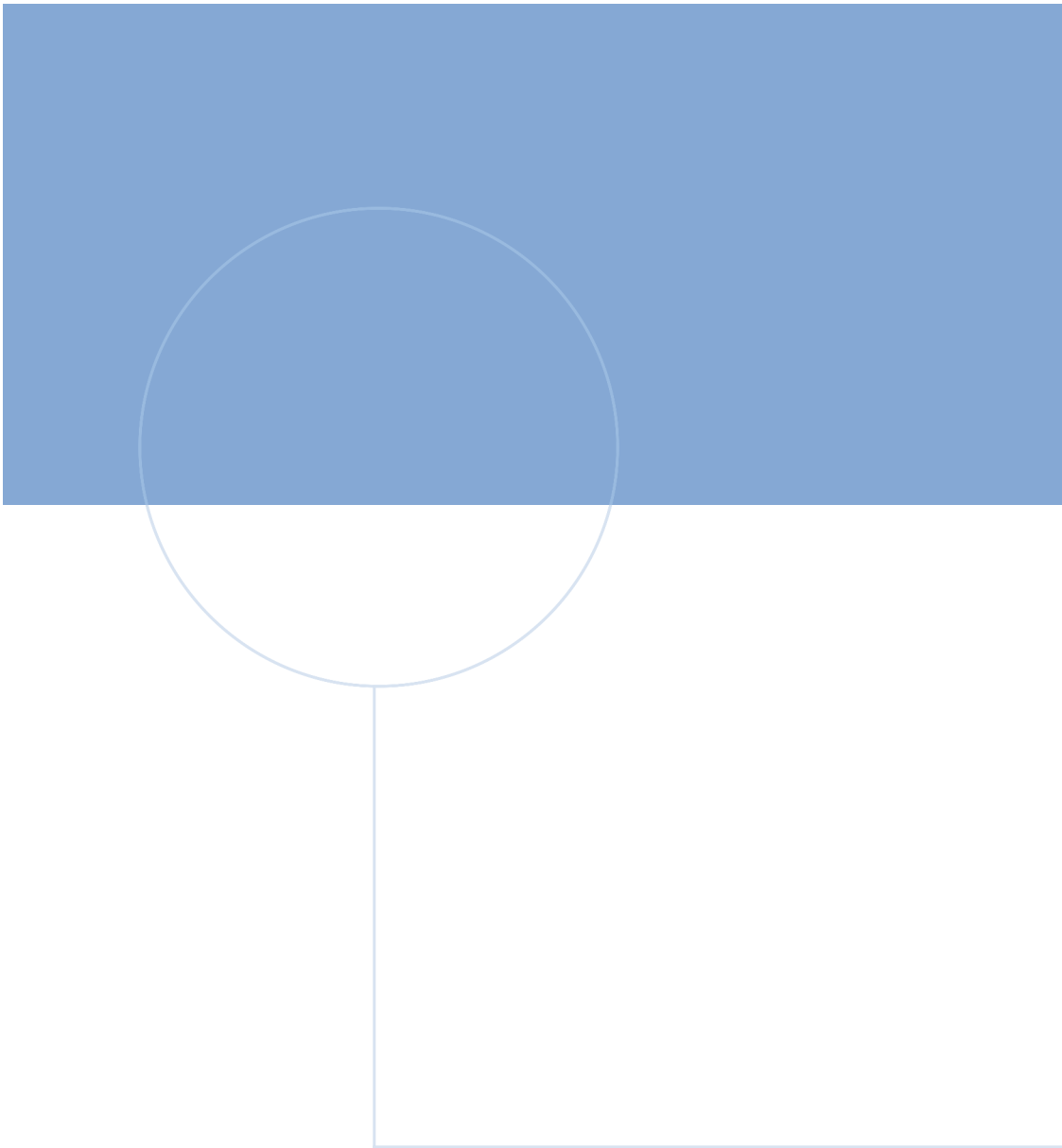
Walsh, P. S., Metzger, D. A., & Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques, 10*(4), 506-513. Retrieved from http://europepmc.org/abstract/MED/1867860

Waples, R. S., Punt, A. E., & Cope, J. M. (2008). Integrating genetic data into management of marine resources: how can we do it better? *Fish and Fisheries, 9*(4), 423-449. doi:https://doi.org/10.1111/j.1467-2979.2008.00303.x

Ward, R. D. (2000). Genetics in fisheries management. *Hydrobiologia, 420*(1), 191-201. doi:10.1023/A:1003928327503

Willcox, S., Moltschaniwskyj, N. A., & Crawford, C. M. (2008). Population dynamics of natural colonies of *Aurelia* sp. scyphistomae in Tasmania, Australia. *Marine Biology, 154*(4), 661-670. doi:10.1007/s00227-008-0959-2

Wirth, T., & Bernatchez, L. (2001). Genetic evidence against panmixia in the European eel. *Nature, 409*(6823), 1037-1040. doi:10.1038/35059079



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