Master's thesis

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Distribution, interspecific variation and habitats of scyphozoan polyp colonies in Trondheimsfjorden

Master's thesis in Ocean Resources
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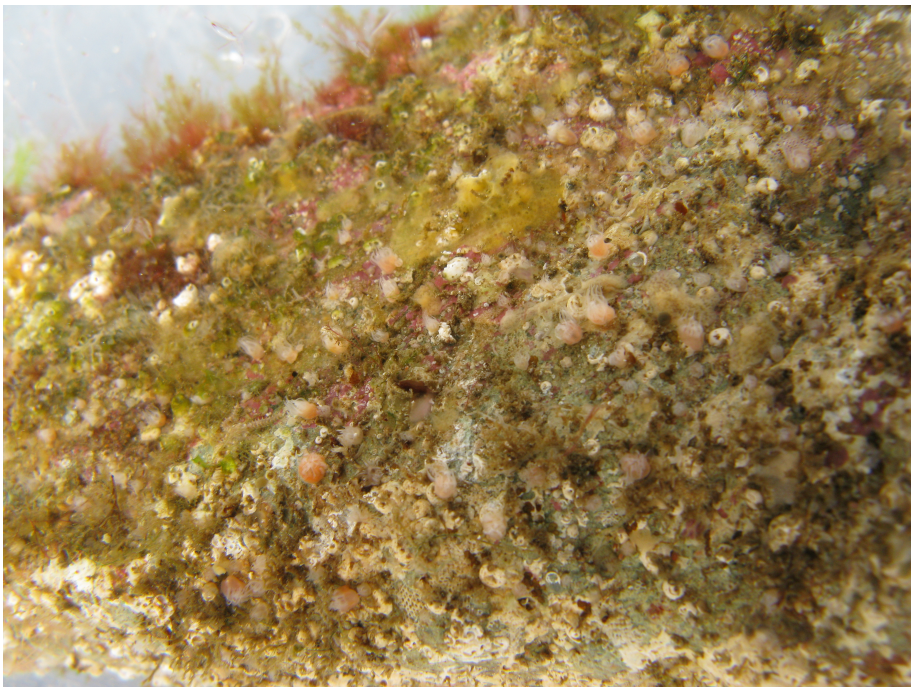

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Kunnskap for en bedre verden

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M.E.R

The picture on the front page depicts polyps on a rock found on sampling station MAU2. Photo: M. E. Rekstad

Abstract

Jellyfish blooms continue to be a topic of economic and ecological concern. There is still an ongoing debate if the frequency and intensity of jellyfish blooms is increasing or not. Combined with the discrepancy of data regarding jellyfish blooms, and the potential to exploit jellyfish as a useful resource, the EU-funded project GoJelly and similar efforts are now trying to increase our knowledge on jellyfish ecology, population dynamics and the factors promoting their occurrence. Particularly on the importance of scyphozoan polyp ecology, which is presently a scarcely investigates subject compared to other common benthic organisms.

Thus, the aim of this master thesis was to investigate the ecology of scyphozoan polyp colonies within and outside of Trondheimsfjorden. More specifically, to study their distribution, interspecific variation, habitats, microhabitats and the biotic interactions between polyps and other settling epibionts. In order to achieve the listed goals, a field survey was performed and settling plates were deployed at several stations along a transect within and outside of Trondheimsfjorden.

The occurrence of *Aurelia aurita* polyp colonies were confirmed throughout the whole transect, while polyps of *Cyanea capillata* and *Cyanea lamarckii* were not found. Shallow, embayed and sheltered littoral zones were viable *A. aurita* polyp habitats. The structural and surface characteristics of *Ascidia mentula* (solitary ascidian), *Pomatoceros triqueter* (polychaete) and dead *Balanus balanoides* (barnacle) shells promoted viable polyp microhabitats. The blue mussel *Mytilus edilus* proved to be a potential competitor of space and food towards other settling epibionts, including *A. aurita* polyps.

Sammendrag

Manetoppblomstringer er fremdeles et emne med høy økonomisk og økologisk betydning. Det er også en pågående debatt om hvorvidt frekvensen og intensiteten av maneoppblomstringer øker eller ikke. Kombinert med tvetydige data om manetoppblomstringer, og potensialet av å utnytte maneter som en nyttig ressurs, har ført til at det EU finansierte prosjektet GoJelly og lignende innsatser nå forsøker å øke vår kunnskap om manetøkologi, populasjonsdynamikk og faktorer som promoterer dem. Særlig med tanke på økologien av stormanerpolypper som til nå er lite undersøkt i forhold til andre vanlige bentiske organismer.

Derfor var målet med denne masteroppgaven å undersøke økologien til stormanetpolypper i Trondheimsfjorden og området utenfor fjorden. Mer spesifikt, å studere deres distribusjon, interspesifikk variasjon, habitater, mikrohabitater, samt biotiske interaksjoner mellom polypper og andre epibionter. For å fullføre disse målene ble en feltundersøkelse iverksatt og «settling plates» ble plassert på flere stasjoner langs en transekt av Trondheimsfjorden og området utenfor fjorden.

Aurelia aurita polyppkolonier ble bekrefter langs hele transekten, mens polypper av *Cyanea capillata* og *Cyanea lamarckii* ble ikke funnet. Små, grunne og lite eksponerte bukter i littoralsonen var levedyktige habitater for *A. aurita* polypper. Strukturen og overflaten til *Ascidia mentula* (sjøpung), *Pomatoceros triqueter* (børstemark), og døde *Balanus balanoides* (rur) promoterte levedyktige mikrohabitater. *Mytilus edilus* (blåskjell) framsto som en potensiell konkurrent for mat og plass, mot andre epibionter, inkludert polypper.

Index

Ascidia mentula	Solitary ascidian
Aurelia aurita	Scyphozaon
Balanus balanoides	Barnacle
BLAST	Basic local alignment search tool
Botryllus schlosseri	Compound ascidian
Cyanea capillata	Scyphozoan
Cyanea lamarckii	Scyphozoan
Ciona intestinalis	Solitary ascidian
Encystment	The process of forming podocyst or planulacyst from polyp and planula larva, respectively
Excystment	The process of forming polyp or planula larva from podocyst and planulacyst, respectively
Jassa falcata	Tube dwelling amphipod
MAU1-3	Stations on Mausund
Mytilus edilus	Bivalve
PCA	Principal component analysis
PCR	Polymerase chain reaction
Planulacyst	Chitin-covered cyst of planula larva
Podocyst	Chitin-covered cyst of polyp
Pomatoceros triqueter	Tube dwelling polychaete
PVC	Polyvinyl chloride. Common plastic material
Scyphopolyp	Scyphozoan polyp
SLE1	Station in Hopavågen, Agdenes
SLE2	Station in mouth of Trondheimsfjorden, Agdenes
SLE3	Station on north side of Agdenes
Spirorbis sp.	Genus of polychaetes
TBS	Station close to TBS (Trondheim Biological Station)
VER	Station close to Verdal

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1 Introduction

A jellyfish bloom is a natural occurrence where large amounts gelatinous plankton aggregate in the water column [3]. These aggregations are caused by a rapid population growth over shorter time frame, although their size, impact, longevity and frequency have been reported to vary [4]. Regarding frequency and intensity, jellyfish blooms have been reported to occur over a variety of temporal scales, ranging from weekly to decadal [4]. There is also an ongoing debate if the frequency and intensity in some instances are increasing, or if the interpretation is an artefact of natural oscillations [5, 6, 7, 8]. In the scenario were the frequency and intensity is increasing, the potential ecological and economic damage would be a topic of increasing concern. As an example with both ecological and economic aspects — one of the leading explanations for the possible increase of jellyfish blooms is because of an imbalance in the natural ecosystem caused by overfishing on predators of jellyfish and zooplanktivorous fish [9]. The main prey item for jellyfish is zooplankton, making them a potential competitor to zooplanktivorous fish [6]. Some species of jellyfish also prey on ichtyoplankton making them a potential predator of fish [6]. By overfishing and reducing fish stocks to an over exploited or depleted level, humans are removing the competitors and predators of jellyfish, giving jellyfish free reign to expand and prosper. This can lead to enhanced feedback loop where overfishing leads to increased jellyfish blooms, increased competition, and increased predation on fish. Ultimately reducing an overfished fish stock's ability to recuperate [9].

Jellyfish are mostly viewed as an economic nuisance rather than a valuable ecosystem service. Jellyfish blooms are known to kill fish in aquaculture fishing pens, clog fishing nets and cooling water intakes in power plants, and interfere with tourism because of the increased stinging risk [6]. In contrast to the Orient where the demand for jellyfish as a food product is increasing [10], most western countries view jellyfish as an unappealing food item of low nutritional value, thus treated as an inconvenient bycatch rather than a source of income [11]. The model produced by Graham et al. (2014) on the economic impact of jellyfish blooms in the future, predicts that "costly adaptive strategies will outpace the beneficial services if jellyfish populations continue to increase in the future". However, they also argue that the negative perception of jellyfish blooms has deterred our knowledge on their true value in terms of ecosystem services [12]. Recently, the perspective on jellyfish as an ecosystem service is indeed changing with recent research on jellyfish

as a source of antioxidant peptides [13], nano-particle filters [14], fertiliser [15] and more. Similarly, the perspective of jellyfish blooms' ecological significance is also changing. Previously, gelatinous plankton was often described as a trophic dead end in the marine food web [16]. However, Hays et al. (2018) used modern techniques like stable isotope analysis on predator tissue, and metabarcoding of predator gut content, demonstrating that many taxa routinely consume jellyfish, shedding new light on their importance in the ecosystem [16].

This change of perspective and the need to gain more knowledge about the ecological and economic significance of jellyfish blooms is what spawned the EU funded research project GoJelly (2018-2022). The main goal of GoJelly is to find new ways to exploit jellyfish blooms as an ecosystem service in a sustainable way, and to increase our knowledge on jellyfish bloom formation. This master thesis is part GoJelly work package 2 (WP2) - "Driving mechanisms and predictions of jellyfish blooms" which aims to identify abiotic and biotic trigger mechanisms that cause jellyfish blooms formation, duration and intensity of key jellyfish species from the Mediterranean Sea to the Norwegian Sea.

One of the driving mechanisms behind jellyfish blooms that has previously been neglected, but is now receiving more attention is the importance of the scyphozoan polyp (herafter referred to as polyp) life stages [4, 17]. Most scyphozoan species have a meropanktonic life cycle that include sexually reproductive pelagic stages (ephyrae, medusae, planulae larvae) and asexually reproductive benthic stages (polyps, strobilae) (Figure 1) [18]. Of particular interest is the cosmopolitan species Common Jellyfish (*Aurelia aurita*), the cold water boreal species Lion's Mane Jellyfish (*Cyanea capillata*), and the northern European seas endemic Blue Jellyfish (*Cyanea lamarckii*). These are part of the target species in GoJelly WP2, and are also the target species of this master thesis [4, 19, 20]. Given that the polyp life stages is a fundamental part of the scyphozoan life cycle, it is necessary to have thorough and accurate knowledge on polyp ecology, distribution and abundance if we are to make accurate jellyfish bloom predictions. Our knowledge on polyp ecology, distribution and abundance is significantly less established compared to other common benthic invertebrates like barnacles, bivalves, polychaetes and ascidians [4, 21, 22]. Polyps are often ignored and rarely found or identified because of their small size and tendency to inhabit shaded crevices and down facing sides of surfaces [4, 19, 23].

Lucas et al. (2012) has compiled an extensive review of the known ecology of polyps and narrowed down the most important mechanics of sustaining polyp and medusae populations as (1) recruitment, settlement and metamorphosis of planulae larvae into polyp, (2) survival and longevity of polyps (3), and (4) strobilation and recruitment of ephyrae [4].

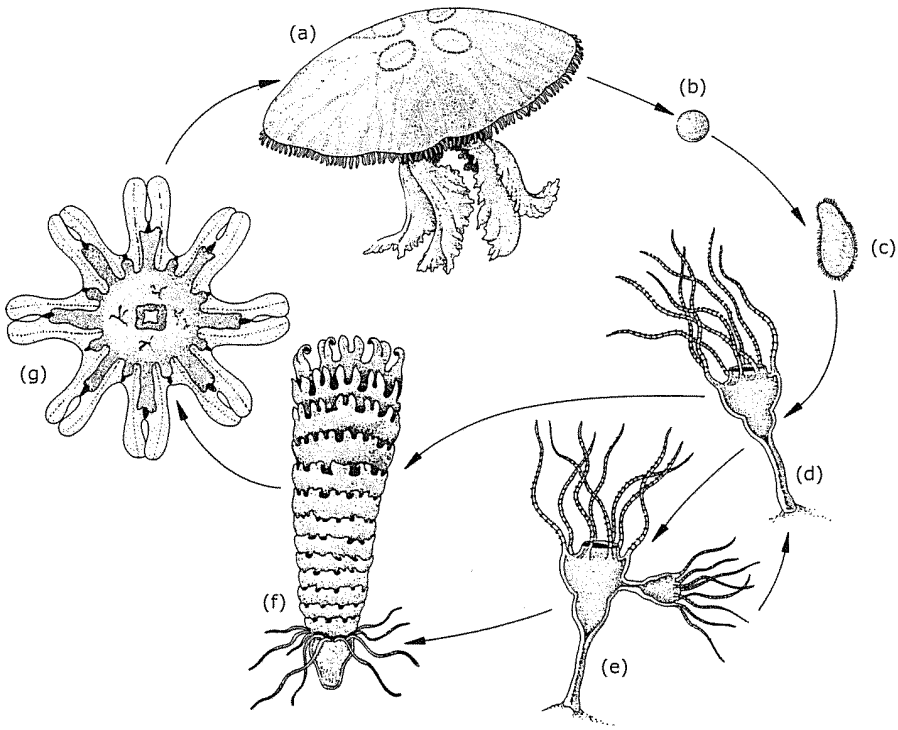


Figure 1: Illustration of the life cycle of the scyphozoan species *Aurelia aurita*. A fertilized egg (b) in the medusa develops into a planula larva (c). The larva settles onto a suitable hard surface and develops into a polyp (d). The polyp can clone itself by budding (c) and metamorphose into a strobila (f). The strobila eventually release its strobila discs e.i. the ephyrae larvae (g). The ephyra larva (g) will then grow into a new medusa (a). Modified illustration by Bayer & Owre (1968) [1].

As for recruitment in temperate waters such as Norwegian Sea, the spawning of planulae larvae is an annual occurrence between 1 and 5 months over the summer period [4, 24]. Scyphozoan planulae larvae is leicotrophic (larvae that only receive nutrition from their yolk sac) and *A. aurita* larvae has been observed to settle after one day, *C. capillata* two days, and *C. lamarckii* after four days [4, 25]. However, the estimated survival period of scyphozoan planulae larvae is longer, possibly up to one week based on the research by Schneider and Weisse (1985) [26]. The sensory capacity of scyphozoan planulae larvae is limited to mechanoreceptive and chemoreceptive cells [27]. They are able to determine the suitability of a settling place based on physical cues such as, gravity, light, and roughness of substratums [4, 28, 29, 30], and chemical cues such as chemicals released from bacterial film, conspecifics, competitors and predators [4, 24, 25].

Overall, polyps typically settle in shaded and sheltered environments on the down facing side of rough substrates [19, 23, 28]. Polyps have been found on a various types of artificial substrates like wood, granite, glass, polymers, iron and natural substrates like rocks, mussels, barnacles, ascidians, polychaetes, and macroalgae [4, 23, 25]. It has been theorised that an increase of introduced artificial substrates by coastal development could be the mechanism behind increased jellyfish bloom intensity and frequency, due to increased availability of down facing surfaces associated with marinas, wavebreakers, wind parks and more [31]. Thus, several studies have explored if there is preference between different substrates, particularly between artificial and natural ones [4, 25, 28]. In a laboratory experiment by Holst and Jarms (2007), artificial substrates such as concrete, machined wood, polythylene and glass were preferred over a natural substrate, namely mussel shells. However, similar quantifiable studies of substrate preference between artificial and natural substrates have not been conducted *in situ*. In addition, our knowledge on the distribution and abundance of polyps in *in situ* is significantly lacking, thus the true effect of coastal development is difficult to estimate. Another aspect of polyp ecology that is lacking, are studies on the potential biotic interactions between polyps and other benthic organisms. Predators of polyps *in situ* has been observed, namely the diatary specialist nudibranch *Coryphella verrucosa* [19, 24], but also non-selective feeders such as caprellid amphipods, pycnogonids and decapods [4, 32]. While direct competition for space and resources between polyps and other sessile epibionts such as mussels, barnacles, polychaetes and ascidians are rarely observed, it is assumed that they are competitively superior to polyps [4, 33].

In light of the limited knowledge on many aspects of polyp ecology, there is room for improvement. Thus, the aim of this thesis is to investigate and expand upon our knowledge on scyphozoan polyp ecology. More specifically, the aims of

this thesis is to study (1) distribution and interspecific variation of polyp colonies within and outside of Trondheimsfjorden, (2) study the natural habitats and microhabitats of polyps within and outside of Trondheimsfjorden, and (3) study the possible biotic effect and interactions between polyps and other sessile epibionts.

2 Methods

2.1 Study Area

The study areas analysed in this study were situated on several locations in Trondheimsfjorden. From the innermost part of the fjord to the outer part of Trondheimsleia (the area between Mausund, Frøya and Hitra and the mainland). (Figure 2).

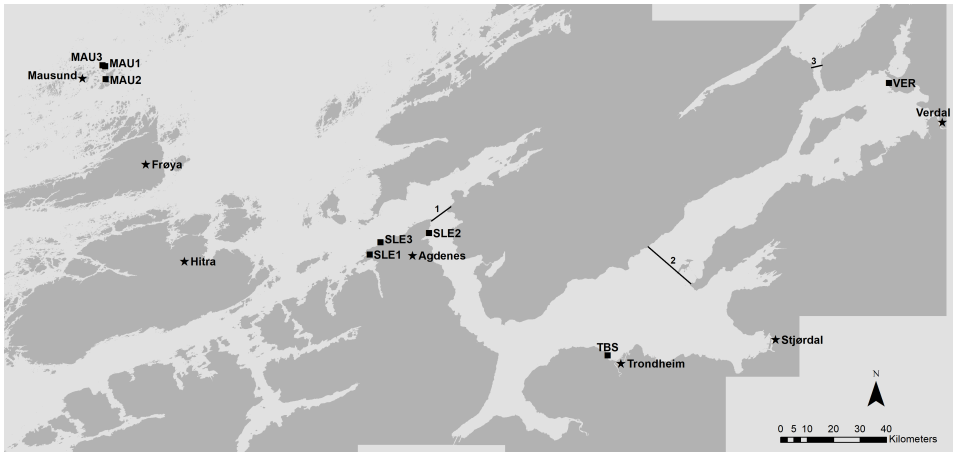


Figure 2: Trondheimsfjorden, Trondheimsleia and island group Hitra, Frøya and Mausund. Settling plate rigs where deployed at MAU1, SLE1 and TBS. Field survey was conducted at MAU1-3, SLE1-3, TBS and VER. Sills are indicated by the black lines. 1: Agdenes sill, 2: Tautra sill, 3: Skarnsund sill. Mapdata from Kartverket [2].

2.1.1 Trondheimsfjorden

Trondheimsfjorden is a fjord situated at 63°north on the west coast of Norway. It is 126 km long, has a volume of 235 km^2 and an average depth of 165 m making it the third longest and seventh deepest fjord in Norway. There are three main sills that define the different areas of Trondheimsfjorden. The Agdenes sill (1), separates the fjord from the outer sea. Two additional sills, the Tautra sill (2) and the Skarnsund sill (3), divides the fjord into three basins. From the outermost to the innermost basin they are called Ytterfjorden, Midtfjorden and Beistadfjorden (Figure 2) [34].

Water mixing in Trondheimsfjorden is affected by wind, river run-off, tidal energy and inflow from the North Atlantic current and the Norwegian coastal current. The sills in Trondheimsfjorden are quite deep, making water exchange with the outer sea relatively easy. The bottom water in Trondheimsfjorden is usually

exchanged twice a year from Atlantic and Norwegian coastal waters. First, from February to May-June there is an inflow of high salinity Atlantic deep water that produces a new layer of bottom water in the fjord, driving the old water out of the fjord. Secondly, in the late summer period, there is an inflow of 32-34 saline Norwegian intermediate coastal water that mixes into the bottom water and slowly exchanges the old water [35].

Rivers have a large effect on surface water mixing in Trondheimsfjorden when considering the multiple river sources [35]. Since Trondheimsfjorden is relatively wide, wind is also a big source of surface water mixing [35]. However, the tidal energy has been measured to be 100 to 1000 times larger than the combined power of wind and river energy sources, making tidal energy the most significant source of surface water mixing. Results from a tidal prism method by Jacobsen et al. (1983) showed that of the total tidal volume, 47% passes from the Ytterfjorden basin to the Midtfjorden basin, while 16% reaches all the way to the Beistadfjorden basin [35].

Also of interest to this study is the area outside of Trondheimsfjorden. Here lies the strait called Trondheimsleia, this strait is located between mainland the island group of Hitra, Frøya and Mausund. Mausund is the most northerly and exposed of the islands (Figure 2). Mausund is usually addressed as a single island, but is in fact a cluster of several islands islets and will be addressed as such in this study. In contrast to Trondheimsleia and Trondheimsfjorden, Hitra, Frøya and Mausund, are particularly exposed to strong currents and winds. This region is also known for its abundant kelp forests and high primary production [36].

2.1.2 Stations

The main stations of this study were at Mausund (MAU1-3), Agdenes (SLE1-3), Trondheim Biological station (TBS) and Verdal (VER). The stations were selected in order to provide a transect across Trondheimsfjorden, and to compare how different hydrographies, topographies and species compositions could affect the polyp colonies. Settling plate rigs where deployed at MAU1, SLE1 and TBS. Field surveys stations were in the littoral zones (more specifically the lower intertidal fringe and the subtidal zone) of MAU1-3, SLE1-3, TBS and VER.

MAU1 was located inside a bay on the North side of the northernmost island of Mausund. As described, Mausund is regularly exposed to strong winds and currents. The bay that MAU1 was located in, was thought to be more sheltered from the strong currents, but this theory was later rebutted. Also, in contrast to the topography of the stations in Trondheimsfjorden, the littoral zone of MAU1 was relatively short and steep. MAU2 was located on the northern side of an island south of MAU1. Similar to MAU1, MAU2's littoral zone was short and steep and mod-

erately exposed to stronger currents. MAU3 was located on the northern side of the same island as MAU1 in a narrow embayment. This embayment was less steep and dominated by large kelp and thus less exposed to strong currents relative to MAU1-2.

SLE1 was located in a sheltered lagoon called Hopavågen on the western side of Agdenes. Water exchange between the lagoon and the outer sea is limited by a narrow passage called Straumen, making water mixing largely dependent on the water inflow and outflow of the tidal cycle, thus strong currents were negligible. A quick survey was performed close to SLE1 outside of Hopavågen close to a breakwater and was called SLE1 (b). SLE2 was located close to Breivika Camping and the Agdenes sill inside the mouth of the fjord. The currents at this relatively narrow passage in the fjord are strong but SLE2 was protected by large rocks, kelp and the Breivika camping wavebreakers. SLE3 was located in a shallow bay on the northern side of Agdenes. This bay was sheltered from stronger currents due to a long sand flat and large abundances of kelp.

TBS was located in the middle of the Ytterfjorden basin and right outside of Trondheim biological station (TBS) close to the city of Trondheim. More specifically in an embayment with macroalgae canopies. This station was affected by water mixing and currents that are typical for Ytterfjorden.

VER was located in a small shallow embayment in inner part of Midtfjorden basin and close to the mouth of Borgefjorden (a side inlet to Trondheimsfjorden) also called Straumen, a source of strong currents during high and low tide. Even though VER1 was close to Straumen, the small embayment was relatively sheltered from the strong current.

2.2 Field survey - determining prevalence, seasonality, and habitat of polyp colonies

2.2.1 Location selection and duration of field survey

Initially, the stations (Table 2, Figure 2) where picked specifically with the intention of finding polyp colonies in order to confirm their presence across a larger transect of Trondheimsfjorden. Previous research on *in situ* polyp findings provided the following characteristics that would increase the probability of finding polyps: (1) Sheltered embayments with macroalgae canopies [19], with (2) sufficient hard substrates able to provide shaded microhabitats [4, 23]. After promising results, polyp habitat were also studied at these stations. The stations were screened for polyps monthly from mid-March 2018 to late May 2018 (Table 2).

2.2.2 Sampling and examination procedure

For the purpose of examining the occurrence of polyp colonies, various kinds of artificial and natural hard substrates in the selected littoral zones were examined (Table 2). The hard substrates were fully or partially submersed in seawater and obtained from approximately 0.1 m to 3 m depth. The samples were obtained by hand, with a rake or with a shovel, using snorkelling and wading equipment. The substrates were then examined on site using transparent plastic containers.

The substrates were examined by the following procedure. Material type was determined (1). Each individual polyp was counted (2). Only substrates with polyps were used in the dataset. The location (i.e. underside, hole, crevice) of polyp clusters on the hard substrate were reported (3). The developmental stage of every polyp was examined and categorised as polyp or strobila in order to evaluate the development stage of the polyp colonies (4). Random polyp individuals from 0.5 mm to 2 mm in size were fixed in ethanol (96%) for molecular species identification (5). If possible, polyp species were identified on site by examining released ephyrae morphology traits as described by Holst (2012) [37]. In order to describe the habitat and possible biotic influences on the polyp colonies, hoop plots were applied at each station (6). The hoop plots consisted of a hula-hoop-ring (1 m diameter) with weights attached. Species observed inside the hoop plot were identified to the lowest possible taxonomic level or functional group. The abundance of each species inside the hoop plot was scaled with the following levels: 0 (none), 1 (very low), 2 (low) 3 (moderate), 4 (high), 5 (very high).

2.3 Settling plate experiment

2.3.1 Settling plate rigs setup and duration

Settling plate rigs were deployed at MAU1, SLE1, and TBS. Each settling plate rig contained a total number of six settling plate units as shown in Figure 3. Design and construction of the settling plates were inspired by the SETL plate design used in the ANEMOON SETL project [23]. The settling plate units were constructed using bricks (285 mm x 85 mm x 85 mm), PVC plates (140 mm x 140 mm x 5 mm) and zip ties. The settling plates were moored to the rigs from floats at 1 m depth and 3 m depth and with approximately 1 m between each float, making three replicates at 1 m and 3 m (Figure 3). Examination of the settling plate rigs were performed monthly on MAU1, SLE1, TBS from April 2018 to early October 2018 (Table 3). After the first observation of polyps, examination of the settling plates ended and the rigs were taken ashore and processed in order to compare the final settling epibiont coverage and polyp abundance on the settling plates at the different stations.

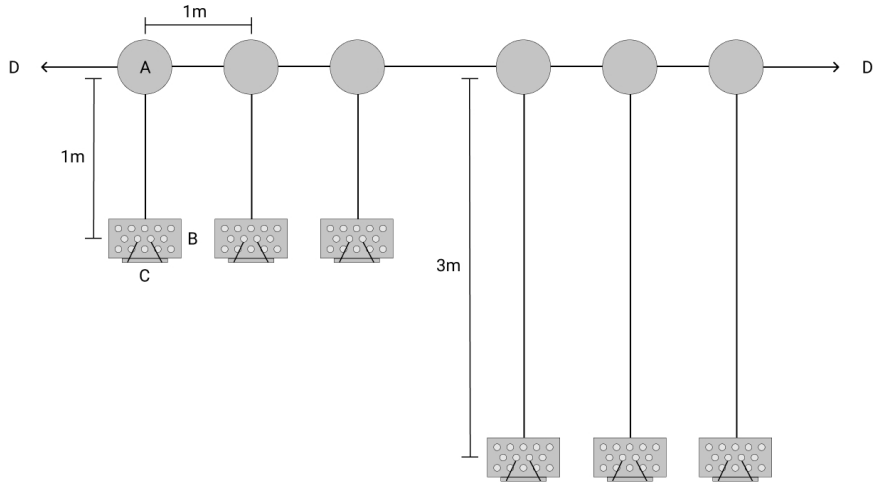


Figure 3: Settling plate rig sketch. A = float, B = brick, C = plate, D = mooring points.

2.3.2 Settling plate examination and processing procedure

From deployment to extraction, the settling plates were examined monthly. Using snorkelling equipment and underwater photography, the settling plates remained fully submerged in seawater when examined. The settling plates were examined for the presence of settling polyps and other settling epibiont species. During the season at each examination, other settling epibiont species were identified to the lowest possible taxonomic level or functional group, and their scale of coverage on the brick and PVC plate from 0 (none) to 5 (very high) was reported. After the first observation of polyps on SLE1 19.09.18, each settling rig (SLE1, MAU1, TBS) was retrieved and processed. The state of other settling epibiont species during the final examination were documented with photography and their percent area coverage on the settling plate, was calculated using ImageJ 1.8.0 software (U.S. Department of Health and Human Services) [38]. All species where treated as a single layer on a two-dimensional plane, even though some plates at TBS had several layers of species. Thus, a mix of dead *Balanus balanoides* (barnacle) and live/dead *Mytilus edilus* (bivalve) were treated as one layer, and one variable. The polyps were counted using stereo microscopy (1-16x). Random polyp individuals from 0.5 mm-2 mm in size were fixed in ethanol (96%) for molecular species identification.

2.4 DNA extraction and sequencing

DNA was extracted from 121 out of 270 field survey polyp samples and 72 and 195 settling plate polyp samples, by randomly selecting 1-10 samples based on station and morphology traits (colour, size).

DNA was extracted with Chelex rapid boiling procedure. Pieces or whole individuals were dried in Eppendorf tubes (1 ml). 50 µL Chelex ((6%), tris (50 mM) and EDTA (0,5 mM)) was added, heated to 96°C for 10min, and centrifuged at 15000 rpm for 10min. The supernatant (DNA) was used for the polymerase chain reaction (PCR) amplification. Similar to the protocol of van Walraven et al. (2016) on scyphozoan DNA extraction and amplification, scyphozoan specific COI primers were used to amplify the COI gene, namely,
ScyCOI forward primer (5-CTATACTTAATATTTGGTGCYTTTTC-3) and
ScyCOI reverse primer (5-AAATGTTGGAATARTATTGGRTCTCCT-3) (Table 1) [23]. PCR amplification started with 5 min at 98°C, followed by 40 cycles of 10 s at 60°C, 1 min at 72°C and finally 5 min at 72°C. Samples which were unsuccessful with scyphozoan specific COI primers, were amplified using Folmer universal COI primers [39],
LCO1490 (5-GGTCAACAAATCATAAAGATATTGG-3) and
HC02198(5-TAAACTTCAGGGTGACCAAAAAATCA-3).
Here, PCR amplification started with 5 min at 98°C, followed by 40 cycles of 10 s at 60°C, 1 min at 72°C and finally 5 min at 72°C.

The quality of the PCR products were analysed using gel-electrophoresis. PCR products with positive results were cleaned using Illustra™ GFX™ PCR DNA and Gel Band Purification Kit and protocol, then shipped to Macrogen Europe (Netherlands) for sequencing. Forward and reverse sequences were cleaned using Chromas 2.6.6 software (Technelysium Pty Ltd) [40], and combined using Contig Assembly Program (CAP) accessory application [41] in BioEdit Sequence Alignment Editor 7.0.5 software [42]. The sequences were then compared with existing nucleotide sequences in a collection of databases using Basic Local Alignment Search Tool (BLAST) in order to identify the polyp species [43].

Table 1: Compound and their respective volume used in the PCR master mix

Compound	volume (µL)
Forward primer	1
Reverse primer	1
dNTP	0.6
DMSO	0.4
Thermo Scientific Phire Hot Start II DNA polymerase	0.4
Thermo Scientific 5x Phire Reaction Buffer	4
dH2O	11.6
DNA supernatant	1

2.5 Statistics

A principal component analysis based on the epibiont coverage variables, was computed in R using Non-metric Multidimensional Scaling with the `metaMDS` function in the R package Vegan [44]. One settling plate replicate was lost from both MAU1 and SLE1 and was thus not part of the calculation. Also, the most shallow settling plate replicate at TBS1 sustained damage from contact to the sea bottom during the season resulting in a settling plate devoid of epibionts and polyps (Figure 12). This was deemed a major outlier in the dataset and thus excluded from the PCA. A PCA plot was then generated in R using the loading scores of the "points"-object from the PCA (Figure 9). The segments representing the variable loading scores were generated using the "species"-object loading scores. A layer encircling the different observations of each station, and a layer for each depths were added. A summary of species and their respective scales was summarised in a stacked bar plot by calculating the mean abundance levels of species from each hoop plot at each station (Figure 4).

3 Results

3.1 Field survey

The field survey provided a qualitative overview of the polyp colonies within and outside of Trondheimsfjorden (Table 2). Polyps were found on 70 substrates of varying material type on nine stations within and outside of Trondheimsfjorden. The polyps on MAU3, SLE2, SLE3, TBS, and VER were observed in embayments with macroalgae canopies at 0.2-1.5 m depth on the down facing side of rocks, a concrete slab, and two occurrences on kelp (MAU3) (Figure 6a). The polyps on MAU1 and MAU2 were observed inside cracks of the rocky bottom at 0.2-2 m depth on the down facing side of rocks. The polyps on SLE1 were observed in a sheltered lagoon at 1-2 m depth on the down facing side and inside bricks, cinder blocks and a glass bottle, on an iron plate, on polychaete tubes of *Pomatoceros triqueter* (Figure 6b), and on rocks. Strobilae where only found earlier in the season from mid March to early May, but were no longer present in late May. The molecular species identification using scyphozoan specific COI primers returned matches of *Aurelia aurita* from MAU2, MAU3, SLE1-3, TBS, VER, and matches of *Aurelia sp.* from SLE1 and SLE2 (accession numbers in Table 4). The molecular identification using universal COI primers returned matches of *Ascophyllum nodosum* (MH309539.1, MH309680.1) (brown macroalgae) (3) from TBS, *Alteromonas sp.* (CP018023.1) (bacteria) (2) from SLE1, *Dexamine thea* (KT209105.1) (amphipod) (1) from MAU3, *Glycinde armigera* (KT989325.1) (polychaete) (2) from MAU2-3, *Ancylis badiana* (KM573396.1) (moth) (1) from MAU2, and were not included in Table 2.

The hoop plots provided an overview of the species and functional group composition of each station (Figure 4). MAU2 and MAU3 were similar in species variation but differing in abundance with the most noticeable groups being, moderate (2.5) and high (4.0) *Spirorbis sp.* (tube dwelling polychaete), moderate (3) and very high (5) coralline algae, moderate (3) and very low (1) green filamentous algae, and low (2) and very high (5) brown macroalgae, and moderate (3) and high (4) brown filamentous algae, respectively. TBS and MAU2-3 shared some species variation and abundances with the most noticeable groups being, moderate (3) Hydrozoa, moderate (3.2) *Spirorbis sp.*, and very high (5) brown macroalgae, while dissimilarities to MAU2-3 was its occurrences of red film algae (1.5) and red macroalgae (1.5). VER was dominated by algae and gastropods with the most no-

Table 2: Results from the field survey. Station name, coordinates of station, depth of sampling, sampling date, substrate type, number of substrates, number of polyps, number of strobilae and species as derived from the BLAST results. Sampling dates and site were substrate type varied have a summarised **Substrate total** row. Only substrates with polyps were used in the dataset. Number of substrates does not equal the number of substrates examined. *Identified by released ephyrae from strobilae.

Station	Coordinates	Depth (m)	Date	Substrate type	Number of substrates	Number of Polyps	Number of Strobilae	Polyp species (# BLAST results)
MAU1	63°52'34.23"; 8°38'29.07"	1	10.04.2018	Rock	3	53	24	*Aurelia aurita* *
MAU2	63°51'23.66"; 8°38'34.62"	0.2-2	29.05.2018	Rock	3	103	0	*Aurelia aurita* (5)
MAU3	63°52'39.26"; 8°37'56.08"	0.4	29.05.2018	Kelp	2	23	0	*Aurelia aurita* (3)
SLE1	63°35'34.98"; 9°32'23.28"	1-2	14.03.2018	Brick	3	14	5	*Aurelia aurita* (4)
				Rock	1	6	5	
				Glass bottle	1	4	3	
				Iron plate	1	24	0	
				Substrate total	**6**	**48**	**13**	
		1-2	12.04.2018	Brick	3	20	0	*Aurelia aurita* (4)
				Cinder block	3	71	0	*Aurelia aurita* (5)
								Aurelia sp. (4)
				Rock	1	3	0	*Aurelia aurita* (1)
				Substrate total	**7**	**94**	**0**	
		1	30.05.2018	Rock	5	36	0	*Aurelia aurita* (4)
								Aurelia sp. (1)
SLE1 (b)		0.5	13.04.2018	Rock	1	2	0	*Aurelia aurita* (2)
SLE2	63°37'32.21"; 9°44'29.33"	0.5-1	31.05.2018	Rock	4	66	0	*Aurelia aurita* (5)
								Aurelia sp. (7)
SLE3	63°36'41.37"; 9°34'36.62"	0.2-1	13.04.2018	Rock	2	39	16	
TBS	63°26'25.41"; 10°20'54.83"	0.2-0.5	06.04.2018	Rock	4	80	13	*Aurelia aurita* (2)
				Concrete	1	15	5	
				Substrate total	**5**	**95**	**18**	
			04.05.2018	Rock	1	30	0	*Aurelia aurita* (3)
			25.05.2018	Rock	2	81	0	
VER	63°51'3.18"; 11°18'16.41"	0.1-1	15.03.2018	Rock	17	214	27	*Aurelia aurita* (6)
		0.1-1.5	02.05.2018	Rock	7	98	6	*Aurelia aurita* (3)

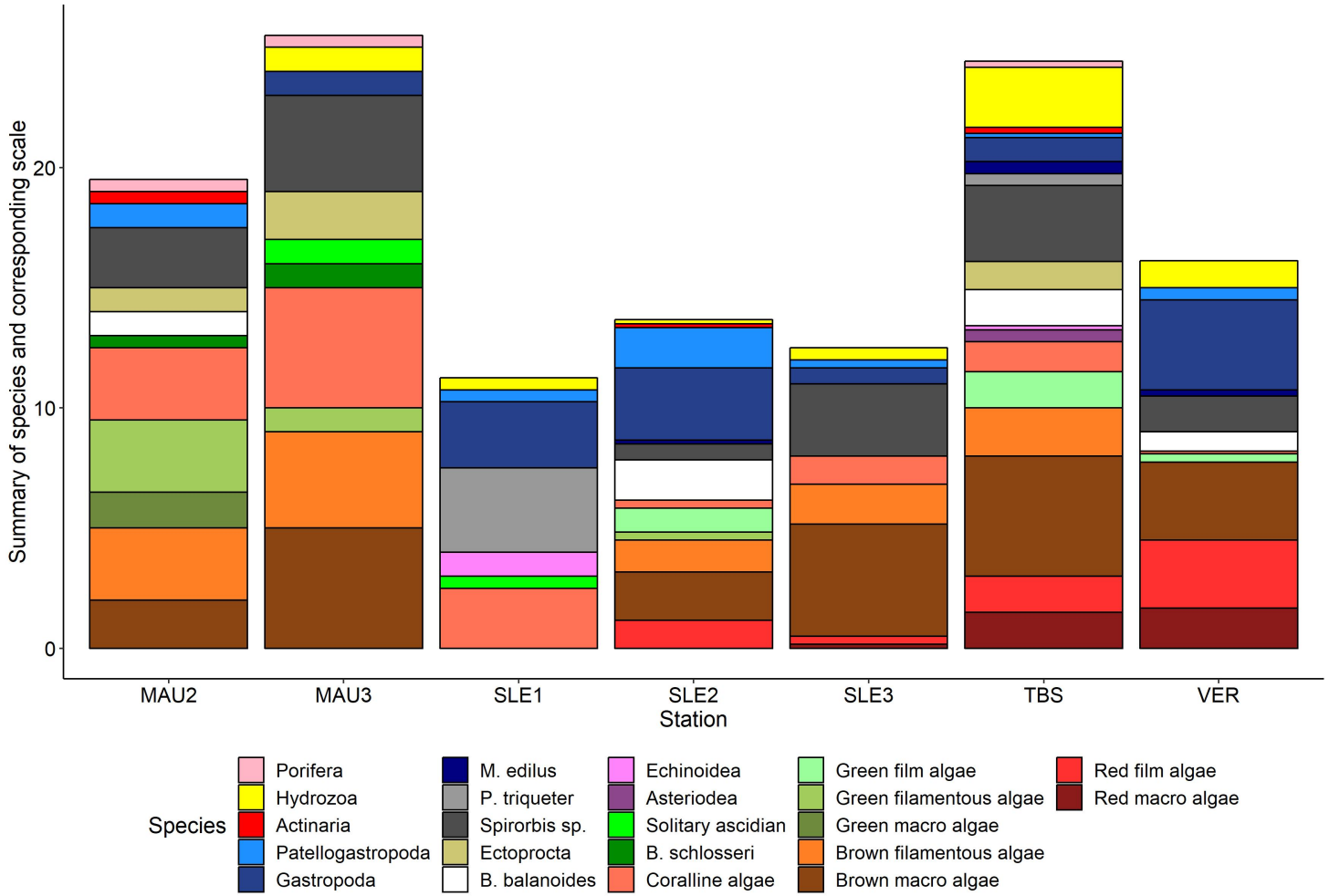
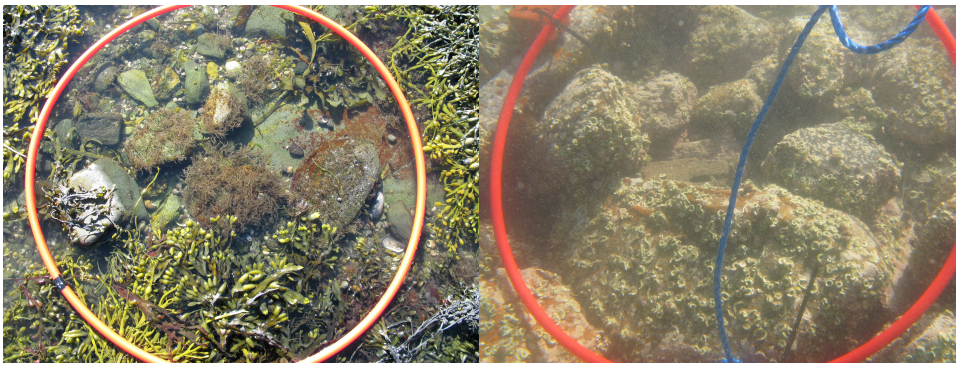


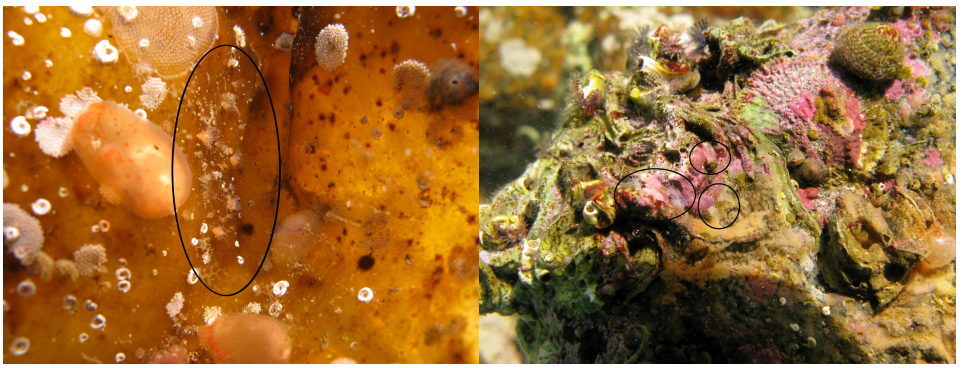
Figure 4: An overview of the species and functional group composition of MAU2, MAU3, SLE1, SLE2, SLE3, TBS and VER.

ticeable groups being high (3.8) Gastropoda, moderate (3.3) brown macroalgae, moderate (2.8) red film algae and low (2) red macroalgae (Figure 5a). SLE1 was uniquely different from the other stations with the most noticeable species being moderate (2.75) Gastropoda, high (3.5) *P. triqueter*, and moderate (2.5) corraline algae (Figure 5b). SLE2 had a high species variation albeit in small abundances and was mostly dominated by moderate (3) Gastropoda, small amounts of brown filamentous algae (1.3) and brown macroalgae (2). SLE3 was mostly dominated by moderate (3) *Spirorbis sp.* and very high (4.7) brown macroalgae.



(a) **(b)**

Figure 5: (a) Hoop plot from VER and an example of a polyp habitat with a very high abundance of macroalgae. (b) Hoop plot from SLE1 and an example of a polyp habitat without any macroalgae and dominated by *P. triqueter*.



(a) **(b)**

Figure 6: (a) Polyp microhabitat on kelp lamina from MAU3. Other species on the lamina are bryozoa, *Spirorbis sp.*, *A. mentula* and compound ascidians. (b) Polyp microhabitats in *P. triqueter* tube crevices, covered with coralline algae on a rock from SLE1. Polyps are encircled. Photo: M. E. Rekstad

3.2 Settling plate experiment

The settled polyp abundance varied greatly between stations, but also between the settling plate units with one standard deviation exceeding the mean (Table 3). The 3 m SLE1 settling plates had the highest amount of settled polyps with an average of 239.3 (±145.3) polyps per plate, while the 1 m SLE1 settling plates had no polyps. 1 m TBS settling plates had an average of 74.5 (±91.2) polyps per plate and 3 m TBS settling plates had an average of 24.3 (±13.9) polyps per plate. There were no polyps on the MAU1 settling plates, both on the 1 m and 3 m plates. The abundance of polyps on the different substrates were highest on the PVC plate material (Figure 8a) at all depths and all locations, followed by *Ascidia mentula* (solitary ascidian) at SLE1 (Figure 7a) and *Balanus balanoides* (barnacle) at TBS (Figure 7b), and finally a few polyps were present on *Ciona intestinalis* (solitary ascidian) at SLE1 and *Mytilus edilus* at TBS. The molecular species identification returned 25 matches of *A. aurita* from 3 m SLE1, 13 matches of *A. aurita* from 1 m TBS, while 3 m TBS had 18 matches of *A. aurita* and 1 match of *Aurelia sp.* (Table 3) (accession numbers in Table 5).

Table 3: Station, depth and retrieval date of the settling plate rigs. The third column shows a summary of polyp abundance between the different stations and depths. The fourth column contains percentage distribution of polyps on the different substrates. The fifth column contains the most likely species match from the BLAST sequence comparison and the number of matches.

Station	Depth(m)	Retrieval date	Mean polyp abundance (±SD)	Polyps on substrate distribution (%)	Polyp species (# BLAST results)
MAU1	1	10.10.18	0		
	3		0		
SLE1	1	19.09.18	0		
	3		239 (±145)	PVC plate (88)	*Aurelia aurita* (25)
				A. mentula (12)	
				C.intestinalis (0.1)	
TBS	1	25.09.18	75 (±91)	PVC plate (79)	*Aurelia aurita* (13)
				B. balanoides (21)	
				M. edilus (0.6)	
	3		24 (±14)	PVC plate (74)	*Aurelia aurita* (18)
				B. balanoides (22)	
				M. edilus (4)	*Aurelia sp.* (1)

The PCA plot of the epibiont coverage variables is provided in Figure 9. The points representing the TBS settling plates are separated from the rest of the points on dimension 1, by the debris-mix variable. Similarly, the points representing the MAU1 settling plates are separated from the points representing the SLE1 settling plates on dimension 2. The MAU1 points are pulled by the *Botryllus schlosseri* (compound ascidian), Bryozoa, compound ascidian, *Jassa falcata* (tube dwelling amphipod) (Figure 8b), and "no coverage" variables. The SLE1 points are pulled by the *A.*

(a) **(b)**

Figure 7: (a) Polyp on *A. mentula* from a SLE1 settling plate. Other species on the plate are *P. triqueter*, *C. intestinalis*, and hydrozoa. (b) Polyp on a barnacle shell of dead *Balanus balanoides* from a TBS settling plate. Polyps are encircled. Photo: M. E. Rekstad

mentula, *C. intestinalis*, *M. edilus* and *P. triqueter* variables. In summary, the points clustered into three groups that resonated with the three stations, first on dimension 1 and then dimension 2, pulled by the different epibiont coverage variables.

Figure 9 (b) shows the encircling of the different depths. The 1 m circle envelops most of the 3 m circle indicating that the variation between the two depths are small, but also that the variation of epibiont coverage is higher on 1 m. Also, there is a positive relationship between high polyp abundance on SLE1 and the epibiont coverage of *A. mentula* and *P. triqueter*, and the positive relationship between low polyp abundance on SLE1 settling plates and epibiont coverage of *C. intestinalis* and *M. edilus*.

(a)

(b)

Figure 8: (a) settling plate surface with visible *B. balanus* imprints, with polyps located on imprints (circle) and PVC material. (b) Settling plate depicting typical ebiont coverage on MAU1, leathery brown tube material are the tubes of *J. falcata*. Photo: M. E. Rekstad

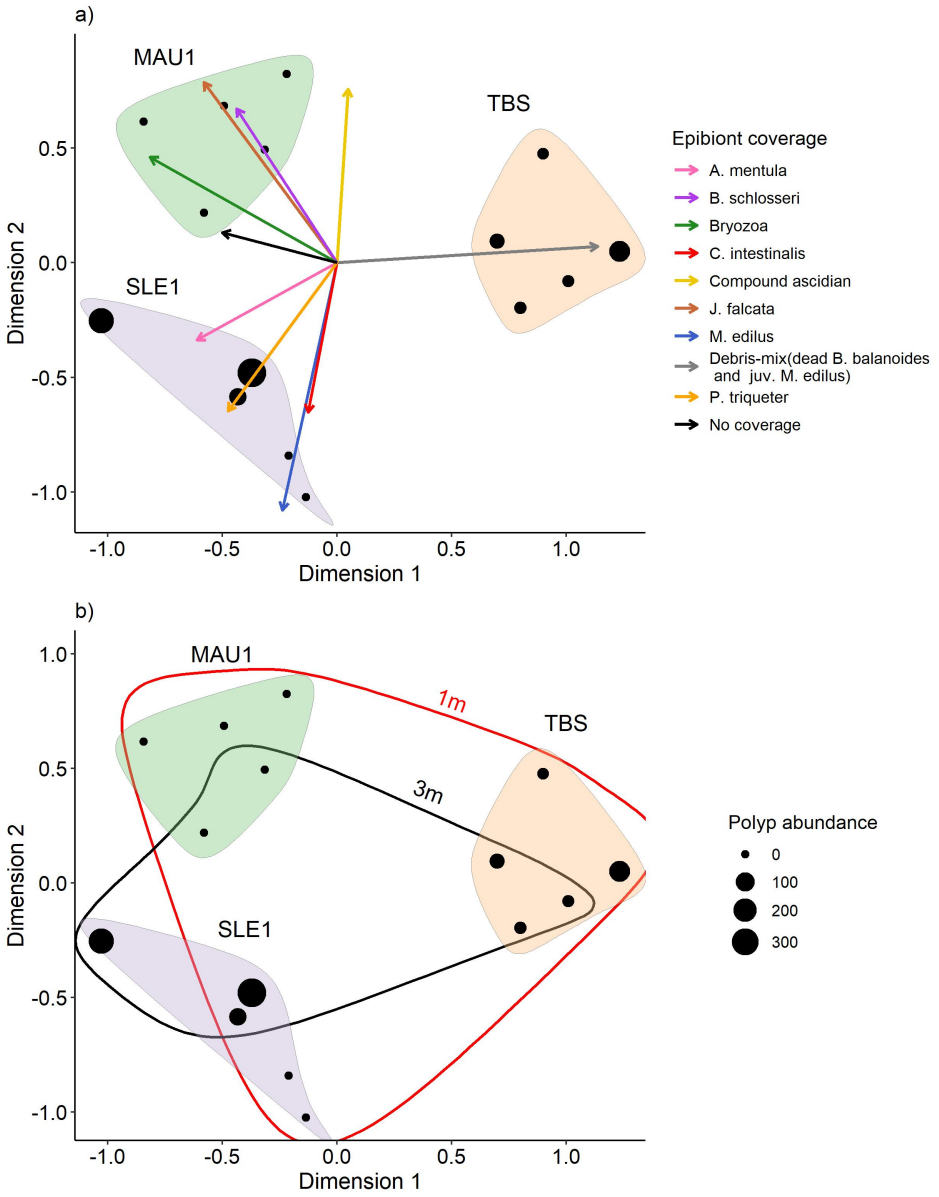


Figure 9: Principal component analysis of the epibiont and substrate coverage variables of the settling plates. Dots represents the settling plate replicates. Dot size increase with high polyp abundance. Each location is encircled and annotated. The vectors (segments) in plot (a) represent the epibiont coverage variable loading scores from PCA. Depths are encircled on plot (b).

4 Discussion

Methodology

The methodology of this study was developed largely due to the known difficulties of studying polyps *in situ*. Conducting a field survey was an objective since the inception of this study, but there was an uncertainty of whether a field survey would provide sufficient data given the known difficulties of actually finding polyps *in situ*. Given the success of previous research using settling plates in studying polyps [23, 24, 45, 46, 47], the same method was incorporated into this study as part of the data gathering process, and as a backup in the scenario were the field survey proved inadequate. The use of settling plates as a data gathering tool was indeed successful. The increasing amount epibiont growth during the season was topic of concern since this could hinder the polyp settlement completely. However, the other epibiont species provided a valuable opportunity to study the possible biotic influences of other epibiont species on polyp settling success, as well as being indicative of the environment the settling plates inhabited.

The structural integrity of the settling plate rigs remained mostly intact during the experiment. The settling plate unit designed by Van Walraven et al. (2016) (SETL plate, ANEMOON SETL project) [23] by itself were fully functional and remained undamaged throughout the season. However, the settling plate rig design had its strengths and weaknesses. Two settling plates units were lost, one from SLE1 and one from MAU1, because of structural damage to the settling plate unit attachment point. The structural damage was caused by friction between the rope knot and zip ties. The zip ties were meant to retain the knots and the settling plate units spatial distribution on the settling plate rig. The use of zip ties for this purpose was a poor design choice and could have been avoided. Using rope exclusively when constructing such a settling rig is recommended, and the use of zip ties should be avoided altogether, mainly because of its lack of elasticity, and potential structural damage to the ropes.

Using floats made it possible to retain the depth of each settling plate during the tidal cycle, but the design was possibly more complex than necessary. A simpler rig without floats, or a rig with more replicates per rope, could potentially provide more replicates with equivalent use of space and materials. Patchy distribution is common among benthic species, and is likely also one of the elements causing variation among the settling plate epibionts (including polyps) [48]. More replicates would provide a more accurate PCA and could possibly reduce the overall variation

of the settling plate data, as was apparent by the high standard deviation of polyps on the settling plates.

The original intent of the field survey was to report the occurrence of polyps in the study area, and sample them for molecular species identification and population genetics (a GoJelly WP2 objective), if polyps were indeed located. Despite the known uncertainties of finding polyps *in situ*, several polyps were located on several stations during the first field survey trails. After locating sufficient amounts of polyps to confirm their occurrence on several stations, additional measures were implemented, in order to study polyp abundance and distribution, and their habitat. It was not possible to quantify the abundance and distribution of polyps, based on the qualitative nature of the previous data gathering process of the first field survey trials. For example, the number of substrates checked was not reported, the substrates checked were not randomly distributed per station, no transects or quadrants were used. Thus, in order to quantify polyp abundance and distribution, the use of hoop plots as a quantification tool was implemented.

The original intent and procedure using the hoop plots was to quantify polyp abundance and distribution in a given area, and to quantify the habitat of the polyps by reporting other species observed in the hoop plot. However, this procedure proved difficult for several reasons. First, the process was relatively time consuming for two persons, resulting in a difficult balance of time management between objectives. Time spent using hoop plots meant less time spent gathering sufficient data on the population genetics of the polyps, which are one of the main objectives of GoJelly WP2. Second, given how polyps are usually located on down facing surface of substrates, invasive measures such as removing substrates from the plots was required in order to locate them. This meant that heavy or practically immobile substrates would remain unexamined inside the hoop plot, thus would not provide an accurate estimate of polyp abundance and distribution. Third, the topographies of the stations were generally uneven and varied, tidal levels during examination varied, and the patchy distribution of benthic organisms is equally true of polyps *in situ*, overall making standardisation between hoops and stations very challenging.

Overall, prior knowledge on correct assessment of benthic habitats and ecology *in situ* was not sufficient, resulting in a time consuming and uncertain data gathering process. Thus, the hoop plot protocol was simplified, and only used as a data gathering tool in order to assess the species composition on the different stations. However, the methodology behind the species composition assessment was relatively imprecise, i. e. grading species abundance on a level scale from 0 (none) to 5 (very high) based upon assessor's taxonomic knowledge and observation skills *in*

situ. In hindsight, a more precise method could have been, to apply many random hoop plots to a given site and then photograph them. Apply a standardised grid of points to the photograph, then identify and count all the organisms marked by a point. This procedure would save time in the field, while also reducing variation by potentially providing more replicates.

The DNA sequencing procedure inspired by the protocol of van Walraven et al. (2018) [23] was successful. However, nine samples failed to amplify using the scyphozoan specific COI primers, but were successfully amplified using universal COI primers. The matches did not return as scyphozoans but as other various marine organisms. It is possible that organisms sharing a morphological resemblance to polyps were sampled by accident, thus serving as a possible explanation for why the scyphozoan specific primers did not work. However, none of the matches from the universal primers sequences resembled polyp morphology. Thus, it is likely that the actual gut content of the potentially misinterpreted organisms represents the universal primer results. The identity of the misinterpreted organisms remains speculative, but some suggestion are hydrozoans or anemones, which are typical groups sharing a similar morphology to polyps. Another possibility for error during the molecular analysis could be that no material from the target species of the scyphozoan specific primers were present during the initial scyphozoan specific PCR amplification due to human error. Thus, when the universal primers were used, background DNA from organisms in the medium might have been picked up during the universal primer PCR amplification.

Interspecific variation among polyps within and outside Trondheimsfjorden

The rest of the organisms sampled from the field survey and settling plates were successfully amplified using the scyphozoan specific COI primers, and returned as matches of *A. aurita* or *Aurelia sp*. Thus, the polyps identified in this study can be considered as either *A. aurita* or *Aurelia sp.* Polyp distribution in the littoral zone within and outside of Trondheimfjorden was evidently dominated by *A. aurita*. Similar to the findings of van Walraven et al. (2016) in the southern North Sea [23], no polyps of *Cyanea capillata* and *Cyanea lamarckii* were found. Their occurrence within and outside of Trondheimsfjorden remain a mystery. Given the fact that *C. capillata* medusae were observed on GoJelly cruises in Trondheimsfjorden June 2018, and *C. lamarckii* was observed once at SLE2 in September 2018 (unpublished data). It is likely that these medusae originate from polyp colonies in Trondheimsfjorden, close-by, or passively drifted into the Trondheimsfjorden ecosystem from longer distances e.g. Norwegian Sea and North Sea. There are some possible explanations for why *C. capillata* and *C. lamarckii* polyps were not discovered in

the study area.

First, *A. aurita*, *C. capillata* and *C. lamarckii* could all have different life cycles. They can spawn at different times of the year, resulting in different planulae settling, polyp development, encystment, excystment, and strobilation seasons. In theory, the abundance of each species' polyps are highest during the post-settlement and polyp development period, but will be reduce in numbers throughout the season due to predation, mortality, intraspecific and interspecific competition [4]. It is possible that the temporal window of *A. aurita* post-settlement period matched the timing of the present study, relative to that of *C. capillata* and *C. lamarckii*. Thus, increasing the probability of finding *A. aurita* relative that of *C. capillata* and *C. lamarckii*.

However, there is a great temporal variation in the reported scyphozoan spawning seasons, particularly relative to the global spatial distribution of *A. aurita* [4]. Gröndahl (1988) investigated the life cycle of *A. aurita*, *C. capillata* and *C. lamarckii* in Gullmarfjorden on the Swedish west coast from 1982 to 1986 [24]. His findings demonstrated that *A. aurita* spawn from June to October, *C. capillata* from July to December, and *C. lamarckii* from May to August [24]. For *A. aurita* and *C. capillata*, these observations match the present study, as the first sexually mature *A. aurita* and *C. capillata* medusae was observed from July 2018 (unpublished data). Gröndahl (1988) observed *A. aurita* polyp settlement from August to October, while *C. capillata* polyps was only found once *in situ* on a settling plate in November, and no *C. lamarckii* polyps were found at all [24]. For *A. aurita*, these observations match the present study, as polyps were observed on the settling plates September 2018. However, all *A. aurita* polyps in the present field survey were observed from March 2018 to May 2018. Given that the *A. aurita* polyps found during the field survey was observed before the annual settling season observed in the present study and by Gröndahl (1988), it is likely that the polyps observed in field survey settled sometime during August to September 2017. This indicates that *A. aurita* polyps survive either in their polyp or podocysts (chitin-covered encystment of polyps) form throughout the whole year in the littoral zone of Trondheimsfjorden, thus more likely to match the temporal window of this field survey.

Given the limited observations of *C. capillata* and *C. lamarckii* polyps *in situ* it is difficult to estimate their survival rate during the same time frame [4, 24]. However, if in fact the temporal window of *C. capillata* and *C. lamarckii* polyps settlement and strobilation is shorter due to mortality, it is indeed possible that they do not match temporal window of this field survey. In addition, *Cyanea sp.* is the only known genus of scyphozoans to form chitin-covered cysts of planulae (planulacysts) [49, 50]. Podocysts and planulacysts are significantly more inconspicuous than regular polyps, thus even more likely to be overlooked than regular polyps *in*

situ. It is thus possible that they were in fact present in the temporal window of the field survey but were overlooked as planulacysts or podocycsts. Similarly, it is possible to overlook *A. aurita* as podocysts. However, given the abundance of regular *A. aurita* polyps found in this study it is safe to conclude that sufficient *A. aurita* polyps exists as regular polyps from March to September, in order to discover *A. aurita* polyp colonies.

Second, *C. capillata* and *C. lamarckii* polyps may not colonise the same habitats surveyed during this study. Van Walraven et al. (2016) argue that the scyphozoan species could have different environmental preferences in terms of salinity [23]. Experimental studies by Holst and Jarms (2010) demonstrated that *C. capillata* and *C. lamarckii* polyps can survive salinities down to 12, and *A. aurita* polyps survived salinities down to 8 [50]. Survival and settlement at these extreme salinities suggest they are capable of inhabiting estuaries [23, 50]. Even though *A. aurita* survived at lower salinity, Holst and Jarms (2010) demonstrated that strobilation of *C. capillata* polyps were 90% for all salinity experiments (32-12) [50]. In contrast, *A. aurita* and *C. lamarckii* had 11% and 16% strobilation at salinity 12, respectively [50]. It is speculative if portions of the *C. capillata* medusae in Trondheimsfjorden originate from estuaries in of Trondheimsfjorden, but given their low salinity tolerance displayed in the experiments by Holst and Jarms (2010) [50], it may be worth investigating. Van Walraven (2016) also argues that based on Hay et al. (1990) findings on the offshore distribution of *C. capillata* and *C. lamarckii* could infer that the species may prefer more saline and deeper waters [20, 23]. Indeed, it is possible that *C. capillata* and *C. lamarckii* polyps inhabit greater depths. Similar to this study, most trials of locating polyps *in situ* are generally executed at shallower depths along the coast, because of the difficulties of exploring polyps at greater depths [4]. If they do indeed inhabit greater depths it not surprising why they remain undiscovered, and is certainly worth exploring further.

Habitats of A. aurita polyp colonies within and outside of Trondheimsfjorden

This study has confirmed the occurrence of several *A. aurita* polyp colonies across a larger transect within and outside of Trondheimsfjorden. The findings of this study show that the sheltered littoral zones along the coast, within and outside of Trondheimsfjorden are viable habitats for *A. aurita* polyps.

The polyps seemed to favour areas with reduced current velocity, since MAU3, SLE1-3, TBS and VER were sheltered from strong currents due to dense growth of macroalgae and topographical characteristics relative to embayments and lagoons. The exceptions were MAU1-2 which were, relative to MAU3 and the other stations, located in a less embayed and topographically steep area. Thus, MAU1-2 were relatively exposed to stronger currents, although *A. aurita* polyps on these particular stations were found in sheltered and shaded microhabitats, namely underneath

rocks located inside cracks of the rocky bottom of the stations.

The epibiont species growing on the MAU1 settling plates reflected the strong current patterns on Mausund. More specifically by the low amount of epibiont coverage compared to the other stations and by the tube dwelling amphipod *Jassa falcata*, an organism that inhabits strong current systems and is a common fouling organism on ships [51]. The strong currents and lack of bigger epibionts able to provide shaded microhabitats on MAU1, are likely the reasons for a reduced settlement of *A. aurita* polyps on the MAU1 settling plates. A pattern that provides some evidence that *A. aurita* polyps do not inhabit more exposed habitats.

There were no obvious depth preference patterns for *A. aurita* polyps in the intertidal and shallow subtidal zones. Polyps inhabited the whole depth range from 0.1 m to 3 m with no significant difference in both the field survey and on settling plates. The difference in polyp abundance between the 1 m and 3 m settling plates are more likely related to the different microhabitats created by the different epibionts that were present on the settling plates. However, the findings of *A. aurita* polyps on VER at 0.1 m depth during low tide was unexpected. Vast number of polyps were present on this shallow transition between the lower intertidal zone and subtidal zone on VER. The dense aggregation of macroalgae in the area formed a layer of emersed macroalgae and tide pools during low tide. Polyps were located on rocks in the tide pools and on rocks underneath the layer of emersed macroalgae. The latter rocks were partly emersed and the polyps survived, probably due to the humid microhabitat provided by the layer of macroalgae. It was unexpected to observe polyps in this relatively shallow environment. In addition, tide pools environments are often associated with a high variability of temperature, salinity and oxygen [52]. However, Holst and Jarms (2010) salinity experiments (see above) demonstrated that different salinity levels has minimal effect on polyp survival rate [25]. In addition, experiments by Ishii et al. (2008) and *in situ* observations by Ishii and Katsukoshi (2010), demonstrated that polyps can survive and may even favour near-hypoxic conditions relative to that of other sessile epibionts [33, 53]. Their findings and the observations of the present study is further evidence of *A. aurita* polyps' ability to survive in shallow and extreme environmental conditions.

In terms of species composition in the benthic habitats, the most apparent similarities was the moderate amount of filamentous algae, macroalgae, coralline algae, *Spirorbis* sp. and gastropods. As discussed earlier, characteristics that would increase the probability of finding polyps were: (1) Sheltered embayments with macroalgae canopies [19], with (2) sufficient hard substrates able to provide shaded microhabitats [4, 23]. Thus, when scouting for suitable field survey sites, narrow

embayments and small bays with visible dense macroalgae canopies were obvious targets of interest. The high amount of macroalgae displayed in the species composition survey, indicates that dense macroalgae canopies are viable habitats of *A. aurita* polyps. Östman (1997) theorised that polyp abundance is higher in sheltered and shallow areas containing macroalgae canopies, were medusae is likely to be trapped [19]. Trapped sexually mature medusae are likely to release planulae within their vicinity, and this is likely one of the mechanisms that led to increased polyp abundance in the habitats of this study.

However, the species composition at SLE1 demonstrates that polyps do not exclusively inhabit dense macroalgae aggregations. This habitat (Hopavågen) is characterized by some macroalgae canopies, patches of seagrass in-between [54] and big patches of filamentous algae at the mouth of the lagoon (Straumen). The station SLE1 in particular, did not have any vast canopies of macroalgae. SLE1 is located in a sheltered lagoon (Hopavågen) with current patterns that resemble the circular current patterns of a small embayment, with wind and tidal currents from Straumen as the only sources of current. The presence of polyps at SLE1 is further evidence that *A. aurita* polyps prefer more sheltered, low current systems. While also demonstrating that macroalgae are not a fundamental component of a polyp habitat, but can indirectly positively affect polyp colonisation by promoting an environment with reduced current velocity, or by trapping sexually mature medusae.

Microhabitats and biotic interactions of A. aurita polyps

Similar to the findings of van Walraven et al. (2016), *A. aurita* polyps were present on a variety of natural and artificial substrates [23]. Given the qualitative nature of the present field survey, and the high variation of polyp abundance on the settling plate epibionts, it is still difficult to estimate if there is a preference between artificial and natural substrates *in situ*. Thus, it is equally difficult to estimate the significance of artificial substrates introduced in coastal development, without quantitative research exploring this particular subject.

However, this study has expanded our knowledge on the cryptic nature of *A. aurita* polyp microhabitats, and the overall factors that could promote polyp settlement. The abundance of *Pomatoceros triqueter* tubes on SLE1 was dense and covered almost every substrate found in the area. The tubes visibly altered the surface of the substrates by creating crevices and concave imprints from the tube loops. *A. aurita* polyps were frequently observed inside these crevices and loops created by the tubes. It is likely that *P. triquter* tubes promote viable microhabitats because of its structure and rough surface. Although there was no direct observations of polyps on *P. triquer* tubes on the SLE1 settling plates, there was a positive relationship between polyp abundance and *P. triqueter* surface coverage from the PCA.

Coralline algae were an abundant substrate on most of the stations. Polyps were often observed on coralline algae such as *Lithothamnion sp.* and *Phymatolithon sp.* Coralline algae can provide a rough surface suitable for polyp settlement on a hard substrate that is otherwise too smooth.

On the settling plates, there was a positive relationship between high polyp abundance and surface coverage of *Ascidia mentula*, *Pomatoceros triqueter*, and debris of *Balanus balanoides*, and a positive relationship between low polyp abundance and surface coverage of *Mytilus edilus* and *Ciona intestinalis*. *A. mentula* is a solitary ascidian with a cartilaginous body and a leathery tunic cuticle [55]. *A. mentula* is thus a suitable polyp settling surface given its rough and hard surface, as was observed on the 3 m SLE1 settling plates with an average of 12% polyps located on individuals *A. mentula*. In addition, *A. mentula* provide a three-dimensional space on a two-dimensional surface, creating polyp microhabitats with hiding spaces between individuals of *A. mentula*, and the substrate it is attached to. In contrast, *C. intestinalis* is a solitary ascidian with a soft, gelatinous and contractile body and a smooth tunic cuticle [56]. Dissimilar to *A. mentula*, the smooth surface of *C. intestinalis* is not considered as an ideal surface for polyp settlement, as was observed from the 3 m SLE1 settling plates with an average 0.1% polyps located on individuals of *C. intestinalis*. Similar to *A. mentula*, *C. intestinalis* also provide a three-dimensional space, however its sessile characteristics are different and possibly more disruptive. Whereas *A. mentula* is practically a stationary and immobile object, *C. intestinalis* is able to retract its body when disturbed, creating a more unstable environment and microhabitat. These particular features of these solitary ascidians are possible explanations to why *A. mentula* surface coverage had a positive relationship with high polyp abundance, while *C. intestinalis* surface coverage had a positive relationship with low polyp abundance on the SLE1 settling plates.

From previous studies, there are several observations of polyps attached to shells of *M. edilus* [19, 23]. The percentage of polyps located on *M. edilus* in this study was low, relative to other epibionts (see above). In total, only 0.6% and 4% of the polyps at 1 m and 3 m depth respectively, were observed on *M. edilus* on the TBS settling plates. No polyps were observed on *M. edilus* on the SLE1 settling plates. The surface of *M. edilus* shells in this study were smooth and slick, possibly because of their juvenile nature, as they were first observed late June at both stations. It can be speculated that these three months old *M. edilus* provide less sufficient settling surface, than possibly older *M. edilus* from the research by van Walraven et al. (2016) and Östman (1997) [19, 23].

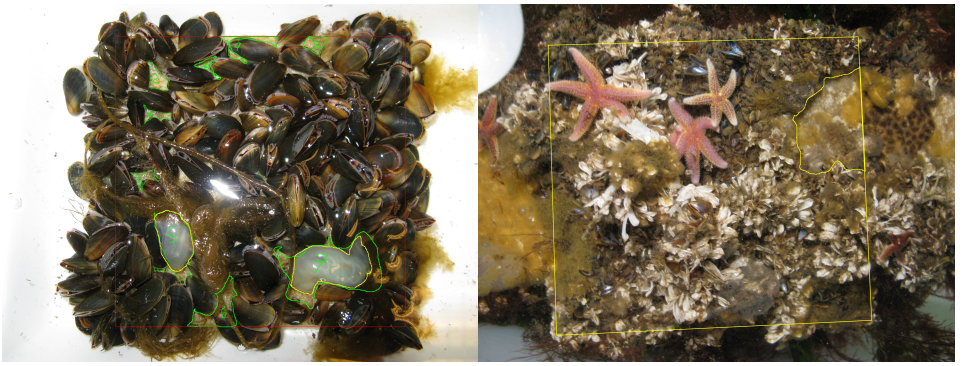
In contrast, the space competition from *M. edilus*, was a lot more evident from the findings of this study. It was apparent from the SLE1 settling plates that a

surface dominated with *M. edilus* had a positive relationship with low polyp abundance. The strong competitive potential of *M. edilus* can be explained by the mussel's substrate attachment mechanism. In contrast to *A. mentula* and *C. intestinalis*, when *M. edilus* attaches to a surface, it produces strong adhesive filaments called byssus threads [57]. The 1 m SLE1 settling plate surface were covered by such byssus threads (Figure 11a). Prior to attaching new byssus threads, the mussel scrubs the substrate surface with its foot, removing potential obstructions such as weakly attached epibionts and dirt particles [57]. This behaviour is likely to increase the competitive potential of *M. edilus* for space in comparison to other epibionts such as polyps. The byssus threads themselves are possibly another hindrance to polyp settlement, given the uneven and unstable surface byssus threads provide. Also, during the examination, when detaching the mussels in order to locate polyps in between the mussels, a removed mussel pulled a sizeable portion of its byssus threads with it, and possibly polyps attached to the same byssus threads. The actual polyp abundace on the TBS settling plate could thus be underestimated. However, the latter theory is less likely, as no polyps were present on *M. edilus* or the SLE1 settling plates they were attached to. To summarise, the high capacity of *M. edilus* to compete for space is likely the reasons for the reduced settlement of other epibionts including polyps in the vicinity of *M. edilus* on SLE1.

Similarly Ishii and Katsukoshi (2010) observed that *A. aurita* polyps may be exposed to space competition from *Mytilus galloprovincialis* in Tokyo Bay. The upper layer of the examined pylon was mainly occupied by other species such as *M. galloprovincialis*, relative to the near-hypoxic bottom layer were *A. aurita* polyps were abundant. The findings implied that *A. aurita* were outcompeted for space by the other sessile epibionts in the upper layer, in contrast to the bottom layer were *A. aurita* polyps were able to survive due to their high hypoxic tolerance relative to that of other sessile epibionts.

It is important to note that *M. edilus* from the SLE1 and TBS settling plates were treated as two different variables, somewhat altering the true loading score of *M. edilus* in the PCA as a single species. The different layers were treated as such because their compositions were different. The *M. edilus* layer on SLE1 was interpreted as purely *M. edilus* (Figure 10a), and were relative to TBS (Figure 10b), larger and healthier. In contrast, the layer on TBS consisted of two layers, an inner layer of dead *B. balanoides* and outer layer with patches of dead and alive *M. edilus* (Figure 11b). During the examination, these two layers were impossible to separate in a sensible way, thus the two layers were treated as one layer and one variable.

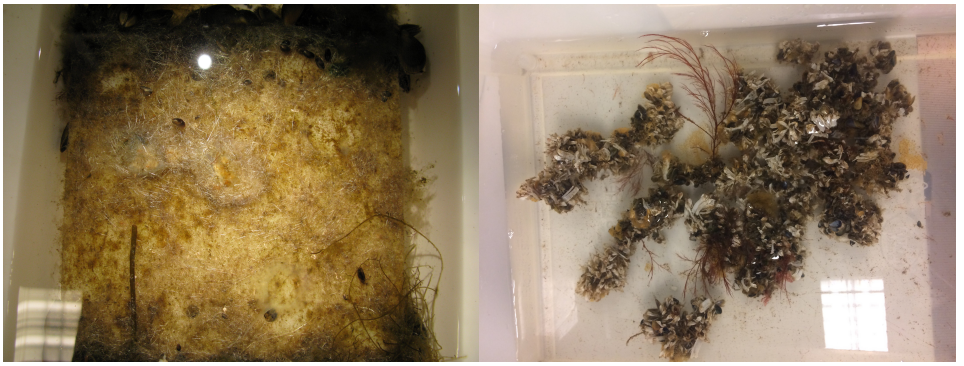
The inner layer of dead *B. balanoides* enabled the settlement of *A. aurita* polyps on the TBS settling plates. A significant proportion of the polyps on the TBS settling plates were located on the shells of dead *B. balanoides* and PVC plate material, and



(a) **(b)**

Figure 10: (a) SLE1 1 m settling plate depicting *M. edilus* size and coverage (18.08.19) vs. TBS (b) with visibly smaller *M. edilus* and patchy coverage (25.09.18). Photo: M. E. Rekstad

some of which were located inside visible imprints of a detached *B. balanoides*. This suggests that they were in fact located inside the base of dead *B. balanoides*. The findings indicate that the inner layer of shells from dead *B. balanoides* provided an optimal microhabitat for *A. aurita* polyps, and protected them from the space competition pressure of *M. edilus*, that was evident on SLE1.



(a) **(b)**

Figure 11: (a) Settling plate surface covered in *M. edilus* byssus threads on SLE1 settling plate. (b) Chunks of dead and alive *M. edilus* and shells of dead *B. balanoides* tethered together by byssus threads. Photo: M. E. Rekstad

Based on the current results, it would be interesting to further investigate the development and survival rate of the polyps colonising the TBS settling plates. Settlement of juvenile *B. balanoides* was discovered late May 2018, while juvenile *M. edilus* was first discovered late June 2018 settling on top of *B. balanoides*. The high mortality of *B. balanoides* was likely the result of starvation, caused by

feeding competition from the newly settled *M. edilus*. Indeed, the high mortality of *B. balanoides* and the seemingly smaller *M. edilus* relative to SLE1 was indicative of competition between the two species. Given that the polyps were located underneath *M. edilus* patches, it is possible that the polyps over time would deteriorate and starve similar to the fate of *B. balanoides*. It is entirely possible that all of the species discussed in this chapter (*A. mentula*, *C. intestinalis*, *M. edilus*, *B. balanoides*) are in direct competition for food and space with *A. aurita* polyps. In addition, these species could also prey on *A. aurita* planulae larvae due to their filter-feeding mode. However, the significance of such a feeding competition and predation on *A. aurita* polyps is difficult to speculate further without sufficient data exploring this particular subject.

5 Conclusion

This study has confirmed the occurrence of several of *Aurelia aurita* polyp colonies across a larger transect within and outside of Trondheimsfjorden. However, the occurrence of *Cyanea capillata* and *Cyanea lamarckii* polyp colonies in the same area remain unsolved. Differing life cycle patterns and habitats relative to *A. aurita* are possible explanations to why *C. capillata* and *C. lamarckii* polyps were not found.

A. aurita polyps were present on a variety of natural and artificial substrates, but estimation of substrate preference *in situ* requires further quantitative research on the subject. The findings demonstrated that sheltered littoral zones along the coast within and outside of Trondheimsfjorden are viable habitats for *A. aurita* polyps. *A. aurita* polyps seemed to prefer shallow and embayed areas with reduced current velocity and macroalgae canopies. Macroalgae canopies in the littoral zone could possibly promote polyp settlement by reducing current velocity and by trapping sexually mature medusae.

In situ observations and principal component analysis (PCA) of settling plate epibionts coverage relative to polyp abundance, provided evidence of viable polyp microhabitats and interspecific space competition between settling epibionts. The roughness and structure of corraline algae and polychaete tubes created by *Pomatoceros triqueter* promoted viable microhabitats for *A. aurita* polyps. The solitary ascidian *Ascidia mentula* promoted viable *A. aurita* polyp habitats, due to its immobile cartilaginous body and leathery tunic cuticle, in contrast to another solitary ascidian *C. instestinalis*, due to its soft contractile body and smooth tunic cuticle. The blue mussel *Mytilus edilus* proved to be a potential competitor of space and food towards other sessile epibionts, including *A. aurita* polyps. The shells of the barnacle *Balanus balanoides* provided sheltered polyp microhabitats, and protection from the space competition of *M. edilus*.

To conclude, this study has provided more insight on the cryptic nature of *A. aurita* ecology, while the occurrence of *C. capillata* and *C. lamarckii* remain unsolved. Blooms of *A. aurita* are likely to occur frequently in Trondheimsfjorden due to the regional reproduction patterns and the settling of polyps in the coastal area of the fjord. Whether the local polyps promote blooms of medusae in the fjord is still subject to further population genetic investigations, which is an ongoing GoJelly objective.

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Appendix

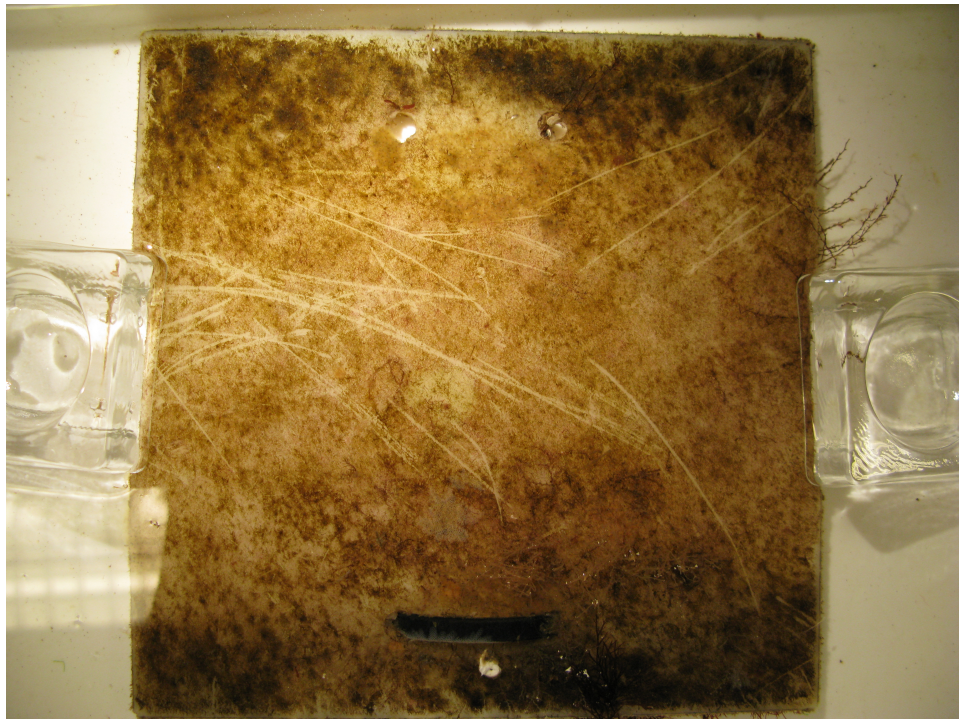


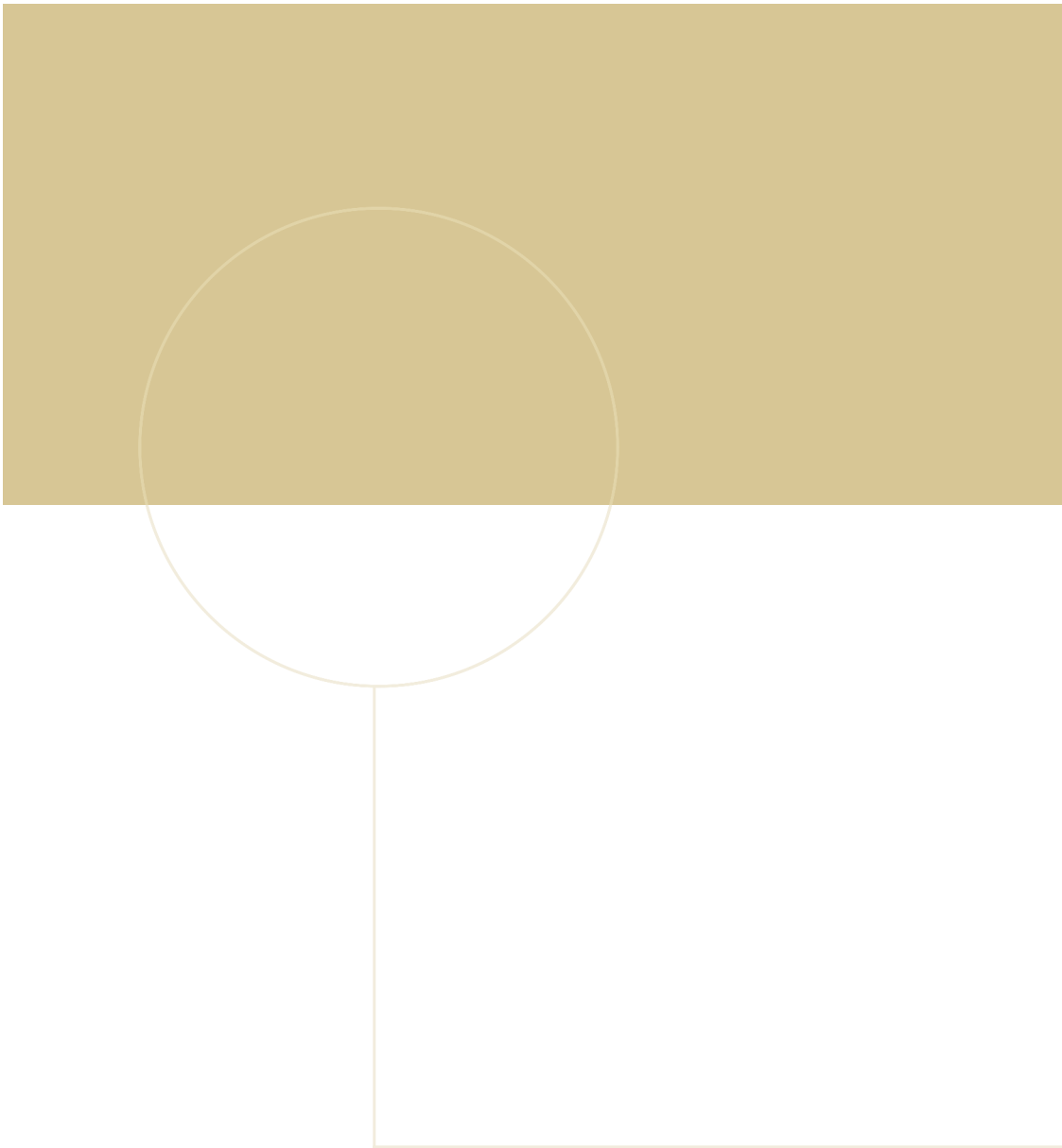
Figure 12: (a) 1 m settling plate from TBS removed from PCA, visibly damaged from sea bottom contact.

Table 4: BLAST results from the field survey samples. BLAST date: 18.04.2018

Sample ID	Location	Sampling Date	Species	Query Cover	Match	Accession number
100	SLE1	12.04.2018	Aurelia aurita	100 %	100.00%	KJ026305.1
101	SLE1	12.04.2018	Aurelia sp.	100 %	99.84%	HQ913940.1
110	SLE1	12.04.2018	Aurelia aurita	100 %	99.82%	MG935022.1
111	SLE1	12.04.2018	Aurelia aurita	100 %	99.66%	KY564361.1
113	SLE4	13.04.2018	Aurelia aurita	100 %	99.69%	MG935022.1
114	SLE4	13.04.2018	Aurelia aurita	100 %	99.69%	MG935022.1
12	VER	15.03.2018	Aurelia aurita	100 %	100.00%	KY564361.1
13	VER	15.03.2018	Aurelia aurita	100 %	99.62%	KY564361.1
13B	VER	15.03.2018	Aurelia sp.	100 %	99.84%	HQ913940.1
143	VER	02.05.2018	Aurelia aurita	100 %	100.00%	KX691612.1
144	VER	02.05.2018	Aurelia aurita	99 %	99.84%	KY564361.1
145	VER	02.05.2018	Aurelia aurita	100 %	100.00%	KY564361.1
14	VER	15.03.2018	Aurelia aurita	100 %	99.69%	MG935022.1
14B	VER	15.03.2018	Aurelia sp.	100 %	99.84%	HQ913940.1
15	VER	15.03.2018	Aurelia aurita	100 %	99.84%	KJ026296.1
180	TBS	04.05.2018	Aurelia aurita	100 %	100.00%	KY564361.1
181	TBS	04.05.2018	Aurelia aurita	100 %	99.84%	KY564361.1
184	TBS	04.05.2018	Aurelia aurita	100 %	99.52%	KY564361.1
19	VER	15.03.2018	Aurelia aurita	100 %	99.82%	MG935022.1
19B	VER	15.03.2018	Aurelia aurita	100 %	99.84%	KJ026305.1
203	MAU2	29.05.2018	Aurelia aurita	100 %	99.52%	KY564361.1
204	MAU2	29.05.2018	Aurelia aurita	99 %	99.68%	KY564361.1
205	MAU2	29.05.2018	Aurelia aurita	100 %	99.51%	KY564361.1
206	MAU2	29.05.2018	Aurelia aurita	100 %	99.82%	KY564361.1
20	VER	15.03.2018	Aurelia aurita	100 %	99.74%	MG935022.1
225	MAU2	29.05.2018	Aurelia aurita	100 %	99.68%	MG935022.1
232	SLE1	29.05.2018	Aurelia aurita	100 %	100.00%	KY564361.1
234	SLE1	29.05.2018	Aurelia aurita	100 %	99.53%	MG935022.1
235	SLE1	29.05.2018	Aurelia aurita	100 %	99.68%	MG935022.1
245	SLE1	30.05.2018	Aurelia aurita	100 %	99.42%	KY564361.1
246	SLE1	30.05.2018	Aurelia aurita	99 %	100.00%	KY564361.1
247	SLE1	30.05.2018	Aurelia aurita	100 %	99.83%	KY564361.1
249	SLE1	30.05.2018	Aurelia aurita	100 %	100.00%	KY564361.1
251	SLE1	30.05.2018	Aurelia sp.	100 %	99.68%	HQ913940.1
253	SLE2	31.05.2018	Aurelia aurita	100 %	99.65%	MG935022.1
254	SLE2	31.05.2018	Aurelia aurita	100 %	99.64%	KJ026309.1
256	SLE2	31.05.2018	Aurelia aurita	100 %	99.83%	MG935022.1
257	SLE2	31.05.2018	Aurelia aurita	100 %	99.83%	MG935022.1
258	SLE2	31.05.2018	Aurelia sp.	100 %	99.84%	HQ913940.1
259	SLE2	31.05.2018	Aurelia sp.	99 %	99.84%	HQ913940.1
260	SLE2	31.05.2018	Aurelia sp.	100 %	99.83%	HQ913940.1
262	SLE2	31.05.2018	Aurelia aurita	100 %	100.00%	KX691612.1
266	SLE2	31.05.2018	Aurelia sp.	99 %	99.84%	HQ913940.1
267	SLE2	31.05.2018	Aurelia sp.	100 %	99.84%	HQ913940.1
268	SLE2	31.05.2018	Aurelia sp.	100 %	99.84%	HQ913940.1
269	SLE2	31.05.2018	Aurelia sp.	100 %	99.84%	HQ913940.1
2	SLE1	14.03.2018	Aurelia aurita	100 %	100.00%	KJ026293.1
50	TBS	06.04.2018	Aurelia aurita	100 %	98.65%	KX691612.1
52	TBS	06.04.2018	Aurelia aurita	100 %	100.00%	KJ026339.1
6	SLE1	14.03.2018	Aurelia aurita	100 %	99.82%	KJ026309.1
79	SLE1	12.04.2018	Aurelia aurita	100 %	99.53%	KJ026309.1
7	SLE1	14.03.2018	Aurelia aurita	100 %	99.53%	KJ026309.1
80	SLE1	12.04.2018	Aurelia aurita	100 %	99.53%	KJ026309.1
82	SLE1	12.04.2018	Aurelia aurita	100 %	99.69%	KJ026309.1
85	SLE1	12.04.2018	Aurelia sp.	100 %	99.84%	HQ913940.1
86	SLE1	12.04.2018	Aurelia sp.	100 %	99.84%	HQ913940.1
87	SLE1	12.04.2018	Aurelia aurita	100 %	99.37%	KJ026319.1
88	SLE1	12.04.2018	Aurelia aurita	100 %	100.00%	KJ026309.1
89	SLE1	12.04.2018	Aurelia aurita	100 %	99.67%	KJ026319.1
8	SLE1	14.03.2018	Aurelia aurita	100 %	99.66%	KJ026309.1
97	SLE1	12.04.2018	Aurelia sp.	100 %	99.84%	HQ913940.1
99	SLE1	12.04.2018	Aurelia aurita	100 %	99.84%	MG935022.1

Table 5: BLAST results from the settling plate samples. BLAST date: 18.04.2018

Sample ID	Location	Species	Query cover	Match	Accession Number
9B	SLE1	Aurelia aurita	100 %	100.00%	KY564361.1
5B	SLE1	Aurelia aurita	99 %	99.84%	KY564361.1
12B	SLE1	Aurelia aurita	99 %	99.84%	KY564361.1
15B	SLE1	Aurelia aurita	99 %	99.84%	KJ026292.1
22B	SLE1	Aurelia aurita	100 %	100.00%	MG935022.1
24B	SLE1	Aurelia aurita	100 %	99.68%	KY564361.1
25B	SLE1	Aurelia aurita	75 %	99.84%	KJ026339.1
26B	SLE1	Aurelia aurita	75 %	99.84%	KJ026339.1
35B	SLE1	Aurelia aurita	100 %	99.68%	MG935022.1
37B	SLE1	Aurelia aurita	100 %	99.84%	KY564361.1
42B	SLE1	Aurelia aurita	99 %	100.00%	KX691612.1
43B	SLE1	Aurelia aurita	100 %	99.84%	KX691612.1
49B	SLE1	Aurelia aurita	100 %	99.52%	KJ026309.1
51B	SLE1	Aurelia aurita	99 %	99.68%	KJ026309.1
60B	SLE1	Aurelia aurita	100 %	99.84%	KY564361.1
62B	SLE1	Aurelia aurita	100 %	99.84%	KX691612.1
63B	SLE1	Aurelia aurita	99 %	99.84%	KX691612.1
70B	SLE1	Aurelia aurita	100 %	100.00%	KX691612.1
74B	SLE1	Aurelia aurita	99 %	100.00%	KY564361.1
86B	SLE1	Aurelia aurita	100 %	100.00%	KY564361.1
92B	SLE1	Aurelia aurita	100 %	99.84%	KJ026309.1
133B	SLE1	Aurelia aurita	99 %	99.84%	KX691612.1
82B	SLE1	Aurelia aurita	99 %	99.84%	MG935022.1
59B	SLE1	Aurelia aurita	99 %	99.84%	MG935022.1
115B	SLE1	Aurelia aurita	99 %	99.84%	MG935022.1
17A	TBS1	Aurelia aurita	99 %	99.84%	MG935022.1
43A	TBS1	Aurelia aurita	99 %	99.68%	MG935022.1
47A	TBS1	Aurelia aurita	100 %	99.84%	KJ026319.1
48A	TBS1	Aurelia aurita	100 %	100.00%	MG935022.1
49A	TBS1	Aurelia aurita	0 %	99.67%	KJ026305.1
50A	TBS1	Aurelia aurita	100 %	99.52%	KJ026309.1
52A	TBS1	Aurelia aurita	100 %	99.53%	KX691612.1
53A	TBS1	Aurelia aurita	100 %	99.52%	MG935022.1
55A	TBS1	Aurelia aurita	100 %	99.52%	MG935022.1
56A	TBS1	Aurelia aurita	100 %	99.68%	KJ026310.1
59A	TBS1	Aurelia aurita	100 %	99.36%	KJ026293.1
60A	TBS1	Aurelia sp.	100 %	99.84%	HQ913940.1
62A	TBS1	Aurelia aurita	98 %	99.84%	MG935022.1
61A	TBS1	Aurelia aurita	98 %	99.84%	MG935022.1
51A	TBS1	Aurelia aurita	100 %	99.52%	KJ026350.1
1A	TBS1	Aurelia aurita	100 %	99.68%	KJ026293.1
2A	TBS1	Aurelia aurita	100 %	99.84%	MG935022.1
3A	TBS1	Aurelia aurita	99 %	99.84%	KY564361.1
5A	TBS1	Aurelia aurita	100 %	99.84%	KJ026285.1
8A	TBS1	Aurelia aurita	99 %	99.68%	KX691612.1
11A	TBS1	Aurelia aurita	100 %	99.68%	MG935022.1
13A	TBS1	Aurelia aurita	99 %	99.68%	MG935022.1
14A	TBS1	Aurelia aurita	99 %	99.84%	KX691612.1
15A	TBS1	Aurelia aurita	100 %	99.84%	KJ026310.1
16A	TBS1	Aurelia aurita	99 %	99.68%	MG935022.1
19A	TBS1	Aurelia aurita	100 %	99.68%	KX691612.1
26A	TBS1	Aurelia aurita	99 %	99.84%	MG935022.1
27A	TBS1	Aurelia aurita	100 %	99.84%	KJ026305.1
28A	TBS1	Aurelia aurita	99 %	99.52%	KJ026305.1
35A	TBS1	Aurelia aurita	99 %	99.67%	KJ026293.1
37A	TBS1	Aurelia aurita	100 %	99.84%	KJ026293.1
42A	TBS1	Aurelia aurita	100 %	99.68%	KJ026319.1



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