Bachelorthesis

Assessing the influence of depth and substrate type on the population growth of colonial tunicate Didemnum molle

Beurteilung des Einflusses der Tiefe und des Substrattypes auf das Populationswachstum des kolonialen Manteltieres Didemnum molle

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1 Abstract

2 Zusammenfassung

3 Introduction

3.1 Location of the study

Figure 1. Map of Thailand. The red arrow shows the island of Koh Tao. Retrieved from monkeetime.com

The study was performed on Koh Tao, a small Island situated in the Western Gulf of Thailand in the province of Surat Thani. Koh Tao is approximately 19 km² in size (Scott, 2014). The study was carried out between May and August 2015 at two different sites off the southeast coast of Koh Tao. The study site, Suan Olan, is located off the southern coast of Ao Leuk Bay (Scott, 2014).

Suan Olan is dominated by both metal and concrete artificial reef structures atop an almost entirely sandy bottom substrate. The coral reef of the bay is a healthy fringing reef (Cabral, 2014) and terminates roughly 50 meters from land and at a depth of approximately 10 meters (Scott, 2014).

3.2 Main threats to Koh Tao's coral reefs

Coral reefs are some of the most diverse and productive ecosystems on Earth, estimated to host at least 25% of all described marine species, including fishes, reptiles, mollusks, crustaceans, sponges and worms. By hosting so many different organisms and thus creating a high amount of marine biodiversity, coral reefs are immensely important to global ecosystem health (Reaka-Kudla, 2001; Scott, 2014). In addition, they play a vital role in many human communities: more than 100 countries have coastlines containing coral reefs, and nearly 8% of the world's population live within 100 km of a coral reef. By breaking the waves during storms, coral reefs play a major role in coastal protection by preventing coastal erosion, flooding and loss of property (Moberg and Folke, 1999).

Coral reefs are also very important for the regulation of atmospheric gases. They secrete calcium carbonate skeletons, created from dissolved carbon dioxide. Therefore serving as a natural carbon sink, they play a major role in atmospheric carbon sequestration and climate change stabilization (Scott, 2014).

Coral reefs across the globe are highly threatened due to both natural and anthropogenic stressors. Natural threats include climate change, tropical storms and other natural catastrophes, and coral diseases. These natural risk factors are often induced and aggravated by human activities, including over-fishing, marine and terrestrial pollution, coastal development, and agricultural activities (Bryant *et al*., 1998; Kent, 2008). Even though these threats have large impacts on the coral reefs, they are not covered in this study.

3.2.1 Coral bleaching and tourism

Corals are colonial animals that live symbiotically with photosynthetic algae, which provide the corals with up to 97% of its energy and/or their pigments (Brown, 1997). When stressed, corals eject their algae in an event called coral bleaching, which leads to a loss of energy production and color. A coral in such a state is not yet dead, but significantly weakened.

Figure 2. Bleached massive coral of the genus *Symphillia* with partial algal overgrowth. Retrieved from noaa.gov.

Since 1979, where the first bleaching event was described, six other mass bleaching events were recorded throughout various sites in the Caribbean, Indian and Pacific Oceans (Brown, 1997). Two bleaching events in 1998 and 2010 were particularly damaging for the corals in the Gulf of Thailand, from which a lot of corals didn’t recover (Wilkinson, 2008; Scott, 2014). This includes Tien Og bay, a bay that lies southwest of the study site Suan Olan, which succumbed to high levels of mortality during the 1998 bleaching event and has subsequently shown little recovery in the shallows and slow recovery at relative depth. The exact trigger for such bleaching events are varied and difficult to define. For many studies in Thailand, climate change and elevated sea water temperatures have been identified as the primary causes (Brown, 1997). Coral bleaching is a significant threat to coral reefs worldwide, including those on Koh Tao.

Another major threat to Koh Tao's coral reefs is tourism. Despite its size, the island is one of Thailand's most popular tourist destinations, attracting over 400,000 tourists annually that primary participate in marine recreation such as SCUBA diving and snorkeling (SATLP, 2009; Scott, 2009). The tiny island hosts at least 67 permanent dive centers in addition to daily visitors from the neighboring islands of Koh Samui and Koh Phangan (Scott *et al.*, 2014). Although the tourism is one of the major contributors to the economy of Koh Tao and Thailand in general (World Travel and Tourism Council, 2014), it also bears adverse impacts on local coral reefs (Larpnun *et al*., 2011, Hein, 2013; Couture, 2013). Development and deforestation lead to sedimentation of the reefs, which can smother coral colonies and otherwise reduce the amount of light available for photosynthesis. In addition, few wastewater treatment systems exist on the island, meaning the sewage from the many resorts and restaurants flow directly into the marine environment (Romeo, 2014).

3.2.2 Fouling organisms and epibiosis

High tourism and rapid development, combined with limited enforcement of environmental regulations, have led to increased levels of nutrients and pollutants in the water that cause direct damage to the coral reefs (Scott, 2014). In addition, high nutrient loads can stimulate the growth of certain fouling organisms (Wilkinson, 2008).

An organism is considered fouling when it overgrows a surface or a substrate rapidly or over a large scale (Wahl, 2009). In marine systems, it is not uncommon for a fouling organism to overgrow other living organisms instead of hard substrates, in which case the fouling organism is considered epibiotic. Epibiosis is a typical aquatic phenomenon, that is considered a direct consequence of surface limitation and results in non-symbiotic, non-parasitic association between two or more living organisms. The substrate organism is considered the basibiont, while the organism attached to the basibiont is referred to as the epibiont (Harder, 2009; Wahl, 2015).

On Koh Tao, the three most abundant fouling organisms are algae, sponges and tunicates (Romeo, 2014). In this study, these organisms are not only considered as fouling organisms, but also as epibionts; the corals constitute the basibionts.

The growth of benthic algae can be stimulated by an increase in nutrients in the water. This increased nutrient load offers a competitive advantage to algae over coral as the former overgrows, shades, and physically damages the latter (Lapointe, 1997). Koh Tao, like many degraded coral reefs systems, has experienced increases of macro-algal cover. Although bleaching events and other disturbances occur often, most reefs around the island have been able to recover. The site at Tien Og, however, has shown little resilience to the island's stressors and has not been able to recover as fully (Scott, 2014). One of the potential reasons is an imbalance in the nutrient cycling in the ecosystem, caused by waste water discharge from local resorts and as well as deforestation and the associated erosion. This may have caused a relatively high macro-algal biomass, which overgrows the substrate and out-competes any coral recruits in the area. As a result, there has been little recovery since the last bleaching event in 2010 (Scott, 2014).

Figure 3. Coral of the genus Acropora with partial overgrowth by sponges (genus unknown). Picture taken on Koh Tao by Chad Scott.

In addition to interactions with algae, corals are often in contact with coral reef sponges that are considered to be important space competitors. This competition between corals and sponges often result in an overgrowth of sponges and therefore damage to the coral (Aerts, 1996). Although sponges do not possess any special competitive organs, they are thought to use toxic substances in interaction with corals to gain their position on the substrate (Jackson & Buss, 1975). This competition gains importance when it comes to nutrient enriched waters, as it is thought to be on Koh Tao: by accelerating primary production of the water column, an increase in nutrients not only enhances the growth of algae, but also favors filter-feeding organisms, such as sponges.

3.2.3 Tunicates as fouling organisms

While many studies concentrate on the macro-algal dominance over corals, only a few studies have discussed the competition between corals and tunicates and the pattern of tunicate abundance (Dijkstra, 2006). In the last decade, there have been many reports of rapid spread of several tunicates, more specifically of the class Ascidiacea, in various tropical regions (Bak *et al.*, 1996; Lambert, 2002). A long-term study in Curacao showed an increase of 900% over 15 years (from 1978 to 1993) of the ascidian *Trididemnum solidum*, thus becoming a possibly superior space competitor over corals (Bak *et al*., 1996).

A distinction is drawn between native- and invasive-dominated communities. Particularly invasive ascidians are a growing concern for ecologists and natural resource managers (Dijkstra, 2006), since they are able to cause significant changes in the structure and composition of marine communities (Lambert, 2001). An example is *Didemnum vexillum*, an established invasive colonial ascidian in Georges Bank, (Massachusetts, USA), which has had a significant impact on the composition of the benthic community by out-competing other epi-faunal and macro-faunal taxa (Lengyel, 2009).

Invasive ascidians can be transported to new areas by ship, either as larvae in ballast water, as juveniles and adults attached to boat hulls (Lambert, 2001), or through aquaculture (Dijkstra *et al*., 2006). Once established in these new locations, they can persist and rapidly become dominant members of the community (Lambert and Lambert, 2003).

The reasons for an increase of abundance of tunicates in deteriorating reef environments are various and not well understood. But several studies suggest a correlation between the increase of the tunicate population and the increase in nutrients in the water. An increase in nutrients would not only stimulate the growth of algae and sponges, but may also favor tunicates (Pastorok and Bilyard, 1985). A long-term study on the ecology of the Hawaiian coral reefs has shown a variety of invertebrates, including sponges and tunicates, outcompeting transplanted corals that were formerly the foundation of fringing reefs (Maragos, 1972). A short-term study about the effect of sewage pollution, carried out by Barnes (1973), showed that the most common response to sewage loading was an increase in benthic algae, sponges and tunicates, among others. More recent studies amplify this theory: For example, a 2008 study in Eilat (Israel) conducted by Schenkar showed an overgrowth of the colonial ascidian

Botryllus eilatensis on dead coral skeletons and artificial substrates. Increased anthropogenic activity that led to an increase in nutrients, was thought to create favorable conditions for this ascidian, providing it with a competitive space advantage over corals (Schenkar, 2008).

Natural phenomena, such as hurricanes, may also produce favorable conditions for ascidians over corals. A study on Swains Island (American Samoa) describes a significant increase in population of the ascidian *Diplosoma similis* (Vargas-Angel *et al*., 2008). The study suggests that this increase is a secondary effect of a hurricane that occurred in 2004 that caused coral habitat fragmentation, and reduced the live coral population. This decrease of coral population created an advantage for the ascidians, which rapidly colonized the open spaces, preventing the coral population from recovering (Vargas-Angel *et al*., 2008).

Although most studies about growing populations of ascidians mainly suggest negative effects, a study in the Gulf of Maine proposes a positive effect on the marine community. Four invasive colonial ascidians are well established in the Gulf of Maine, providing a new source of prey for some species, including seastar and snail species. An increase in prey availability has the potential to increase predator abundance that can affect community-wide interactions. An example would be a greater abundance of the predator *Henricia*, a seastar feeding of ascidians. This increase in predation may result in a decrease in sponge abundance, currently overgrowing the coral reef (Dijkstra *et al*., 2006).

3.3 Test organism: *Didemnum molle*

3.3.1 Classification

Didemnum molle is a marine species of the phylum Chordata, the subphylum Tunicata (also known as Urochordata), the class Ascidiacea and the family Didemnidae (Herdmann, 1886). The general name "tunicate" is derived from the tunic, a body envelope composed of polysaccharides that form a flexible skeleton to the animal.

3.3.2 Biology and physiology of the tunicate *Didemnum molle*

Although ascidians can be solitary or colonial, the species of *Didemnum molle* is most often observed as a sessile colony. In this case, the colony is composed of a number of genetically identical zooids adhering to the substrate, arranged in loose circles, rows, or dense clusters. Each zooid can have a diameter between 1 to 10 centimeters and has many inhalant (also called oral) siphons, distributed across the entire body of the tunic (**Figure 6, number 1**), and a single large exhalant (also called atrial) siphon based on the top of the body (**Figure 6, number 3**) (Schenker et al., 2015).

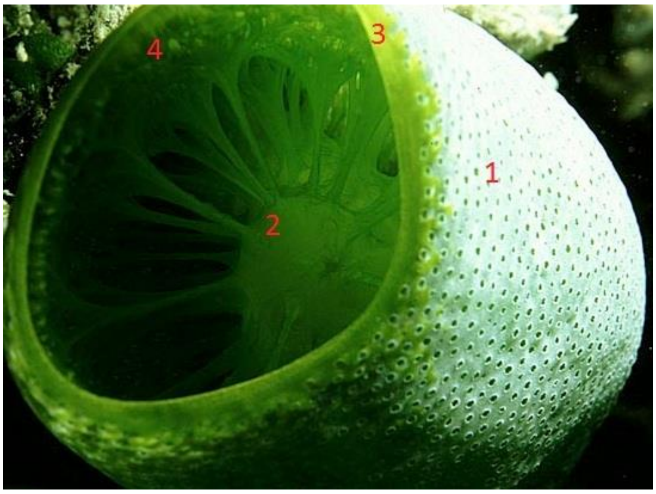

Figure 6. Zooid of ascidian *Didemnum molle*. This figure shows a close-up picture of the ascidian *D. molle* with four of the important body structures numbered from 1 to 4. 1: Tunic with many inhalant siphons 2: Branchial sac 3: Exhalant siphon at the top of the body 4: Mucus net. Picture taken in Indonesia from Arjan Gittenberger. Retrieved from ascidians.com.

Most of the species of the Didemnidae family, including *Didemnum molle*, live symbiotically with photosynthetic cyanobacteria. These cells are found on the wall of the atrial siphon and contain as the pigment chlorophylle b, giving them a green or blue color (Lamare, Vaulot and André, 2014).

Ascidians filter their food via the inhalant siphons that draw water into the branchial sac (**Figure 6, number 2**). Here, food items (such as microalgae and phytoplankton) are filtered through a mucus net (**Figure 6, number 4**), produced by the endostyle. Food particles are trapped on the wall of the branchial sac while water and feces are expelled through the atrial siphon (Schenker et al., 2015).

Figure 7. Colony of ascidian Didemnum molle. This colony is composed of approximately 65 zooids. Picture taken on Koh Tao by Rahul Mehrotra.

Ascidians, such as Didemnum molle, reach sexual maturity when only a few weeks old and have a rapid growth rate. The reproduction can be sexual or asexual. Asexual reproduction means that the colony extends through budding. A new bud will form and actively feed while the zooid from which it emerged gradually regresses. Sexual reproduction occurs internally since all ascidians are hermaphrodites, meaning that they both have male and female gonads. The formed larva is then released through the exhalant siphon (Lamare *et al.*, 2014). The larvae produced in sexual reproduction are generally short-lived, swimming for only a few hours before attaching to the substrate. This short, free-swimming larval stage likely does not last long enough for larvae to be carried great distances by ocean currents. Asexual reproduction and fragmentation (whether by natural or anthropogenic causes), play a much bigger role in the spread of the Didemnum genus. The budding often creates long tendrils, which can break off from the parent colonies and be transported long distances by ocean currents (Lengyel, 2009).

3.3.3 Observations on competition between corals and *Didemnum molle* on Koh Tao

During the last few years, local reports observed an increase in the abundance of the tunicates species *Didemnum molle* throughout many locations of the island of Koh Tao. These appear to be most significantly concentrated at the edge of the reef, in sandy areas and on artificial reef structures (Scott, 2015, personal comment).

Figure 4. Colony of ascidian *Didemnum molle* growing on glass bottle nurseries. This colony is growing on an artificial reef made up of glass bottles, hindering the growth of the corals. Picture taken on Koh Tao by Rahul Mehrotra.

There have been no tunicate abundance or distribution studies on the island thus far, nor are there tunicate monitoring programs, which makes it difficult to describe the exact reasons for this population increase (Scott, 2015, personal comment). As mentioned above, an increase in nutrients and habitat fragmentation appear to correlate with an increase in ascidian abundance, though this has yet to be formally described for Koh Tao.

Local observations indicate furthermore that the tunicate abundance shows strong seasonal variation, with a larger population during the summer months (Mehrotra, 2015, personal comment). This seasonal change of abundance has not been formally described, but may be impacted by changes in seawater temperatures. Studies in the Gulf of Maine (USA) and Eilat (Israel), have shown a seasonal change of population of ascidians, with a higher abundance during the summer and a high mortality during the winter months (Dijkstra *et al*., 2006; Schenkar, 2008). It appears that seawater temperature plays a significant role in the dynamics of marine communities and influences these seasonal changes (Dijkstra *et al*., 2006; Schenkar, 2008).

Further informal observations describe *Didemnum molle* not simply covering hard artificial and natural substrates, hindering coral growth and recruitment, but also over-growing the corals and leading to coral mortality (Scott, 2015, personal comment).

Figure 5. Colony of ascidian *Didemnum molle* on mushroom coral *Fungia fungites*. This colony is growing on and in between several mushroom corals of the species *Fungia fungites*. Picture taken on Koh Tao by Chad Scott.

3.4 Aim of Study

It appears that ascidian abundance can differ greatly between man-made and natural substrates, though few studies have directly tested the influence of the substrate type on community development (Chase *et al*., 2015). Many artificial coral reef structures have been deployed around the island of Koh Tao, made up of concrete, metal, glass, and various plastics (e.g. PVC). Artificial reefs have the potential to restore marine habitats that have been heavily damaged – in the case of Koh Tao, both through natural phenomena and as a result of extensive tourist activity (Chase *et al*., 2015). Unfortunately, some of these artificial reef structures (primarily large concrete blocks) on Koh Tao could not be used as intended due to widespread tunicate growth. Informal observations on Koh Tao suggest that the substrate type is an influential factor on the tunicate growth and indicate that the tunicates grow abundantly in areas where artificial reefs are present, especially around the metal structures and on the concrete structures.

The first purpose of this study is therefore to explore if the ascidians *Didemnum molle* exhibit preference for a certain type of substrate by examining both recruitment and growth on a variety of natural and artificial materials often found on Koh Tao's reefs. The artificial materials tested included all those being used in artificial reefs or coral nurseries on Koh Tao: concrete, steel rebar, PVC, glass and synthetic rope. The natural materials tested included a dead mushroom coral and the shells of a giant clam.

The second purpose of the study is to determine if depth is an influential factor on the population growth of the tunicates. Informal observations suggest that the depth does influence the tunicate population growth and that tunicates recruit heavily at a depth between 8-12m. To test the impact of depth on tunicate recruitment and growth, this study conducted transect surveys beginning at a shallow natural reef and continuing to a deeper sandy area.

To determine whether the substrate type and the depth have an impact on the tunicate growth and settlement is important not only for the construction and placement of artificial reef structures, but may also help further scientific studies as the findings may impact experimental conclusions (Chase *et al*., 2015).

4 Material and Methods

4.1 Materials

4.1.1 Substrate Survey

Three 100x100cm quadrats were constructed from PVC pipes and plastic mesh. Seven samples of different substrate types, each approximately 20x20cm, were placed in each quadrat and secured to the plastic mesh to ensure that they did not move. As mentioned, five of the substrate types were artificial (concrete, rope, PVC, steel rebar and glass) and selected due to their use in local artificial reef structures. The remaining two samples were natural

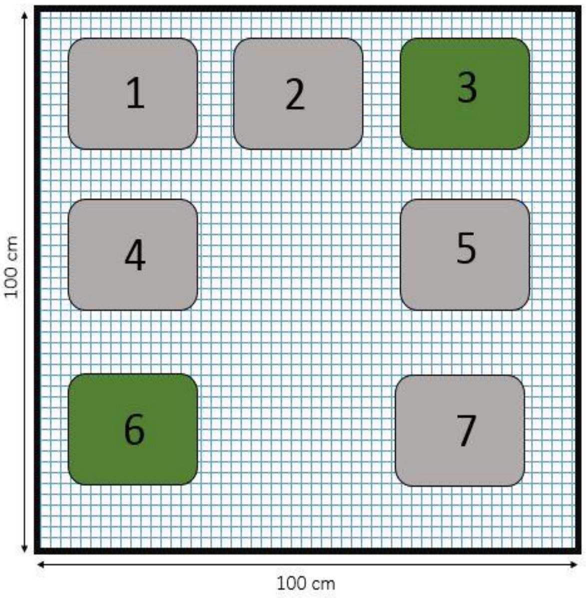

Figure 8. Schematic picture of the quadrat used for the substrate survey. Each side was 100 cm. Seven substrate samples were used, 20x20 cm each, numbered from 1 to 7. They were attached to a mesh, here represented by the blue pattern. The grey quadrats represent the artificial substrates; the green quadrats represent the natural substrates. 1: Steel rebar; 2: Concrete; 3: Dead mushroom coral; 4: Rope; 5: PVC; 6: Giant clam shell; 7: Glass bottles.

substrate found in the area: dead mushroom coral (*Fungia sp.)* and the shells of a giant clam (*Tridacna sp*.).

Concrete, synthetic rope, steel and glass were used to represent the artificial reefs of the area that are built with these four substrate types. The PVC was chosen to add another artificial substrate type. The concrete samples had been submerged in-situ for over a year before being used for this study. This allowed for the concrete chemistry to equilibrate and better represent the long term substrate quality of the artificial structures. Fresh concrete could falsify the results, as chemicals could be released during the survey and may interfere with organisms settling on it. All substrate samples were cleared of any epifauna prior to use in this study.

The dead mushroom coral samples were chosen because tunicates have been observed recruiting on them. The giant clam shells were also chosen to simulate natural substrate and were shells that had undergone natural mortality. They had been reared in-situ for over a year prior to mortality and use in this study. As with concrete, any epifauna living on the shells were removed by hand before being used in this study.

Additionally, the green mesh on which the samples were secured was also monitored throughout the study.

4.1.2 Depth Survey

This study was carried out with an additional 100x100cm quadrat made from PVC pipes. This quadrat was divided into 9 identical quadrats of 33,3x33,3cm each. Five of these 9 smaller quadrats were used to count the number of zooids on the transect line (pic. 4.1).

A dive computer was used to monitor the depth, while a GPS receiver was used to mark the exact beginning and ending coordinates of the transect lines. Google Earth Pro was used for visualization of the GPS points.

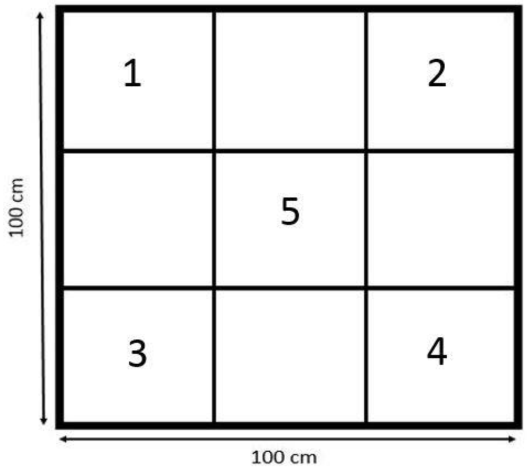

Figure 9. Schematic picture of the quadrat used for the transect lines. Each side was 100 cm. The smaller quadrats 1-5 were used to count the number of zooids.

4.2 Methods

4.2.1 Substrate survey

The three quadrats with substrate samples were positioned at different locations in Suan Olan on 23-06-2015. The first quadrat was placed in the natural reef at a depth of 5 meters, the second one was placed in a sandy area at a depth of 10 meters and the third quadrat was placed in the deeper sand at a depth 15 meters.

A total of four data collections was performed, with at least one week in between each sampling. The data collection consisted of a repeated measurement counting on every substrate sample.

The mesh on which the samples were secured was also monitored throughout the study and taken into account for the statistical analysis.

4.2.2 Depth Survey

The transect lines began in the shallows of the natural reef (4-7m), continued through the sandy area beyond the reef and ended in the deeper muck (14-16m). The number of zooids was counted every five meters using the aforementioned subdivided quadrat. The depth was monitored every five meters as well. The general type of environment (natural reef, sand or artificial reef) was monitored as additional information, but was not included in the statistical analysis.

A total of three transect surveys were performed. The first was performed on 25-06-15 and was 180-meter long. The second was performed on 02-07-15 and was 300-meter long. The third was performed on 09-07-15 and was 300-meter long.

The GPS coordinates of the starting and ending points of the transect lines were collected by mounting a handheld GPS unit on a floating raft with vertical estimation of start points marked below the surveyor. These points were then input into Google Earth Pro for visualization

(**Figure 10**). The GPS coordinates can be taken off **Supplemental Table 12**. The geographic coordinates of the third transect line could not be taken due to unsuitable weather conditions.

Figure 10. Map of Ao Leuk Bay, representing the starting and ending points of the transect lines. The northern line represents the first transect line, with 1-A being the starting point and 1-B being the ending point. The southern line represents the second transect line, with 2-A being the starting point and 2-B being the ending point. The geographic coordinates can be taken off Supplemental Table 12. The geographic coordinates of the third transect line, conducted further south of second transect line, could not be collected due to unsuitable weather conditions, and is therefore not represented in this figure.

4.3 Statistical analyses

4.3.1 Substrate Survey

A Levene-test and additional F-tests were conducted to test the homogeneity of variance. The results showed a *P* value under 0,05, showing an unequal variance and distribution of data.

The small sample size of the data was an additional factor as to why a normal distribution was rejected. Therefore, a nonparametrical Kruskal-Wallis-test was conducted. Two post hoc tests (Turkey-HSD and Scheffé) were performed for pair-wise comparison of all samples. A *P* value below 0,05 was seen as a significant difference.

All tests were carried out with the program SPSS.

4.3.2 Depth Survey

The data showed different distributions for the two variables, and thus a generalized linear model with a Poisson distribution and a log-link was conducted. Within this model, an omnibus-test and a Wald-chi-square-test were carried out to determine if there is a significant connection between the depth and the number of tunicates within the three transect lines. The Wald-chi-square-test was followed by a more detailed analysis of the parameters. This analysis offered additional information on the slope and the axis intercept of the data. A positive regression coefficient would mean that the population grows with the depth. Furthermore, an Omnibus-test, a Wald-chi-square-test and a more detailed analysis of the parameters were conducted for each transect line individually.

A *P* value below 0,05 was seen as a significant difference.

These tests, too, were carried out with the program SPSS.

5 Results

5.1 Substrate Survey

5.1.1 Data results

The rope samples showed a total number of 9 zooids (2 adults, 7 juveniles) after the 5-week-survey: the first data collection showed 1 adult tunicate. An additional adult zooid was counted during the second data collection. The third data collection showed 7 zooids (4 adults, 3 juveniles). The last data collection counted therefore an addition of 2 zooids (**Table 1**, column 2).

The PVC samples showed a total number of 6 zooids (2 adults, 4 juveniles) after the 5-week-survey: the first data collection showed 1 adult zooid, which was not seen during the second data collection anymore. No tunicate settlement was observed during this week. The third data collection showed 5 zooids (2 adults, 3 juveniles). An addition of 1 zooid was counted during the last data collection (**Table 1**, column 3).

The glass samples hosted a total number of 8 juvenile zooids after the 5-week-survey: No tunicate settlement was observed during the first two weeks of survey, but the third data collection showed 2 zooids (1 adult, 1 juvenile). An addition of 6 zooids was observed during the last data collection (**Table 1**, column 4).

The concrete samples showed a total number of 12 zooids (1 adult, 11 juveniles) after the 5-week-survey: the first data collection produced 6 juvenile zooids and the double amount was observed during the second data collection. 10 juvenile zooids were observed during the third data collection, and an addition of 2 zooids were counted during the last data collection (**Table 1**, column 5).

The giant clam shell samples hosted a total number 27 juvenile zooids after the 5-week-survey: 11 juvenile zooids were observed during the first data collection, 20 juvenile zooids during the second data collection, 19 juvenile zooids during the third data collection, and an addition of 6 juvenile zooids during the last data collection (**Table 1**, column 6).

The mushroom coral samples hosted a total number of 24 juvenile zooids after the 5-week-survey: 10 juvenile zooids were observed during the first data collection, 13 zooids (1 adult, 12 juveniles) were observed during the second data collection, 14 juvenile zooids during the third data collection and an addition of 8 juvenile zooids were counted during the last data collection (**Table 1**, column 7).

The steel samples counted a total number of 24 juvenile zooids as well: no tunicate settlement was observed during the first two weeks of survey, but during the second data collection, 12 juvenile zooids were observed and the third data collection showed 14 juvenile zooids. An addition of 10 juvenile zooids were counted during the last data collection (**Table 1**, column 8).

The mesh hosted a total number of 35 zooids (26 adults, 9 juveniles) after the 5-week-survey: 15 zooids (12 adults, 3 juveniles) were observed during the first data collection, 19 zooids (14 adults, 5 juveniles) were counted during the second data collection, 27 zooids (21 adults, 6 juveniles) were counted during the third data collection and an addition of 8 zooids was seen during the last data collection (**Table 1**, column 9).

Table 1. Number of individuals of the ascidian *Didemnum molle* counted during a 5-week-survey on 8 different substrate types. Each of the seven substrate types had three replications. The results of each replicate were added up. This table shows the sum of individuals counted on the samples. The original data, which includes all replications, can be taken off the Supplemental Table 1. The counting was a repeated measurement counting. Each data collection had approximately one week in between them, numbered from 1-4, with 1 being the data collection after one week of survey. The exact dates of the data collections can also be taken off the Supplemental Table 1. GCS is the abbreviation for Giant clam shell and MC Mushroom coral. The cursive numbers represent adult individuals, whereas the rest are juvenile individuals.

Data collection	Rope	PVC	Glass	Concrete	GCS	MC	Steel	Mesh	Total
1	***1***	***1***	0	6	11	10	0	***12***; 3	44
2	***2***	0	0	12	20	***1***; 12	12	***14***; 5	78
3	***4***; 3	***2***; 3	***1***; 1	10	19	16	14	***21***; 6	100
4	***2***; 7	***2***; 4	8	***1***; 11	27	24	24	***26***; 9	145

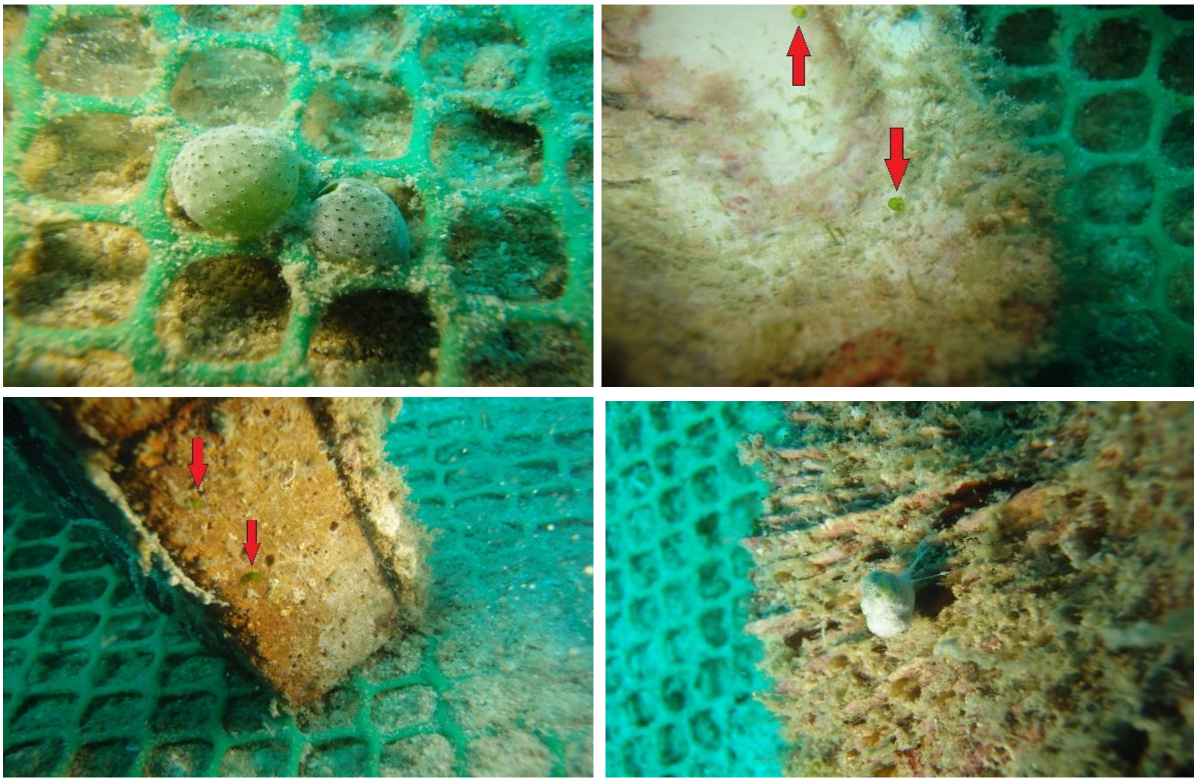

Figure 10. Settlement of *Didemnum molle* after two weeks of survey. This figure shows two juvenile zooids settled on the mesh (upper left picture), two juvenile zooids on the giant clam shell (upper right picture), two juvenile zooids on the side of the concrete block (lower left picture) and one juvenile zooid settling on the mushroom coral (lower right picture). These pictures were taken during the first data collection, on 30.06.2015 by Rahul Mehrotra and Rebecca Danieli.

The giant clam shell showed the most number of individuals settled on it (with a total of 27 individuals), followed by the mushroom coral (with a total of 24 individuals). Even though the steel sample only showed tunicate growth after 3 weeks of survey, it showed an equal number of individuals as the mushroom coral samples (24 individuals). The samples with the least tunicate growth were the rope, the PVC and the glass (**Table 2**).

Table 2. Ranking of the substrate types in descending order after 5 weeks of survey. This table presents the ranking of the substrate types, excluding the mesh, with number 1 being the substrate type with most individuals settled on it and 6 the substrate type with the least. The last column lists the total number of individuals of each substrate type after 5 weeks of survey.

Ranking in descending order	Substrate type	Total number of individuals
1	Giant clam shell	27
2	Mushroom coral; Steel	24; 24
3	Concrete	18
4	Rope	9

5	Glass	8
6	PVC	6

Figure 11. The three steel samples used for the substrate survey after two weeks of survey. All three steel samples showed sponge settlement on the sides and the edges.

5.1.2 Statistical results

The Levene-test showed a *P* value of 0,00, which means that there is an unequal variance and distribution of data (**Supplemental Table 2**). Additional F-tests supported these results by also producing *P* values under 0,05 (**Supplemental Table 3**).

The non-parametrical Kruskal-Wallis test produced an asymptotic significant value of 0,162 (**Supplemental table 4**), which is higher than the significance level of 0,05, which means that it can't be considered as a significant number. However, the statistical analysis showed that there is a graduated growth of the tunicates (**Figure 12**) and the raw data indicates a preference for certain types of substrate. Therefore, post hoc tests were conducted to conduct pair-wise comparisons to identify if there are significant differences between each substrate samples individually.

The Turkey-HSD post-hoc test showed several pair-wise significant differences. The most significant difference is between mesh and glass, with a *P* value of 0,003. The positive mean difference (5,73) indicates that there were significantly more individuals of *D. molle* on the mesh than on the glass samples. The mesh also showed a significant difference with the PVC samples, with a P value of 0,004 and a mean difference of 5,60, which indicates again more individuals on the mesh. Furthermore, the mesh shows a significant difference with the rope, with a *P* value of 0,012 and a mean difference of 5,13. The last significant difference is between the giant clam shells and the glass, with a *P* value of 0,049 and a mean difference of 4,47, indicating a higher abundance of individuals on the giant clam shells.

The Scheffé post-hoc- test only showed one significant difference between the mesh and the glass, with a *P* value of 0,045 and a mean difference of 5,73, indicating more individuals on the mesh.

Table 3. Significant differences between substrate types resulting from pair-wise comparison with the two post-hoc-tests Turkey-HSD and Scheffé. Substrate type I was compared to Substrate Type II. The mean difference indicates which substrate type has a higher abundance of individuals. A positive mean difference indicates a higher abundance on the Substrate type I. A P value below 0,05 was seen as a significant difference.

Post-Hoc-Test	Substrate type I	Substrate type II	Mean difference (I – II)	*P* value
Turkey-HSD	Mesh	Glass	5,73	0,003
	Mesh	PVC	5,60	0,004
	Mesh	Rope	5,13	0,012
	Giant Clam Shell	Glass	4,47	0,049
Scheffé	Mesh	Glass	5,73	0,045

As mentioned above, a graduate population growth was observed, represented on Figure 12. The significant differences resulting from the Turkey-HSD post-hoc-test can be observed on this graph as well. A big interval can be seen between the mesh (green) and the rope (light blue), the PVC (orange) and the glass (light grey).

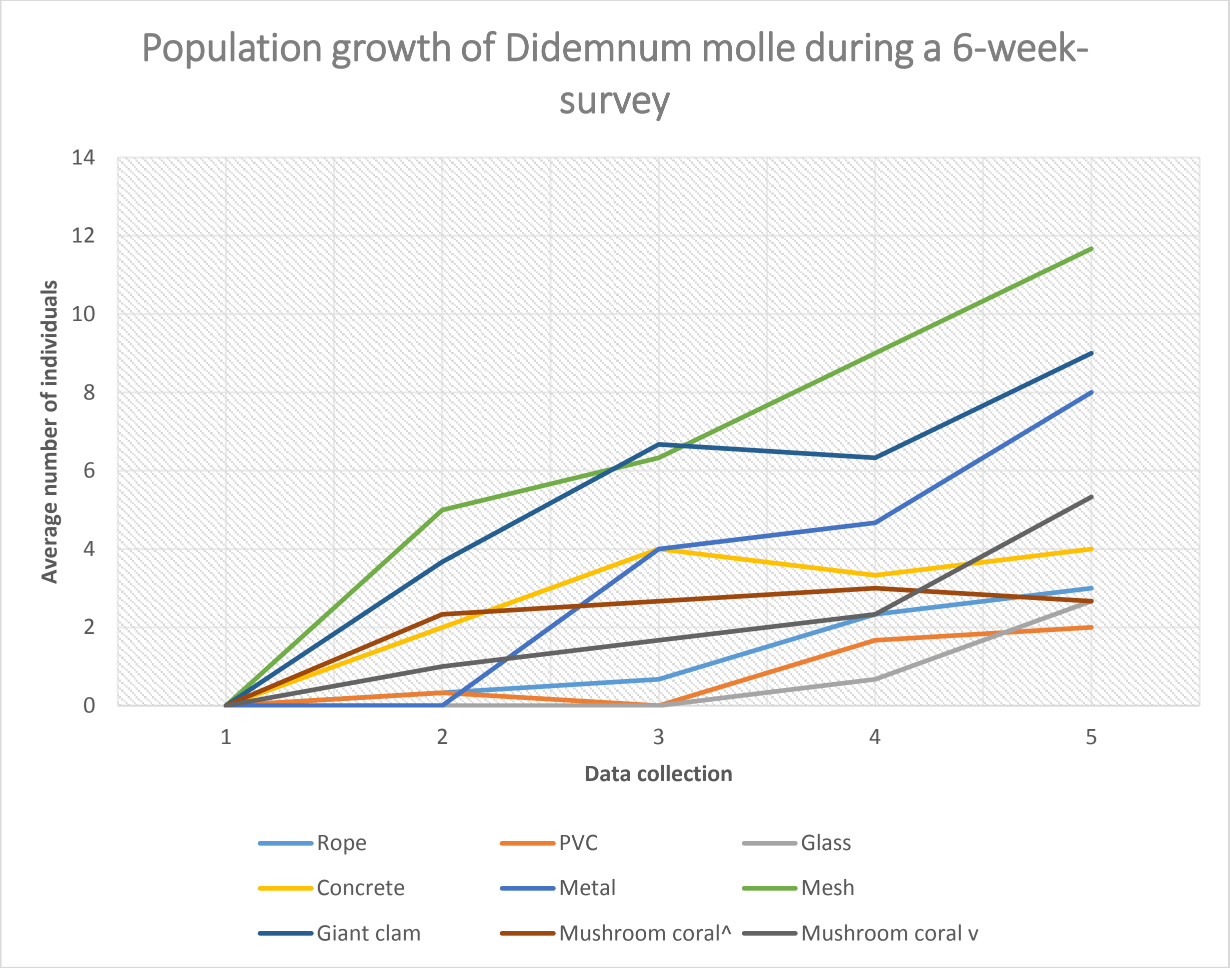

Figure 12. Population growth of Didemnum molle during a 6-week-survey. This figure shows the population growth of the ascidian *Didemnum molle* during the 6-week-survey. The x-axis shows the five data collections. The numbers 1-5 represent the dates on which the measurements were carried out (1 = 23.06.15; 2 = 30.06.15; 3 = 09.07.15; 4 = 23.07.15; 5 = 05.08.15). The y-axis represents the average number of individuals, obtained by the descriptive statistical analysis of the raw data. “Mushroom coral ^” means the upper side of the coral and “mushroom coral v” the underside.

5.2 Depth Survey

5.2.1 Data results

The first transect started in the natural reef at a depth of 5,2 meters and ended in the sand at a depth of 12,4 meters.

A total average number of 281,2 individuals of the ascidian *Didemnum molle* was observed throughout this transect line. The zooids were present between a depth of 8,3 meters and a depth of 12,3 meters, although the majority was observed between a depth of 9,5 meters and a depth of 12,3 meters. The first individuals were observed after 50 meters at a depth of 8,3 meters (2 individuals). The highest number of individuals on a transect point was an average of 37,6 zooids, counted after 115 meters at a depth of 9,7 meters (**Table 4).**

Even though the first zooids were observed in the natural reef, most of them were present beyond the reef (**Table 4**) and all adhering to sand particles.

Table 4. Results of the first transect line for the Depth Survey. The average number of individuals of the ascidian *D. molle* is shown in the second column. They were counted every five meters, represented in the first column. The depth was monitored throughout the whole transect. The general environment is listed in the last column.

Transect points [m]	Average number of individuals	Depth [m]	Environment
0	0	5,2	Natural reef
5	0	5,4	Natural reef
10	0	5,8	Natural reef
15	0	6,4	Natural reef
20	0	6,4	Natural reef
25	0	6,5	Natural reef
30	0	7	Natural reef
35	0	7,3	Natural reef
40	0	7,6	Natural reef
45	0	8	Natural reef
50	2	8,3	Natural reef and sand
55	4,4	8,3	Natural reef and sand
60	0	8,5	Natural reef and sand
65	0	8,7	Natural reef and sand
70	0	8,9	Natural reef and sand
75	0	9	Natural reef and sand
80	0,2	9,2	Natural reef and sand
85	0	9,2	Natural reef and sand
90	0,2	9,3	Natural reef and sand
95	0	9,3	Natural reef and sand
100	0	9,4	Sand
105	0	9,5	Sand
110	3,6	9,5	Sand
115	37,6	9,7	Sand
120	33	10	Sand
125	11	10,3	Sand
130	10	10,4	Sand
135	29	10,7	Sand
140	27,4	11	Sand
145	7,8	11,4	Sand
150	28,6	11,5	Sand

155	16,4	11,6	Sand
160	16,8	11,9	Sand
165	17,6	12	Sand
170	17	12,3	Sand
175	18,6	12,3	Sand
180	0	12,4	Sand
Total average number of individuals	281,2		

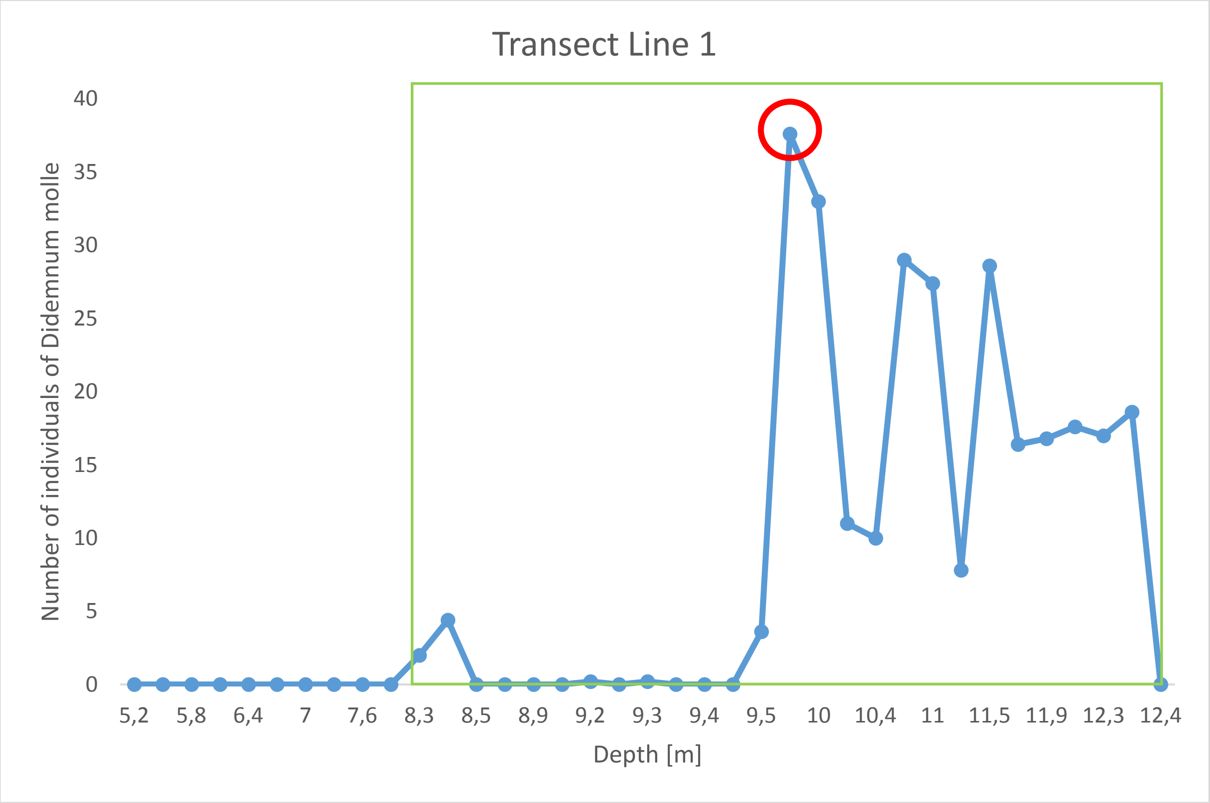

Figure 13. Representation of the first transect line, carried out for the Depth Survey. This figure shows the number of individuals of the ascidian Didemnum molle counted during the first transect line. The x-axis shows the depth in meters. The y-axis represents the number of individuals. The red circle represents the highest number of individuals counted on one transect point. In this case, 37,6 individuals were counted at a depth of 9,7 meters. The green rectangle comprises the area in which zooids were observed. In this case, between a depth of 8,3 meters and a depth of 12,4 meters.

The second transect started in the natural reef at a depth of 7,1 meters and ended in the sand at a depth of 15,3 meters.

A total average number of 29,2 individuals was observed throughout this transect line. The zooids were present between a depth of 11,3 meters and a depth of 12,6 meters. The first individuals were observed after 130 meters at a depth of 11,3 meters (0,6 individuals). The highest number of individuals on a transect point was an average of 17,4 zooids, counted after 135 meters at a depth of 11,7 meters (**Table 5**).

Shortly after the natural reef, between a depth of 9,6 meters and a depth of 11 meters, artificial reefs (made up off concrete and metal) were present (**Table 5**). Even though the zooids were observed close to these structures, all of them were adhering to sand particles.

Table 5. Results of the second transect line for the Depth Survey. The average number of individuals of the ascidian *D. molle* is shown in the second column. They were counted every five meters, represented in the first column. The depth was monitored throughout the whole transect. The general environment is listed in the last column.

Transect points [m]	**Average number of individuals**	**Depth [m]**	**Depth/m**
0	0	7,1	Natural reef
5	0	7,1	Natural reef
10	0	7,2	Natural reef
15	0	7,4	Natural reef
20	0	7,5	Natural reef
25	0	7,8	Natural reef
30	0	8	Natural reef
35	0	8,1	Natural reef
40	0	8,3	Natural reef
45	0	8,5	Natural reef
50	0	8,9	Sand
55	0	9	Sand
60	0	9,2	Sand
65	0	9,4	Sand
70	0	9,6	Artificial reef (concrete)
75	0	9,7	Artificial reef (concrete)
80	0	9,7	Artificial reef (concrete)
85	0	9,9	Artficial reef (metal)
90	0	10	Artficial reef (metal)
95	0	10	Artficial reef (metal)
100	0	10,1	Sand
105	0	10,5	Artficial reef (metal)
110	0	10,6	Artficial reef (metal)
115	0	10,6	Artficial reef (metal)
120	0	10,9	Artficial reef (metal)
125	0	11	Artficial reef (metal)
130	0,6	11,3	Sand
135	17,4	11,7	Sand
140	7,2	12,1	Sand
145	1,6	12,4	Sand

150	0	12,5	Sand
155	0	12,5	Sand
160	1,6	12,5	Sand
165	0	12,5	Sand
170	0	12,5	Sand
175	0,4	12,6	Sand
180	0	12,6	Sand
185	0	12,6	Sand
190	0	12,6	Sand
195	0	12,6	Sand
200	0,2	12,6	Sand
205	0,2	12,6	Sand
210	0	12,7	Sand
215	0	12,8	Sand
220	0	12,8	Sand
225	0	13	Sand
230	0	13,4	Sand
235	0	13,7	Sand
240	0	13,8	Sand
245	0	13,8	Sand
250	0	13,9	Sand
255	0	14,1	Sand
260	0	14,3	Sand
265	0	14,7	Sand
270	0	14,7	Sand
275	0	14,8	Sand
280	0	15	Sand
285	0	15,2	Sand
290	0	15,2	Sand
295	0	15,3	Sand
300	0	15,3	Sand
Total average number of individuals	29,2		

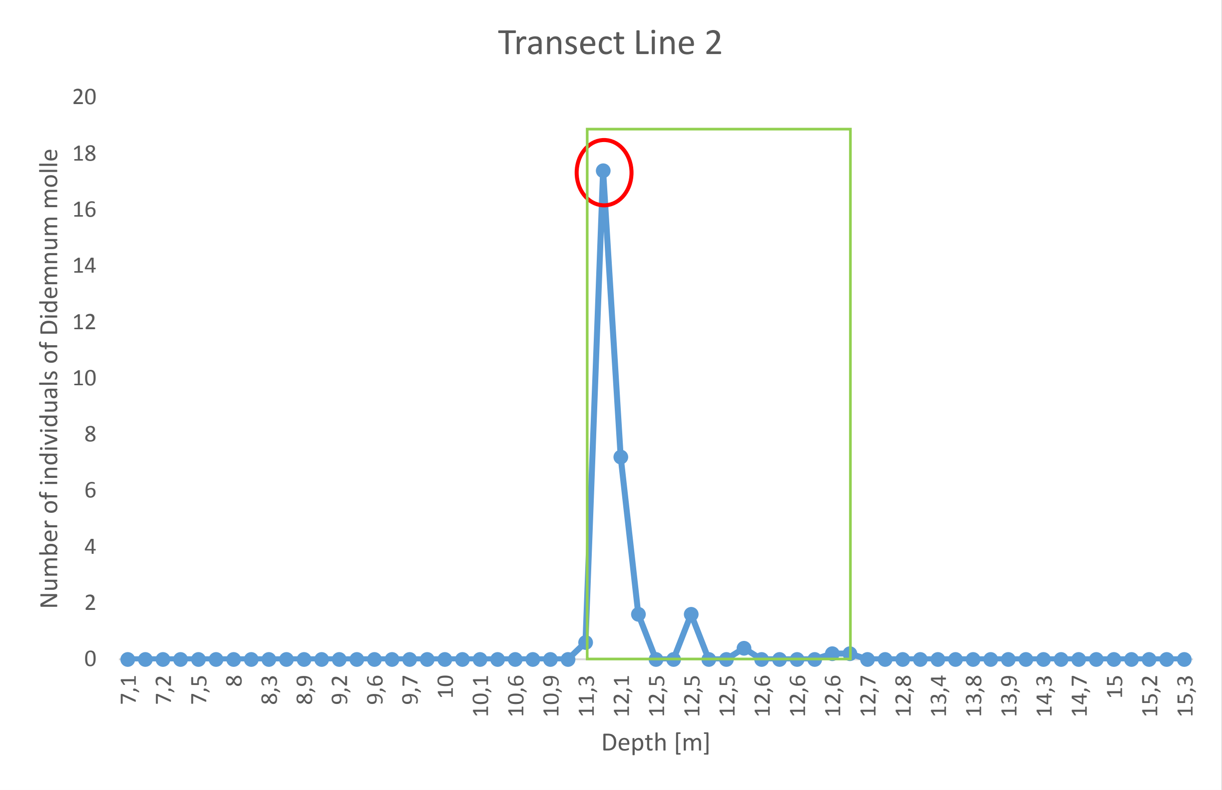

Figure 14. Representation of the second transect line, carried out for the Depth Survey. This figure shows the number of individuals of the ascidian Didemnum molle counted during the second transect line. The x-axis shows the depth in meters. The y-axis represents the number of individuals. The red circle represents the highest number of individuals counted on one transect point. In this case, 17,4 individuals were counted at a depth of 11,7 meters. The green rectangle comprises the area in which zooids were observed. In this case, between a depth of 11,3 meters and a depth of 12,6 meters.

The third transect line started in the natural reef at a depth of 6,3 meters and ended in the sand at a depth of 15,2 meters.

A total average number of 8,2 individuals was observed throughout this transect line. Zooids were present between a depth of 13,6 meters and a depth of 14,4 meters. The first zooids were observed after 180 meters at a depth of 13,6 meters (0,8 individuals). The highest number of individuals on a transect point was an average of 2,4 zooids, counted after 255 meters at a depth of 14,4 meters (**Table 6**).

Most of the observed individuals were present in the sandy area, but an average number of 2,4 zooids was observed near the artificial reefs (after 255 meters of transect and a depth of 14,4 meters) (**Table 6**). Nevertheless, all of the individuals were adhering to sand particles.

Table 6. Results of the third transect line for the Depth Survey. The average number of individuals of the ascidian *D. molle* is shown in the second column. They were counted every five meters, represented in the first column. The depth was monitored throughout the whole transect. The general environment is listed in the last column.

Transect points [m]	Average number of individuals	Depth [m]	Environment
0	0	6,3	Natural reef
5	0	6,5	Natural reef
10	0	6,5	Natural reef
15	0	6,6	Natural reef
20	0	7	Natural reef
25	0	7,2	Natural reef
30	0	7,3	Artifcial reef (concrete)
35	0	7,3	Artifcial reef (concrete)
40	0	7,7	Sand
45	0	8	Sand
50	0	8,4	Sand
55	0	8,4	Sand
60	0	8,6	Sand
65	0	8,7	Sand
70	0	9	Natural reef
75	0	9,2	Natural reef
80	0	9,4	Sand
85	0	9,6	Sand
90	0	9,7	Sand
95	0	9,8	Sand
100	0	9,8	Sand
105	0	10,1	Sand
110	0	10,2	Sand
115	0	10,5	Sand
120	0	11	Sand
125	0	11,5	Sand
130	0	11,8	Sand
135	0	12	Sand
140	0	12,4	Sand
145	0	12,6	Sand
150	0	13	Sand
155	0	13,2	Sand
160	0	13,4	Sand
165	0	13,5	Sand
170	0	13,5	Sand
175	0	13,5	Sand
180	0,8	13,6	Sand
185	2,2	13,6	Sand
190	0,2	13,6	Sand
195	0,2	13,7	Sand

200	0,2	13,7	Sand
205	0,4	13,8	Sand
210	1,8	13,8	Sand
215	0	13,9	Sand
220	0	14	Sand
225	0	14	Sand
230	0	14	Sand
235	0	14,1	Sand
240	0	14,1	Artificial reef (concrete)
245	0	14,1	Artificial reef (concrete)
250	0	14,1	Artificial reef (concrete)
255	2,4	14,4	Artificial reef (concrete)
260	0	14,5	Artificial reef (concrete)
265	0	14,7	Artificial reef (concrete)
270	0	14,7	Artificial reef (concrete)
275	0	14,8	Sand
280	0	15	Sand
285	0	15	Sand
290	0	15,1	Sand
295	0	15,2	Sand
300	0	15,2	Sand
Total average number of individuals	8,2		

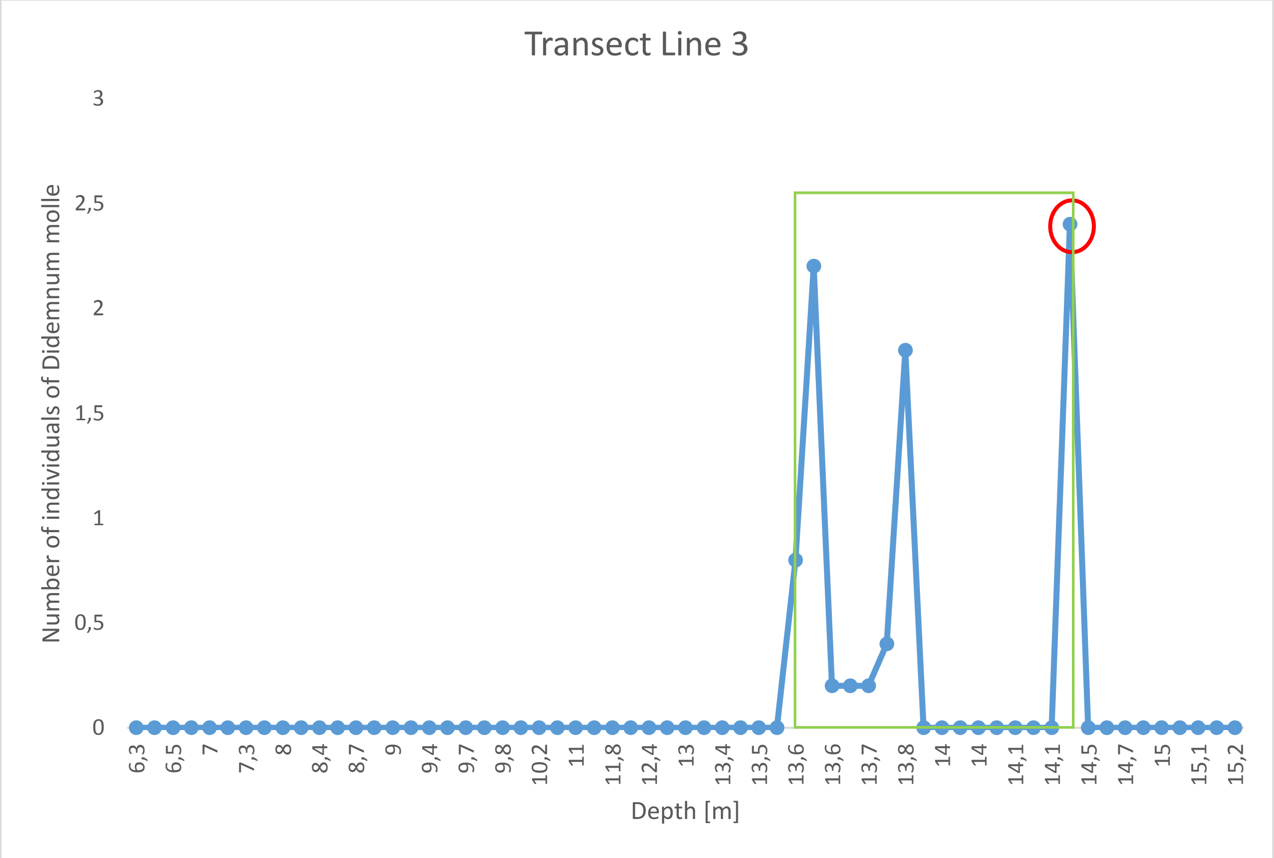

Figure 15. Representation of the third transect line, carried out for the Depth Survey. This figure shows the number of individuals of the ascidian Didemnum molle counted during the third transect line. The x-axis shows the depth in meters. The y-axis represents the number of individuals. The red circle represents the highest number of individuals counted on one transect point. In this case, 2,4 individuals were counted at a depth of 14,4 meters. The green rectangle comprises the area in which zooids were observed. In this case, between a depth of 13,6 meters and a depth of 14,4 meters.

5.2.2 Statistical results

The first Omnibus test showed a *P* value of 0,0, which is seen as a significant difference (**Supplemental Table 6**). The Chi-Square test supported these results by producing a *P* value of 0,0 (**Supplemental Table 7**). The more detailed analysis of the parameters also showed a P value of 0,0 and additionally a regression coefficient of 0,112 (**Supplemental Table 8**).

Furthermore, an Omnibus-test, a Wald-chi-square-test and a more detailed analysis of the parameters were conducted on each transect line individually.

The Omnibus-test showed significant numbers for all transect lines. A *P* value of 0,0 was produced for the first transect line, a *P* value of 0,038 was produced for the second transect line, and a *P* value of 0,0 was produced for the third transect line (**Table 7**).

The Wald-chi-square-test showed significant numbers for all transect lines as well. A *P* value of 0,0 was produced for the first transect line, a *P* value of 0,040 was produced for the second transect line and a *P* value of 0,0 was produced for the third transect line (**Table 7**).

The more detailed analysis of the parameters supported these results, by showing the same significant numbers as the Wald-chi-square-test did. In addition, it showed positive regression coefficients for all transect lines. The first transect line produced a regression coefficient of 0,537, the second transect line produced a coefficient of 0,075 and the third transect line produced a coefficient of 0,500 (**Table 7**).

Table 7. Results of the Omnibus-test, the Wald-chi-square-test and the detailed analysis of the parameters for each transect lines individually. The third column shows the P values. A P value below 0,050 was seen as a significant number. The last column shows the regression coefficient, conducted in the detailed analysis of the parameters. A positive regression was interpreted as a population growth with the depth.

Statistical Test	Data Set	P Value	Regression Coefficient
Omnibus-test	Transect Line 1	0,0	
	Transect Line 2	0,038	
	Transect Line 3	0,0	
Wald-chi-square-test	Transect Line 1	0,0	
Data Set	Transect Line 2	0,040	
	Transect Line 3	0,0	
Detailed analysis	Transect Line 1	0,0	0,537
Data set	Transect Line 2	0,040	0,075
	Transect Line 3	0,0	0,500

6 Discussion

6.1 Substrate Survey

The purpose of this study was to determine if the substrate type is an influential factor on the population growth of the tunicates. This result can be important for the construction and placement of artificial reef structures and may help further scientific studies (Chase *et al.*, 2015). The informal observations on Koh Tao, suggesting that the substrate type is an influential factor and that the tunicates show a bigger growth on artificial reefs made up off concrete, have not been confirmed. The results of this study do not show a significant difference of tunicate growth between the different substrate types.

The observations showed that all samples have been populated at some point during the 5-week-survey with a high colonization and growth rate. The exact number cannot be calculated though due to several unknown parameters. No studies have tested the colonization rate of this tunicate species and therefore the colonization rate probability, needed to calculate the colonization rate, cannot be defined.

The two natural substrate types were the most populated, with the steel samples showing an equal number of tunicates as the mushroom coral, followed by the concrete samples. If the natural substrate types are generally preferred by the tunicates, cannot clearly be stated. Even though the number of tunicates was higher on the natural substrate types, the distribution appeared to have been random. Only one significant difference can be considered in this study, being between the giant clam shell and the glass samples (with a *P* value of 0,049). The significant differences occurred from the post-hoc-tests in the statistical analysis between the mesh and the glass, the mesh and the PVC, and the mesh and the rope, cannot be used for a statement because of the higher size of the mesh surface.

The samples with the least tunicate growth were the rope, the PVC and the glass samples. All three of these substrate types have a smooth surface and lack therefore the grip that substrates as the giant clam shell, the mushroom coral, the steel and the concrete provide. This is also thought to be the reason of the significant difference of the number of tunicates between the giant clam shell and the glass samples.

All steel samples showed sponge growth. As mentioned in 3.2.2., sponges are considered fouling organisms and are therefore thought to have prevented the tunicates to properly settle on the steel samples. Competition among benthic space occupiers impacts the recruitment, growth and mortality and alter their population dynamics (Chadwick, Morrow, 2010). It is therefore presumed that a higher number of tunicates would have been counted on the steel samples if the sponges would not have been present.

Most of the tunicates though were seen on the mesh on which the substrate samples were attached (a total of 35 individuals) instead of on the actual samples. This may have been caused by the fact that the samples were too small, compared to the mesh, and did therefore not offer the populations a big enough settlement space. Additionally, the substrate samples are thought to have been too close to each other. These might also be part of the reasons why no colonies were formed during the survey. A total of 145 individuals were counted but all of them seemed to have settled solitarily, even though this species mostly appears as a colony. Intraspecific competition is also thought to be an influential factor in this matter. A high density has been proven to have negative impacts on larval settlement and juvenile mortality of colonial ascidians (Hurlbut, 1991).

6.2 Depth Survey

The purpose of this study was to determine if depth is an influential factor on the population growth of the tunicates. This result can be important for the construction and placement of artificial reef structures and may help further scientific studies (Chase *et al.*, 2015). The informal observations on Koh Tao, suggesting that the depth is an influential factor and the tunicates favor a depth between 8-12 meters, have only partially been confirmed. The results of this study confirm that the depth is an influential factor, but show a tunicate population between a minimum depth of 8,3 meters and a maximum depth of 14,4 meters.

The influence of the depth on the population growth has been confirmed through the statistical analysis of the data. The first set of the analysis, comparing the three transects to determine a connection between the depth and the number of tunicates, showed significant *P* values. This indicates that there is a connection between the depth and the population growth of the tunicates. The positive regression coefficient resulting from the analysis (with a value of 0,012) indicates that the tunicate population grew with depth.

These two statements can only be acknowledged though because the second set of the statistical data analysis showed significant *P* values as well as positive regression coefficients for each transect line individually. This ensures that each transect line shows the significant difference of population growth depending on the depth as well as a growth of population with depth.

Attention is drawn to the statement that the population of the tunicates grows with depth, which only applies to an area with a maximum depth of 15,3 meters. This statement cannot be transferred to an area with a higher maximum depth. Further tests with deeper areas would have to be carried out to assess this information.

7 Conclusion

As mentioned above, other studies have suspected a difference in abundance between man-made and natural substrates (Chase *et al*., 2005). In this study, the substrate type is not being considered an influential factor on the population growth of the colonial tunicate *Didemnum molle*. But the results do show a tendency of the tunicates preferring certain types of substrates, suggesting therefore a more detailed and ameliorated survey. Improvements would be to use bigger samples of the substrate types and more space in between each of them to avoid intraspecific competition. Space competition with other marine organisms can be prevented by removing fouling organisms (such as alga and sponges) as soon as they appear near or on the substrate samples. The mesh, on which many tunicates settled, should also be tested by being a substrate sample itself and the same size as the other samples. Another way of attachment would therefore have to be designed to ensure that the samples do not move.

Even though the Depth Survey was a success by confirming the statement that the depth is an influential factor on the population growth, improvements in the methodology could be made to support these findings. More replications of transect lines would lead to a better assessment of this influence. These transect lines would ideally be taken off the whole bay to have a better evaluation of the entire area. To have an even better estimation, several identical surveys should be made in multiple bays around the island of Koh Tao. Longer transect lines, assessing consequently deeper depths, should also be conducted to confirm or disprove the statement that the tunicate population grows with depth.

As mentioned in 3.4., artificial reefs can have a great impact in the restoration of marine habitat that have been heavily damaged. But some of these artificial reefs structures could not be used due to an overgrowth of tunicates. Even though exact numbers cannot be calculated in this case, the results of this study suspect that the tunicate *Didemnum molle* has a higher colonization and growth rate than corals. This makes them important space competitors, hindering corals to properly grow and expand.

A monitoring program, conducting population surveys on the tunicate *Didemnum molle*, could provide estimations on the tunicate abundance and distribution around the island. Additionally, the correlation between the increase of nutrients and the increase in ascidian abundance still has to be formally described for Koh Tao. These informations could help prevent an overgrowth of this fouling organism and therefore ensure the successful rehabilitation of degraded coral reefs.

This study suggests that artificial reefs should be avoided being deployed within a depth between 8 meters and 15 meters. Structures that are already in this area and threatened by an overgrowth of tunicates, should be relocated to shallower depths, where the tunicate abundance is shown to be very small to non-existent. Even though the depth is presumably not the only influential factor on the population growth of tunicates, this approach would ensure that corals and tunicates are not present in the same areas. This would prevent a space competition between both species, stimulating therefore the coral spread and thereby helping maintain the marine biodiversity created by coral reefs.

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9 Supplements

Supplemental Table 1. Data collection results for the Substrate Survey. This table includes the results of all five data collections of the 6-week Substrate Survey, numbered from 1 to 5, followed by their exact date. Three replications were made, named R1, R2 and R3, during which the number of individuals of the ascidian *Didemnum molle* were counted and listed in this table. The blue numbers mean that the individuals were adults, whereas the black numbers represent juvenile individuals.

Data collection	**Rope**	**PVC**	**Glass**	**Concrete**	**Giant Clam**	**Mushroom coral**	**Metal**	**Mesh**
1								
30.06.15								
R1	0	0	0	0	0	0	0	0
R2	1	0	0	1	11	1; 5	3	8; 1
R3	0	1	0	5	4	7	0	4; 2
2								
09.07.15								
R1	0	0	0	0	0	0	0	0
R2	2	0	0	2	11	1; 5	3	11; 2
R3	0	0	0	10	9	7	9	3; 3
3								
23.07.15								
R1	0	0	0	0	0	0	0	0
R2	2	2	1	2	10	8	5	9; 1
R3	2; 3	3	1	8	9	8	9	12; 5
4								
05.08.15								
R1	0	0	0	0	0	0	0	0
R2	2; 5	2; 2	7	1; 4	17	17	13	17; 3
R3	2	2	1	7	10	7	11	9; 6

Supplemental Table 2. Test of homogeneity with Levene-Test for the Substrate Survey. The last column shows the *P* value resulting from the Levene-Test.

Depending variable: Number of zooids			
F	df1	df2	*P* value
4,151	44	90	0,0

Supplemental Table 3. Test of homogeneity with additional F-Test for the Substrate Survey. The last column shows the P value resulting from the F-Test.

Depending variable: Number of zooids					
	Square sum	**df**	**Mean of squares**	**F**	**P value**
Contrast	448,415	4	112,104	7,613	0,0
Error	1325,333	90	14,726		

Supplemental Table 4. Comparison of samples with the non-parametrical Kruskal-Wallis-Test for the Substrate Survey. The asymptotic significance shows the P value. A P value below 0,05 was seen as a significant difference.

	Rank		
Substrate		**N**	**Mean rank**
Number of zooids	Rope	15	60,13
	PVC	15	55,20
	Glass	15	51,20
	Concrete	15	72,40
	GianClam	15	82,17
	Mushroom^	15	67,83
	Mushroomv	15	69,47
	Metal	15	69,47
	Mesh	15	84,13
	Total	135	
Statistic for test			
		Number of zooids	
Chi-Square		11,758	
df		8	
Asymptotic significance		0,162	

Supplemental Table 5. Pair-wise comparison of data with two post-hoc-Tests for the Substrate Survey. This table includes both post-hoc-tests, Turkey-HSD and Scheffé. A *P* value below 0,05 was seen as a significant difference. The red numbers show the *P* values that are considered showing a significant difference between the substrate samples.

Substrate			**Mean difference (I-J)**	**Standard error**	***P* value**	**95% confidence interval**	
						Upper limit	**Under limit**
Tukey-HSD	**Rope**	PVC	0,47	1,401	1,000	-3,99	4,92
		Glass	0,60	1,401	1,000	-3,85	5,05
		Concrete	-1,40	1,401	0,985	-5,85	3,05
		GiantClam	-3,87	1,401	0,143	-8,32	0,59
		Mushroom^	-0,87	1,401	0,999	-5,32	3,59
		Mushroomv	-0,80	1,401	1,000	-5,25	3,65
		Metal	-2,07	1,401	0,864	-6,52	2,39
		Mesh	-5,13*	1,401	0,012	-9,59	-0,68
	PVC	Rope	-0,47	1,401	1,000	-4,92	3,99
		Glass	0,13	1,401	1,000	-4,32	4,59
		Concrete	-1,87	1,401	0,919	-6,32	2,59
		GiantClam	-4,33	1,401	0,063	-8,79	0,12
		Mushroom^	-1,33	1,401	0,989	-5,79	3,12
		Mushroomv	-1,27	1,401	0,992	-5,72	3,19
		Metal	-2,53	1,401	0,677	-6,99	1,92
		Mesh	-5,60*	1,401	0,004	-10,05	-1,15
	Glass	Rope	-0,60	1,401	1,000	-5,05	3,85
		PVC	-0,13	1,401	1,000	-4,59	4,32
		Concrete	-2,00	1,401	0,884	-6,45	2,45

	GiantClam	-4,47*	1,401	0,049	-8,92	-0,01
	Mushroom^	-1,47	1,401	0,980	-5,92	2,99
	Mushroomv	-1,40	1,401	0,985	-5,85	3,05
	Metal	-2,67	1,401	0,613	-7,12	1,79
	Mesh	-5,73*	1,401	0,003	-10,19	-1,28
Concrete	Rope	1,40	1,401	0,985	-3,05	5,85
	PVC	1,87	1,401	0,919	-2,59	6,32
	Glass	2,00	1,401	0,884	-2,45	6,45
	GiantClam	-2,47	1,401	0,708	-6,92	1,99
	Mushroom^	0,53	1,401	1,000	-3,92	4,99
	Mushroomv	0,60	1,401	1,000	-3,85	5,05
	Metal	-0,67	1,401	1,000	-5,12	3,79
	Mesh	-3,73	1,401	0,176	-8,19	0,72
GiantClam	Rope	3,87	1,401	0,143	-0,59	8,32
	PVC	4,33	1,401	0,063	-0,12	8,79
	Glass	4,47*	1,401	0,049	0,01	8,92
	Concrete	2,47	1,401	0,708	-1,99	6,92
	Mushroom^	3,00	1,401	0,453	-1,45	7,45
	Mushroomv	3,07	1,401	0,422	-1,39	7,52
	Metal	1,80	1,401	0,933	-2,65	6,25
	Mesh	-1,27	1,401	0,992	-5,72	3,19
Mushroom ^	Rope	0,87	1,401	0,999	-3,59	5,32
	PVC	1,33	1,401	0,989	-3,12	5,79
	Glass	1,47	1,401	0,980	-2,99	5,92
	Concrete	-0,53	1,401	1,000	-4,99	3,92
	GiantClam	-3,00	1,401	0,453	-7,45	1,45
	Mushroomv	0,07	1,401	1,000	-4,39	4,52
	Metal	-1,20	1,401	0,995	-5,65	3,25
	Mesh	-4,27	1,401	0,071	-8,72	0,19
Mushroomv	Rope	0,80	1,401	1,000	-3,65	5,25
	PVC	1,27	1,401	0,992	-3,19	5,72
	Glass	1,40	1,401	0,985	-3,05	5,85
	Concrete	-0,60	1,401	1,000	-5,05	3,85
	GiantClam	-3,07	1,401	0,422	-7,52	1,39
	Mushroom^	-0,07	1,401	1,000	-4,52	4,39
	Metal	-1,27	1,401	0,992	-5,72	3,19
	Mesh	-4,33	1,401	0,063	-8,79	0,12
Metal	Rope	2,07	1,401	0,864	-2,39	6,52
	PVC	2,53	1,401	0,677	-1,92	6,99
	Glass	2,67	1,401	0,613	-1,79	7,12
	Concrete	0,67	1,401	1,000	-3,79	5,12
	GiantClam	-1,80	1,401	0,933	-6,25	2,65
	Mushroom^	1,20	1,401	0,995	-3,25	5,65
	Mushroomv	1,27	1,401	0,992	-3,19	5,72
	Mesh	-3,07	1,401	0,422	-7,52	1,39
Mesh	Rope	5,13*	1,401	0,012	0,68	9,59
	PVC	5,60*	1,401	0,004	1,15	10,05

		Glass	5,73*	1,401	0,003	1,28	10,19
		Concrete	3,73	1,401	0,176	-0,72	8,19
		GiantClam	1,27	1,401	0,992	-3,19	5,72
		Mushroom^	4,27	1,401	0,071	-0,19	8,72
		Mushroomv	4,33	1,401	0,063	-0,12	8,79
		Metal	3,07	1,401	0,422	-1,39	7,52
Scheffé	**Rope**	PVC	0,47	1,401	1,000	-5,20	6,13
		Glass	0,60	1,401	1,000	-5,06	6,26
		Concrete	-1,40	1,401	0,998	-7,06	4,26
		GiantClam	-3,87	1,401	0,479	-9,53	1,80
		Mushroom^	-0,87	1,401	1,000	-6,53	4,80
		Mushroomv	-0,80	1,401	1,000	-6,46	4,86
		Metal	-2,07	1,401	0,974	-7,73	3,60
		Mesh	-5,13	1,401	0,115	-10,80	0,53
	PVC	Rope	-0,47	1,401	1,000	-6,13	5,20
		Glass	0,13	1,401	1,000	-5,53	5,80
		Concrete	-1,87	1,401	0,986	-7,53	3,80
		GiantClam	-4,33	1,401	0,311	-10,00	1,33
		Mushroom^	-1,33	1,401	0,999	-7,00	4,33
		Mushroomv	-1,27	1,401	0,999	-6,93	4,40
		Metal	-2,53	1,401	0,913	-8,20	3,13
		Mesh	-5,60	1,401	0,056	-11,26	0,06
	Glass	Rope	-0,60	1,401	1,000	-6,26	5,06
		PVC	-0,13	1,401	1,000	-5,80	5,53
		Concrete	-2,00	1,401	0,978	-7,66	3,66
		GiantClam	-4,47	1,401	0,269	-10,13	1,20
		Mushroom^	-1,47	1,401	0,997	-7,13	4,20
		Mushroomv	-1,40	1,401	0,998	-7,06	4,26
		Metal	-2,67	1,401	0,886	-8,33	3,00
		Mesh	-5,73*	1,401	0,045	-11,40	-0,07
	Concrete	Rope	1,40	1,401	0,998	-4,26	7,06
		PVC	1,87	1,401	0,986	-3,80	7,53
		Glass	2,00	1,401	0,978	-3,66	7,66
		GiantClam	-2,47	1,401	0,925	-8,13	3,20
		Mushroom^	0,53	1,401	1,000	-5,13	6,20
		Mushroomv	0,60	1,401	1,000	-5,06	6,26
		Metal	-0,67	1,401	1,000	-6,33	5,00
		Mesh	-3,73	1,401	0,531	-9,40	1,93
	GiantClam	Rope	3,87	1,401	0,479	-1,80	9,53
		PVC	4,33	1,401	0,311	-1,33	10,00
		Glass	4,47	1,401	0,269	-1,20	10,13
		Concrete	2,47	1,401	0,925	-3,20	8,13
		Mushroom^	3,00	1,401	0,798	-2,66	8,66
		Mushroomv	3,07	1,401	0,777	-2,60	8,73
		Metal	1,80	1,401	0,989	-3,86	7,46

	Mesh	-1,27	1,401	0,999	-6,93	4,40
Mushroom^	Rope	0,87	1,401	1,000	-4,80	6,53
	PVC	1,33	1,401	0,999	-4,33	7,00
	Glass	1,47	1,401	0,997	-4,20	7,13
	Concrete	-0,53	1,401	1,000	-6,20	5,13
	GiantClam	-3,00	1,401	0,798	-8,66	2,66
	Mushroomv	0,07	1,401	1,000	-5,60	5,73
	Metal	-1,20	1,401	0,999	-6,86	4,46
	Mesh	-4,27	1,401	0,333	-9,93	1,40
Mushroomv	Rope	0,80	1,401	1,000	-4,86	6,46
	PVC	1,27	1,401	0,999	-4,40	6,93
	Glass	1,40	1,401	0,998	-4,26	7,06
	Concrete	-0,60	1,401	1,000	-6,26	5,06
	GiantClam	-3,07	1,401	0,777	-8,73	2,60
	Mushroom^	-0,07	1,401	1,000	-5,73	5,60
	Metal	-1,27	1,401	0,999	-6,93	4,40
	Mesh	-4,33	1,401	0,311	-10,00	1,33
Metal	Rope	2,07	1,401	0,974	-3,60	7,73
	PVC	2,53	1,401	0,913	-3,13	8,20
	Glass	2,67	1,401	0,886	-3,00	8,33
	Concrete	0,67	1,401	1,000	-5,00	6,33
	GiantClam	-1,80	1,401	0,989	-7,46	3,86
	Mushroom^	1,20	1,401	0,999	-4,46	6,86
	Mushroomv	1,27	1,401	0,999	-4,40	6,93
	Mesh	-3,07	1,401	0,777	-8,73	2,60
Mesh	Rope	5,13	1,401	0,115	-0,53	10,80
	PVC	5,60	1,401	0,056	-0,06	11,26
	Glass	5,73*	1,401	0,045	0,07	11,40
	Concrete	3,73	1,401	0,531	-1,93	9,40
	GiantClam	1,27	1,401	0,999	-4,40	6,93
	Mushroom^	4,27	1,401	0,333	-1,40	9,93
	Mushroomv	4,33	1,401	0,311	-1,33	10,00
	Metal	3,07	1,401	0,777	-2,60	8,73

Supplemental Table 6. Omnibus-Test within the generalized linear model with a poisson distribution and a log-link for the Depth Survey. The last column shows the *P* values. A *P* value below 0,05 was seen as a significant difference.

Likelihood-Quotient-Chi-square	df	*P* value
141,750	1	0,0

Supplemental Table 7. Wald-Chi-Square-Test within the generalized linear model with a poisson distribution and a log-link for the Depth Survey. The last column shows the *P* value. A *P* value below 0,05 was seen as a significant difference.

Source	Wald-Chi-Square	df	*P* value
(constant term)	65,865	1	0,0

Depth_m	140,945	1	0,0

Supplemental Table 8. Detailed analysis of the parameters for the Depth Survey. The last column shows the *P* value. A *P* value below 0,05 was seen as a significant difference. The second column shows the regression coefficient. A positive regression coefficient was interpreted as a population growth with depth.

			95% Wald-confidence interval		**Hypothesis test**		
Parameter	**Regression coefficient**	**Standard error**	**Lower value**	**Upper value**	**Wald-Chi-Square**	**df**	***P* value**
(constant term)	0,877	0,1081	0,665	1,089	65,865	1	0,0
Depth_m (scale)	0,1121^{3}	0,0094	0,093	0,130	140,945	1	0,0

Supplemental Table 9. Second Omnibus-Test within the generalized linear model with a poisson distribution and a log-link for the Depth Survey. The last column shows the *P* values. A *P* value below o,05 was seen as a significant difference.

Data Set	**Likelihood-Quotient-Chi-Square**	**df**	***P* value**
Transect Line 1	1243,884	1	0,000
Transect Line 2	4,315	1	0,038
Transect Line 3	43,173	1	0,000

Supplemental Table 10. Second Wald-Chi-Square-Test within the generalized linear model with a poisson distribution and a log-link for the Depth Survey. The last column shows the *P* value. A *P* value below 0,05 was seen as a significant difference.

Data set		**Wald-Chi-Square**	**df**	***P* value**
Transect Line 1	(constant term)	92,531	1	0,000
	Depth_m	954,046	1	0,000
Transect Line 2	(constant term)	0,001	1	0,978
	Depth_m	4,211	1	0,040
Transect Line 3	(constant term)	18,259	1	0,000
	Depth_m	18,538	1	0,000

Supplemental Table 11. Second detailed analysis of the parameters for the Depth Survey. The last column shows the *P* value. A *P* value below 0,05 was seen as a significant difference. The third column shows the regression coefficient. A positive regression coefficient was interpreted as a population growth with depth.

				95 % Wald-confidence interval		**Hypothesis-test**		
Data Set		**Regression coefficient**	**Standard error**	**Lower value**	**Upper value**	**Wald-Chi-Square**	**df**	***P* value**
Transect Line 1	**(Constant term)**	-1,871	0,1945	-2,252	-1,490	92,531	1	0,000
	Depth_m (Scale)	0,537 1^3	0,0174	0,503	0,571	954,046	1 1	0,000
Transect Line 2	**(Constant term)**	-0,012	0,4462	-0,887	0,862	0,001	1	0,978
	Depth_m (Scale)	0,075 1^3	0,0368	0,003	0,148	4,211	1 1	0,040
Transect Line 3	**(Constant term)**	-6,868	1,6073	-10,18	-3,718	18,259	1	0,000
	Depth_m (Scale)	0,500 1^3	0,1161	0,727	18,538	18,538	1 1	0,000

Supplemental Table 12. Geographic coordinates collected during the Depth Survey. This table shows the geographic coordinates of the starting and ending points for the first two transect lines conducted during the Depth Survey. The coordinates of the third transect line could not be taken due to unsuitable weather conditions. N represents North. E represents East.

Transect Line	**Starting Point**	**Ending Point**
1	N 10.06880 E 099.84031	N 10.06963 E 099.84169
2	N 10.06831 E 099.84038	N 10.06955 E 099.84287

10 Acknowledgments