

**HYDROMEDUSAE BLOOMS AND SEASONAL BIODIVERSITY
CHANGES IN GALVESTON BAY**

An Undergraduate Research Scholars Thesis

by

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TABLE OF CONTENTS

	Page
ABSTRACT	1
ACKNOWLEDGMENTS	2
SECTION	
I. INTRODUCTION	3
Hydromedusae Overview.....	3
Ecological Importance of Hydromedusae.....	4
The Gulf of Mexico and Hydromedusae Blooms	4
Environmental Conditions that May Affect Hydromedusae Abundance and Diversity.....	5
II. METHODOLOGY	7
Sampling	7
Temperature and Salinity Data	7
Photographs and DNA Extraction	7
Polymerase Chain Reaction and Purification	8
BLAST Sequencing	8
III. RESULTS	10
Hydromedusae Abundance and Water Temperature Data.....	10
Hydromedusa Diversity	12
IV. CONCLUSION.....	17
Effects of Temperature/Salinity on Abundance.....	17
Seasonality of Species.....	17
Possible Sources of Error.....	18
Future Research	18
REFERENCES	20
APPENDIX.....	23

ABSTRACT

Hydromedusae Blooms and Seasonal Biodiversity Changes in Galveston Bay

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Jellyfish of the class Hydrozoa (phylum Cnidaria) in the Gulf of Mexico are greatly understudied despite the fact that they are top predator and may have a significant ecological impact on fisheries and marine plankton in general. Medusae of the class Hydrozoa were collected every other day from October 2016 to February 2017 at the boat basin at the Texas A&M University at Galveston campus. Hydromedusae were isolated and examined for morphological characters. Each medusa was photographed, and DNA was extracted from every collected medusa. The mitochondrial 16S gene was amplified and sequenced, and the sequences were analyzed and compared with available sequences in a public repository, such as GenBank. Results illustrated that the abundance of Hydromedusae was not significantly correlated to water temperature, but was significantly correlated to salinity. Species diversity was varied throughout the sampling period, exhibiting the greatest amount of diversity in the Fall. The goal of this project is to contribute to long-term monitoring to assess the diversity and temporal fluctuations of the Hydromedusae population in Galveston Bay, and will be continued to obtain further information about the frequency and intensity of Hydromedusae blooms.

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Finally, thanks to our parents for their encouragement and their patience.

SECTION I

INTRODUCTION

Jellyfish play a vital role in marine ecosystems worldwide and are part of a vast category of gelatinous plankton. Gelatinous zooplankton comprises four distinct groups based on morphology and life history: Hydrozoa, Scyphozoa, Cubozoa, and Ctenophora (Marshall, 2008). Hydromedusae, belonging to class Hydrozoa, phylum Cnidaria, are some of the most abundant planktonic invertebrates, despite their diminutive size. Although Hydrozoa proliferate extensively in aquatic ecosystems, their small size and numerous species lead to underrepresentation in scientific studies (Miglietta *et al.*, 2008). Hydromedusae display a life cycle with benthic and planktonic stages: the benthic polyp, the non-feeding larva planula, and the planktonic medusa (or jellyfish). Misidentification proves common during hydromedusae observation due to the difficulty in distinguishing species, especially at the juvenile stage of development (Boero and Bouillon, 1987; 1993).

Hydrozoa Overview

Over 3,700 species have been identified within the class Hydrozoa, each possessing unique and intricate life cycles (Bouillon *et al.*, 1936). Class Hydrozoa exhibits a life cycle that alternates between benthic and medusa forms, with both sexual and asexual reproductive methods. The medusa stage of the life cycle is the reproductive and pelagic feeding stage. This distinguishes Hydrozoans from other invertebrate groups in which the larval phase is planktonic and the adults are benthic (Miglietta and Cunningham, 2012). As a group, Hydrozoans are

morphologically very diverse. Ecologically, both the benthic colonies and the planktonic medusa are predators, and consume a wide variety of planktonic organisms, including fish larva, protozoans, crustaceans, and generally larvae of most invertebrates. Because they are top predators, Hydrozoa and their medusae play a vital role in ocean ecology and marine food webs (Bouillon *et al.*, 2006).

Ecological Importance of Hydromedusae

The importance of investigating dynamics of jellyfish populations is substantiated by the increasing concern regarding degeneration of marine ecosystems. Jellyfish aid in the passage of energy from low to high trophic levels and supply the oceanic food chain with substantial organic material (Allen *et al.*, 2008; Boero *et al.*, 2008). As observed jellyfish populations increase in frequency and number, so too does the concern over possible effects of their blooms on marine ecosystems (Condon *et al.*, 2013). Due to the lack of investigation into the irregularity of timing and size of jellyfish blooms as well as the interaction between gelatinous zooplankton and the benthic environment, no real method exists for predicting cycles in jellyfish population dynamics. The specific causation of abnormal blooms has been linked to abiotic factors including human intervention, climate change, and alterations within the ecosystem (Boero *et al.*, 2008).

The Gulf of Mexico and Hydromedusae Blooms

Hydromedusae are abundant worldwide, undergo blooms, and eat virtually any larva. Medusae can also compete with other marine organisms, and the overabundance of jellyfish during blooms poses a threat to the stability of the trophic levels. The most obvious impact of

jellyfish on the food web is that of the fish stocks, both through competition for food and predation on fish larvae. A recent study conducted in China in 2010 (Qiu, 2014) reported that the rate of jellyfish blooms has increased, possibly in response to changes in ocean temperature. Thriving populations of jellyfish were also seen in areas devoid of higher trophic-level organisms (such as overfished basins) and areas with a high amount of nutrient runoff from agriculture and human activity (Qiu, 2014). One recent study examined jellyfish collection data from 1940 to 2010, indicating that increased frequency in blooms showed a 20-year cycle (Condon *et al.*, 2013). Most of the studies have, however, focused on the medusa of the order Scyphozoa, which are conspicuous and easier to study due to their big size. Very few studies have focused on Hydromedusae. However, Hydromedusae can be extremely abundant, go through population blooms, which frequency and triggers are mostly unknown (Miglietta *et al.*, 2008). Thus, understanding their plankton dynamics and assessing the frequency and composition of these blooms is extremely important, especially in systems such as the Gulf of Mexico, which represents one of the more productive fishing grounds of the world (Oceana, 2010).

Environmental Conditions that May Affect Hydromedusa Abundance and Diversity

Although there is limited information available regarding the impact of water temperatures on the overall proliferation of Hydrozoans, long-term studies have been completed to record jellyfish abundance in correlation with climate fluctuations. Generally, life cycle functions such as sexual and asexual reproduction have been found to increase in response to warmer water temperatures, in addition to widening their distributions and extending their

seasonality (Purcell, 2005). Specifically, temperature has been shown to correlate with local hydromedusae blooms of six hydromedusae species (Purcell, 2005). According to Purcell (2012), the dissemination, length, and timing of jellyfish blooms may be linked to warming of ocean waters. In regions with strong upwelling, such as the Pacific coast of Panama, hydromedusae abundance was shown to correlate with upwelling events, hinting to the possibility that productivity (associated with the upwelling waters) may have an impact on jellyfish blooms (Miglietta *et al.*, 2008).

Similar to temperature, salinity influences the life histories of hydromedusae; the extent of that effect is dependent on the range in salinities of a particular body of water. Estuaries, which possess fluctuating salinity levels, have a dramatic effect on reproductive rates and population size (Purcell, 2015). Studies have found that the species richness of hydroids fluctuates in response to salinity, specifically illustrating that salinity is correlated to species richness. Species that can tolerate a narrow salinity range are gradually replaced by species that can endure the fluctuation (Kinne 1956a, b; 1957, 1958; Boero, 1984). Environmental factors such as water temperature and salinity could play a role in both the abundance and diversity of hydroid species and, therefore, in the frequency and magnitude of their medusa blooms.

Ecological elements of gelatinous zooplankton have not been widely studied, but investigations suggest both biotic and abiotic causations. To obtain a more accurate model for anticipating the appearance of large-scale jellyfish blooms, efforts must be made to accumulate long-term data on species diversity, seasonality, and frequency of jellyfish blooms.

SECTION II

METHODOLOGY

Sampling

Hydromedusae were collected using plankton tows at the docks at Texas A&M University at Galveston campus. The planktonic samples were collected using a 100 micron net, 90 cm long, with a 30 cm mouth. Sampling was conducted three to four times per week, two tows per day, from September 2016 to February 2017. Each sampling day was conducted in the morning and was consistently carried out by towing the net six times alongside the 156 meters of the dock.

Temperature and Salinity Data

Daily water temperature (in °C) during sampling days was obtained from the National Oceanic and Atmospheric Administration Tides & Currents website. Daily water salinity (in ppt) was obtained from Amelia McAmis in the Quigg Lab at Texas A&M University Galveston campus, as well as from the Galveston Bay Foundation website.

Photographs and DNA Extraction

In the laboratory, plankton was examined under a Leica M80 Stereomicroscope and the hydromedusae were isolated using a micropipette. Each hydromedusa taken from the sample was anesthetized using menthol crystals, photographed using a Leica MC170 HD camera mounted on the Stereoscope, and analyzed with the software Leica Application Suite. DNA was extracted

using standard extraction protocol (Zietara, 2000). The protocol is as follows: 8 μ L distilled water, 1 μ L PCR buffer, incubated at 90°C for 10 minutes, 1 μ L Proteinkinase, incubated for 30 minutes at 55°C followed by 10 minutes at 90°C.

Polymerase Chain Reaction and Purification

The ~600bp fragment of the large ribosomal subunit of the mitochondrial RNA (18S rRNA, 16S) was amplified using primers SHA and SHB (Miglietta *et al.*, 2008). The protocol is as follows: 12.5 μ L of Green GoTaq PCR primer, 11 μ L of distilled water, 0.5 μ L of each primer, and 0.5 μ L of DNA. The mitochondrial 16S is considered the barcoding molecule for Hydrozoa and has been extensively used to identify species boundaries within this group (Miglietta *et al.*, 2009; 2015). The PCR product was run through a 2% agarose gel stained with Sybersafe at 110V for 15 minutes to determine the presence/absence of DNA. A 1kb ladder was used as reference. After DNA amplification was confirmed, samples were purified using exoSAP-it digestion (from Affimetrix).

The purification process is as follows: A 10 μ L quantity of the PCR product was mixed with 2 μ L of ExoSAP-IT reagent for a combined 12 μ L reaction volume, incubated at 37°C for 15 minutes to degrade remaining primers and nucleotides, incubated again at 80°C for 15 minutes to inactivate ExoSAP-IT reagent. Purified DNA was sent to the genomic core facility at Texas A&M University Corpus Christi for sequencing.

BLAST Sequencing

When sequenced DNA was returned, Geneious-software version R9 (Kearse *et al.*, 2012) was utilized for sequence clean up, and BLAST was used for species identification. In BLAST,

parameters that were taken into account were: E-value, query percentage, and identity percentage. Results from the BLAST analysis are shown in Table 1 (see Appendix).

SECTION III

RESULTS

Hydromedusae Abundance and Water Temperature Data

Sampling period started on 10/3/2016 and ended on 2/10/2017.

There were 12 sampling days in October, 10 sampling days in November, 3 sampling days in December, 6 in January, and 5 in February. The lower numbers of sampling days in December and January is due to a combination of holiday break and bad weather.

Temperature: The water temperature data (obtained from NOAA Tides & Currents) fluctuated slightly, but did not change drastically between sampling days. Figure 1 depicts a gradual downward trend in degrees Celsius from October until mid-December, where the temperature was more consistent. The temperature peaked at 27.8° C on 10/07/2016, and dropped as low as 14.2° C on 01/30/2017, a range of 13.6° C. The trend in water temperature depicts higher temperatures overall in October and November than the period from December until February.

Salinity: The salinity data (obtained from Quigg, 2017 and Galveston Bay Foundation) fluctuated noticeably twice during October, stayed consistent in November, fluctuated noticeably at least 4 times during December and January, and once in February. The salinity peaked at 36.04 ppt on 10/12/2016, and dropped as low as 14.1 ppt on 10/19/2016, a range of 21.94 ppt. The overall trend illustrates higher salinity measurements in January and February than the sampling days in October until December.

Hydromedusae: At the conclusion of the sampling period, it was found that the amount of

individual hydromedusae obtained from each water sample oscillated significantly between the sampling dates, as seen in Figure 1. The total number of hydromedusae collected per sampling day ranged from zero individuals to a maximum of 51 individuals. The overall average number of medusa collected/day was 20.8 with standard deviation of 13.3. There were 9 days in which there were more than 30 hydromedusae, and seven days where there were fewer than five hydromedusae (see Figure 1).

Of the 750 medusae collected, 543 were collected in the Fall and 212 in the Winter months. During the fall, the average medusae/day was 24.7, while in the winter it was 14.1. The minimum number of medusae/day was 5 in the fall and 0 in the winter. Finally, the maximum number of medusae per day was 51 in the fall and 40 in the winter.

We tested for correlation between hydromedusa abundance and both temperature and salinity. Correlation between number of medusa/day and temperature was 0.42 and between number of medusa/day and salinity was 0.02.

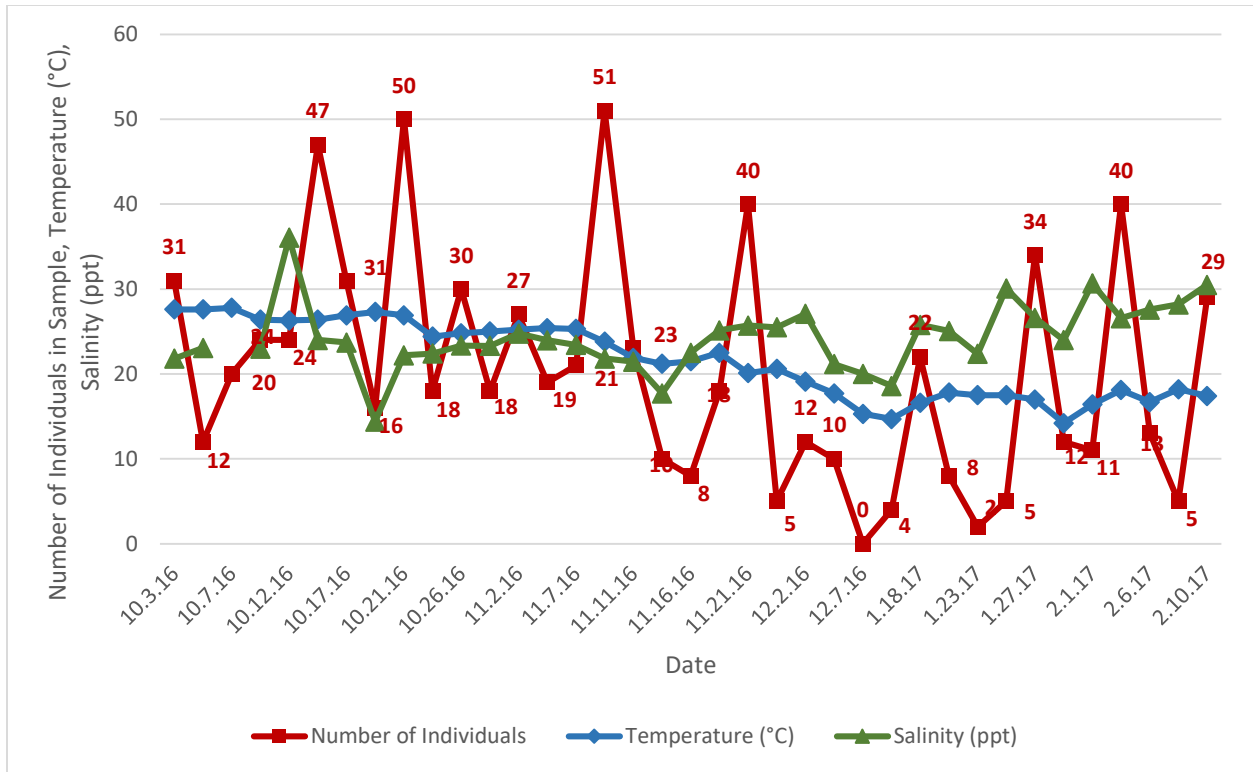


Figure 1: Number of hydromedusae observed each sampling date, along with water temperature in degrees Celsius and salinity in ppt.

Hydromedusa Diversity

At the end of the sampling period, there were 23 total Hydromedusa species collected, and variation in species diversity from month to month. In October (Figure 2), there were 15 species collected, and the most abundant species were *Clytia gracilis* (16 individuals), *Malagazzia carolinae* (41 individuals), *Obelia bidentata* (17 individuals), *Obelia dichotoma* (36 individuals), and *Malagazzia* sp. (33 individuals). There were 15 species collected in November (Figure 3), and the most abundant species were *Malagazzia* sp. (37 individuals), and *Obelia dichotoma* (16 individuals). In December (Figure 4), there were 3 species collected, and the most abundant species were *Bougainvillia triestina* (6 individuals), and *Obelia dichotoma* (5

individuals). There were 6 species collected in January (Figure 5), and the most abundant species was *Obelia dichotoma* (9 individuals). In February (Figure 6), there were 5 species collected, and the most abundant were *Bougainvillia triestina* (9 individuals), and *Obelia dichotoma* (18 individuals). The most and least common species collected throughout the sampling period are illustrated in Figures 7 and 8. Images of common species from each month are pictured in Figures 9-13 (see Appendix).

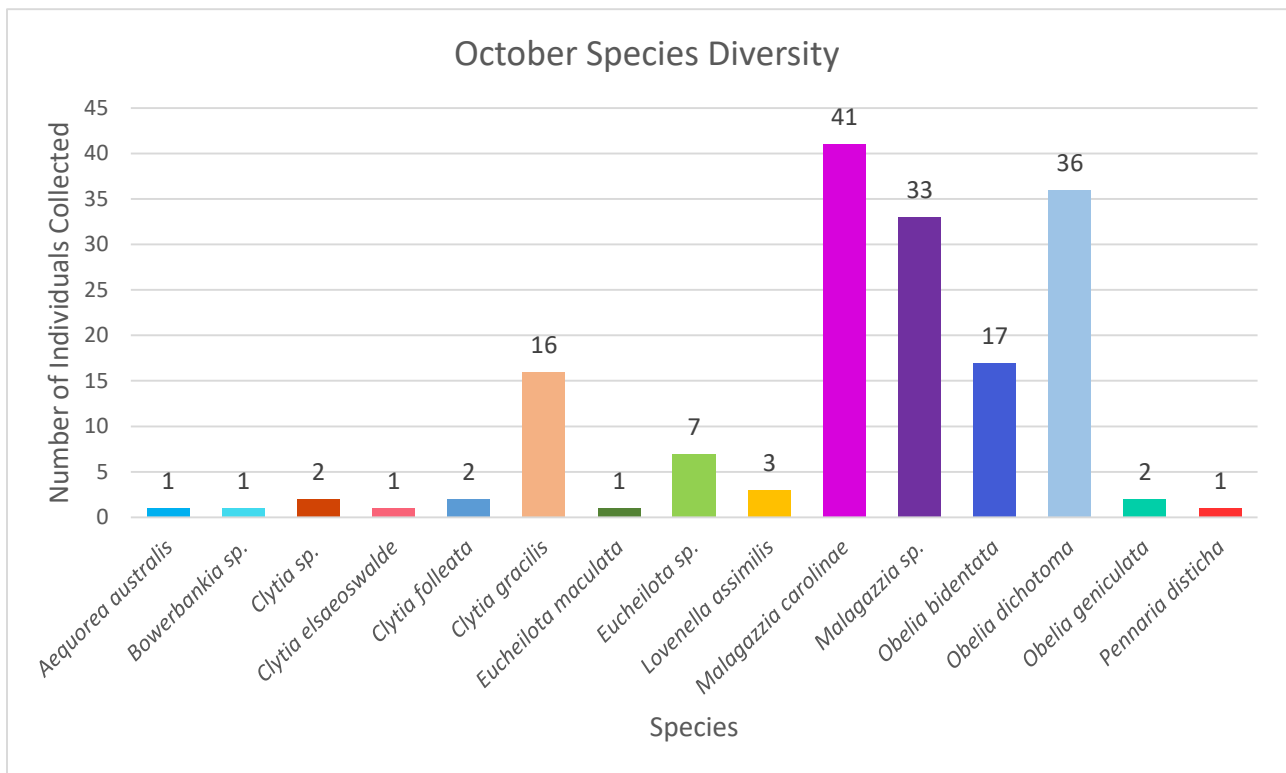


Figure 2. Total number of individual Hydromedusae collected from each species during the month of October.

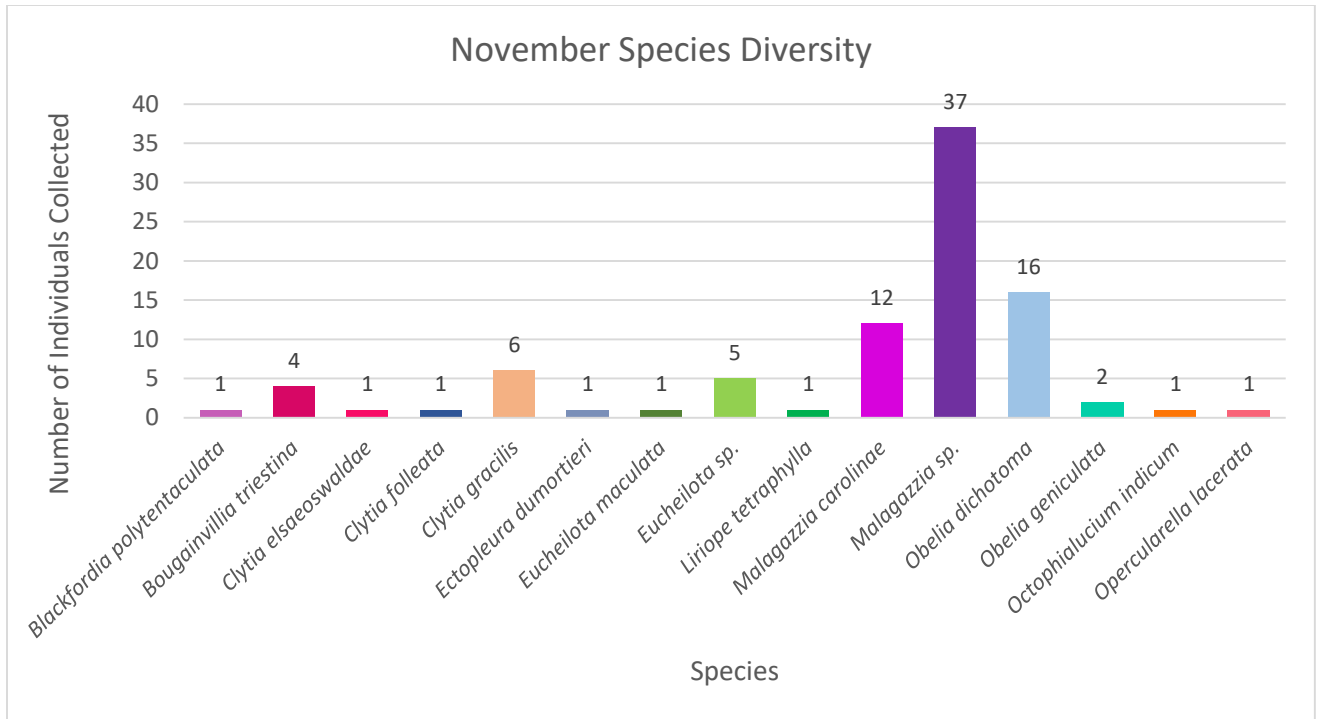


Figure 3. Total number of individual Hydromedusae collected from each species during the month of November.

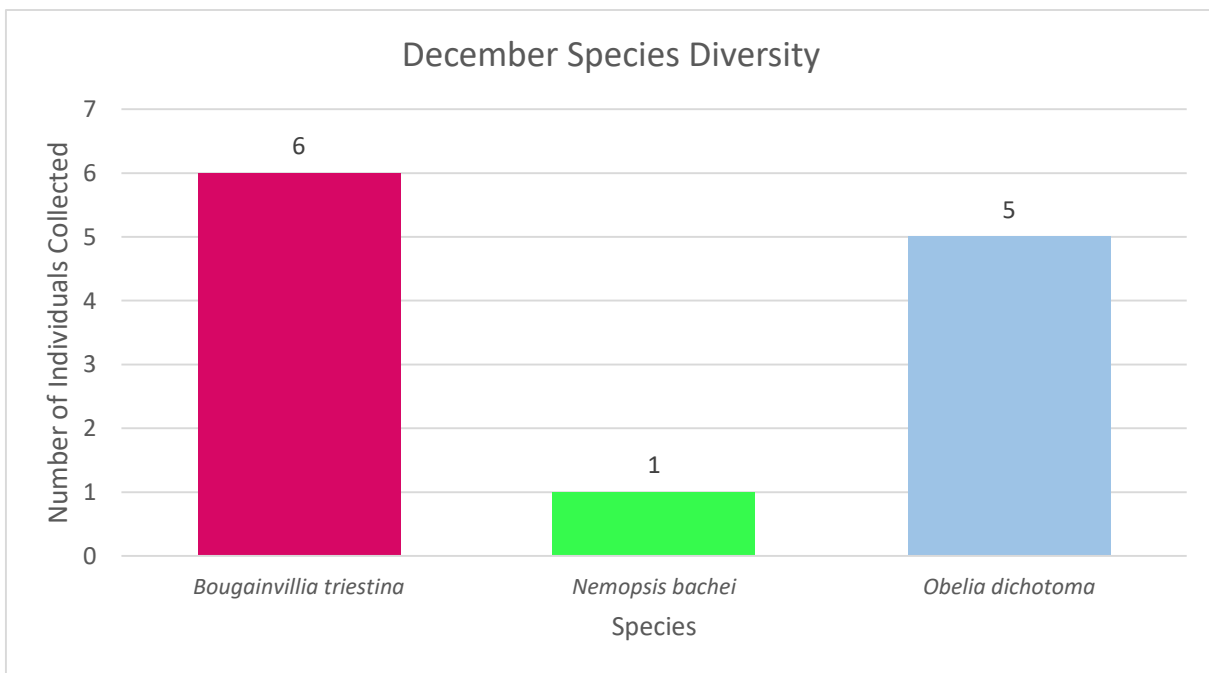


Figure 4. Total number of individual Hydromedusae collected from each species during the month of December.

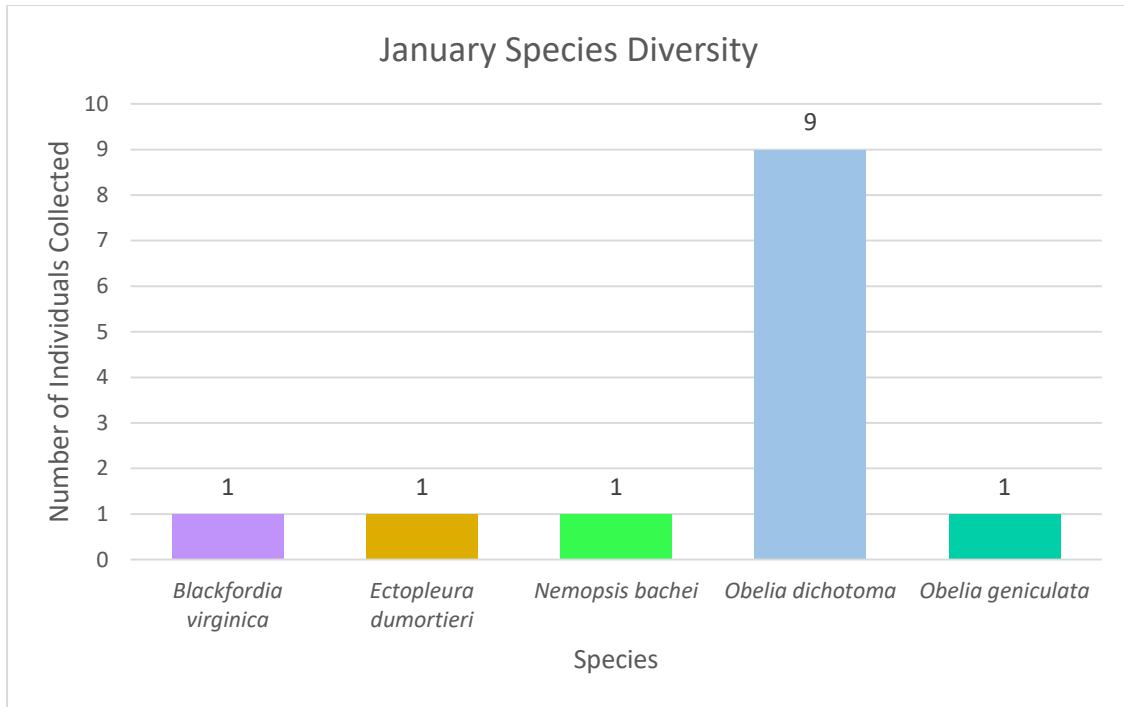


Figure 5. Total number of individual Hydromedusae collected from each species during the month of January.

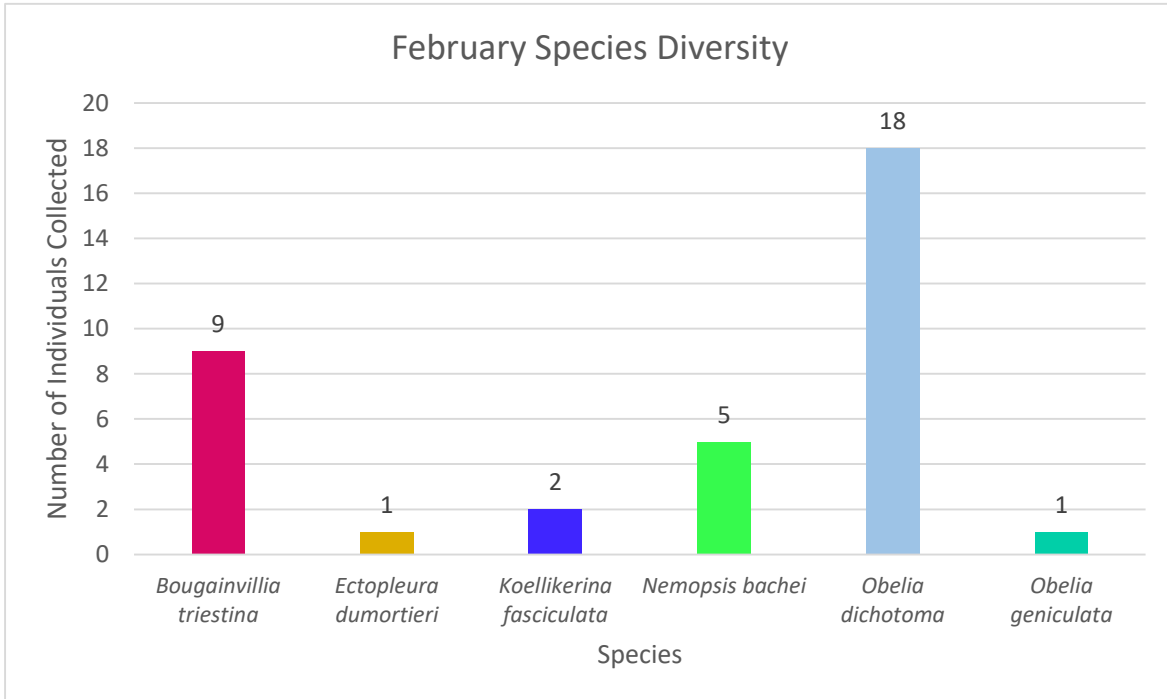


Figure 6. Total number of individual Hydromedusae collected from each species during the month of February.

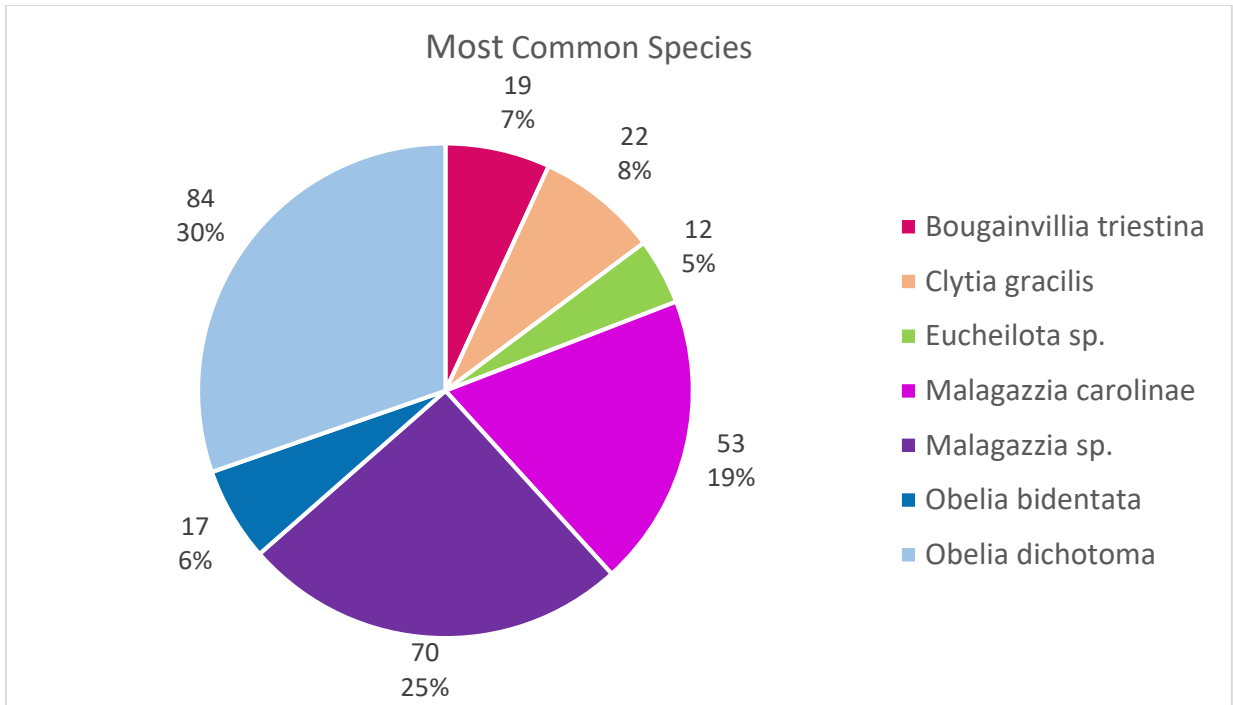


Figure 7. Percentages of the most common Hydromedusa species collected from October to February.

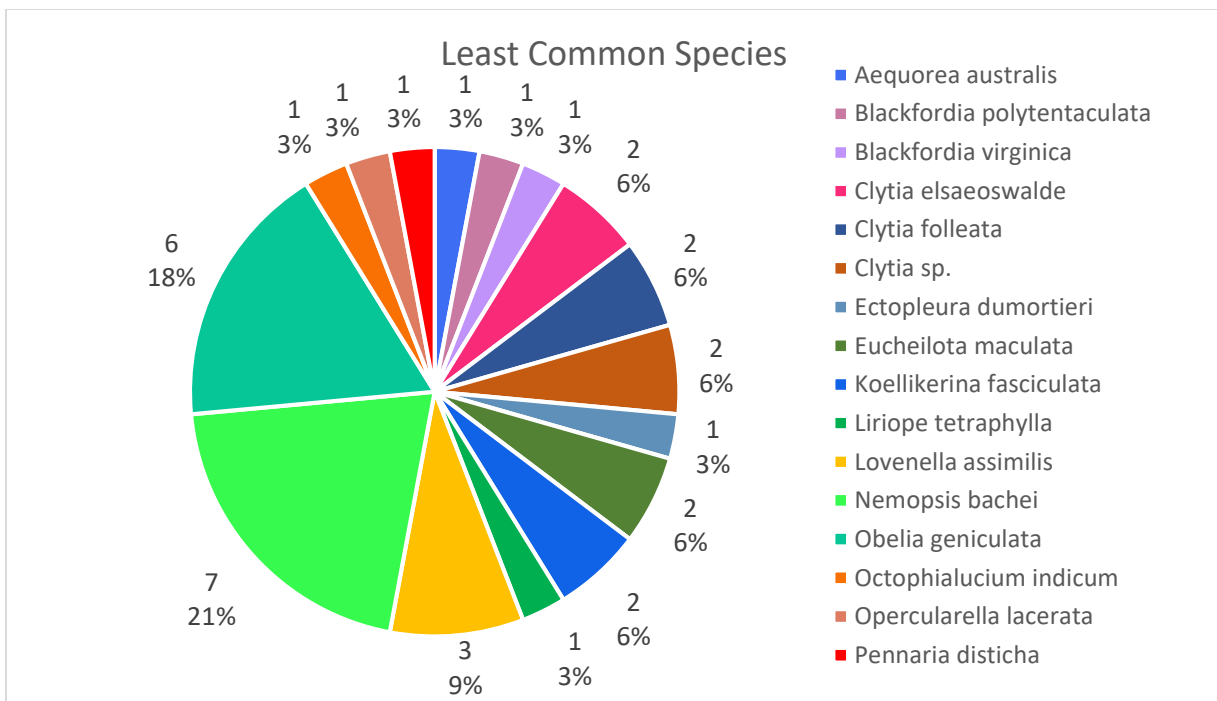


Figure 8. Percentages of the least common Hydromedusa species collected from October to February.

SECTION IV

CONCLUSION

Effects of Temperature/Salinity on Abundance

In this investigation, statistical analysis was performed to determine the correlation of both water temperature and salinity to Hydromedusae abundance within the sampling period. It was found that there was not a significant correlation between medusa abundance and water temperature (0.42) and salinity (0.02). The overall number of Hydromedusae collected in the Fall (543) was much greater than the total number collected in the Winter (207).

Seasonality of Species

The species collected during the sampling period differed greatly between the Fall and Winter months, with more species collected in October and November than December to February. This indicates that different factors trigger medusa production by the polyps in different species, with some species reproducing during the cold season and some during the warm season. The most commonly collected species during the sampling period were *Obelia dichotoma* and *Malagazzia* sp.. As one of the most abundant species of Hydrozoans, *Obelia dichotoma* has a wide geographic range that spans most of the world's oceans, and its polyp stage inhabits a variety of solid surfaces (Orejas *et al.*, 2013). Although *Malagazzia* sp. was only collected in October and November, it still comprised a large portion of the total species abundance. *Malagazzia* sp. is found in the Atlantic Ocean, Indian Ocean, and Pacific Oceans, most commonly found in the Indo-Pacific region (OBIS, 2017).

Possible Sources of Error

Some of the individual medusae collected and for which the DNA was extracted did not yield good quality DNA and the 16S gene could not be amplified. For some of the amplified DNA, sequencing did not work. This means that although 750 Hydromedusae samples were collected, we have a complete sequence for only about 500. The sequencing failure rate could have impacted the species diversity, as some species may have been more prevalent than is actually represented in this data but were not included in the diversity results because they could not be identified.

Future Research

This investigation is only a portion of a larger effort to study jellyfish and their blooms in the Galveston Bay. Environmental factors analyzed in this study are temperature and salinity. However, a wider variety of environmental factors may be involved in triggering medusa blooms. Parameters that will be analyzed in the future are: primary productivity levels, dissolved oxygen percentage, and overall weather patterns. Further statistical analysis will also be performed to test for possible correlation between bloom frequency and intensity and the environment. Species identification will also be re-evaluated using phylogenetic methods and analysis of morphological traits on pictures taken on live animals of each species.

Because this research is an extension of a study that has been performed previously, it could prove valuable to associate the current results to results from years past to note any common trends in Hydromedusae abundance and diversity. Comparing aspects such as the overall abundance, number and frequency of blooms, and species richness could help determine

whether or not these values seem to be changing over time. Since literature concerning Hydromedusae is so limited (Miglietta *et al.*, 2008), creating a database specifically for the observed Hydromedusa species in the Gulf of Mexico could be a beneficial method for tracking the diversity and seasonality of these species over time. Environmental information could supplement the Hydromedusa data in illustrating the fluctuations of blooms, and help build a model for predicting bloom occurrence. If the dynamics between Hydromedusae and the environment are successfully monitored over an extended period, valuable information about the effects of factors such as climate change could be better understood.

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APPENDIX

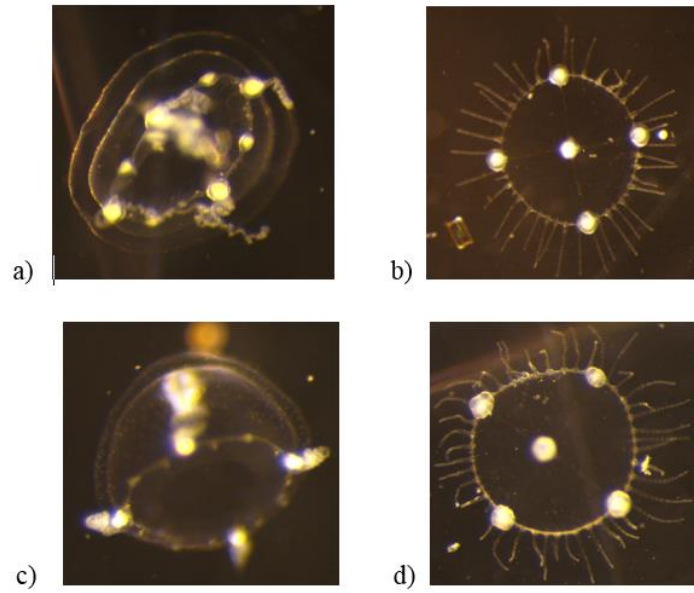


Figure 9. Most common species collected during the month of October. **a)** *Malagazzia carolinae* (10/14/16); **b)** *Obelia dichotoma* (10/14/16); **c)** *Malagazzia* sp. (10/24/16); **d)** *Obelia bidentata* (10/21/16).

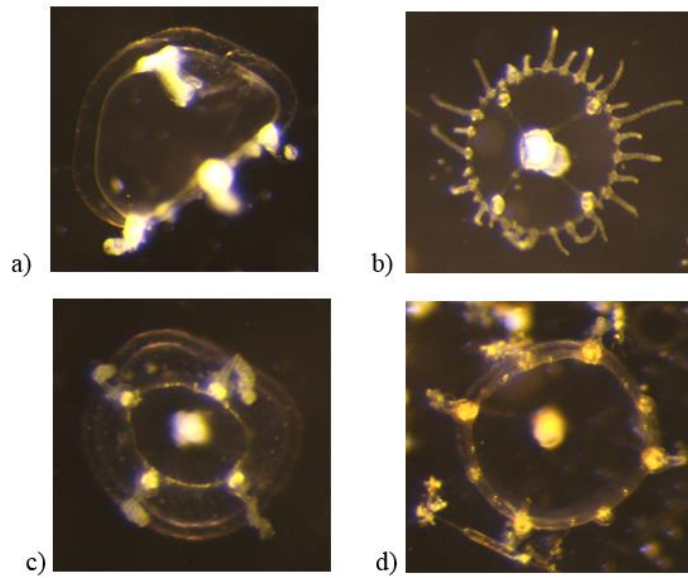


Figure 10. Most common species collected during the month of November: **a)** *Malagazzia* sp. (11/4/16); **b)** *Obelia dichotoma* (11/4/16); **c)** *Malagazzia carolinae* (11/9/16); **d)** *Clytia gracilis* (11/2/16).

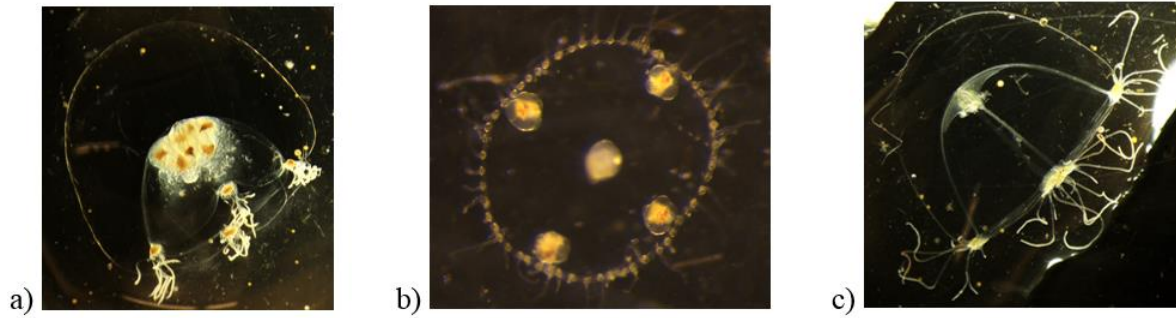


Figure 11. Species collected during the month of December: **a)** *Bougainvillia triestina* (12/5/16); **b)** *Obelia dichotoma* (12/2/16); **c)** *Nemopsis bachei* (12/5/16).

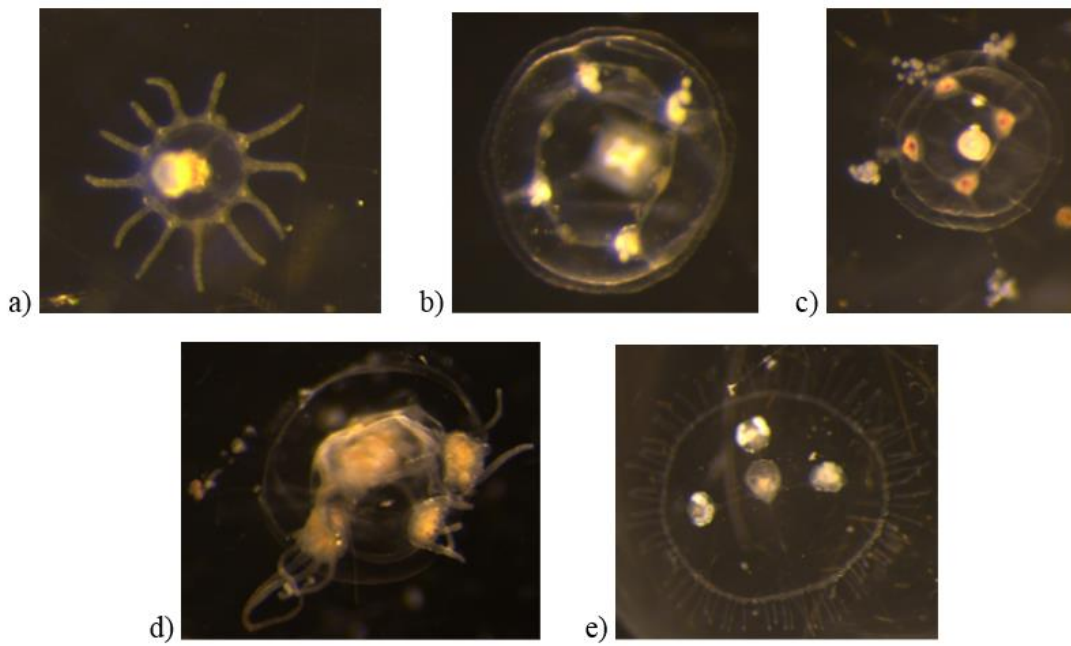


Figure 12. Species collected during the month of January: **a)** *Obelia dichotoma* (1/18/17); **b)** *Blackfordia virginica* (1/20/17); **c)** *Ectopleura dumortieri* (1/18/17); **d)** *Nemopsis bachei* (1/27/17); **e)** *Obelia genculata* (1/30/17).

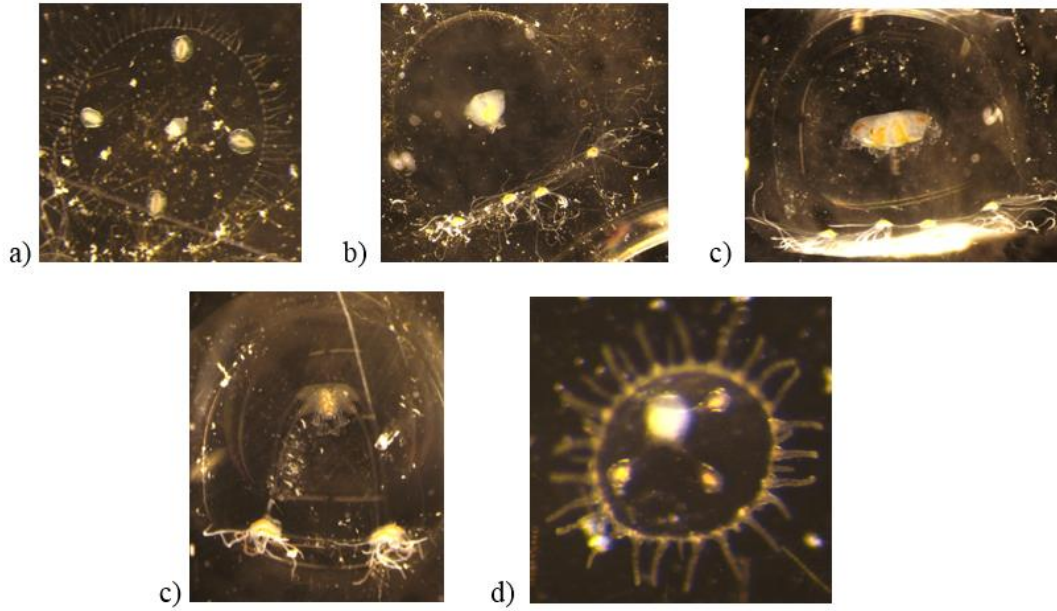


Figure 13. Species collected in the month of February: **a)** *Obelia dichotoma* (2/3/17); **b)** *Bougainvillia treistina* (2/3/17); **c)** *Koellikerina fasciculata* (2/3/17); **d)** *Nemopsis bachei* (2/3/17); **e)** *Obelia geniculata* (2/3/17).

Table 1. BLAST results, including sample number, species identified, percent identity, percent query cover, and E-value

Sample Name	Species Name	ID %	Query Cover %	E-value
10.03.16.10	Malagazzia sp.	96.50%	86.99%	0
10.03.16.17	Malagazzia carolinae	96.4	87.54	0
10.03.16.18	Clytia gracilis	93.1	97.59	0
10.03.16.21	Malagazzia sp.	96.5	85.74	0
10.03.16.22	Malagazzia sp.	96.5	85.6	0
10.05.16.4	Lovenella assimilis	91.7	97.75	0
10.05.16.6	Obelia dichotoma	99.8	100	0
10.05.16.7	Obelia dichotoma	99.8	98.07	0
10.05.16.8	Lovenella assimilis	91.8	97.32	0
10.05.16.9	Obelia dichotoma	99.8	98.07	0
10.05.16.10	Clytia gracilis	93.5	95.75	0
10.05.16.11	Obelia dichotoma	99.5	99.02	0
10.05.16.12	Obelia dichotoma	99.8	100	0
10.07.16.3	Clytia gracilis	93.5	100	0
10.07.16.4	Malagazzia carolinae	96.5	86.15	0
10.07.16.5	Malagazzia carolinae	96.5	86.01	0
10.07.16.6	Clytia sp.	99.8	94.11	0
10.07.16.7	Obelia dichotoma	99.5	98.24	0
10.07.16.8	Obelia bidentata	99.1	100	0

10.07.16.11	<i>Obelia dichotoma</i>	99.6	100	0
10.07.16.12	<i>Obelia dichotoma</i>	99.8	100	0
10.07.16.14	<i>Malagazzia carolinae</i>	96.2	86.61	0
10.07.16.18	<i>Malagazzia carolinae</i>	96.3	85.84	0
10.07.16.19	<i>Malagazzia carolinae</i>	96.5	85.33	0
10.07.16.20	<i>Malagazzia carolinae</i>	96.2	86.56	0
10.10.16.1	<i>Malagazzia carolinae</i>	97	82.66	0
10.10.16.2	<i>Malagazzia carolinae</i>	96.3	84.56	0
10.10.16.3	<i>Malagazzia carolinae</i>	96.3	86.59	0
10.10.16.5	<i>Bowerbankia</i> sp.	87.8	98.91	1.01E-146
10.10.16.6	<i>Malagazzia carolinae</i>	96.5	86.01	0
10.10.16.7	<i>Obelia dichotoma</i>	99.8	98.39	0
10.10.16.8	<i>Malagazzia carolinae</i>	96.5	85.46	0
10.10.16.9	<i>Malagazzia carolinae</i>	95.9	86.77	0
10.10.16.13	<i>Malagazzia carolinae</i>	96	86.89	0
10.10.16.14	<i>Clytia gracilis</i>	93	100	0
10.10.16.16	<i>Obelia dichotoma</i>	99.7	98.23	0
10.10.16.21	<i>Lovenella assimilis</i>	91.7	99.47	0
10.10.16.22	<i>Pennaria disticha</i>	100	96.06	0
10.12.16.5	<i>Malagazzia carolinae</i>	96.5	85.46	0
10.12.16.6	<i>Malagazzia carolinae</i>	95.9	85.81	0
10.12.16.7	<i>Obelia dichotoma</i>	100	91.37	0
10.12.16.8	<i>Obelia dichotoma</i>	100	100	0
10.12.16.9	<i>Obelia bidentata</i>	99.3	100	0
10.12.16.10	<i>Malagazzia carolinae</i>	96.3	84.35	0
10.12.16.11	<i>Malagazzia carolinae</i>	96.5	86.15	0
10.12.16.12	<i>Malagazzia carolinae</i>	96.3	85.69	0
10.12.16.13	<i>Malagazzia carolinae</i>	96.2	86.74	0
10.12.16.15	<i>Obelia dichotoma</i>	100	100	0
10.12.16.17	<i>Obelia dichotoma</i>	93.2	100	0
10.12.16.18	<i>Malagazzia carolinae</i>	96.2	86.28	0
10.12.16.19	<i>Obelia dichotoma</i>	99.8	98.55	0
10.12.16.20	<i>Malagazzia carolinae</i>	96.2	86.71	0
10.12.16.21	<i>Clytia gracilis</i>	92.7	98.05	0
10.12.16.22	<i>Malagazzia carolinae</i>	96.1	86.25	0
10.14.16.1	<i>Clytis folleata</i>	97.9	92.23	0
10.14.16.2	<i>Malagazzia carolinae</i>	96.3	85.84	0
10.14.16.3	<i>Malagazzia carolinae</i>	96.3	84.06	0
10.14.16.4	<i>Clytia</i> sp.	90.2	95.23	0
10.14.16.5	<i>Malagazzia carolinae</i>	96.4	92.72	0
10.14.16.8	<i>Malagazzia carolinae</i>	96.3	92.21	0
10.14.16.9	<i>Malagazzia carolinae</i>	96.1	92.83	0
10.14.16.10	<i>Malagazzia carolinae</i>	96.3	86.04	0
10.14.16.12	<i>Malagazzia carolinae</i>	96.2	92.72	0
10.14.16.15	<i>Clytia gracilis</i>	90.1	99.47	0
10.14.16.16	<i>Clytis folleata</i>	98.5	99.09	0
10.14.16.18	<i>Clytia gracilis</i>	92.8	97.88	0

10.14.16.20	<i>Obelia dichotoma</i>	99.6	100	0
10.14.16.23	<i>Clytia gracilis</i>	92.9	99.82	0
10.14.16.25	<i>Malagazzia carolinae</i>	96.5	93.04	0
10.14.16.34	<i>Obelia dichotoma</i>	99.8	100	0
10.17.16.1	<i>Malagazzia sp.</i>	96.1	85.39	0
10.17.16.4	<i>Malagazzia carolinae</i>	96.2	94.3	0
10.17.16.5	<i>Eucheilota sp.</i>	92.7	98.09	0
10.17.16.8	<i>Clytia gracilis</i>	93.4	99.27	0
10.17.16.9	<i>Clytia gracilis</i>	92.5	100	0
10.17.16.11	<i>Malagazzia sp.</i>	96.3	92.19	0
10.17.16.12	<i>Malagazzia carolinae</i>	96	93.95	0
10.17.16.15	<i>Aequorea australis</i>	99.8	94.73	0
10.17.16.16	<i>Malagazzia carolinae</i>	96	92.27	0
10.17.16.18	<i>Malagazzia sp.</i>	96.1	84.55	0
10.17.16.19	<i>Malagazzia carolinae</i>	96.2	92.21	0
10.17.16.21	<i>Malagazzia carolinae</i>	96.4	94.49	0
10.17.16.29	<i>Malagazzia sp.</i>	96.8	85.48	0
10.19.16.1	<i>Clytia gracilis</i>	99.8	100	0
10.19.16.2	<i>Obelia dichotoma</i>	99.6	100	0
10.19.16.5	<i>Clytia gracilis</i>	99.6	98.76	0
10.19.16.7	<i>Clytia gracilis</i>	98.4	99.11	0
10.19.16.8	<i>Obelia dichotoma</i>	99.8	100	0
10.19.16.11	<i>Obelia dichotoma</i>	100	99.61	0
10.19.16.12	<i>Malagazzia sp.</i>	96.3	85.69	0
10.19.16.14	<i>Obelia dichotoma</i>	99.8	100	0
10.19.16.18	<i>Malagazzia carolinae</i>	96.2	94.34	0
10.19.16.19	<i>Malagazzia carolinae</i>	96.4	92.55	0
10.21.16.1	<i>Eucheilota maculata</i>	89.9	97.17	0
10.21.16.2	<i>Malagazzia sp.</i>	96.1	86.76	0
10.21.16.4	<i>Obelia bidentata</i>	99.3	100	0
10.21.16.9	<i>Obelia bidentata</i>	99.3	100	0
10.21.16.11	<i>Eucheilota sp.</i>	91.8	100	0
10.21.16.12	<i>Eucheilota sp.</i>	91.4	100	0
10.21.16.13	<i>Obelia bidentata</i>	99.7	100	0
10.21.16.14	<i>Obelia dichotoma</i>	98.9	100	0
10.21.16.15	<i>Malagazzia sp.</i>	96.3	92.21	0
10.21.16.16	<i>Obelia dichotoma</i>	100	100	0
10.21.16.17	<i>Obelia dichotoma</i>	100	100	0
10.21.16.18	<i>Obelia bidentata</i>	99	100	0
10.21.16.20	<i>Obelia dichotoma</i>	100	100	0
10.21.16.21	<i>Obelia bidentata</i>	99.3	100	0
10.21.16.22	<i>Obelia dichotoma</i>	99.7	100	0
10.21.16.23	<i>Obelia dichotoma</i>	97.9	100	0
10.21.16.24	<i>Eucheilota sp.</i>	92.9	97.92	0
10.21.16.25	<i>Eucheilota sp.</i>	91.4	100	0
10.21.16.26	<i>Obelia dichotoma</i>	99.8	100	0
10.21.16.27	<i>Eucheilota sp.</i>	91.4	100	0

10.21.16.28	Malagazzia sp.	96.3	85.84	0
10.21.16.29	Malagazzia sp.	96.3	86.19	0
10.21.16.30	Malagazzia carolinae	96.4	94.33	0
10.21.16.31	Malagazzia carolinae	96.3	92.03	0
10.21.16.33	Obelia dichotoma	100	100	0
10.21.16.34	Obelia bidentata	99.3	100	0
10.21.16.35	Obelia bidentata	99.2	100	0
10.21.16.36	Obelia bidentata	99.3	100	0
10.21.16.38	Obelia bidentata	99.3	100	0
10.21.16.39	Obelia dichotoma	99.8	100	0
10.21.16.40	Obelia geniculata	95.4	99.65	0
10.21.16.41	Obelia bidentata	99.3	100	0
10.21.16.42	Obelia bidentata	99.3	100	0
10.21.16.43	Malagazzia sp.	96.1	85.84	0
10.21.16.44	Obelia dichotoma	100	100	0
10.21.16.45	Obelia bidentata	99.3	100	0
10.21.16.46	Obelia bidentata	99.3	100	0
10.21.16.48	Obelia geniculata	95.1	100	0
10.21.16.49	Obelia bidentata	99.3	100	0
10.24.16.1	Malagazzia sp.	96.3	85.84	0
10.24.16.2	Malagazzia sp.	96.3	85.99	0
10.24.16.4	Malagazzia sp.	96.3	86.19	0
10.24.16.5	Malagazzia sp.	96.3	84.35	0
10.24.16.7	Malagazzia sp.	96.3	85.84	0
10.24.16.8	Malagazzia sp.	96.3	86.5	0
10.24.16.13	Malagazzia sp.	96.3	85.84	0
10.24.16.18	Malagazzia sp.	96.3	85.84	0
10.26.16.2	Malagazzia carolinae	96.3	98.35	0
10.26.16.5	Obelia dichotoma	99.8	100	0
10.26.16.11	Malagazzia sp.	96.3	86.19	0
10.26.16.12	Clytia elsaeoswaldae	96.5	95.76	0
10.26.16.14	Malagazzia sp.	96.3	85.84	0
10.26.16.15	Clytia sp.	100	94.41	0
10.26.16.16	Obelia dichotoma	99.8	100	0
10.26.16.19	Malagazzia sp.	96.3	85.99	0
10.26.16.20	Malagazzia sp.	96.3	86.19	0
10.26.16.21	Malagazzia sp.	96.3	86.15	0
10.26.16.22	Malagazzia sp.	96.7	83.3	0
10.26.16.26	Obelia dichotoma	100	100	0
10.31.16.2	Malagazzia sp.	96.3	85.84	0
10.31.16.3	Obelia dichotoma	99.8	100	0
10.31.16.4	Malagazzia sp.	96.3	86.63	0
10.31.16.5	Obelia dichotoma	99.8	100	0
10.31.16.6	Eucheilota sp.	92.6	97.92	0
10.31.16.7	Malagazzia sp.	96.3	86.3	0
10.31.16.9	Malagazzia sp.	96.1	85.69	0
10.31.16.11	Clytia gracilis	100	99.82	0

10.31.16.12	Malagazzia sp.	96.3	86.15	0
10.31.16.16	Malagazzia sp.	96.3	86.3	0
10.31.16.17	Clytia gracilis	93.6	97.67	0
10.31.16.18	Obelia dichotoma	99.1	100	0
11.2.16.2	Eucheilota maculata	90.1	98.22	0
11.2.16.3	Malagazzia sp.	96.3	86.45	0
11.2.16.5	Clytia gracilis	98.4	99.82	0
11.2.16.6	Malagazzia sp.	96.3	86.45	0
11.2.16.8	Opercularella lacerata	93.2	93.42	0
11.2.16.9	Malagazzia sp.	96.3	86.19	0
11.2.16.20	Clytia gracilis	93.1	100	0
11.2.16.22	Bougainvillia triestina	89	98.95	0
11.2.16.23	Clytia gracilis	93.1	98.53	0
11.2.16.24	Clytia gracilis	98.4	100	0
11.2.16.25	Obelia dichotoma	99.8	100	0
11.4.16.1	Malagazzia sp.	96.1	85.84	0
11.4.16.2	Malagazzia sp.	96.3	86.19	0
11.4.16.4	Malagazzia sp.	97	83.48	0
11.4.16.7	Malagazzia sp.	96.3	85.99	0
11.4.16.10	Malagazzia sp.	96.3	85.84	0
11.4.16.12	Obelia dichotoma	99.8	100	0
11.4.16.13	Obelia dichotoma	99.8	100	0
11.4.16.18	Obelia dichotoma	99.1	100	0
11.7.16.1	Clytia folleata	99.8	88.28	0
11.7.16.3	Malagazzia sp.	96.3	85.99	0
11.7.16.5	Octophialucium indicum	94.6	97.2	0
11.7.16.6	Malagazzia sp.	95.9	83.74	0
11.7.16.8	Clytia elsaeoswaldae	96.5	95.91	0
11.7.16.9	Malagazzia sp.	96.3	85.39	0
11.7.16.10	Malagazzia sp.	96.3	85.39	0
11.7.16.11	Clytia gracilis	90.9	100	0
11.7.16.14	Obelia dichotoma	99.8	100	0
11.7.16.16	Obelia dichotoma	99.8	100	0
11.7.16.18	Malagazzia sp.	96.3	84.2	0
11.7.16.20	Clytia gracilis	99.6	100	0
11.7.16.21	Malagazzia carolinae	96.3	95.28	0
11.9.16.1	Bougainvillia triestina	89.1	99.65	0
11.9.16.3	Malagazzia sp.	96.3	85.69	0
11.9.16.5	Malagazzia sp.	96.3	85.69	0
11.9.16.9	Eucheilota sp.	92.7	98.95	0
11.9.16.10	Malagazzia carolinae	96.4	94.17	0
11.9.16.11	Malagazzia carolinae	96.5	93.21	0
11.9.16.19	Eucheilota sp.	92.7	97.92	0
11.9.16.22	Malagazzia sp.	96.3	85.69	0
11.9.16.24	Malagazzia sp.	96.3	85.09	0
11.9.16.26	Malagazzia sp.	96.3	85.39	0
11.9.16.28	Malagazzia sp.	96.3	85.69	0

11.9.16.31	<i>Euceilota</i> sp.	92.7	98.08	0
11.9.16.32	<i>Euceilota</i> sp.	92.7	97.91	0
11.9.16.34	<i>Malagazzia</i> sp.	94.9	84.55	0
11.9.16.37	<i>Malagazzia carolinae</i>	96.4	97.44	0
11.9.16.39	<i>Malagazzia carolinae</i>	96.3	93.81	0
11.9.16.40	<i>Malagazzia</i> sp.	96.7	83.83	0
11.9.16.43	<i>Malagazzia</i> sp.	97	83.04	0
11.9.16.45	<i>Malagazzia</i> sp.	96.3	84.35	0
11.9.16.46	<i>Malagazzia</i> sp.	96.3	84.2	0
11.9.16.48	<i>Malagazzia</i> sp.	96.3	84.49	0
11.9.16.51	<i>Malagazzia carolinae</i>	96.5	94.19	0
11.11.16.1	<i>Malagazzia carolinae</i>	96.5	94.19	0
11.11.16.3	<i>Malagazzia</i> sp.	96.3	84.35	0
11.11.16.4	<i>Malagazzia</i> sp.	96	83.71	0
11.11.16.6	<i>Malagazzia</i> sp.	96.3	84.35	0
11.11.16.7	<i>Malagazzia</i> sp.	96.3	84.84	0
11.11.16.8	<i>Malagazzia</i> sp.	96.3	84.35	0
11.11.16.9	<i>Malagazzia</i> sp.	96.3	85.69	0
11.11.16.10	<i>Malagazzia</i> sp.	96.3	84.2	0
11.11.16.13	<i>Malagazzia carolinae</i>	96.6	93.84	0
11.11.16.19	<i>Blackfordia polytentaculata</i>	99.4	85.51	0
11.11.16.20	<i>Malagazzia</i> sp.	95.9	85.84	0
11.11.16.21	<i>Malagazzia</i> sp.	96.3	85.14	0
11.14.16.1	<i>Malagazzia</i> sp.	96.3	84.35	0
11.14.16.2	<i>Malagazzia</i> sp.	96.7	83.83	0
11.14.16.3	<i>Malagazzia carolinae</i>	96.5	94.02	0
11.14.16.4	<i>Malagazzia carolinae</i>	96.5	92.88	0
11.14.16.5	<i>Malagazzia carolinae</i>	96.6	94.17	0
11.14.16.7	<i>Malagazzia</i> sp.	96.3	84.49	0
11.14.16.10	<i>Malagazzia carolinae</i>	96.2	97.44	0
11.16.16.1	<i>Obelia dichotoma</i>	99.8	100	0
11.16.16.2	<i>Liriope tetraphylla</i>	96.9	98.11	0
11.16.16.3	<i>Bougainvillia triestina</i>	89.1	99.31	0
11.16.16.7	<i>Obelia dichotoma</i>	99.7	100	0
11.21.16.3	<i>Euceilota</i> sp.	92.8	100	0
11.21.16.9	<i>Obelia dichotoma</i>	100	100	0
11.21.16.10	<i>Obelia dichotoma</i>	99.8	100	0
11.21.16.15	<i>Obelia dichotoma</i>	98.5	100	0
11.21.16.20	<i>Ectopleura dumortieri</i>	96.3	99.12	0
11.21.16.25	<i>Obelia dichotoma</i>	98.4	100	0
11.21.16.26	<i>Obelia dichotoma</i>	98.6	100	0
11.21.16.32	<i>Obelia dichotoma</i>	99.5	100	0
11.21.16.35	<i>Obelia dichotoma</i>	98.4	100	0
11.21.16.40	<i>Obelia dichotoma</i>	100	100	0
11.30.16.2	<i>Obelia geniculata</i>	95.2	99.64	0
11.30.16.3	<i>Obelia geniculata</i>	95.2	100	0
11.30.16.5	<i>Bougainvillia triestina</i>	88.9	99.13	0

12.2.16.1	Obelia dichotoma	99.8	100	0
12.2.16.2	Obelia dichotoma	99.5	100	0
12.2.16.3	Obelia dichotoma	99.8	100	0
12.2.16.4	Obelia dichotoma	99.8	100	0
12.2.16.7	Obelia dichotoma	99.7	100	0
12.2.16.10	Bougainvillia triestina	89.1	99.12	0
12.5.16.1	Bougainvillia triestina	88.9	99.47	0
12.5.16.3	Bougainvillia triestina	88.9	100	0
12.5.16.4	Bougainvillia triestina	89.1	98.8	0
12.5.16.5	Nemopsis bachei	99.2	85.54	0
12.5.16.6	Bougainvillia triestina	89	99.13	0
12.5.16.8	Bougainvillia triestina	88.9	100	0
1.18.17.4	Ectopleura dumortieri	93.8	100	0
1.18.17.8	Obelia dichotoma	99	100	0
1.18.17.11	Obelia dichotoma	98.8	100	0
1.18.17.15	Obelia dichotoma	98.9	100	0
1.18.17.17	Obelia dichotoma	98.8	100	0
1.18.17.20	Obelia dichotoma	99.3	100	0
1.18.17.22	Obelia dichotoma	99.1	99.29	0
1.20.17.8	Blackfordia virginica	99.4	100	0
1.27.17.7	Obelia dichotoma	99	100	0
1.27.17.10	Obelia dichotoma	99.5	100	0
1.27.17.11	Obelia dichotoma	98.1	100	0
1.27.17.24	Nemopsis bachei	99.6	85.53	0
1.30.17.12	Obelia geniculata	94.2	100	0
2.3.17.1	Bougainvillia triestina	88.8	99.12	0
2.3.17.2	Nemopsis bachei	88.1	100	0
2.3.17.3	Obelia dichotoma	99.8	100	0
2.3.17.4	Obelia dichotoma	99.8	100	0
2.3.17.5	Nemopsis bachei	90.6	85.11	2.05E-178
2.3.17.7	Bougainvillia triestina	89.3	99.12	0
2.3.17.8	Obelia dichotoma	99.1	100	0
2.3.17.9	Obelia dichotoma	100	100	0
2.3.17.11	Bougainvillia triestina	89	99.12	0
2.3.17.12	Nemopsis bachei	99.6	85.61	0
2.3.17.13	Bougainvillia triestina	88.9	99.12	0
2.3.17.14	Bougainvillia triestina	88.9	99.82	0
2.3.17.15	Bougainvillia triestina	88.9	99.12	0
2.3.17.17	Koellikerina fasciculata	89.5	96.83	0
2.3.17.18	Bougainvillia triestina	88.9	99.12	0
2.3.17.19	Koellikerina fasciculata	89.4	97	0
2.3.17.20	Nemopsis bachei	91.1	83.92	0
2.3.17.21	Bougainvillia triestina	88.9	99.13	0
2.3.17.22	Obelia dichotoma	99.8	100	0
2.3.17.23	Obelia dichotoma	98.6	100	0
2.3.17.24	Bougainvillia triestina	88.9	99.12	0
2.3.17.25	Nemopsis bachei	99.4	86.07	0

2.3.17.27	Obelia dichotoma	100	100	0
2.3.17.29	Obelia dichotoma	99.1	100	0
2.3.17.30	Obelia dichotoma	99.6	100	0
2.3.17.31	Obelia dichotoma	99.8	100	0
2.3.17.32	Obelia dichotoma	99.6	100	0
2.3.17.33	Obelia dichotoma	98.6	100	0
2.3.17.34	Obelia dichotoma	99.8	100	0
2.3.17.35	Obelia dichotoma	99.8	100	0
2.3.17.36	Obelia geniculata	95.2	99.82	0
2.3.17.37	Obelia dichotoma	99.8	100	0
2.3.17.38	Obelia dichotoma	99.8	100	0
2.3.17.39	Obelia dichotoma	99.8	100	0
2.3.17.40	Obelia dichotoma	99.8	100	0
2.6.17.5	Ectopleura dumortieri	94.9	100	0