NaGISA Informational Packet

2014 NaGISA General All-Purpose Information

History

Natural Geography In-Shore Areas (NaGISA) is a global marine diversity study of the world's coastline. The NaGISA project is one of fourteen current field projects of the Census of Marine Life (CoML). It is a collaborative effort aimed at inventorying and monitoring the biodiversity in the narrow inshore zone of the world's oceans at depths of less than 20 meters, which is the area people know best and impact most. This is reflected in the Japanese word NaGISA, referring to the narrow coastal zone where land meets the sea.

NaGISA holds a unique position in the Census of Marine Life as an ambassador project, linking CoML to local interests. It is an exercise in international cooperation and capacity building. NaGISA will complete an equatorial longitudinal gradient from the east coast of Africa to the Palmyra Atoll and a pole-to-pole latitudinal transect from the northern coast of Alaska to Antarctica's McMurdo Sound. NaGISA employs a simple, cost-efficient and intentionally low-tech sampling protocol that can be adopted by many research groups and countries, and encourage local community involvement. The ultimate goal is a series of well-distributed standard transects from the high intertidal zone to 20 meters water depth around the world, which can be repeated over a 50-year or even greater time frame.

Professor Yoshihisa Shirayama of Kyoto University established this program in the year 2000. Our high school, Niceville Senior High School, joined NaGISA in 2003. Our involvement began when the Teacher of the Gifted at NHS, Mr. Richard Hernandez, traveled to Japan for the Japan Fulbright Memorial Fund Master Teacher program. Since then, our teams and our coffers have grown, but we are far from being able to operate at maximum efficiency due to insufficient funds and a few taxonomic keys. We hope to continue our collaboration with other Florida communities, and communities worldwide.

The history of the NaGISA project in Northwest Florida starts in the summer of 2003. Having been involved in the Fulbright Memorial Fund (FMF) Teacher Program (now the Japan Fulbright Memorial Fund), Richard A. Hernandez, the Teacher of the Gifted from Niceville High School, applied for and was accepted by the FMF Master Teacher Program. This is a program that establishes a collaborative relationship between schools in Japan and counterpart schools in the United States. Niceville High School was paired with the Wakayama Prefectural Tanabe Commercial High School. During the summer, the United States traveling teacher visited and worked in the Japanese school for four weeks.

It was during this timeframe, in July 2003, that Mr. Hernandez was taken on a tour of the area surrounding the City of Tanabe. One stop was at the Seto Marine Biological Research Laboratory of Kyoto University. He was given a thorough tour of this facility by the director, Dr. Yoshihisa Shirayama. Upon the conclusion of the tour, Dr. Shirayama, Mr. Hernandez, and Takamasa Yamamoto, the Vice-Principal of the Tanabe Commercial High School, discussed the NaGISA project. Mr. Hernandez, however, had first heard of this project in 2000. It was during this discussion that Dr. Shirayama invited Niceville High School to participate in the project. The NaGISA program, Mr. Hernandez learned, was under the CoML group (Census of Marine Life). While CoML was a census that only lasted ten years, the NaGISA program was scheduled to last for 50 years, starting in 2000. The invitation was based on the fact that the project was on such a massive scale; it would be very difficult for universities alone to adequately survey the entire coast line of the planet. Participation by high schools would also permit students to have an opportunity to not only be involved in an extremely worthwhile project but also be exposed to real world science and collection techniques so they might see some of the practical applications of the concepts, which until then had only been theories and lectures in the classroom. Additionally, the Gulf Coast was not represented in NaGISA at this time, and the primary purpose of NaGISA is education. On behalf of Niceville High School, Mr. Hernandez accepted the offer.

As a result, NaGISA NW Florida became a test case to see if a high school would have the ability to interpret the NaGISA protocol, amass the expertise and equipment necessary to do a collection and analysis, and train the students sufficiently to ensure everything was accomplished at a standard that was acceptable at the international science research level. In July 2004, the FMF again provided assistance by creating a special program in which high school students and teacher from the United States exchanged with students and teachers from a high school in Japan. Niceville High School and Tanabe Commercial High School were chosen for this pilot project of the Fulbright Memorial Fund Collaborative School Science Network (FMF CSSN). The special collaborative project chosen for this pilot program was NaGISA. Five students and two teachers traveled to the Seto Marine Biological Research Laboratory in Shirayama, Japan where they were given personal instruction by Dr. Shirayama and his staff on collection and analysis techniques associated with the NaGISA project.

In October 2004, five students and two teachers from the Tanabe Commercial High School traveled to work with the students and faculty at Niceville High School. During that visit, they participated in a NaGISA collection and analysis at the NaGISA NW Florida collection site. From the beginning, Niceville High School has worked very hard to learn everything possible about the NaGISA program and has taken steps to improve collection and analysis procedures with each collection. The program now has expanded to include two more high schools on the north Florida coast.

Originally, there were only two protocols used in the NaGISA collection to obtain specimens, the Sea Grass Protocol and Rocky Shores protocol. Since neither of these applied to our area, we invented our own protocol, the Sandy Beach protocol which is now used world-wide. It has been fully endorsed by the NaGISA program and will continue to be used for years to come. It is a combination of the Sea grass and Rocky Shores, but it has been continually adapted to best fit a sandy beach type environment. After three years of collection and analysis, refinements of technique and training, the results are conclusive that with the right relationship with a sponsoring university, high schools can effectively be a part of the NaGISA project and take their place with university level researchers.

The Niceville High School NaGISA program is completely student run. Generally, 5 gifted students opt to become NaGISA Externs. Normally, these 5 students are in their senior year; however in some years there are 4 senior NaGISA Externs and 1 NaGISA Extern in the junior class. We allow one junior to become a NaGISA extern so he or she can learn the system and lead the other NaGISA Externs in the following year. It helps tremendously to have a student who has previous experience in running the program instead of 5 completely new Externs. The externs take care of all NaGISA related business, including public presentations and collection preparations. The externs are constantly working on preparing for the next collection, but intense preparations do not begin until 5 weeks before the collection date.

Niceville High School’s NaGISA Trips (in chronological order)

All information should be reviewed on the Niceville High School NaGISA Website:

[*http://nagisa.gknu.com*](http://nagisa.gknu.com)

|  |  |
| --- | --- |
| Japan - 2003 Kyoto University | See the above information concerning the beginning of Niceville High School’s NaGISA Program. |
| Japan - 2004 Kyoto University | Here the Niceville High School and the Tanabe Commercial High School (of Japan) teams are heard instructions from Dr. Yoshihisa Shirayama (the Principal NaGISA Investigator) on what specimens might be encountered in the coming collections.  This was conducted during the 1st Japan Fulbright Memorial Fund Collaborative School Science Network exchange at Kyoto University’s Seto Marine Biological Research Laboratory in July 2004 |
| Japan – 2006 Kobe | The Niceville High School NaGISA team was invited to participate in the 1st NaGISA World Conference in Kobe, Japan in October 2006.  Eight students and seven faculty members traveled to this conference where the students made two poster presentations.   At the conference, the Niceville High School team was welcomed as full participants.  Based on the quality of the presentations made by the students and their ability to answer all questions, the team was invited to send a group to Tanzania to establish a NaGISA High School Initiative and collection site in the area of Kizimkazi. |
| Tanzania - 2007 Kizimkazi | Overall, the trip was a huge success. The Tanzanian students really picked up on NaGISA procedures quickly and were incredibly motivated about all aspects of the project. There is no question that the student to student teaching technique we used was very effective.From the Niceville High School perspective, it was very interesting to see the differences in our shorelines. Our Northwest Florida shore could be referred to as an aquatic desert. We have very little biodiversity on our coast, whereas, comparatively, the shore at Kizimkazi is essentially an aquatic rainforest with seemingly endless amounts of creatures filling the tidal pools.Again, in our view, both sides gained a vast amount of knowledge not only about science, but also about the cultural similarities and differences. Our trip to Tanzania has most definitely helped expand the NaGISA high school initiative, and has created a new sampling site for future NaGISA collections. We have helped instill an interest in science which will permanently impact the lives of the Tanzanian students just as their generosity, enthusiasm, positive outlook on life and selflessness will have a lasting influence on all of us from NHS.As a result of the success of this Tanzania trip, we feel that NaGISA now has a blueprint for further expansion of the NaGISA high school initiative. The students and faculty of Niceville High School stand ready to assist with further expansions in any part of the world. |
| Crete - 2009 Heraklion | Important aspects include:* Visiting and working in Hellenic Center for Marine Research
* Learning how to more accurately identify organisms
* Establishes a high school NaGISA collection site in Crete
 |
| Egypt - 2010  Sharm el-Sheikh | On Tuesday, January 5, 2010, the Niceville High School NaGISA Team began their collection on the Red Sea at Nabq National Park in Sharm el Sheikh, Egypt. We worked with Dr. Knowlton, NaGISA International Program Director, as well as Dr. Kimani, NaGISA Indian Ocean Coordinator, and Dr. Mohammed, a professor at Suez Canal Univeresity, to learn the official sea grass protocol. After learning the protocol, the students were put in charge of teaching the protocol to a select group of students from Sharm el Sheikh College, a local K-12 school. The weather was not perfect, so we chose to send our four strongest divers, Heather Caulkins, Montana Hobbs, Shreyans Patel, and Alexis Reddington, to collect an example sample so the Sharm students could see the analysis process. We also successfully obtained five samples from the 1 meter depth with Dr. Kimani and his colleagues. Due to a lack of supplies, we were only able to sort through the sample and separate all the visible fauna.The second day was heavily concentrated in collecting the dive samples. We sent out all other divers in two different rounds, 7 meters and 15 meters. In the 15 meter dive, Alexis Reddington, Tony Rustin, and Buddy Jackson, were able to bring up five successful samples. The second dive team performed the 7 meter dive. This dive team included Heather Caulkins, Desiree Babin, and TJ Brown. They were able to successfully collect and bring up four samples. The majority of the samples were analyzed, and the organisms found were properly preserved. The specimens included Macrofauna and sea grass.          The third day consisted of additional analysis at the hotel. The samples from a rocky shores collection were taken and analyzed with the assistance of Dr. Kimani and his colleagues. We were successfully able to collect all organisms out of the samples using forceps and to preserve them all. |
| Turkey - 2011  IstanbulTurkey - 2011  Istanbul | **On July 10, 2011,** we traveled across the Bosphorus to get to the Black Sea on the Asian side of Turkey. We traveled by bus with several students from Fisheries High school to the town of Riva and a beach nearby. Along the way, we had a chance to see how popular the beaches are with the people of Istanbul. The town and road leading to the town were packed with cars all waiting in line to get to the beach on a Sunday afternoon. When we arrived at the beach, we met with seven other students from a private high school and the University of Istanbul to complete the beach collection and one meter transect phase. Our team demonstrated the protocol and provided intensive instruction and practical experience for the Turkish students. We showed a power point with English and Turkish translations as well as went through the protocol in a large group and in small groups. After the beach phase, we moved to the one meter transect, and again, provided instruction and practical experience. As a result of the local environment, it was necessary to do both the Sandy Beach and the Rocky Shores Protocols. The collection went perfectly. The students were all able to understand and complete the protocol. **On July 11, 2011,** we split into two groups: Beach Analysis and Dive Collection. The divers went back to the beach to finish their collection, and the beach and 1 meter people went back to the Fisheries High School to start the analysis procedure on the samples from the previous day’s collection. The analysis was successful; however, it was made particularly challenging due to the age of the Fisheries High School’s microscopes. We did classify the organisms down to the proper class.The dive portion was also challenging.  Due to a failure incommunication, it was not made clear how important it was to conduct dive operations from the rented boat.  The dive master and his assistant had planned to do the collection by a shore dive.  This would have been difficult for the five meter depth and impossible for the ten and fifteen meter depths.  After consultation, a solution was reached.  All the equipment and people were rowed out to the boat in a small zodiac boat.  This required a great many trips and great skill on the part of the Turkish lifeguard doing the rowing. Once everyone was on board the boat, it was quite clear that the boat was a little small for dive operations.  At this point there was no other option but to do the best possible.  The dive master and his assistant performed Herculean tasks to get all the gear assembled and ready.  Once this was accomplished, the divers performed the dive protocol starting at fifteen meters.  The collection portion of the dive at this depth and later at the other two depths was tremendous.  All five quadrates were performed correctly and the samples were recovered.  At the five meter depth, the rocky shores protocol was necessary.  At the other depths they used the Sandy Beach protocol.One interesting point…we were told that almost no one dives in the Black Sea because there is not sufficient interest or infrastructure to support such activities.  Consequently, our Niceville High School dive team can claim the fact that they are among elite company in that few have done what they have done! **On July 12, 2011,** we completed the last segment of the NaGISA Analysis.  After a very successful dive, there was a wealth of specimens available to process and analyze.  This time the Turkish students had a chance to become even more familiar with the procedures, and they did a great deal of processing, analyzing, and classifying the specimens. By this time the two teams, Niceville High School and the students from the two Turkish High Schools with the university participants, had bounded into a single cohesive and effective team.  More samples were processed and analyzed than any collection ever at Niceville High School.  This was a testimony to the efficiency of this combined team.  As a result of this effort,there is every chance that a lasting effort to continue thiscollection site on the Black Sea may be pursued in the future. The American students and the Turkish Students exchanged contact information as well so they could continue their friendship across the big pond once the American students returned home to Florida. |
| Costa Rica - 2013 Cahuita | **Our expedition to Costa Rica was an eventful learning experience. During spring break of 2013, our team traveled to Cahuita, Costa Rica in order to extend the NaGISA program to Central America. The Costa Rican students quickly learned the protocol, and together we were able to complete a successful collection and analysis. Because the school we collaborated with did not have many of the necessary supplies, we gave them our equipment so that they could continue to program after we left. In addition to creating a new NaGISA collection site in Costa Rica, students also created life-long friendships with the locals at Cahuita.** |

Structure of NaGISA

NaGISA Extern Positions

 Head Student Coordinator

 Beach Team Coordinator

 Head of Beach

 Head of Analysis

 Dive Team Coordinator

The Niceville High School NaGISA program is completely student run. From organizing collections to management of the program, the NaGISA Externs are the leaders of the NaGISA Team. Generally, 3 “baby” externs from the junior class are chosen in the spring to shadow and train under the current externs of that year. The baby externs are in charge of learning about each of their prospective positions during the spring collection. This is their “training” period when the senior externs pass down any relevant knowledge and information. Once the next school year starts, the new group of externs is assigned main roles in the NaGISA Program. While every extern is extremely important to the program as a whole, they each have their special skills that they are most familiar with. For example, an appropriate extern is designated Head of Analysis due to their experience in the lab and familiarity with the nuances of analysis procedures. Finally the junior extern from the previous spring is selected as the Head Student Coordinator, who is the leader of the program. For this reason, one sophomore is usually selected each spring to become an extern the next fall. By including a junior each year into the NaGISA Externs, a significant amount of knowledge is saved from the previous year and is passed on when the current senior externs graduate.

These past two school years, the NaGISA program has new system of externship positions in place. The program currently consists of 8 externs instead of the usual 5, 3 senior externs, 4 junior externs, and 1 sophomore extern. This change was imposed to give more responsibility to the students. Additional extern roles include a Head of Training, Equipment Manager, Media Specialist, Relations Manager / Analysis Assistant, and a Quality Control / Dive Assistant.s

The chart below provides details about specific positions of the NaGISA Externs:

* Leads the dive collection
* Puts together dive boxes
* Sets up dive team meetings
* Finds dive boats
* Finds dive masters
* Puts together dive teams
* Demonstrates collection protocol
* Monitors weather
and dive conditions
to ensure safety
* Gives safety briefing day of collection
* Leads beach collection
* Puts together collection boxes
* Organizes the beach collection
* Writes up student collection bus list
* Finds teachers to mentor the collection
* Demonstrates collection protocol
to beach team the day of collection
* Responsible for setting up lab prior
to analysis
* Responsible for ordering analysis supplies
* Responsible for keeping microscopes and analysis equipment in
working order
* Responsible for compiling collection data
* Keeps record of all specimens preserved
* Keeps track of all samples
* Analyzes water turbidity, salinity, and dissolved oxygen

NaGISA Sampling Protocol

*for sandy beach coastal areas*

**Shoreline**

1. Cautiously approach your designated site.



*Take care not to disturb it by making any unnecessary footprints in the areas where you will be placing your quadrats and taking samples. Never cross the transect line!*

1. Place the 1x1 meter quadrat (grid) on any random point along the main transect line. Be sure to place the bottom edge of the quadrat along the transect line. Try to avoid allowing too much water wash into the grid. *See diagram.*



*There are two flagged poles on opposite ends of the beach. This line is your main transect line; it stretches 30 meters.*

1. Next, unscrew the lid of your 2cm Sample jar and place it in the lower right hand corner of your 1x1 meter quadrat grid.

1. Send your team’s runner to get the photographer. The photographer will take a picture of the 1x1meter quadrat (grid). Make sure the photographer takes the photograph from directly above the quadrat grid. The grid’s square shape must not be distorted in order to be able to determine the relative size of objects.

*Note: You will not be taking samples or organisms from the 1x1meter quadrat (grid).You will only photograph it.*

1. Place the 50x50cm quadrat adjacent to the upper left corner of the 1x1meter quadrat (grid). *See diagram.*
2. Kneel down in front of the 50x50cm quadrat. Remove any visible organisms on the surface and place them in the container marked with YOUR CORRECT QUADRAT AND TIME and “**Beach 50cm Sample.**”
3. Next, gently sift through the sand with your hands and look for any additional organisms that may be *just below* the surface of the sand. Remove these organisms and place them in the correct container as well.

*You do not need to “dig” into the sand. Simply glide your hands through the surface to uncover any living organisms that may be hidden in the sand. You should not dig, only sift.*

1. Once you are done collecting organisms, your team’s runner should immediately bring the container to the ice cooler. Do not place the sample jars in the cooler. Hand it to the specimen curator, and he or she will place it in the correct place.
2. Place the 15cm Cylinder next to the upper left corner of the 50x50cm quadrat.



1. Next, you need the 2cm Core. Take the 2cm Core and place it next to the upper left of the 15cm Cylinder. *See diagram for details.*
2. Press the 2cm Core down into the sanduntil the sand reaches the black line that is marked on the side.
3. Pull the 2cm Core straight out of the sand.

*Holding your thumb over the hole on the top may help keep the contents from falling out of the 2cm Core. If the contents of the core fall out, try to take another sample in an undisturbed area near your first attempt.*

1. Empty all of the contents from the 2cm Core into the container marked “**Beach (SHORELINE) 2cm Core Sample.**” Your team’s runner should immediately bring the container to the ice cooler.

*If necessary, use the wash bottle filled with salt water to rinse out the insides of the core into the container.*

1. Now, go back to the 15cm Cylinder that you previously placed. Kneel down next to the Cylinder and press it down in the sand. It is easiest to use a combination of pushing down with a twisting-motion.

\*\**Be sure to push the 15cm Cylinder until the sand reaches the black line that is marked on the side of the cylinder. This line marks a depth of 10cm\*\**

1. Next, use the digging tools and your hands to dig away the areas around the 15cm Cylinder. Slide the board or plate underneath the 15cm Cylinder and carefully lift.
2. Send your team’s runner to get the photographer. The photographer will take a picture of the entire 15cm Cylinder and its contents while you are holding it in the air.
3. Once your cylinder has been photographed, place the entire sample into the large bucket.

*If necessary, use the wash bottle filled with salt water to rinse out the insides of the cylinder into the large bucket.*

1. Add approximately 1 liter of water from the clear plastic jug into the bucket containing the 15cm Cylinder Sample. Then, gently swirl around the sand and water with your hands for 10-20 seconds.

*As you swirl the water and sand in the bucket, macro fauna will float to the surface of the water, so be careful not to crush any of the organisms.*

1. Next, you need to sift the 1 liter of water through the 500micron net for any macro fauna organisms. Slowly tip the edge of the bucket over the 500micron net and allow only the water to pour into the 500micron net.

*You may discard the water that pours through the net. You only need to keep the organisms that do not sift through the net.*

1. Repeat steps 18 and 19 for a total of 3 times. Once you have swirled and sifted 3 times, you may discard any sand that remains in the plastic bucket.

*You may have to make multiple runs back to the ocean when you run out of water while swirling and sifting.*

1. Next, find the container marked “**Beach (SHORELINE) 15cm Cylinder Sample.**”



1. Take the wash bottle filled with gulf water. Turn the 500micron net over so that the side that holds the organisms is facing the sampling container. Aim the wash bottle at the side of the net that does not contain organisms. Spray the net until all of the visible organisms have been washed into the sampling container. This process is called “back-rinsing.”

*You may now discard any remaining sand left in the bucket. The only organisms that you need to keep and place in the container are located on the 500micron net.*

1. Once you have back-rinsed all of the organisms into the plastic or glass container, your team’s runner should immediately bring the container to the ice cooler.

NaGISA Laboratory Analysis Procedure

*for all samples found on the shoreline and at the depths
of 1meter, 5meters, 10meters, 15 meters, and 20 meters.*

**Process for analyzing 2cm Core Samples – (micro fauna organisms)**

1. Remove the lid to the container labeled 2cm Core Sample.
2. Add enough gulf water to the container to cover the entire sand sample and approximately 2cm above the sand.
3. Pick up the container and gently swirl the water around. Use a stirring rod to swirl the sand around. Swirl the contents of the container for approximately 10-20 seconds. This will loosen any microscopic organisms free from the sand.
4. Hold the 63micron net over the empty bucket. Slowly tip the edge of the plastic container over the 63micron net and allow only the water to pour into to the net. Do not allow any of the actual sand to fall onto the net, only the water needs to be poured through. *(See diagram)*

*Do not discard the water that pours through the net. This gulf water may
be recycled and used for the next sifting. At the end of this process, you
only need to keep the organisms that do not sift through the 63micron net.*

1. Carefully set aside the 63micron net for the next sift.
2. Repeat steps 2 through 4 for a total of 3 times. (You may re-use the same gulf water from step #2 that is in the large petri dish). During your third swirl and sift, add fresh water to the sample instead of gulf water.

*\*\*Adding fresh water may harm some of the organisms. However, it forces
some types of organisms to release themselves from the sand grains.\*\**

1. Once you have swirled and sifted 3 times, you may discard any sand that remains in the sample container.
2. Take the wash bottle filled with fresh water. Turn the 63micron net over so that the side that holds the organisms is facing the petri dish. Aim the wash bottle at the side of the net that does not contain organisms. Spray the net until all of the visible organisms have been washed into the *medium-sized* petri dish. This process is called “back-rinsing.” *(See diagram)*
3. Using the long pipette, transfer approximately 3-5 mL (1 full pipette) of the sample from the medium-sized petri dish to the smaller petri dish. Place the small petri dish under a dissecting scope for observation.

\*\**Note: 1 full transfer pipette = 3mL – 5mL\*\**

1. Remove any organisms that you can see in the petri dish using the small pipette or inoculating loop. Place these organisms on well slides with only one organism per slide. Then place a cover slip over the well.

*\*\*Some samples may not contain any organisms at all.
However, this is highly uncommon so look carefully.\*\**

1. Once you have isolated a single organism, place the slide under a compound microscope for further observation. Using the compound microscope and the taxonomic guides provided, identify the organism in the slide and record its taxonomic levels on your data sheet. Be sure to input the data into the correctly labeled data sheet in your transect line’s folder.

*\*\*Note: When entering data into the Microsoft Publisher data sheet, you only
need to fill out columns “D” through “J.” In other words, you only need to fill out
the Kingdom, Phylum, Class, Order...etc. columns of the data sheet. If you
know the common name of the organism, please fill out column “C” as well.*

1. Next, record the amount of organisms of this exact type that you found in the sample. Record this data into the data sheet on the computer as well.
2. Once you have identified the organism and counted it, carry it on the well slide to the processing station and place it in your designated area. Using the slide labels, label each slide with Transect Line number, Sample type, Depth, and the furthest taxonomic level that you identified the organism as.

*\*\*Example: Line #2, 2cm Core, 5meters: Order: Osteroidea\*\**

1. Repeat steps 9-13 for a total of three times. For this entire sample, you will need to have removed a total of three full pipettes from the medium sized petri dish. After you have removed a total of this amount, you may discard what is left of the sample.
2. Once you are done collecting data from the sample, bring the empty sample container to the processing station. Rinse the container and place it in the storage box.

**Process for analyzing 15cm samples (macrofauna organisms)**

1. Transfer all of the contents of the 15cm Cylinder Sample container into a medium-sized or large petri dish.
2. Using the long pipette, transfer approximately 3-5mL (1 full pipette) of the sample from the medium or large petri dish to the smaller petri dish. Place the small petri dish on a dissecting scope for observation.

\*\**Note: 1 full transfer pipette = 3mL – 5mL\*\**

1. Remove any organisms that you can see in the petri dish using the small pipette or inoculating loop. Place these organisms on well slides with only one organism per well slide. Place a cover slip over the well of the slide.

*\*\*Some samples may not contain any organisms at all.*

*However, this is highly uncommon so look carefully.\*\**

1. Once you have isolated a single organism, place the slide under a compound microscope for further observation. Using the compound microscope and the taxonomic guides provided, identify the organism in the slide and record its taxonomic levels on your data sheet.
2. Next, record the amount of organisms of this exact type you found in the sample.
3. Once you have identified the organism and counted it, carry it on the well slide to the processing station and place it in your designated area. Using the slide labels, label each slide with Transect Line number, Sample type, Depth, and the furthest taxonomic level that you identified the organism as.

*\*\*Example: Line #2, 2cm Core, 5meters: Order: Osteroidea\*\**

1. Repeat steps 2-6 for a total of three times. For this entire sample, you will need to have removed a total three full pipettes from the medium-sized or large-sized petri dish. After you have removed a total of this amount, you may discard the entire remaining sample.
2. Bring the empty sample container to the processing station. Rinse the container and place it in the storage box.

**Process for analyzing the 50cm Survey Samples (macrofauna organisms)**

1. Remove any organisms that you can see in the plastic 50cm Survey Sample container using the small pipette, inoculating loop, or your hands (if the organism is large). Place these objects on a well slide or a small or large petri dish.
2. Using the dissecting microscope and the taxonomic guides provided, identify the organism in the slide and record its taxonomic levels on the data sheet provided.
3. Next, record the amount of organisms of this exact type that you found in the sample.
4. Once you have identified the organism and counted it, bring it on a small petri dish, well slide, or a large petri dish (if the organism is very large) to the processing station and place it in your designated area. You must label each slide with Transect Line number, Sample type, Depth, and the furthest taxonomic level that you identified the organism as.

*\*\*Example: Line #2, 2cm Core, 5meters: Order: Osteroidea\*\**

1. Continue removing and identifying any organisms that you find in the sample container until there are none left. Once you have done this, bring the empty sample container to the processing station. Rinse the container and place it in the storage box.

NaGISA Sampling Protocol

*for sandy beach coastal areas*

**Dive**

1. Cautiously approach your designated site.

*Take care not make any disturbances in the areas where
you will be placing your quadrats and taking samples.*

1. Place the 1x1 meter quadrat (grid) on any random point along the main transect line. Be sure to place the bottom edge of the quadrat along the transect line. *See diagram.*

*There are two flagged poles on opposite ends of the beach.
This line is your main transect line, it stretches 30 meters.*

1. Next, take your labeled card and place it in the lower right hand corner of your 1x1 meter quadrat grid.

1. Get the dive photographer. The photographer will take a picture of the entire frame of the 1x1meter quadrat (grid). Make sure the photographer takes the photograph from directly above the quadrat grid. The grid’s square shape must not be distorted in order to be able to determine the relative size of objects.

*Note: You will not be taking samples or organisms from the 1x1meter quadrat (grid).You will only photograph it.*

1. Place the 50x50cm quadrat adjacent to the upper left corner of the 1x1meter quadrat (grid). *See diagram.*
2. Remove any visible organisms on the surface and place them in the plastic bag labeled accordingly.
3. Next, gently sift through the ocean floor with your hands and look for any additional organisms that may be *just below* the surface of the sand. Remove these organisms and place them in the plastic bag as well.

*You do not need to “dig” into the sand. Simply glide your hands*

*through the surface to uncover any living organisms that*

*may be hidden in the sand. You should not dig, only sift.*

1. Place the 15cm Cylinder next to the upper left corner of the 50x50cm quadrat.
2. Next, you need the 2cm Core. Take the 2cm Core and place it next to the upper left of the 15cm Cylinder. *See diagram for details.*
3. Press the 2cm Core down into the sanduntil the sand reaches the black line that is marked on the side.
4. Pull the 2cm Core straight out of the sand.

*Scoop sideways to help keep the contents from falling out of the 2cm Core. If the contents of the core fall out, try to take another sample in an undisturbed area near your first attempt.*

1. Place the 2cm Core and its contents in the appropriately labeled plastic bag.



1. Now, go back to the 15cm Cylinder that you previously placed. Kneel down next to the Cylinder and press it down in the sand. It is easiest to use a combination of pushing down with a twisting-motion.

\*\**Be sure to push the 15cm Cylinder until the sand
reaches the black line that is marked on the side
of the cylinder. This line marks a depth of 10cm\*\**

1. Next, use the digging tools and your hands to dig away the areas around the 15cm Cylinder. Slide the board or plate underneath the 15cm Cylinder and carefully lift.
2. The photographer will take a picture of the entire 15cm Cylinder and its contents while you are holding it in the water.
3. Once your cylinder has been photographed, place the entire sample into the appropriately labeled plastic bag.

Preservation Protocol

1. A slide or Petri dish with an organism will be sent to the preservation station. It should have a label with the following information: sample depth, quadrat number, sample type, dead or alive, photographed status and organism name.
2. Be sure that the sample has been photographed before continuing
3. On a spreadsheet, list the following information: tag field number, site information (depth/ quadrat/ core sample), organism, photography status, living status, and, if needed, DNA subsample.\*\*
	1. field number: NAG for NaGISA, 2 digits indicating the month, 2 digits indicating the year, - (hyphen), sample number

Ex. NAG0914-27 indicates NaGISA collection during September, 2014, sample #27

* 1. site information: quadrat number, depth, sample type

Ex. Q2 10 m 15 cm

* 1. organism: taxonomic identification of the organism

Ex. Polychaete

(FURTHER CLASSIFIED EXAMPLE)

* 1. photography status: marked “yes” if photographed, marked “no” if not
	2. living status: list alive or dead, depending upon whether the sample was alive or dead **when it was brought to the preservation station**.

*\*\*DNA subsample status: indicate whether a DNA sample will be taken (this is
done so if the organism is still alive, is dead within a few minutes, or is relatively large; otherwise, the DNA will have deteriorated and a DNA sample will be useless). The DNA subsample will be received with the label. It will be marked by an “S”\*\**

 Ex. Polychaete (S) (DNA SUBSAMPLE IF IT IS UNIQUE)

1. Make a label with the field tag number (NAG0914-27) to go inside the preserved bottle. The label must be written with a number 2 pencil on a small slip of parchment or archival paper. Pen ink or normal printer ink will bleed out into the preserved sample, rendering the label useless. If a DNA subsample is being taken, the label must be indicated by an “s” after the field number.

Ex: NAG0909-27 (S)

1. Carefully insert the label into a 2 mL screw cap bottle. (substitute 2 mL bottle for a larger bottle if needed for this and the next steps)
2. Fill the 2 mL bottle with the fixation medium. If a DNA subsample is being taken, use 2 of the 2 mL bottles with the fixation medium (one bottle for the original sample and one for the subsample). Again, use formalin if the sample is a worm, Ethyl alcohol for anything else.
3. Take a DNA subsample of the specimen.
	1. If there is no DNA subsample being taken, skip this step.
	2. For a DNA subsample, place the specimen in a Petri dish and add a bit of Magnesium Chloride (skip this step if the sample is not moving or barely moving). This puts the specimen to sleep.
	3. Using a scalpel, remove a small piece of soft tissue.
4. Place the specimen in the correctly labeled 2 mL bottle. Place any DNA subsamples in correctly labeled 2 mL bottles.
5. Screw on the cap, sealing the 2 mL bottle.
6. Place the sticker label on the vial (cut the label if it is too large).
7. Set aside in a container and repeat for the next sample.

Water Analysis Protocol

**Collection**

GPS

1. Turn on the GPS by holding the bottom right light bulb button
2. Go to the screen with the latitude and longitude coordinates by tapping the top right button several times
3. Record the coordinates for each quadrat
4. Turn of the GPS by holding the bottom right light bulb button

Dissolved Oxygen CHEMets Kit

1. Get water from the ocean using the vial (go out far and try to get water with few bubbles) and fill to the 25 ml line
2. Break ampoule in the vial, swirl for 10 seconds
3. Invert back and forth until color changes
4. Compare to color table
5. Record data in ppm (parts per million)

Refractometer salinity

1. Clean the refractometer
2. Place a drop of water on the blue glass and close to the top
3. Look through the scope and record the salinity
4. Clean the refratometer

Temperature

1. Get the thermometer
2. Go out far in the water
3. Hold the thermometer underwater for at least 20 seconds
4. Record temperature

Salinity Titration Test Kit – procedures in the kit

Dissolved Oxygen Test Kit – procedures in the kit

**Analysis**

To begin water analysis, calibrate the probes. You will only be using the Turbidity, Salinity and Dissolved Oxygen.

Plug the LabPro into the computer under the Logger Pro program. Use the connector with the square plug for the LabPro and the regular USB plug for the computer.

Turbidity

1. Connect the turbidity sensor to the Lab Pro. (green box)
2. Start Logger Pro 3.6.0, select new from the menu. Select it again and the Turbidity sensor should now be identified.
3. Calibrate the sensor (Hover over “**Calibrate”** and click LabPro: 1CH1: Turbidity)
	* 1. Let the Turbidity sensor warm up for five to ten minutes in order to establish a stable voltage.
		2. Place the 100 NTU Turbidity Standard cuvette and gentle invert in a few times to mix up anything that could have settled. (**DO NOT SHAKE: This will cause bubble that will effect your readings)**
		3. Align the arrow on the cuvette with the arrow inside the sensor.
		4. Close the lid.
		5. Click “**Calibrate Now”**
		6. Enter **100** as the value for NTUs in the **First Calibration Point,** press enter.
		7. Remove the Turbidity Standard (100 NTU cuvette) from the sensor.
		8. Fill the empty cuvette with distilled water to the line. Make sure the meniscus is touching the top of the designated line.
		9. Align the arrow on the cuvette with the arrow inside the sensor.
		10. Close the lid.
		11. Enter **0** as the value for NTUs in the **Reading 2**, press enter.
4. Dump out the distilled water and fill it with the test sample water.
5. Click **“Experiment”**, then **“Start Collection”**
6. Wait for the registering value to level out and that will be your data. Do not wait too long or the suspended sediment will settle to the bottom.
7. When finished, (after 2 or 3 minutes or whenever the NTU value stabilizes), click **“Experiment”**, then **“Stop Collection”**, then “**Clear Latest Run”**.
8. After recording the data, repeat from Step 4.

Salinity

1. Connect the salinity probe to the Lab Pro.
2. Select new from **“File”**. Select it again and the Turbidity sensor should now be identified.
3. Salinity probe does not require calibration, you may now use it to test the water sample.
4. Rinse the tip of the probe with distilled water.
5. Insert the tip into the sample and gentle swirl.
6. Once the reading stabilizes, that will be your data

Dissolved Oxygen (optional)

1. Get the CHEMets Kit.
2. Put on gloves and safety goggles.
3. Fill the sample cup with 25 mL of the first sample that will be tested.
4. Place the ampoule, tip first, into the sample cup. Carefully break the tip of the ampoule. The ampoule will fill with the sample. (There will be a bubble at the top)
5. Invert the ampoule several times. **DO NOT SHAKE** (the bubble will flow from end to end).
6. Dry the ampoule using a paper towel. Let the ampoule stand for 2 minutes before matching the sample.
7. Using the color standards, carefully match the ampoule with the first sample to one of the standards to find the best match.
8. Repeat steps 3-7 for each depth that has a sample.