**PROTOCOL**
**for**
**SORTING OF PLASTICS and PLANKTON**
**in ALGALITA FOUNDATON'S**
**SEA WATER SAMPLES**
**from**
**North Pacific Gyre, Summer 2000**

Following is a protocol for sorting of plastic and plankton in sea water samples collected from the North Pacific Gyre aboard the sailing vessel "Aguita" in summer 2000. This protocol is intended as a step-by-step procedure for lab technicians to use in analyzing these samples, and should be followed closely to yield consistent results from one technician to another. The goal of this careful analysis is to accurately characterize the plastic and plankton found during this expedition.

## General:

Following are some general guidelines for each technician to follow as he or she analyzes each sample:

- quality is much more important than quantity;

- there is no time limit; accuracy is paramount;

- record all results of your analysis as you move through the protocol (i.e., write down the weights and quantities as you complete those steps);

- use the sample data sheet for notes of general work performed and specific questions that may arise as the work proceeds;

- clean-up equipment and your work area as you move through the protocol;

- label all analyzed samples with the following data:
  - sample station no.
  - sample weight
  - sample sieve size-class

- give sorting-sheets and data sheets to supervisor at end of each work day;

- store processed samples in locker in a box labeled with sample station number.

## **Equipment Needed:**

Following is the equipment needed to analyze each sample:

- light ring (6” magnifying glass with a light around perimeter)
- dissecting microscope
- scale sensitive to 1/1000 gram
- set of 7 Tyler nesting sieves
- several 200 to 333 micron screens micron straining sieves, 6”diameter (for draining samples)
- several 200 to 333 micron screens micron straining sieves, 2” diameter (for blotted wet weight of plankton)
- eye droppers, various sizes
- teaspoons
- tablespoons
- 3 wash bottles filled with tap water, sea water, and 50% isopropyl alcohol
- fine-nosed and snub-nosed tweezers
- tags for putting in alcohol-preserved samples
- petri dishes, 3” to 6”diameter and in 1/4” to 1” deep (glass and plastic)
- watch glasses, approx. 4” in diameter
- fine-meshed, round pieces of screening material, approx. 2” in diameter
- sorting sheets and data sheets (used to record results of analysis)

## Steps to Analyze Samples:

Following are the steps to analyze each sample.

1. Sample Preparation:
   Goal: to drain away alcohol from sample material.

   a) drain sample through 200 to 333
   b) rinse off particles adhering to the immersed label with sea water wash bottle
   c) place label in small petri dish and set aside
   d) rinse drained plastic and plankton into large petri dish with spray from wash bottle
   e) spray screen with sea water to eliminate any plastic bits that may cling to screen and sides of cylinder; rinse plastic bits into large petri dish
   f) if process above does not result in adequate liquid, add sufficient sea water to petri dish to float all plastic bits -- do not overfill

2. Gross Separation:
   Goal: To segregate largest bits of plastic.

   a) place petri dish from #1, above, under light ring
   b) using tweezers, remove all pieces of plastic >5 mm (1/2 cm. - you will soon recognize these pieces, which will be sieved later if not removed at this point).
   c) rinse off any residual smaller plastic bits adhering to the >5mm ones with sea water wash bottle
   d) place rinsed, large bits of plastic on round plastic screen on watch glasses and set aside for later drying and counting
   e) using spoon or eye dropper, remove all remaining plastic from plankton that is easily visible. Start at center of petri dish and move out to sides. Start at surface and then look deeper. When light ring magnifier is no longer adequate to see plastic bits, transfer balance of sample into several 1/4" deep petri dishes for sieving later on.

3. Plankton Separation:
   Goal: To segregate plankton from smaller fragments of plastic.

   a) view each 1/4" deep petri dish of sample material under dissecting scope - start with 15 power scope first
   b) use tweezers, eye dropper or spoon to lift all plastic bits into separate petri dish for subsequent sieving, drying, and weighing.
   c) rinse remaining plankton through 200-333 mesh sieve to remove sea water and then wash into original sample jar with isopropyl alcohol wash bottle.
   d) add sufficient alcohol to completely immerse plankton; replace label and cap.

4. Screening of Plastic:
   Goal: To segregate plastic bits into 7 different size classes.

   a) obtain all plastic fragments (except those >5mm taken from sample material earlier) and prepare to sieve for size classing
   b) arrange 7 Tyler sieves in descending size of mesh ( smallest mesh on bottom)
   c) place stacked sieves on edge of sink or on the edge of a box over asphalt outside
   e) pour sample material that remains after step nos. 2 and 3, above, through sieves
      - rinse out all particles into sieve array carefully with tap water
   e) gently pour tap water through sieves to promote accurate settling of different-sized plastic bits into their appropriate-sized sieve
      - do not pour water too quickly to avoid breaking-up plastic
   f) separate the 7 sieves to inspect
   g) advise supervisor if you notice any plankton (clear, gelatinous material) in any of the sieves
   h) gently squirt plastic bits to one small area on the side of each of the sieves (squirt from backside of screens)

5. Drying of Screened Plastic:
   Goal: To dry out screened plastic for subsequent weighing.

   a) set oven to 60° C (~3.3 on oven scale)
      - wait until thermometer reaches desired temperature

   b) place all 7 rinsed screens from step #4, above, into oven
      - lift 1 or 2 screens into oven at a time (okay to stack on each other)
      - put 3 screens on one shelf
      - put 4 screens on another shelf
      - close oven door between transfers to maintain temperature in oven
   c) place watch glasses into oven; these are the >5 mm plastic bits on top of round plastic screens (from step #2)
   d) dry samples at 60°C for ~1 hour:
      - inspect samples for moisture at the end of 1 hour
      - dry over 1 hour if samples still show any moisture; check every 15 minutes and remove when no moisture visible
      - notify supervisor if temperature varies >5 degrees above or below 60° C level

6. Weighing of Dried Plastic:
   Goal: To determine dry weight of plastic.

   a) turn on scale 30 minutes prior to weighing
   b) weigh each small petri dish or weighing paper to obtain tare weight
   c) transfer dried plastic bits from each of 7 sieves into a petri dish or weighing paper
      - use brush or scraper to very carefully accomplish transfer
      - take care not to lose any little plastic bits, as they will be light from drying and can easily pop loose
      - brush from top edge of sieve to bottom edge to transfer
   d) label each dish or paper with:
      - sieve size class
      - sample station no.
   e) inspect each sample under dissecting scope and remove any brush hairs
   f) weigh dish or paper containing plastic to obtain gross weight
   g) subtract tare weight from gross weight to obtain net weight of plastic:

      (gross weight) less (tare weight) = net weight

   h) record net weight on sorting sheet
   i) transfer weighed plastic sample to sample jar
      - do under light ring to avoid loosing any sample material
   j) put label IN sample jar:
      - sieve size class
      - sample station no.
      - net weight of plastic
   k) put label ON sample jar, showing same data as above

7. Sorting of Plastic:
   Goal: To determine types and colors of plastic in each sample.

   a) place contents of each labeled jar from step #6, above, in a large petri dish
      - put just one jar's contents (one size class) in dish at a time
      - place dish under light ring to analyze
   b) types: using tweezers or push points, sort each sample per types in sorting sheet (attached) – move them to different areas of the petri dish for each type
      - record no. of plastic bits in each type on sorting sheet
   c) colors: using tweezers, sort each sample per colors in sorting sheet (attached)
      - record no. of plastic bits in each color on sorting sheet
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8. Weighing of Plankton:
   Goal: To determine blotted wet weight of plankton.

   a) turn on scale 30 minutes prior to weighing
   b) weigh a 333 micron sieve to determine tare weight
   d) take result of step #3, above, and pour through 333 micron screen
      - rinse all organisms into small 2" weighing sieve with alcohol wash bottle

   d) place screen containing sample material on dry paper towel for 10 minutes
   e) weigh screen containing sample material to obtain gross weight

   f) subtract tare weight from gross weight to obtain net weight of plankton:

      (gross weight) less (tare weight) = net weight

   g) record net weight on sorting sheet
   h) rinse weighed plankton off of screen into sample jar:
      - use isopropyl alcohol to rinse screen
   i) put alcohol label IN sample jar:
      - sample station no.
      - net weight of plankton
   j) put label ON sample jar, showing same data as above

9. Sorting of Plankton:
   Goal: To determine type of plankton in each sample.

   a) take small sub-sample of result of step #3, above, and place in small petri dish and then under most powerful scope in lab
   b) sort by type of organism per sorting sheet for plankton
   c) consult with supervisor if you are unsure of classification of any organism
   d) count and record numbers of each plankton class

10. Give Results of Sample Analysis to Supervisor at End of Each Day's Work

# SAMPLE DATA SHEET
## for ALGALITA FOUNDATON'S SEA WATER SAMPLES from North Pacific Gyre, Summer 2000

Name of Lab Technician:_____

Date of analysis:_____ Sample Station No._____

Work done today on sample:

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Notes (problems, unusual observations, ideas, etc.):

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