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SUBJECT: **CMarZ sample collection protocol**

FROM: **Ann Bucklin, University of Connecticut**

The *Census of Marine Zooplankton* (CMarZ) is working toward a taxonomically comprehensive assessment of biodiversity of animal plankton throughout the world ocean. The project goal is to produce accurate and complete information on zooplankton species diversity, biomass, biogeographical distribution, genetic diversity, and community structure by 2010. Our taxonomic focus is the holozooplankton, including ~6,800 described species in fifteen phyla; our expectation is that at least that many new species will be discovered as a result of our efforts.

Following taxonomic identification of specimens, a DNA sequence is determined for a 660-base pair portion of the mitochondrial cytochrome oxidase I (mtCOI) gene; multiple mtCOI sequences are included as necessary to reflect intraspecific variation. Different genes are used for some groups or species, as needed. Samples collected with minimum damage to the specimens are preferred (i.e., short tows taken in good weather). Samples must be preserved immediately upon collection. Only those individuals that are ALIVE up to the moment of preservation should be used. DNA is destroyed by enzymes immediately upon the death of the organism.

CMarZ seeks zooplankton collections from any region of the world oceans; samples must be preserved especially for molecular analysis following the instructions provided here. In most cases, we would greatly appreciate your assistance in providing specimens identified to species, with the identification confirmed by a taxonomic authority. In this case, please send 20 to 30 individuals (adult females are preferred). Specimens should be shipped in glass vials of appropriate size, capped with a plastic top that will not leak or allow evaporation. Please include a label inside the vial; use a label without ink markings and write all collection information in pencil.

For gelatinous zooplankton groups, specimens should be placed individually in vials. Generally, 5 to 10 individuals per species are desired. Assuming the taxonomic identification of the specimen is definitive, the size and maturity of the specimen does not matter. If collections are made from diverse regions, we prefer to have specimens a given species from a selection of sites.

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PROTOCOLS FOR COLLECTION AND PRESERVATION OF CMarZ SAMPLES

Please follow these instructions carefully. Unless samples are carefully preserved, specimens will yield no DNA for our analyses. This protocol is also available on the project website at http://www.CMarZ.org.

Steps 1 – 3 for Crustacean and other zooplankton

1) Samples should be collected using nets with appropriate size mesh. Samples need not be quantitative; non-quantitative portions of samples are acceptable. We recommend removing fish from the samples.

2) Immediately after collection, drain samples of excess seawater (using a sieve with mesh of same size - or smaller - as the net).

3) Wash the sample into a glass jar using 95% un-denatured (i.e., drinkable) ethyl alcohol. Add additional 95% ethyl alcohol to fill the jar. NOTE: there must be 3 to 4 times more alcohol than plankton volume.

Steps 1 – 3 for Gelatinous zooplankton

1) Some gelatinous zooplankton may be collected in nets; others will require special collection methods (submersible, ROV, divers). Samples need not be quantitative; non-quantitative portions of samples are acceptable.

2) For very large individuals, tissue can be excised prior to preservation. Different vials should be used for each individual (or colony). If portions of animals are preserved, care should be taken to avoid non-cellular regions (e.g., inner bell matrix). Wash the sample into a glass jar using 95% un-denatured (i.e., drinkable) ethyl alcohol. Add additional 95% ethyl alcohol to fill the jar, ensuring 3 to 4 times more alcohol than plankton volume.

3) Gelatinous zooplankton specimens that disintegrate in alcohol can still be used for molecular analysis. If the specimen has disintegrated, DO NOT change the alcohol, since the DNA will have dissolved or remained in flocculent material. Note that gelatinous specimens can also be placed in cryovials and flash frozen in liquid nitrogen. Label the cryovials with an indelible felt-tip pen, including species name, collection date, and geo-reference coordinates.

Steps 4 – 7 for all CMarZ samples

4) NOTE: ONLY 95% UNDENATURED ETHYL ALCOHOL CAN BE USED TO PRESERVE CMarZ SAMPLES. PLEASE DO NOT USE 100% ETHANOL. DO NOT USE DENATURED ALCOHOL. If you are not sure which alcohol to use, please ask us for specific information and suggestions for vendors in your region.

5) Place a label inside the jar or vial, writing in pencil. Unprinted labels are preferred since the ink may dissolve in the alcohol. Use small labels made from acid-free paper. (We have discovered that some labels change the sample pH significantly, especially in small volumes). Sample pH should remain close to pH 8.0. Please note collection information desired: cruise (ship and cruise name or number); collection date and local time; georeference coordinates (latitude/longitude); station or tow number; net and mesh size.

6) After 24 hours, drain off alcohol and replace with fresh alcohol. Continue to change the alcohol every 24 to 48 hours, until the fluid remains clear and free of debris.

7) Ship samples to Ann Bucklin. For shipping, please ship in the smallest vials possible. Fill the vials FULL to the top with alcohol, since the postal services do not promise to keep packages upright! Please ensure that samples will not be exposed to extreme heat (over 30^{o} C) at any time during shipment. Costs of sample shipment will be paid or reimbursed upon request.

MICROSCOPIC EXAMINATION OF SPECIMENS FOR MOLECULAR ANALYSIS

When microscopic examination is required for species identification of copepods, euphausiids, or gelatinous forms, please take special care in order to allow later molecular analysis of these individuals. View the specimens in 95% ethyl alcohol; do not move them to water or other fluid. Do not use stains or other treatments. Avoid dissection; if necessary, use sterilized tools that have never been exposed to formalin; do not use the same tools for multiple individuals. Do not allow the alcohol to evaporate or become warm; minimize light exposure by limiting both duration and intensity.

If such handling is not possible to allow identification, consider examining only some individuals of each sample carefully. Keep these and send the others individuals of that sample to us for molecular analysis.

SHIPMENT OF ALCOHOL-PRESERVED SAMPLES TO THE USA

For alcohol-preserved samples, international and domestic (USA) hazardous material shipping regulations MUST BE FOLLOWED EXACTLY. Shipments of flammable liquids may be made according to the Excepted Quantity provision of the International Air Transport Association (IATA) and U.S. Department of Transportation (DOT) regulations. ***Shipments made under this exception must only be made by a person trained to use IATA Dangerous Goods Regulations and/or U.S. DOT Hazardous Material Regulations.*** *Please see the CMarZ sample shipping protocol on the project website.*

THANK YOU VERY MUCH!

Please don't hesitate to contact me if you have any questions or concerns about collection, preservation, and shipment of CMarZ samples.

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