



Ocean Process Analysis Laboratory
Institute for the Study of Earth, Oceans, and Space
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SUBJECT: ZooGene: zooplankton collections for molecular analysis

FROM: Ann Bucklin, University of New Hampshire

ZooGene is an international partnership to create a database of DNA type sequences for calanoid copepods and euphausiids (see <http://www.ZooGene.org>). ZooGene has recently expanded to include gelatinous zooplankton groups. Following taxonomic identification of specimens, a DNA type 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.

Crustacean zooplankton: The ZooGene project seeks zooplankton collections containing calanoid copepods and euphausiids 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) in a small glass vial (5 - 20 ml) 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.

Gelatinous zooplankton: ZooGene has recently expanded its taxonomic range to include gelatinous zooplankton groups, and we are focusing on ctenophores, planktonic cnidarians, and larvaceans, among others. Please let us know of any samples or specimens you may be able to collect, and we can determine which ones are of most use for us. 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, but this is not necessary.

PROTOCOLS FOR COLLECTION AND PRESERVATION OF ZooGene 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.ZooGene.org>.

Steps 1 – 3 for Crustacean zooplankton

- 1) Samples should be collected using nets with 100 um to 333 um mesh for copepods, and 333 to 550 um mesh for euphausiids. Samples need not be quantitative; non-quantitative portions of samples are acceptable.
- 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. Samples can be split to keep plankton biovolume to one-third or one-fourth of the jar volume. We recommend removing fish from the samples.

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. Whether entire individuals or excised tissue is preserved, 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. NOTE: there must be 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 ZooGene samples

- 4) NOTE: ONLY 95% UNDENATURED ETHYL ALCOHOL CAN BE USED TO PRESERVE ZOOGENE 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 and 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. Follow the Guidelines for the Shipment of Excepted Quantities of Flammable Liquids. DO NOT ship via airmail; use a carrier that accepts hazardous materials such as DHL, FedEx, etc. 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.

THANK YOU VERY MUCH!

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

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Guidelines for the Shipment of Excepted Quantities of Flammable Liquids

International and domestic hazardous material shipping regulations protect individuals and property involved in the shipment of hazardous materials. 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.***

Note: flammable liquids are not permitted in checked baggage, carry-on baggage or airmail.

Packaging

Packaging for excepted quantities of flammable liquids must have three basic components:

1. **Inner packaging**, such as a vial, tube, jar, etc. Do not completely fill inner packagings; allow space for liquid expansion. Liquids must not completely fill inner packagings at a temperature of 55°C (130°F). Closures of inner packagings must be held securely in place with tape, wire, metal crimps or other positive means.
2. **Intermediate packaging**, such as a ziplock or other plastic bag. Intermediate packaging must contain enough absorbent material to absorb all contents.
3. **Outer packaging**, such as a cardboard (fibreboard) box. Flammable liquids may not be shipped in envelopes, Tyvek® sleeves, or other non-rigid mailers. The dimensions of the outer box must be at least 100 mm on two of three sides.

Quantity Limits

95% ethanol is a flammable liquid in hazard class 3, packing group II. As such, each inner packaging may contain not more than 30 mL. Each outer package may contain not more than 500 mL.

Example: If you are shipping 10 mL vials of 95% ethanol, you may put up to 50 vials in one box.

Documentation

The “Nature and Quantity of Goods” box of the air waybill must include the words “Dangerous Goods in Excepted Quantities.” See attached DHL air waybill.

Labeling

An Excepted Quantities of Dangerous Goods Label must be completely filled out and affixed to the outer container. For 95% ethanol check the box for Class 3 material and write the UN number UN 1170. The attached label may be used, but it must be printed in color. Consider printing it on an adhesive backed label. Its overall dimensions must be at least 100mm X 100mm.



Process and Track your shipment online: <http://www.dhl-usa.com>

1-800-CALL-DHL in USA only

Shipment Air Waybill
(Non negotiable)

778 0344 795

ORIGIN

DESTINATION CODE

1 Payer account number and shipment value protection details

Charge to Shipper Receiver 3rd Party Cash
 Check
 Credit Card

Payer Account No. _____

Shipment Value Protection (see reverse)

Yes Declared Value for Carriage (in US \$) _____

Not all payment options are available in all countries.

2 From (Shipper)

Shipper's Account Number _____ Contact Name _____

Shipper's Reference (up to 35 characters) _____

Company Name _____

Address _____

Post/ZIP Code (required) _____ Phone, Fax, or E-mail (required) _____

3 To (Receiver)

Company Name _____

Contact Name _____

Delivery Address **DHL Cannot Deliver to a PO Box**

Country _____

Post/ZIP Code (required) _____ Phone, Fax, or E-mail (required) _____

4 Shipment Details

Total Number of Packages	Total Weight If DHL Express Document packaging used, enter XD.	Dimensions (in inches)			Height
		Pieces	Length	Width	
		@	x	x	x
		@	x	x	x
	lbs	@	x	x	x

5 Full Description of Contents

Give Content and Quantity **DHL Does Not Transport Cash**

Dangerous Goods in Excepted Quantities

6 Dutiable Shipments Only (Customs requirement)

Attach the original and four copies of a Commercial Invoice or Pro Forma.
Export License No./Symbol (if applicable) Receiver's VAT/GST or Shipper's EIN/SSN

Value for Customs (in US \$) _____ Schedule B Number / Harmonized Code (if applicable) _____

TYPE OF EXPORT Permanent Repair/Return Temporary

Destination Duties/Taxes If left blank, Receiver pays duties/taxes.

Receiver Shipper Other _____
Specify approved account number

The commodities, technology or software to be exported from the U.S. are in compliance with the U.S. Bureau of Export Administration. Diversion to countries contrary to U.S. law prohibited.

7 Shipper's Authorization (signature required)

I/we agree that DHL's standard terms apply to this shipment and limit DHL's liability for loss or damage to U.S. \$100. The Warsaw Convention may also apply (see reverse). I/we authorize DHL to complete other documents necessary to export this shipment. I/we understand that Shipment Value Protection is available on request, for an extra charge. I/we agree to pay all charges if the recipient or 3rd party refuses to pay. I/we understand that DHL DOES NOT TRANSPORT CASH.

8 Products & Services

DOMESTIC EXPRESS	DP GLOBAL MAIL
<input type="checkbox"/> U.S. Express Envelope	<input type="checkbox"/> Priority
<input type="checkbox"/> USA Overnight	<input type="checkbox"/> Standard
<input type="checkbox"/> Other.....	<input type="checkbox"/> IPA
	<input type="checkbox"/> ISAL
	<input type="checkbox"/> DomFlats
WORLDWIDE EXPRESS	
<input type="checkbox"/> Int'l Express Envelope	
<input type="checkbox"/> Non-Dutiable <input type="checkbox"/> WorldFreight	
<input type="checkbox"/> Dutiable <input type="checkbox"/> Other.....	
Service Options (extra charges may apply)	
<input type="checkbox"/> Saturday Delivery <input type="checkbox"/> Special Pickup.....	
<input type="checkbox"/> Hold For Pickup* <input type="checkbox"/> Delivery Notification	
<small>*US deliveries only</small>	
<small>Other.....</small>	
<small>Not all products or service options are available to/from all locations.</small>	

DIMENSIONAL/CHARGEABLE WEIGHT

lbs

SERVICES CHARGES

SERVICES	CHARGES
Drop Box #	TOTAL

TRANSPORT COLLECT STICKER No.

PAYMENT DETAILS (Check, Card No.)

No.: _____

Type Expires _____

Auth. _____

PICKED UP BY _____

Route No. _____

DHL Worldwide Express, Inc. 50 California Street, Suite 500, San Francisco, CA 94111

Original (DHL Billing) Copy

DHL WORLDWIDE EXPRESS USA

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Complete sections 1-8. You are making 4 copies, please press hard.

1A 0402

1B 0402

002

DANGEROUS GOODS IN EXCEPTED QUANTITIES

This package contains dangerous goods in excepted small quantities and is in all respects in compliance with the applicable international and national government regulations and the IATA Dangerous Goods Regulations

Signature of Shipper

Title

Date

Name and address of Shipper

This package contains substance(s) in Class(es)
(check applicable box(es))

Class:

2

3

4

5

6

8

9

and the applicable UN Numbers are: