University of Oregon
Zebrafish International Resource Center

TITLE: Procedure for Shipment of Juvenile or Adult Zebrafish

SUBJECT AREA: Zebrafish Distribution

Summary:
Distribution of juvenile or adult zebrafish at the ZIRC requires special packaging conditions to insure safe delivery. The procedure can be simple, however, the protocol may require adjustment depending on the specific courier and/or the customs requirements for international shipments, which vary by country. International shipments should be sent on a Monday or Friday to allow time for customs clearance (1-3 days) and to avoid weekend delivery.

Supplies (see vendor information on page 10-11):

- Boxes: Styrofoam box nested in a cardboard box
- Fish container options:
    - Plastic bags (square bottom and non-square bottom) are used for juvenile or adult fish. Standard bag size for up to 20 fish is 10" X 18".
    - Smart Bottles
        - 1/2 gallon (2-liter) for 5 fish
        - 1 gallon (4-liter) for 10-20 fish
    - Cubitainers
        - 4-liter for 10-20 fish
- ClorAM-X Ammonia Binder
- Pure oxygen (not required, but recommended)
- Rubber bands (ideally small)
- Labels: Live fish labels (see below), address and courier labels
- Styrofoam packing peanuts, avoid using dissolvable starch peanuts
- Heat Packs: 20, 40 & 60-70 hours, for shipment during low temperature seasons
- Customs documents for international distribution (pages 7-9)

Detailed Procedure for Packaging of Juvenile or Adult Fish:

1. Determine the preferred fish containment container, plastic bag, Smart Bottle, Cubitainer, or other similar container (see 3 or 4 below).
2. Write the fish information on the plastic bag or container (line name and allele, # of adults and date of birth; see image 2).
3. Using a plastic bag(s) for fish containment (image 2):
    a. Fill the square bottom bag 1/3 of the way with fish water (approximately 1.5 liters fish water).
    b. Add ClorAM-X ammonia binder to the bag (2.4 ml ClorAM-X per 1.5 liters of fish water). Add fish to the bag.
    c. For US or priority overnight shipments, place 20 fish/bag (1.5 liters of water); for international or 2-3 days shipments, place 10 fish/bag (1.5 liters water) unless pure oxygen is used.
    d. Close the bag quickly trapping as much air as possible. Grasp the top of the bag, close it off and twist. Twist tightly, keeping significant air pressure in the bag so that it looks like a cushion. Check the bag to see that it is approximately 2/3 air, 1/3 water. Do not blow into the bag to add more air. If needed, reopen the bag and try to

capture more air. If possible, use pure oxygen as the air source to allow for higher densities of fish per bag.

e. Seal the bag by knotting the top or twisting the bag so that the top is folded over and can be secured with a rubber-band (see image 2 below). You want the bag to remain tight. Once closed, gently tip the bag over to make sure it doesn't leak at the closure. This step is very important to ensure safe arrival without any leaks.
f. Place the bag of fish into another plastic bag (square bottom not required), to double bag the fish. Line up the bags by their seams so that they are similarly oriented. Suck the air out of the second bag in the event that the inner bag leaks. Close the outer bag by knotting it or using a rubber-band.

4. Using a Smart Bottle or like container for fish containment (images 3):
   a. Expand the Smart Bottle.
   b. Fill a 1/2 gallon bottle with 600 ml of fish water or the 1 gallon bottle with 1800 ml.
   c. Add ClorAM-X ammonia binder to the container (0.60 ml per 600ml fish water).
   d. Place a wide neck funnel in the container opening and add 5 fish to the 1/2 gallon bottle or 10-20 fish per 1 gallon bottle.
   e. If possible, add pure oxygen to the container until it is ballooned and quickly tighten the lid.
   f. If pure oxygen is not available, use an empty Cubitainer to add air and pressurize the container. **See instructional video for demo, https://youtu.be/1aqFnMiyNoQ**).
   g. The bottle should expand like a balloon (image 3). This step is critical for safe transport of the fish.
   h. Gently turn the container upside down to check for leaking. Make sure the lid is on tight and the valve closed.

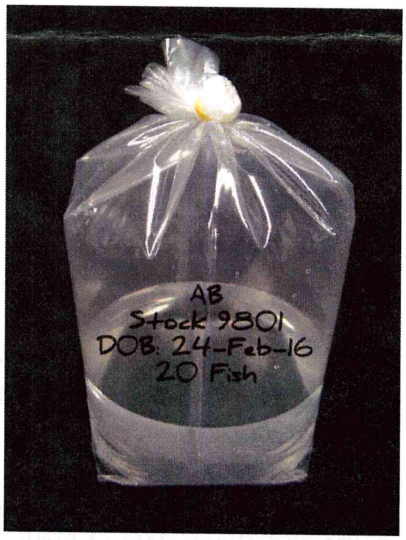
5. Place the bag(s) or container(s) of fish in the shipping box and fill up the remaining space with Styrofoam packing peanuts (see image 4). Close the box and seal with sturdy packing tape.

6. During low temperature months, heat packs should be placed in the box to keep the fish warm. Start by punching a small hole in the center of the Styrofoam shipping box lid and tape two heat packs to the underside of the lid covering the hole (see additional instructions below, image 5).
   a) 20 hour – all shipments, plus
   b) 40 hour – domestic shipments
   c) 60-70 hour – international shipments

7. Place the required labels and documents on the outside of the box (image 6): live fish labels, address label and customs documents for international distribution. Note: The standard customs paperwork for international distribution is listed below, however, the countries listed below have specific customs requirements that necessitate the use of a specialized animal courier. ZIRC currently used World Courier for these countries. The fees for a typical World Courier shipment are approximately $1500 USD. Countries currently requiring a specialized animal courier include:
   - Austria, Australia, China, Germany, Ireland, Italy, New Zealand, Spain, Saudi Arabia, Singapore, all South American countries, South Korea, Sweden, and the United Kingdom

Packaging Procedure Summary

It is very important to pack embryos for shipment in an insulated container. Zebrafish require warm temperatures for normal development, and these conditions need to be met during shipping as well.

Use a thick Styrofoam box nested inside a cardboard box. The interior box is very important since this is what helps keep the fish warm. The interior Styrofoam box should fit the cardboard box perfectly. Attach shipping labels to the outside of the box. The pictured box is available at: http://www.thermosafe.com/ or VWR.	 **Image 1**
Plastic bag containment: • Label the bag(s) with the line name and allele, date of birth, number of # of fish and any other necessary information. • Fill a square bottom plastic bag with 10-20 fish per 1.5 L of fish water. • Add ClorAM-X ammonia binder (2.4 ml ClorAM-X / 1.5 liters of water). • Close the bag trapping as much air as possible (use pure oxygen if possible) and seal with a rubber band. Knot the bag closure to prevent leakage. • Place the bag of fish inside another bag (non-square bottom) to prevent leakage.	 **Image 2**

Smart Bottles – 1 or 4-liter :

- 1/2 Gallon Bottle (5 fish):
  - Fill with 600 ml fish water.
  - Add ClorAM-X ammonia binder (0.60 ml per bottle).
- 1-Gallon Bottle (10-20 fish):
  - Fill with 1800 ml fish water.
  - Add ClorAM-X (2.4 ml per bottle).
- Use a wide neck funnel to add fish to the bag.
- If possible, add pure oxygen to pressurize the bottle and close immediately.
- If pure oxygen is not available, pressurize the container using tubing and an another bottle or Cubitainer (**see instructional video**, **https://youtu.be/1aqFnMiyNoQ**).
- The Smart Bottle should feel firm/rigid after adding air.
  - **Note:** *This step is critical to insure safe transport of the fish and prevent gas bubble disease.*
  - *Make sure lid is on tight and valve closed.*
- Label the bottle(s) with the line name and allele, date of birth, number of # of fish and any other necessary information.

Image 3

- Place the bag or container(s) of fish into a Styrofoam box and fill the remaining space with packaging peanuts. *Important: Do not use packaging peanuts that breakdown in water.*

Image 4

- Place the Styrofoam lid on the box. The lid helps insulate the fish and prevent water from leaking outside of the box should the bag leak during transit.
- **Note:** In the low temperature months, heat packs are recommended for warmth. Punch a small hole in the center of the Styrofoam lid and tape a 20 hour and a 40 (domestic) or 60-70 hour (international) activated heat pack on the underside of the lid covering the hole. Note, the image only shows one heat pack, but we recommend a short term and long term heat pack per box.

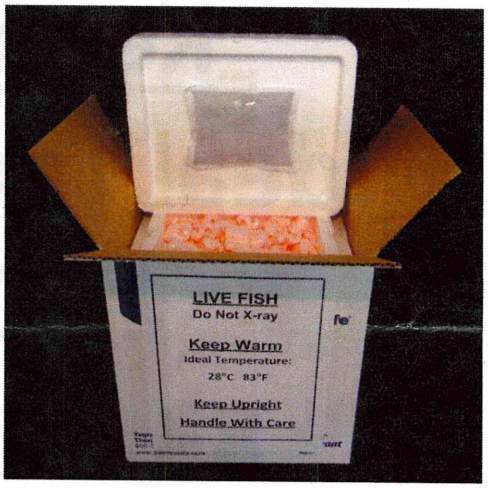


Image 5

- Close the cardboard box and tape shut.
- Affix the address label to the top of the box. Customs documents should also be affixed for international shipments. Most couriers provide clear adhesive pouches for these documents.

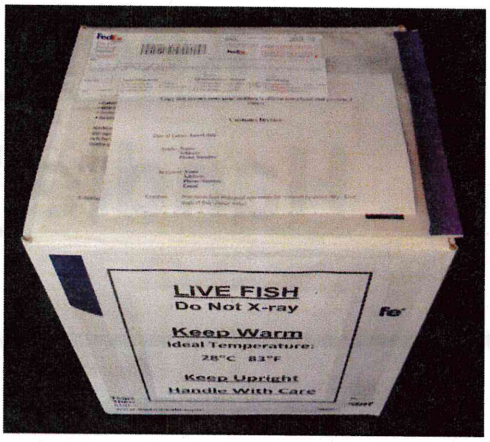


Image 6

Live Fish
Do Not X-ray

Keep Warm
Ideal Temperature
28 Celsius
83 Fahrenheit

Keep Upright
Handle With Care

International Customs Invoice

Copy this invoice onto your institute's official letterhead and provide 3 copies

Customs Invoice

Date of Export: **Insert date**

Sender: **Name**
Address
Phone Number

Recipient: **Name**
Address
Phone Number
Email

Contents: Non-hazardous biological specimens for research purposes only. Live tropical fish (*Danio rerio*).

Reason for Sending: The contents are intended for scientific research and are a gift from **Sender Name** to **Recipient Name**.

Declared Value: **$5.00 USD or equivalent currency**

Country of Origin: Insert Country

These commodities are licensed for the ultimate destination shown. I, **Senders Name**, the undersigned, do certify that this statement is both true and correct.

_____

Insert Sender Name Here

International Customs Statement

Copy this statement onto your institute's official letterhead

Date: **Insert date**

To Customs Officials:

The consignment contains non-hazardous, non-infectious biological material(s). Material does not come from a facility where work with viruses affecting livestock and avian species is conducted. The contents are to be used for biomedical research purposes only. The contents have no commercial value and are not for resale. For the purpose of Customs the nominal value is **$5.00 USD or equivalent currency**. **PLEASE DO NOT X-RAY.**

Detailed Description: Non-hazardous biological specimens for research purposes only. Live tropical fish (*Danio rerio*).

Country of Origin: **Insert Country**

Consigned To: **Recipient Name**
Telephone:
Fax:

I certify that this is true and correct.

Sign here

Sender Name

Sender Address
Telephone Number

International Customs Health Letter

Copy this letter onto your institute's official letterhead

Declaration of Health

Identification

Contents of Shipment: Live fish adults and embryos - list only what applies

Species:	*Danio rerio*
Common name:	Zebrafish
Description:	This is a tropical, freshwater aquarium species commonly used as a model organism for genetic and biomedical research.
Age:	Adults (6 months); Embryos (2 days) - list only what applies
Number:	Adults (8); Embryos (400) - list only what applies

Place of Production

Aquaculture Facility:
Address:

Destination

Name:
Address:

Phone (office):
Importing Country:

Declaration

- The Zebrafish is not an endangered species.
- The animals were bred in a controlled environment at **ENTER INSTITUTION NAME, CITY and COUNTRY HERE.**
- For biomedical research, scientific, and lab testing purposes only.
- Material does not come from a facility where work with viruses affecting livestock and avian species is conducted.
- Pathogen free.

Does NOT include any Equine, Ruminant, Swine or Avian Species or Materials (such as transport media or stabilizers).

Country of Origin:

I certify that this is true and correct.

Signature: _____ Date: _____

Type Sender Name Here

Vendor Information for Fish Shipping Materials

1. Boxes
   - VWR International, https://us.vwr.com
     - Small box dimensions 8" L X 6" W X 9" H
       - Catalog number 14100-412
     - Medium box dimensions 12" L X 10" W X 7" H
       - Catalog number 14100-402
     - Extra-large box dimensions 22" L X 15" W X 15" H
       - Catalog number 15714-704
   - Superior Shipping, https://superiorshippingsupplies.com/
     - Multiple sizes available

2. Smart Bottles (for juvenile or adult fish) - **Recommended** (easier to use and tightly seal)
   - Smart Bottle: https://smartbottleinc.com/
     - Smart Bottle, 1/2 Gallon - up to 5 fish
       - SKU: 1112, https://smartbottleinc.com/product/1-2-gallon/
     - Smart Bottle, 1 Gallon - for 10 to 20 fish
       - SKU: 1114, https://smartbottleinc.com/product/1-gallon/
     - Note: Smart Bottles do not come with spigot caps/faucets. These will need to be purchased separately (see #5 below).

3. Plastic bags (for juvenile or adult fish)
   - Gulf Coast Plastics for bulk quantity, https://www.gulfcoastplastics.com/
   - Superior Shipping, https://superiorshippingsupplies.com/
   - Check any pet store for small quantities
   - Average size for holding 10-20 fish (10" X 18" (.003 mil))
   - Note: Square bottom bags can be purchased from a vendor or made using a plastic heat/bag sealer.

4. Cubitainers
   - United States Plastic Corp., http://www.usplastic.com
     - Cubitainer, 4-Liter (1 Gallon)
       - Catalog number 75077

5. Spigot Caps/Faucet
   - Best Containers: https://www.bestcontainers.com/
     - Cap/Faucet for Cubitainers or Smart Bottles
       - Item number 400-4401, https://www.bestcontainers.com/400-4401.html

6. ClorAM-X (Ammonia binder)
   - Reed Mariculture, http://www.reedmariculture.com/product_cloram_x.html
   - Check any local pet store for ammonia binder

7. Pure Oxygen
   - Pentair, http://pentairaes.com/
     - Oxygen Regulator
       - Part number OX3
     - Oxygen Hose
       - Part number OX4
     - Air Nozzle
       - Part number OX11
     - Air Filling Kit
       - Part number OXF1
   - Oxygen Tank – purchase or rent form a local distributor

8. Heat Packs
   - 20 & 40 hour packs for domestic (overnight) shipments
   - 20 & 60 hour packs for international shipments
   - Heat Pack Sources:
     - http://heatpacksource.com/
     - https://www.tsksupply.com/
   - For the UK: https://heatpacksuk.co.uk/

9. Bag clippers
   - Hand Tool and Rubber Bands
     - https://www.farmstore.com/product/economy-castration-bander/?gclid=EAIaIQobChMI6frRq7ye-AIVgMLCBB38nQIZEAQYBSABEgJPpvD_BwE
     - https://www.farmstore.com/product/ideal-castration-bands-100-ct/
   - Mechanical Clipper
     - https://www.tippertie.com/en/website-library/home/products/clipping/table-clippers/econo-line