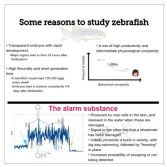

Working

Extraction of alarm substance in zebrafish

Rhayra Xavier do Carmo Silva[1], Sueslene Prado Rocha[2], Monica Gomes Lima-Maximino[2], Caio Maximino[3]

[1]Universidade Federal do Pará, [2]Universidade do Estado do Pará, [3]Universidade Federal do Sul e Sudeste do Pará

Sep 20, 2018 dx.doi.org/10.17504/protocols.io.tr3em8n

Fish behavior and physiology

Caio Maximino
Universidade Federal do Sul e ...

ABSTRACT

Alarm substance, or *Schrekcstoff*, is an mixture of compounds produced by specialized club cells in the epidermis of Ostariophysan fish. When lesioned, these cells release the substance, which acts as an aversive olfactory stimulus capable of evoking intense behavioral responses (alarm reaction) in other fish in the shoal (Maximino et al. 2018). Alarm substance is used as an aversive stimulus to study fear-like behavior in Ostariophysan - especially in zebrafish. The present protocol describes a technique to easily extract alarm substance for acute exposure.

TAGS

Zebrafish

Alarm substance

Show tags

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Maximino, C., Silva, R. X. do C., Campos, K. dos S., Oliveira, J. S. de, Rocha, S. P., Pyterson, M. P., ... Maximino, M. L. (2018). Sensory ecology of Ostariophysan alarm substances. Preprints.Org. https://doi.org/10.20944/preprints201803.0279.v1

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Guidelines for humane euthanasia must be followed, and animals should NEVER be euthanized without deep anesthesia. See Matthews & Varga (doi: 10.1093/ilar.53.2.192) for a review.

MATERIALS

NAME	CATALOG #	
Distilled Water		Contributed by users
15 ml sterile falcon tubes and rack		Contributed by users
scalpel blades		Contributed by users
Tweezers		Contributed by users
Ice		Contributed by users
Glass Petri dishes 90 x 15 cm		Contributed by users
Graduated Plastic Beakers, 2000ml	BK044-2K.SIZE.1	by Bio Basic Inc.
Cotton Gauze Swabs (10 x 10cm x 8 ply)	G5201 Emh40/0404	Contributed by users
Disposable pasteur pipettes	EA61.1	by Carl Roth
Graduated measuring cylinder 10 ml	View	Contributed by users

NAME	CATALOG #	
Aquarium thermometer	View	Contributed by users

SAFETY WARNINGS

1. Alarm substance extraction begins by cutting the fish skin with a scalpel; therefore, care must be taken to avoid accidents
2. All manipulations must be made using surgical gloves, to reduce the risk of contamination by aquatic bacteria (e.g., Mycobacterium marinum)

Preparation

1 Separate a donor fish from the home tank and transport to the experiment area

Anesthesia

2 Make crushed ice using cubes made from mineral water

REAGENT
Ice
Contributed by users

3 Fill a 2L beaker with water from the fish facility

REAGENT
Graduated Plastic Beakers, 2000ml
by Bio Basic Inc.
Catalog #: BK044-2K.SIZE.1

4 Bring the water temperature to 17 °C by adding the ice chips

5 Use a net to transfer the fish from the container to the cold water

6 Slowly add ice chips to the beaker to bring the temperature down to 12 °C. The animal is considered anesthetized when it no longer reacts to gentle manipulation.

Alarm substance preparation

7 Transfer the anesthetized fish to a Petri dish

REAGENT
Glass Petri dishes 90 x 15 cm
Contributed by users

8 Quickly decapitate the animal using a scapel; discard the head

9 Remove excess blood using a swab

REAGENT
Cotton Gauze Swabs (10 x 10cm x 8 ply)
Contributed by users
Catalog #: G5201 Emh40/0404

10 Transfer the body to another Petri dish

11 Using a scalpel, make 15 superficial cuts in both sides of the trunk

REAGENT
scalpel blades
Contributed by users

12 Wash the body with 10 ml ddH2O

REAGENT
Distilled Water
Contributed by users

13 Discard the body

14 Remove scales and other impurities from the solution

15 Transfer 7 ml from the solution to a Falcon tube

REAGENT
15 ml sterile falcon tubes and rack
Contributed by users

16 Store on ice or refrigerator for up to 4 h.