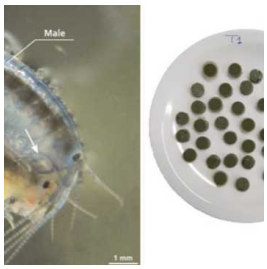
Jun 11, 2024

Assessing Fluoranthene Impact on Initiation of Feeding Response: A Time-to-Event Experiment with Parhyale hawaiensis



DOI
dx.doi.org/10.17504/protocols.io.5qpvok93zl4o/v1

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Protocol Citation: Ibrahim Lawan 2024. Assessing Fluoranthene Impact on Initiation of Feeding Response: A Time-to-Event Experiment with Parhyale hawaiensis. **protocols.io https://dx.doi.org/10.17504/protocols.io.5qpvok93zl4o/v1**

Protocol status: Working
We use this protocol and it's working

Created: June 10, 2024

Last Modified: June 11, 2024

Protocol Integer ID: 101540

Keywords: Feeding behaviour, Fluoranthene, chemosensory response, Parhyale hawaiensis, time-to-event (TTE), behavioural ecotoxicology, fluoranthene on aquatic organism, fluoranthene impact on initiation, assessing fluoranthene impact, fluoranthene exposure, event experiment with parhyale hawaiensi, influence of fluoranthene, valuable insights into the behavioural ecotoxicological impact, behavioural ecotoxicological impact, employing parhyale hawaiensi, aquatic organism, fluoranthene, aromatic hydrocarbon, dynamic chemosensory response, organism survival, feeding response, parhyale hawaiensi, polycyclic aromatic hydrocarbon, model organism, essential for organism survival, timing of crucial behavioural response, organism

Disclaimer

The protocol provided here is intended for educational purposes only and should be conducted in appropriate laboratory settings. The developers of this protocol are not liable for any damages or consequences arising from its use. Users should ensure compliance with relevant safety regulations and ethical guidelines when conducting experiments involving live organisms.

Abstract

This protocol delineates a controlled laboratory experiment employing *Parhyale hawaiensis*, an amphipod crustacean, as a model organism to assess the influence of Fluoranthene, a polycyclic aromatic hydrocarbon (PAH), on the dynamic chemosensory responses of *P. hawaiensis*. Using a time-to-event (TTE) methodology, we examine how Fluoranthene exposure affects the timing of crucial behavioural responses essential for organism survival and reproduction. This protocol offers comprehensive step-by-step instructions for conducting TTE experiments, providing valuable insights into the behavioural ecotoxicological impacts of Fluoranthene on aquatic organisms.

Materials

1. Laboratory culture tanks with *P. hawaiensis*
2. Stereomicroscope
3. Glass beakers (1-liter)
4. Exposure medium (reconstituted seawater)
5. Dimethyl sulfoxide (DMSO, solvent)
6. Fluoranthene (5g/L) stock solution
7. Fluoranthene working solutions:
    a. Control (0 µg/L)
    b. Low (10 µg/L)
    c. Medium (50 µg/L)
    d. High (250 µg/L)
8. Aeration system
9. Y-maze Petri dishes (70 ml)
10. Freshly prepared feed (standardised diet)
11. Timer or stopwatch
12. Mortality recording sheets
13. Laboratory notebook

Troubleshooting

Introduction

1 Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants originating from diverse anthropogenic sources like oil spills and combustion processes (Diamond et al., 2003; Hylland, 2006). Fluoranthene, a notable PAH, has garnered attention due to its persistence, bioaccumulation potential, and adverse effects on aquatic organisms (Behera et al., 2018). However, the influence of Fluoranthene on the chemosensory behaviour of aquatic organisms, particularly epibenthic amphipods, remains poorly understood. Specifically, the influence of environmentally relevant PAHs, like Fluoranthene, on chemosensory systems and potential alterations in ecological fitness are areas requiring further investigation. Chemosensation plays a pivotal role in various behaviours of crustaceans, including amphipods, such as food location, predator detection, mate finding, and physiological indicators (Hardege et al., 2022). We conducted a controlled laboratory experiment to address this knowledge gap using *P. hawaiensis* as our model organism. Employing a time-to-event (TTE) methodology, which provides a robust framework for examining the effects of environmental stressors on aquatic organism behaviour and physiology, we aimed to elucidate the impact of Fluoranthene on the chemosensory responses of *P. hawaiensis* and develop a protocol for TTE analysis in behavioural ecotoxicology. TTE experiments offer crucial insights into how pollutants influence the timing of specific behavioural responses, such as feeding or mating activities, which are vital for the survival and reproduction of organisms in aquatic ecosystems. Thus, this protocol outlines step-by-step procedures for conducting TTE experiments using *P. hawaiensis* as a model organism and Fluoranthene as the toxicant of interest.

Objective:
To investigate the impact of Fluoranthene on the dynamic chemosensory responses of *P. hawaiensis* and establish a protocol for time-to-event (TTE) analysis in behavioural ecotoxicology.

1. Collection and Sexing:

2

- Collect adult *P. hawaiensis* individuals from controlled laboratory culture tanks.
- Sex the organisms under a stereomicroscope to ensure precise identification of males and females.

Sexing male and female *P. hawaiensis*

2. Acclimation:

3

- Acclimate the organisms separately for 48 hours to establish consistent baseline conditions prior to the experiment.

3. Exposure Setup:

4

- Prepare stock solutions of Fluoranthene at 5g/L concentration
- Prepare four different environmentally relevant concentrations of Fluoranthene (0, 10, 50, and 250 µg/L) in 1-litre glass beakers containing 500 ml of exposure medium.
- Ensure continuous aeration to maintain uniform exposure conditions.
- Conduct exposures for one week in a static renewal system without feeding to stimulate appetite.

4. Mortality Assessment:

5

- Monitor mortality daily over the exposure period by visually inspecting each experimental container and removing dead individuals.
- Record mortality counts for each treatment group.

	Treatment	Mortality_count	Sex	Toxicant_Conc_µg/L
	Control	0	Male	0

	Control	0	Male	0
	...	...	...	...
	Low	1	Male	10
	Low	0	Male	10
	...	...	...	...
	Medium	3	Male	50
	Medium	3	Male	50
	..	...	...	...
	High	3	Male	250
	High	5	Male	250
	...	...	...	...
	Control	0	Female	0
	Control	0	Female	0
	...	...	...	...
	Low	1	Female	10
	Low	2	Female	10
	...	...	...	...
	Medium	4	Female	50
	Medium	5	Female	50
	...	...	...	...
	High	6	Female	250
	High	7	Female	250
	...	...	...	...

Table of Sample Mortality Recording Sheet

5. Y-maze Experiment Setup:

6

- After the exposure period, select at least **twelve (12)** surviving amphipods from each treatment group.
- Transfer the selected organisms individually to clean, reconstituted seawater in 70 ml Y-maze Petri dishes.

6. Acclimation to Y-maze:

7

- Allow the organisms to acclimate for at least three (3) minutes in one arm of the maze.

7. Feeding Response Recording:

8

- Introduce the freshly prepared feed tab to the other arm of the Y-maze.
- Record the time each organism responds to the feed within a **30-minute** window.
- **NB:** Response is actively visiting and starting feeding on the provided feed within the allotted timeframe.
- Censor organisms failed to respond within the **30-minute** period from the analysis.

Y-Maze with *P. hawaiensis* under time-to-respond to feed presence analysis

8. Data Collection:

9

- Record mortality counts, feeding response times, toxicant concentrations, and sex for each organism.

	Experiment_ID	Treatment	Organism_ID	Sex	Toxicant_Conc_µg/L	Start_Time	End_Time	Response_Time	Status

	1	Control	1	Male	0	1000	1006	6	1
	1	Control	2	Male	0	1000	1004	4	1
	1	Control	3	Female	0	1000	1007	7	1
	1	Control	4	Female	0	1000	1008	8	1
	...	...	...	...	...	...	...	...	...
	2	Low	4	Female	10	1100	1108	8	1
	2	Low	5	Female	10	1100	1109	9	1
	2	Low	6	Male	10	1100	1110	10	1
	2	Low	7	Male	10	1100	1111	11	1
	...	...	...	...	...	...	...	...	...
	1	Medium	1	Male	50	1003	1015	12	1
	1	Medium	2	Male	50	1003	1015	12	1
	1	Medium	3	Female	50	1003	1016	13	1
	1	Medium	4	Female	50	1003	1018	15	1
	...	...	...	...	...	...	...	...	...
	2	High	4	Male	250	1103	1133	30	0
	2	High	5	Male	250	1103	1122	19	1
	2	High	6	Female	250	1103	1125	22	1
	2	High	7	Female	250	1103	1123	20	1
	...	...	...	...	...	...	...	...	...

Table for Recording Time to Respond to Feed Experiment

a. Experiment_ID: This column identifies the experiment number or batch in which the data was collected. Each unique experiment is assigned a specific ID.
b. Treatment: This column describes the treatment condition applied to the organisms. It typically includes labels such as "Control" or specific treatment names, indicating whether the organisms were exposed to a toxicant or kept as controls.
c. Organism_ID: This column assigns a unique identifier to each individual organism involved in the experiment. It helps in tracking the responses of each organism across different conditions.
d. Sex: This column indicates the sex of the organism, categorised as "Male" or "Female."
f. Toxicant_Conc_µg/L: This column specifies the toxicant concentration (Fluoranthene) the organisms were exposed to.
g. Start_Time: This column records the start time of the observation period for the time-to-event (TTE) experiment.
h. End_Time: This column notes the end time of the observation period or the event of interest, such as feeding response.
i. Response_Time: This column calculates the time the organism takes to respond to the feed, which is the difference between the End_Time and Start_Time.
j. Status: This column indicates the status of the response, typically coded as binary values where "1" represents an event occurrence (e.g., successful initiation of feeding) and "0" represents a censored event (e.g., no response within the allotted time).

9. Data Analysis:

10

- To assess the effects of fluoranthene exposure on P. hawaiensis, perform appropriate statistical analyses, such as Kaplan-Meier estimates or Cox proportional hazards models time-to-event (TTE) analysis for feeding response time and Poisson logistic regression for mortality count.
- Explore relationships between toxicant concentrations, sex, mortality, and feeding response times using appropriate statistical software, such as R.
- Discuss any sex-specific differences observed in the responses.

10. General Considerations:

11 a. Standardise all experimental conditions, including temperature, lighting, and water quality parameters, to minimise variability.
b. Conduct pilot experiments to optimise the protocol and troubleshoot any potential issues.
c. Ensure the ethical treatment of organisms throughout the experiment, following institutional guidelines and regulations.

Conclusion

12 This protocol provides a comprehensive framework for conducting time-to-event experiments in aquatic ecotoxicology, specifically focusing on the behavioural responses of *P. hawaiensis* to Fluoranthene exposure. By following these standardised procedures, this protocol contributes to advancing robust analysis of behavioural ecotoxicology data and gaining insights into the impacts of pollutants on aquatic organism behaviour and survival.

Protocol references

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