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Cost-effective Approach for Assessing Chemosensory Response in Parhyale hawaiensis: A Time-to-Event Experiment

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We use this protocol and it's working

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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

Understanding the impact of environmental contaminants on chemosensation is crucial for assessing the ecological fitness of aquatic organisms. Here, we present a comprehensive protocol for investigating the chemosensory responses of *Parhyale hawaiensis* to food cues following exposure to Phenanthrene (Phe), a model polycyclic aromatic hydrocarbon (PAH). Using a time-to-event experimental design, we assess the organism's response time to fresh feed in a Y-maze setup under different exposure conditions. This protocol offers a simple approach for conducting cost-effective behavioural ecotoxicology and contributes to our understanding of the interactive effects of contaminants on aquatic organisms.

Materials

a) *Parhyale hawaiensis*
b) Reconstituted seawater
c) Phenanthrene (Phe)
d) Microplastics:
a. Polyamide (PA)
b. Polyethylene (PE)
c. Polyethylene terephthalate (PET)
e) Acetone
f) Freshly prepared feed
g) 70 ml petri dishes (Y-maze apparatus)
h) Stopwatch or timer

Introduction

1 Chemosensation plays a crucial role in the survival and ecological interactions of aquatic organisms, enabling them to detect and respond to chemical cues in their environment. This chemical communication is used by crustaceans, including amphipods, for various behaviours such as food location, predator detection, mate finding, and physiological indicators (Hardege et al., 2022). However, exposure to environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs) can disrupt chemosensory processes, potentially impairing vital behaviours such as foraging and predator avoidance in amphipods (Diamond et al., 2003). *Parhyale hawaiensis*, a tropical marine amphipod, relies on chemosensation for various behaviours crucial to its survival (Paris et al., 2022)., making it an excellent model organism for studying the impact of contaminants on chemosensory function. This protocol aims to establish a simple and cost-effective standardised methodology for assessing chemosensory responses in *P. hawaiensis* using a time-to-event experimental approach, with a focus on the effects of Phenanthrene (Phe) alone and in combination with microplastics (MPs).

Step I - Preparation of Exposure Medium:

2
- Prepare 500 ml seawater in four 1 L glass beakers for each treatment group.
- Prepare stock solution of phenanthrene (Phe) and microplastics (MPs) in carrier solvent (acetone in this case) (**NB:** this is for insoluble substances).
- Add appropriate concentrations of Phe (fixed at 50µg/L) and MPs (fixed at 500µg/L) to respective containers (**NB:** adjust this step based on your preferred toxicants and the determined effective concentrations).
- Ensure thorough mixing to achieve homogeneity.

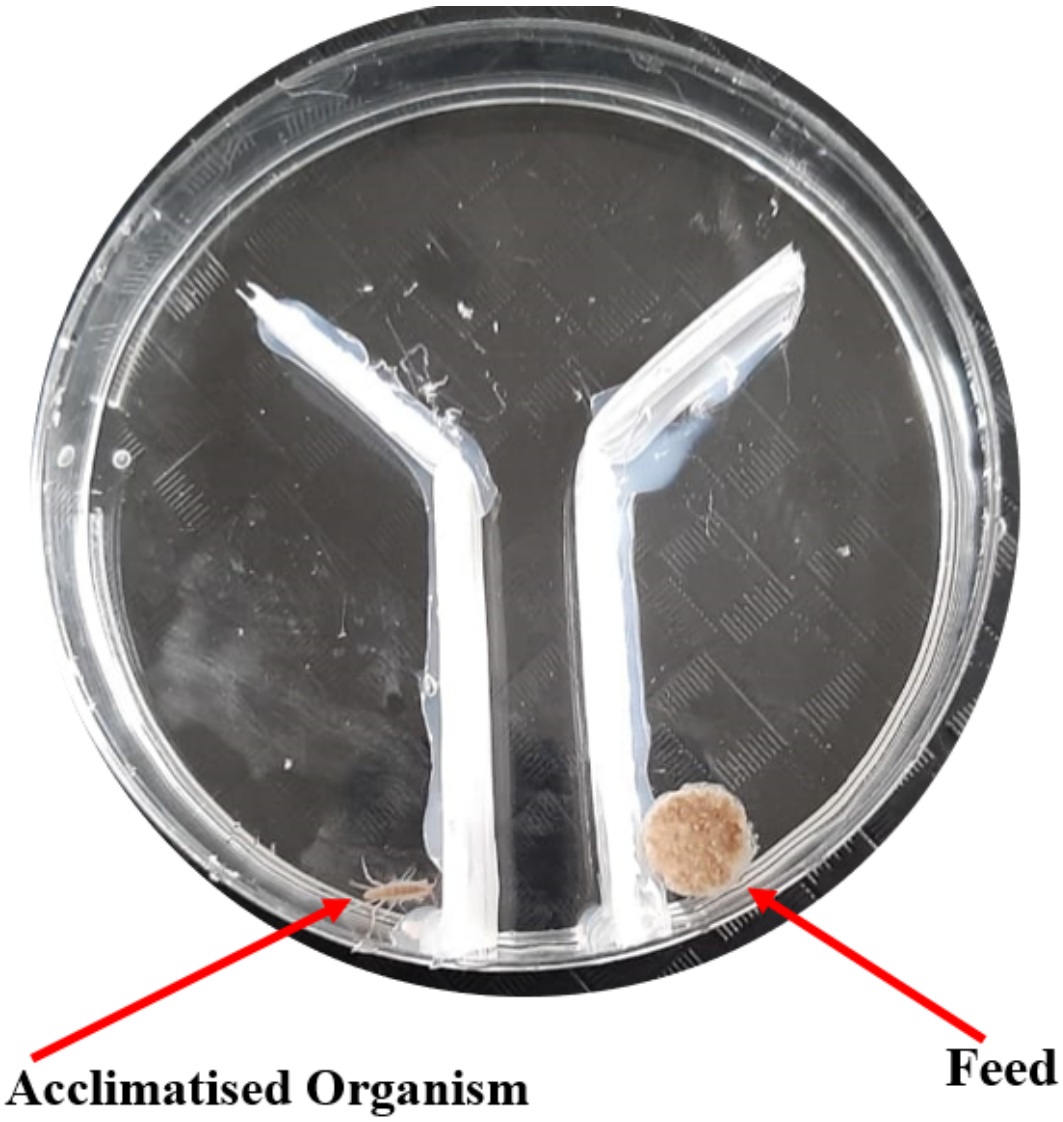
Step II - Acclimation of Parhyale hawaiensis:

3
- Collect and distribute organisms in 1 L glass beakers containing reconstituted seawater.
- Acclimate the organisms for **3 days** under controlled conditions.
- After 3 days, transfer organisms to individual containers with exposure medium and conduct long-term (21-day) exposure.
- After **21 days**, stop feeding and keep the living *P. hawaiensis* in the exposure medium for one week to stimulate appetite.

Step III - Time-to-Event Experiment Setup:

4
- Prepare the Y-maze apparatus (70 ml petri dish) and fill it with clean, reconstituted seawater.
- Introduce organisms into the Y-maze and allow acclimation in one arm of the maze for **5 minutes.**

- Introduce freshly prepared feed in the other arm and record the start time.



Y-Maze containing *P.hawaiensis* and feed

Step IV - Observation and Recording:

5

- Observe the behaviour of each organism and record the time taken to respond to the feed.
- Set a maximum observation time of **30 minutes** per trial.
- If an organism fails to respond within the allotted time, record it as censored.

	Treatment	Replicate	Organism_No	Sex	Start_Time	End_Time	Time	Status

	Seawater	1	1	Male	1000	1005	5	1
	Seawater	2	2	Female	1030	1034	4	1
	...	...	...	...	...		...	...
	Solvent	1	9	Female	1000	1009	9	1
	Solvent	2	10	Male	1030	1033	3	1
	...	...	...	...	...	...	...	...
	PA	1	17	Male	1000	1005	5	1
	PA	2	18	Female	1030	1039	9	1
	...	...	...	...	...	...	...	...
	PE	1	25	Male	1000	1011	11	1
	PE	2	26	Female	1030	1038	8	1
	...	...	...	...	...	...	...	...
	PET	1	33	Female	1000	1007	7	1
	PET	2	34	Male	1030	1036	6	1
	...	...	...	...	...	...	...	...
	Phe	1	41	Male	1000	1030	30	0
	Phe	2	42	Female	1030	1054	24	1
	...	...	...	...	...	...	...	...
	PA+Phe	6	54	Male	1105	1122	17	1
	PA+Phe	7	55	Female	1135	1156	21	1
	...	...	...	...	...	...	...	...
	PE+Phe	1	57	Female	1000	1022	22	1
	PE+Phe	2	58	Male	1030	1049	19	1
	...	...	...	...	...	...	...	...
	PET+Phe	1	65	Female	1000	1019	19	1
	PET+Phe	2	66	Male	1030	1053	23	1
	...	...	...	...	...	...	...	...

Recording table

In this table:

- "**Treatment**" indicates the different treatment levels to which the organisms were exposed.
- "**Replicate**" specifies the replicate number for each treatment.
- "**Organism_No**" represents the unique identifier for each individual Parhyale hawaiensis in the experiment.
- "**Sex**" denotes the sex of each organism (Male/Female).
- "**Start_Time**" denotes the time at which the feeding response observation began.
- "**End_Time**" represents the time at which a particular organism responded to the food (maximum of 30 minutes after the start time).

- "**Time**" indicates the total time a particular organism spent before responding to the food.
- "**Status**" indicates whether the organism has responded (1) to the feed within the 30-minute observation period and denoted with 1 or not responded and denoted with 0 (i.e., censored).

NB: You can adjust the number of replicates and customise other aspects of this table to fit your specific experimental design and data recording needs.

Step V - Data Analysis:

6
- Compile response times from all trials for each treatment group.
- Perform statistical analysis (e.g., Kaplan-Meier OR Cox regression survival analysis) to compare response times between treatment groups.
- Assess significance levels and generate survival curves to visualise differences in chemosensory responses.

Step VI - Interpretation and Reporting:

7
- Interpret findings in the context of exposure to a preferred toxicant.
- Discuss implications for chemosensory function and feeding behaviour in *Parhyale hawaiensis*.

Conclusion

8 This protocol provides a simple, cost-effective and standardised methodology for investigating the chemosensory responses of *Parhyale hawaiensis* in the presence of Phenanthrene (Phe) and microplastics (MPs). By employing a time-to-event experimental design, researchers can accurately assess the impact of contaminants on feeding behaviour and chemosensation in aquatic organisms. The insights gained from this study contribute to our understanding of chemosensory endpoints in ecotoxicology and offer valuable implications for assessing the ecological risks associated with environmental contaminants.

Protocol references

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