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Assessing Growth Parameters in amphipod, *Parhyale hawaiiensis*: Weighing and Measuring Length

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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

The developed protocol provides a standardised method for precisely determining the weight and length of *Parhyale hawaiiensis*, a crucial model organism in ecotoxicology and ecophysiology research. By employing precise techniques with analytical scales and imaging software, researchers can obtain reliable data to investigate the effects of environmental stressors on aquatic ecosystems.

Materials

Materials used in this protocol include:

1. Sartorius digital analytical scale (0.01 ± 0.02 mg)
2. Small dry, clean weighing boat or petri dish
3. Liquepipette pipette (127-P330-000)
4. ImageJ software
5. Graph sheet

Introduction

- 1 *Parhyale hawaiiensis* is a circumtropical marine amphipod crucial for epibenthic communities in tropical marine ecosystems (Poovachiranon et al., 1986). This amphipod has gained recognition for its sensitivity to environmental stressors and ecological significance, making it a promising model organism for tropical marine studies (Dos Santos et al., 2022) and a valuable laboratory model for various disciplines (Paris et al., 2022). To fully utilise *P. hawaiiensis* as a global model for ecotoxicology and ecophysiology research, it is essential to establish a standardised and precise protocol for accurately measuring its weight and length. Doing so will enable researchers to collect accurate data and investigate the impact of environmental stressors on aquatic ecosystems, ultimately advancing our understanding of the ecotoxicological effects of key environmental contaminants.

Weighing *Parhyale hawaiiensis* Wet Weight to Measure Growth Parameters

- 2 To measure the weight of the amphipod, *Parhyale hawaiiensis*, a Sartorius digital analytical scale (0.01 ± 0.02 mg) that can measure small masses with a high degree of accuracy was used. Here is a general protocol for weighing *P. hawaiiensis*:
- 3 Obtain a small dry, clean weighing boat or petri dish.
- 4 Turn on the analytical balance and allow it to stabilise for a few minutes.
- 5 Place the small weighing boat on balance and tare it (i.e., set it to zero) or record the weight of the boat.
- 6 Cut the tip of a 127-P330-000 aliquette pipette at 3 - 4 mm
- 7 Gently use the cut pipette and transfer the specimen (a group of 4 to 6 amphipods, based on preference) into the weighing boat on the scale.
- 8 Use another aliquette pipette to remove all the water from the boat and around the amphipods.
- 9 Record the weight of the amphipods. OR



- 10 Subtract the weight of the empty weighing boat from the combined weight of the boat and the specimen to obtain the weight of the amphipods.
- 11 Expose the organisms for a specified experimental period, such as one to two weeks.
- 12 Obtain a new specimen (a group of 4 to 6 amphipods, based on preference) of the organisms at the end of the exposure.
- 13 Measure the weight of the specimen.
- 14 Compare the weight of the specimen from the start and end of the exposure to analyse growth parameters. OR
- 15 Repeat the process at several time points to determine the overall growth rate of the amphipods over time.
- 16 Repeat the process for the additional specimens if needed.
- 17 **NB:** Handling the amphipods gently and with care and keeping their environment as stable as possible are essential to avoid stress or causing any damage during the process. Also, it is crucial to keep the weighing boat and scale clean to avoid contaminating the specimen.

Measuring the Length of *Parhyale hawaiiensis* to Analyse Growth Parameters

- 18 To measure the body length of a curved amphipod crustacean such as *Parhyale hawaiiensis*, ImageJ software was used in a slightly different approach when measuring a straight-line length. Therefore, the body length was calculated as the distance along the dorsal side from the base of the first pair of antennae to the base of the telson, as suggested by (Asochakov, 1994). Here are the steps to measure the length of *P. hawaiiensis* using ImageJ:
- 19 Place a clean and dry petri dish on a graph sheet.
- 20 Cut the tip of a 127-P330-000 liquipette pipette at 3 - 4 mm

- 21 Use the cut pipette to gently transfer the specimen (a group of 4 to 6 amphipods, based on preference) into the petri dish.
- 22 Use another liquid pipette to remove all the water from the petri dish and around the specimen.
- 23 Take an image of the specimen and open it in ImageJ.
- 24 Use the "Set Scale" function in ImageJ to set the correct pixel-to-length conversion. To do this, select the "straight line" tool from the toolbar, measure a known length of a reference object in the image (such as a 10 mm size from the graph in the picture) and select "Analyze", then set the scale as 10 mm known size. ImageJ will then adjust the length measurements accordingly.
- 25 Right-click and select the "segmented line" tool from the toolbar.
- 26 Carefully trace the curved body of *Parhyale* from the base of the first pair of antennae to the base of the telson with the "segmented line" selection tool. Try to follow the curve body as closely as possible without including overlapping areas.
- 27 Right-click at the last point to drop the "segmented line" selection tool and select "Analyze", and then "Measure" to obtain the length of the amphipod.
- 28 Repeat the process for the additional amphipods from the specimen.
- 29 Once done with all the amphipods from the specimen, select "Analyze" from the top menu and then "Measure". This will open a window that displays various measurements of the selections, including the lengths. Copy the length measurements of all the amphipods from the specimen into Excel and take the average as the length of a particular specimen. Or save the measurements by selecting the desired output format.
- 30 **NB:** To avoid stress or damage, the amphipods must be handled gently and with care, and their environment must be kept as stable as possible.

Conclusion

- 31 In summary, the developed protocol offers a robust and systematic approach for accurately determining the weight and length of *Parhyale hawaiiensis*, an amphipod that is crucial for ecotoxicological research. By employing a Sartorius digital analytical scale

with high precision and ImageJ software for weight and length measurements, respectively, this protocol ensures the reliability and consistency of data acquisition. The method highlights the importance of handling amphipods with care to minimise stress and maintain environmental stability throughout the procedure, thus reducing the likelihood of any potential harm to the organisms. Additionally, the protocol provides clear and concise step-by-step instructions, which facilitates the ease of replication and application in research settings. Overall, this protocol serves as a useful tool for scientists aiming to assess growth parameters and analyse the effects of environmental stressors on aquatic organisms, contributing to advancements in the field of aquatic ecotoxicology.

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