Amphipod size variation and substrate color preferences in coastal habitats: an experimental study in Ujung Genteng, West Java

Majariana Krisanti[1*], *Ali* Mashar[1], *Inna* Puspa Ayu[1], *Achmad* Farajallah[2], *Ananingtyas*[3], *Mita* Aprilia[1], *Jauhar* Zainal Arifin[4], *Reza* Zulmi[1], *Vendi* Eko Susilo[5], *Tazkiah* Aulia Putri[6], *Irwan* Iskandar[7], *Musti'atin*[7], and *Yusli* Wardiatno[1]

[1]Department Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, IPB University, Dramaga Bogor 16680, Indonesia
[2]Department Biology, Faculty of Mathematic and Natural Sciences, IPB University, Dramaga Bogor 16680, Indonesia
[3]Faculty of Agriculture, Djuanda University, Bogor 16720, Indonesia
[4]Graduate Student of Coastal and Marine Resources Management, Department Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, IPB University, Dramaga Bogor 16680, Indonesia
[5]Doctoral Student of Animal Biosciences Study Program, Department Biology, Faculty of Mathematic and Natural Sciences, IPB University, Dramaga Bogor 16680, Indonesia
[6]Magister Student of Animal Biosciences Study Program, Department Biology, Faculty of Mathematic and Natural Sciences, IPB University, Dramaga Bogor 16680, Indonesia
[7]Mining Engineering, Faculty of Mining and Petroleum Engineering, Institut Teknologi Bandung (ITB), Bandung 40132, Indonesia

Abstract. Amphipods are key detritivores in coastal ecosystems, and their behaviour and habitat selection can offer insights into their survival strategies. This study aims to investigate the substrate colour preferences and size variation in *Traskorchestia georgiana*, an amphipod species inhabiting sandy beaches. Experiment was done in August 2024 at coastal habitat of Ujung Genteng, Sukabumi, West java. Through a series of controlled experiments, amphipods were exposed to four different substrate colours (yellow, pink, green, and light blue) to assess their preferences and size distribution with Kruskal-Wallis and Friedman Test. Although statistical analysis indicated no significant colour preferences ($p > 0.05$), observable trends suggested a higher average number of amphipods on the pink substrate compared to the light blue. Additionally, size variation among the amphipods did not correlate with substrate colour. These findings suggest that other factors, such as substrate texture or moisture content, may play a more important role in habitat selection for these species. The study contributes to a broader understanding of amphipod behavior and provides insights into coastal ecosystem management and conservation, particularly in the context of habitat restoration efforts.

[*] Corresponding author: majariana.krisanti@apps.ipb.ac.id

1 Introduction

Amphipods are an order of crustaceans commonly found in marine, estuarine, and freshwater environments [1], playing an important role in aquatic ecosystems. They are known for their ecological diversity. There are 10,590 recognized amphipod species in marine, brackish, freshwater, and terrestrial environments [2]. Amphipods are found in a wide range of habitats from sandy beaches to rocky intertidal zones [1]. They play a vital role in the food chain of ecosystems, particularly as detritivores, helping recycle organic matter and serving as food for predators such as fish and seabirds [1 ,3]. Additionally, amphipods contribute to the overall health of aquatic ecosystems by facilitating nutrient cycling, supporting biodiversity, and acting as bioindicators that reflect the quality and changes in their habitats [4]. Their presence and abundance can provide crucial information regarding environmental conditions, such as pollution levels and habitat stability [5]. For example, amphipods have been used to monitor pollution levels in coastal habitats [3].

Amphipods exhibit a range of visual adaptations, such as sensitivity to light intensity and color, that play a crucial role in their habitat selection and predator avoidance strategies. Their vision is relatively well-developed compared to other crustaceans, enabling them to perceive differences in light intensity and colour, which is essential for selecting optimal substrates for camouflage [6]. Amphipods use visual ability to identify substrates that match their body coloration, enhancing their ability to blend in and reduce predation risk. This behaviour could happen in species inhabiting intertidal zones, where the dynamic nature of light and substrate requires precise matching to maintain effective camouflage [7]. Differences in substrate preference among amphipod species might be linked to variations in their visual capabilities.

Understanding substrate color preferences in these species is crucial for several reasons. First, color preferences may reveal how a species optimizes its camouflage strategies to avoid predation. Selecting substrates that match their body color can enhance their survival from predation by birds, fish, and other visual predators. Additionally, studying substrate color preferences can provide insights into habitat selection and behavior [8]. Amphipods are bioindicators of environmental health due to their sensitivity to habitat changes and pollution [5]. By understanding how substrate color affects their preferences, we can better predict their responses to changes in coastal environments, such as beach erosion, habitat restoration, or artificial substrate use [9].

This study aims to identify whether sand hoppers exhibit substrate color preferences on sandy beaches. It also seeks to evaluate whether body size differences are associated with the color of the substrate they select.

2 Methods

2.1 Location and species

The experiment was conducted on a supratidal sandy beach at Ujung Genteng (GPS coordinates -7.357744249034122, 106.40320405795556), West Java, Indonesia (Fig. 1), in August 2024. In this study, we focus on species *Traskorchestia georgiana* (Fig. 2) [10]. *T. georgiana*, is widely distributed across coastal environments and can be found on a variety of coastal substrates. They tend to exhibit variable coloration, possibly reflecting its adaptability to different substrates [8].

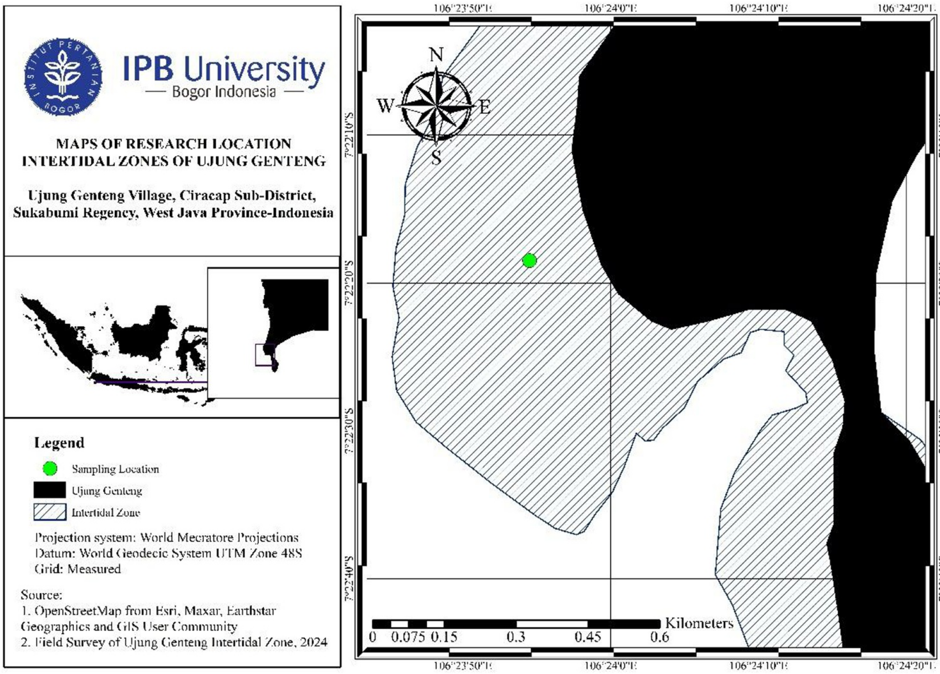

Fig. 2. Research location map (Credit: Arifin, August 2024).

Fig. 2. *Traskorchestia georgiana*. (Photo credit: Krisanti, September 2024).

2.2 Experimental design

Four containers of different colours (yellow, pink, green, and light blue) were filled with sea water and randomly placed on the beach (Fig. 3). The containers were left for 10 minutes to attract the amphipods jump into the water. The Amphipods that stay in the container were counted and collected for body length measured. The experiment was repeated four times (four replicates), with the container order changed for each replicate.

In addition to counting the number of amphipods from each species attracted to each colour, the sizes of individual amphipods were measured in millimeters (mm) using Olympus SZ61 stereo microscope connected to a Beta microscope camera and BetaView program.

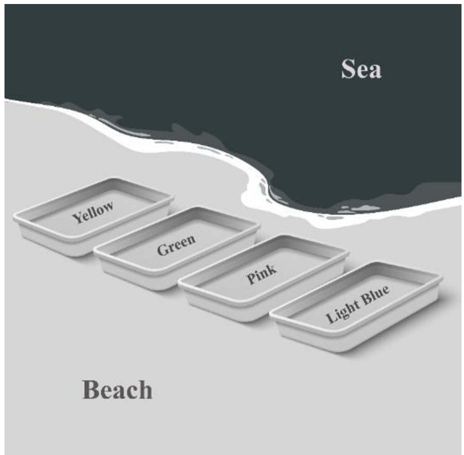

Fig. 3. Container arrangement on the beach. (Credit: Krisanti, September 2024).

2.3 Statistical analysis

Two primary statistical tests were used:

1. Kruskal-Wallis Test to compare substrate colour preferences and to analyze the size differences based on substrate colour. Since the data on amphipod abundance and sizes may not fulfill the normality or equal variance assumptions necessary for parametric tests, the Kruskal-Wallis Test serves as a strong alternative. This test allows for the determination of significant differences in the medians of amphipod abundance and sizes across various substrate colors without relying on a specific distribution. This adaptability is especially beneficial in ecological research, where data can often be skewed or contain outliers [11].
2. Friedman Test to evaluate the effect of replicates on the results. This non-parametric statistical test evaluates differences in treatments across multiple test attempts, making it ideal for analyzing repeated measures or matched groups [12].

3 Results and discussion

3.1 Results

The experiment showed pie chart (**Fig 4.**) illustrates the average number of amphipods found on substrates of different colours: Yellow, Pink, Green, and Light Blue. Based on the chart, the following observations can be made:

- The **Pink substrate** has the highest average number of amphipods, represented by the largest segment of the pie chart, which is labelled as **7**.
- The **Green substrate** is the second most preferred, with an average number of **6** amphipods.
- The **Yellow substrate** follows with an average of **5** amphipods.
- Lastly, the **Light Blue substrate** has the lowest average, with **4** amphipods observed.

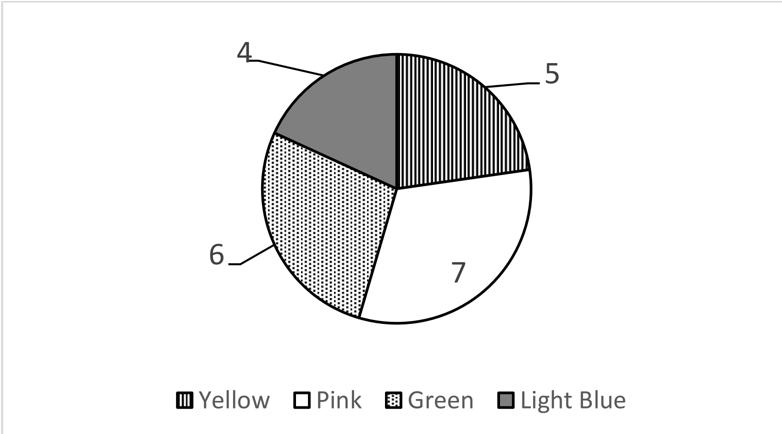

Fig. 4. Average number of *T. georgiana* in each substrate colour**.**

The distribution of amphipods across the four substrate colours (Yellow, Pink, Green, and Light Blue) showed a trend in which the pink substrate attracting the highest average number of individuals, while the Light Blue substrate the lowest. The differences in average abundance across the substrates could indicate varying suitability or preference due to factors such as camouflage effectiveness, temperature, or other environmental conditions related to substrate colour.

Although these patterns suggest potential preferences, statistical tests Kruskal-Wallis Test did not reveal significant differences in substrate color preferences for *T. georgiana* ($p = 0.613$). The Friedman Test ($p = 0.182$) also showed no significant differences across replicates. This condition suggesting that the observed variations could be due to random fluctuations rather than true substrate preference

From all specimens, we measure total body length that can be seen in Fig. 5. Body length of *T. georgiana* from pink and green substrate have a wider spread of data, indicating more variability in body length measurements compared to yellow and light blue substrate. The median of total body length from yellow, pink, green and light blue substrate respectively were 8.96 mm, 10.18 mm, 9.03 mm, and 9.41 mm. Body length from pink substrate has the highest median value, while yellow has the lowest.

Despite some differences in medians and ranges, all four categories show a relatively similar range, with most body lengths falling between 6 mm and 15 mm. This plot is effective in highlighting the variability within each category, as well as comparing the central tendency (median) across the four groups.

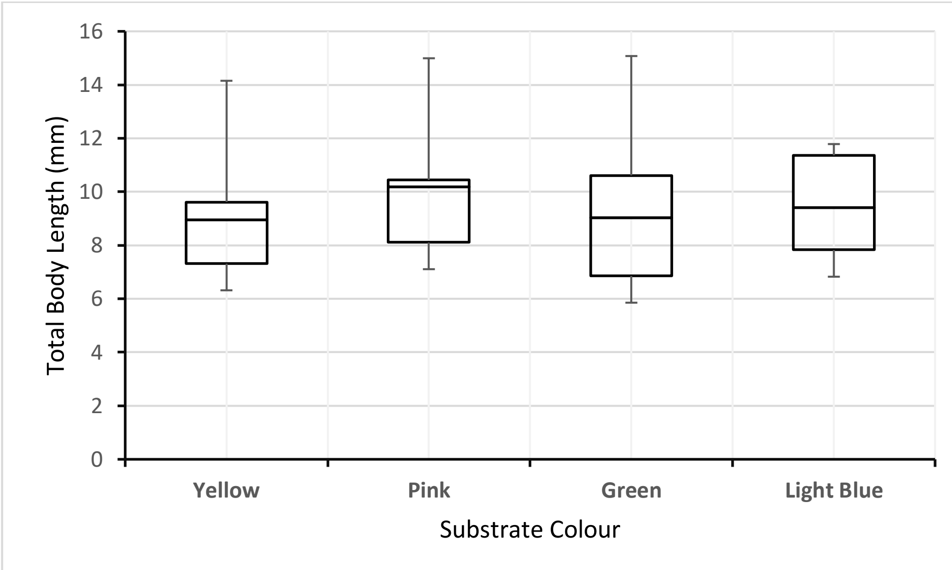

Fig. 5. Total body length of *T. georgiana* in each substrate colour**.**

3.2 Discussion

The results of this study provide insights into the substrate colour preferences of *Traskorchestia georgiana* and highlight patterns in the body lengths of individuals found on different substrates. Although statistical analyses revealed no significant differences in substrate preferences, some trends were observed that merit discussion.

The distribution of amphipods across the four different coloured substrates (Yellow, Pink, Green, and Light Blue) demonstrated a noticeable trend in which the pink substrate supported the highest average number of individuals (31%), while the Light Blue substrate had the lowest. This suggests that amphipods may be more attracted to the pink substrate due to factors such as better camouflage, suitability for temperature regulation, or other environmental cues related to the colour.

The distribution of amphipods across different colored substrates, with the pink substrate supporting the highest average number and the Light Blue supporting the lowest, can be explained by their negative phototactic behavior. Amphipods tend to avoid light and prefer darker or duller environments [13]. Lighter-colored substrates reflect more light, making them appear brighter, while darker substrates absorb most of the light, giving them a more muted or dull appearance [14]. In this case, Light Blue substrates reflect more lighter and thus seem less appealing to amphipods. In contrast, darker or less reflective colors like Pink absorb more light, giving them a duller appearance that offers better shelter and camouflage, making these substrates more attractive habitats for amphipods.

While the pink substrate showed a higher average presence of amphipods, it is essential to note that these trends were not statistically significant. Previous studies suggest that color may play a role in camouflage and predator avoidance; however, more targeted research is required to confirm whether such factors influence habitat selection for *T. georgiana*. When juvenile amphipods are released, they start off transparent but develop the distinct pink coloration of adults by consuming coral tissues. This feeding behavior enables them to imitate the color of their host. Evidence of their ingestion of coral tissues is found in the sclerites from the host present in the amphipods' digestive tracts. Additionally, amphipods display protective behaviors by deterring predatory nudibranchs that approach the coral colony, thereby protecting their host [15].

However, it is important to note that the Kruskal-Wallis's test (p = 0.613) and the Friedman test (p = 0.182) indicated that these differences were not statistically significant. This implies that, although we observed variations in the number of amphipods on different substrates, these differences could be due to random variability rather than a true preference for one colour over another. Further studies with larger sample sizes or under different environmental conditions could help clarify whether substrate colour plays a definitive role in habitat selection for *T. georgiana*.

The body lengths of *T. georgiana* amphipods from each substrate also provided interesting results. Amphipods from the Pink and Green substrates exhibited a wider spread of body length measurements, indicating more variability in size compared to those from the Yellow and Light Blue substrates. The median body length from the pink substrate was the highest (10.18 mm), while the yellow substrate had the lowest median body length (8.96 mm). Despite these differences, the overall range of body lengths across all substrates remained fairly consistent, with most individuals falling between 6 mm and 15 mm.

The observed variability in body length across different substrate colours showed wider ranges on pink and green substrates, with a higher median length on the pink substrate. However, the lack of statistical significance indicates that these differences are not conclusive. Further research with larger sample sizes or under controlled environmental conditions may help clarify any potential links between body size variation and substrate colour.

Although the statistical analyses did not show significant differences in substrate preference or body length distribution across substrate colours, the trends observed in the data suggest that colour may still play a role in habitat selection for *T. georgiana*. The amphipods' preference for the pink substrate, in particular, could be worth further investigation, especially in relation to environmental factors such as light, temperature, and camouflage.

Future research could explore the role of additional variables, such as substrate texture, moisture levels, or temperature gradients, in driving habitat selection. Additionally, a larger sample size or experiments conducted over longer periods might reveal more definitive patterns of preference or variability in body length. It would also be valuable to investigate the underlying biological mechanisms, such as visual cues or physiological responses, that could influence the interaction between amphipods and substrate colour.

In conclusion, while this study did not reveal statistically significant substrate preferences or body size variations, the observed trends provide a foundation for further exploration into the ecological interactions between *T. georgiana* and its environment. Understanding these interactions could contribute to a broader understanding of amphipod habitat selection and the factors influencing their distribution in natural ecosystems.

Relevance to Marine Biology Knowledge Development in Indonesia

This experiment offers significant value for the development of marine biology knowledge in Indonesia, a country with one of the longest coastlines and the richest marine biodiversity in the world. Indonesia's coastal ecosystems are vital both ecologically and economically, supporting diverse species that contribute to fisheries, tourism, and coastal protection. Amphipods, though small, play a crucial role in the functioning of these ecosystems as detr itivores and as prey for larger species [16]. Understanding their behavior, including how they interact with their environment, is fundamental for coastal ecosystem management and conservation.

Furthermore, given the growing pressures on Indonesia's coastal areas from human activities—such as habitat destruction, pollution, and climate change—having detailed knowledge of the habitat preferences of species like amphipods can help inform coastal management strategies [17]. This study, although focusing on a seemingly narrow topic of

substrate color preferences, contributes to a broader understanding of how species may or may not rely on specific environmental factors when selecting habitats. This kind of ecological insight is critical as Indonesia continues to implement conservation initiatives like the establishment of marine protected areas (MPAs) and the restoration of degraded coastal ecosystems.

In the context of habitat restoration, especially in initiatives aimed at rebuilding beach ecosystems or using artificial substrates, understanding how organisms like amphipods interact with their environment can guide restoration efforts. For instance, if certain species prefer specific substrate types for reasons other than color—such as texture or moisture content—this information could be applied to create more suitable environments that support a full range of biodiversity.

As Indonesia continues to develop its marine research infrastructure and seeks to balance conservation with economic development, studies like this one provide the groundwork for more sophisticated models of species behavior and ecosystem interactions. Expanding this knowledge base is key to sustaining the health of Indonesia's coastal and marine ecosystems in the face of ongoing environmental changes.

4 Conclusion

This study found no statistically significant preferences for substrate colour on sandy beaches for *Traskorchestia georgiana*. While observable trends suggested a higher attraction to the pink substrate, these were not confirmed by statistical analysis, indicating that the species may prioritize other factors, such as substrate texture or moisture, in habitat selection. Further studies are recommended to explore these factors under varied environmental conditions.

Acknowledgement

Directorate General of Higher Education, Research, and Technology Ministry of Education, Culture, Research, and Technology in accordance with the 2024 Research Program Implementation Contract Number: 027/E5/PG.02.00.PL/2024 dated 11 June 2024.

References

1. E.E. Ruppert and R.D. Barnes, Invertebrate Zoology, 6th Ed., (Saunders College Publishing, Forth Worth, (1994)
2. WoRMS Editorial Board. World Register of Marine Species. Available from https://www.marinespecies.org at VLIZ. Accessed 2024-10-10. doi:10.14284/170 (2024)
3. C.J. Ritter and D.G. Bourne, Marine amphipods as integral members of global ocean ecosystems, Monograph. J. Exp. Mar. Biol. Ecol. **572**, 151985 (2024) https://doi.org/10.1016/j.jembe.2023.151985
4. J.A. Pechenik, *Biology of the Invertebrates*. 7th Ed. McGraw-Hill Education. (2014)
5. C. Navarro-Barranco, M. Ros, J.M. Tierno de Figueroa and J.M. Guerra-García, Marine Crustaceans as Bioindicators: Amphipods as Case Study, In book: The Natural History of Crustaceans, Vol.9: Fisheries and Aquaculture Publisher: Oxford University Press. (2020) DOI: 10.1093/oso/9780190865627.003.0017
6. E. Naylor, Chronobiology of Marine Organisms. Cambridge University Press. (2011).
7. M. Stevens and G.D Ruxton, The key role of behaviour in animal camouflage, Biol. Rev. **94**, pp.116–134. 116, DOI: https://doi.org/10.1111/brv.12438 (2019)

8. L. Fanini and J. Lowry, Coastal talitrids and connectivity between beaches: A behavioural test, J. Exp. Mar. Biol. Ecol. **457**: 120–127 (2014) http://dx.doi.org/10.1016/j.jembe.2014.04.010
9. K. L. Sobocinski, J. R. Cordell and C. A. Simenstad, Effects of Shoreline Modifications on Supratidal Macroinvertebrate Fauna on Puget Sound, Washington Beaches, Estuaries and Coasts **33**:699–711 (2010) DOI: https://doi.org/10.1007/s12237-009-9262-9
10. J.W. Chapman, The Light and Smith manual, chapter 39: intertidal invertebrates from central California to Oregon, 4th ed. University of California Press, Berkeley, CA, (2007)
11. P.E. McKnight and J. Najab, Kruskal-Wallis test, in *The Corsini Encyclopedia of Psychology*, 1-1 (2010) https://doi.org/10.1002/9780470479216.corpsy0491
12. M.R. Sheldon, M.J. Fillyaw, and W.D. Thompson, The use and interpretation of the Friedman test in the analysis of ordinal-scale data in repeated measures designs, Phys. Ther. Res. Int. **1**(4): 221–228 (1996) https://doi.org/10.1002/pri.66
13. M.D. Kennedy and V.P. Connaughton, Differential effects of fluoxetine on the phototactic behavior of 3 amphipod species (Crustacea; Amphipoda), Environ. Toxicol. Pharmacol. **93**: 103889 (2022) https://doi.org/10.1016/j.etap.2022.103889
14. N. Schaefera, M.J. Bishop, A.B. Bugnot, B. Herbert, A.S. Hoeye, M. Mayer-Pintof, C.D.H. Sherman, C. Foster-Thorpe, M.L. Vozzo, and K.A. Dafforn, Variable effects of substrate colour and microtexture on sessile marine taxa in Australian estuaries, Biofouling **40**(2): 223–234 (2024) https://doi.org/10.1080/08927014.2024.2332710
15. G. Caulier, J.F. Hamel, E.A. Hendrycks, K.E. Conlan, and A. Mercier, Mutualistic relationship between the amphipod *Stenula nordmanni* (Stephensen, 1931) and the nephtheid coral *Gersemia rubiformis* (Ehrenberg, 1834), Symbiosis **85**: 93–104 (2021) https://doi.org/10.1007/s13199-021-00800-5
16. C.J. Ritter and D.G. Bourne, Marine amphipods as integral members of global ocean ecosystems, J. Exp. Mar. Biol. Ecol. **572**: 151985 (2024) https://doi.org/10.1016/j.jembe.2023.151985
17. P. Parretti, M. Ros, I. Gestoso, P. Ramalhosa, A.C. Costa, and J. Canning-Clode, Assessing biotic interactions between a non-indigenous amphipod and its congener in a future climate change scenario, Aquat. Invasions **16**(2): 186–207 (2021) https://doi.org/10.3391/ai.2021.16.2.01