Journal of Environmental Studies [JES]
An International Journal
https://jesj.journals.ekb.eg/
doi: 10.21608/JESJ.2024.270871.1070

Variations in shell morphology of freshwater mussels *Anodonta anatina* and *Unio tumidus* (Bivalvia: Unionidae) from Lake Polvijärvi, Finland

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Citation: *Abdelsaleheen, O. (2024). Variations in shell morphology of freshwater mussels Anodonta anatina and Unio tumidus (Bivalvia: Unionidae) from Lake Polvijärvi, Finland, Vol. 33(1): 1-6.*

Article Information

Received 17 Feb. 2024,
Revised 22 Feb. 2024,
Accepted 26 Feb. 2024,
Published online March 2024

Abstract: Freshwater mussels play an important role in aquatic ecosystems and have been the subject of numerous ecological and taxonomical studies. The present study investigated the shell morphology of *A. anatina* and *U. tumidus* with focusing on the width-length, height-length, and width-height relationships in Lake Polvijärvi, Finland. Both species revealed distinct external shell morphologies, where *A. anatina* exhibited an elongated shell shape with a slightly curved dorsal margin and a smooth surface, while *U. tumidus* had a shorter and flat shell with a more swollen umbo region and a rough surface. The correlation analysis demonstrated strong positive relationships between width-length, height-length, and width-height variables for both species ($R^2 \geq 0.82$). Linear regression models accurately described these relationships indicating that shell width and height increased consistently as shell length increased. Both of *A. anatina* and *U. tumidus* exhibited positive slopes in the width-length relationship suggesting a widening of shells with increasing length, however, *A. anatina* had a higher intercept than *U. tumidus* indicating a wider shell at a given length in comparison to *U. tumidus*. In the height-length relationship, both species showed positive relationships, but *U. tumidus* exhibited a steeper slope indicating a faster rate of increasing shell height with increasing length compared to *A. anatina*. These findings contribute to understand the patterns of shell morphology for both species; and the observed species-specific dependence have implications for their growth, proportions, ecological adaptations, and shedding light on their ecological niches and evolutionary dynamics.

Keywords: Unionids, Morphometry, Duck mussel, Swollen River mussel.

Introduction

Freshwater mussels are a diverse and ecologically important group of bivalves found in rivers, lakes, and streams around the world (**Strayer, 2008**). They are known for their unique life cycle, which involves a parasitic larval stage known as the glochidium; and for their ability to filter large quantities of water and improve its quality (**Strayer, 2008; Soliman *et al.*, 2016**). They play a vital role in aquatic ecosystems as they help to maintain water quality by filtering out particles and organic matter, and by removing excess nutrients such as nitrogen and phosphorus (**Vaughn & Spooner, 2006; Strayer, 2008**). In addition, they are an important food source for many aquatic and terrestrial animals, including humans (**Howard & Cuffey, 2006; Abdelsaleheen, 2019**). In addition to their ecological importance, freshwater mussels also have cultural and economic significance in many regions around the world. They have been used for food, tools, and jewelry for thousands of years (**Howard & Cuffey, 2006; Abdelsaleheen, 2019**). Freshwater mussels are currently facing a variety of threats, such as habitat loss, pollution, and overharvesting, which have led many species to be endangered or threatened. Therefore, conservation efforts are needed to protect them and their habitats (**Auld *et al.*, 2013; Qu *et al.*, 2018; Ferreira-Rodríguez *et al.*, 2019; Abdelsaleheen, 2023**). Unionids undergo significant changes in their shell morphology, as they grow from juveniles to adults, and these changes can be influenced by a range of environmental and genetic factors (**Rhoads & Pannella, 1970; Inoue *et al.*, 2013; Guarneri *et al.*, 2014; Soliman *et al.*, 2019**). For instance, in some bivalve species, the shape of the shell changes as the animal transitions from a larva to a sessile adult. This change is thought to be driven by the need to anchor the animal to the substrate,

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and to protect it from predators (Soroka *et al*., 2021). Changes in the shell morphology may also occur when mussels reach their sexual maturity and begin able to reproduce. Moreover, shell morphology can be influenced by food availability and interspecific competition. For instance, in areas with limited food resources, mussels may develop thicker shells to provide additional protection against predators (Vereycken & Aldridge, 2023). Similarly, in situations where multiple bivalve species coexist, competition for resources can lead to changes in shell morphology to reduce overlap in resource use (Lopes-Lima *et al*., 2021).

The study of population-level variation in shell morphology can provide insights into the factors that shape mussel populations over time, as well as the role of shell morphology in local adaptation and speciation, i.e., it can help in identifying the threatened populations under different environmental stressors (Soroka *et al*., 2021). It can also shed light on the evolutionary history of bivalves and the factors that have contributed to their diversification over time (Lopes-Lima *et al*., 2021). The variation in shell shape among mussels can also have important implications for their ecological role. For instance, some studies have shown that shell morphology can affect a bivalve's ability to filter water and remove harmful pollutants (Vereycken & Aldridge, 2023). Additionally, shell shape can influence the mussel's ability to anchor itself to the substrate, which has implications for its feeding and movement (Béguinot, 2018). Understanding this variation is crucial for assessing the health of mussel populations and their ecological roles.

Anodonta anatina and *Unio tumidus* are two common species of freshwater mussels in European rivers and lakes (Abdelsaleheen *et al*., 2021; Abdelsaleheen *et al*., 2022; Abdelsaleheen, 2023; Abdelsaleheen *et al*., 2023). Both species are known to exhibit considerable morphological variation in their shells, but the extent and causes of this variation are not well understood. They are among the most imperiled groups of freshwater organisms in Europe with declining populations due to a range of threats, including habitat loss, over-exploitation, pollution, eutrophication, loss of fish hosts, and the introduction of invasive species (Auld *et al*., 2013; Qu *et al*., 2018; Ferreira-Rodríguez *et al*., 2019; Abdelsaleheen, 2023). In the face of these threats, there is an urgent need to better understand the details of the morphological variation of these species for developing effective conservation and management strategies, as well as understanding the role of these important organisms in freshwater ecosystems (Soroka *et al*., 2021). Therefore, this study aims to investigate the shell morphology of *Anodonta anatina* and *Unio tumidus* and compare the shell characteristics, including length, width, size, shape, and surface features, between both species.

Materials and methods

Sampling and species identification

A total of 160 specimens each of *A. anatina* and *U. tumidus* were collected from Lake Polvijärvi in Eastern Finland during June and July 2022. The samples included both live and dead mussels. The identification of the two mussel species, *A. anatina* and *U. tumidus*, was based on a combination of external and internal shell characteristics, following the taxonomic keys developed by (Kennard *et al*., 1925; Ellis, 1978). All experiments were made with the consent of the national committee for animal experimentation (permission CSRE-30-23).

For each mussel specimen, the length (L) (the maximum distance between the anterior and posterior margins of the shell), the width (W) (the maximum distance between the outer surfaces of the valves), and the height (H) (the maximum distance between the umbo and the ventral surface margin) were measured to the nearest 0.1 mm using digital Vernier caliper (Fig. 1). The relationships between shell morphology variables (width-length, height-length, and width-height) were figured and the regression equations were calculated as follows: $Y = a + b X$; where "a" (intercept), and "b" (slope).

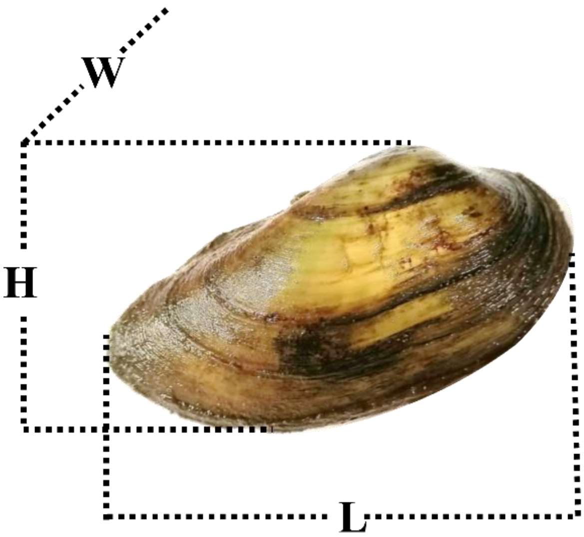

Figure (1): Schematic representation illustrating the measurements of freshwater mussel's shell (L = Shell length; H = Shell height; W = Shell width).

Statistical analysis

Pearson's correlation was used to check the correlation between shell morphology variables, including width-length, height-length, and width-height relationships. The significant differences of linear relationships between both species were tested by using the linear mixed model. The statistical significance differences were tested by the statistical software SPSS program (IBM software version 27.0). The differences between the mean values were deemed statistically significant if $p < 0.05$.

Results

External shell morphology in *A. anatina* and *U. tumidus*

The external shell morphology of the two mussel species, *A. anatina* and *U. tumidus*, is distinct and can be used as a reliable characteristic for their identification. The shell of *A. anatina* has an elongated shape with a slightly curved dorsal margin, and a smooth surface. The dorsal and ventral valve margins diverge towards the posterior with a swelling appearance in the dorsal region, located slightly posterior to the umbo. This swelling is sometimes referred to as the "umbo swelling". Another characteristic feature of *A. anatina* is the presence of wavy umbonal rugae, which are raised ridges or folds on the shell surface near the umbo. The color of the shell can range from dark brown to greenish brown (Fig. 2A&B). In contrast, the shell of *U. tumidus* is relatively short and squat with a more swollen umbonal region, and a straight dorsal margin. Moreover, the hinge length anterior to the umbonal swelling is shorter than *A. anatina*, and the ventral valve margin is convex. The surface of the shell is rough with prominent concentric ridges and radial furrows. The color of the shell can range from dark brown to greenish yellow (Fig. 2C&D).

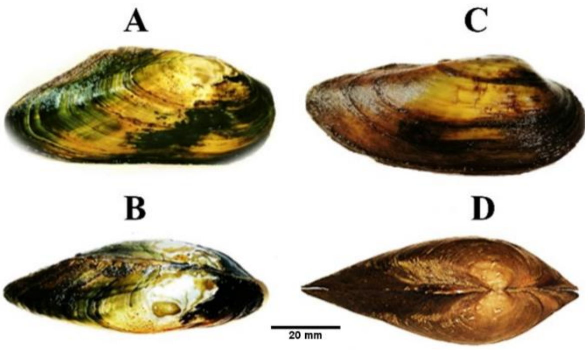

Figure (2): Lateral and dorsal view for the shell of *A. anatina* (A& B), and *U. tumidus* (C&D). Scale bar = 20 mm.

Comparative analysis of shell morphology in *A. anatina* and *U. tumidus*

The relationships between shell morphology variables (width-length, height-length, and width-height) were strongly positively correlated for *A. anatina* (R^2= 0.82; R^2= 0.89; R^2= 0.87; $p < 0.001$); and *U. tumidus* (R^2= 0.95; R^2=0.96; R^2= 0.94; $p < 0.001$), respectively. The relationships were best described by linear functions indicating that the width and height of the shells increased consistently with the increase in the shell length for both species. These findings are represented in Figures 3, 4 & 5.

In terms of the width-length relationship using regression analyses (Fig. 3), *A. anatina* exhibited a slope of 0.31, while *U. tumidus* showed a slightly higher slope of 0.34 ($p > 0.05$). These results indicate that both species display positive slopes and a tendency for the shells to wide as the shell length increases. However, there is a notable difference in the intercept values where *A. anatina* has a significantly higher intercept (1.43) than *U. tumidus* (0.58) ($p < 0.05$) suggesting that *A. anatina* tends to have a considerably wider shell compared to *U. tumidus* at a given length.

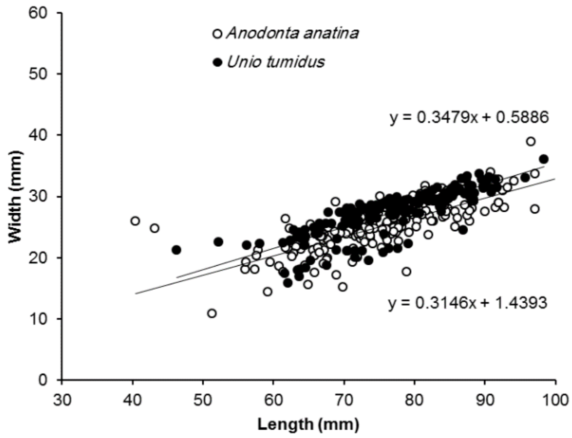

Figure (3): The width-length relationship for *A. anatina* and *U. tumidus* shows the Linear regressions for *A. anatina* (The upper line) and *U. tumidus* (lower line).

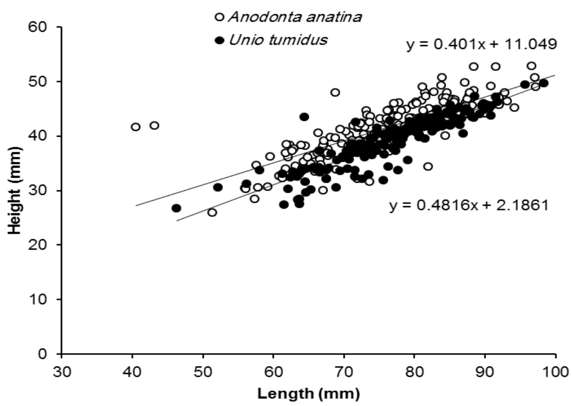

Figure (4): The height-length relationship for *A. anatina* and *U. tumidus* shows the Linear regressions for *A. anatina* (The upper line) and *U. tumidus* (lower line).

The height-length relationship showed that *A. anatina* has a slope of 0.40 and an intercept of 11.04, while *U. tumidus* shows a steeper slope (0.50) and lower intercept (2.18) (Fig. 4; $p < 0.05$). These results suggest that *U. tumidus* tends to have an elongated shell length in comparison to *A. anatina* for a given length, i.e., *U.*

tumidus has a faster rate of increase in shell height as length increases in comparison to *A. anatina*. Moreover, the examination of the width-height relationship revealed that *A. anatina* has a slope of 0.72 and an intercept of -0.95, while *U. tumidus* has a slightly lower slope (0.65) and an intercept (-1.76) (Fig. 5). These findings indicate that both species tend to widen their shells as the shell height increases.

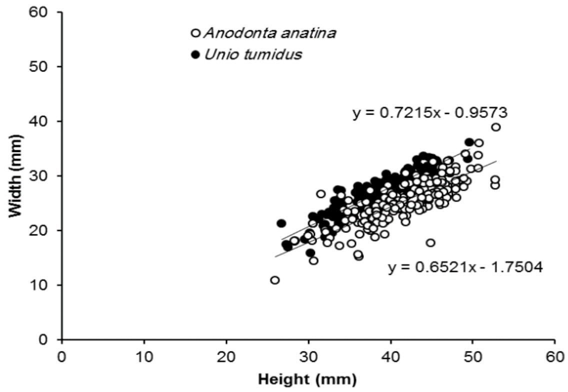

Figure (5): The width-height relationship for *A. anatina* and *U. tumidus* shows the Linear regressions for *A. anatina* (The upper line) and *U. tumidus* (lower line).

Discussion

The shell morphology of freshwater mussels (Unionidae) has long been a subject of scientific inquiry as it provides essential insights into their biology, ecology, and adaptation to their respective habitats (**Haag 2012; Graf, 2013**). Accurate species identification, including shell morphology, is critical for effective conservation management (**Haag & Williams, 2014; Lopes-Lima *et al.*, 2017**). The clear differences in shell length and other morphological features between *A. anatina* and *U. tumidus* in the present study underscore the importance of considering shell characteristics in taxonomic assessments and monitoring programs.

Generally, shell morphology of freshwater mussels is species- and habitat dependent (**Keogh *et al.*, 2023**). Indeed, the current study revealed interesting patterns of shell morphology in *A. anatina* and *U. tumidus*, particularly in the width-length relationship. Both species exhibited slightly similar slopes that indicates a widening trend as shell length increased for both species. However, the striking difference in intercept values suggested that *A. anatina* consistently possessed wider shells compared to *U. tumidus* for a given length. Therefore, the significant difference in shell morphology between *A. anatina* and *U. tumidus* in Lake Polvijärvi might return to different environmental or biological factors as parasites prevalence, where infected mussels have flattened (thinner) shell in comparison to healthy mussels (**Müller *et al.*, 2015**)., e.g., healthy *U. tumidus* has wider shell than infected *A. anatina* by parasites in Lake Viinijärvi (**Abdelsaleheen, 2023; Abdelsaleheen *et al.*, 2023)**. On the other hand, the reproduction activity might reflect on the shell morphology, where mussels with higher reproduction activity have wider shells. To this end, the shell of *A. anatina* might be wider than *U. tumidus* in Lake Polvijärvi because of higher reproduction activity in *A. anatina* or parasites prevalence in *U. tumidus* or other factors that need more studies. This observation aligns with the previous studies that documented a significant variation in shell shape and size among freshwater mussel species (**Haag & Williams, 2014; Lopes Lima *et al.*, 2017**).

Several studies observed variations in the shell thickness, shell shape, shell sculpture, growth rate, and overall development of freshwater mussels between the same species across different lake-stream habitats, such as *A. anatina* and *U. tumidus* in Lake Polvijärvi (the current study) have different shell morphology in comparison to the same species at Lake Viinijärvi (**Abdelsaleheen, 2023**), and National Nature Reserve, Wicken Lode, United Kingdom (**Aldridge, 1999**); or between different species at the same habitat, as *Pyganodon grandis* and *Lampsilis radiata siliquoidea* in USA (**Keogh *et al.*, 2023**); *Anodonta cygnea*, *Pseudanodonta complanate*, *Anodonta anatina*, *Unio pictorum* and *Unio tumidus* in National Nature Reserve, Wicken Lode, U. K. (**Aldridge, 1999**). Likewise, the b value (0.72) of the width-height relationship was higher for *A. anatina*, the present study, compared to previously studied mussel species, such as *A. anatina*, *P. complanate*, *U. tumidus*, *P. corrugate*, and *P. favidens*, and lower than *A. cygnea and U. pictorum* (**Aldridge 1999; Ramesha & Thippeswamy, 2009; Thippeswamy *et al.*, 2014**). Similarly, *U. tumidus* in the present study exhibited a higher b value (0.65) compared to *P. complanate*, *P. corrugate*, and *P. favidens*, and lower than *A. anatina*, *A. cygnea*, *U. pictorum*, and *U. tumidus* (**Aldridge, 1999; Ramesha & Thippeswamy, 2009; Thippeswamy *et al.*, 2014).**

It is essential to consider the potential environmental drivers behind the observed shell morphology. Previous research has demonstrated that the influence of environmental factors such as water chemistry, flow velocity, and substrate type on mussel shell shape (**Seed 1968; Krapivka *et al.*, 2007**). Shell morphology often holds ecological significance for freshwater mussels. The shape and size of the shell can impact burying behavior, resistance to predation, and interactions with their environment (**Beadman *et al.*, 2003; Johnson, 2020**). In the current results, the difference in shell width between *A. anatina* and *U. tumidus* could reflect distinct ecological strategies or selective pressures, potentially related to feeding, habitat, or predation dynamics. These factors were not directly assessed, and future investigations should explore whether the difference in shell morphology between *A. anatina* and *U. tumidus* populations returns to reproduction activity, parasites prevalence, or other environmental factors.

Conclusion

The comparative study on the shell morphology of *A. anatina* and *U. tumidus* provides valuable insights into their distinct characteristics and growth patterns. Both species exhibit positive correlations between shell width, height, and length, indicating consistent increases in these dimensions as they grow, however, *A. anatina* having a wider shell compared to *U. tumidus* at a given length. These findings contribute to our understanding of shell morphology and highlight the importance of considering multiple parameters when studying freshwater mussels. Further research is needed to explore the functional implications of these morphological patterns and their ecological adaptations, and to explore the genetic and environmental factors that underlie the observed shell variations.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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