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Mussels with luggage: the influence of artificially attached "backpack" devices on mussel movement behavior

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Abstract

Background Freshwater mussels are important keystone and indicator species of aquatic ecosystems. Recent advances in sensor technology facilitate applications to individually track mussels and to record and monitor their behavior and physiology. These approaches require the attachment of sensor devices as "backpacks" to the outer shell surface. The interpretation of such data makes it necessary to understand the influence of these attachments on the horizontal and vertical movement behaviors of freshwater mussels. Over a series of mesocosm experiments, this study systematically investigated the effects of three size- and wiring-specific variants of artificially attached backpacks on the horizontal and vertical movement behavior of *Anodonta anatina*.

Results Across all experiments, equipping mussels with backpacks did not result in a significant influence on horizontal movement for any of the backpack variants. In contrast to this finding, the big backpacks with a high ratio between backpack volume and mussel length resulted in a significantly negative effect on vertical movement, indicating a potential for adverse effects of such devices on mussels, especially in natural settings.

Conclusions The findings of this study show that assessing the effects of attached devices on mussels requires a species-specific evaluation of potential impacts on the endpoints of interest. Especially for vertical movement patterns, selection of the smallest available devices appears mandatory.

Keywords Freshwater mussels, Ecological indicators, Biological early warning systems, Backpacks, Sensors, Behavior, Burrowing

Background

Freshwater mussels provide a wide range of essential functions to aquatic ecosystems, including nutrient cycling, filtration of large water volumes [1], biodeposition of particulate matter from the water column [2, 3], as well as sediment mixing [4, 5]. Due to their relative sensitivity and high conservation status, many species of freshwater mussels are considered target species for conservation and indicator species for water and habitat quality [6, 7]. The occurrence of freshwater mussels has also been seen as a trigger for increased taxon richness of other invertebrates in aquatic ecosystems [8], albeit this role may be impaired in intensively used catchments [9]. Additionally, due to their sensitivity, sedentary lifestyle, and filtering of large volumes of water, the behavior of mussels is increasingly being used for biomonitoring of water quality [10, 11].

Freshwater mussel populations continue to decline in Europe [6, 12–15] as well as globally [16], requiring increasing conservation efforts to sustain populations and their functions in aquatic ecosystems. A better understanding of the specific reactions of the mussel to environmental changes is essential for the assessment

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and the further improvement of conservation measures efficiency. This typically includes (a) the implementation of suitable population monitoring strategies in the respective habitats, (b) in situ exposure experiments or (c) systematic laboratory-based investigations to determine the effects of individual stressors. Therefore, modern technology is increasingly applied:

Commonly used for monitoring purposes are very small technological devices such as Passive Integrated Transponder tags (PIT tags) (e.g., [17–21]). These are attached to the mussels for long-term use, e.g. for the purpose of recapture [19]. Since these devices do not need to transfer information continuously, e.g. via mobile networks, no battery and no transfer device are needed. The cylindrical PIT tags are available in different sizes, e.g. by the company Biomark (Idaho, USA), in a length ranging from 8 mm (Mini HPT8 PIT Tag) to 32 mm (HDX32 PIT Tag). For the assessment of direct reactions of mussels to environmental changes, the valve gaping behavior can be measured in situ by devices such as Hall effect sensors (e.g., [10, 11, 22, 23]). The sensors are usually connected to a data-logging device outside of the water via cable, but future applications will include wireless devices [24, 25]. The latter devices contain a battery, a microcontroller, a Hall-effect sensor, a clock, and a radio and antenna to transmit data and are hence bigger ([24], approximately 4 cm × 3 cm × 1 cm) than wired or passive devices like PIT tags. Hall sensors can also be used to study behavioral reactions under controlled conditions, most commonly by using a wired connection to a data logger in a laboratory setting [2, 11, 26]. The size of these sensor devices can vary between 3 mm and 2 cm (e.g., [2, 11, 22, 26–29].

In addition to using mussel sensors in the context of bioindication of water quality [10], there is also an increasing number of supportive breeding programs for endangered species [30–32] that benefit from monitoring released captive-bred specimens. For instance, stocking measures to augment declining wild populations demand a subsequent monitoring of survival, growth and dispersal of captive-bred mussels, which demands respective tools for identification and tracking.

Because backpacks are increasingly being used in conservation studies, there is considerable information on how to attach them to the mussels' shells [33]. However, there is no published information on the influence depending on the backpack's size and only few information available from the literature on the influence of backpacks on mussels' movement behavior [34]. A substantial body of literature confirms that, although adult mussels are sedentary, horizontal and vertical movement behaviors are ecologically relevant and can be influenced in various ways [35–47]. For example, horizontal and vertical movement behaviors are important for adapting to resource availability [43] as well as to floods and droughts [48, 49] and burrowing behavior can be considered an indicator of population health [50]. Consequently, knowledge of the effects of backpacks on mussels is crucial for avoiding bias in behavioral studies or adversely affecting conservational efforts. However, to the best of our knowledge, no study has yet systematically tested the effects of various types of sensors attached as "backpacks" to living mussels on their behavior.

To fill this knowledge gap, this study's objective was to investigate the effects of backpacks on the horizontal and vertical movement behavior of the freshwater mussel species *Anodonta anatina*. For this, three different variants of dummy backpacks were used, which were designed based on different functional devices, including two wireless and one wired variant. For each of the backpack variants, it was hypothesized that (1) distances covered during horizontal movement and (2) vertical movement, including positioning and burrowing, do not differ significantly between mussels with and without attached backpacks.

Methods

For the investigation of mussel movement and burrowing behavior a mesocosm setup was chosen, to combine the advantages of controlled conditions with the advantages of considering the bigger picture of realistic environmental conditions [51].

Experimental setup

The series of experiments (see experimental design below) were conducted in mesocosms. In total, 5 mesocosms were operated in parallel, of which 4 were used for experiments and 1 was used to shelter the remaining mussels. Each of the 130 L-mesocosm-tanks (78 × 56 × 43 cm SAMLA Box, IKEA, Hofheim-Wallau, Germany) contained 10 cm of washed sand substratum (0.1–0.9 mm granularity Rosi's Rosnerski Aquariensand, Königslutter, Germany) and had a continuous water flow resulting in full water exchange about every hour. The water was pumped from the river Moosach and was led through aquaria filter foam pads (30—10 pores per inch) before flowing into the mesocosm. The filter foam pads did not remove all particles from the water but reduced them to slow down the clogging of water tubes that were used for water transport. Each of the experimental mesocosms were framed by LED stripes below the water level, to minimize reflections on the water surface. The lights remained turned on continuously, to enable continuous recordings. The mesocosms were filmed from above via two Raspberry Pi cameras (Raspberry Pi 3B + with 5MP NoIR camera for Raspberry, Raspberry Pi Foundation in association with Broadcom Inc., Cambridge, United Kingdom). At random points in time (see experimental design below), each mesocosm was additionally equipped with 4 GoPro cameras, one in each corner of the mesocosm, totaling 16 GoPros per experimental run.

After each experimental run, mussels were put into the additional mesocosm and the 4 experimental mesocosms were cleaned thoroughly, including draining all water, washing the sand substratum, flushing the inflow water tubes and scrubbing the walls of the mesocosm tanks to allow the next experimental run to start with optimal light and contrast conditions.

Experimental design

Backpack variants

Three different backpack variants were tested. All backpacks were designed and produced by ECOSOPH GmbH (Garching, Germany), imitating functioning devices from their portfolio in size and weight. The backpacks were 3D-printed in the form of cuboids and filled with epoxy resin to imitate the weight of the devices including the technical components (for specifications regarding size and weight see Table 1). No technologically relevant components were incorporated into the backpacks used for this study.

The big backpacks (see Fig. 1A) were designed according to functioning prototypes of wireless sensor devices from ECOSOPH GmbH. The original functioning prototypes include a Hall sensor device, an accelerometer, a device for wireless data transfer, and a power source in the form of a rechargeable battery. These prototypes exemplify devices that need to store or transmit data without a wired connection to an external data storage or data transfer device, as, e.g., used for tracking nitrate in the Mississippi [25].

The small wired backpacks (see Fig. 1C) were designed according to functioning prototypes of wired sensor devices from ECOSOPH GmbH. The original functioning prototypes included a Hall sensor device and an accelerometer, but no internal data transfer device or power source. These were added externally through wiring. Usually, wired devices with similar functionality are smaller than the here used but due to the need for proper mounting, the size of the actual devices can be increased by covering them with an adhesive, as, e.g., for using an accelerometer to study valve gaping behavior [27].

The small unwired backpacks (see Fig. 1B) are based on the same prototype as the small wired backpacks but without wiring and are supposed to represent small actively communicating backpacks (e.g., [52]) and passively communicating devices (e.g. PITtags). As for the wired variant, the passively communicating and the unwired actively communicating equivalents are smaller than the here used device, but due to covering in adhesive, the overall size of the attachment can increase compared to the bare device. Hence, this variant is an over-approximation of currently used passively communicating devices and this needs to be considered for the interpretation of the experimental results. In addition to testing the influence of small wireless devices, using this backpack variant in addition to the wired variant also allows for testing the effect of wiring.

For reference (see Fig. 1D), mussels were equipped with fabric tape (for details see mussel handling).

Experimental runs

For each backpack variant, 2 experimental runs were conducted. In each experimental run, all 4 mesocosms were used, each containing 3 mussels with backpacks and 3 reference mussels, totaling in 24 mussels per experimental run and hence 48 mussels per test of backpack variant. For the experimental runs for small unwired backpacks (SU1 & SU2), the same mussels were used as for small wired backpacks (SC1 & SC2). Therefore, one batch of mussels was first equipped with unwired backpacks (SU1) and later wiring was added (SC2) and one batch of mussels was first equipped with wired backpacks (SC1) and later the wiring was removed (SU2). Since no technologically relevant parts were used in the backpacks, the wiring was simply glued on and cut off the backpacks.

Table 1 Specifications of the three different backpack variants used in the different experimental runs

Experimental runs	BU1 & BU2	SU1 & SU2	SC1 & SC2
Backpack variant	Big backpack	Small backpack	Small backpack
Wiring	No	No	0.5 m
Length × Height × Width	4.1 × 1.7 × 3.4 cm	2.4 × 1.0 × 1.8 cm	2.4 × 1.0 × 1.8 cm
Volume	23.7 cm^3	4.3 cm^3	4.3 cm^3
Weight	24.95 g	5.70 g	5.70 g

For each experimental run, all 4 mesocosms were used

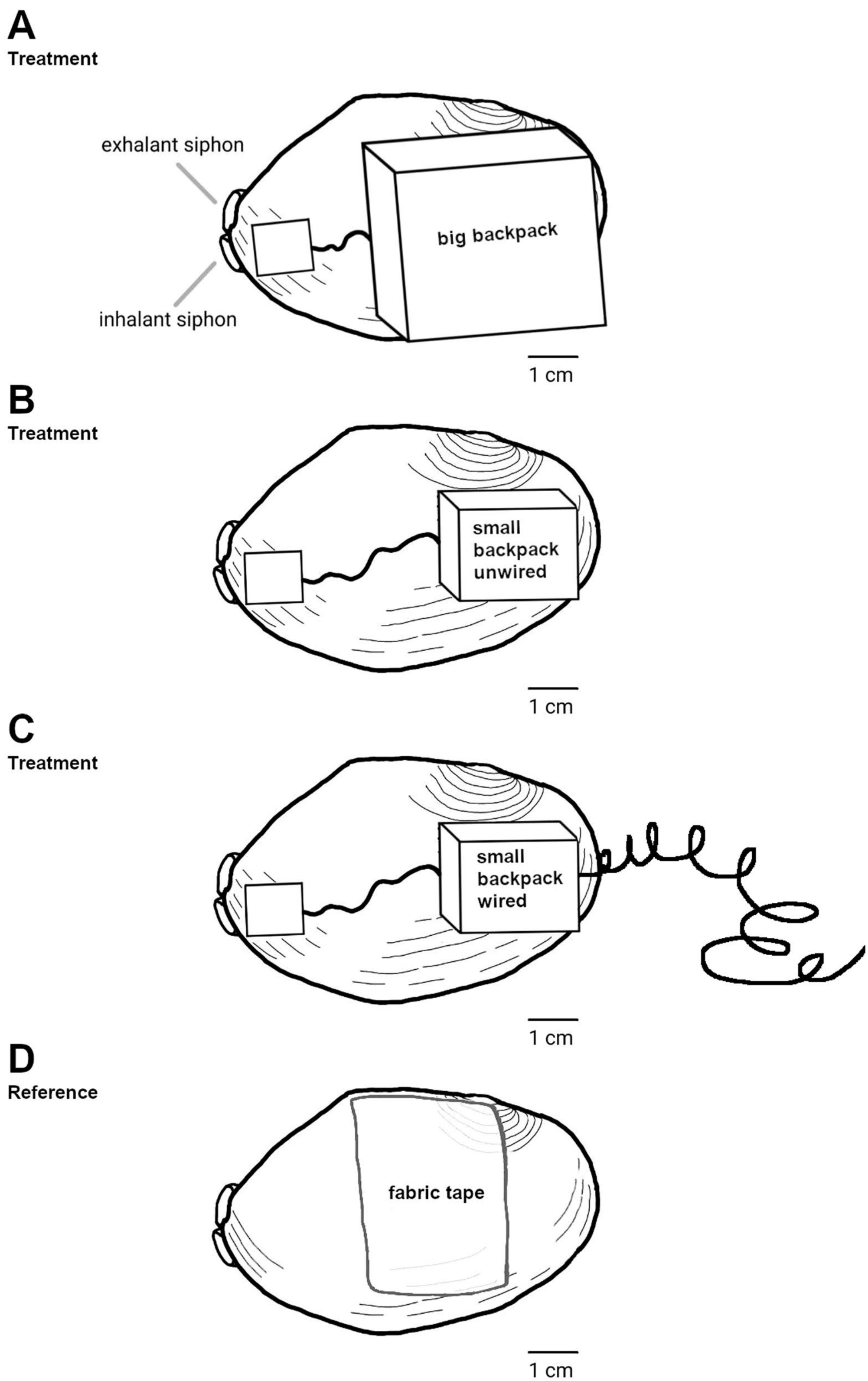

Fig. 1 Schematic of mussels with three types of backpacks (Treatment) and with fabric tape (Reference). **A** Big backpack (4.1 × 1.7 × 3.4 cm) imitating a wireless, multi-measurement device, including a device for data transfer and a rechargeable battery. **B** Small wireless backpack (2.4 × 1 × 1.8 cm) imitating a wireless (passive) device. **C** Small wired backpack (2.4 × 1 × 1.8 cm) without a data transfer device or an included power source. **D** Fabric tape for color coding in reference mussels

Data acquisition

All experimental runs were recorded for 15 h by the cameras installed above the mesocosm tanks with a framerate of 1 fps in the first experimental run (SU1) and 2.3 fps in the remaining five experimental runs (SU2, SC1, SC2, BU1, BU2). The initially very low framerate was meant to reduce the need for memory space but due to difficulties in the automatized analysis of horizontal movement, the framerate had to be increased. GoPro cameras were positioned in the mesocosms at random points of time, to capture random samples of burrowing depth and upright behavior. Each time the GoPros were inserted, they captured 1 picture per minute for approximately 2 h (the duration of the battery lifetime). Data from two of these insertions (=2×2 h) were used for the examination of vertical movements.

For each mussel, the backpack volume was divided by the mussel's shell length and the value was used as parameter "Proportion backpack volume by mussel length".

Mussel handling

This study used 96 mussels of the species *Anodonta anatina* (Linnaeus, 1758) purchased via NatureHolic GmbH (Mannheim, Germany). The mussels averaged 8.7 ± 1.2 cm in length and 5.4 ± 0.8 cm in width (mean ± standard deviation). The mussels were acclimatized for 7 days prior to their first experimental run in the acclimatization/waiting tank.

For each experiment, the mussels were handled on the day of the start of the experiment to attach backpacks or fabric tape or to attach/cut off wiring. For handling, mussels were put in a small basin that was filled approximately 1 cm with water. Within approximately 2 h, all mussels for the upcoming experimental run were either equipped with the according backpack variant (on one half of the shell) or with fabric tape (on both halfs of the shell). Both were attached to the shell with Dupla® Plant-Fix since it was successfully tested as an adhesive for tagging mussels [33]. Each mussel was taken out of the water basin, carefully wiped clean and slightly dried with a paper towel before attaching the respective backpack or fabric tape as shown in Fig. 1. Afterwards, mussels were kept on a dry paper towel for approximately 5 min for first hardening of the adhesive before being put back into the water basin.

Before each experimental run, the mussels were individually distributed in a lateral position in the mesocosms, with the backpack facing towards the water surface. For experimental runs with wiring, additionally the cables were attached to the edges of the mesocosm tanks.

The mussels that were not participating in an experimental run were held in the acclimatization/waiting tank, where they were put into the substratum with their siphons pointing towards the ceiling in approximately an angle of 45°. Most mussels remained in this position throughout their acclimatization/waiting period.

Data processing and analysis

Horizontal movement

Total distance moved horizontally by each mussel was quantified with the video tracking software EthoVision XT 11.5 (Noldus, Wageningen, The Netherlands) for a total duration of 15 h. For this, each mussel within a mesocosm-tank was marked with a color. This was achieved by colored backpacks and colored fabric tape so that each mussel could be individually identified and tracked within one mesocosm-tank by the software. For optimal tracking results, the number of colors (and hence mussels) had to be limited to 6 per mesocosm tank. Total horizontal distance moved within the 15 h of recording were reported for each mussel individually, resulting in 129 valid observations in total.

Vertical movement

Vertical movement was examined based on the 2×2 h of images recorded by the GoPro cameras. For this, two behavioral endpoints were observed: whether a mussel was in an upright position and burrowing depth. To examine whether a mussel was upright, mussels were categorized as (1) lying if one half of the shell was still in the initial position or (2) upright if it had changed its position such that the siphons were pointing up. This information was summarized over the 2×2 h of image recordings, and it was evaluated for each individual mussel, whether it had been observed upright at any point of time (=1) or not at all (=0), resulting in a binary dataset with 128 valid observations in total.

For burrowing depth, mussels were categorized in five states: (1) state "0", indicating that the mussel was completely on the sediment surface; (2) state "0.33", indicating that the mussel was buried to a depth of about one-third of its shell; (3) state "0.5", indicating that the mussel was half buried into the substratum; (4) state "0.66", indicating that the mussel was buried to a depth of about two-third of its shell, and (5) state "1", indicating that the mussel was completely buried in the substrate. Based on these observations, the mean proportion of shell burrowed was calculated for each mussel individually for each of the two image recordings, resulting in a numerical dataset with 256 valid observations in total.

Statistical analysis

Statistical analysis was conducted with R 4.4.1 [53]. To test the hypothesis that there are no significant effects of backpacks on horizontal and vertical movement behavior, zero-inflated generalized linear mixed models from the R-package *glmmTMB* [54] were used. The models contained subsets of the fixed factors backpack variant, proportion (backpack volume divided by mussel length), and shell length. Additionally, the models contained subsets of the random factors mussel ID, mesocosm tank, and experimental run. In the supplementary material, the different models used are described in detail (see supplementary Table S1). For horizontal movement, the total distance for each individual mussel within the 15 h of recordings was used as the response variable. For burrowing, the mean proportion of shell burrowed in the substratum for each individual mussel for each of the two recordings (2 × 2 h) were used as a response variable (data range from 0 to 1). For upright data, it was examined for each individual mussel, whether it showed an upright position at any time during the 4 h of recordings and each individual mussel received the score 1 (if it was observed upright at any time) or 0 (if it was not observed upright at all), resulting in a binary response variable.

The assumptions for the models in terms of data distribution of residuals, homogeneity of variances and outliers were evaluated based on the R-package *DHARMa* [55].

Results

Of the 96 mussels used in the experiments, only one specimen died (SC1, treatment) and all expected behavioral endpoints (horizontal movement, burrowing to different extents, and upright mussels) could be recorded and analysed with the methods presented in this study. For details on the models used for statistical analysis, see supplementary Table S1.

The mean ratio between backpack volume and mussel length was 0.5 ± 0.1 (mean ± SD) for small backpacks and 2.8 ± 0.3 for big backpacks.

Horizontal movement

There were no statistically significant differences in the horizontal movement of reference (22 ± 33 cm, mean ± SD) and treatment (15 ± 17 cm) mussels, independent of whether the mussels were equipped with small (12 ± 13 cm) or big (21 ± 20 cm) backpacks (for data distribution see Fig. 2 top).

Different combinations of fixed effects in the generalized linear mixed model met the criteria of the *DHARMa* package for the given dataset (for details see supplementary Table S1). In all tested variants that contained shell length as a fixed effect, the effect of shell length on horizontal distance was significant (e.g., model variant H1: $\beta = 0.37$, SE = 0.14, $p = 0.011$), whereas backpacks did not have significant effects on horizontal movement in any of the model variants.

Vertical movement—burrowing depth

Reference mussels burrowed approximately 26% ± 21% (mean ± SD) of their shell into the substratum, while treatment mussels burrowed approximately 22% ± 20%. From treatment mussels, those equipped with big backpacks burrowed approximately 13% ± 16%, those with small unwired backpacks 24% ± 16%, and those with small wired backpacks 29% ± 25% (see Fig. 2 mid).

In 32% of the observations in the reference group, the burrowing depth was less than 1/3 of the shell (52% in the experimental runs BU1 and BU2, 33% in SU1 and SU2, and 32% in SC1 and SC2) and 60% of the observations were ≥ 1/3 and ≤ 2/3 (48% in BU1 and BU2, 68% in SU1 and SU2, and 66% in SC1 and SC2), while 1 entirely burrowed specimen was identified (SC).

In the treatment groups, 48% of the observations of burrowing depth were smaller than 1/3 of the shell (73% in experimental runs BU1 and BU2, 34% in SU1 and SU2, 32% in SC1 and SC2), 51% of the observations were ≥ 1/3 and ≤ 2/3 (27% in BU1 and BU2, 66% in SU1 and SU2, and 63% in SC1 and SC2), and 2 entirely burrowed specimen were identified (SC).

The results show visible differences between the experimental runs with big backpacks (BU1 + BU2) and the experimental runs with small backpacks (SU1, SU2, SC1, and SC2), with a tendency of reduced burrowing depth for big backpacks.

Different models showed different significant effects, but all of the models did not meet the *DHARMa* criterion of a non-significant result in the Kolmogorov–Smirnov test. In two models, the effect of big backpacks on burrowing depth was significant (e.g., model variant B4: $\beta = -0.09$, SE = 0.04, $p = 0.030$), and in one model the effect of the proportion (backpack volume divided by mussel length) was significant (model variant B6: $\beta = -0.03$, SE = 0.01, $p = 0.024$). Furthermore, two models showed no significant effects (model variant B1 and B2), both including a combination of backpack variant and proportion as a fixed effect.

Vertical movement—upright position

Whether a mussel was observed to be upright was significantly negatively impacted by big backpacks and a high ratio between backpack size and mussel length (see Fig. 2 bottom). For 8% of reference and 23% of treatment mussels (17% of mussels with small and 33% of mussels with big backpack), no upright position was observed during the recording periods.

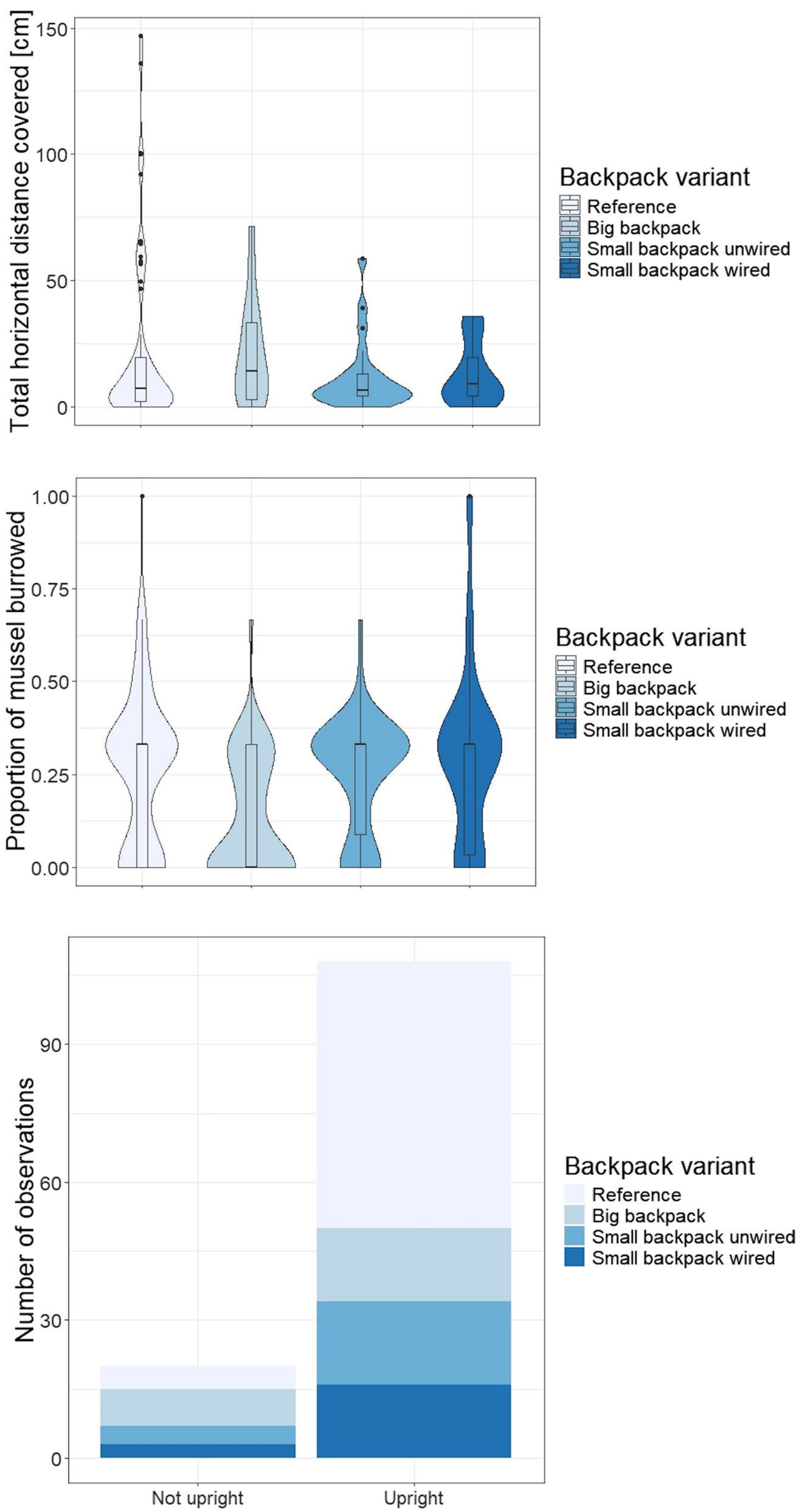

Fig. 2 Data distribution for the three different parameters measured, differentiating data in reference, mussels with big backpacks, small unwired backpacks, and small wired backpacks. Top: Horizontal movement; violine plots including boxplots for total horizontal distance covered. Mid: Burrowing; violine plots including boxplots for the proportion of mussel shell burrowed in the substratum. Bottom: Upright; bar plot for upright observations

Different combinations of fixed effects in the generalized linear mixed model met the criteria of the *DHARMa* package for the given dataset (for details see supplementary Table S1). Two models showed a significant effect of big backpacks (e.g., model variant U4: $\beta = -1.66$, $SE = 0.73$, $p = 0.022$) and two models showed a significant effect of proportion (backpack volume divided by mussel length, e.g. model variant U3: $\beta = -0.54$, $SE = 0.24$, $p = 0.025$) on observing mussels in an upright position. Furthermore, three models did not show any significant results (model variants U1, U2, and U7). These models either combined both of the above significant parameters as fixed variables (model variants U1 and U2) or used none of them (U7). Both—significant effect by big backpacks and significant effect by proportion—indicate a decrease in the odds of observing mussels in the upright position with increasing backpack size or a higher ratio of backpack volume to mussel length.

Discussion

This study provides a first characterization of the effects of three different backpack variants on the horizontal and vertical movement behavior of the freshwater mussel *Anodonta anatina*. This information is relevant to future research on the use of mussels as indicators of water quality, as well as for mussel tagging in ecological studies, and for releases of mussels in the context of conservation. While the backpacks did not influence horizontal movement behavior, the vertical movement behavior was affected by big backpacks.

The attachment of external devices on the shells of mussels is required for a range of different investigational purposes (e.g., [19, 25, 56]). Those attached backpacks can vary in size and shape and consequently might influence the natural behavior of the mussel. As demonstrated in this study, a relatively large backpack with a sensor volume-to-shell length ratio of approximately 2.8 has a significant influence on vertical movement. Such large backpacks, with an approximate ratio > 2.8 can comprise sensor-containing devices [24, 25, 56], but this size ratio can also be reached for instance if large PIT tags or radio transmitters are used on relatively small mussels [17].

The implications of these findings are particularly relevant for the use of backpacks under natural conditions. The ability to move vertically and burrow into the substratum is an important behavioral trait of freshwater mussels [41, 43, 46]. For example, Lymbery et al. [49] showed that burrowing is one relevant factor for the survival of *Westralunio carteri* in drying rivers in southwestern Australia. Analogously, weakly buried mussels were shown to be more likely to get dislodged after a major flood event and get washed out to areas that fall dry as soon as the water level drops. They can hence suffer from desiccation as demonstrated by *Margaritifera margaritifera* in the Scottish river Kerry [48]. Additionally, it was proposed that vertical and horizontal movement could be important for reproductive activity (increased amounts of population found on sediment surface) and the trade-off between resource availability (low degree of burrowing) and control of infestation by *Dreissena polymorpha* (high degree of burrowing) [43]. Also, Diggins and Stewart [57] hypothesized that species that burrow deeply could be less affected by predation from muskrats.

Since movement behavior is of high relevance for freshwater mussels, it is usually a parameter observed during monitoring and conservation efforts. Especially since the use of backpacks in natural habitats is important for mussels released after captive breeding [30] or translocation [19], which are both of major relevance for endangered species, a negative impact on movement behavior by the used backpacks might be impairing the targeted aim and hence should be revised carefully. Transponder backpacks are, for example, applied to study species dispersal, habitat choice and stocking success (e.g., [18, 56]) and the data derived is used to draw conclusions on the effectiveness of the measure.

According to the results presented here, the observed behavior for equipped mussels might not be applicable to unequipped mussels, as the backpacks might alter the mussels' behavior of interest, as shown here for vertical movement. If burrowing depth is decreased and proper anchoring in the substratum is impaired, this might lead to involuntary horizontal movement (e.g., by discharge), which, erroneously, might be observed as active horizontal movement while naturally, the mussels would not have moved as far. Especially in high flood events, mussels might also get washed out to unfavorable areas, as described above [48]. All three factors—limited transferability of data, erroneous movement measurements, and dislodgement with potentially fatal consequences—can directly influence the success of conservation efforts by leading to incorrect conclusions about different measures and/or directly reducing the positive effects of the efforts.

Based on the results of this study, no negative impact is expected for the use of small devices like PIT-tags with a sensor volume-to-shell length ratio ≤ 0.5. Nevertheless, Wilson et al. [34] found a negative short-term effect of PIT tags on mussels, which they explained by handling effects instead of an influence by the device itself. Since in the present study all mussels (Treatment and Reference) were handled in the same way, the differences in behavior can directly be related to the influence of the backpacks in our dataset. Still, it is important to consider handling as an additional stressor when equipping mussels, which might not directly be connected to the used backpack type but might also adversely affect the animals under natural conditions.

While vertical movement was significantly affected by big backpacks, no effects were observed for horizontal movement. This indicates that the size of the tested backpacks and their additional weight is likely not influencing the ability of mussels to move towards or away from bank areas, but attached objects may still result in additional resistance and energy consumption when attempting a change of position. This should be tested in future studies by varying the weight of backpacks without varying the size and relating the backpack weight to mussel weight, as it was done here for backpack and mussel size. Horizontal movement should not be impaired in a natural setting by the size range of backpacks tested here. Still, under conditions different from those tested here, e.g. under higher mussel density, backpacks might lead to alterations by, e.g., hooking into each other and impeding horizontal movement.

Backpacks are used not only under natural but also under artificial conditions. The latter are used for the investigation of behavioral reactions to single stressors (e.g., [2, 11]) and for the development of biological early warning systems, e.g., based on valve-gaping behavior (e.g., [11, 58–61]). Here, vertical and horizontal movement are usually restricted by design, by affixing mussels to solid objects, which reduces noise in the dataset that is produced by other behavioral traits than the ones of interest [23]. Hence, the results of the present study are not directly applicable to this area of backpack use. Still, for artificial conditions that allow for movement, e.g. in mesocosm setups, the effect of backpacks on the movement behavior plays a greater role than for mussels under fixation. As under natural conditions, the transferability of results to unequipped mussels is questionable without baseline data and hence preliminary or parallel baseline data acquisition is recommended, i.e. examining the behavior of interest of mussels with backpacks vs. mussels without backpacks within the given setup. Still, the emerging field of biological early warning systems is also tested in these setups (e.g., [22, 24, 26, 62]). Here, data transferability to unequipped mussels usually does not play a role because only equipped mussels can be used for this purpose. Within the experiments, only data from equipped mussels is used and hence all systems involved—e.g., AI applications, algorithms, etc.—are adapted to backpack-impacted mussel behavior and no comparisons to unequipped mussels are necessary. Nevertheless, the long-term effects of backpacks on the performance and fitness of the used animals should be tested, as changes in fitness might also alter behavioral responses to stressors, and hence, the reliability of the early warning system might decrease over time.

Since significant effects on vertical movement were only found for big backpacks or increasing the ratio between backpack volume and mussel length, but not for small (wired) backpacks, the use of smaller, if necessary wired devices should be preferred over big backpacks to minimize negative effects. While in general, technological progress contributes to a continuous reduction in the size of devices, increasing backpacks are also under development. This is caused by the increasing amounts of measurement devices that are combined for behavior observation of mussels and the need for wireless devices (e.g., [24], containing a battery, a microcontroller, a Hall-effect sensor, a clock, and a radio and antenna to transmit data). This holds especially true for applications in natural habitats where external energy sources cannot be provided and/or individuals need to be observed site-independent. To make these devices applicable in natural settings without taking the risk of negative impact and/or misleading results, it might be a solution to use mussels of larger size, to reduce the backpack-to-mussel-size-ratio. Still, this should be tested in advance.

In summary, the findings of this study contribute knowledge on the impact of attached backpacks on mussel movement. The wide range of applications of backpacks ranging from monitoring of single behavioral traits in fixated mussels for water quality monitoring, to tracking of mussels released into the wild for conservation purposes, requires a target-specific evaluation of tolerable impacts of backpacks for the respective research question. Especially for applications in natural environments and due to the variety of applicable backpacks for natural habitats (e.g., [56, 63, 64]), determining baseline data with the backpack of interest on the mussel species of interest within a mesocosm setup as presented in this study is advisable before applying such approaches.

The results presented here provide a direct comparison between equipped and unequipped mussels in a test scenario. To account for additional factors that most likely will have an influence on the mussel behavior, such as different substratum types, light regimes, flow conditions, mussel densities and mussel species (of different size/shape/sculpture), further experiments need to be conducted by customizing the mesocosm setup accordingly. With some further adaptations, the observation of additional parameters like valve-gaping behavior, excretion, reproduction attempts, movement speed/movement time, etc., could be assessed to gain even more information on the potential influence of backpacks on mussel behavior and physiology. Additionally, a setup as used here would allow for the assessment of the influence of species interactions on mussel movement and physiology, for example, by introducing native as well as invasive predators (as, e.g., in [65]) or threatening/competing invasive species like *Dreissena polymorpha* [66] to the mesocosm tanks. The setup is also suitable for collecting baseline data before an experiment in a natural setting, i.e. comparing the behavior of interest between equipped and unequipped mussels. With such a preliminary experiment, data gathered from equipped mussels could be corrected for a "backpack-impact" to make the results applicable to unequipped mussels.

If used carefully, backpacks will continue to contribute to the understanding of mussel behavior and relevant stressors and hence will enable stakeholders to implement meaningful measures to sustain the populations of these ecologically highly relevant animals.

Conclusion

This study provides an experimental setup suitable for assessing the effects of three backpack variants on the horizontal and vertical movement behaviors in freshwater mussels. (Relatively) big backpacks significantly affect the vertical, but not the horizontal movement of mussels. These results have implications for future studies because of the importance of mussel movement in ecological processes such as feeding, reproduction, and predator avoidance. Because of the negative effect of (relatively) big backpacks, future studies should assess the importance of the potential impact by the used backpack on their study aims. If the impact might corrupt the results, baseline data should be collected, and the smallest backpack that will obtain the desired information should be used to reduce the potential for effects on movement patterns and resulting physiological and ecological effects, even if the decrease in size makes wiring necessary.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-024-00976-9.

Additional file 1.

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Author contributions
Conceptualization: SB, JG, KD; Methodology: SB, JG, KD; Data acquisition: KD, SB; Investigation: KD, SB, JG; Illustration: KD, SB; Validation: SB, JG; Supervision: JG; Funding acquisition: SB, JG; Writing—original draft: KD; Writing—review and editing: JG, SB. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare no competing interests.

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