Breathing apparatus or just life buoy for a drowning?

The function of elongated pronotum in groundhoppers (Orthoptera, Tetrigidae) under water

Musiolek D., Kuřavová K. & Kočárek P.

Polyneoptera Research Group, Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10, Slezská Ostrava

Introduction

There are various modifications of respiratory system in aquatic insects. Many species use plastron (physical gills) – tiny air-store bubble surrounding insect body acting as oxygen source. Oxygen dissolved in water is gradually diffusing into the plastron, thus enable insect to staying in the water for longer time. Another insects use air tubes, tracheal gills or exposed body surface for breathing.

Orthoptera are mainly terrestrial. Truly aquatic species are rare, but there are many species closely connected with water habitats.

Groundhoppers (Tetrigidae) are small, inconspicious insects with elongated pronotum dorsally covering thorax and abdomen. Some species are semi-aquatic. They can actively move on or under water (swimming, diving). When submerged, the space between pronotum and abdomen is filled with air bubble. The underwater behaviour and breathing in groundhoppers was not studied in detail till this time.

groundhopper *Tetrix subulata*

Questions and aims

What is the meaning of the air bubble fixed under groundhoppers pronotum in the water?

air buble fixed under pronotum

Presumption:

The bubble forms plastron → groundhoppers will survive for longer time in oxygenated water than in anoxic water

The bubble facilitate swimming → individuals with bubble will coordinate movement in/on the water and float more efficiently compared to individuals without bubble.

Particular aims:

- Does the survival differ between groundhoppers with elongated vs. shortened pronotum in oxygenated and anoxic water?
- Depend the groundhoppers survival on the pronotum length and/or on the sex?
- Does the pronotum length influence underwater movement?

Methods

We used *Tetrix subulata* (Linnaeus, 1758) as model semi-aquatic species. This groundhopper inhabits wet habitats with sparse vegetation (riparian zones, river and pool banks, etc.). We used macropronotal individuals with normal pronotum length (with air bubble) and individuals with artificially shortened pronotum (> 50 % pronotum length cutted), thus not creating air bubble.

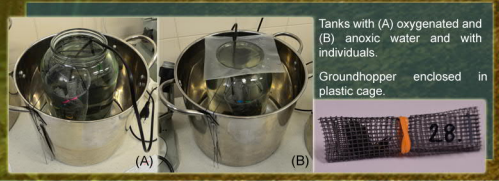
Float-behavioural study:

- Pilot study of diving and swimming was realized with both elongated-pronotum and shortened-pronotum individuals (i.e. with and without air bubble). Observations took place in water-filled glass reservoir.
- We recorded movement pattern, floating ability and diving time. The pilot study didn't provide enough data for statistical analysis. Extensive study will be accomplished during next season.

Survival study:

- The study of underwater survival of groundhoppers was performed in laboratory conditions. We used anoxic water (O_2 conc. 17.2 ± 1%; temp. 20.12 ± 0.36 °C) and oxygenated water (O_2 conc. 78.8 ± 3.9%; temp. 20.23 ± 0.64 °C).
- Each individual was enclosed in small perforated plastic cage and fully submerged in water. Individuals were taken out of water in one hour period (1st to 10th hour, than in 22nd hour) and placed into vial with food (moss, detritus). Survival was determined 24 h after taking groundhopper out of water (active movement, feeding and presence of feces acted as survival indicator).
- Concurrently we performed control measurement with individuals not submerged in the water.
- Treatments (i.e. individuals with and without air bubble, in anoxic and oxygenated water, and in control) were represented by 20 individuals in each time interval.

Kaplan-Meier survival function estimations were used for data analysis. Treatment levels were compared by *Log-rank tests*. Variables effects were tested by *Cox proportional hazard models (method „efron")*.



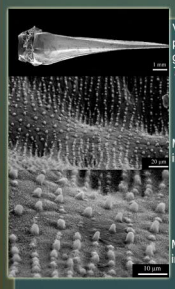
Tanks with (A) oxygenated and (B) anoxic water and with individuals.

Groundhopper enclosed in plastic cage.

Results

Float-behavioural study:

Based on pilot study we found that underwater movement of individuals with artificially shortened pronotum was uncoordinated. Non-moving groundhoppers dropped to the reservoir bottom and only active kicking with hind legs enabled them reaching water surface. Individuals with normal elongated pronotum moved horizontally and vertically by using hind legs (kicking). Non-moving individuals passively floated near the water surface (see video).



Ventral view on pronotum of groundhopper *T. subulata*.

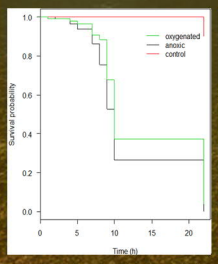
Morphology on the inner side of pronotum.

Microstructures on the inner side of pronotum.

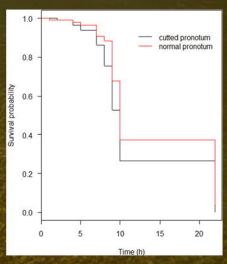
Float-behavioural study on video, webside prg.osu.cz/galerie - Zoodny2017 or take a photo QR code

Survival study:

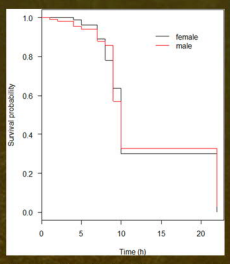
Groundhoppers in the water suffered from higher mortality risk than groundhoppers in control (n = 330; $\chi 2 = 45.7$; $p < 0.01$; Graph 1). Survival probability was not influenced by O_2 concentration in the water (n = 220, $\chi 2 = 2.7$; $p = 0.09$; Graph 1). Pronotum shortening didn't influence the groundhoppers survival probability in the water (n = 220, $\chi 2 = 1$; $p = 0.31$; Graph 2). The underwater survival probability didn't differ between sexes (n = 220, $\chi 2 = 0$; $p = 0.91$; Graph 3). The total mortality of groundhoppers was 0.9 % (1/110) in control, and 34.5 % (76/220) in the water. Only 1 individual (male with shortened pronotum in oxygenated water) survived from 20 individuals submerged in the water for extreme period of 22 h. Neither body weight, nor pronotum length significantly influenced groundhoppers underwater survival.



Graph 1: Kaplan-Meier survival probability curves for groundhoppers submerged in oxygenated water (78.8 ± 3.9% O_2), in anoxic water (17.2 ± 1% O_2) and in control without water.



Graph 2: Kaplan-Meier survival probability curves for groundhoppers submerged in the water. Comparison of individuals with elongated („natural") pronotum and individuals with artificially shortened pronotum.



Graph 3: Kaplan-Meier survival probability curves for groundhoppers submerged in the water. Sexes comparison.

Conclusion

Based on the survival analysis we suggest that air-bubble hold under the groundhoppers pronotum don't allow underwater breathing. Temporary submerged individuals apparently suffer from hypoxia. Nevertheless they can survive several hours under water. The air-bubble under pronotum probably facilitate active movement under water. Especially it acts as „buoy" for reaching water surface and leaving water. Beside the underwater behaviour and survival we observed also pronotum morphology using SEM. We found tubercle-like microstructures on the inner surface of pronotum. These structures probably participate in air-bubble formation. We plan to finish the float-behavioural study in the season 2017 and perform additional non-invasive experiments concerning underwater behaviour of groundhoppers.

The study was supported by University of Ostrava institutional grant SGS20/PřF/2016.