

Air-entrapping capacity in the hair coverage of *Malacosoma castrensis* (Lasiocampidae: Lepidoptera) caterpillar: a case study

Alexander Kovalev¹, Manuela Rebora², Gianandrea Salerno³ and Stanislav Gorb¹

¹ Department of Functional Morphology and Biomechanics, Zoological Institute, Kiel University, Am Botanischen Garten 1-9, 24118 Kiel, Germany

² Dipartimento di Chimica, Biologia e Biotechnologie, University of Perugia, Via Elce di Sotto 8, 06121 Perugia, Italy

³ Dipartimento di Scienze Agrarie, Alimentari e Ambientali, University of Perugia, Borgo XX Giugno, 06121 Perugia, Italy

Corresponding author:

Alexander Kovalev

E-mail: akovalev@zoologie.uni-kiel.de

ORCID

Alexander Kovalev 0000-0002-9441-5316

Manuela Rebora 0000-0002-4271-6336

Gianandrea Salerno 0000-0002-8357-7598

Stanislav Gorb 0000-0001-9712-7953

Abstract

The moth *Malacosoma castrensis* (Lasiocampidae) is commonly found along the Northern Germany coasts whose habitat is mainly represented by salt marshes subjected to sea level variations. Surprisingly, terrestrial caterpillars can withstand many hours being flooded by the seawater. The ability to withstand periods of submersion in a terrestrial insect raises the problem of respiration related to avoiding water percolation into the tracheal system. In the present study, we investigated under laboratory conditions the role of water-repellent cuticle structures in oxygen supply in caterpillars of *M. castrensis* submerged in water. For this purpose, air-layer stability tests using force measurements, and micromorphology of cuticle structures using SEM and fluorescence microscopy were performed.

A plastron appeared when a caterpillar is under water. Plastron stability, its' gasses composition, and internal pressure were estimated. The plastron is stabilized by long and rare hairs, which are much thicker than the corresponding hairs of aquatic insects. Thick and stiff hairs with sclerotized basal and middle regions protrude into the water through plastron – water interface, while substantial regions of thin and flexible hairs are aligned along the plastron – water interface and their side walls can support pressure in plastron even below atmospheric pressure. Additional anchoring points between hair's stalk and microtrichia near to the hair base provide enhanced stiffness to the hair layer and prevent hair layer from collapse and water entering between hairs. Advancing contact angle on hairs is more than 90°, which is close to the effective contact angle for the whole caterpillar.

Keywords: cuticle, hydrophobicity, plastron, air-retaining

Introduction

On the basis of fossil records it is generally agreed that the basal forms of modern insects were terrestrial and that aquatic insects are derived from terrestrial precursors secondarily invading aquatic environments (Wootton, 1988; Lancaster and Downes, 2013; Dijkstra et al., 2014). The transition from a terrestrial habitat to an aquatic one presents a range of specific problems for insects and requires numerous adaptations in their mechanisms of thermo- and osmoregulation, locomotion, feeding, and respiration.

Aquatic insects belong to 12 orders which invaded water multiple times. Among them, Ephemeroptera, Odonata, Plecoptera, Trichoptera, and Megaloptera are almost exclusively restricted to freshwater at their larval stage. Approximately 30% of Diptera (the largest group), over 10% of the suborder Heteroptera, 3% of Coleoptera, and very small proportions of Neuroptera, Orthoptera, Hymenoptera, and Lepidoptera are aquatic (Dijkstra et al., 2014). This last order is primarily terrestrial but there are a few species mainly belonging to Pyralidae and Crambidae with aquatic larvae, which show filamentous gills on their body (Stoops et al., 1998; Vallenduuk and Cuppen, 2004).

The ground lackey, *Malacosoma castrensis* (Linnaeus, 1758) (Lepidoptera: Lasiocampidae) is widespread in Europe and temperate Asia (Tshistjakov, 1998; Zolotuhin, 1992) (Fig. 1A). It can be found in terrestrial regions, but tends to the tidal regions. In Europe, for example, it is missing in the southernmost regions (but occurs numerously in the northern Mediterranean), and the polar zone (Southern Scandinavia) (www.pyrgus.de/Malacosoma_castrensis_en.html, 2020) (Fig. 1B). It is apolyphagous species inhabiting warm nutrient-poor grasslands. The adults do not feed, while caterpillars forage on a wide range of herbaceous plant species belonging to different families. The species has only one generation per year and overwinters at the egg stage. Larvae from overwintering egg masses laid around the stem of herbaceous plants hatch in early spring with the emergence and expanding of buds on host plants

(Karimpour, 2018). The young larvae are gregarious and, as in other Lasiocampidae, form larval masses living in conspicuous tents of silk used as shelters and molting sites. This moth is commonly found along the Northern Germany coasts whose habitat is mainly represented by salt marshes subjected to sea level variations. Here caterpillars feed on coastal plants, such as Sea Plantain (*Plantago maritima* L.), Common Sea-Lavender (*Limonium vulgare* Miller) and Sea Wormwood (*Artemisia maritima* L.). In consideration of the habitat of this species, caterpillars can withstand many hours being flooded by the seawater.

The ability to withstand periods of submersion in a terrestrial insect raises the problem of respiration related to avoiding water invasion into the tracheal system. Some aquatic insects, such as marine Heteroptera, living at the boundary between air and water, and resisting immersion for short, but regular periods of time, developed a cuticle that is rich of dense setae inserted in sockets and short subcellular filiform protuberances (microtrichia) able to retain a thin layer of air on the cuticle during submersion (Perez-Goodwyn, 2009; Balmert et al., 2011).

In the present study, we investigated under laboratory conditions water-repellent cuticle structures, which allow caterpillars of the terrestrial moth *M. castrensis* to retain atmospheric oxygen when submerged in water. For this purpose, the air-layer stability tests using force measurements were performed. To analyze the structure of caterpillar hairs in relation to their possible involvement in air retaining, we performed an ultrastructural analysis with Scanning Electron Microscope (SEM). The presence and distribution of the elastic protein resilin as well as distribution of sclerotization degree in the cuticle of these structures were revealed by fluorescence microscopy.

Material and Methods

Insects

Two caterpillars of the ground lackey *M. castrensis* belonging to the penultimate and ultimate stage were collected in June 2017 at the North Sea shore in the salt marsh vegetation near to Westerhever (Schleswig-Holstein, Germany) on sea grasses (*Seriphidium maritimum*, *Limonium vulgare*) growing in the area around the lighthouse. This area is flooded twice a day for one to approximately six hours. Length and weight of the caterpillars were 44.6 mm and 46.7 mm, 0.797 g and 0.839 g, respectively.

Buoyancy force measurements

The experiments set up for a caterpillar buoyancy characterization consisted of a force transducer FORT-10 (10 g capacity; World Precision Instruments Inc., Sarasota, FL, USA) connected to a MP 100 amplification and digitalization system (Biopac Systems Ltd, Goleta, CA, USA) (Gorb et al. 2010; Zheden et al. 2015). Data were recorded using AcqKnowledge 3.7.0 software (Biopac Systems Ltd, Goleta, CA, USA). A caterpillar was attached to the force transducer using a metal wire (Fig. 1C,D). It was submerged then for some time (from 1 min to 13 h) under the water. The animals were alive after all the submerging experiments carried out. The buoyant force was measured during this procedure. Six separate experiments on two individual animals were performed. The variations of the measured force arise because of the changes in the volume of the air layer surrounding the caterpillar.

Scanning electron microscopy

Air-dried samples of the hairs of *M. castrensis* caterpillar were mounted on aluminum holders, sputter-coated with gold-palladium (10 nm) and studied using a scanning electron microscope Hitachi S-4800 (Hitachi High-Technologies Corp., Tokyo, Japan) at an acceleration voltage of 3 kV. The mixture of signals from upper and lower detectors was used to produce images. For further details of the sample preparation and the mounting for SEM imaging see Gorb and Gorb (2009).

Fluorescence microscopy

Fluorescence microscopy was used to reveal some differences in the molecular composition along the caterpillar setae, which basically are cuticular outgrowths. Different cuticle constituents of insect cuticle are well known to have a spectrally specific autofluorescence. In contrast to tanned sclerotized cuticle, which usually demonstrates red-fluorescence by 488 nm excitation, a rubber-like cuticular protein resilin possesses a narrow band autofluorescence at around 420 nm by 405 nm excitation (Andersen, 1964). For photobleaching prevention setae were mounted in a water-soluble medium (Moviol) on a glass slide and covered with a coverslip (Haas et al., 2000). Autofluorescence images were obtained using a conventional fluorescence microscope Zeiss Axioplan (Oberkochen, Germany) equipped with AxioCam MRc camera and light source with HBO 100 W lamp. The autofluorescence was registered in three wavelengths bands: UV band (excitation 320–380 nm, emission 420–470 nm), green (excitation 450–490 nm, emission >520 nm), and red (excitation 535–560 nm, emission >590 nm). Fluorescence images, taken in three different spectral ranges, were superimposed and assigned to the three color channels, in order to reveal local differences in the cuticle composition.

Results

Buoyancy force measurements

An unusual behavior of *M. castrensis* caterpillars was observed in the field. Unlike caterpillars of other species from this genus that immediately leave their plant substrate, when submerged, *M. castrensis* caterpillars stop moving clinging to the plant. During the high tide at North Sea coasts, they can survive being submerged under salt water for some (1-8) hours twice a day without the presence of specialized gill, further called plastron. This observation let us assume, that the caterpillars have specific adaptations to survive tide periods and cling to the plant, in order to prevent to be taken and drifted away by the sea.

In the buoyancy experiment, the forces during submerging of the caterpillar under sea water were recorded. This force (F_m) is the sum of push out (F_p) and gravitational (F_g) forces: $F_m = F_g - F_p$ (Fig. 2). Since the gravitational force is constant (Fig. 2A and the left diagram in Fig 2B), the measured force curve characterizes the change in the push out force, which reaches its maximum at some particular dip depth (Fig. 2A and the middle diagram in Fig 2B). When the caterpillar is completely under water, the push out force remains constant, and tiny force variations are correlated with the change in the volume of the caterpillar and air bubble around it, which can be recognized because of the total internal reflection from water/air interface when the observation angle exceeds 49° (mirror-like reflection in Fig. 1D).

The volume of caterpillars and air around it can be estimated from Archimedes' principle. After long time under water, this volume was $960.6 \pm 3.0 \mu\text{l}$ (mean \pm std. error) (Fig. 2A). It was calculated from the caterpillars' length ($44.71 \pm 0.05 \text{ mm}$) under an assumption that the caterpillar has a cylindrical shape, and diameter-to-length ratio (or projection area to length squared ratio) equals to 0.117 (Fig. 1A). Effective density can be calculated as mass-to-

volume ratio. For the two investigated caterpillars their effective densities were 818 and 856 kg/m³. The maximum caterpillar's volume immediately after submerging it in water was 1335 μ l: assuming homogeneous distribution of the air around the caterpillar body, it corresponds to 468 μ m thick air layer. From two long lasting experiments, disappeared air volumes were 237 μ l (within 46 min experiment) and 276 μ l (within 13 h experiment), which correspond to 298 μ m and 359 μ m of the air layer thickness, respectively. Such air loss is known to happen, since air layer under water is at higher than atmospheric pressure, and therefore it is in a metastable state. The air permanently diffuses into / out of water through air – water interfaces. The diffusional dissolution rate of the plastron gas (ratio of the gas volume loss per surface area per time) was very different in different experiments (0.5 to 7.8 mmol·m⁻²·h⁻¹) as estimated from the linear regression of the time-volume decay (Fig. 2C).

Other mechanism of the air loss from the plastron is a spontaneous quick (within one second) bubbles separation (Fig. 2A). The mean bubbles diameter was 5.34±0.30 mm, which is comparable with the caterpillar diameter and corresponds to the effective air layer thickness change in ~100 μ m. The air bubbles separate from plastron within 10 min after caterpillar submerging, geometric mean time 110 s. In one of the experiments, we observed that a bubble 2 mm in diameter joined the plastron. The bubbles like this often appeared on the vessel walls and bottom, when the ambient temperature increased. However, the gas volume change in the plastron, because of the temperature variation in the laboratory during experiments (less than 4 °C), was negligible.

Some interesting events of the sudden volume increase and further exponential decay to the previous volume value were observed for the first hour of the caterpillar submergence (Fig 2C). The mean volume variation was 88±32 μ l, the geometric mean recovery time was 14.0 s (4.2 to 319.0 s). After the first hour of caterpillar under water, similar but slower volume variations were observed with almost linear volume increase and following exponential

volume decay (Fig 3A). For two of such events, the mean volume increase speed was 71 $\mu\text{l}/\text{hour}$, the mean volume variation was 39 μl , and the geometric mean recovery time was 20 min.

During dipping a cylinder with its side in water, the menisci may produce forces comparable with the gravitational forces. General push out (buoyant) force, which act on a cylindrical body, is determined by the following equation:

$$F_p = F_t + F_b = 2L\gamma\sin\varphi_w + \rho gRL(R\varphi_c + (2h - R\cos\varphi_c)\sin\varphi_c), \quad (1)$$

where F_t is the surface tension force, F_b is the buoyancy force (the difference between forces acting on lower and upper surfaces of the cylindrical body), L is the cylinder length, γ is the surface tension ($\gamma=73.3$ mN/m, calculated according to Naya et al. (2014)), φ_c and φ_w are the water contact angle and the angle between water meniscus at certain depth h and a vertical line, ρ is the water density (for sea water $\rho=1022$ kg/m³, calculated according to an equation presented in Fofonoff et al. (1984)), g is the gravitational acceleration ($g = 9.813$ m/s²), R is the radius of the cylinder (Fig. 3). After a straight forward conversion of equations presented in Zheng et al. (2009) φ_w dependency on h can be expressed as:

$$\varphi_w = tg^{-1}\left(\frac{h\sqrt{4\gamma/(\rho g)-h^2}}{h^2-2\gamma/(\rho g)}\right) + \pi/2 + \pi\theta(h - \sqrt{2\gamma/(\rho g)}), \quad (2)$$

where θ is the step function ($\theta(x) = 0, x \leq 0; \theta(x) = 1, x > 0$).

The push out force reaches its maximum at some particular dip depth (Fig. 2A,B). From the known caterpillar dimensions and the maximum value of the push out force the effective water contact angle on the caterpillar can be estimated using equations 1 and 2. The effective contact angle for a caterpillar was equal to $98.3 \pm 11.7^\circ$.

Setae morphology

The hairs (setae) at their base are cylindrical and supplemented by microtrichia pointed to the tip of the hair (Fig. 4A,E,F). The very top of the hairs is flattened and slightly coiled, has no microtrichia (Fig. 4E,G). The longest hair length was around 3 mm (Fig. 1D). Diameter in the middle area of hairs strongly varies from 7 to 25 μm , therefore their shape varies from filiform to stiff and straight correspondingly.

The basis of hairs demonstrates enhanced autofluorescence in the red spectral range (emission $>590\text{ nm}$), while the tip of hairs demonstrates enhanced autofluorescence in the blue spectral range (emission 420-470 nm) (Fig. 4B,D). The receding and advancing contact angles are slightly below and slightly above 90° respectively (Fig. 1D).

Discussion

Air-retaining surfaces are known from different arthropods, aquatic birds, aquatic plant surfaces, and some other organisms (Barthlott et al., 2010; Ditsche-Kuru et al., 2011; Marx and Messner, 2012; Ditsche et al., 2015; see review in Barthlott et al., 2016). Among insects, according to the different grade of adaptation to aquatic habitats (species living close to water in air, living in a middle point between water and air, and species living under water), different levels of air-retention capability can be envisaged (Perez-Goodwyn, 2009; Balmert et al., 2011). However, primarily terrestrial species usually do not possess such highly specialized surfaces. This study reports on air-entrapping capacity in the hair coverage of the primarily terrestrial caterpillar *M. castrensis*. This adaptation provides some selective advantage to this species in occupying intertidal habitats at the North Sea.

In aquatic insects plastron is supported by dense layer of specialized thin hairs, while the hairs in caterpillars are much thicker, have higher aspect ratio, and less dense. Caterpillar hairs

were previously demonstrated to provide several different functions. They simply build physical barrier as a defense mechanism against natural enemies, as it was shown for caterpillars of *Lemyra imparilis* (Butler) (Lepidoptera: Erebidae) and *Lymantria dispar japonica* Motschulsky (Lepidoptera: Erebidae), which hairs' length is of high importance in defense against predation by a carabid beetles (Sugiura et al., 2014). Additionally, both hairs' length and thickness are important factors in defense of *L. dispar japonica* against oviposition by the endoparasitoid *Meteorus pulchricornis* (Wesmael) (Hymenoptera: Braconidae) (Kageyama et al., 2016). The hairs may be involved in the perception of the surrounding medium motion, as previously reported for the cabbage moth caterpillar *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae) that can sense sound stimuli at low frequency and response with a defensive reaction (Markl et al., 1975). Finally, the hairs might prevent drowning of caterpillars, as it was shown for *L. imparilis* (Meyer-Rochow, 2016).

Most terrestrial insects placed under water start to actively move trying to reach air-water interface. In caterpillars of tidal *M. castrensis*, other specific behavior is observed: once under water, they cling to the substrate with their attachment devices and stay motionless (S.G. personal observations). For the caterpillars, it is obviously advantageous, to stay through the high tide on the host plants. Since the body density of *M. castrensis* is about 840kg/m^3 , if they are released from the plant, they would stay on the water surface. Floating on the water surface is a high risk for the caterpillars, because they may be driven by water into the open sea or collected by water birds.

Interestingly, the caterpillar radius almost equals to the capillary length (2.7 mm in our case). It may be related to the smallest shear forces on a cylinder with radius equal to capillary length produced by water running on the hydrophobic plant surface (e.g. *A. maritima*), when water level increases during tide. To prevent caterpillar suffocation under water, it should possess a kind of a plastron for effective CO_2 exchange. Assuming the volume of tracheal

system in *M. castrensis* caterpillars is similar to that previously studied in *Manduca sexta* (L.) (Lepidoptera: Sphingidae), which is ~7.5% of the caterpillar volume (Helm et al., 2013), we can approximate the tracheal volume for our caterpillars as 72 μ l. A plastron is obviously present in *M. castrensis* caterpillars under water. The volume of the plastron is decreasing with time. The maximal measured air volume disappeared from the plastron was 375 μ l, which is about five times the tracheal volume.

There are two main mechanisms of the plastron volume decay: bubbles separation and air diffusion from plastron, where the air pressure is higher than the atmospheric one, through the water. Bubbles separated from plastron reduce its volume and the time caterpillar may stay under water.

The effective diffusion constant for the air leakage from the plastron could be estimated from the Henry's and Fick's first laws (Henry, 1803; Fick, 1855). Assuming, that a stable concentration gradient of dissolved gases is formed in a thin water layer close to the plastron, the following equation for the gas transfer rate, v , through gas/water interface could be written:

$$v = ADH_{cp} \frac{p_{pl} - p_{atm}}{\delta}, \quad (3)$$

where A is the surface area of the gas/water interface, D is the diffusion constant of gas in water, H_{cp} is the gas solubility constant, δ is the diffusion layer thickness, $p_{atm/pl}$ is the partial pressure of a gas in atmosphere/plastron. The gas transfer rates at some interesting parameter combinations calculated using Eq. 3 are presented in the Table 1. The diffusion layer thickness was taken equal to 0.5 mm, according to Seymour et al. (2015), all the parameters were normalized for experimental conditions at 20 °C.

During respiration oxygen in the plastron is converting at a constant rate into carbon dioxide. Carbon dioxide has transfer rate comparable with that for oxygen at ~1 kPa partial pressure

difference (Tab. 1). The CO₂ transfer rate at 1 kPa partial pressure is also comparable with minimal CO₂ emission rate in e.g. *M. sexta*, ~3 μmol/h (0.2-2 g body weight). At such metabolism rate, the oxygen from the plastron and tracheal system together is enough just for one hour respiration without gas exchange between plastron and its' surrounding water. This means that permanent oxygen diffusion into plastron is taking place, Fig. 5. Stationary O₂ partial pressure should be above 2 kPa (see Seymour et al., 2015) to maintain the oxidative process in mitochondria.

Assuming stationary gas concentrations and diffusion, the whole nitrogen should diffuse out of the plastron (and the plastron should disappear) in about 7.6 hour. Since caterpillar in our experiment possessed plastron even after 13 hours under water, a cuticular hydrophilic structure should exist, which can suspend the gas water interface at the pressure in plastron, which is less than 93.7 kPa (for 101.3 kPa atmospheric pressure). Therefore in the case of *M. castrensis* caterpillars one may consider the presence of an incompressible physical gas gill (Seymour et al., 2013). Summing up, the oxygen permanently diffuses into the plastron and is converting during respiration into CO₂, which diffuses out of the plastron. Nitrogen basically diffuses out of the plastron until the pressure drops below atmospheric pressure (Fig.5).

The gas transfer rates in tidal sea should be much higher, since the diffusion layer thickness depends on the water flow velocity and already for ~6 mm/s velocity it is 5 times less than for standing water (Seymour et al., 2015).

One event of the sudden significant gas volume increase (around 90 μl, Fig. 2C) cannot be related to muscle contraction or to the change in internal hydrostatic pressure, but may be associated to bacterial activity in the caterpillar gut. However small reversible changes of the caterpillar volume at different time intervals, Fig. 2A, might be related to the tonal muscle contraction.

At the initial stage of the caterpillar dipping the hairs on the cuticle of *M. castrensis* are responsible for enlarged volume of the plastron and prevent the contact of water with the animal body, Fig. 1D. The hairs have advancing contact angle more than 90° , which explains the high value of the effective macroscopic contact angle of caterpillars during dipping, 98° . The plastron volume is also stabilized by hairs. Thick and stiff hairs protrude into the water through plastron – water interface and stabilize it at shear stress in turbulent water flow. The middle and basal regions of the hairs are rigid, because they are thickened and sclerotized (Fig. 4D, red fluorescence). Thin hairs are more flexible. Especially flexible are their distal part, which is flattened and demonstrate bluish autofluorescence (Fig. 4B), that indicates on the resilin (rubber-like soft protein) enrichment (Haas et al., 2000). Since thin hairs tips are extremely flexible and advancing contact angle is more than 90° , they cannot penetrate the plastron – water interface. Substantial regions of thin hairs are aligned along the plastron – water interface and can support menisci with even negative curvature (when the pressure in plastron is below atmospheric pressure) resembling hair-like structures (trichomes) on the leaves of underwater fern *Salvinia oblongifolia* (Barthlott et al., 2010; Ditsche et al., 2015). Additionally, interlocking of hair's stalk and tip with microtrichia located near to the hair base (Fig. 4E,F) provides enhanced stiffness to the hair layer and prevent hair layer from collapse and water entering between hairs. The twist of the flattened hairs' tip ensures its proper orientation for interlocking with microtrichia and is responsible for reduction of the contact area of the tip with water. Anisotropic orientation of microtrichia presumably hinders the water meniscus motion into direction to the hair base, since the water-air interface is disturbed by microtrichia tips. Therefore the motion of water-air interface against the microtrichia requires more energy than its motion along the microtrichia or equivalent conical structure, or even along a smooth hair. Microtrichia on a hair along the water-air interface increase the interface deformation.

Conclusion

Caterpillars of *M. castrensis* may be regarded as terrestrial animals, however possessing specific structures and behavioral adaptations let them survive on plants in a tide zone. It is still open which other adaptations for this specific habitat they have. Here we investigated some properties of the plastron, which allows caterpillars of *M. castrensis* to survive many hours under water. *M. castrensis* plastron is different from the typical plastron of aquatic insects. In the majority of aquatic insects, the plastron is supported by dense layer of specialized thin hairs, whereas the hairs in the caterpillars are much thicker, have higher aspect ratio, and are much less dense. The caterpillar plastron is stabilized by hairs sidewalls similar to those of aquatic insects described in Flynn et al. (2008). The pressure in the caterpillar plastron can be less than 92 % of atmospheric pressure. The plastron may withstand both hydrostatic and hydrodynamic pressure in tide water currents.

However, future studies on different larval instars of *M. castrensis*, as well as on other closely-related species of *Malacosoma* moths that do not live in such a specific habitat as *M. castrensis* should be performed, in order to understand selective advantages of the morphological and behavioral features of these caterpillars in further detail. Also, exploring caterpillars of other lepidopteran species, living in the habitat similar to *M. castrensis*, would be important to reveal convergent evolution of hairy cover of the larval body.

Similar to other superhydrophobic plastron-bearing biological surfaces (Busch et al. 2019) our results on the caterpillar plastron may find its applications in marine technology due to its ability to maintain air bubbles for drag reduction and anti-fouling. Additionally, as suggested by Shirtcliffe et al. (2006), submerged cavities consisting of specially designed hydrophobic material might provide oxygen necessary to run fuel cells to supply power to small underwater vehicles through a mechanism analogous to plastron respiration.

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Tab. 1 Particular gas transfer rates through gas/water interface.

gas	H_{cp} , $\mu\text{mol}/(\text{Pa}\cdot\text{m}^3)$	D , m^2/s	$p_{pl}-p_{atm}$, kPa	v , $\mu\text{mol}/\text{h}$
CO ₂	330	$1.92\cdot 10^{-9}$	1	3.36
O ₂	12	$2.1\cdot 10^{-9}$	-19.3	-2.58
N ₂	6.4	$1.88\cdot 10^{-9}$	18.3	1.17

H_{cp} and D values were taken from Sander et al. (2015) and Ferrel et al. (1967), respectively (at 737 mm² interface area).

Figures

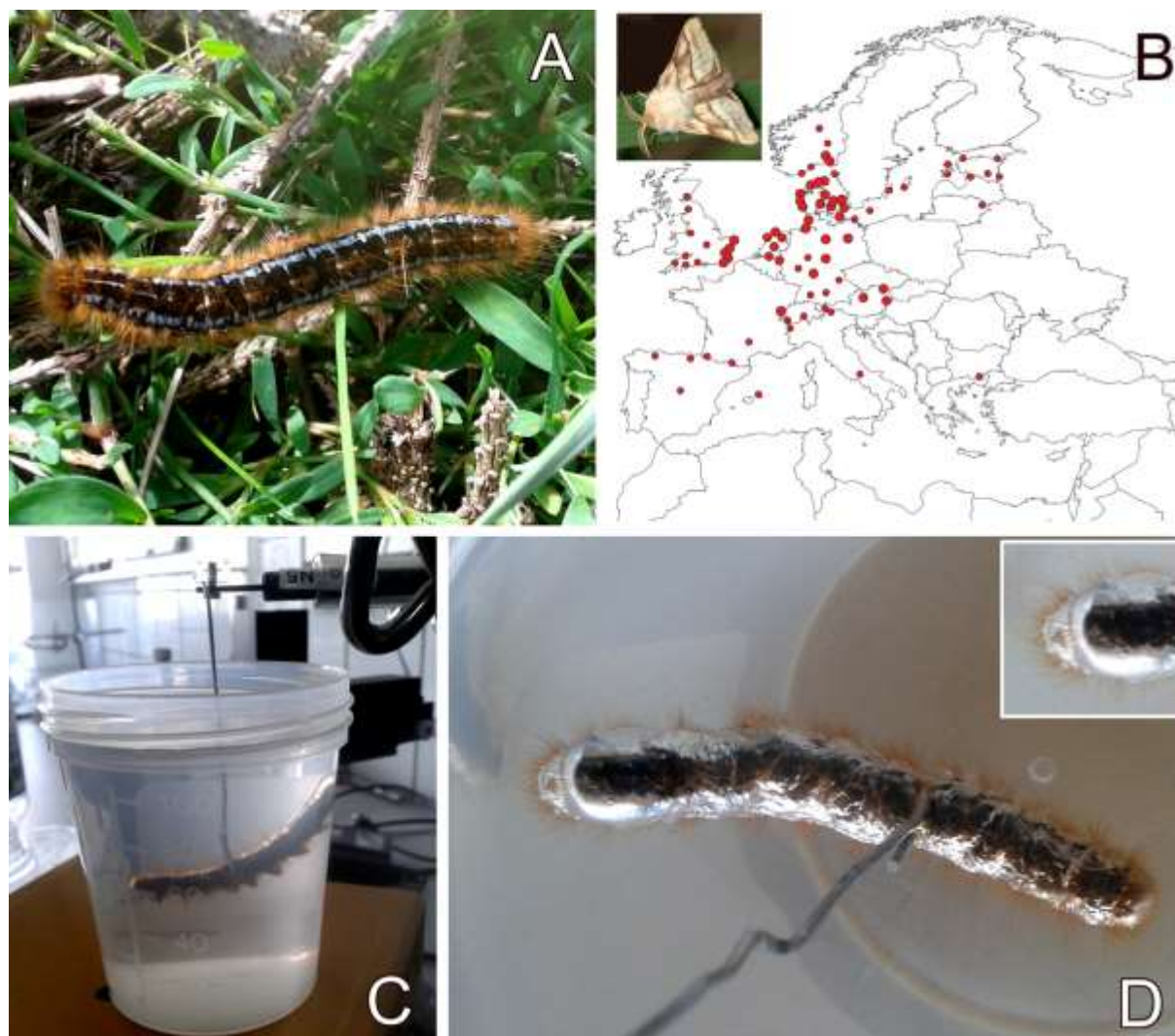


Fig. 1. (A) *Malacosoma castrensis* caterpillar in its natural habitat. (B) Distribution areal (data from www.beachexplorer.org/arten/malacosoma-castrensis-larva/verbreitung, 2020). Inset in B shows an adult moth. (C) Experimental setup for measuring buoyancy force. (D) Buoyancy measurement, view from above. Inset in D shows an enlarged image of the caterpillars' head one minute after beginning of the experiment.

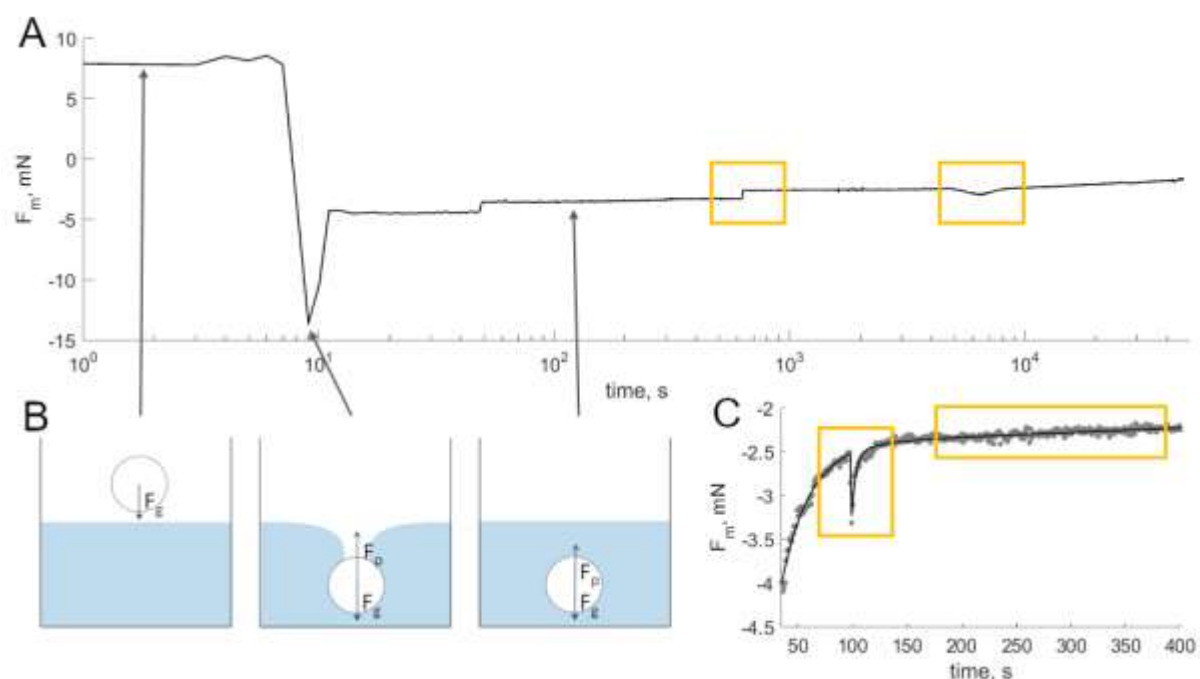


Fig. 2. A typical buoyancy experiment. Force dynamics during the experiment (A) is presented in logarithmic time scale. The force-time curve with the three, indicated by arrows, important phases of the dip process: (1) caterpillar has no contact with water, (2) highly curved meniscus is formed around the caterpillar body, (3) caterpillar is completely under the water (B). The circle corresponds to the cross section of the caterpillar body. F_g and F_p are the gravitational and push out forces, respectively. A sudden force jump and a slow reversible force change are marked with (correspondingly left and right) boxes on the force-time curve. A section of the force-time curve shown in (C) represents a slow exponential force relaxation (volume decay), when caterpillar was completely under the water (the right subplot in B). Measurements are shown as gray dots, fit is shown as a solid line. Left and right boxes mark a fast relaxation process with small amplitude and a linear force increase (volume decay), respectively.

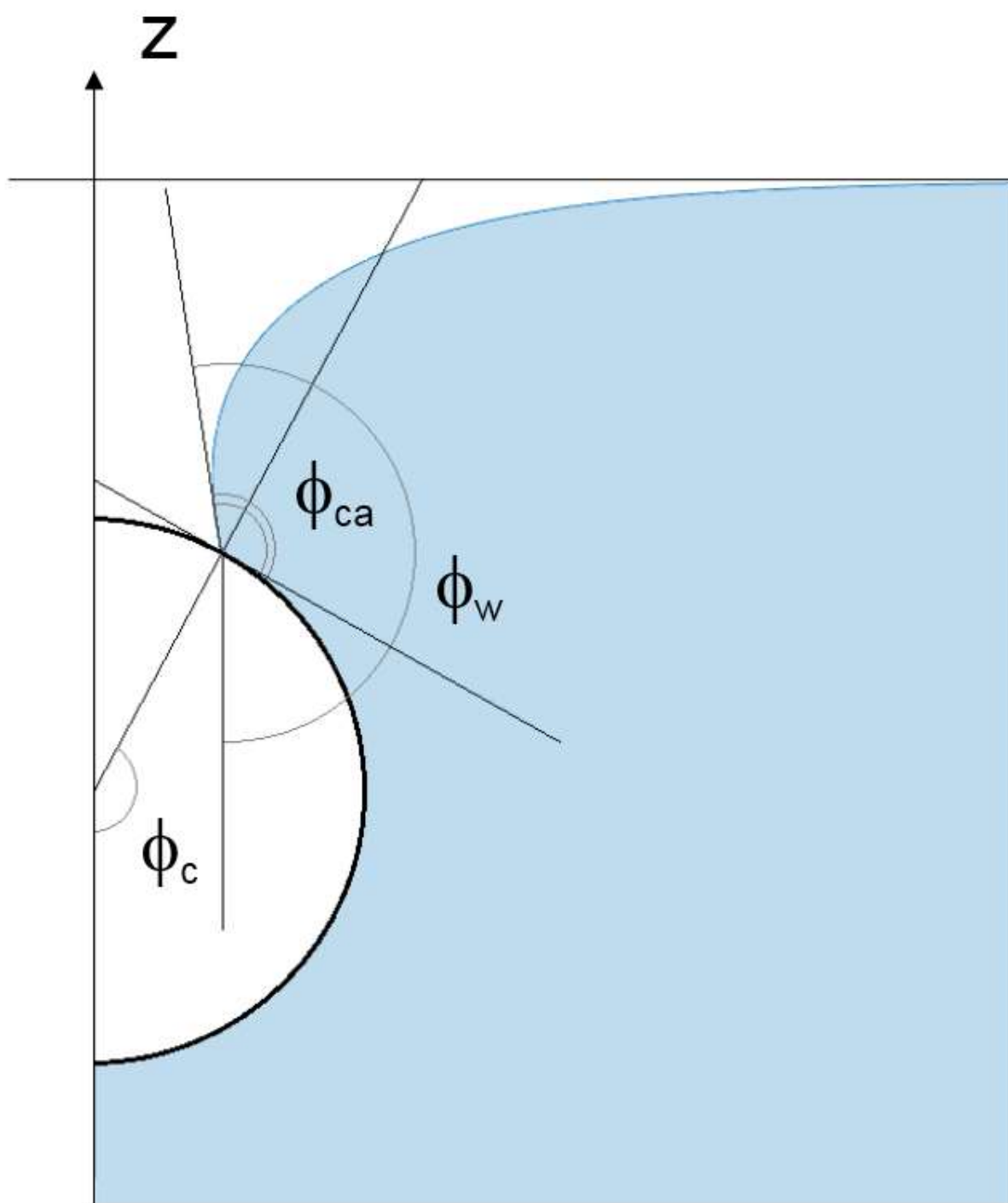


Fig. 3 Cylindrical body (just one half is shown) submerged in water (shown in blue). Undisturbed water level is at $z=0$, ϕ_{ca} is the water contact angle, ϕ_c is the angle between z -axis and a line going through the center of cylinder and the point of the water meniscus contact on the cylinder, ϕ_w the angle between water meniscus surface and a vertical.

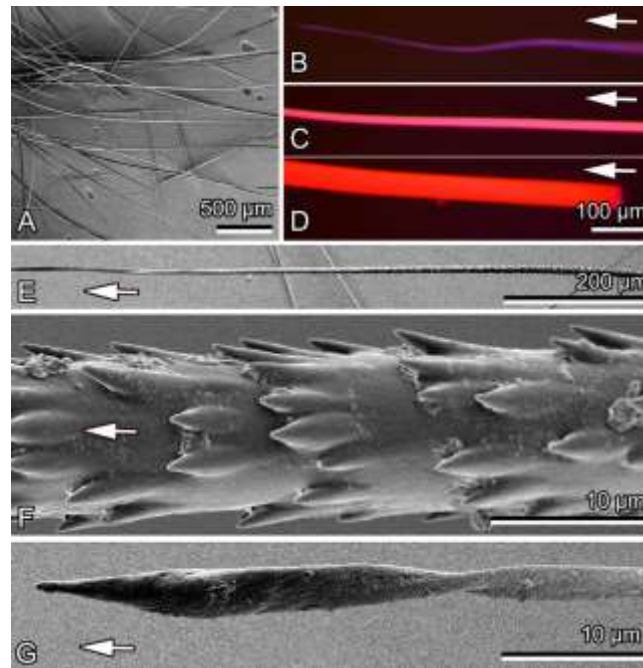


Fig. 4 Setae from the *M. castrensis* caterpillar body (A). B, G. Setal tips. C, E. Middle region of the seta. D, F. Basal region of the seta. A, E-G. Scanning electron microscopy (SEM) images. B-D. Composed autofluorescence images. Arrows indicate distal direction.

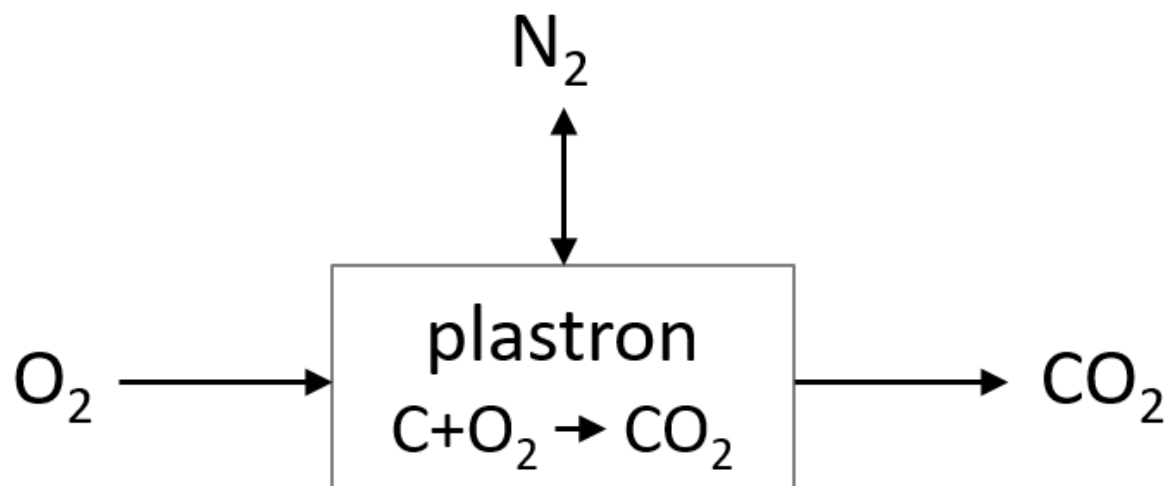


Fig. 5 Gas balance in the plastron depends on respiration and gas exchange between plastron and its' surrounding water.