Air Bells of Water Spiders are an Extended Phenotype Modified in Response to Gas Composition

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ABSTRACT   The water spider *Argyroneta aquatica* (Clerck) is the only spider that spends its whole life under water. Water spiders keep an air bubble around their body for breathing and build under-water air bells, which they use for shelter and raising offspring, digesting and consuming prey, moulting, depositing eggs and sperm, and copulating. It is unclear whether these bells are an important oxygen reservoir for breathing under water, or whether they serve mainly to create water-free space for feeding and reproduction. In this study, we manipulated the composition of the gas inside the bell of female water spiders to test whether they monitor the quality of this gas, and replenish oxygen if required. We exchanged the entire gas in the bell either with pure O_2, pure CO_2, or with ambient air as control, and monitored behavioural responses. The test spiders surfaced and replenished air more often in the CO_2 treatment than in the O_2 treatment, and they increased bell building behaviour. In addition to active oxygen regulation, they monitored and adjusted the bells by adding silk. These results show that water spiders use the air bell as an oxygen reservoir, and that it functions as an external lung, which renders it essential for living under water permanently. *A. aquatica* is the only animal that collects, transports, and stores air, and monitors its property for breathing, which is an adaptive response of a terrestrial animal to the colonization of an aquatic habitat. *J. Exp. Zool. 307A:549–555, 2007.*   © 2007 Wiley-Liss, Inc.

The water spider *Argyroneta aquatica* is the only spider that spends its entire life under water (Grothendieck and Kraus, '94), and it shows specific adaptations to this mode of life. The abdomen and legs bear hairs helping to keep an air bubble around the body, which is used to breathe under water, and helps to transport air from the surface down to their habitat (Ehlers, '39). Due to the spiders' breathing activity and passive diffusion of gases from the bubble into the water, water spiders have to surface regularly to renew the air captured around their opisthosoma (Braun, '31). The oxygen concentration inside this bubble decreases constantly, hence *A. aquatica* breathes air of lower oxygen content than other spiders (Braun, '31; Crome, '52).

Surfacing is not only energetically costly for water spiders (Schütz and Taborsky, 2003), but also bears predation risk. To reduce these costs, *A. aquatica* builds air bells under water, which are a unique structure in animals. The bells are usually located between water plants, and fixed with spider thread to plants or stones (Wesenberg-Lund, '39; Crome, '51; Heinzberger, '74). Starting with a small web and collecting air from the surface, air collecting, transporting it down to the bell, and

enlarging the bell is repeated until the bell is large enough for its various purposes (Braun, '31). When transporting air down to the bell, water spiders show a special transport behaviour. They hold their hind legs backwards around the abdomen, which enlarges the transportable air volume. Air bells are also of crucial importance for offspring development (Braun, '31). Females, which are smaller than males (Schütz and Taborsky, 2003, 2005), defend their broods from predators and renew the air in the bell containing the cocoon. Water spiders also digest and consume their prey, moult, deposit eggs and sperm (the male transfers its sperm from the genital opening on the opisthosoma to its pedipalps), and copulate there (Wesenberg-Lund, '39; Heinzberger, '74).

As under-water air bells are "multi-purpose rooms", it is unclear whether they serve for breathing, or are only important to create water-free space to feed, and reproduce. As the gas exchange of O_2 and CO_2 is the same inside the bell as in the air bubble around the spiders' body, air bells have been referred to as "physical trachea" or external lungs (Schollmeyer, '13; Turner, 2000), but this has never been tested. We examined the function of under-water air bells in laboratory experiments by asking whether *A. aquatica* monitor the quality of the gas inside the bell (CO_2 or O_2 content), and observing how they react to respective changes.

Some invertebrates were found to monitor the air condition in their microhabitats (Diesel, '97). For example, wasps, bees, and bumblebees regulate air temperature in their nests by metabolic heat production, fanning, and water evaporation (Gibo et al., '74; Heinrich, '84; Martin, '90). Honeybees *Apis mellifera* possess a CO_2 receptor (Lacher, '64), and by fanning behaviour they regulate the CO_2 concentration in their hives very efficiently (Seeley, '74). To test whether *A. aquatica* monitor the quality of the gas composition inside their air bells, we manipulated the gas composition of air bells and recorded the spiders' behaviour. In laboratory experiments, we exchanged the entire volume of the bell with pure O_2, pure CO_2, or with ambient air as a control gas, and tested whether and how the gas quality in the bell would influence the spiders' behaviour.

Four possible functions of under-water bells for water spiders result in different predictions concerning the spiders' behaviour in response to our experimental manipulations: (1) the bells are not important for breathing but serve merely to create a water-free space allowing spiders to feed and reproduce. In this case spiders should not react differently to the CO_2, O_2, and control treatments.

(2) The bells serve for breathing, but the oxygen level in the bell is just a by-product of the normal breathing activity, i.e. spiders do not monitor and actively regulate the oxygen content inside the bell. In this case spiders should increase activity after CO_2 treatment in comparison to the control and O_2 treatments, but they should not increase surfacing and air replenishment in the bell. (3) The bells serve for breathing, and spiders actively regulate the oxygen content inside the bell. If so, we expect the spiders to increase activity, surfacing, and air replenishment in the bell after CO_2 treatment in comparison to the control treatment, and a reduction of these activities against the control situation in the O_2 treatment. (4) The bells serve for breathing, and in addition to active oxygen regulation of gas in the bell the spiders also monitor and adjust the silk structure of the bells in response to gas concentrations. In this case, we expect that in addition to more surfacing and air replenishment in the CO_2 treatment, spiders should also increase bell-building behaviour.

MATERIALS AND METHODS

Experimental set-up

We used eight adult females, which were wild caught animals from the Lobau near Vienna, Austria, and their offspring. Although both sexes in *A. aquatica* build under-water air bells, we only used females in our experiments, because for females air bells play a more important and manifold role than for males (Schütz and Taborsky, 2003). Females stay inside their air bells for a large proportion of times and they also care for the brood there, whereas males are more active and rove around extensively (Schütz and Taborsky, 2003, 2005).

Each test spider was kept in a separate glass jar and its cephalothorax width was determined as a measure of body size (see Foelix, '92; Lang, 2001). Clear plastic tanks ($15 \times 15 \times 20$ cm) were used for the experiments. The tank bottom was covered with gravel, and two water plants (*Cryptocoryne sp.*) with one leaf each were placed in the middle of the tank. Test spiders were put singly into these tanks, one day before manipulating the underwater bells. We added three *Asellus aquaticus* as food. Each spider was tested in three different treatments in a randomized sequence. We sucked out the air of the bell completely with a syringe, and noted the gas volume in the bell. Then we refilled the bell with the same volume of pure CO_2,

pure O_2, or ambient air (referred to as CO_2, O_2, or control treatment). The silk structure contracted when the air was sucked from the bell, but we could always inject exactly the same volume back into the bell. All treatments were performed between 1300 and 1700 h. The whole tank was illuminated, and videotaped continuously with a time-lapse recorder, which was set to condense real time by a factor 10. The behaviour of each spider was recorded for 21–28 h in each treatment, before we put the spiders back into their holding tanks. There were at least 3 days between subsequent experimental treatments of the same spider. Since the partial pressures of O_2 and CO_2, respectively, in manipulated air bubbles are higher than in the surrounding water, the gases dissolve in the water because of hydrostatic pressure and surface tension (Turner, 2000). Therefore, we observed the spiders' behaviour only for 1 day, because later they probably face normal conditions again (see Turner, 2000). The following behaviours were recorded: "surfacing", which is part of the normal breathing activity without bringing the collected air into the bell. The spiders rise to the surface, stick their abdomens out of the water, move their hind legs above the surface, and dive down at the same time. "Air replenishment", which is the transport behaviour to collect air for the bell. The spiders swim to the surface, and cross their two pairs of hind legs above the back side of the abdomens so that the legs form a frame above the water (Schollmeyer, '13). With the air that adheres to the hairs inside of the leg frame and the opisthosoma, which is larger than the regular air bubble for breathing, the spider dives down to the bell where it releases the air. "Bell building", which consists of building new bells or fixing or enlarging air bells by weaving activity. "Locomotion", which includes swimming and walking under water. Water spiders rarely swim in the water freely because they are highly buoyant (Schütz and Taborsky, 2003). They mainly walk along structures or spider thread, which they spin between air bells, plants, and debris. "Catching and handling of prey", which includes hunting, killing, and transporting prey to the bell, and digesting the prey inside the bell.

Video analysis and statistics

Each video-tape was analyzed twice. The data were analyzed with non-parametric statistics because of small sample sizes. In O_2 experiments

spider activity was found increased only in the first hour after manipulation, in control experiments this effect lasted for 2 hr, and in CO_2 experiments the spiders were more active during the first 4 hr (see Fig. 1). Therefore, for comparison of the spiders' behaviour between treatments, we tested for differences separately in the first hour and in the first four hours after manipulation. (1) The total duration of activity in each hour (min/h) was determined over the entire observation period to test whether the spiders' activity changed with time following the manipulations. Total activity included surfacing, air replenishment, other locomotion, air bell building behaviour, and catching and handling of prey. We included only those spiders in our analysis from which we had data from all three treatments. We used Spearman rank correlation analyses to test for a change of mean activity per hour. With Friedman ANOVAs we tested whether the spiders' activity differed between treatments in the first hour and within the first four hours after manipulation; differences between pairs of treatments were subsequently tested with Friedman
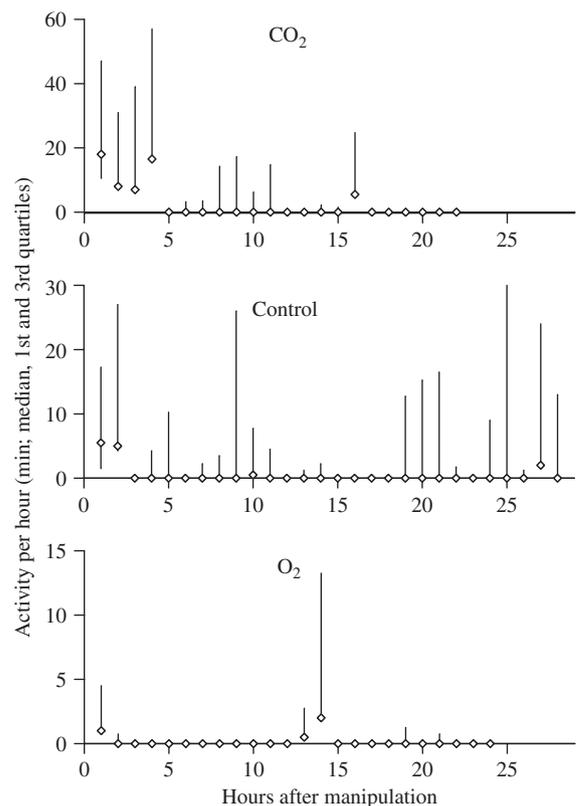

Fig. 1. Hourly activity after three different manipulations (minutes; medians, and quartiles of eight females). Note different scales on ordinates.

Post-hoc multiple-comparison tests ($\alpha = 0.05$; Conover, '80). (2) Surfacing, air replenishment, and air bell-building behaviour were measured for the first four hours following manipulations and compared between treatments. At 5-min intervals we checked whether females were surfacing, air replenishing, or building air bells. Then we compared the numbers of 5-min scans in which the respective behaviours occurred between treatments. For the analysis of air replenishment, we only included behaviour shown at the experimentally manipulated air bell and not at any newly built bell. To compensate for missing data, we extrapolated to the total observation period (4 hr) in two cases, in which the manipulated bell was only tended for 80 and 120 min, respectively. We used Friedman ANOVAs to test whether the frequencies of surfacing, air replenishment, and bell building behaviour differed between treatments, and with Friedman Post-hoc multiple-comparison tests ($\alpha = 0.05$ and 0.01; Conover, '80) we checked for differences between pairs of treatments. Within each treatment, we tested whether the spiders' behaviour was related to their body size using Spearman rank correlations.

RESULTS

Total activity per hour

Activity change with time after manipulation

Only in the CO_2 treatment, the median activity in minutes per hour decreased significantly with time after manipulation (Spearman rank correlations: CO_2: $r_s = -0.642$, $P = 0.002$, $n = 21$ h; control $r_s = -0.02$, $P = 0.921$, $n = 28$ hr; O_2: $r_s = -0.272$, $P = 0.198$, $n = 24$ hr; Fig. 1).

Activity differences between treatments

Between the treatments, spider activity did not differ significantly in the first hour following manipulations (Friedman test, $N = 8$ spiders, $df = 2$, $\chi^2 = 3.25$, $P = 0.197$), but the total activity shown in the first 4 hr after manipulation differed significantly between treatments (Friedman test, $N = 8$ spiders, $df = 2$, $\chi^2 = 7.75$, $P = 0.021$; Fig. 2). Total activity in the first 4 hr was higher in the experiment with CO_2 exchange than in the O_2 and control treatments (Friedman Post-hoc multiple-comparison tests, $P < 0.05$), but there was no significant difference between the control group and the O_2 treatment group (Friedman Post-hoc-Multiple-Comparison-Test, $P > 0.05$).
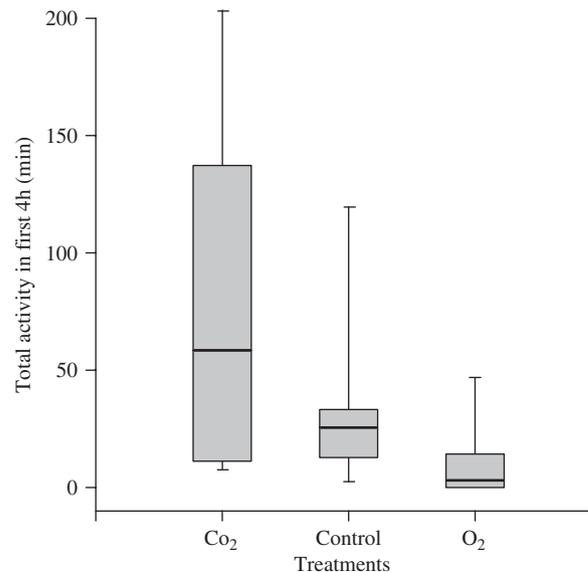

Fig. 2. Total activity shown during the first 4 hr after bell manipulation (minutes; box plots with medians, quartiles, minimum, and maximum of eight females).

Surfacing, air replenishment, and air-bell building behavior

The frequencies of surfacing differed significantly between treatments (Friedman test, $N = 8$ spiders, $df = 2$, $\chi^2 = 8.087$, $P = 0.018$). In the CO_2 treatment, spiders surfaced more often than in the O_2 and control treatments (Friedman Post-hoc multiple-comparison tests, $P < 0.01$; Fig. 3). Treatments did not differ significantly when comparing the frequencies of air replenishment (Friedman test, $N = 8$ spiders, $df = 2$, $\chi^2 = 2.69$, $P = 0.261$; Fig. 3), whereas the time spent in building behaviour at the manipulated air bells differed between treatments (Friedman test, $N = 8$ spiders, $df = 2$, $\chi^2 = 7.583$, $P = 0.023$; Fig. 3). Spiders showed more building behaviour in the CO_2 treatment than in the O_2 treatment (Friedman Post-hoc multiple-comparison test, $P < 0.05$), but there was no significant difference between the control group and the O_2 and CO_2 treatments (Friedman Post-hoc multiple-comparison tests, $P > 0.05$). One spider built a new air bell after 80 min in the CO_2 treatment, and another one did so after 120 min in the O_2 treatment.

Relationship between behaviour and body size

Total activity exhibited in the experimental period of the first 4 hr after manipulation did not
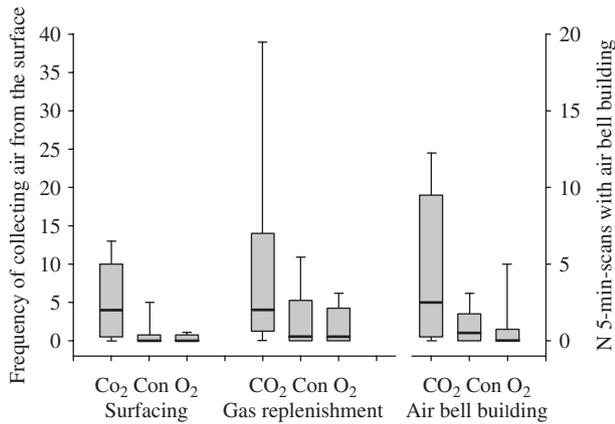
Fig. 3. Frequencies of collecting air at the surface to replenish gas in the bell or just to breathe ("surfacing"), and number of 5-min scans with building behaviour under different treatments. Box plots like in Figure 2. (Con = Control)

relate to female body size within treatments (Spearman rank correlations, $0.036 < r_s < 0.198$; $0.67 < P < 0.939$). Only in the control treatment, there was a trend that larger females surfaced more often than smaller ones (Spearman rank correlation, $r_s = 0.659$, $P = 0.076$, $n = 8$), and neither air replenishment ($r_s = 0.364$, $P = 0.376$) nor bell building ($r_s = 0.289$, $P = 0.487$) correlated with female size. In the CO_2 and O_2 treatments, no behaviour was related to female size (Spearman rank correlations, $0.006 < r_s < 0.596$; $0.119 < P < 0.989$).

DISCUSSION

Our experimental manipulation of the gas composition of under-water air bells significantly affected the spiders' behaviour. In all treatments, spider activity increased directly after manipulation, and did not differ significantly between treatments during the first hour after manipulation. This suggests that manipulation per se was responsible for this immediate effect. Overall, in the CO_2 treatment activity increase was much higher than in control or O_2 treatments, and this effect lasted the longest. Spider activity decreased again in the fifth hour after CO_2 application, which suggests that by then the oxygen levels had been sufficiently replenished in their bells. Pure CO_2 is toxic for arthropods, and is often used as an anaesthetic. However, if the increased activity of the spiders in the CO_2 treatment was due to its toxic effect, the change in activity should have peaked immediately after the manip-

ulation, i.e. within the first hour, which was not the case.

Water spiders showed not only more surfacing in the CO_2 treatment, but also increased bell building behaviour. The frequency of air replenishment did not differ significantly between treatments, but it showed high variance and the statistical power was low (Fig. 3). The observation that both in the CO_2 and O_2 treatments, one spider built a new air bell after 80 (CO_2) and 120 (O_2) min, respectively, may be due to the disturbance of the spiders by the manipulations. Regarding the hypotheses outlined above, our results suggest that the spiders monitored the quality of the gas in their bells, and actively improved the oxygen content required. In addition, they adjusted the silk structure of their bells in response to gas concentrations, i.e., hypothesis (4) seems to apply. The fact that the spiders did not react differently to the O_2 and control situations may indicate that they measured CO_2 levels in their bells rather than O_2. This should be further studied in a future experiment, where air bell atmosphere will be replaced either by oxygen or nitrogen.

Spider behaviour did not significantly relate to female body size. Only in the control treatment, larger females tended to surface more often than smaller ones, which might reflect a higher oxygen demand of the larger females. The manipulations seemed to affect spiders of all sizes in a similar fashion, because there was no indication of a relationship between body size and behaviour in the O_2, and CO_2 treatments.

To our knowledge *A. aquatica* is the only animal to collect, transport, store, and monitor air for breathing under water, which appears to be a necessary consequence of the colonization of a new environment. Species colonizing new environments are likely to encounter ecological conditions different from those to which their ancestors were adapted, and need to find new ways to sustain vital functions, e.g. feeding and reproduction in the new environment (Diesel, '92). For example, the bromeliad crab, *Metopaulias depressus*, breeds in rainwater filled leaf axils of bromeliads (Diesel, '92). Maternal care, consisting of removing leaf litter, maintaining ventilatory water current, and deposing snail shells in the nursery axil, is necessary to maintain oxygen, pH, and calcium levels optimal for the development of larvae (Diesel and Schuh, '93).

Under-water air bells can be viewed as an extended phenotype, an attribute of the organism,

which is not confined to the individual body bearing the responsible coding genes (Dawkins, '82). By structurally modifying the environment, *A. aquatica* construct and adaptively modify under-water bells and their gas content to support and maintain their internal physiology. Turner (2000) suggested to extend animal physiology beyond the conventionally defined boundaries of the organism, and defined results from adaptive modifications of the environment as ''external physiology''.

Spider webs have been described as external phenotypes with the main purpose of food gathering and protection of adults and/or young (Hansell, 2004). Another example of a woven structure that is used as an external organ for respiratory gas exchange is the bubble nest of spittlebugs (Turner, 2000). These homopterans are parasites of plant vascular tissue, and their diet of xylem sap is virtually all amino acids. The spittle nest is produced from xylem sap mixed with secretions from glands of the bug, and serves as an accessory gas exchange organ that promotes the exchange of ammonia rather than oxygen (Turner, 2000). Larvae of the wasp *Argiotypus* also use air bubbles as accessory gills (von Frisch and von Frisch, '74). *Argiotypus* lays its eggs on a caddis fly larva, which constructs water filled shelters coated with tiny bits of gravel. During the last larval instar stage, *Argiotypus* larvae become air breathers, and produce a web that shuts the open ends of the hosts' shelter, and evacuates the water from the now closed cocoon, creating an air space for breathing (von Frisch and von Frisch, '74).

Our results show that water spiders respond to gas conditions in their under-water bells, and actively monitor the quality of the bell atmosphere. We conclude that air bells are used as external oxygen reservoirs, and do not just serve as a water-free space for feeding and reproduction. The bells function as an external lung (Schollmeyer, '13; Heinzberger, '74), and hence are essential for permanently living under water (Braun, '31). An earlier study showed that individuals without air bells survived 3–5 days when prevented from surfacing, and 3 weeks under the same conditions when they had an air bell (Schollmeyer, '13).

ACKNOWLEDGMENTS

LITERATURE CITED

Braun F. 1931. Beiträge zur Biologie und Atmungsphysiologie der *Argyroneta aquatica* Cl. Zool Jb Syst 62:175–262.

Conover WJ. 1980. Practical nonparametric statistics. New York: John Wiley and Sons.

Crome W. 1951. Die Wasserspinne. Die neue Brehm-Bücherei 44:3–46.

Crome W. 1952. Die Respirations- und Cirkulationsorgane der *Argyroneta aquatica* Cl. Araneae Wiss Z Humboldt Univ Berlin 2:53–83.

Dawkins R. 1982. The extended phenotype: the gene as the unit of selection. New York: W.H. Freeman.

Diesel R. 1992. Managing the offspring environment: brood care in the bromeliad crab, *Metapaulias depressus*. Behav Ecol Sociobiol 30:125–134.

Diesel R. 1997. Maternal control of calcium concentration in the larval nursery of the bromeliad crab, *Metopaulias depressus* Grapsidae. Proc R Soc Lond B 264:1403–1406.

Diesel R, Schuh M. 1993. Maternal care in the bromeliad crab *Metopaulias depressus* Decapoda: maintaining oxygen, pH and calcium levels optimal for the larvae. Behav Ecol Sociobiol 32:11–15.

Ehlers M. 1939. Untersuchungen über Formen aktiver Lokomotion bei Spinnen. Zool Jb Syst 72:373–499.

Foelix RF. 1992. Biologie der Spinnen. Stuttgart: Georg Thieme Verlag.

Gibo DL, Yarascav RM, Dew HE. 1974. Thermoregulation in colonies of *Vespula arenaria* and *Vespula maculata* Hymenopterea, Vespidae under normal conditions and under cold stress. Can Entomol 106:503–507.

Grothendieck K, Kraus O. 1994. Die Wasserspinne *Argyroneta aquatica*: Verwandtschaft und Spezialisation Arachnida, Araneae, Agelenidae. Verh Naturwiss Ver Hamburg B:259–273.

Hansell MH. 2004. Animal architecture. Oxford: Oxford Animal Biology Series.

Heinrich B. 1984. Strategies of thermoregulation and foraging in 2 vespid wasps, *Dolichovespula maculata* and *Vespula vulgaris*. J Comp Physiol 154:175–180.

Heinzberger R. 1974. Verhaltensphysiologische Untersuchungen an *Argyronetha aquatica* Cl. Wuppertal, Friedrich-Wilhelms-Universität zu Bonn. PhD. Thesis. p. 1–93.

Lacher V. 1964. Elektrophysiologische Untersuchungen an einzelnen Rezeptoren für Geruch, Kohlendioxid, Luftfeuchtigkeit und Temperatur auf den Antennen der Arbeitsbiene und der Drohne *Apis mellifica* L. Z Vergl Physiol 48: 587–623.

Lang GHP. 2001. Sexual size dimorphism and juvenile growth rate in *Linyphia triangularis* Linyphiidae, Araneae. J Arachnol 29:64–71.

Martin SJ. 1990. Nest thermoregulation in *Vespa simillima*, *Vespa tropica* and *Vespa analis*. Ecol Entomol 15: 301–310.

Schollmeyer A. 1913. *Argyroneta aquatica*. Biologie mit besonderer Berücksichtigung der Atmung. Ann Biol Lacustre 6:314–338.

Schütz D, Taborsky M. 2003. Adaptations to an aquatic life may be responsible for the reversed sexual size dimorphism in the water spider, *Argyroneta aquatica*. Evol Ecol Res 5:105–117.

Schütz D, Taborsky M. 2005. Mate choice and sexual conflict in the size dimorphic water spider, *Argyroneta aquatica* Araneae: Argyronetidae. J Arachnol 33:767–775.

Seeley TD. 1974. Atmospheric carbon-dioxode regulation in honeybee *Apis mellifera* colonies. J Insect Physiol 20:2301–2305.

Turner JS. 2000. The extended organism. Cambridge, MA: Harvard University Press.

von Frisch K, von Frisch O. 1974. Animal architecture. New York: Harcourt Brace Javanovich.

Wesenberg-Lund C. 1939. Biologie der Süßwassertiere. Wien: Springer. p 595–619.