Journal of Insect Physiology 97 (2017) 38–44

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Metabolism and gas exchange patterns in *Rhodnius prolixus*

Pablo E. Schilman *

Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Biodiversidad y Biología Experimental, Buenos Aires, Argentina
Instituto de Biodiversidad y Biología Experimental y Aplicada, CONICET-UBA, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form 12 July 2016
Accepted 3 August 2016
Available online 3 August 2016

Keywords:
Wigglesworth
Respirometry
Gas-exchange patterns
Temperature

ABSTRACT

Insect's metabolic rate and patterns of gas-exchange varies according to different factors such as: species, activity, mass, and temperature among others. One particular striking pattern of gas-exchange in insects is discontinuous gas-exchange cycles, for which many different hypotheses regarding their evolution have been stated. This article does not pretend to be an extensive review on the subject, rather to focus on the work performed on the haematophagous bug *Rhodnius prolixus*, a model organism used from the mid XX century until present days, with the great influence of Wigglesworth and his students/collaborator's work. I have no doubt that the renovated field of insect gas-exchange has a bright future and will advance at large gaits thank to the help of this model organism, *R. prolixus*, whose entire genome has recently being unraveled.

1. Metabolic rate: modulation and measurements

The metabolic rate of an animal is the rate at which the metabolic energy is consumed under certain circumstances, namely the "cost of living". In other words the metabolic rate is given by the sum of all biochemical reactions that occur inside the animal. Among other things, the metabolic rate of an animal is modified by the activity, the mass of the animal, the temperature, whether or not it is digesting food, the time of day, etc. (Randall et al., 2002).

The metabolic rate can be measured by direct calorimetry, *i.e.*, measuring the total heat produced by an animal in units of calories min^{-1} or kilojoules min^{-1}, or by indirect calorimetry. One technique of indirect calorimetry is to measure the gas exchange between the animal and the environment by respirometry. For example the consumption of O_2, in units such as $\mu l\ O_2\ min^{-1}$ or the production of CO_2, expressed for example in $\mu l\ CO_2\ min^{-1}$ or both. Another way to measure the CO_2 produced is by the injection of doubly radiolabeled water ($^3H_2{}^{18}O$) in animals and subsequently collecting blood samples at different time intervals. The difference in the rates of oxygen and hydrogen lost are due to CO_2 production since O_2 is lost from the animal as CO_2 and H_2O, and H_2 is lost as H_2O only. For a complete summary of the technique and it's applications see the standard reference work of Speakman (1997) for doubly radiolabeled water technique or Lighton (2008) for gas exchange measurements.

The doubly labeled water method is particularly useful for measuring average metabolic rate (it is called "field metabolic rate") over relatively long periods of time (a few days or weeks), in subjects for which other types of direct or indirect calorimetric measurements of metabolic rate would be more difficult or impossible. It has the advantage of estimating the energetic cost of living under natural conditions. On the contrary, it has the disadvantage that it is not useful for individual insects. In addition, you need to recapture the animal in healthy conditions after weeks in the wild, therefore it is an average measurement and it does not allow calculation of the energetic cost of particular activities, *e.g.*, walking, carrying a load, flying, etc.

Most insects have aerobic metabolism, even under high energetic expenditures activities such as flight, thus measuring O_2 consumption or CO_2 production, or both is a good indirect method for calculating the metabolic rate. In addition, in part due to their small size, insects have low metabolic rates and consequently small volumes of gas exchange, thus measuring CO_2 production in real time is easier and more feasible than O_2 consumption. This is because: 1) CO_2 analyzers are at least an order of magnitude more sensitive than O_2 analyzers and 2) it is possible to scrub all CO_2 from incurrent air and measure tiny concentrations above a *ca.* zero background, but it is impossible to scrub all O_2 from incurrent air and obtain meaningful aerobic metabolic information. Therefore, it is impossible to measure small concentrations (few ppm) of O_2 from a huge background under normoxic condition, *i.e.*, *ca.* 210,000 ppm of O_2, due to the low signal to noise ratio.

* Address: Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Biodiversidad y Biología Experimental, Laboratorio de Ecofisiología de Insectos, Instituto de Biodiversidad y Biología Experimental y Aplicada – IBBEA, CONICET-UBA, Ciudad Universitaria, Pabellón II, Ciudad Autónoma de Buenos Aires C1428EHA, Argentina.
E-mail address: schilman@bg.fcen.uba.ar

http://dx.doi.org/10.1016/j.jinsphys.2016.08.002
0022-1910/

However, to convert O_2 consumption or in particular CO_2 production to power (*e.g.*, μWatt) requires the knowledge of respiratory quotient (RQ). RQ can be taken from literature or measured, for example using the stop-flow technique, which is a useful technique for measuring, among other things, O_2 consumption in small insects. Briefly, a chamber is flushed with ambient air or air free of CO_2 and water, the insect is placed inside the chamber and after a time (minutes or hours, depending on the chamber's and insect's size, temperature, etc.) air from the chamber is passed through the analyzers and the O_2 consumption or CO_2 production rates are calculated based on the concentrations and time period that the insect spends on the chamber (Fig. 1). While it is not necessary for RQ measurements, to actually measure gas exchange under these conditions, the flow rate through the analyzers must be known. This allows the y axis in Fig. 1 to be in units such as ml of gas per minutes (multiplying flow rate through the analyzer, *e.g.*, in ml min^{-1} * the CO_2 concentration expressed as a fraction). If the x axis is in minutes, integrating the peak gives gas exchange in ml. Dividing by the time of chamber closure gives gas exchange rate, *e.g.* ml/time. For advantages and disadvantages of different techniques see Lighton (2008) and Lighton and Hasley (2011).

For the reasons given above, most metabolic studies, particularly those done with such small animals as individual *Drosophila* (mass *ca.* 1 mg) are conducted by the stop-flow or constant volume techniques (exception *e.g.*, Lighton, 2007; Lighton and Schilman, 2007; Schilman et al., 2011). This technique has poor temporal resolution because integrates averages of catabolic flux rates over periods of an hour or more, during which bursts of activity may lead to serious measurement overestimates (Lighton et al., 2001 and references therein). In contrast, flow-through respirometry, although far more demanding of instrumentation stability and resolution, serves to minimize these errors (Lighton, 2008), and combined with activity monitoring allows the assessment of the standard metabolic rate (SMR) and the relation between MR and activity as well dynamics changes on the pattern of gas exchange (Fig. 2).

In order to have an "instantaneous", or at least a measurement with a good temporal resolution using open-flow respirometry, it is important to take into account the lag time of the system and the time-constant of the respirometry chamber. Lag time is the time required for changes in gas concentrations inside the respirometry chamber to reach the analyzer. It is a function of the air flow rate as well as the tubing volume between the chamber and the analyzer. It is best calculated empirically by introducing a brief and sharp peak of gas (*e.g.*, CO_2) in the respirometry chamber and then measure the time for the change to appear on the analyzer, *i.e.*, CO_2 analyzer. On the other hand, the time-constant of the respirometry chamber is the time required for a step change in rate of respiratory gas exchange to reach 63% of its final value within the chamber. It is calculated by the ratio between the volume of the chamber and air flow rate (*e.g.*, the time constant of a chamber with a volume of 400 ml, through which air flows at 200 ml min^{-1}, is 2 min). Time constants of 1 or less are adequate for a proper temporal resolution of the signal. In addition, the mixing characteristics of a gas stream within a chamber can be mathematically corrected. This wash-out phenomena correction, response correction or instantaneous correction can be done by the original Z-transform method (ZT) (Bartholomew et al., 1981) or by two new methods, which are based on modifications of the original ZT method: the extension of the ZT method (EZT), and the generalized ZT method (GZT) (Pendar and Socha, 2015).

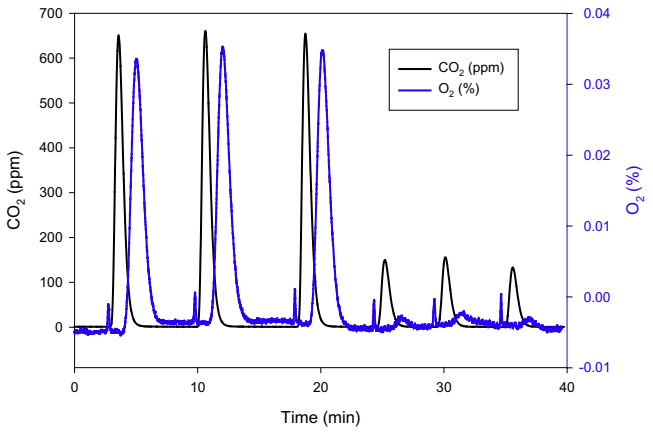

Fig. 1. CO_2 production (black) and O_2 consumption (blue) from *Drosophila melanogaster* measured by stop-flow technique. Three last peaks are controls (injected air from empty syringes), which values should be subtracted from the measurements (three first peaks; unpublished results). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

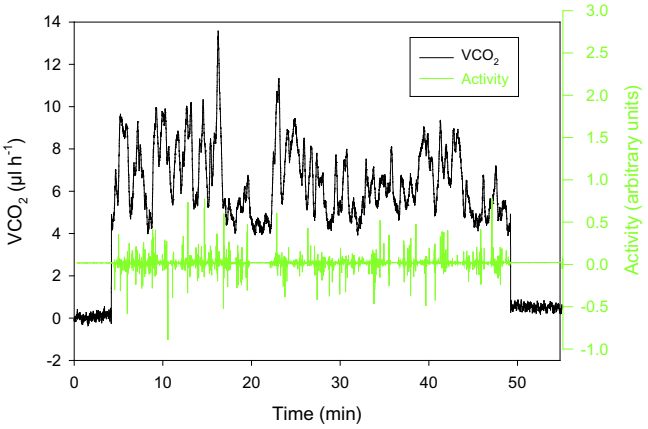

Fig. 2. Real-time recording of CO_2 production (μl h^{-1}) and activity (arbitrary units) as a function of time (min) from an individual fly, *Drosophila melanogaster* (mass = *ca.* 1 mg) at 25 °C. Note that the activity, dropping close to zero (no fluctuations) at about the 20 min mark, is accompanied by a corresponding drop in VCO_2 and SMR can be calculated (unpublished results).

2. Patterns of gas exchange in insects

In small animals, such as insects, the tracheal respiratory system allows rapid gas exchange between the cells and the atmosphere. Such rapid gaseous diffusion together with active convective ventilation (Socha et al., 2010; Harrison et al., 2013) allows insects to achieve high rates of gas exchange. For example, when leafcutter ants are cutting leaves, gas exchange increases 31-fold compared to rest (Roces and Lighton, 1995) or 20–100 times when insects are flying (Casey, 1989; Casey and Ellington, 1989; Ellington et al., 1990). The latter not only includes a higher O_2 demand of flight muscle, but also an increase for large changes in thorax temperature during flight (Harrison and Fewell, 2002).

While respiration is continuous in cells, in some insects under certain situations (for example at rest) measured as a whole organism, the gas exchange pattern is discontinuous. The intervals between bursts of gas exchange can be of only few seconds to several hours, depending on the insect (species, adult or larvae, temperature, etc.).

Also, insects are able to survive in extreme conditions of high temperature and low humidity, such as in most deserts of the earth. This feature results from the properties of the cuticle, composed of hydrocarbons and waxes that make it less permeable to water and reducing desiccation. This also contributes to the imper-

meability to exchange gases through the cuticle. However, when insects open their spiracles to gas-exchange, there is a huge increase of water loss (Fig. 3). Hence controlling spiracles opening is critical for the insects' survival.

The phenomenon of discontinuous gas exchange cycle (DGC) was discovered in the mid twentieth century (Punt, 1950), and it was described in detail in the 1950's and 1960's, among others by Schneiderman and collaborators with a butterfly pupae (*e.g.*, Levy and Schneiderman (1966a,b)). Overall, "classical" DGC is composed by three phases or periods. A period called C (for closed), when spiracles are closed and no gas exchange between the atmosphere and the animal occurs. During this phase the tissues of the insect consume endotracheal O_2 and cell respiration produces CO_2, which is partly absorbed by extracellular fluids (Hetz et al., 1993). Thus, the endotracheal O_2 partial pressure (PO_2) decreases and tracheal air pressure becomes lower than atmospheric pressure. Upon reaching a low value of PO_2, the central nervous system of the insect, repeatedly opens and closes the spiracles for short periods of time. This second period is called F (for flutter). During this phase, atmospheric air enters the trachea by difference in PO_2 without excessive respiratory water loss. Meanwhile CO_2 continues to accumulate, yet partly absorbed by the hemolymph and buffered. When this accumulation reaches a certain value, the spiracles open (without intervention of the central nervous system) and release the accumulated CO_2 to the atmosphere. This phase is called O (for open). For details see extensive and excellent reviews about DGC in Lighton (1994, 1996) and a more recent one of Quinlan and Gibbs (2006).

It may be noted that there are two of the three periods described with very low (in F) or null (in C) water loss through the spiracles. Therefore, the phenomenon of discontinuous gas exchange was initially associated with the need to reduce water loss when insects exchange gases with the atmosphere. In other words, the concept that DGC *per se* plays a role on water economy by reducing respiratory water loss was first suggested by Buck and colleagues in the 50' (Buck et al., 1953; Buck and Keister, 1955).

Although the "water saving hypothesis" was dominant for decades, other alternative hypotheses for the evolution of the DGC existed. One of these alternative hypotheses was the "strolling arthropod" hypothesis stated by Miller in mid 70', who proposed that keeping the spiracles closed prevents infection by parasites (Miller, 1974). This hypothesis did not receive much attention or experimental testing except for Harrison et al. (2001). The theory that evolution of gas exchange in insects would have resulted in the DGC due to the advantages offered for survival was then developed. Subsequent research however, yielded conflicting results,

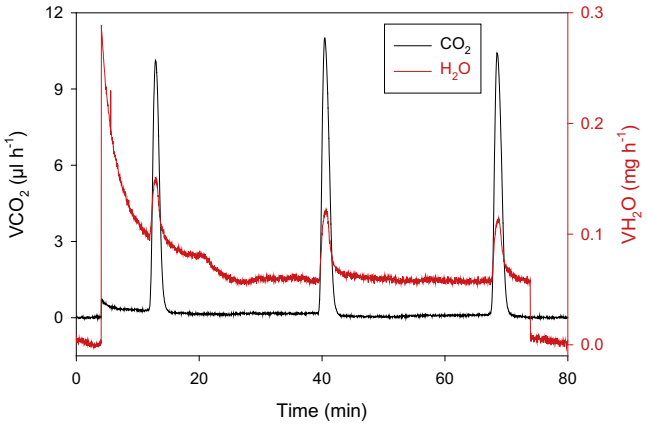

Fig. 3. Real-time recording of CO_2 production (µl h^{-1}) and H_2O release (mg h^{-1}) as a function of time (min) from an individual worker ant, *Pogonomyrmex californicus* (mass = 6.96 mg) at 20 °C. Note CO_2 and H_2O vapor emission peaks synchronization due to respiratory water loss. Water loss between peaks corresponds to cuticular water loss rate (modified from Schilman (2007)).

enabling the first question of this theory. For example while the termite *Prorhinotermites simplex* employs DGC in dry air, but not in humid one (Slama et al., 2007), supporting the water saving hypothesis, DGC does not cease in *Rhodnius prolixus* in humid air (Contreras and Bradley, 2009) or tenebrionid beetles from xeric environments such as *Eleodes obscura* always showed continuous gas-exchange (Schilman et al., 2008), which contrasts to the expectations deriving from the hygric hypothesis.

In the early 1990s, John Lighton proposed an alternative hypothesis for the evolution of the DGC in insects, the "chthonic hypothesis" (Lighton and Berrigan, 1995; Lighton, 1996). This alternative hypothesis, which received much attention, proposed that DGC developed in insects living under high levels of CO_2 (hypercapnia) and low O_2 (hypoxia) like ants and beetles with subterranean habits. Keeping the spiracles closed (during the C phase) would increase CO_2 and decrease O_2 endotracheal levels in order to increase gradients for gas exchange, making the gas exchange with the environment by diffusion more efficient.

While "chthonic hypothesis" proposed that DGC serves to improve gas exchange, the more recent "oxidative stress hypothesis" (Bradley, 2000; Hetz and Bradley, 2005) proposed that DGC limits the uptake of O_2. High concentrations of O_2 have negative or toxic effects, not only for insects but for animals or cells in general. Hetz and Bradley showed that endotracheal O_2 concentration in the pupae of the giant moth *Attacus atlas* was kept constant at a value of *ca.* 5%. by varying the frequency of spiracles opening (Hetz and Bradley, 2005).

In addition, Chown and Holter (2000) proposed the "emergent properties" hypothesis. This is a non-adaptive hypothesis based on the modulation of spiracles opening by levels of CO_2 and O_2 or the interaction of two set points (CO_2 and O_2), which recently received support from results of a simple computational model of two interacting feed-back loops (Grieshaber and Terblanche, 2015).

Another non-adaptive hypothesis was the "neural hypothesis" (Matthews and White, 2011). This hypothesis suggests that DGC emerges in periods of reduced brain activity, when modulation of opening spiracles responds to tracheal CO_2 and oxygen partial pressures via segmental ganglia through the feedback system described by Förster and Hetz (2009). The neural hypothesis received support from well-designed experiments from Matthews and White. They clearly demonstrated brain activity, instead of insect metabolic rate, is correlated with insect respiratory pattern (Matthews and White, 2013).

In addition, John Lighton, Alex Kaiser and the author of this article suggest that the tenebrionid beetle, *Eleodes obscura* cannot exchange respiratory gases discontinuously because of a morphological constraint (small tracheal or spiracular conductance). This "conductance constraint hypothesis" may help to explain the otherwise puzzling phylogenetic patterns of continuous vs. discontinuous gas exchange observed in tracheate arthropods (Schilman et al., 2008).

It is worthy to be noted that all these hypotheses are not mutually exclusive and more than one can be applied.

Although the respiratory pattern on insects' gas exchange has been extensively described, explanation for their occurrence has been controversial. Different hypotheses to explain the evolution of different patterns of gas exchange have been stated. For a more complete discussion of the different hypothesis of DGC see Chown et al. (2006) and Contreras et al. (2014).

3. Wigglesworth's contributions to insect's pattern of gas exchange

There was a lot of work done by Wigglesworth and his students on development including some interesting results on

morphogenesis of the tracheal system, the control on its distribution and responses to oxygen deficit (Locke, 1958a, 1958b; Wigglesworth, 1954, 1959). However, I will focus on the control of gas exchange patterns. Most of the credit for the detailed description and the initial unraveling of the underlying mechanism of the remarkable pattern of discontinuous gas exchange observed in some insects laid on the pioneer work of Levy and Schneiderman (1966a, 1966b). A senior study by Wigglesworth (1935) in the flea, showed the modulation of spiracular opening by gas composition. Particularly, in his Fig. 4H (Wigglesworth, 1935), it is clear that the closed phase of the last abdominal spiracle lasts longer in pure oxygen. More than 30 years later, Levy and Schneiderman (1966a) demonstrated a similar phenomenon, but did not cite the Wigglesworth's 1935 study. Working with the silkworm pupae, Levy and Schneiderman observed an increase in the period of spiracular constriction with increasing ambient O_2 (PO_2 = 60%), and hypothesized a theoretical spiracular response in pure O_2. This phenomenon of modulation of spiracular opening by gas composition, in particular the constriction of the spiracles by infusion of pure oxygen was used by John Lighton and the author of this article based on an inspired idea of Lighton to develop a technique to discern between spiracular and cuticular water loss in insects showing continuous gas exchange; the hyperoxic switch method (Lighton et al., 2004; Schilman et al., 2005). Coincidentally, in the same year (2004) and in the same journal (The Journal of Experimental Biology) Gibbs and Johnson published another technique that also enabled to distinguish between cuticular and respiratory water loss in insects with continuous gas exchange: the regression method (Gibbs and Johnson, 2004). The advance in the measurements resolution due to improvement of equipment during the last 20 years, together with the development of new techniques greatly aided to the advance in understanding the relation between the pattern of gas exchange and the water balance of insects.

4. *Rhodnius prolixus* as a model for metabolism and patterns of gas exchange

Insect metabolic rates are highly variable and are affected by environmental, behavioral, developmental and evolutionary factors. Among these factors are: the temperature, activity, insect's mass, nutritional and reproductive status (Waters and Harrison, 2012). Very few studies address the question of the costs of reproduction. One of them was performed by Davey in 1993. Davey completed his BSc in Zoology and MSc in Insect Physiology at the University of Western Ontario in Canada. He then travelled to England to study at Cambridge with Wigglesworth as advisor for a PhD in the reproductive physiology of *Rhodnius prolixus* or in other words, the "Wigglesworth Bug". In his paper from 1993, Davey calculated the metabolic rate of reproduction of mated and virgin *R. prolixus* females at 28 °C with a standard Warburg differential respirometer. In both virgin and mated females, O_2 consumption rises immediately after feeding and then slowly declines to prefeeding levels after 10–12 days. However, mated females maintain their O_2 consumption higher than virgin females during the period of egg production. In addition, O_2 consumption of ovariectomized females did not reach same levels as control females did, however they exhibit the same peak just after feeding. This increase is absent in allactomized females, indicating a role of the juvenile hormone on the oxidative metabolism (Davey, 1993).

In a series of papers published in the mid-late sixties/start of the seventies, Okasha studied the effect of high, but sub-lethal temperatures in different processes of *Rhodnius prolixus* larvae, including the O_2 consumption as an indirect measure of metabolic rate.

Okasha also carried out his Ph.D. and received a Ph.D. degree from the University of Cambridge under supervision of Professor Sir Vincent B. Wigglesworth. It is noteworthy that in all the papers published from his Ph.D. thesis work, Okasha was the only author.

In a Nature paper from 1968, Okasha showed that *R. prolixus* N4 larvae failed to moult at temperatures higher than 35 °C due to a disturbance of hormone balance. For various stages of *R. prolixus*, Okasha exposed them to 36.5 °C just after feeding for different periods of time and then placed them at 28 °C (optimum temperature). He observed a delay in moulting, reduction of sensory bristles, diuresis problems, despite apparently normal function of the Malpighian tubules. He concluded that high temperature interfered with protein synthesis (Okasha, 1964). The latter study, besides of being under the supervision of Wigglesworth, it was partially based in a previous work from Wigglesworth himself (1937), where he showed a two-fold increase of O_2 consumption five days after injury (decapitated) due to the growth activity in the epidermis during wound healing. This increase in O_2 consumption ceased at 36.5 °C (Wigglesworth, 1937).

Growth and development ceased in *Rhodnius prolixus* larvae placed immediately after feeding at sub-lethal high temperatures (Okasha, 1964, 1968a; Wigglesworth, 1972). Cessation of growth in *Rhodnius* at high temperatures was also combined with inhibition of the secretion of the brain hormone and consequently of the moulting hormone, cell division in the epidermis and protein synthesis (Okasha, 1968a, 1968b, 1968c).

At 25 °C, larval stages of *Rhodnius* showed an increase (4–5 times) or peak in O_2 consumption after feeding. In 4th stage, 13–15 days after feeding there was a second peak of O_2 consumption when ecdysis occurred. The latter increase in O_2 uptake was due to protein synthesis; namely, increase in cell numbers, and muscle formation of chitin in the cuticle (Zwicky and Wigglesworth, 1956). Similarly, at 28 °C, *R. prolixus* larvae exhibited an increase of O_2 consumption following feeding with a peak on day 12 associated with ecdysis. However, at 36.5 °C, the O_2 consumption of *R. prolixus* 1 day after feeding was about two-fold that measured at 28 °C, and decreased over time (at 36.5 °C) instead of increasing like it occurred at 28 °C. When insects were moving from 36.5 to 28 °C at 11 days after feeding, there was a delay of *ca.* 4 days and O_2 consumption began to increase. However, in unfed *R. prolixus* larvae the O_2 consumption remained fairly constant and it was about two-fold higher at 36.5 compared to 28 °C. The experiments described in this work clearly showed the high level of general

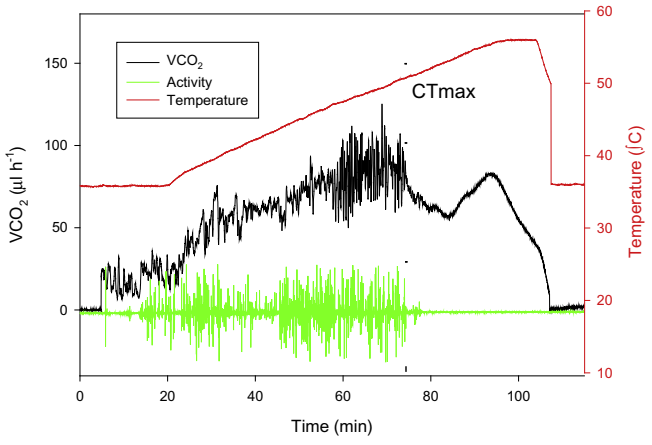

Fig. 4. Thermo-limit respirometry. Real-time recording of CO_2 production expressed in µl h^{-1} (black), activity in volts (green) and temperature in °C (red) as a function of time in minutes from an unfed fifth-instars of *Rhodnius prolixus* (mass = 29.9 mg) at temperatures ranging from 10 to 35 °C by five degrees steps. The equilibration temperature (right red scale) was 35.79 °C. The ramping rate was 0.25 °C min^{-1}. Beginning and ending of recording are baselines taken with the empty chamber (modified from de la Vega et al. (2015)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

metabolism associated with temperature, growth and development processes, which is reduced when these processes cease, *e.g.*, at 36.5 °C (Okasha, 1968d).

Decapitation of *R. prolixus* larvae 1 day after feeding resulted in an increase of O_2 consumption to similar levels of fed larvae related with wounding and synthesis of proteins. Injury did not elevate the metabolism of intact larvae, which was already high due to the moulting process (Okasha, 1970).

Besides the effects of temperature on the hormonal regulation of *R. prolixus*, temperature affects and modifies many processes that would be very interesting to investigate. In particular, since *R. prolixus* like most invertebrates is ectothermic, an increase in environmental temperature speeds up biochemical reactions, permitting a rise in activity and growth rates, which in turn brings an increase in metabolic and nutrient transformation rates. Consequently, it could be expected an increase on biting rate with a concomitant boost in the transmission of infectious diseases in the case of a disease vector like *R. prolixus*. This effect of temperature on vector's metabolic rates should be seriously taken into account when formulating mathematical models to predict disease transmission in a global climate change scenario (Rolandi and Schilman, 2012). One approach could be by measuring the temperature coefficient (Q_{10}) for the different stages of *R. prolixus*

Although *R. prolixus* is a model organism in insect physiology and during the last 50 years, a lot of work has been done in relation to temperature, some standard measurements of thermal tolerance (CTmin, CTmax, upper lethal temperature and chill coma recovery time) have only been recently done (de la Vega et al., 2015). Future research, on the effects of acclimation and hardening to thermotolerance measurements should be performed. Moreover, the actual knowledge of insect's heat shock proteins function (King and MacRae, 2015) together with complete genome sequence of *R. prolixus* (Mesquita et al., 2015) would allow to design and perform a strong-inference experimental approach, *e.g.*, with RNAi injection.

R. prolixus suffer mainly from great thermal stress not by the environmental temperature change, since they inhabit the tropics with no extreme temperatures, but by the large amount of "hot" blood ingestion (up to 10 times their mass for a N5) from endothermic animals that can change the bug's body temperature many degrees. Future research, should focus on finding the existence of differential expression of genes related to heat tolerance like heat shock proteins at the moment of ¨hot¨ blood ingestion or a different mechanism as evaporative cooling, as it was found in another hematophagous insect, the mosquito *Anopheles stephensi* (Lahondère and Lazzari, 2012).

Thanks to technical improvements of the last 20 or 30 years, it is now possible to measure CO_2 production from individual *R. prolixus* in real time. These improvements allow, among other things, to measure the energetic cost associated with different activities such as walking and flying (for dispersal), reproduction, feeding on different hosts, etc., and their consequences in terms of individual fitness. All these are very interesting questions on the evolutionary, ecological and epidemiological perspective to address using *R. prolixus* as a model organism.

In addition, during the last 10 years, Tim J. Bradley and collaborators have placed *Rhodnius prolixus* again on the spot-light as a model organism to study the patterns of gas exchange. Dr. Bradley worked with Simon Maddrell a former Ph.D. student of Sir Vincent Wigglesworth.

R. prolixus is a good model for studying insect metabolism and patterns of gas exchange because it has a low and constant metabolic rate indicated by its CO_2 production.

In a series of papers, Bradley and collaborators studied the effect of metabolic rate (modulated by feeding or temperature), as well as air humidity on respiratory patterns. Working on 5th instar nymphs of *R. prolixus* the authors showed metabolic scope values of almost 8 and 14 (Bradley et al., 2003; Heinrich and Bradley, 2014), while Rolandi and collaborators (2014) working with *R. prolixus* males registered a postprandial metabolic scope of 2. These higher values found by Bradley and collaborators could be explained by the larger amount of blood ingested by nymphs compared with adult males, together with the effects of other physiological processes such as development, which occur following feeding. Fed *R. prolixus* showed similar metabolic rates compared to other species of insects. However, fasted *R. prolixus* showed lower metabolic rates than other insects' species, but always higher than other haematophagous such as ticks (Lighton and Fielden, 1995). More recently, and for the first time in any haematophagous arthropod, the cost of haematophagy was assessed in *R. prolixus* (Leis et al., 2016). The authors found an increase of up to 17-fold in the metabolic rate during feeding, as well as a change in the respiratory pattern and respiratory quotient (RQ). They observed a decrease in the mean RQ from 0.83 in resting bugs to 0.52 during feeding (Leis et al., 2016). This phenomenon of changing RQ to a rare value of *ca.* 0.5 deserves further study.

In addition, using *Rhodnius prolixus* males at two different temperatures (25 and 35 °C) and nutritional status (1 or 21 days post-ingesta), Contreras and Bradley (2009) tried to provide a mechanistic explanation for the variations in insect respiratory patterns. The authors proposed that gas-exchange pattern of the insect was a function of the amount of endotracheal PO_2 and the aerobic metabolic rate. The closed (C) phase was used to lower endotracheal O_2 levels. As metabolic rate increased, the C phase shortened and disappeared leading to a cyclic pattern. Further metabolic rate increases shortened the flutter phase until its elimination which led to a continuous gas-exchange pattern. Same authors, using the haematophagous bug, *Rhodnius prolixus* and the hissing cockroaches, *Gromphadorhina portentosa* tried to determine the effect of temperature on metabolic rate (Fig. 5). Both species, *G. portentosa* and fasted *R. prolixus*, have low and relatively constant metabolic rates making them suitable for direct control of metabolic rate by temperature variations. Contreras and Bradley objectively determined periods of spiracular closure using the hyperoxic switch method (Lighton et al., 2004) and observed whether changes in metabolic rates were correlated with length of spiracular closure (Contreras and Bradley, 2010).

They observed at low temperatures, long periods of spiracular closure, giving as a result a DGC pattern. Increasing the tempera-

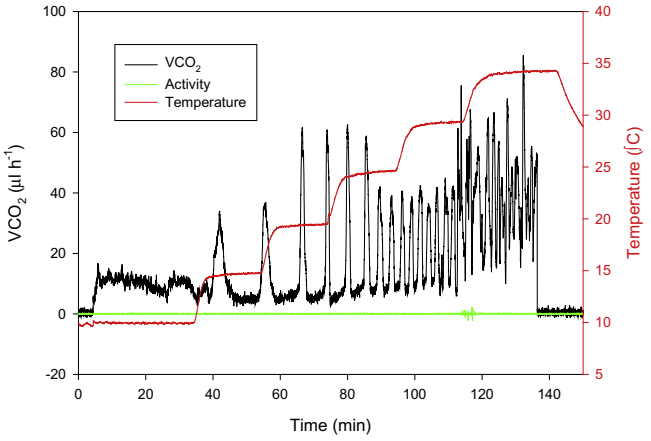

Fig. 5. Real-time recording of CO_2 production expressed in µl h^{-1} (black), activity in volts (green) and temperature in °C (red) as a function of time in minutes from a *Rhodnius prolixus* female (mass = 80.9 mg) at temperatures ranging from 10 to 35 °C by five degrees steps. Note changes in gas-exchange pattern by temperature. Beginning and ending of recording are baselines taken with the empty chamber (unpublished results). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ture produced a cyclic pattern, while further increase in temperature resulted in a continuous pattern of gas-exchange (Contreras and Bradley, 2010). They proposed that the gas exchange patterns of insects are not discrete respiratory forms, but instead are a continuum that reflects a balance between oxygen demand and oxygen supply (Contreras and Bradley, 2010). However, more recently Heinrich and Bradley (2014) showed that the transition of *R. prolixus* between respiratory patterns depended on the metabolic increase, *i.e.*, by temperature or feeding, suggesting that factors other than the metabolic rate itself influence respiratory patterns. In other words, gas exchange pattern of *Rhodnius* depends on both metabolic rate and environmental temperature. Insects at high temperatures abandoned DGC at lower metabolic rates compared to individuals at lower temperatures (Heinrich and Bradley, 2014). Similarly, Rolandi et al. (2014) found in *Rhodnius* males that temperature and activity affected the change from a cyclic to a continuous gas-exchange pattern, but not the level of starvation (Rolandi et al., 2014). These results may support the "neural hypothesis" of DGC, however future experiments should be designed specifically to test this hypothesis.

Other relevant questions relate to the metabolic scaling. In particular, how metabolic rate changes with the mass of the animal. For that question, *R. prolixus* is a suitable model because is a hemimetabolous insect, all stages sharing similar requirements and ecological niche including being obligatory hematophagous. Consequently there is a wide range of masses from first stage to adults to be tested.

5. Final remarks

It is always amazing to realize that only one person, *i.e.*, Professor Sir Vincent B. Wigglesworth, and one model organism, *i.e.*, the haematophagous bug *Rodnius prolixus*, could contribute so dramatically to insect physiology.

Although great advances in understanding the control and evolution of respiratory patterns of gas exchange on traqueate arthropods have been made, *R. prolixus* will continue giving us information and new questions to investigate. In particular, the recent sequence of the complete genome of *R. prolixus* (Mesquita et al., 2015) will bring a set of new questions and genetic tools that would move forward the field of insect physiology in great quantitative and qualitative ways that can only be compared with the huge advance produced by Sir Wigglesworth and his collaborators in mid XX century. Thus, I dedicate this article to Sir Vincent Wigglesworth, whose research in insect physiology and endocrinology over more than six decades illuminated the subject and inspired generations of researchers.

Abbreviations

RQ = respiratory quotient
DGC = discontinuous gas-exchange cycle
SMR = standard metabolic rate
PO_2 = O_2 partial pressure
VCO_2 = rate of CO_2
VH_2O = rate of H_2O
CTmax = critical thermal maxima
CTmin = critical thermal minimum

Acknowledgements

The author thanks C.R. Lazzari for the invitation to this special issue of the Journal of Insect Physiology, C. Rolandi for data of Fig. 5 and graphical abstract, G. Pontes and R. Sinistro for the picture of *R. prolixus* and to A. Marin-Burgin and A. Dyzenchauz for critical reading of the manuscript. The author also heartily thanks J.R.B. Lighton for his generosity and vast knowledge of respirometry, which I likely get a little. Past and present financial support by DAAD/Germany and ANPCyT, UBA and CONICET/Argentina (PIP-112-201301-00550). The manuscript greatly improves by the constructive comments of two anonymous reviewers.

References

Bartholomew, G.A., Vleck, D., Vleck, C.M., 1981. Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. J. Exp. Biol. 90, 17–32.
Bradley, T.J., 2000. The discontinuous gas exchange cycle in insects may serve to reduce oxygen supply to the tissues. Am. Zool. 40, 952.
Bradley, T.J., Brethorst, L., Robinson, S., Hetz, S., 2003. Changes in the rate of CO_2 release following feeding in the insect *Rhodnius prolixus*. Physiol. Biochem. Zool. 76, 302–309.
Buck, J., Keister, M., 1955. Cyclic CO_2 release in diapausing *Agapema* pupae. Biol. Bull. 114, 118–140.
Buck, J., Keister, M., Specht, H., 1953. Discontinuous respiration in diapausing *Agapema* pupae. Anat. Rec. 117, 541.
Casey, T.M., 1989. Oxygen consumption during flight. In: Goldsworthy, G.J., Wheeler, C.H. (Eds.), Insect Flight. CRC Press, Boca Raton, Florida.
Casey, T.M., Ellington, C.P., 1989. Energetics of insect flight. In: Wieser, W., Gnaiger, E. (Eds.), Energy Transformations in Cells and Animals. George Thieme Verglag, Stuttgart, pp. 200–212.
Chown, S.L., Holter, P., 2000. Discontinuous gas exchange cycles in *Aphodius fossor* (Scarabaeidae): a test of hypotheses concerning origins and mechanisms. J. Exp. Biol. 203, 397–403.
Chown, S.L., Gibbs, A.G., Hetz, S.K., Klok, C.J., Lighton, J.R.B., Marais, E., 2006. Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. Physiol. Biochem. Zool. 79, 333–343.
Contreras, H.L., Bradley, T.J., 2009. Metabolic rate controls respiratory pattern in insects. J. Exp. Biol. 212, 424–428.
Contreras, H.L., Bradley, T.J., 2010. Transitions in insect respiratory patterns are controlled by changes in metabolic rate. J. Insect Physiol. 56, 522–528.
Contreras, H.L., Heinrich, E.C., Bradley, T.J., 2014. Hypotheses regarding the discontinuous gas exchange cycle (DGC) of insects. Curr. Opin. Insect Sci. 4, 48–53.
Davey, K.G., 1993. Oxygen consumption and egg production in the blood feeding insect *Rhodnius prolixus*. Invertebrate Reprod. Dev. 23, 45–49.
de la Vega, G., Medone, P., Ceccarelli, S., Rabinovich, J., Schilman, P.E., 2015. Geographical distribution, climatic variability and thermo-tolerance of Chagas disease vectors. Ecography 38, 851–860.
Ellington, C.P., Machin, K.E., Casey, T.M., 1990. Oxygen consumption of bumblebees in free forward flight – no power curve. Nature 347, 472–473.
Förster, T., Hetz, S.K., 2009. Spiracle activity in moth pupae: the role of oxygen and carbon dioxide revisited. J. Insect Physiol. 56, 492–501.
Grieshaber, B.J., Terblanche, J.S., 2015. Computational model of insect discontinuous gas exchange: a two-sensor, control systems approach. J. Theor. Biol. 374, 138–151.
Gibbs, A.G., Johnson, R.A., 2004. The role of discontinuous gas exchange in insects: the chthonic hypothesis does not hold water. J. Exp. Biol. 207, 3477–3482.
Harrison, J.F., Camazine, S., Marden, J.H., Kirkon, S.D., Rozo, A., Yang, X., 2001. Mite not make it home: tracheal mites reduce the safety margin for oxygen delivery of flying honeybees. J. Exp. Biol. 204, 805–814.
Harrison, J.F., Fewell, J.H., 2002. Environmental and genetic influences on flight metabolic rate in the honey bee, *Apis mellifera*. Comp. Biochem. Physiol. A 133, 323–333.
Harrison, J.F., Wasserthal, L.T., Chapman, R.F., 2013. Gaseous exchange. In: Simpson, S.J., Douglas, A.E. (Eds.), The Insects: Structure and Function, fifth ed. Cambridge University Press, New York, pp. 501–545.
Heinrich, E., Bradley, T.J., 2014. Temperature-dependent variation in gas exchange patterns and spiracular control in *Rhodnius prolixus*. J. Exp. Biol. 217, 2752–2760.
Hetz, S.K., Bradley, T.J., 2005. Insects breathe discontinuously to avoid oxygen toxicity. Nature 433, 516–519.
Hetz, S.K., Wasserthal, L.T., Hermann, S., Kaden, H., Oelßner, W., 1993. Direct oxygen measurements in the tracheal system of lepidopterous pupae using miniaturized amperometric sensors. Bioelectrochem. Bioenergy 33, 165–170.
King, A.M., MacRae, T.H., 2015. Insect heat shock proteins during stress and diapause. Annu. Rev. Entomol. 60, 59–75.
Lahondère, C., Lazzari, C.R., 2012. Mosquitoes cool down during blood feeding to avoid overheating. Curr. Biol. 22, 40–45.
Leis, M., Pereira, M.H., Casas, J., Menu, F., Lazzari, C.R., 2016. Haematophagy is costly: respiratory patterns and metabolism during feeding in *Rhodnius prolixus*. J. Exp. Biol. http://dx.doi.org/10.1242/jeb.120816.
Levy, R.I., Schneiderman, H.A., 1966a. Discontinuous respiration in insects – III. The effect of temperature and ambient oxygen tension on the gaseous composition of the tracheal system of silkworm pupae. J. Insect Physiol. 12, 105–121.
Levy, R.I., Schneiderman, H.A., 1966b. Discontinuous respiration in insects – IV. Changes in intra-tracheal pressure during the respiratory cycle of silkworm pupae. J. Insect Physiol. 12, 465–492.

Lighton, J.R.B., 1994. Discontinuous ventilation in terrestrial insects. Physiol. Zool. 67 (1), 142–162.
Lighton, J.R.B., 1996. Discontinuous gas exchange in insects. Annu. Rev. Entomol. 41, 309–324.
Lighton, J.R.B., 2007. Hot hypoxic flies: whole-organism interactions between hypoxic and thermal stressors in *Drosophila melanogaster*. J. Therm. Biol. 32 (3), 134–143.
Lighton, J.R.B., 2008. Measuring Metabolic Rates: A Manual for Scientists. Oxford University Press, Oxford.
Lighton, J.R.B., Berrigan, D., 1995. Questioning paradigms: caste-specific ventilation in harvester ants, *Messor pergandei* and *M. julianus* (Hymenoptera: Formicidae). J. Exp. Biol. 198, 521–530.
Lighton, J.R.B., Brownell, P., Joos, B., Turner, R., 2001. Low metabolic rate in scorpions: implications for population biomass and cannibalism. J. Exp. Biol. 204 (3), 607–613.
Lighton, J.R.B., Fielden, L.J., 1995. Mass scaling of standard metabolism in ticks: a valid case of low metabolic rates in sit-and-wait strategists. Physiol. Zool. 68 (1), 43–62.
Lighton, J.R.B., Hasley, L.G., 2011. Flow-through respirometry applied to chamber systems: pros and cons, hints and tips. Comp. Biochem. Physiol. A. 158, 265–275.
Lighton, J.R.B., Schilman, P.E., 2007. Oxygen reperfusion damage in an insect. PLoS One 2, e1267.
Lighton, J.R.B., Schilman, P.E., Holway, D.A., 2004. The hyperoxic switch: assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. J. Exp. Biol. 207, 4463–4471.
Locke, M., 1958a. The formation of tracheae and tracheoles in *Rhodnius prolixus*. Q. J. Microsc. Sci. 99, 26–46.
Locke, M., 1958b. The coordination of growth in the tracheal system of insects. Q. J. Microsc. Sci. 99, 373–391.
Matthews, P.G.D., White, C.R., 2011. Discontinuous gas exchange: is it all in their heads? Am. Nat. 177, 130–134.
Matthews, P.G.D., White, C.R., 2013. Reversible brain inactivation induces discontinuous gas exchange in cockroaches. J. Exp. Biol. 216, 2012–2016.
Mesquita, R.D., Vionette-Amaral, R.J., Lowenberger, C., Rivera-Pomar, R., Monteiro, P., Minx, F.A., et al., 2015. Genome of *Rhodnius prolixus*, an insect vector of Chagas disease, reveals unique adaptations to haematophagy and parasite infection. Proc. Natl. Acad. Sci. U.S.A. 112, 14936–14941.
Miller, P.L., 1974. Respiration: aerial gas transport. In: Rockstein, M. (Ed.), Physiology of Insecta, . second ed., IV Academic Press, New York, pp. 345–402.
Okasha, A.Y.K., 1964. Effects of high temperature in *Rhodnius prolixus* (Stål.). Nature 204, 1221–1222.
Okasha, A.Y.K., 1968a. Effects of sub-lethal high temperature on an insect, *Rhodnius prolixus* (Stål.) – I. Induction of delayed moulting and defects. J. Exp. Biol. 48, 455–463.
Okasha, A.Y.K., 1968b. Effects of sub-lethal high temperature on an insect, *Rhodnius prolixus* (Stål.). II. Mechanism of cessation and delay of moulting. J. Exp. Biol. 48, 465–473.
Okasha, A.Y.K., 1968c. Effects of sub-lethal high temperature on an insect, *Rhodnius prolixus* (Stål.). III. Metabolic changes and their bearing on the cessation and delay of moulting. J. Exp. Biol. 48, 475–486.
Okasha, A.Y.K., 1968d. Changes in the respiratory metabolism of *Rhodnius prolixus* as induced by temperature. J. Insect Physiol. 14, 1621–1634.
Okasha, A.Y.K., 1970. Injury metabolism in an insect, *Rhodnius prolixus*. J. Insect Physiol. 16, 1579–1585.
Pendar, H., Socha, J.J., 2015. Estimation of instantaneous gas exchange in flow-through respirometry systems: a modern revision of Bartholomew's Z-transform method. PLoS One 10 (10), e0139508. http://dx.doi.org/10.1371/journal.pone.0139508.
Punt, A., 1950. The respiration of insects. Physiol. Comp. Oecol. 2, 59–74.
Quinlan, M.C., Gibbs, A.G., 2006. Discontinuous gas exchange in insects. Respir. Physiol. Neurobiol. 154, 18–29.
Randall, D., Burggren, W.W., French, K., Eckert, R., 2002. Eckert Animal Physiology. W.H. Freeman, p. 120.
Roces, F., Lighton, J.R.B., 1995. Larger bites of leaf-cutting ants. Nature 373, 392–393.
Rolandi, C., Schilman, P.E., 2012. Linking global warming, metabolic rate of hematophagous vectors, and the transmission of infectious diseases. Front. Physiol. 3, 75.
Rolandi, C., Iglesias, M.S., Schilman, P.E., 2014. Metabolism and water loss rate of the haematophagous insect *Rhodnius prolixus*: effect of starvation and temperature. J. Exp. Biol. 217, 4414–4422. http://dx.doi.org/10.1242/jeb.109298.
Schilman, P.E., 2007. La respiración de los artrópodos con tráqueas. Ciencia Hoy 17 (100), 22–26.
Schilman, P.E., Lighton, J.R.B., Holway, D.A., 2005. Respiratory and cuticular water loss in insects with continuous gas exchange: comparison across five ant species. J. Insect Physiol. 51, 1295–1305.
Schilman, P.E., Kaiser, A., Lighton, J.R.B., 2008. Breathe softly, beetle: continuous gas exchange, water loss and the role of the subelytral space in the tenebrionid beetle, *Eleodes obscura*. J. Insect Physiol. 54, 192–203.
Schilman, P.E., Waters, J.S., Harrison, J.F., Lighton, J.R.B., 2011. Effects of temperature on responses to anoxia and oxygen reperfusion in *Drosophila melanogaster*. J. Exp. Biol. 214, 1271–1275.
Slama, K., Sobotnik, J., Hanus, R., 2007. Respiratory concerts reveals by scanning microrespirography in a termite *Prohinotermes simplex* (Isoptera: Rhinotermitidae). J. Insect Physiol. 53, 295–311.
Socha, J.J., Forster, T.D., Greenlee, K.J., 2010. Issues of convection in insect respiration: Insights from synchrotron X-ray imaging and beyond. Respir. Physiol. Neurobiol. 173S, S65–S73.
Speakman, J.R., 1997. Doubly Labelled Water: Theory and Practice. Chapman & Hall, London, ISBN 0-412-63780-4.
Waters, J.S., Harrison, J.E., 2012. Insect metabolic rates. In: Sibly, R.M., Brown, J.H., Kodric-Brown, A. (Eds.), Metabolic Ecology: A Scaling Approach. John Wiley & Son Ltd, pp. 198–211 (Chapter 16).
Wigglesworth, V.B., 1935. The regulation of respiration in the flea, *Xenopsylla cheopis*, Roths. (Pulicidae). Proc. R. Soc. Lond. Ser. B 118, 397–419.
Wigglesworth, V.B., 1937. Wound healing in an insect (*Rhodnius prolixus* Hemiptera). J. Exp. Biol. 14, 364–381.
Wigglesworth, V.B., 1954. Growth and regeneration in the tracheal system of an insect, *Rhodnius prolixus* (Hemiptera). Q. J. Microsc. Sci. 95, 115–137.
Wigglesworth, V.B., 1959. The role of the epidermal cells in the 'migration' of tracheoles in *Rhodnius prolixus* (Hemiptera). J. Exp. Biol. 36, 632–640.
Wigglesworth, V.B., 1972. The Principles of Insect Physiology. Chapman & Hall, London.
Zwicky, K., Wigglesworth, V.B., 1956. The course of oxygen consumption during the moulting cycle of *Rhodnius prolixus* Stål (Hemiptera). Proc. R. Entomol. Soc. Lond. Ser. A Gen. Entomol. 31, 153–160.