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„Regulation of nest and body temperature of *Polistes biglumis* and *Polistes gallicus*, two wasps from strongly different climates"

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Statutory Declaration in Lieu of an Oath

I hereby declare in lieu of an oath that I have completed the present Master's thesis entitled "**Regulation of nest and body temperature of *Polistes biglumis* and *Polistes gallicus*, two wasps from strongly different climates**" independently and without illegitimate assistance from third parties. I have used no other than the specified sources and aids. In case that the thesis is additionally submitted in an electronic format, I declare that the written and electronic versions are fully identical. The thesis has not been submitted to any examination body in this, or similar, form.

07.05.2018, Vienna

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(Mag.ª Julia Magdalena Nagy)

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Abstract

Polistes is a widespread taxon, which builds nests without a protective outer layer. This allows non-invasive observation of their thermoregulatory behaviour, however, it makes them much more vulnerable to changing temperatures. Even minimal climatic changes could have a crucial effect on the brood or the adult wasps. This thesis focuses on two species of the genus *Polistes*: *Polistes biglumis* (examined in Obergail, Austria; two nests) and *Polistes gallicus* (examined in Sesto Fiorentino, Italy; four nests). Both species set up their nests outdoor, hardly protected. *P. biglumis* builds the nests east-south-east orientated on stone, to gain the most of the morning sun (radiation) and to profit from heat radiation of the stone during the night. *P. gallicus* on the other hand avoids direct insolation and constructs their nests in shade. For body-surface and nest temperature measurements, infrared thermographic pictures were taken during hot days in summer. Microclimatic data was collected with data loggers.

Results showed that in *P. biglumis* the T_a (ambient air temperature 1-3 m away from nest) and T_a (nest) (ambient air temperature a few centimetres away from nest) were mostly below 35°C, in *P. gallicus* T_a and T_a (nest) were often even higher than 40°C.

Due to either direct sunshine or rising T_a in the morning until 14:00, the nests heated up rapidly. Without active cooling, the brood and the adults would have reached their critical thermal maximum (CT_{max}). It is to mention that in *P. gallicus* generally higher body temperatures (up to 45°C) were measured than in *P. biglumis* (always below 40°C). However, observed cooling events allowed the adult wasps of both species to keep their mean body temperature below their critical thermal limit.

A remarkable finding was that even at high ambient temperatures the adults of both species kept the temperature of their brood below 40°C by active cooling mechanisms. It is believed that this is close to the upper thermal limit for the brood. Keeping them below 40°C proves that the adult wasps of both species are capable of maintaining the highest possible temperatures for the development of their brood as well as preventing overheating at the same time. A fast development of the brood in return guarantees a higher fitness of the colony.

For active nest-cooling two mechanisms were observed. Although both species use both mechanisms, *P. gallicus* was seen to carry more water droplets onto the nest, whereas *P. biglumis* tended to the fanning activity. Which mechanism is preferred, depends on the diverse surrounding air temperature of the given habitat. A convective cooling mechanism like fanning is much more efficient in areas with low T_a and a warm nest, a condition found in *P. biglumis*. The evaporative cooling effect of water is more important in *P. gallicus* as their ambient temperature is generally higher and the difference in temperature to the nest is small.

Zusammenfassung

Polistes zählt zu einem weit verbreiteten Taxon, welches Nester ohne eine schützende Hülle baut. Diese Tatsache erlaubt es, deren thermoregulatorisches Verhalten mit nicht-invasiven Methoden zu studieren. Gleichzeitig macht es *Polistes* aber auch stark von Temperaturschwankungen abhängig und bereits minimale Veränderungen in der Außentemperatur können schwerwiegende Folgen für die Brut und die adulten Tiere mit sich bringen. Diese Arbeit konzentriert sich auf zwei Arten von *Polistes*: *Polistes biglumis* (untersucht in Obergail, Österreich; zwei Nester) und *Polistes gallicus* (untersucht in Sesto Fiorentino, Italien; vier Nester). Beide Arten bauen ihre Nester relativ ungeschützt im Freien. Dabei baut *P. biglumis* ihre Nester Ost-Süd-Ost orientiert auf Stein, um am Vormittag von der Morgensonne (Strahlung) sowie von der Wärmestrahlung des Untergrunds während der Nacht zu profitieren. *P. gallicus* hingegen vermeidet direkte Sonneneinstrahlung und baut ihre Nester meist im Schatten.

Zur Untersuchung der Körperoberfläche und der Nesttemperatur wurden Infrarot Bilder während heißer Sommertage gemacht. Zusätzlich wurden mikroklimatische Daten mittels Daten-Logger gesammelt.

Es konnte gezeigt werden, dass die T_a (Umgebungstemperatur 1-3 m vom Nest entfernt) und die T_a (nest) (Umgebungstemperatur wenige Zentimeter vom Nest entfernt) in *P. biglumis* meistens unterhalb 35°C, bei *P. gallicus* hingegen zeitweise über 40°C lagen.

Aufgrund direkter Sonneneinstrahlung oder steigender T_a von morgens bis ungefähr 14:00 heizten sich die Nester schnell und stark auf. Ohne aktiver Kühlung würden die Brut und die adulten Tiere ihr kritisches thermisches Limit (CT_{max}) erreichen und überschreiten. Hier gilt anzumerken, dass in *P. gallicus* generell höhere Körpertemperaturen (bis zu 45°C) gemessen wurden als bei *P. biglumis* (immer unter 40°C). Beide jedoch schafften es mit den beobachteten Kühlungs-Mechanismen, ihre Körpertemperatur unterhalb des kritischen thermischen Limits zu halten.

Besonders interessant ist das Resultat, dass auch bei sehr hohen Temperaturen die adulten Tiere beider Arten die Temperatur der Brut mittels aktiver Kühlung unterhalb 40°C halten können. Es wird angenommen, dass dies die obere Grenze des thermischen Limits der Brut ist. Das Halten einer bestimmten Brut-Temperatur beweist wiederum, dass die adulten Wespen beider Arten in der Lage sind, ihre Brut bei höchstmöglicher Temperatur konstant zu halten, und dabei gleichzeitig eine Überhitzung zu verhindern. Eine hohe Temperatur wiederum fördert die Entwicklungsgeschwindigkeit der Nachkommen, wodurch die Kolonie eine allgemein höhere Fitness erreicht.

Bei der aktiven Kühlung konnten zwei Mechanismen beobachtet werden. Obwohl beide Arten beide Mechanismen kennen und nutzen, brachte *P. gallicus* häufiger Wassertropfen zum Nest, wohingegen *P. biglumis* mehr fächelte. Welcher Mechanismus genutzt wird, hängt von der jeweiligen Umgebungstemperatur des Verbreitungsgebietes ab. Eine konvektive Kühlung, wie beim Fächeln, ist wesentlich effizienter in Gegenden mit einer niedrigen T_a und einem warmen Nest, Konditionen, die bei *P. biglumis* zutreffen. Der kühlende Effekt der Evaporation von Wasser ist hingegen sinnvoller in Gegenden mit hoher Umgebungstemperatur und einem warmen Nest, wie es bei *P. gallicus* der Fall ist.

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Introduction

Temperature is a very important abiotic factor influencing most of the physiological and biochemical processes of animate beings. In addition to the influence on these processes, temperature influences the distribution of most insects essentially. Polistine wasps are distributed across quite different climates all over the world (Carpenter 1996; Arévalo et al. 2004). As *Polistinae* built an open paper nest, which is connected with just a stem to the ground and lacking an insulating envelope, the development of the brood is highly depending on the surrounding microclimate. Thus, even minimal temperature changes could have a crucial effect on the development and survival of the individuals (Steiner A. 1929; Lorenzi 1986; Arévalo et al. 2004). An investigation of the regulation of the body temperature as well as the nest temperature therefore is, in consideration of climate change, very important, as changes in temperature will affect ecosystems and thus the dispersion and survival of insects (Kovac et al. 2016).

As part of the FWF Project (P 30350, *"Die Auswirkungen des Klimawandels auf die Energetik von primitiv eusozialen Wespen"*) this thesis focuses on two paper wasps, *Polistes biglumis* and *Polistes gallicus* (*Hymenoptera, Polistinae*) of the family *Vespidae*.

Thermoregulation

Thermoregulation can be defined as the possibility to maintain the body temperature, autonomously or at least partly independent of external influences (Heinrich 1993). Thus, thermoregulation of the own body is essential to dial with the thermal environment. It is implicated with endothermic animals, which, on the other hand, means that ectothermic animals, like most insects, are much more depending on their environmental temperature. However, there are some behavioural strategies of ectothermic insects to cope with extreme temperatures. This is essential as they need to be able to maintain their optimal body temperature as well as the optimal temperature for the development of their offspring (Addo-Bediako et al. 2002). Nonetheless, active thermoregulation costs more energy, leading to a possible higher mortality in individuals if changes in ambient temperature occur and they need to actively regulate temperature even more (Angilletta et al. 2002). According to Jones et al (2006) thermoregulation could be divided into two forms: active and passive. Active would describe the regulation of one's own body temperature with physical or chemical activity, the other refers to thermoregulation through nest-site choice or physical structures of the nest (Jones und Oldroyd 2006). In *Polistes* any active heating effort of the adults cannot be kept inside a nest but the produced heat would rather be emitted to the surrounding air, thus they can regulate heat only via nest site choice. However, they actively cool their nests by fanning as well as actively carrying water droplets onto the nest for evaporative cooling. The first does not only cool the nest but the individuals and the brood as well.

Behavioural thermoregulatory strategies

Ectothermic insects rely completely on environmental factors, whereas endothermic insects can generate heat within their own body, usually with muscular activity (Esch et al. 1991; Heinrich 1993). Yet, if insects do not want to completely depend on their given ambient temperature, they need do apply endothermic strategies to maintain their optimal temperature (Heinrich 1993; Addo-Bediako et al. 2002). Insects, like honeybees or wasps for example, that are able to change between ectothermy and endothermy are called heterothermic (Stabentheiner et al. 2012).

The best observed behaviour in producing heat is by flight muscle activity. This allows, for example, bumblebees to maintain a thoracic temperature of 36°C at ambient temperatures close to 0 °C, or honeybees to heat their thorax to more than 35°C at ambient temperatures of 15°C (Heinrich 1974; Stabentheiner et al. 2014). However, the maintenance of one's body temperature does cost energy (Weiner et al. 2010). *Polistes* wasps are only weakly endothermic during foraging, and remain ectothermic during long periods of time on the nest (Kovac et al. 2009; Kovac et al. 2016).

As insects are normally small in size, heat exchange with the environment is quick because of their unfavourable surface to volume ratio. Passive cooling is very easy for them if they rest in shade for short. However, if ambient temperature gets too high, active cooling is necessary, as they do not want to exceed their thermal limits (Heinrich 1974; Kovac et al. 2016).

Evaporative cooling is often a requirement to keep optimal temperature and can, among others, be observed in honeybees during flight. At high ambient temperature honeybees volatilise a liquid from their head and via blood flow cool their thorax, allowing them to fly at temperatures close to their thermal limits (Heinrich 1980; Tomlinson et al. 2012). Nest building insects which disclaim an outer protective layer for the nest face the challenge to cool their nest when ambient temperature rises, as the brood needs to maintain the optimal temperature. Active cooling is even more important if animals have their nests exposed to the sun as it is common in *P. biglumis* (Lorenzi 1986; Stabentheiner personal communication) and *Polistes nimpha* (Cervo und Turilazzi 1985; Kozyra et al. 2016) and sometimes observed in *P. gallicus* (Kovac, Käfer and Stabentheiner, personal communication).

Active nest cooling was already observed by Steiner (1929). He could see *P. biglumis* fan and carry water droplets into the nest as soon as ambient temperature rose. The evaporative cooling effect of water droplets on the nest of *P. gallicus* could be shown by Kovac et al. (2015a).

Experimental Animals

In *Vespula* a non-invasive infrared measurement of nest temperature would not be possible, as the protective layer of the nest would hinder the rays. As *Polistes* do built open nests where the outer layer is missing, it is especially suited for a non-impact thermoregulatory measurement. It allows observations without interfering with their social or their thermoregulatory behaviour (Steiner A. 1929; Sôichi Y. 1969; Stabentheiner und Schmaranzer 1987; Schmaranzer und Stabentheiner 1988; Stabentheiner et al. 2012; Santos et al. 2015).

For this thesis two species of the genus *Polistes* were chosen: *Polistes biglumis* and *Polistes gallicus* (Figure 1). They are closely related species, which differ in their distribution. *P. biglumis* is found mostly in Montane (Alpine) areas, whereas *P. gallicus* is located in Mediterranean climate regions (Fucini et al. 2009; Kovac et al. 2015a).

Figure 1 - Polistes biglumis (left) and Polistes gallicus (right)

Both are primitively eusocial wasps which do not built closed nests, as for example other socials wasps like *Vespula* or *Vespa* do. Accordingly, *P. biglumis* and *P. gallicus* cannot regulate the temperature within their nests, making them highly depending on ambient temperature and nest site choice (Jones and Oldroyd 2006; Weiner et al. 2012). Furthermore, as they are ectotherms they depend even more on their thermal environment though they show weakly endothermic strategies during foraging (Kovac et al. 2015b). This thesis focuses on the daily temperature cycles of the wasps and their nests on warm midsummer days, and a representative active cooling mechanism as the fanning behaviour.

Polistes biglumis

P. biglumis (Figure 1, left) has a dark dorsal side and a lighter ventral side with both sides being sharply separated by colour (Entomologischer Verein Krefeld 2011). They show a relatively small body with two yellow dots seen on the abdomen and a mostly black mesoscutum (Neumeyer et al. 2014). It is one of the nine *Polistes* species in Europe occurring mainly in mountain areas, e.g. in the Alps and in the Appenin (Fucini et al. 2009). As a primitively eusocial wasp, their caste expression is quite flexible and not given physiologically (Fucini et al. 2009; Weiner et al. 2010; Neumeyer et al. 2011). Since it inhabits the cold montane areas, its colony cycle is fairly short, beginning around May and ending the latest in September. One queen is enough for founding a new nest. If this queen loses her nest, however, she does not start a new one but instead takes over another nest, due the short cycle (Seppä et al. 2011). Belonging to the species *Polistes*, the colonies of *P. biglumis* are rather small, consisting approximately of 1-30 individuals, mostly less than 12 (Fucini et al. 2009; Käfer et al. 2015; Stabentheiner, pers. comm.). The thermal ecology of *P. biglumis* was already investigated by Steiner in 1929. With his experiments he was able to prove that *P. biglumis* is capable of maintaining a particular temperature even with intense solar radiation and high ambient temperatures. It was shown that the empty nest is much hotter than the inhabited nest, proving active cooling activities of the adult wasps. Additionally, Steiner observed the fanning mechanism of *P. biglumis* when ambient temperature was high (Steiner 1929).

Polistes gallicus

P. gallicus (Figure 1, right) is a primitively eusocial wasp inhabiting the Mediterranean areas and belongs to the nine *Polistes* species known in Europe (Neumeyer R. et al. 2011). Their hypopygium is black with sometimes a yellow spot at the tip and their mesoscutum is black with two yellow spots (Neumeyer et al. 2014). Living in areas with high ambient temperatures, *P. gallicus* is well adjusted to those, resulting in a reduced metabolism, and it is known to have a thermal maximum of 47.6°C (Kovac et al. 2015a; Kovac et al. 2016). One or more female individuals found a new colony, though only one female functions as egg-layer whereas the others take the position as workers and do not reproduce as long as the dominant female is on the nest (Röseler et al. 1984).

As the adults seek to maintain an optimal temperature for their brood to develop, they try to avoid building their nests exposed to the sun. Since the ambient temperature is already very high in Mediterranean climate zones, direct radiation would heat up the nests too much. Thus, *P. gallicus* usually (but not exclusively) choose their nesting sites in corners, protected from direct sunshine, as well as orientate their nests to the east and therefore avoid the sun during the hottest times of the day (Kovac et al. 2016).

Research locations

The locations for observing *P. biglumis* were Obergail (Lesachtal, Carinthia, Austria) and for *P. gallicus* Sesto Fiorentino (Tuscany, Italy) (Figure 2).

Figure 2 - Places of research - Obergail (left) and Sesto Fiorentino (right)

Obergail (Figure 2, left) is a mountain region ~1160 meters above sea level showing a typical Alpine climate, with relatively warm days and often cold nights during midsummer. As the climate change has a crucial effect on the Alpine regions, animals inhabiting this area will face a hard challenge over the next years. Climate change will attenuate the Alpine day-night-cycle resulting in rising temperatures during the night and a drastically rising temperature during the day. Therefore, insects, which are depending on the ambient temperature, may not be able to inhabit Alpine regions for long, as active cooling mechanisms do cost energy. Alternatively, they could evade to colder mountain regions, but food and water resources may be limited (Kromp-Kolb et al. 2014; Seppä et al. 2011; Tamme 2013).

Sesto Fiorentino (Figure 2, right), only 55 meters above sea level, shows a Mediterranean climate with very high temperatures during midsummer. The temperature does not sink much during the night and rises greatly during the day. In Mediterranean climate zones, insects do not face the problem of cold temperatures but rather of overheating, thus trying to avoid direct radiation (Kovac et al. 2016).

Aim of the study

This thesis aims for showing differences and similarities of the thermoregulatory strategies between *P. biglumis* and *P. gallicus* as they are from very different climate zones, yet belong to the same genus. Since in both the upcoming of their offspring highly depends on the ambient temperature and the nest site choice, it is crucial to know how they cope with high or low temperatures. Due to the fact that both face different challenges in their temperature environment, it is especially interesting to look at their cooling or heating behaviour. Moreover, as accompanying studies to the present thesis try to see how much energy is needed at different temperatures, it is essential to know which cooling or heating behaviour is needed and applied in the field, along with what increased temperatures could imply for insects and their metabolic rate and – in broader sense – for their survival.

Methods

Fieldwork

The fieldwork for this thesis was executed by Anton Stabentheiner, Helmut Kovac and Helmut Käfer. Anton Stabentheiner collected the data from *Polistes biglumis*, whereas Helmut Kovac and Helmut Käfer measured the data from *Polistes gallicus* (Figure 3). They used the thermographic cameras Flir T650sc (resolution 640x480 pixels, sensitivity < 20 mK), or i60 (resolution 180x180 pixels, sensitivity < 100 mK), yet had a different amount of nests and days tested. It is essential to mention that the pictures of both species were captured during hot days in midsummer in order to allow a comparison of their thermoregulatory strategies close to their upper thermal limits.

Figure 3 – Fieldwork set-up in Obergail (left) and Sesto Fiorentino (right). *Red circle = thermocouple; yellow square = sensor, with witch microclimate date was collected; orange rhomb = sensor for air current (only used in* P. biglumis*); blue triangle = reference (only used in* P. gallicus*); camera = Flir T650sc*

In addition to the thermographic pictures, microclimate data was collected with data-loggers (Ahlborn) for ambient temperature and relative humidity in shade (T_a [°C] and rH [%]; with Almemo FH A646-E1 sensor), ambient air temperature close to the nests (T_a nest [°C]; with type K thermocouples), global radiation ([W m^{-2}]; FLA613-GS mini spezial sensor, Ahlborn), and air current within 10-20 cm of the nests ([m s^{-1}]; FVA605-TA5O, Ahlborn). Thermocouple measurements were corrected for the effect of solar radiation.

Obergail (Austria)

Anton Stabentheiner took thermographic infrared pictures in Obergail (Austria) (Figure 4) and collected data from *P. biglumis*. He took pictures with the Flir T650sc as well as the Flir i60. Two different nests (W4/W5) were examined over eight days in total and during the process, one picture every minute was taken. The pictures with the i60 were only taken on one day for each nest (W4: 18.07.2017; W5: 19.07.2017). To show the effect of fanning behaviour on nest temperature, thermographic videos were made at a frame rate of 30 Hz. In order to investigate the diurnal temperature variation one picture was taken every minute with the Flir T650sc, resulting in two days where a distinct day-night cycle was observed. With the i60 two daily cycles from dawn to dusk were measured (~06:00-22:00), measurement intervals ranging mostly from ~10-45 min (in some cases 2 h).

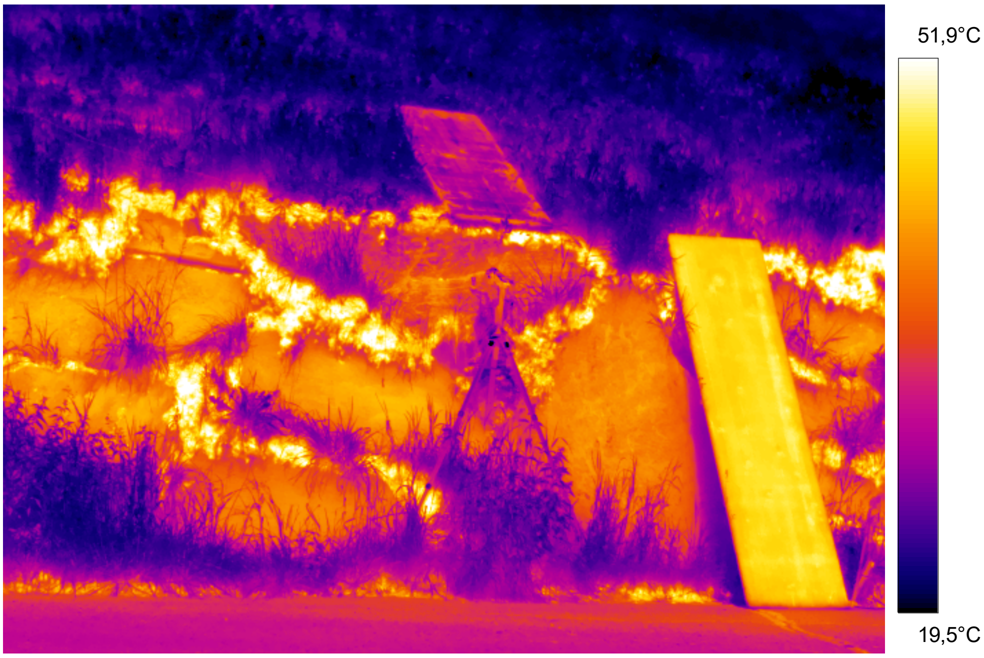


***Figure 4 – Thermal environment of nest W4 and fieldwork set-up of Obergail thermographed with the Flir T650sc** – the tripod of the camera can be seen in the middle, facing the wall on which the nests of* P. biglumis *were examined.*

Sesto Fiorentino (Italy)

Helmut Kovac and Helmut Käfer took the thermographic infrared pictures of *Polistes gallicus* in Sesto Fiorentino (Italy) with the Flir T650sc. They took pictures every minute of four nests in total (N1/N2/N3/N4) over a period of five days but measured only N3 during the night. With *P. gallicus* thermographic videos were recorded as well.

Each day thermographic infrared pictures of a different nest were taken, except with N3 where a day-night-cycle was measured (31.07.2017.-01.07.2017).

Evaluation

The evaluation of the thermographic infrared pictures was done at the University of Graz with Flir ThermaCam Researcher Pro 2.10 software controlled by a proprietary Excel VBA macro, which also extracted the microclimatic data from the logger files.

Graphing was done with Origin (OriginLab), and statistics with Statgraphics 18 software (Statgraphics Technologies). For the final evaluation, the data was divided into three main parts. The temperature of the nests and of the wasps on the nests of both species **(Part One)**, the temperature of the larvae on the nests as well as of the open empty cells and the top of the closed cells with the pupae inside of both species **(Part Two)**, and the cooling effect of fanning of *P. biglumis* **(Part Three)** was quantified.

Part One – Temperature of nests and wasps

Thermograms were evaluated with the program ThermaCam Researcher Pro 2.10 which was controlled by an Excel VBA-Macro to use pre-built evaluation tools (box areas and a polygon) for temperature measurement of the thermographic infrared pictures (Figure 5). In Table 1 the different tools used are listed as well as the measured temperatures (= T). The VBA-Macro also extracted the environmental data from the logger files at the time of thermographic picture evaluation and wrote them into an Excel file, as well as the temperatures measured with the evaluation tools.

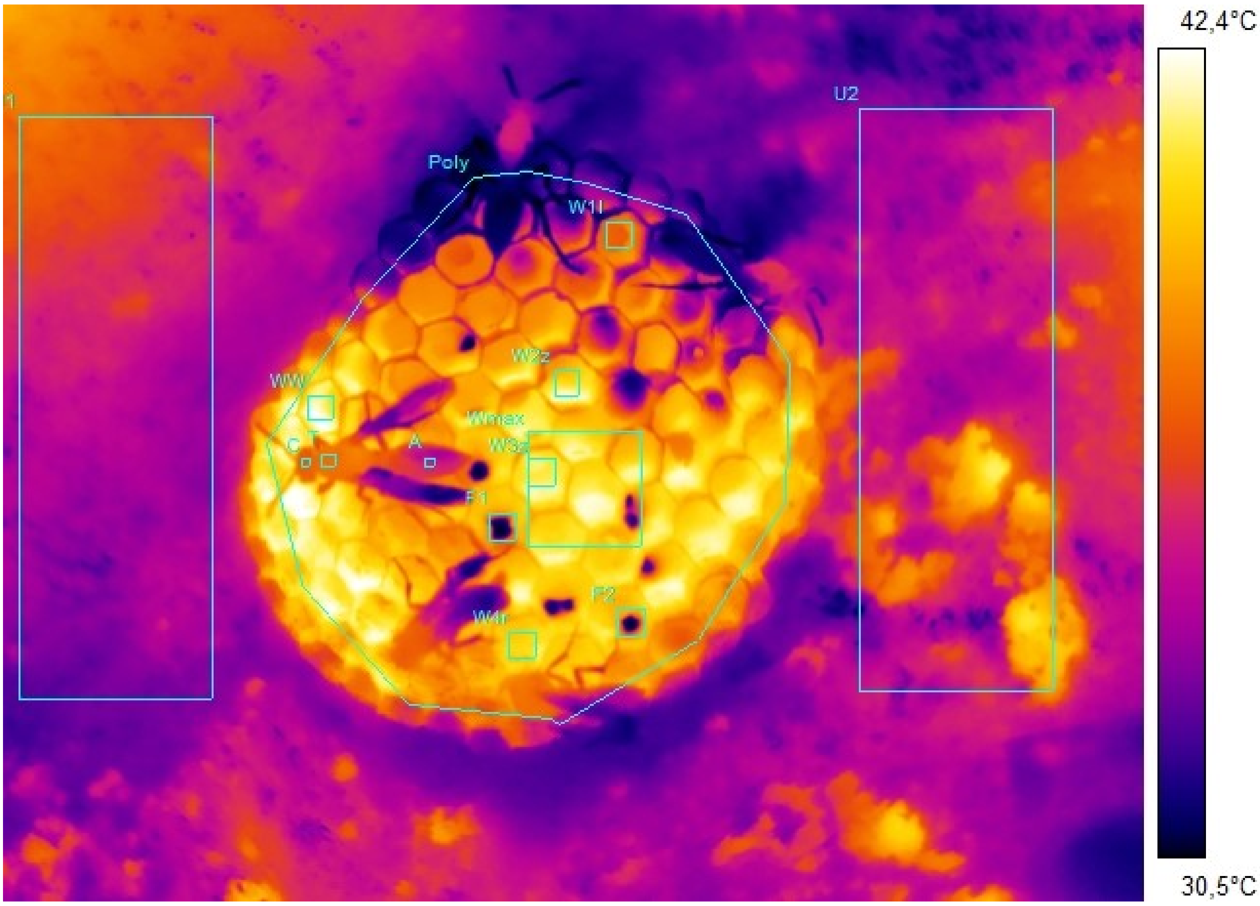


Figure 5 - Evaluation of the thermographic pictures with the program ThermaCam Researcher Pro 2.10. The picture shows the nest W5 of* P. biglumis *during the day (19.07.2017). *The small dark spots on the nest show water droplets for nest cooling by evaporation. At the upper part of the nest a fanning adult can be seen, cooling the nest to around 30°C.*

Each nest and up to five wasps on top of it (if present) were quantified every ten minutes. The size of the specific tools was adjusted when needed. Water drops were measured when present. With *P. biglumis* water droplets were less abundant on the nest than in *P. gallicus* where they could be found almost on every nest during the day.

In *P. gallicus* the placement of the tools "U1" and "U2" was more difficult, as the nest itself was reflected on the rather smooth surface of the side walls and measuring the reflection of the nest would not have been representative for the surrounding substrate temperature. To avoid this error, the tools were reduced in their size and positioned above the nest.

Table 1 – Specific evaluation tools used for temperature measurement in Part One

Tool	***What was measured***		***Tool***	***What was measured***
C	Caput (highest 3 pixels mean T)		Poly	Total nest (mean, maximum, minimum T)
T	Thorax (highest 9 pixels mean T)		Wmax	Nest Maximum (highest 10 pixels mean T)
A	Abdomen (highest 6 pixels mean T)		U1	Substrate 1 – left of nest (mean, max., min. T)
WW	Cell next to measured wasp (mean T)		U2	Substrate 2 – right of nest (mean, max., min. T)
W1l	Cell 1 – left/top border of nest (mean T)		F1	Water drop (coolest 10 pixels mean T)
W2z	Cell 2 – centre of nest (mean T)		F2	Water drop (coolest 10 pixels mean T)
W3z	Cell 3 – centre of nest (mean T)		Ref	Reference radiator (only used for N3 and N4)
W4r	Cell 4 – right/lowest border of nest (mean T)			

After the temperature was measured with ThermaCam Researcher Pro 2.10 the data was edited with Excel and graphs were generated with Origin software.

Part Two – Temperature of larvae and cells

For Part Two the same thermographic pictures as in Part One were used. With the program FLIR Tools the real life pictures were extracted from the thermograms (Figure 6), so the larvae could be seen clearly for measuring purposes. During the day, the temperature was measured every thirty minutes and during the night every sixty minutes. The same tools as in Part One were used for the evaluation in Part Two, but only "WW" (empty cell), "W1l" (larvae 1), “W2z” (sealed cell 1), “W3z” (sealed cell 2), “W4r” (larvae 2) were placed for measurements. The names of the tools as well as the measured temperature were kept the same. After the data had been measured the data was edited in Excel and graphs were generated with the program Origin.

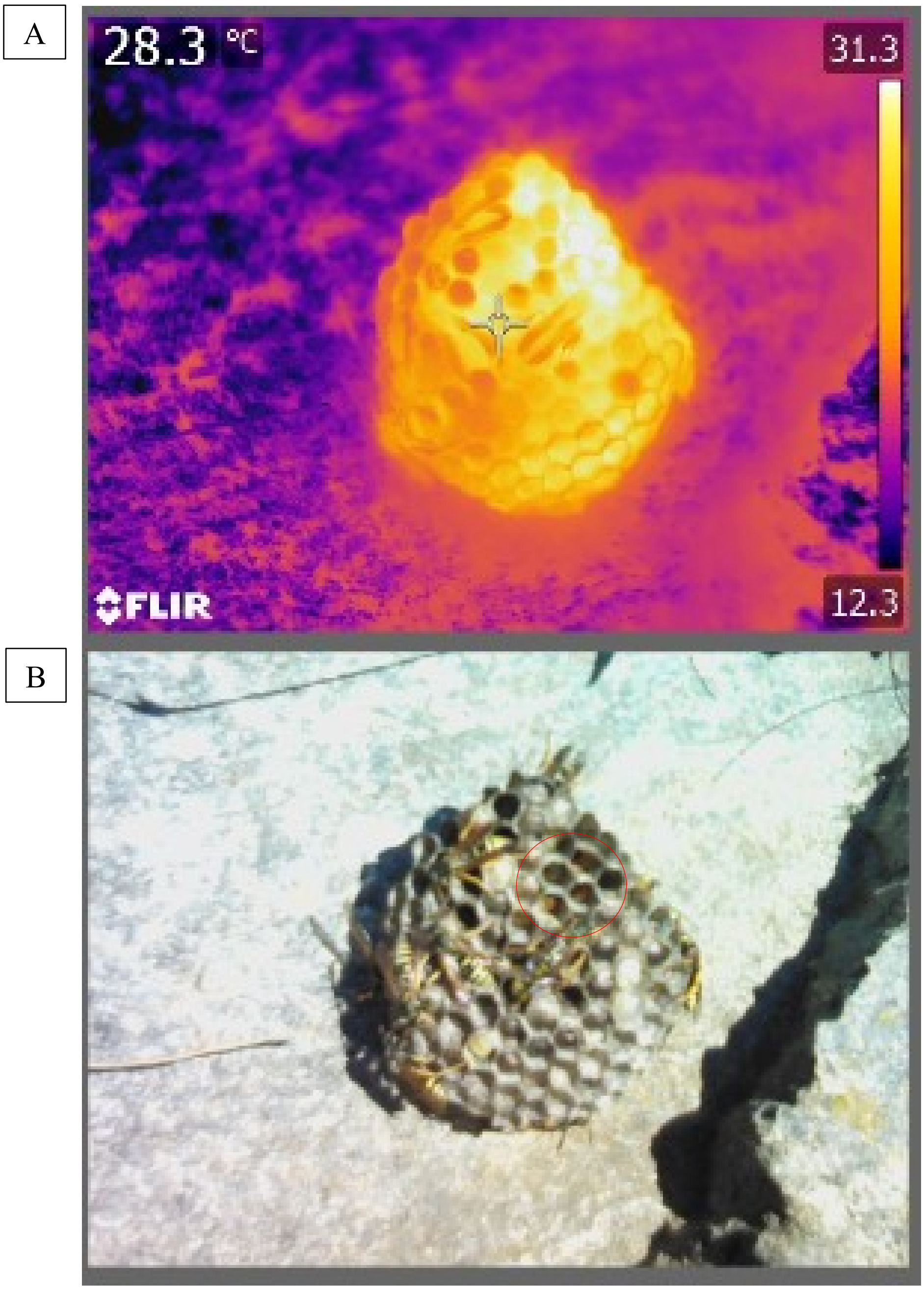


Figure 6 – Sample thermogram (A) and the extracted real life picture (B) of the nest W4 (17.07.2017) of **P. biglumis.** *With help of the program FLIR tools the larvae (red circle) could be identified for measurements. For the evaluation, the same larvae and the same sealed and open cells were used, unless an adult blocked the view. Sealed cells, which appear dark grey, were older, whereas a white cap suggests a freshly closed cell.*

Part Three – Cooling effect of fanning

For the last part, thermographic real-time recordings of *P. biglumis* of the nests W4 and W5 on the days 18.07.2017 (W4) and 20.07.2017 (W5) were used for measuring. For observation of the fanning behaviour new evaluation tools were defined in the VBA Macro (Table 2). The timeline was different, as the fanning activity often only lasted a few seconds. Therefore, the temperatures were measured every ten milliseconds during the fanning process, including several pictures before and after the fanning event to show the actual cooling effect. The latter was yet not always possible, as the fanning individuals moved away from their fanning spot very quickly after they stopped their action and simultaneously another adult started to fan. Some of the tools were placed close to the fanning wasp; others were placed on the opposite side, where the fanning was expected to have a smaller effect. Three fanning individuals were measured for each nest, thus six specimen in total were observed. The fanning individuals are designated as W4/1, W4/2, W4/3 and W5/1, W5/2, W5/3. The results of the fanning events are divided into three graphs always: Graph 1 shows the cells which were close to the fanning individual and which were most affected by the fanning event (= close cells), Graph 2 represents the cells on the opposite side of the fanning individual where the fanning did not have a pronounced effect (= distant cells), and Graph 3 shows the temperature of the fanning individual (= fanning individual) together with the temperature of an additional cell close to the fanner.

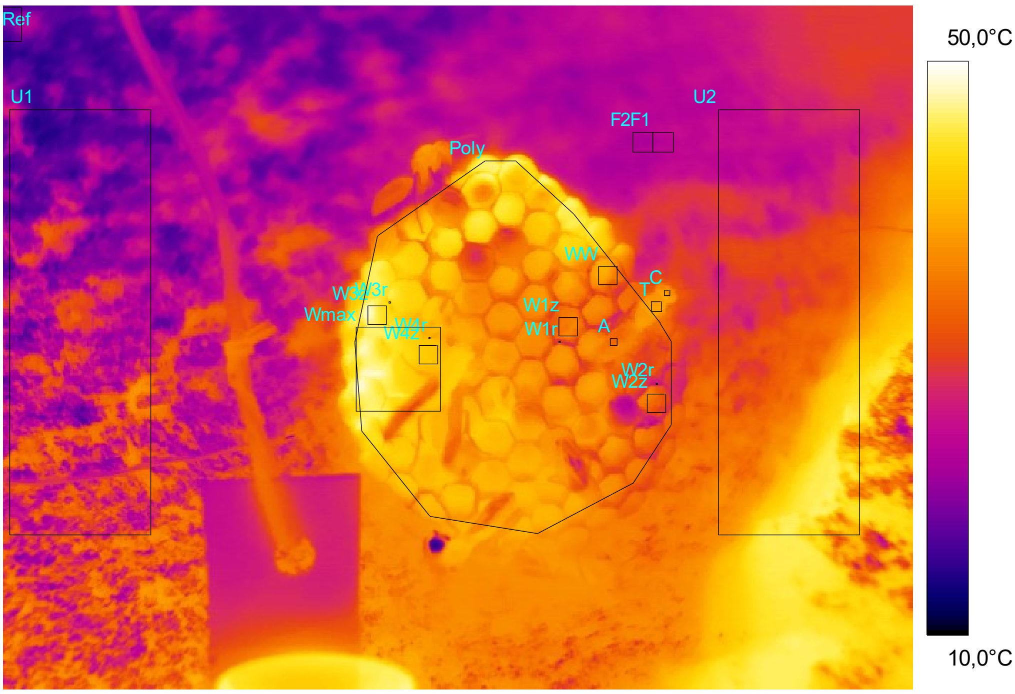


Figure 7 – Placement of the tools in* P. biglumis *for Part Three (fanning events). *Shortcuts are identified in Table 2. W1z, W1r, W2z and W2r are close to the fanning individual whereas W3z, W3r, W4z and W3r are on the opposite side where no effect of fanning was expected.*

Table 2 - Specific evaluation tools used for temperature measurement in Part Three

Tool	***What was measured***		***Tool***	***What was measured***
C	Caput (mean T)		W3z	Cell (center) on opposite side of fanning individual (mean T)
Th	Thorax (highest T)		W4r	Cell (frame) on opposite side of fanning individual (mean T)
Ab	Abdomen (coolest T)		W4z	Cell (center) on opposite side of fanning individual (mean T)
WW	Cell next to measured wasp (mean T)		Poly	Total nest (mean, maximum, minimum T)
W1r	Cell (frame) next to measured wasp (mean T)		Wmax	Nest Maximum (highest 10 pixels mean T)
W1z	Cell (center) next to measured wasp (mean T)		U1	Substrate 1 – left of nest (mean, max., min. T)
W2r	Cell (frame) next to measured wasp (mean T)		U2	Substrate 2 – right of nest (mean, max., min. T)
W2z	Cell (center) next to measured wasp (mean T)		F1	Water drop (coolest 10 pixels mean T)
W3r	Cell (frame) on opposite side of fanning individual (mean T)		F2	Water drop (coolest 10 pixels mean T)

Data was edited in Excel as well and graphs were afterwards again generated with Origin 8.6 32bit.

Results

In total 6 nests over a time period of overall 11 days were measured for Part One (temperature of nests and wasps) and Part Two (temperature of larvae and cells) of the two species *P. gallicus* and *P. biglumis*. Two different nests on 2 days and overall 6 individuals were measured for Part Three (cooling effect of fanning event), but only for one species (*P. biglumis*).

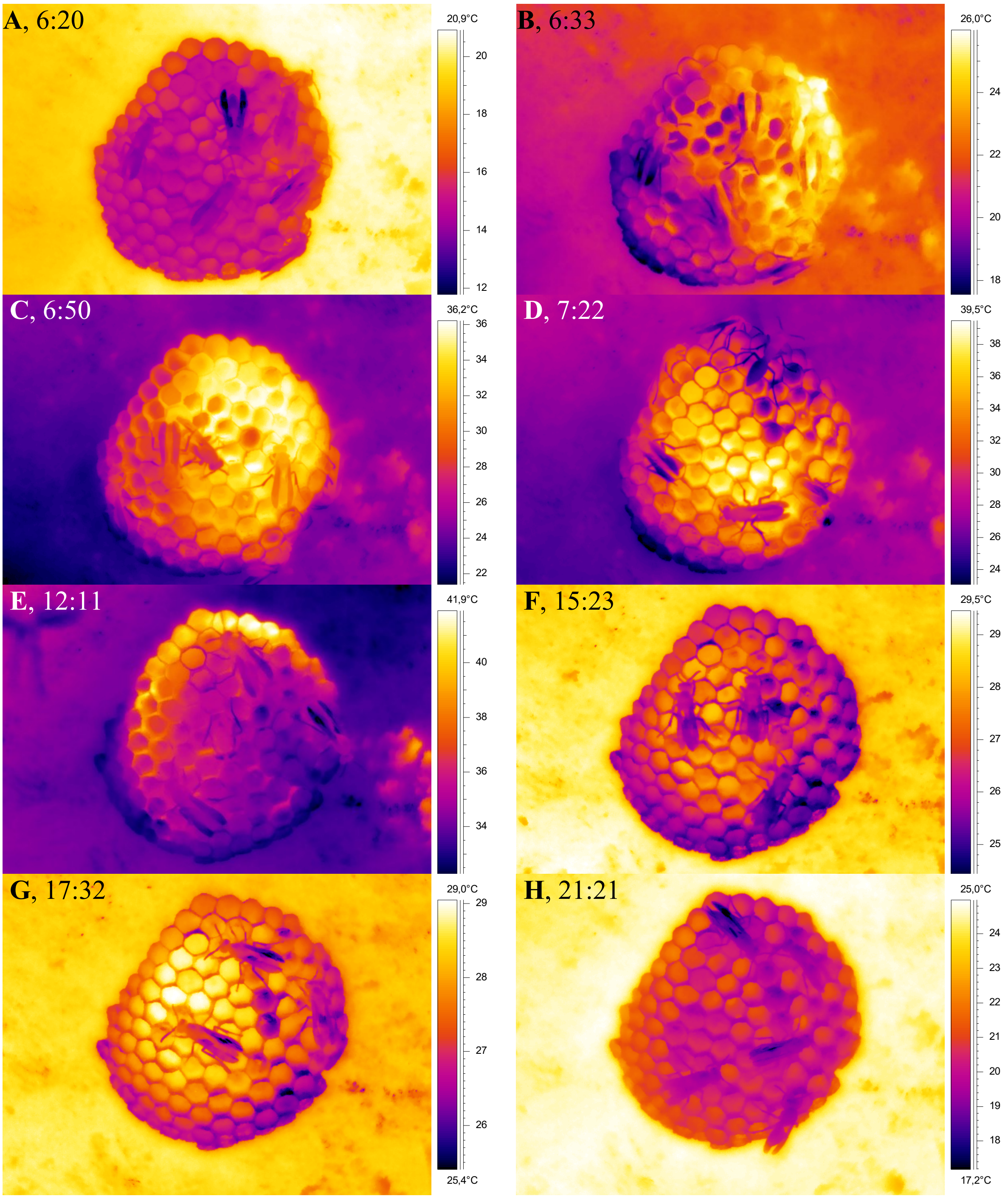


Figure 8 – Thermographic pictures of* P. biglumis *nest W5 during a whole day (19.07.2017), shot with the Flir T650sc. *The pictures show the changing temperature of nest, wasps and substrate during the day, starting at 6:20 before sunrise (A) and ending at 21:21 (H). B, sunrise; D, a fanning wasp can be seen on top (right individual).*

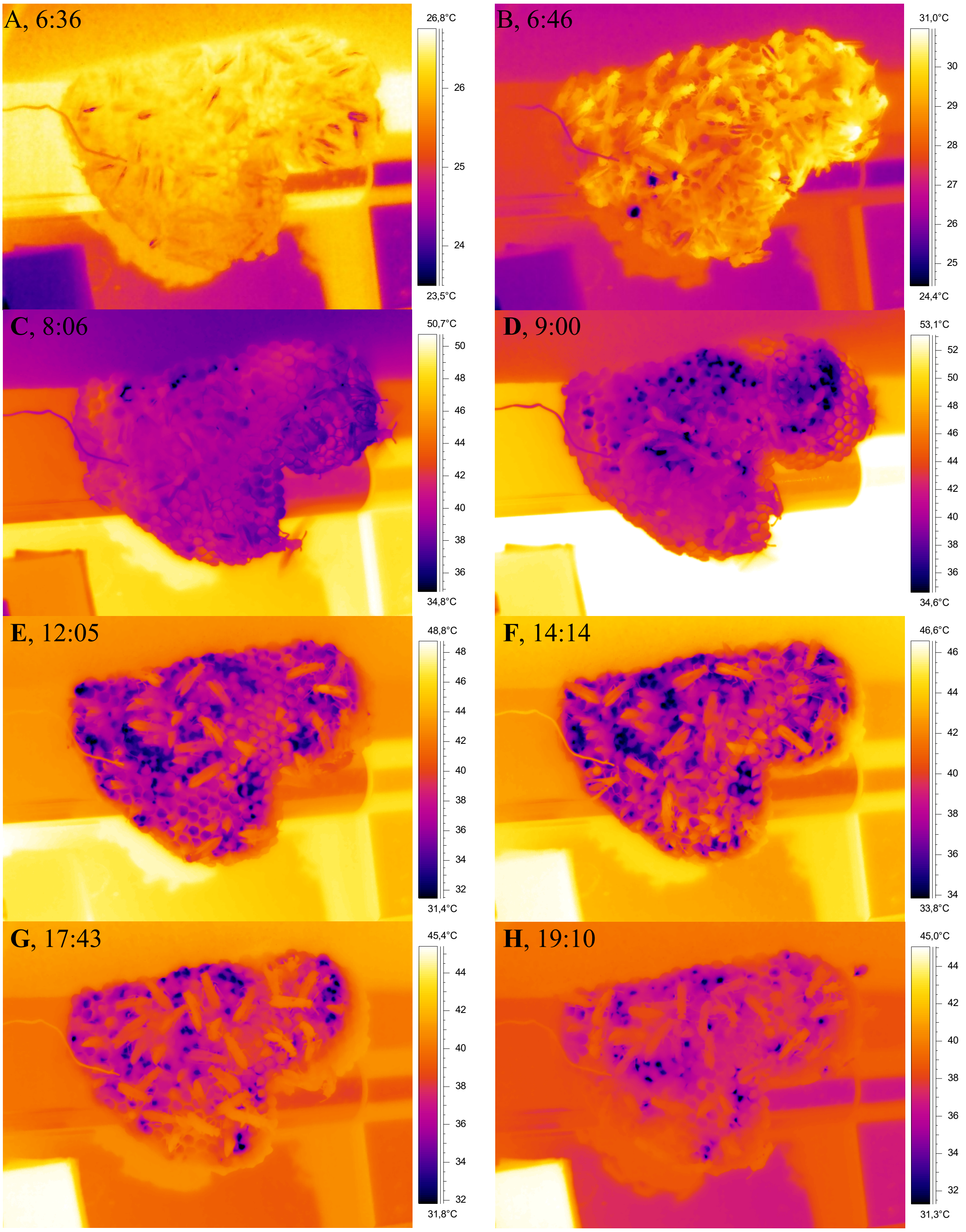


Figure 9 – Thermographic pictures of* P. gallicus *nest W3 during a whole day (01.08.2017), shot with the Flir T650sc. *The thermograms are turned to the right, the here seen upper part is originally on the left side. The pictures show the changing temperature of nest, wasps and substrate during the day, starting at 6:36 before sunrise (A) and ending at 19:10 (H). The dark spots are the water droplets carried onto the nest by the adults. First water drops are seen as soon as temperature rises (sunrise, B). In C, two fanning individuals due high temperatures can be seen on the upper and right ends (original orientation) of the nest.*

For the evaluation the point of origin were the thermographic pictures, showing the changes in temperature across the day (Figure 8). The thermograms (Figure 8, A-H, *P. biglumis*) show

that in the night the temperature on the nest did not go higher than 17°C and stayed colder than the substrate below (here stone), which showed temperatures of up to 20.9°C even without sunshine. Yet as soon as the sun rose at 6:30, the situation changed, as the nest had the highest temperatures with 26.0°C, whereas the stone below stayed cooler. It can be seen that it only needed 13 minutes of sunshine (radiation) to heat the nest from ~17°C to ~25°C. At 6:20 and 6:33, the adults seemed not to move on the nest, as they need warm ambient temperature and an increased body temperature for their activity. At 6:50, however, when the hottest temperatures on the nest were already at 36.2°C, a fast movement of the adults could be observed, resulting in fanning events soon afterwards (Figure 8, D, 7:22). Within one hour, temperature differences of almost 20°C (Figure 8, A to D) were measured on the nest.
The thermograms of *P. gallicus* (Figure 9, A-H) show the nest N3, which was the only of *P. gallicus* directly hit by sunshine. In contrast to *P. biglumis* nest temperature was around 20°C already before sunrise and it rose strongly up to 40°C within an hour (Figure 9, A, C). In *P. gallicus* dark water spots were found on the nest as soon as the sun rose (Figure 9, B). Additionally to the water droplets two fanning individuals are seen at 8:06 (Figure 9, C) when nest temperature rose highly due to direct insolation. Water droplets were carried onto the nest until evening, as the nest was still about 35-38°C even at 19:10 (Figure 8, H).

Statistics

The statistics proved a significant influence of radiation and T_a (nest) on thoracic temperature of both, *P. biglumis* and *P. gallicus* (ANOVA, $p << 0.0001$). However, it was shown that the T_a (nest) (= ambient temperature a few centimetres away from nest) had a greater impact on T_{th} (= thorax temperature of adult wasps) than radiation, in both species.
With the nest temperature, i.e. $T_{cells\ (polygon)}$ (the entire nest surface = polygon) as well as the $T_{cells\ (mean)}$ (mean temperature of 4 individual cells), the same significant influence of radiation and T_a (nest) was found (ANOVA, $p << 0.0001$). Again, the T_a (nest) was the factor influencing the temperature the most (compare Table 3).

It has to be mentioned again, however, that only an abstract of the environmental conditions of a breeding season was given. Statistics could change to some extent if the wasps are observed over a longer period.

***Table 3 – ANOVA results of T_{th} and T_{nest} of* P. biglumis *and* P. gallicus**

ANOVA T_{th} (A = T_a nest; B = radiation); *P. biglumis*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	41485.7	1	41485.7	2832.79	<<0.0001
radiation	6611.63	1	6611.63	451.46	<<0.0001
residuals	25262,4	1725	14.6448		
total (corrected)	158188	1727			
ANOVA $T_{cells\ (mean)}$ (A = T_a nest; B = radiation); *P. biglumis*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	41485.7	1	41485.7	2832.79	<<0.0001
radiation	6611.63	1	6611.63	451.46	<<0.0001
residuals	25262.4	1725	14.6448		
total (corrected)	158188	1727			
ANOVA $T_{cells\ (polygon)}$ (A = T_a nest; B = radiation); *P. biglumis*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	43212.3	1	43212.3	3254.88	<<0.0001
radiation	5710.88	1	5710.88	430.16	<<0.0001
residuals	22888.1	1724	13.2762		
total (corrected)	155241	1726			
ANOVA T_{th} (A = T_a nest; B = radiation); *P. gallicus*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	30768	1	30768	18239.04	<<0.0001
radiation	1435.64	1	1435.64	851.04	<<0.0001
residuals	2626.55	1557	1.68693		
total (corrected)	41651.3	1559			
ANOVA $T_{cells\ (mean)}$ (A = T_a nest; B = radiation); *P. gallicus*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	12633.8	1	12633.8	5908.81	<<0.0001
radiation	880.496	1	880.496	411.81	<<0.0001
residuals	3329.08	1557	2.13813		
total (corrected)	20039.3	1559			
ANOVA $T_{cells\ (polygon)}$ (A = T_a nest; B = radiation); *P. gallicus*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	18309.7	1	18309.7	10394.8	<<0.0001
radiation	1003.04	1	1003.04	569.45	<<0.0001
residuals	2742.55	1557	1.76143		
total (corrected)	26329	1559			

Additionally the statistics proved a significant influence of radiation and T_a (nest) on the temperature of the larvae (T_{larvae}, two individuals measured), the open and empty cells ($T_{cells\ (open)}$, one cell measured) as well as on the sealed cells with the pupae inside ($T_{cells\ (sealed)}$, two cells measured). Here almost all data was highly significant (ANOVA, p << 0.0001), except in the sealed cells of *P. gallicus*, where radiation had a less significant effect on temperature (ANOVA, p = 0.0004). However, in all other, radiation and T_a (nest) showed again a significant influence on the measured temperature (ANOVA, p << 0.0001). Yet, here again the T_a (nest) had a slightly greater impact on the temperature than radiation (compare Table 4).

***Table 4 – ANOVA results of T_{larvae}, $T_{cells\ (open)}$ and $T_{cells\ (sealed)}$ of* P. biglumis *and* P. gallicus**

ANOVA T_{larvae} (A = T_a nest; B = radiation); *P. biglumis*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	6397.15	1	6397.15	342.84	<<0.0001
radiation	864.672	1	864.672	46.34	<<0.0001
residuals	5840.31	313	18.6591		
total (corrected)	26181.3	315			
ANOVA $T_{cells\ (open)}$ (A = T_a nest; B = radiation); *P. biglumis*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	5916.82	1	5916.82	359.3	<<0.0001
radiation	797.876	1	797.876	48.45	<<0.0001
residuals	5154.35	313	16.4676		
total (corrected)	23957.6	315			
ANOVA $T_{cells\ (sealed)}$ (A = T_a nest; B = radiation); *P. biglumis*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	6223.69	1	6223.69	301.57	<<0.0001
radiation	538.358	1	538.358	26.09	<<0.0001
residuals	6459.55	313	20.6375		
total (corrected)	24435.7	315			
ANOVA T_{larvae} (A = T_a nest; B = radiation); *P. gallicus*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	1668.09	1	1668.09	375.12	<<0.0001
radiation	244.152	1	244.152	54.91	<<0.0001
residuals	1080.57	243	4.44679		
total (corrected)	3235.63	245			

ANOVA $T_{cells\ (open)}$ (A = T_a nest; B = radiation); *P. gallicus*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	1912.59	1	1912.59	453.79	<<0.0001
radiation	316.957	1	316.957	75.2	<<0.0001
residuals	1024.16	243	4.21467		
total (corrected)	3547.52	245			
ANOVA $T_{cells\ (sealed)}$ (A = T_a nest; B = radiation); *P. gallicus*					
cause	sum of squares	degree of freedom	av. deviation	F-quotient	p-value
T_a (nest)	1829.72	1	1829.72	283.76	<<0.0001
radiation	82.1848	1	82.1848	12.75	0.0004
residuals	1566.92	243	6.44825		
total (corrected)	3643.99	245			

Part One – Temperature of nests and wasps

***Polistes biglumis* – Nest W4:** The nest W4 of *P. biglumis* (Figure 10 A and B) clearly shows that the T_{max} of the nest rose as soon as radiation hit the nest, reaching temperatures of 46°C. Adults started to fan almost simultaneously to rising radiation while they kept their temperature below 38°C on average. The T_{thorax} (mean) was above the temperature of the substrate only during the day when radiation was high but dropped below it during the night. However, the T_{thorax} (mean) stayed always above the measured temperature of T_a (ambient air temperature measured 1-3 meters afar from nest) and was higher than the air temperature close to the nest (T_a (nest)) during the day and was hardly below it during the night. As soon as direct radiation on the nest decreased, the T_{max} of the nest came closer to the T_{min} of the nest, being only slightly higher than the T_{thorax} (mean) with temperatures below 16°C during the night. T_{mean} (nest) and T_{thorax} (mean) were always close throughout the day-night cycle and only rarely the T_{mean} (nest) was higher. During the day, the T_a and the T_a (nest) stayed below all other measured temperatures, except the T_{min} of the nest. Both were close to T_{mean} (nest) and T_{thorax} (mean) during the night. In total, the T_a (nest) did not rise above 34°C and the T_a was not higher than 30°C on this nest. However, both did not go lower than 9°C. The temperature of the substrate stayed above all other measured temperatures during the night (~ 15°C) (Figure 10, A), but did not go higher than 35°C during the day and stayed below the T_{thorax} (mean), the T_{mean} (nest) mostly and clearly below T_{max} (nest).

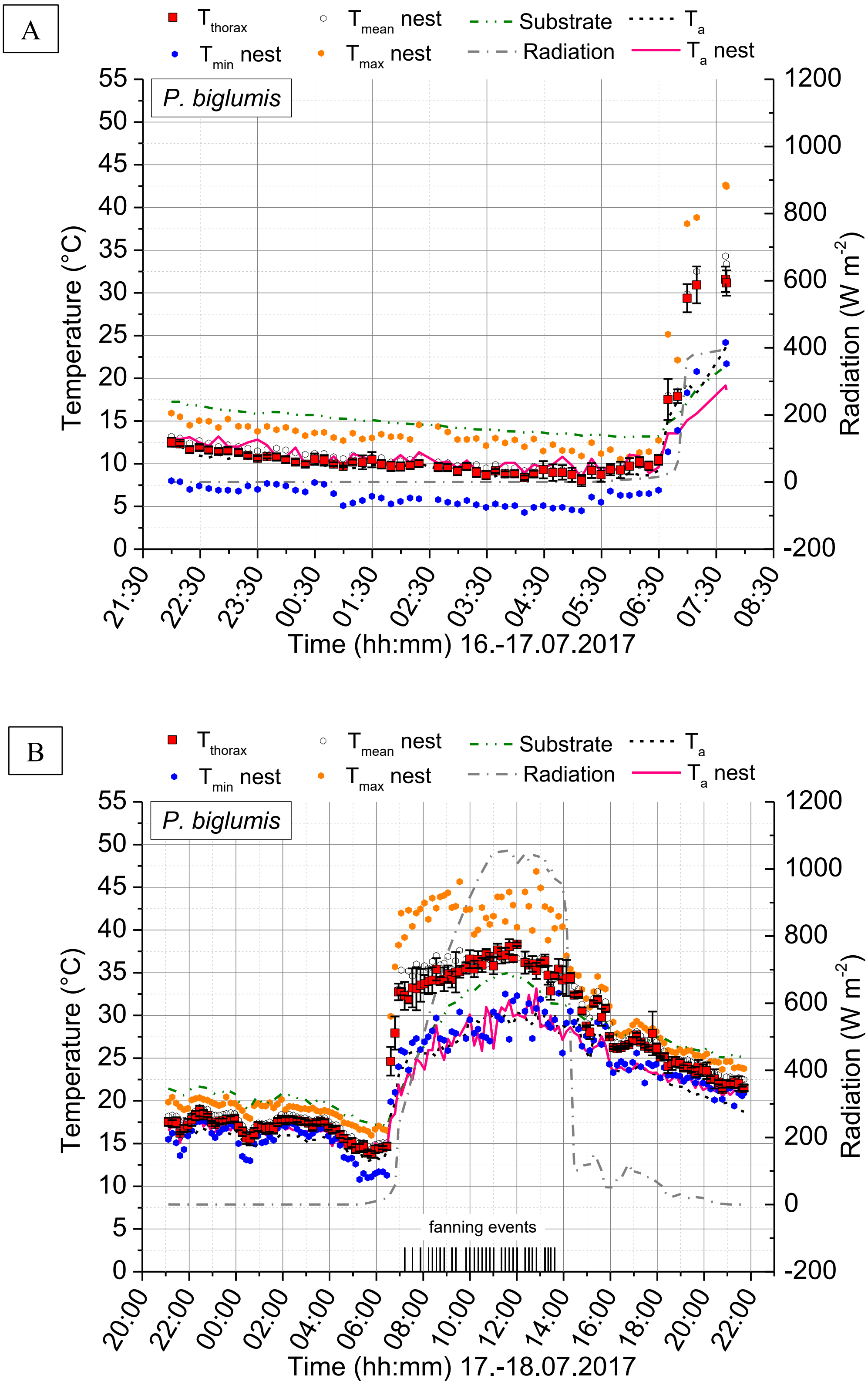


Figure 10 – Temperature changes over 3 days of* P. biglumis *(16.-18.07.2017). The graph shows the data of the nest W4 during the night (A) and during the day (B) thermographed with the Flir T650sc.
T_{thorax} = mean temperature of the thorax of up to five adult individuals; T_{min} = coldest temperature on the nest; T_{mean} = mean of T_{min} and T_{max}; T_{max} = hottest temperature on the nest; substrate = the ground, where the nest was built on (stone); radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest. The observed fanning events are represented by the black bars.

***Polistes biglumis* – Nest W5:** Looking at the nest W5 (*P. biglumis*; Figure 11 A and B), it shows a similar pattern than nest W4. As soon as radiation hit the nest, the T_{max} (nest) rose to 46°C, and stayed above 40°C until around 13:00 when radiation sank again (Figure 8, E and F). Fanning started when the nest was heated up, resulting in fanning activity already around 7:00, simultaneously to sunrise and thus rising radiation. The T_{thorax} (mean) was kept at a mean maximum of 36°C. T_{thorax} (mean) was hardly above the temperature of the substrate during the day when radiation was high, but dropped below it during the night. However, the T_{thorax} (mean) stayed above the measured temperature of T_a always and stayed higher than T_a (nest) during the day and little below it during the night. As soon as direct radiation on the nest fell, the T_{max} of the nest converged to the T_{min} of the nest, but showed a higher difference than in the nest W4 (cf. Figure 10 B and Figure 11 B). The T_{max} of this nest stayed above the temperature of T_{thorax} (mean) during the night, with highest temperatures of 15°C. T_{mean} (nest) and T_{thorax} (mean) were close throughout the day-night cycle and rarely the T_{mean} (nest) was higher with 37°C at most. The T_a and the T_a (nest) stayed below all other measured temperatures, except the T_{min} of the nest, during the day. T_a was below all other measured temperatures (except T_{min}) during the night as well, yet, the T_a (nest) approached the T_{thorax} (mean), being rarely higher (maximum temperature 13°C). In total the T_a (nest) did not rise above 34°C and the T_a was not higher than 30°C on this nest, showing the same pattern as in nest W4. T_a and T_a (nest) dropped to as low as 4°C during the night, which was cooler than in nest W4. The temperature of the substrate stayed above all other measured temperatures during the night (~ 15°C), but did not go higher than 33°C during the day, as it stayed mostly below the T_{thorax} (mean), T_{mean} (nest) and clearly below T_{max} (nest) but was higher than in W4.

The measurements with the Flir i60 (Figure 12, A and B) did not bring as many data points as those with the Flir T650sc because no automated recording was possible, resulting in a less dense day-cycle. At this point it is to remember that the measurements with the Flir i60 were done simultaneously to those of the Flir T650sc (W4 i60: 18.07.2017 / W5 i60: 19.07.2017). Yet, always the other nest was measured with the Flir i60 than with the Flir T650sc (W5 measured with the T650sc meant measurement of W4 with the i60 on one specific day). Nonetheless, the daytime temperature pattern stayed the same as in Figure 10 and Figure 11 and clearly showed an increasing T_{max} (nest) when radiation rose. As there are no late night measurements, nothing can be said about the temperature during this period. Nevertheless, due to the seen day-cycle-data, it is very likely that during the night a same pattern would be seen as well.

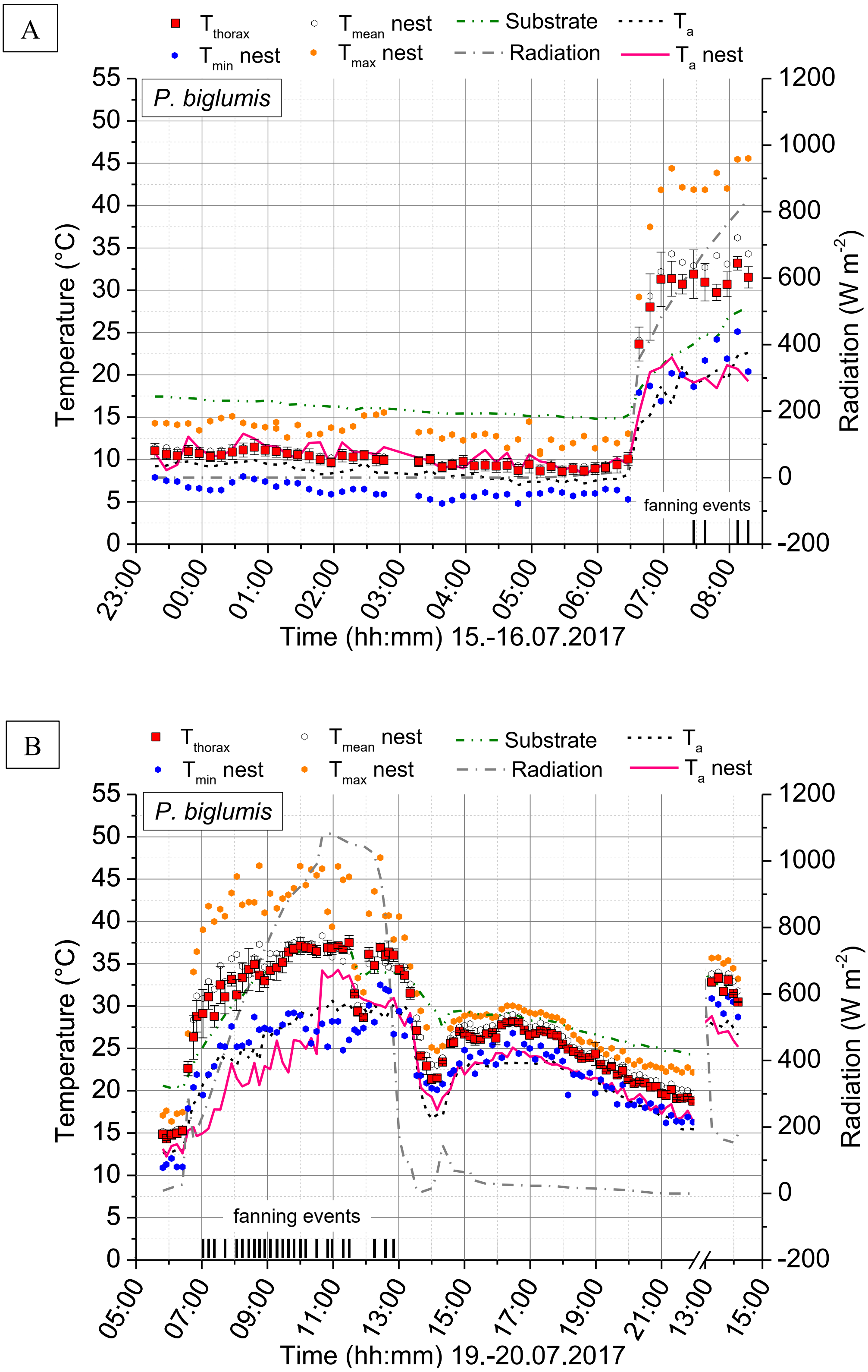


Figure 11 – Temperature changes over 4 days of* P. biglumis *(15.-16.07.2017 and 19.-20.07.2017). The graph shows the data of the nest W5 during the night (A) and the day (B) thermographed with the Flir T650sc.
T_{thorax} = mean temperature of the thorax of up to five adult individuals; T_{min} = coldest temperature on the nest; T_{mean} = mean of T_{min} and T_{max}; T_{max} = hottest temperature on the nest; substrate = the ground, where the nest was built on (stone); radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest. The observed fanning events are represented by the black bars.

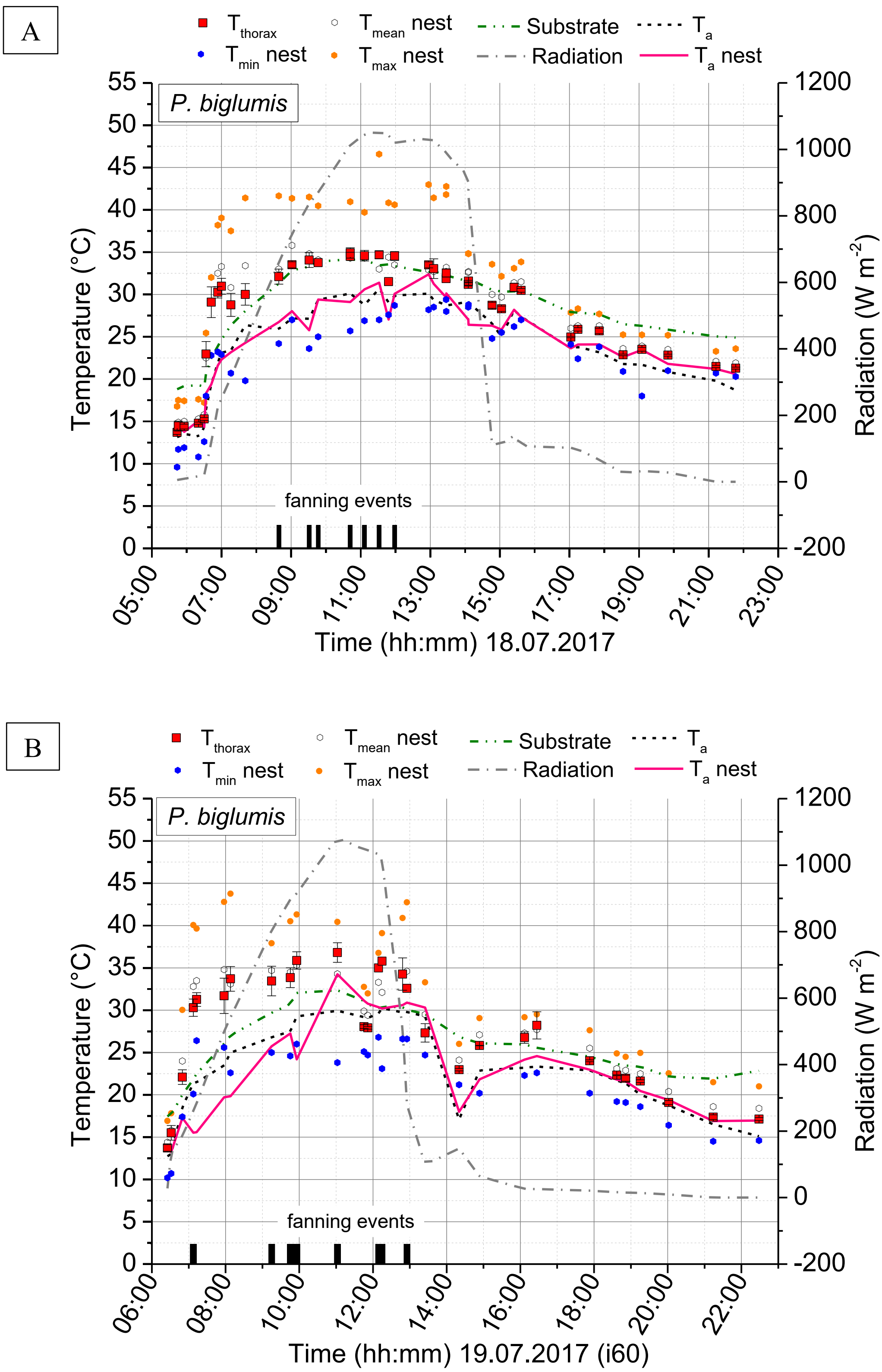


Figure 12 – Temperature changes over 2 days of* P. biglumis *(18.07.2017 and 19.07.2017). The graph shows the data of the nest W4 (A) and W5 (B) thermographed with the Fliri60.

T_{thorax} = mean temperature of the thorax of up to five adult individuals; T_{min} = coldest temperature on the nest; T_{mean} = mean of T_{min} and T_{max}; T_{max} = hottest temperature on the nest; substrate = the ground, where the nest was built on (stone); radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest. The observed fanning events are represented by the black bars.

***Polistes gallicus* – Nests N1, N2:** Looking at the data of *P. gallicus* the first notable difference is that no radiation hit the nests N1 (Figure 13, A) and N2 (Figure 13, B) and no fanning events were observed. In contrast to W4 (Figure 10) and W5 (Figure 11), the radiation only rose to a maximum of 300 W m^{-2} (in W4 and W5 it was around 1000 W m^{-2}). Thus, the temperature did not vary a lot, resulting in a more or less constant temperature distribution during the day. Night measurements do not exist for these nests. However, as in *P. biglumis*, in N1 and N2 the T_a and the T_a (nest) stayed below all other measured temperatures (except T_{min} of the nest). Looking closer to N2 it can be seen that the T_a approached the temperature of the T_{throax} during the afternoon, showing a contrast to *P. biglumis*, where the T_a (nest) came closer.
T_{thorax} (mean) hardly went above 35°C but did not go lower than 23°C on these days. The T_{min} (nest) and the T_{max} (nest) showed no strong differences in nests N1 and N2, as they were close each other throughout the day. The T_{thorax} (mean) and the T_{max} of the nest stayed even closer to each other. The temperature of the substrate was higher than all the other measured temperatures in N1 during noon, and always stayed above the T_a and the T_a (nest). In N2 it was always similar to the T_{max} of the nest, and stayed higher than T_a and T_a (nest) as well.

***Polistes gallicus* – Nests N4:** Looking at N4 (Figure 14) of *P. gallicus* one of the most noteworthy facts is that the temperature of the substrate stayed above all measured temperatures except in the early morning hours, reaching a peak of 50°C. The mean of the T_{thorax} was more uneven than the T_{thorax} at other nests of *P. gallicus*, with coldest temperatures of 30°C and highest temperatures of 45°C. T_{min} (nest) stayed below all measured temperatures, except in the early morning hours.
In contrast to the other nests of *P. gallicus*, in N4 the T_a (nest) was a lot higher than the T_a during the day but did adjust to it in the late afternoon. Starting from a level of 30 °C in the morning T_a (nest) reached at highest 45°. The T_a increased from about 25°C in the morning to 41°C during the day, approaching T_a (nest) in the late afternoon. Notable is that although almost zero radiation hit the nest, the adults started fanning behaviour in the early morning. However, they did not fan for long.
As in N3 (Figure 15), the T_{mean} of the nest stayed below the T_{thorax} (mean), with temperatures of maximal 42°C and minimal 30°C.

***Polistes gallicus* – Nest N3:** Results changed highly as soon as a nest of *P. gallicus* was hit by solar radiation (Figure 15). Although radiation was still not as strong as in *P. biglumis*, the T_{max} of the nest rose up to 55°C, and was thereby almost 10°C hotter than the highest measured temperature in *P. biglumis*. With rising T_{max} of the nest and radiation, *P. gallicus* started to fan just like *P. biglumis* and stopped when radiation fell. The T_{thorax} (mean) did rise to 45°C and did not go below 25°C during night-time on these warm days, being higher than the T_{mean} of the nest during the day and closer to it during the night. The T_{min} of the nest clearly stayed below all other measured temperatures. The T_{max} (nest) approximated T_{min} (nest) during the night but remained higher and sank in parallel with it. Looking at the T_a and the T_a (nest), it can be seen that the T_a surpassed the T_a (nest) around midday on both days but dropped below it during the night, with highest temperatures of 47°C. The T_{min} of the nest oscillated constantly between 27°C and 34°C during the day, and did not drop below 23°C during the night. The temperature of the substrate stayed higher than the T_a (nest) but did not surpass the T_a during midday. With maximal 45°C (Figure 15, B) the substrate was close to the T_{thorax} (mean) but remained always above the T_{mean} of the nest.

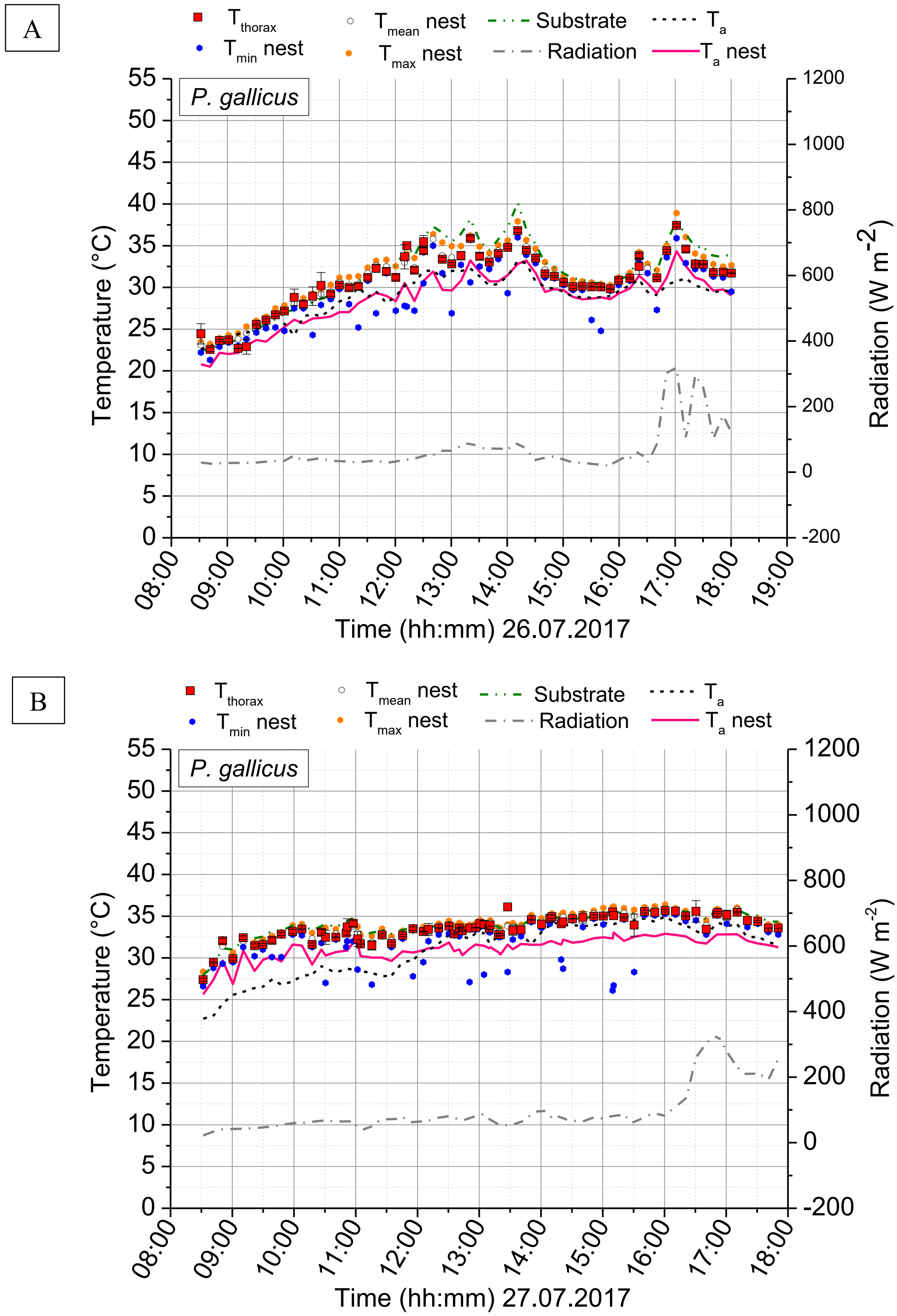


Figure 13 – Temperature changes over 2 days of **P. gallicus** ***(18.07.2017 and 19.07.2017). The graph shows the data of the nest N1 (A) and N2 (B) thermographed with the Flir T650sc.***
T_{thorax} = mean temperature of the thorax of up to five adult individuals; T_{min} = coldest temperature on the nest; T_{mean} = mean of T_{min} and T_{max}; T_{max} = hottest temperature on the nest; substrate = the ground, where the nest was built on (stone); radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest.

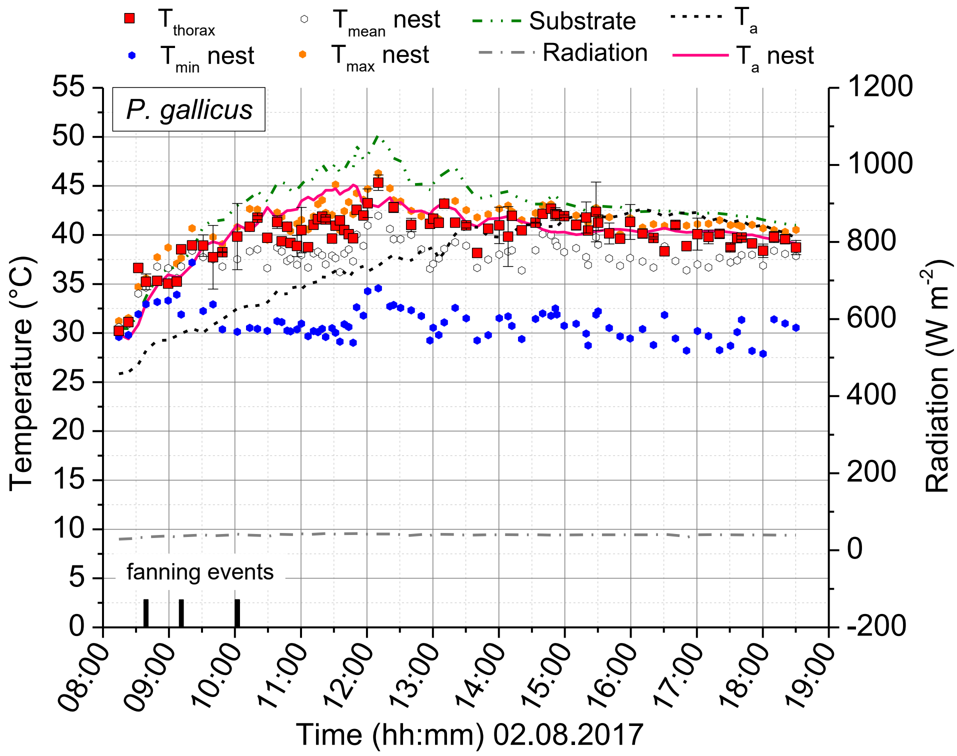


Figure 14 – Temperature changes of* P. gallicus *(02.08.2017). The graph shows the data of the nest N4 thermographed with the Flir T650sc.

T_{thorax} = mean temperature of the thorax of up to five adult individuals; T_{min} = coldest temperature on the nest; T_{mean} = mean of T_{min} and T_{max}; T_{max} = hottest temperature on the nest; substrate = the ground, where the nest was built on (stone); radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest.

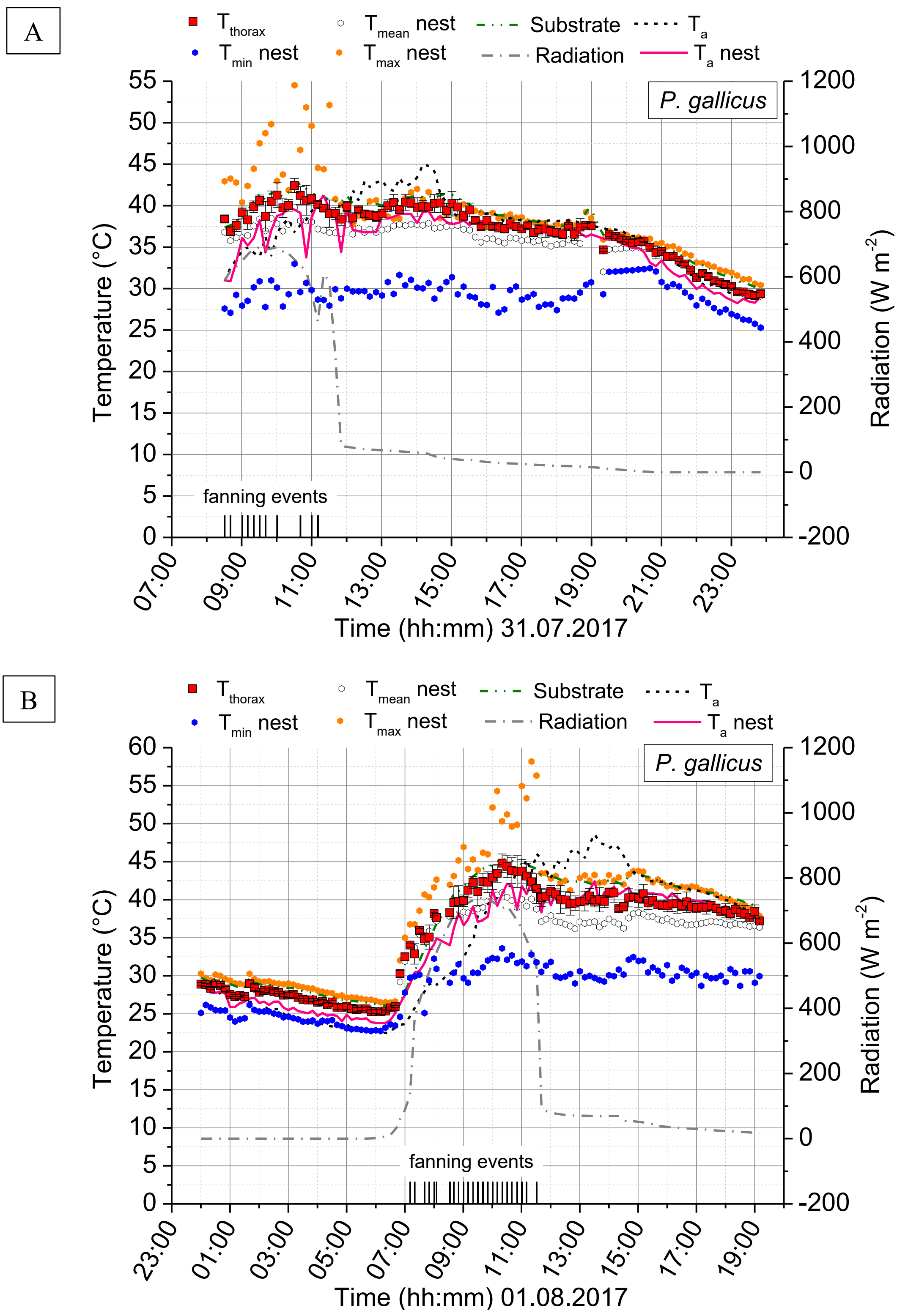
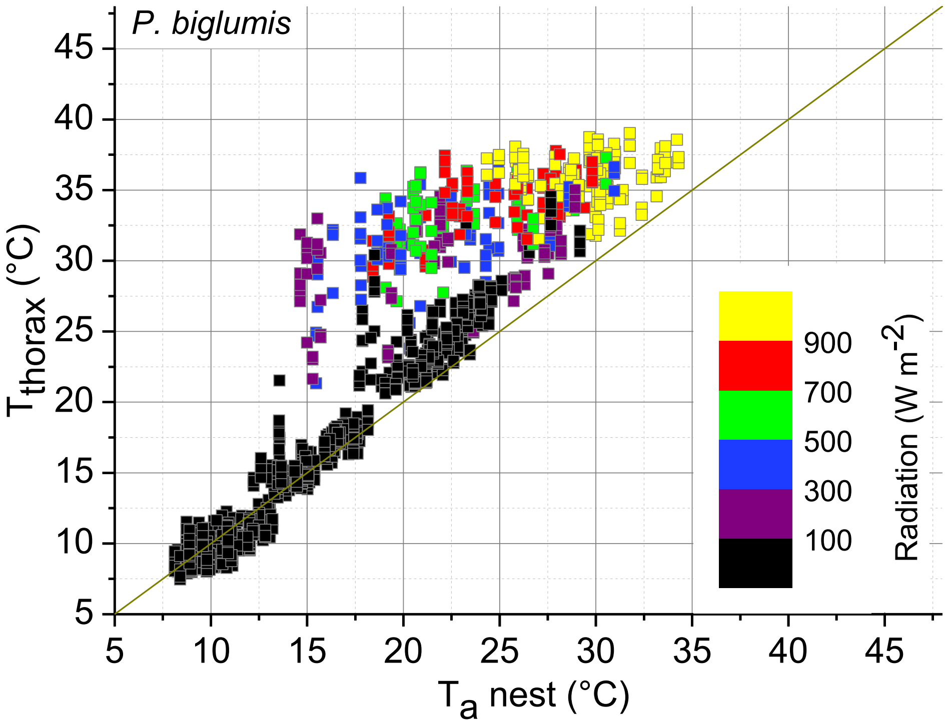


Figure 15 – Temperature changes over 2 days and one night of* P. gallicus *(18.07.2017 and 19.07.2017). The graph shows the data of the nest N3 thermographed with the Flir T650sc.
T_{thorax} = mean temperature of the thorax of up to five adult individuals; T_{min} = coldest temperature on the nest; T_{mean} = mean of T_{min} and T_{max}; T_{max} = hottest temperature on the nest; substrate = the ground, where the nest was built on (stone); radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest.

A



B

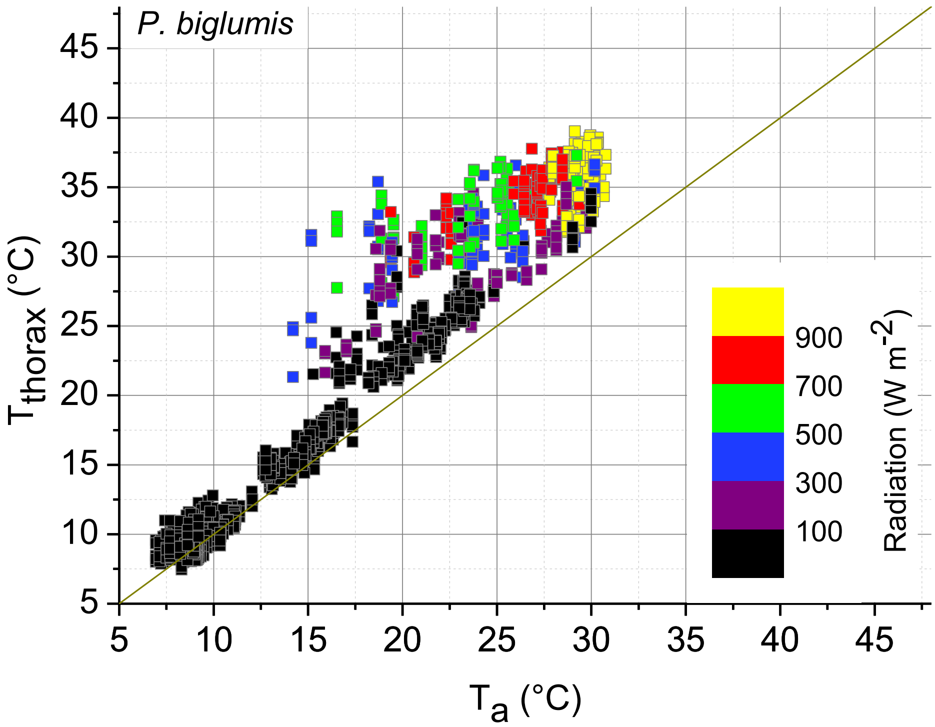


Figure 16 – Surface temperature of the thorax of adults of* P. biglumis *measured at the nest in dependence on ambient air temperature close to the nest (T_a nest) (A) and ambient temperature in shade a few meters away (T_a) (B).

T_{thorax} = temperature of the thorax of all measured adult individuals; radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest; diagonal lines = Isolines: Tth = Ta nest (A) / Tth = Ta (B)

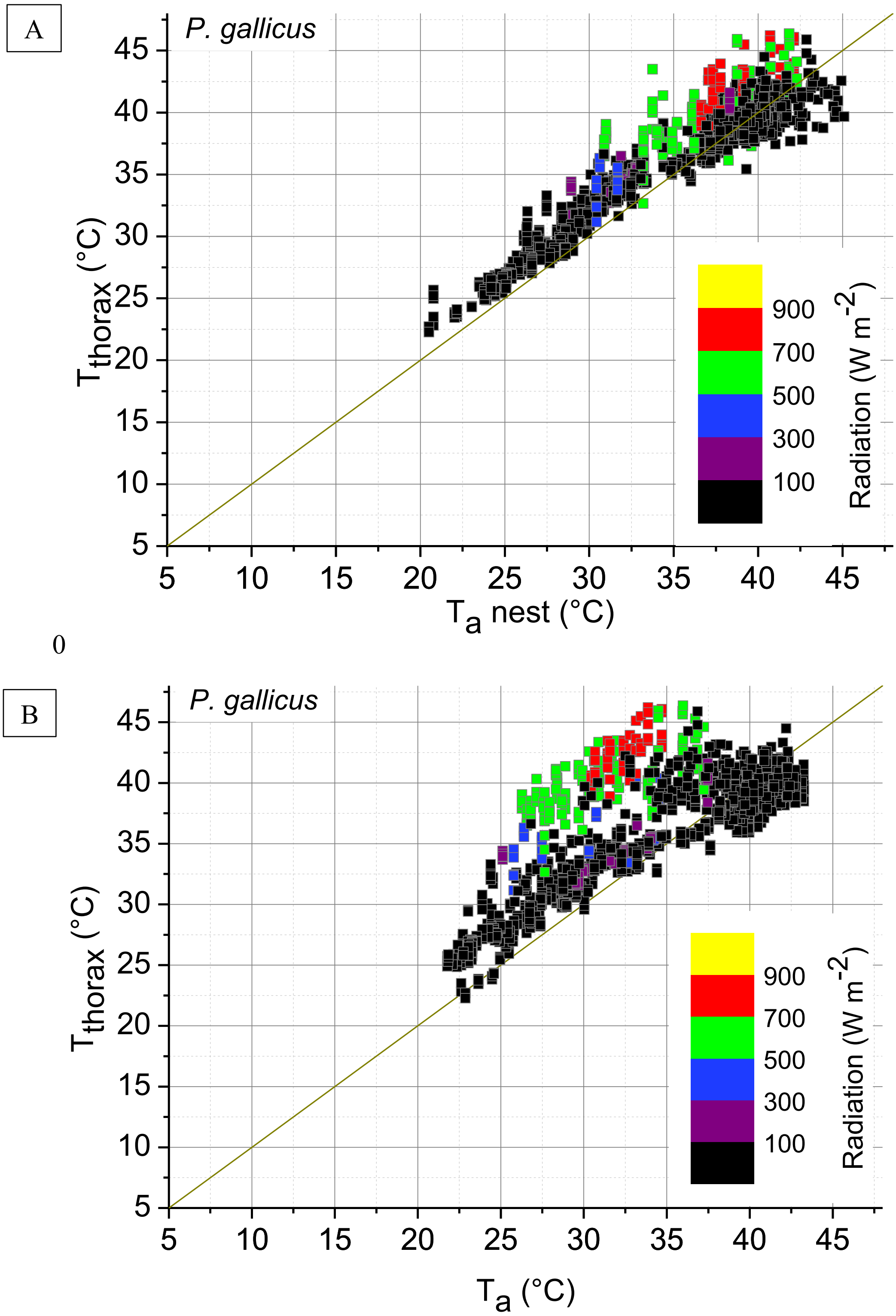


Figure 17 – Surface temperature of the thorax of adults of* P. gallicus *measured at the nest in dependence on ambient air temperature close to the nest (T_a nest) (A) and ambient temperature in shade a few meters away (T_a) (B).

T_{thorax} = temperature of the thorax of all measured adult individuals; radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest; diagonal lines = Isolines: Tth = Ta nest (A) / Tth = Ta (B)

A comparison of the dependence of T_{thorax} on T_a and T_a (nest) between *P. biglumis* (Figure 16) and *P. gallicus* (Figure 17) gives a rough impression of the differences in environmental conditions on warm summer days between Mediterranean and Alpine climate. While in *P. biglumis* the lowest ambient temperatures found were below 10°C, *P. gallicus* was not exposed to temperatures lower than 20°C. Maximum ambient temperatures were by 15 °C higher in the Mediterranean habitat of *P. gallicus.* Global (solar) radiation rose only in *P. biglumis* higher than 900 W m^{-2}. Figures 16 and 17 also demonstrate that ambient temperature measurements close to the nest deviated often considerably from those some meters away.
In shade (radiation < 100 W m^{-2}) the T_{thorax} resembled the T_a (nest) at cool conditions (in *P. biglumis*) or was a few degrees higher than it. At the most extreme T_a (nest) higher than 40 °C which only *P. gallicus* was exposed to, several individuals had a T_{thorax} below T_a (nest) due to cooling efforts (Figure 17).
Solar radiation increased the T_{thorax} considerably (up to 18 °C) above T_a (nest) in *P. biglumis* but T_{thorax} never exceeded 39 °C (Figure 16). In *P. gallicus* the increase of T_{thorax} above T_a (nest) was considerably lower (up to 10 °C) but – due to the generally higher T_a (nest) – reached values as high as 46 °C (Figure 17)!

Part Two – Temperature of larvae and cells

Looking at the results of the temperature measurements one has to keep in mind that only the surface of the cells and larvae was measured. Since a pupa is definitely behind a sealed cell surface, the temperature only represents that of the surface of the cap, not of the pupa itself. The same holds for the larvae, as the animal could have a somewhat different mean body temperature, if the whole animal would have been measured and not only the head of it. Exact measurement of inner body temperature, however, would have meant to kill the brood by insertion of thermocouple needles in their body, which would have prevented recording of daily temperature cycles. It is also important to mention that temperatures of larvae, sealed cells (pupae) and empty cells reported represent only a subset of possible cells, selected in a way to allow investigation of daily temperature changes.

***Polistes biglumis* – Nests W4, W5:** The repetitive day-night pattern of brood and cell temperature can be seen in the measurements of nests W4 and W5. In the nests W4 (Figure 18, A) and W5 (Figure 18, B) the temperature of the larvae did not rise higher than 40°C, with only one individual close to 42°C (W5). The temperature of the larvae stayed above the T_a (nest) and the T_a during the day, yet it converged closer to both during the night but stayed higher. As soon as radiation rose, all measured temperatures increased as well. The empty cells were hardly warmer than the sealed cells during the night and did not surpass them during the day either. Only in W4 during noon, the empty cells achieved temperatures up to 40°C, the hottest measured temperature on the nest.

The measurements with the i60 camera confirmed the daytime temperature changes of nests W4 (Figure 19, A) and W5 (Figure 19, B), though only a less dense measurement was possible. The T_{larvae} stayed the hottest of all measured temperatures during the day, being hotter than the sealed cells almost all time. The T_{larvae} stayed higher than the T_a (nest) and the T_{cells} (empty/sealed) during the day and only came closer to them during the night but never surpassed them.

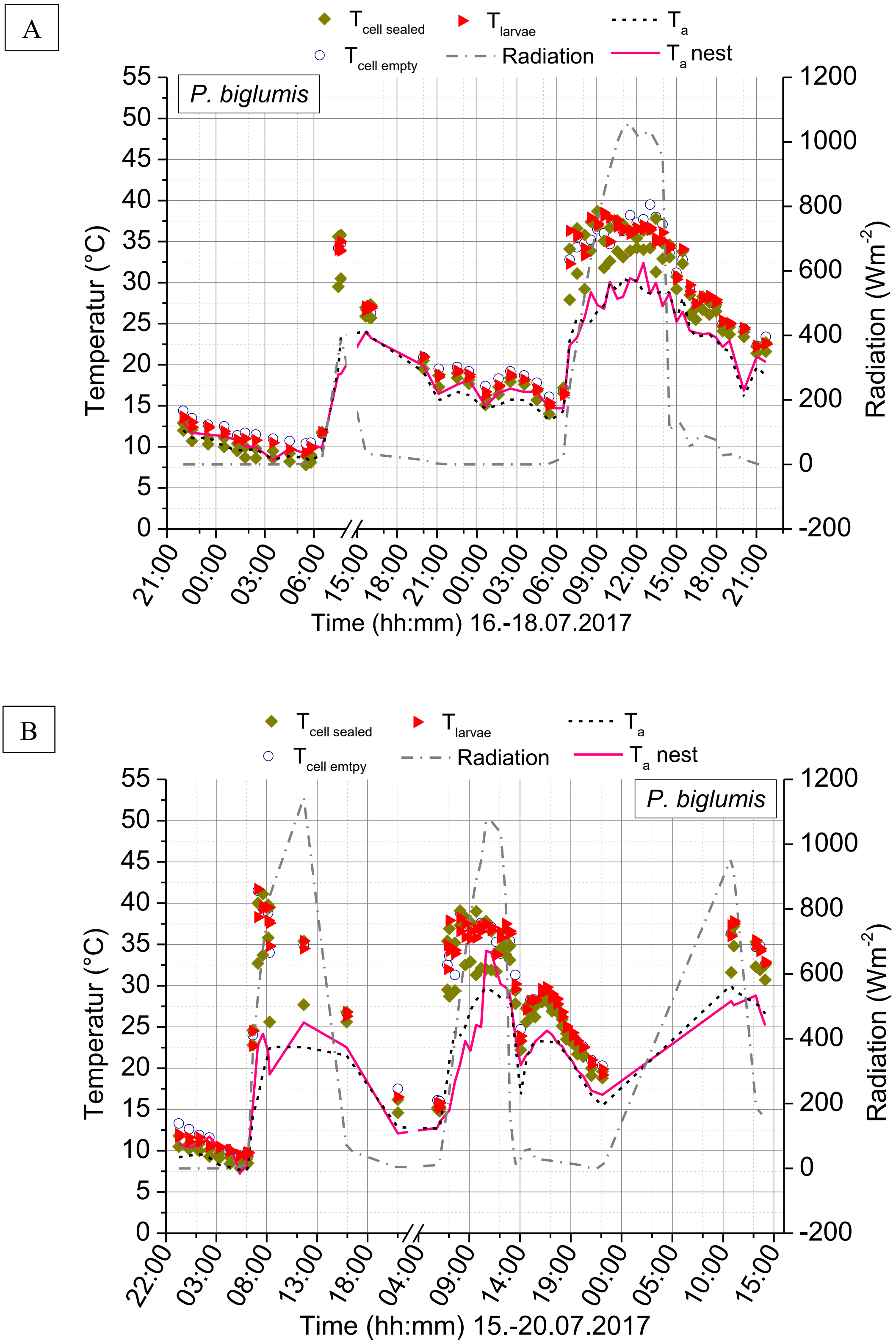


Figure 18 – Temperature changes of* P. biglumis *larvae, pupae (sealed cells) and empty cells (16.-18.07.2017 and 15.-20.07.2017). The graph shows the data of the nest W4 (A) and W5 (B) thermographed with the Flir T650sc.

$T_{cell\ sealed}$ = individual temperature of two sealed cells; $T_{cell\ empty}$ = individual temperature of one open and empty cell; T_{larvae} = individual temperature of two larvae; radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest.

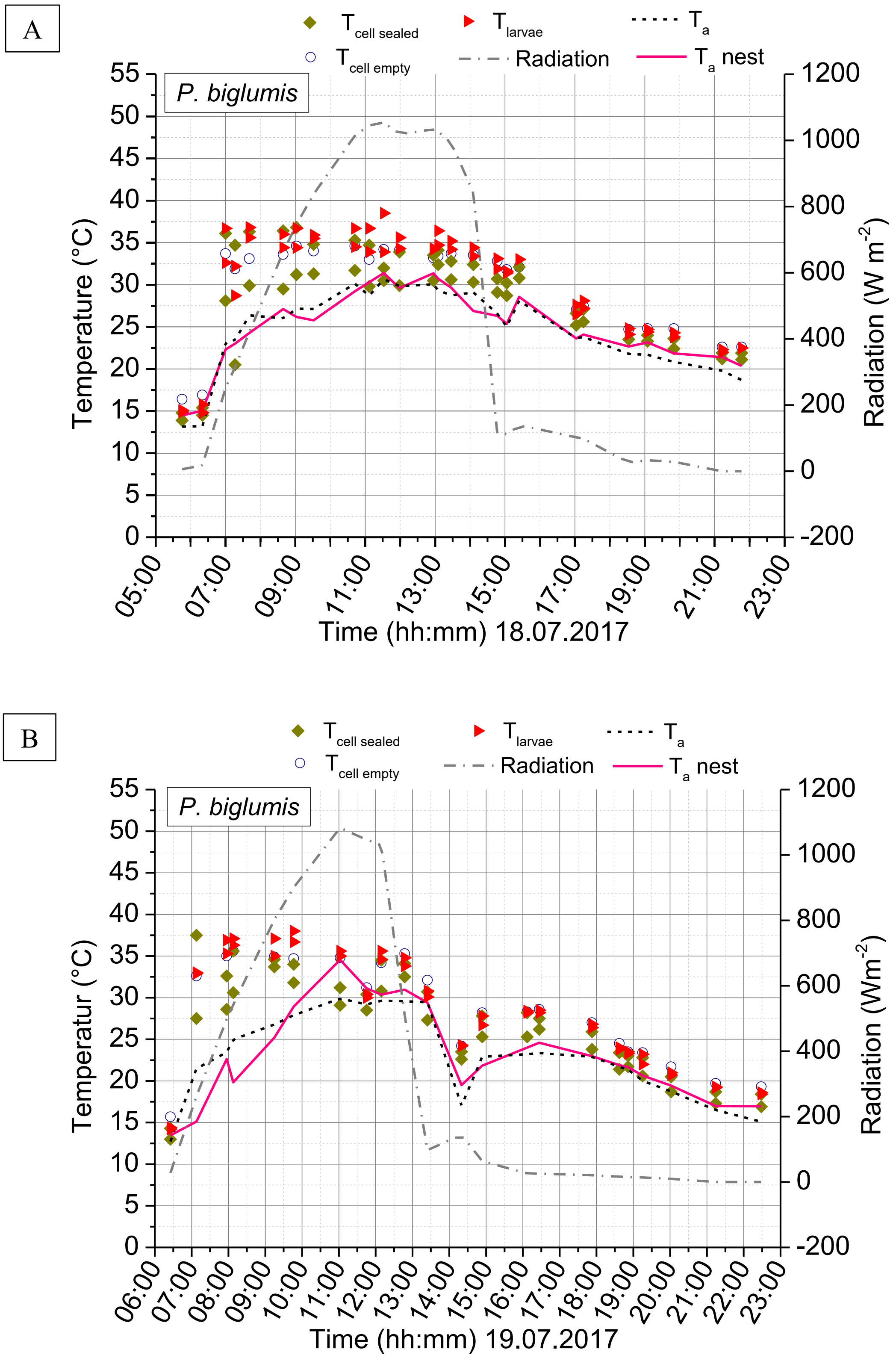


Figure 19 – Temperature changes of* P. biglumis *larvae, pupae (sealed cells) and empty cells (18.07.2017 and 19.07.2017). The graph shows the data of the nest W4 (A) and W5 (B) thermographed with the Flir T650sc. The graph shows the data of the nest W4 (A) and W5 (B) thermographed with the Fliri60.

$T_{cell\ sealed}$ = individual temperature of two sealed cells; $T_{cell\ empty}$ = individual temperature of one open and empty cell; T_{larvae} = individual temperature of two larvae; radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest.

***Polistes gallicus* – Nests N1, N2:** The results of N1 and N2 of *P. gallicus* (Figure 20, A and B) did not show any difference in temperature changes between larvae and cells (sealed/empty). All three were rather close to each other throughout the day, with minimal 23°C and maximal 36°C in N1, and minimal 30°C and maximal of 35°C in N2. This means that the nest N2 showed less temperature changes than N1. However, in both nests the T_a (nest) and the T_a stayed always below the other measured temperatures.

***Polistes gallicus* – Nest N4:** Looking at N4 (Figure 21, A), a different pattern could be observed. The T_a (nest) rose rapidly above all other measured temperatures from late morning to noon, whereas the T_a slowly climbed higher over the day, slightly surpassing the T_a (nest) in the afternoon (starting from ~14:30). The highest measured T_a (nest) was 45°C, the coolest one 30°C. The T_a did not reach such high temperatures, with lowest temperatures of 26°C and highest temperatures of 43°C. Between the highest temperature of T_a (nest) and the highest temperature of T_a a difference of 3°C can be seen. The T_{larvae} stayed more or less constant over the day with temperatures ranging from 30°C to 38°C. Only one larva dropped below 30°C in the early evening. The $T_{cell\ sealed}$ was close to the T_{larvae} in the morning. However, it rose higher than T_{larvae} during the rest of the day, with highest temperatures of 39°C.

***Polistes gallicus* – Nest N3:** N3 (Figure 21, B) showed again a unique pattern. The T_{larvae} stayed the hottest in the morning when the sun was shining on the nest, with temperatures up to 42°C. In the afternoon, however, the T_{larvae} was surpassed by the T_a and slightly by the T_a (nest). During noon (~13:00), the T_a and the T_a (nest) were the highest measured temperatures, whereas both dropped below all other at night.
From morning to evening, $T_{cell\ sealed}$ were cooler than the T_{larvae} and $T_{cell\ open}$, with minimal and maximal temperatures of 30°C and 39°C, respectively. Only one sealed cell reached 43°C. $T_{cell\ open}$ fluctuated with the temperature of the larvae, being only few times higher during the day.

It is again to remember that intense radiation did not reach most nests of *P. gallicus*, except N3, where a maximum of 740 W m^{-2} was measured.

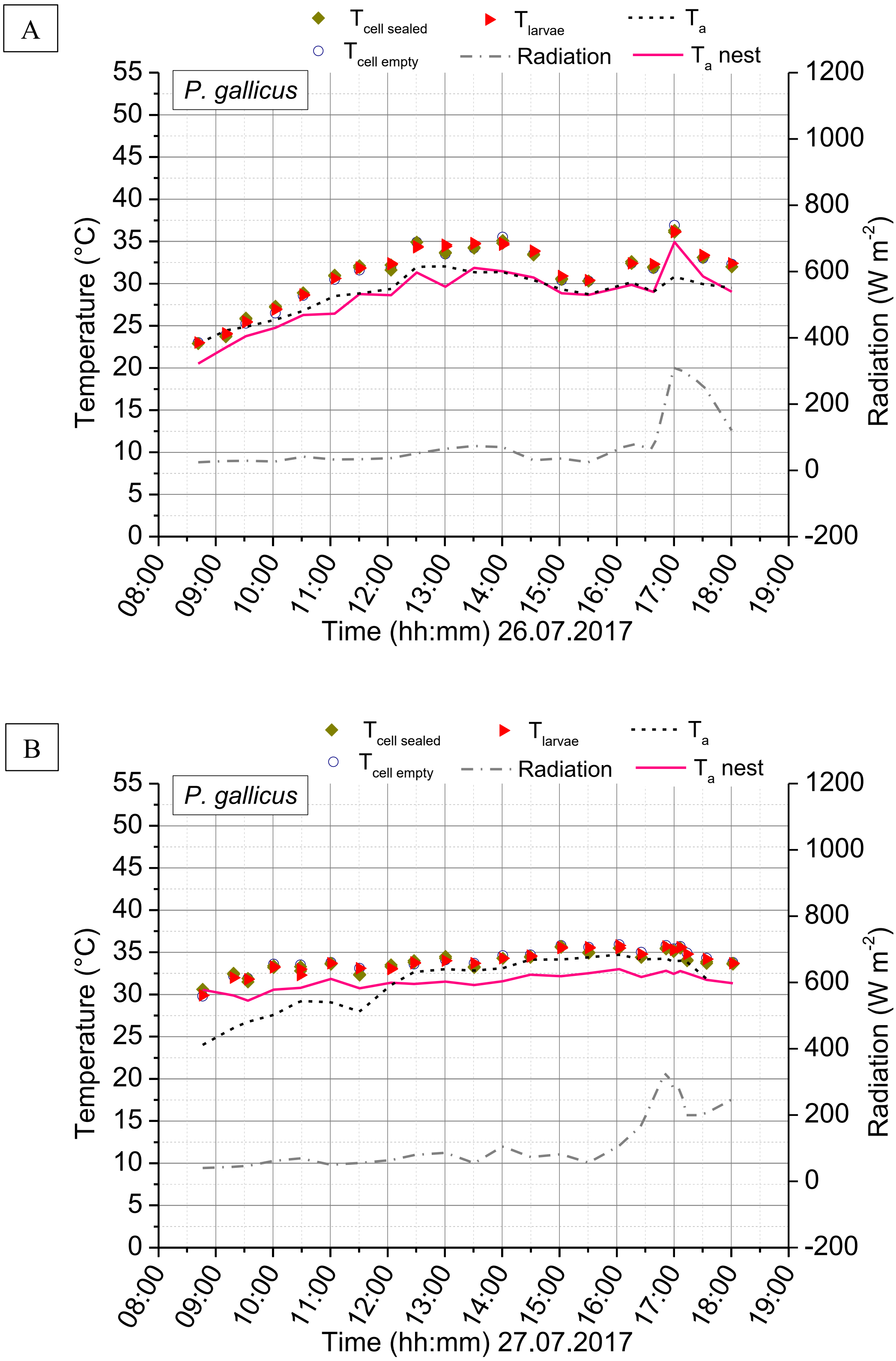


Figure 20 – Temperature changes of* P. gallicus *larvae, pupae (sealed cells) and empty cells (26.07.2017 and 27.07.2017). The graph shows the data of the nest N1 (A) and N2 (B) thermographed with the Flir T650sc. *$T_{cell\ sealed}$ = individual temperature of two sealed cells; $T_{cell\ empty}$ = individual temperature of one open and empty cell; T_{larvae} = individual temperature of two larvae; radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest.*

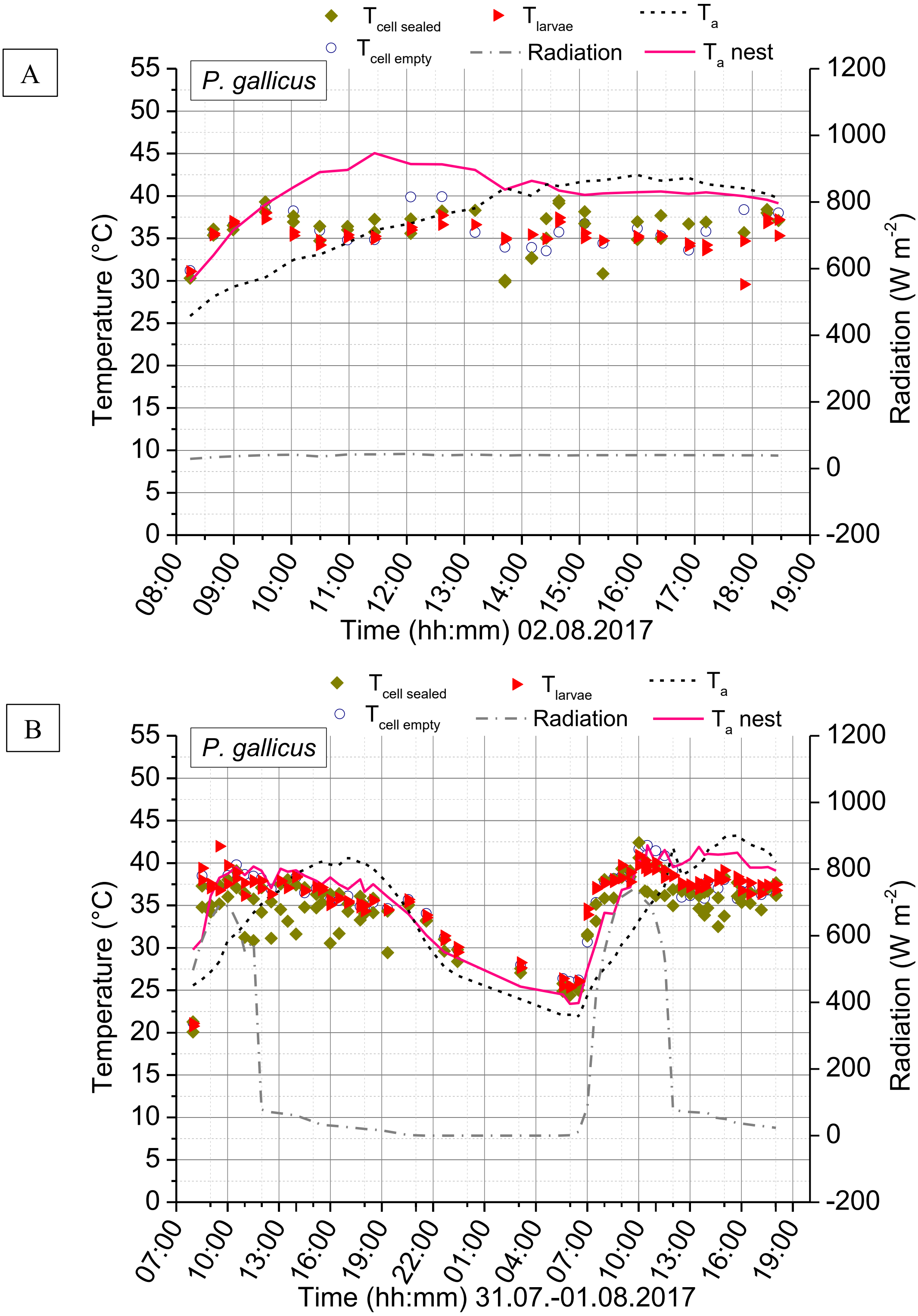


Figure 21 – Temperature changes of* P. gallicus *larvae, pupae (sealed cells) and empty cells (31.07.-01.08.2017 and 02.08.2017). The graph shows the data of the nest N4 (A) and N3 (B) thermographed with the Flir T650sc.

$T_{cell\ sealed}$ = individual temperature of two sealed cells; $T_{cell\ empty}$ = individual temperature of one open and empty cell; T_{larvae} = individual temperature of two larvae; radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest.

***Polistes biglumis* – Nests W4, W5:** Plots of the dependence of the T_{larvae} and $T_{cell\ sealed}$ on T_a (nest) and T_a in *P. biglumis* show that the temperature of the larvae rose with rising radiation but in very most cases was kept below 40 °C by the adults (Figure 22). This was achieved even when the T_a (nest) reached up to 35°C.

The pattern of $T_{cell\ sealed}$ was similar to that of T_{larvae} but showed a much higher dispersion, with temperatures being often lower than T_{larvae} at the same radiation and same T_a (nest). In contrast to T_{larvae} the $T_{cell\ sealed}$ did drop below the isoline more often. Figure 22 again shows that ambient temperature has to be measured close to the nests as well T_a.

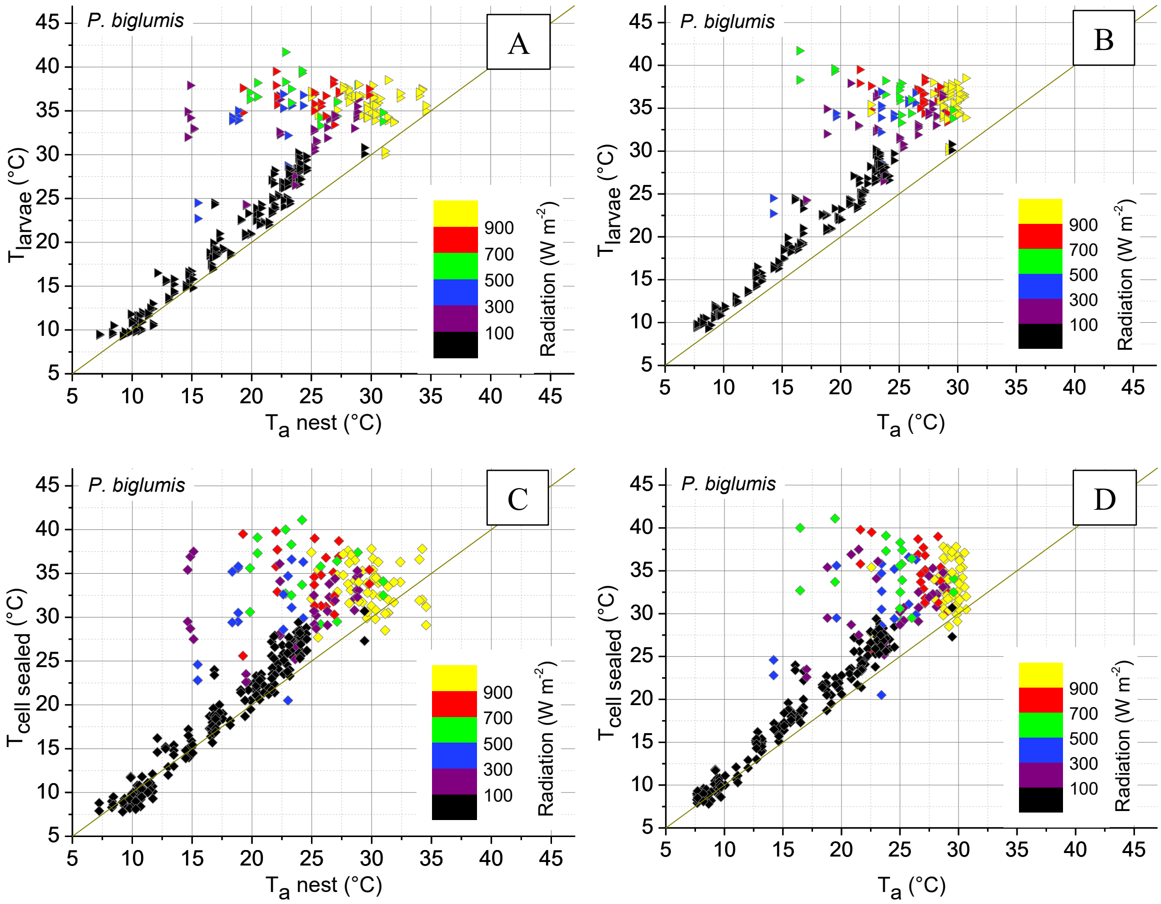


Figure 22 – Surface temperature of larvae and of sealed cells (pupae) of* P. biglumis *measured at the nest in dependence on ambient temperature close to the nest (T_a nest) (A, B) and some meters away (T_a) (C, D).
$T_{cell\ sealed}$ = temperature of all measured sealed cells; T_{larvae} = temperature of all measured larvae; T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest; diagonal lines = Isolines: temperature of y-axis = x-axis.

***Polistes gallicus* – Nests N1, N2, N3, and N4:** Plots of the dependence of the T_{larvae} and $T_{cell\ sealed}$ on T_a (nest) and T_a in *P. gallicus* (Figure 23) showed that T_{larvae} and the $T_{cell\ sealed}$ remained mostly below 40°C like in *P. biglumis*, though ambient temperatures were considerably higher (with a T_a of 43°C and a T_a (nest) up to 45°C) than in the *P. biglumis* measurements (Figure 22). Only in a few cases higher temperatures were measured. At the highest T_a (nest) the T_{larvae} and the $T_{cell\ sealed}$ dropped up to 10°C below the isoline.

In contrast to *P. biglumis,* only in one nest (N3) intense solar radiation reached the nest (Figure 21). Nevertheless, highest $T_{cell\ sealed}$ were reached when radiation was below 100 W m^{-2}. The other way round it was with T_{larvae}, where highest temperatures were achieved when radiation hit the nest (Figure 23).

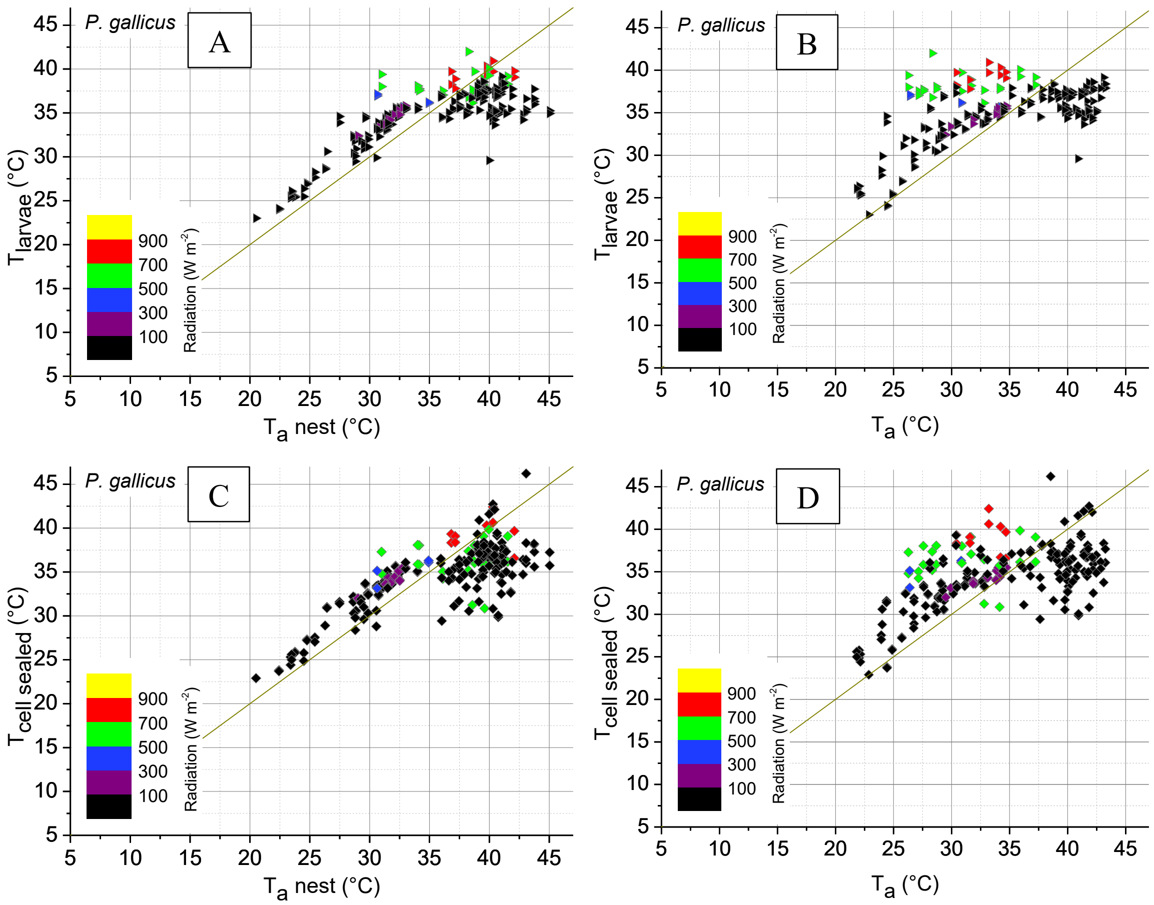


Figure 23 – Surface temperature of larvae and of sealed cells (pupae) of* P. gallicus *measured at the nest in dependence on ambient temperature close to the nest (T_a nest) (A, B) and some meters away (T_a) (C, D). *$T_{cell\ sealed}$ = temperature of all measured sealed cells; T_{larvae} = temperature of all measured larvae; T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest; diagonal lines = Isolines: temperature of y-axis = x-axis.*

Part Three – Cooling effect of fanning

Cooling by fanning was evaluated in *P. biglumis* only. Mostly before fanning events, the adults put their head into empty cells (Figure 24, B) and started to fan right afterwards (Figure 24, C, D). In addition, the fanning individual stayed mainly on the upper part of the nest (compare Figure 8 and Figure 9). Figure 24 shows a complete fanning event. It can be seen that cells are cooled down to even below 30°C, when the nest showed temperatures of maximal 42°C. The cooling effect was clearly seen by the dark spots and cell rims next to the fanning individual (Figure 24, D), which vanished only a few seconds after the wasp stopped its activity (Figure 24, F).

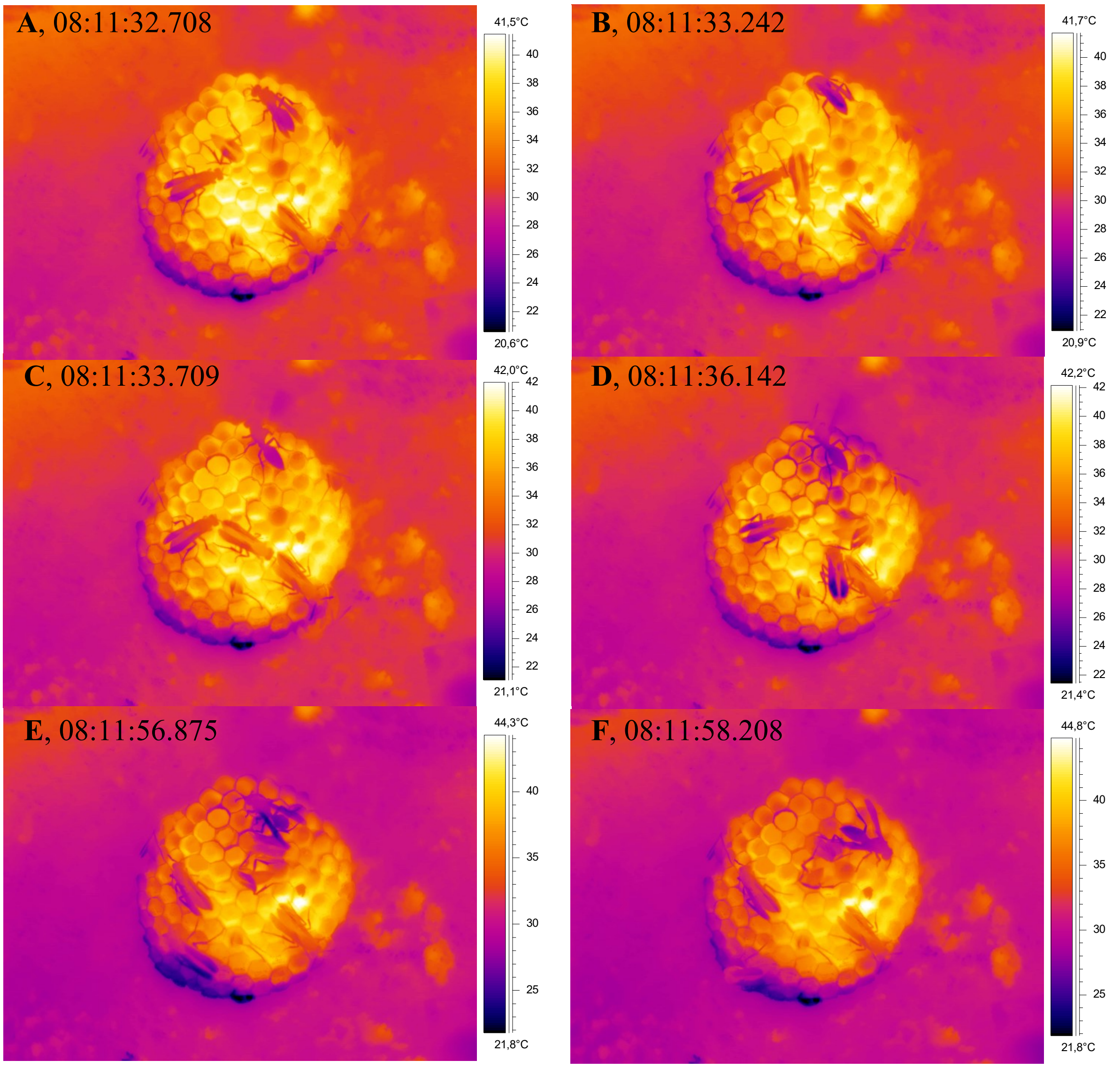


Figure 24 – Thermographic pictures of a fanning event shot with the Flir T650sc on the nest W5 of* P. biglumis** *(total of 25 seconds;* ***19.07.2017). *The pictures show the starting and ending point of a fanning event lasting for a few seconds (C, D). The cooling effect on cells near the fanner can be seen in D. The fanning adult stayed at the upper part of the nest most of the time, putting its head inside a cell before starting the fanning (A, B) and running across the nest after termination (E, F).*

Figure 25 to Figure 42 show detailed analysis of fanning events. Temperatures of cells ($T_{cell\ center}$) and cell borders (common edges of three adjacent cells; $T_{cell\ border}$) close to the fanner and on the opposite part of the comb are compared with the body temperature of the fanners. The graphs show the evaluated temperatures in relation to the T_a (nest) which was (most of the time) the lowest of all measured temperatures. On the other hand the T_{max} of the nest was the hottest measured temperature always.

The asterisks (*) in all graphs (Figure 25 to Figure 42) signal the start as well as the ending of the fanning events. As the wasps were very active during the fanning event, especially during midday, the measured cells had to be changed sometimes. These modifications can be seen in Table 5 (page 71, Appendix). In these cases uneasy fluctuations are the result of the change of the tools during the fanning event. However, this was not the only reason for fluctuations (compare Table 5 with the individual fanning events).

Fanning events on nest W4 (*Polistes biglumis*):

In W4 (Figure 25 to Figure 33), the fanning individuals were measured over a period of three minutes, and all adults fanned consecutively, with one event lasting for about 15 seconds. Three fanning events were evaluated from measurements around midday. Therefore, ambient temperature was rather high and showed a T_a (nest) ranging from 30°C to 32.5°C.

First fanning individual (W4/1): The T_{max} (nest) of the nest W4/1 (Figure 25 to Figure 27) started at 45°C degrees, fell down to 40°C during the fanning event and rose to 45°C again afterwards. The temperature of the close cell centers (Figure 25) dropped from 36-38°C to 33-34°C and stayed below the mean nest temperature (T_{mean} (nest)). The temperature of measured cell borders also decreased but in one cell stayed above the T_{mean} (nest) and did not drop below 36°C. However, comparing the close cells of W4/1 to those of W4/2 and W4/3, it can be seen that only in W4/1 the temperature of the border of one cell remained above the temperature of the center of the cells. The other $T_{cell\ border}$, however, showed the typical pattern, staying below the temperature of the corresponding $T_{cell\ center}$.

Looking at the temperature of the distant cells (Figure 26), cooling was less pronounced but temperatures around the T_{mean} (nest) were observed. However, $T_{cell\ center}$ remained mostly above the T_{mean} (nest). One border sank to 34°C, rose to 37°C and dropped again down to 34°C.

The fanning individual (Figure 27) had a more or less constant temperature of the thorax and showed somewhat more cooling in head and abdomen. All body parts stayed below the temperature of about 39°C of an additionally measured cell close to the wasp (T_{cell}). Of the body parts, T_{thorax} was the hottest, with temperatures above 36°C. $T_{abdomen}$, on the other hand, was the coldest body part, cooling even to below 34°C and was about 3°C below T_{caput}.

Second fanning individual (W4/2): W4/2 (Figure 28 to Figure 30) was the only event, where the temperature of the close cells (Figure 28) dropped below the T_a (nest), with temperatures of even below 30°C. It was observed, however, that only the temperature of the border of the cells went below the T_a (nest), the center of the cells did not cool that much. In addition, the $T_{cells\ border/center}$ stayed below the T_{mean} (nest) and did not rise higher than 37°C. Yet, the temperature of the center of the cells remained hotter than the associated $T_{cell\ border}$.
The distant cells (Figure 29) showed less cooling. Only one $T_{cell\ border}$ fell below 36°C for a short period. It is noticeable that the T_{mean} (nest) stayed colder than the $T_{cells\ border/center}$ most of the time.
The fanning individual (Figure 30) had a more constant temperature during the event, with the T_{thorax} being the hottest, and the T_{abomen} the coldest. In total, the body temperature did not drop below 34°C degrees and did not rise higher than slightly above 38°C during the event.

Third fanning individual (W4/3): The results of W4/3 (Figure 31 to Figure 33) show again that the T_a (nest) was the coldest of all measured temperatures with about 32°C always. Here it could be seen again that the centers of the cells were hotter than the associated borders. The highest temperature of the close cells had a starting point of slightly above 40°C, while the coldest cells started at about 38°C. However, one $T_{cell\ center}$ stayed above the T_{mean} (nest) most of the time, whereas in the other cell $T_{cell\ border/center}$ remained below.
In the distant cells (Figure 32) the cooling effect was only weakly visible. Measured cell temperatures stayed either clearly above or slightly below the T_{mean} (nest). Although the cell temperature remained more or less constant during the event, one $T_{cell\ border}$ fluctuated uneasily up and down, with temperatures between 37°C to 39°C (compare Table 5).
The fanning individual (Figure 33) showed a similar pattern than the other but an even more stable T_{body} with temperatures between 35°C and 38°C, with the coldest temperature at the abdomen and the hottest at the thorax. However, in contrast to the other measurements, the T_{cell} had a strong rise during the end of the event, going up from 35°C to 38°C within milliseconds, which is only partly due to tool changes (compare Table 5).

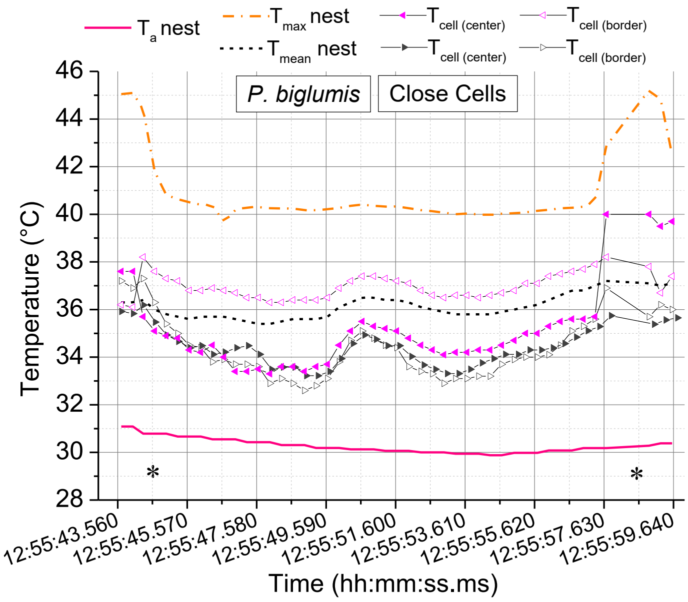


Figure 25 – Fanning event of the first individual of* P. biglumis *(W4/1) on the nest W4 (18.07.2017). Cells close to the fanning individual are shown. *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (magenta ◀ represents W1r/z; dark grey ▶ represents W2r/z, see Table 2)*

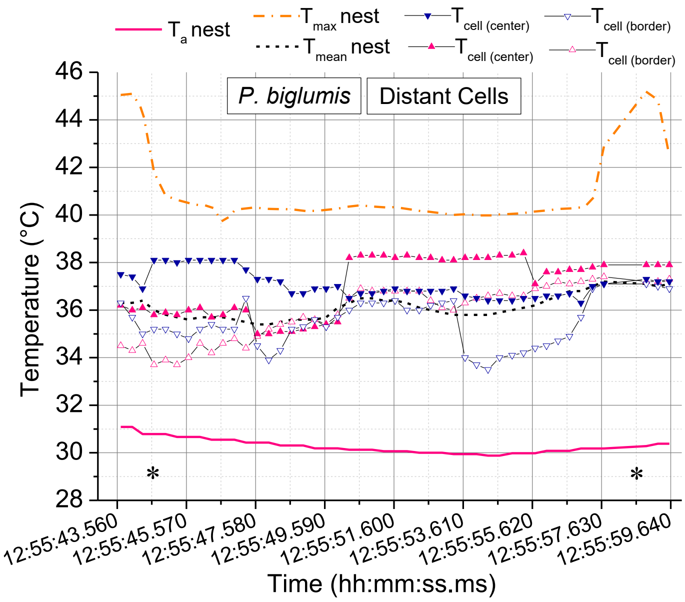


Figure 26 - Fanning event of the first individual of* P. biglumis *(W4/1) on the nest W4 (18.07.2017). Cells on opposite side to the fanning individual are shown. *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (pink ▲ represents W3z/r; royal blue ▼ represents W4r/z, see Table 2)*

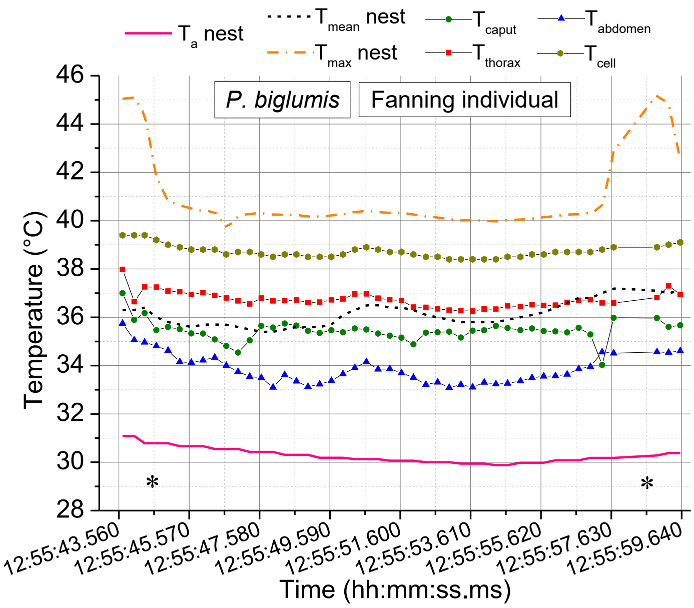


Figure 27 – Fanning event of the first individual of* P. biglumis *(W4/1) on the nest W4 (18.07.2017). Fanning individual is shown. *T_a nest = ambient temperature directly beside the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; T_{caput} = temperature of the caput; T_{thorax} = temperature of the thorax; $T_{abdomen}$ = temperature of the abdomen; T_{cell} = temperature of the center of a cell next to the fanning individual*

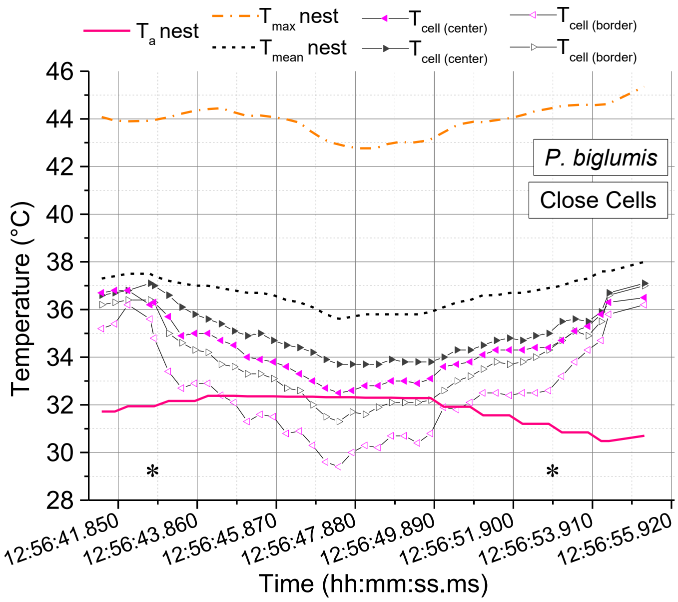


Figure 28 – Fanning event of the second individual of* P. biglumis *(W4/2) on the nest W4 (18.07.2017). Cells close to the fanning individual are shown. *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (magenta ◄ represents W1r/z; dark grey ► represents W2r/z, see Table 2)*

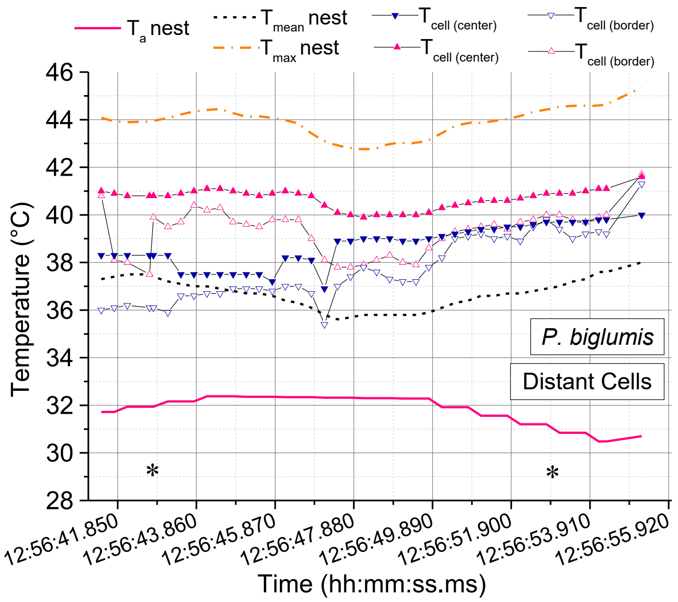


Figure 29 – Fanning event of the second individual of* P. biglumis *(W4/2) on the nest W4 (18.07.2017). Cells on opposite side to the fanning individual are shown. *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (pink ▲ represents W3z/r; royal blue ▼ represents W4r/z, see Table 2)*

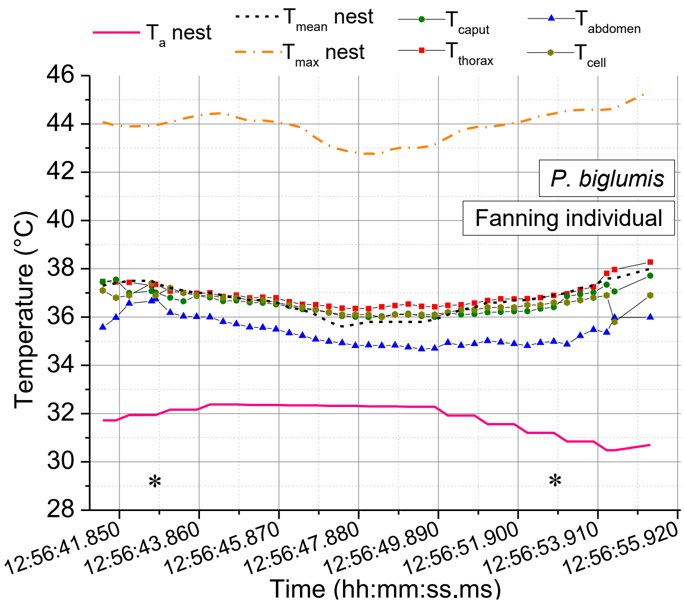


Figure 30 – Fanning event of the second individual of* P. biglumis *(W4/2) on the nest W4 (18.07.2017). Fanning individual is shown. *T_a nest = ambient temperature directly beside the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; T_{caput} = temperature of the caput; T_{thorax} = temperature of the thorax; $T_{abdomen}$ = temperature of the abdomen; T_{cell} = temperature of the center of a cell next to the fanning individual*

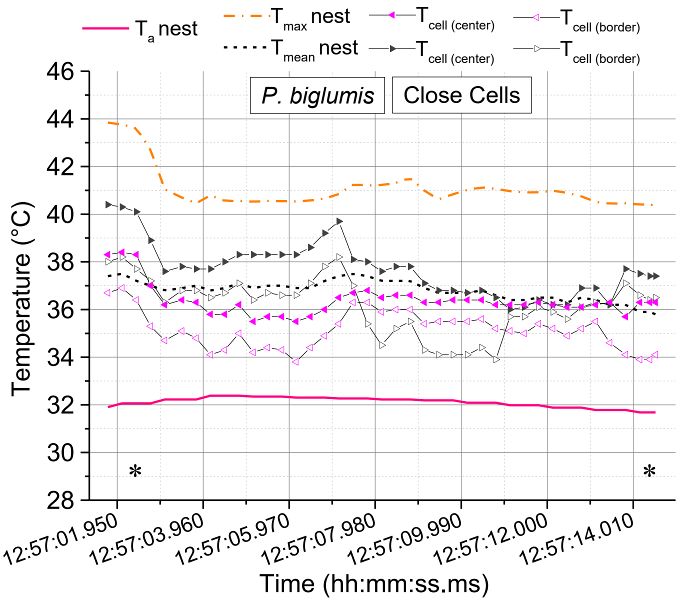


Figure 31 – Fanning event of the third individual of* P. biglumis *(W4/3) on the nest W4 (18.07.2017). Cells close to the fanning individual are shown. *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (magenta ◀ represents W1r/z; dark grey ▶ represents W2r/z, see Table 2)*

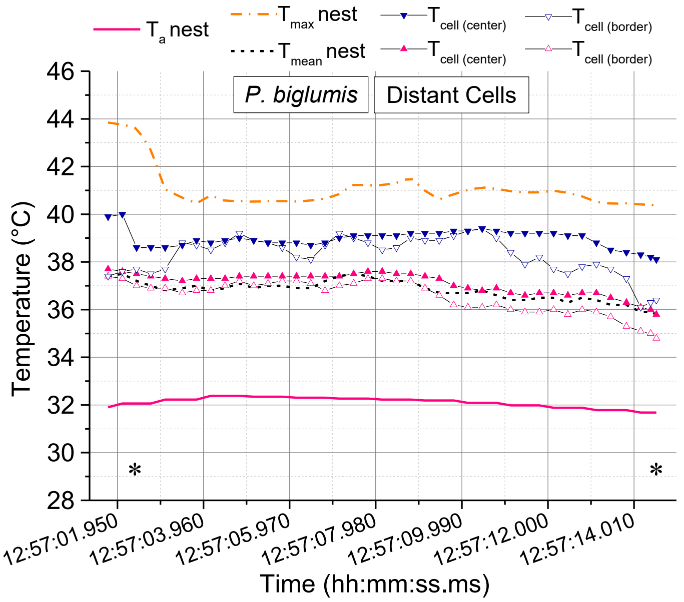


Figure 32 – Fanning event of the third individual of* P. biglumis *(W4/3) on the nest W4 (18.07.2017). Cells on opposite side to the fanning individual are shown. *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (pink ▲ represents W3z/r; royal blue ▼ represents W4r/z, see Table 2)*

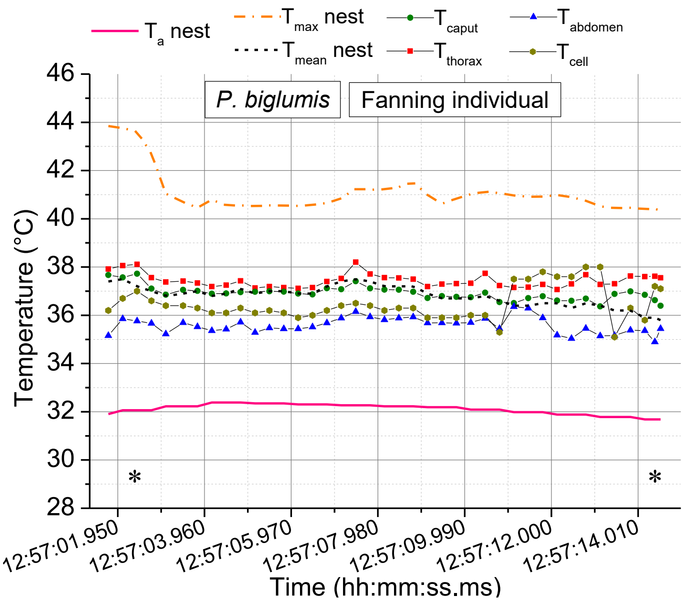


Figure 33 – Fanning event of the third individual of* P. biglumis *(W4/3) on the nest W4 (18.07.2017). Fanning individual is shown. *T_a nest = ambient temperature directly beside the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; T_{caput} = temperature of the caput; T_{thorax} = temperature of the thorax; $T_{abdomen}$ = temperature of the abdomen; T_{cell} = temperature of the center of a cell next to the fanning individual*

Fanning events on nest W5 (*Polistes biglumis*):

Fanning events evaluated from nest W5 (Figure 34 to Figure 42) were measured not at midday but in the morning. Accordingly, the T_a (nest) was considerably lower than in W4, with approximately 18-20°C. The T_{max} of the nest stayed always above all other measured temperatures. Like in W4 the center of the cells was normally hotter than the border except in event W5/1 (Figure 34 to Figure 36).

First fanning individual (W5/1): In event W5/1 (Figure 34 to Figure 36) the T_{max} (nest) remained quite stable between 42°C and 44°C with only one depression below 42°C. The close cells (Figure 34) chosen for evaluation showed a strong difference between the hottest (39°C) and coldest temperature (29°C). The coldest temperature appeared when the T_{max} (nest) was below 42°C.

The distant cells (Figure 35) on the other hand showed, during the event of fanning, a less extreme difference of hot and cold with temperatures between 34°C and 38°C and less fluctuations.

The T_{body} of the first fanning individual (Figure 36) on the nest W5 stayed quite stable. $T_{abdomen}$ was the coldest throughout the event, and T_{thorax} and T_{caput} were close to each other (~32-33°C). However, in contrast to the individuals on W4, T_{thorax} was slightly colder than T_{caput}.

Second fanning individual (W5/2): In W5/2 (Figure 37 to Figure 39) the temperature of T_{max} (nest) and the close cells (Figure 37) dropped as soon as the fanning event started with a temperature fall of 2°C in T_{max} (nest) and even more than 5°C in $T_{cells\ border/center}$. The latter stayed below T_{mean} (nest) throughout the event, rising above it shortly afterwards.

The center of the distant cells (Figure 38) remained constant with temperatures of 38°C and 37°C, close to the T_{max} (nest) and higher than the T_{mean} (nest). The border of the distant cells however, reacted partly to the air flow from the fanner, showing an uneasy pattern. One $T_{cell\ border}$ even fell below the T_{mean} (nest).

The T_{cell} close to the fanning individual (Figure 39) reacted immediately to the cooling airflow but showed a somewhat uneasy pattern with many small changes in temperature, which was not due to tool changes (compare Table 5). It stayed close to T_{caput} and T_{thorax}. As in all other individuals, the $T_{abdomen}$ was the coldest, falling even down to 28°C. The thorax was again slightly warmer than the caput but both changed nearly parallel to each other, with temperatures between 30°C and 32°C.

Third fanning individual (W5/3): In W5/3 (Figure 40 to Figure 42) temperature fell again during the fanning event in the close cells (Figure 40). Starting with temperatures above the T_{mean} (nest), the temperature of the cells (border/center) soon fell below, with a difference to T_{mean} (nest) of up to 6°C. The rapid drop of $T_{cells\ border/center}$ amounted to more than 7°C. Again, the $T_{cell\ center}$ stayed warmer than the $T_{cell\ border}$ most of the time. However, on this nest, one $T_{cell\ border}$ rose sometimes slightly above the $T_{cell\ center}$.

In the distant cells (Figure 41), the $T_{cell\ border}$ and the $T_{cell\ center}$ of the evaluated cells were not affected by the fanning airstream. Temperatures were by several degrees higher than in the close cells, and centers of the cells were warmer than their borders. All temperatures remained above T_{mean} (nest), and only one $T_{cell\ center}$ rose above T_{max} (nest) for a short period.

The fanning individual (Figure 42) displayed the same pattern as the individuals in W4, with $T_{abdomen}$ being the coldest at around 27°C and the T_{caput} the hottest at 30°C, and with the T_{thorax} slightly below T_{caput}. The temperature of the cell close to the fanning individual stayed close to T_{mean} (nest) all the time (~ 35°C).

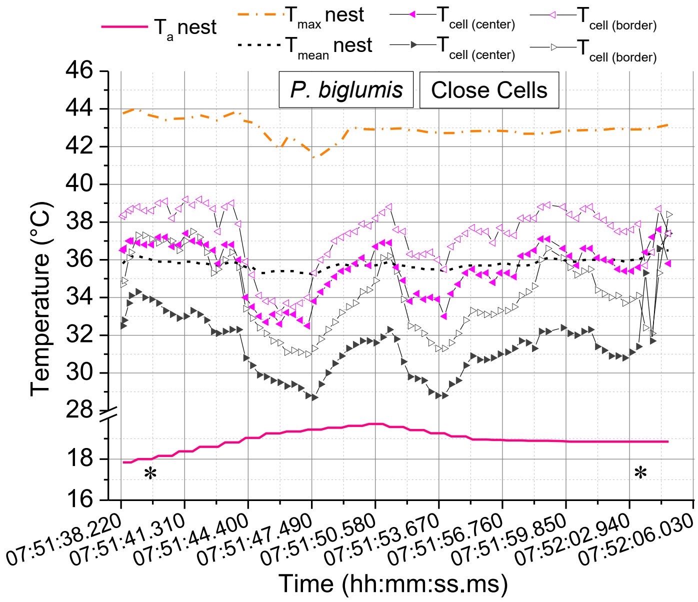


Figure 34 – Fanning event of the first individual of* P. biglumis *(W5/1) on the nest W5 (19.07.2017). Cells close to the fanning individual are shown. *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (magenta ◀ represents W1r/z; dark grey ▶ represents W2r/z, see Table 2)*

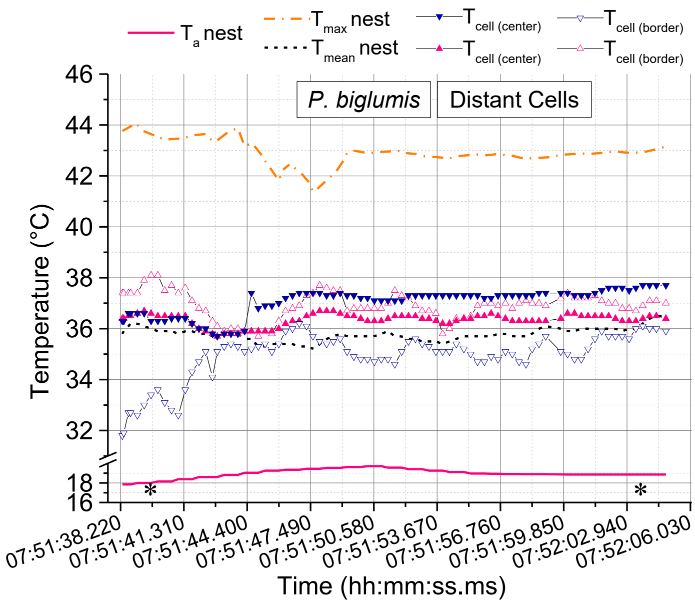


Figure 35 – Fanning event of the first individual of* P. biglumis *(W5/1) on the nest W5 (19.07.2017). Cells on opposite side to the fanning individual are shown. *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (pink ▲ represents W3z/r; royal blue ▼ represents W4r/z, see Table 2)*

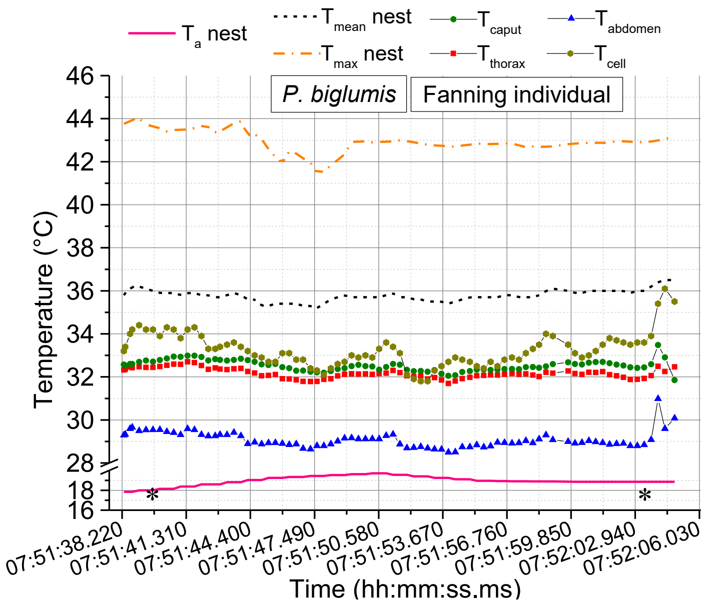


Figure 36 – Fanning event of the first individual of* P. biglumis *(W5/1) on the nest W5 (19.07.2017). Fanning individual is shown. *T_a nest = ambient temperature directly beside the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; T_{caput} = temperature of the caput; T_{thorax} = temperature of the thorax; $T_{abdomen}$ = temperature of the abdomen; T_{cell} = temperature of the center of a cell next to the fanning individual*

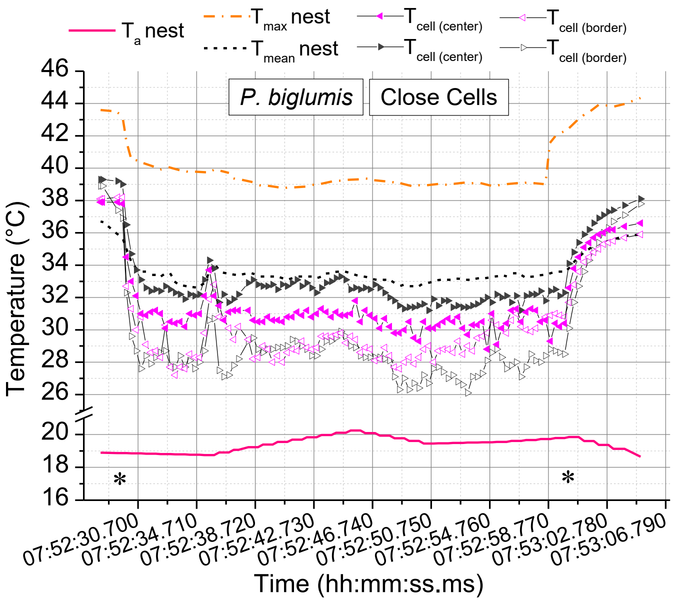


Figure 37 – Fanning event of the second individual of **P. biglumis** ***(W5/2) on the nest W5 (19.07.2017). Cells close to the fanning individual are shown.*** *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (magenta ◀ represents W1r/z; dark grey ▶ represents W2r/z, see Table 2)*

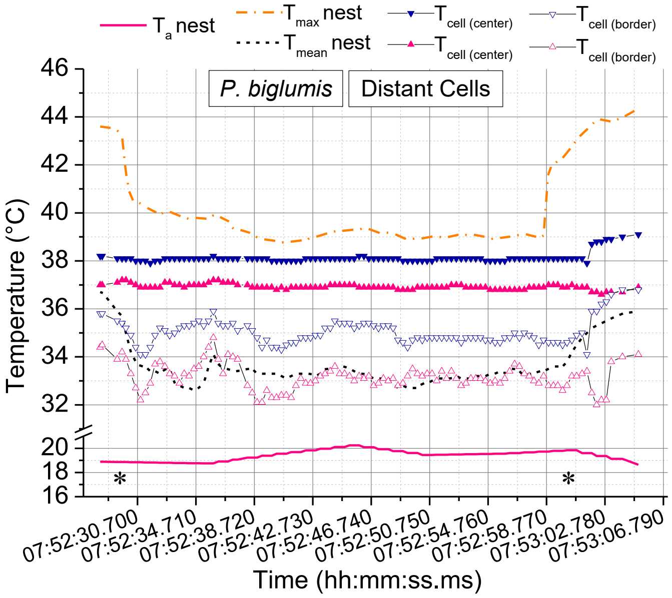


Figure 38 – Fanning event of the second individual of **P. biglumis** ***(W5/2) on the nest W5 (19.07.2017). Cells on opposite side to the fanning individual are shown.*** *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (pink ▲ represents W3z/r; royal blue ▼ represents W4r/z, see Table 2)*

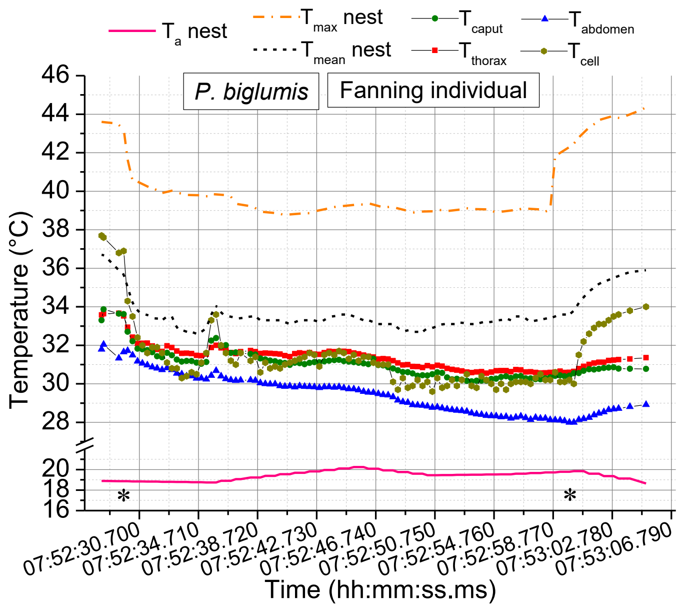


Figure 39 – Fanning event of the second individual of **P. biglumis** ***(W5/2) on the nest W5 (19.07.2017). Fanning individual is shown.*** *T_a nest = ambient temperature directly beside the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; T_{caput} = temperature of the caput; T_{thorax} = temperature of the thorax; $T_{abdomen}$ = temperature of the abdomen; T_{cell} = temperature of the center of a cell next to the fanning individual*

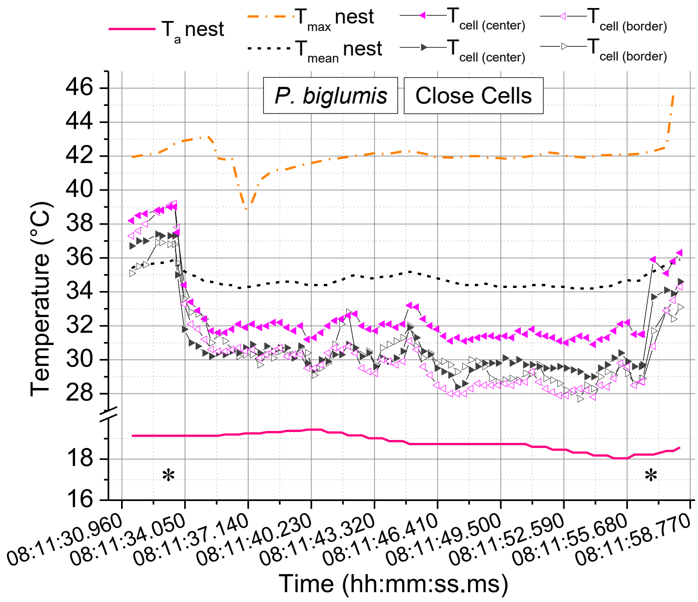


Figure 40 – Fanning event of the third individual of* P. biglumis *(W5/3) on the nest W5 (19.07.2017). Cells close to the fanning individual are shown. *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (magenta ◄ represents W1r/z; dark grey ► represents W2r/z, see Table 2)*

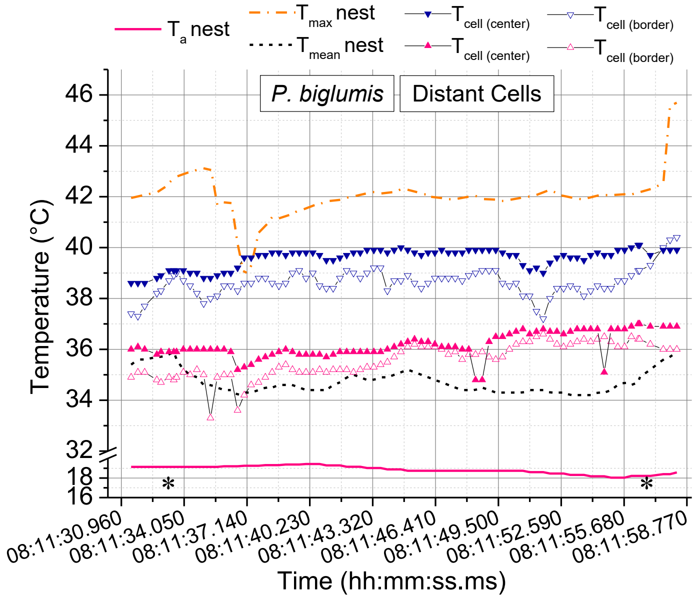


Figure 41 – Fanning event of the third individual of* P. biglumis *(W5/3) on the nest W5 (19.07.2017). Cells on opposite side to the fanning individual are shown. *T_a nest = ambient temperature directly besides the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; $T_{cell\ (center)}$ = temperature of the center of in total two different cells; $T_{cell\ (border)}$ = temperature of the border of the respective $T_{cell\ (center)}$ → each colour represents one center and the associated border (pink ▲ represents W3z/r; royal blue ▼ represents W4r/z, see Table 2)*

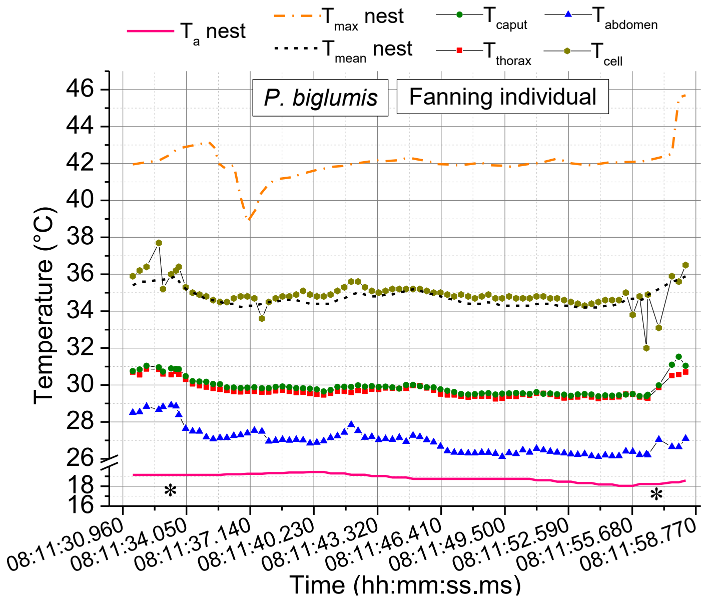


Figure 42– Fanning event of the third individual of* P. biglumis *(W5/3) on the nest W5 (19.07.2017). Fanning individual is shown. *T_a nest = ambient temperature directly beside the nest; T_{max} = hottest temperature on the nest; T_{mean} = mean of T_{min} (not shown) and T_{max} of nest temperature; T_{caput} = temperature of the caput; T_{thorax} = temperature of the thorax; $T_{abdomen}$ = temperature of the abdomen; T_{cell} = temperature of the center of a cell next to the fanning individual*

Discussion

This thesis aim was to show similarities and differences in the two closely related species *P. gallicus* and *P. biglumis* in terms of their thermoregulatory behaviour on hot days. Thus, only an abstract of their behaviour was examined over a short period during summer. The results cannot provide a complete survey of the (micro-) climatic conditions the wasps have to face throughout a season but show an abstract of their thermoregulatory behaviour during critically warm conditions.

This thesis demonstrates that it is important to work with the nests' closest microclimate (in this thesis T_a (nest)) whenever possible. Looking at Figure 16 and Figure 17, for example, it can be seen that the T_a measured farther from the nest may lead to imprecise or even wrong interpretations. Although the T_a was measured very closely to the nest (1-3 meters away) in this thesis, immense temperature differences could be seen compared to the T_a (nest). This example is to show that only a few meters difference of the measured nest-microclimate could mean a strong temperature change for the wasps already (Pincebourde und Casas 2015).

Differences in T_a and T_a (nest), and nest site choice: As already suggested, temperatures vary strongly between the Alpine region and the Mediterranean area. The results proved a very high T_a (~44°C max.) and T_a (nest) (~45°C max.) in *P. gallicus* throughout the day, whereas in *P. biglumis* the T_a (~30°C max.) and T_a (nest) (~35°C max.) was a lot cooler. At night T_a and T_a (nest) never sank below 23°C and 24°C in *P. gallicus* but dropped to as low as 8°C and 10°C in *P. biglumis*, respectively (Figure 16, Figure 17). These differences show why the two species apply different nest-site choice strategies to face their given T_a. In *P. gallicus* the majority of examined nests was built into corners to prevent intense insolation, because radiation would additionally heat the nest and, combined with a high T_a, the nest may reach temperatures above the CT_{max} of the brood (which has to be measured in future investigations) and the adults on top (Figure 15). On the other hand, *P. biglumis* build their nests specifically exposed to the sun (east-south-east) (Lorenzi 1986; Stabentheiner, pers. comm.), providing the nest with solar heat to warm up quickly, which is especially important after cold nights (Figure 10 and Figure 11). This allows the brood to maintain their optimal development temperature although the T_a and T_a (nest) are low. A fast brood development is important, as it guarantees a higher fitness of the colony (Jeanne and Morgan 1992).

As mentioned before, the T_a and T_a (nest) were high in *P. gallicus*, not only during the day but also during the night. In *P. biglumis* both were never that high, but actually did drop strongly during the night. Hence, *P. biglumis* needs a strategy to keep their nests as warm as possible during the night as well, as in Alpine regions temperatures can go close to zero even during midsummer (comp. Figure 10 A and Figure 11 A). The examined nests showed a simple solution to that problem: All nests found in Obergail were built on stone substrate only (46 nests; Stabentheiner, pers. comm.). The results show why the wasps chose that specific substrate. During the night, the stone stayed the hottest of all measured temperatures (up to 7°C higher than T_a (nest)) because of the heat stored during daytime, thus warming the nest from below via heat radiation (Figure 10 A, and Figure 11 A). Thereby the animals on the nest and the brood could maintain a body temperature above the present T_a and T_a (nest).

Cooling behaviour: Although the brood needs a specific temperature to develop and the adults to function, overheating is in both species a problem they face. However, the difference in T_a and T_a (nest) force both species to show an adapted cooling behaviour. The results indicated two different cooling activities in the two paper wasp species. In case of *P. biglumis*, where T_a and T_a (nest) were lower, the most seen cooling behaviour was active fanning. The fanning occurred as soon as the nest was hit by radiation and the nest heated up rapidly (Figure 10, B and Figure 11, B). This specific behaviour was already investigated by Steiner (1929) but he could not measure the true efficiency of it because he only had mercury thermometers at hand. Temperature measurements of the fanning behaviour in this thesis (Figure 25 to Figure 42) could prove a given impact on the temperature of the cells of the nest close to the fanning adult. Although fanning activities were only short, the temperature fall of the cells was up to 4°C and even more (e.g. Figure 40). Interestingly, the wasps put their head into open cells before starting their fanning activity (Figure 8). Not only do they put their head in cells but at the hottest times sometimes also fly shortly above the nest and land again afterwards, a behaviour already reported by Steiner (1929). This leads to the suggestion that the adults measure the temperature inside the cells as well as of the surrounding air close to the nest with their thermoreceptor cells in their antennae (Lacher 1964; Tichy und Loftus 1987; Lorenzo et al. 2013). If they found the temperature to be too hot for their brood, they started the fanning behaviour in the examined area. If more parts of the nest were heated up, other adults started to fan as well. This allowed getting the most of this specific cooling behaviour.

In *P. gallicus,* fanning behaviour was only observed in two nests (N4, Figure 14 and N3, Figure 15) though in one nest (N4) the activity was really short and did not occur at the hottest time of the day, and therefore had a negligible effect. In the other nest, however, the fanning was as long lasting as in *P. biglumis*. In this context, it has to be said that this specific nest was hit by the sun in the morning, resulting in a maximum nest temperature of 50°C (Figure 15)! In this case the normal cooling behaviour of *P. gallicus* by dispersing water on the combs (see below) would not have been enough to cool down the nest. Fanning of air of a similar temperature than that of the nest is probably not effective enough but energetically costly. In this context it is again to mention that this may be the reason why *P. gallicus* tries to avoid direct radiation via the nest site choice.

Normally, *P. gallicus* shows another cooling mechanism (Kovac et al. 2016). The thermographic pictures proved that *P. gallicus* massively transported water onto the nest (Figure 9). The water was not used for drinking purposes but did rather cool the nest, which is possible because of the rather high heat of evaporation of 2.44 kJ/g (at 25 °C) of water. Especially during the morning and noon, the thermographic pictures showed dark water spots all over the nest, leading to the suggestion that this mechanism is much more effective (and important) for *P. gallicus* than the fanning behaviour.

The behaviour of *P. gallicus* would lead to the question of, why *P. biglumis* does not carry so many water droplets onto the nest as well, though they unequivocally do so in sunshine (Figure 5), but amounts are smaller than in *P. gallicus* and drops are sometimes hardly to be seen in the thermograms (Figure 8). This may be, because gathering of water is surely energetically costly (compare costs for flight in Weiner et al. 2012) and probably more costly than fanning. Additionally, the air temperature (T_a (nest)) at *P. biglumis* nests remains usually considerably below the mean nest temperature (T_{nest}) when the sun is shining on them (Figure 10, B; Figure 11, B). The cold air circulating on the nest through the fanning activity cools the cells rapidly (Figure 24; Figure 25 to Figure 42). Therefore, convective cooling by fanning seems more effective in *P. biglumis*, though often not sufficient.

To sum it up, it depends highly on the surrounding temperature, which cooling behaviour would be the most suitable. A high T_a leads to a faster evaporation and promotes evaporative cooling, whereas a cold T_a suggests a strategy, where circulating air cools the nest. However, despite the differences in their climatological challenges, the two species use both strategies on demand. Both *P. gallicus* as well as *P. biglumis* are flexible in their thermoregulatory behaviour and can adjust to changing thermal situations.

However, water supply is crucial in both species. In Obergail, for example, it was seen that none of the inhabited nests of *P. biglumis* were more than about 50 meters away from a persisting water source (Stabentheiner, pers. communication). In *P. biglumis* this was already reported by Steiner (1929).

Brood and body temperature: During the days of observation, *P. gallicus* showed a generally higher thoracic temperature, leading to the suggestion that this species might have a greater tolerance for high temperature than *P. biglumis*. This could be seen as an adaption to their natural habitat, where T_a is all together higher. As Kovac et al. (2016) already have examined, the critical thermal maximum (CT_{max}; the temperature individuals can stand for a short period without damage) of *P. gallicus* is about 47.6°C. With this in mind the results of this thesis proved that *P. gallicus* is able to maintain a body temperature below their CT_{max} even during the hottest times of the day. In *P. biglumis* the CT_{max} is not yet determined but it is believed to be similar to that of *P. dominula*, which would be 47.1°C (Kovac et al. 2016). With this assumption it can be said that *P. biglumis* is capable of keeping their body temperature below their CT_{max} just like *P. gallicus* even under strong insolation.
Still it has to be mentioned that, although *P. biglumis* did not reach temperatures as high as *P. gallicus*, *P. biglumis* was exposed to a stronger variability in temperatures in total. The body temperature of *P. biglumis* varied between 5°C to 35°C (Figure 16) whereas *P. gallicus* showed temperatures from 20°C to 45°C (Figure 17). However, these measurements represent only the variability of temperatures during a few warm summer days. Currently running measurements of environmental temperatures at different nesting sites will show whether this is also true for a whole breeding season (Stabentheiner and Kovac, personal communication). Even though *P. gallicus* showed a higher body temperature, as well as it was exposed to a generally higher T_a (nest), the measured brood temperature stayed just the same as in *P. biglumis*. In both species it rarely exceeded 40°C (Figure 22 and Figure 23). To keep the brood below this limit, *P. gallicus* had to cool the brood considerably (up to 6°C, Figure 21, B) below the surrounding air temperature during the hottest times of a day (Figure 22 and Figure 23). These findings are remarkable, as it proves that both species are capable of maintaining the perfect temperature for the development of their brood although they face considerably differing macroclimatic and microclimatic conditions throughout a year. Since the temperature of the brood stays constant but very high, it is believed to be the upper limit of the normal (medium-term) thermal tolerance of the brood. It is still an open question to be investigated how close to the CT_{max} of the brood the observed maximum brood temperature of about 40°C is. Nevertheless, keeping the brood close to their maximum tolerable temperature allows a fast development of the brood through high metabolism.
Interestingly, the adults were not always capable of keeping their body temperature below 40°, although they were able to do so for the brood. This may reflect a lower thermal

tolerance of the brood, and leads to the suggestion that the upcoming of the brood is so valuable to the adults that they even may take the risk of overheating.

Conclusion

Both species do face different challenges concerning their surrounding temperature. Nesting site selection is the most important measure to counteract these challenges. While *Polistes gallicus* from the Mediterranean climate prefers protection from sunshine, *P. biglumis* from Alpine (Montane) climate seeks the direct sunshine. In this context it was observed that *P. biglumis* build their nests on stone. This way heating radiation stored during daytime prevents the nest temperatures from going too low at night. However, both have strategies to cool their nests if it heats up too much. These strategies are fanning of air across the nest for convective cooling, and spreading of water drops for evaporative cooling. The relative importance of cooling by fanning or with water drops is adapted to the climatic condition at their nesting site. *P. biglumis*, which always builds nests exposed to the sun, shows both behaviours but intense fanning is more important than cooling with drops. *P. gallicus*, however, which mostly (but not exclusively) prefers nesting sites without direct insolation, prefers cooling with water drops. It uses intense fanning only when during warm times solar radiation threatens the nest to be heated to near lethal temperatures. A most remarkable finding is that both species keep their brood around the same temperature and below about 40 °C even during the hottest times of the day, proving that they found the most effective balancing of strategies for cooling in their specific habitat.

List of Figures

List of References

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Appendix

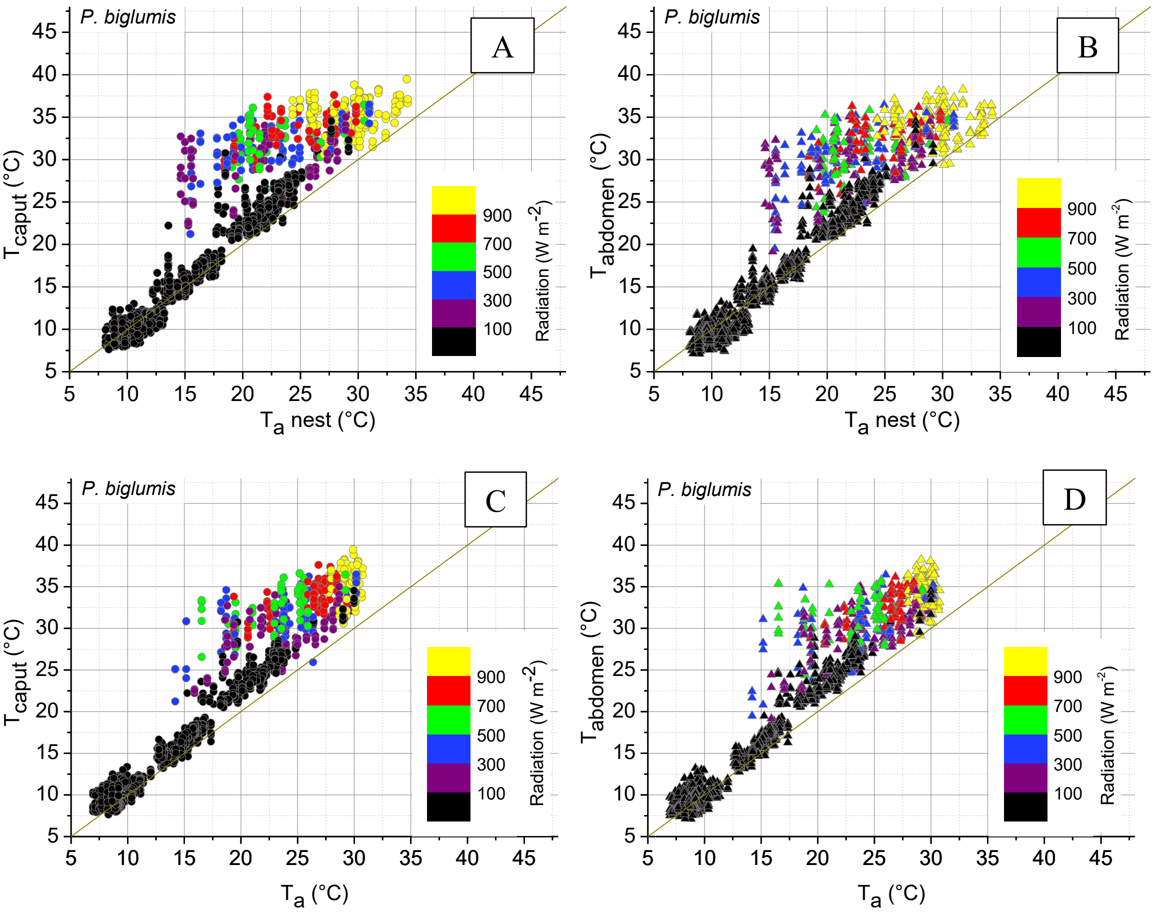

Figure 43 – Surface temperature of the caput (A, C) and abdomen (B, D) of adults of* P. biglumis *measured at the nest in dependence on ambient air temperature close to the nest (T_a nest) (A, B) and ambient temperature in shade a few meters away (T_a) (B, D).

T_{thorax} = mean temperature of the thorax of all measured adult individuals; radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest; diagonal line = Isoline: Tth = Ta nest (A, C) / Tth = Ta (B, D)

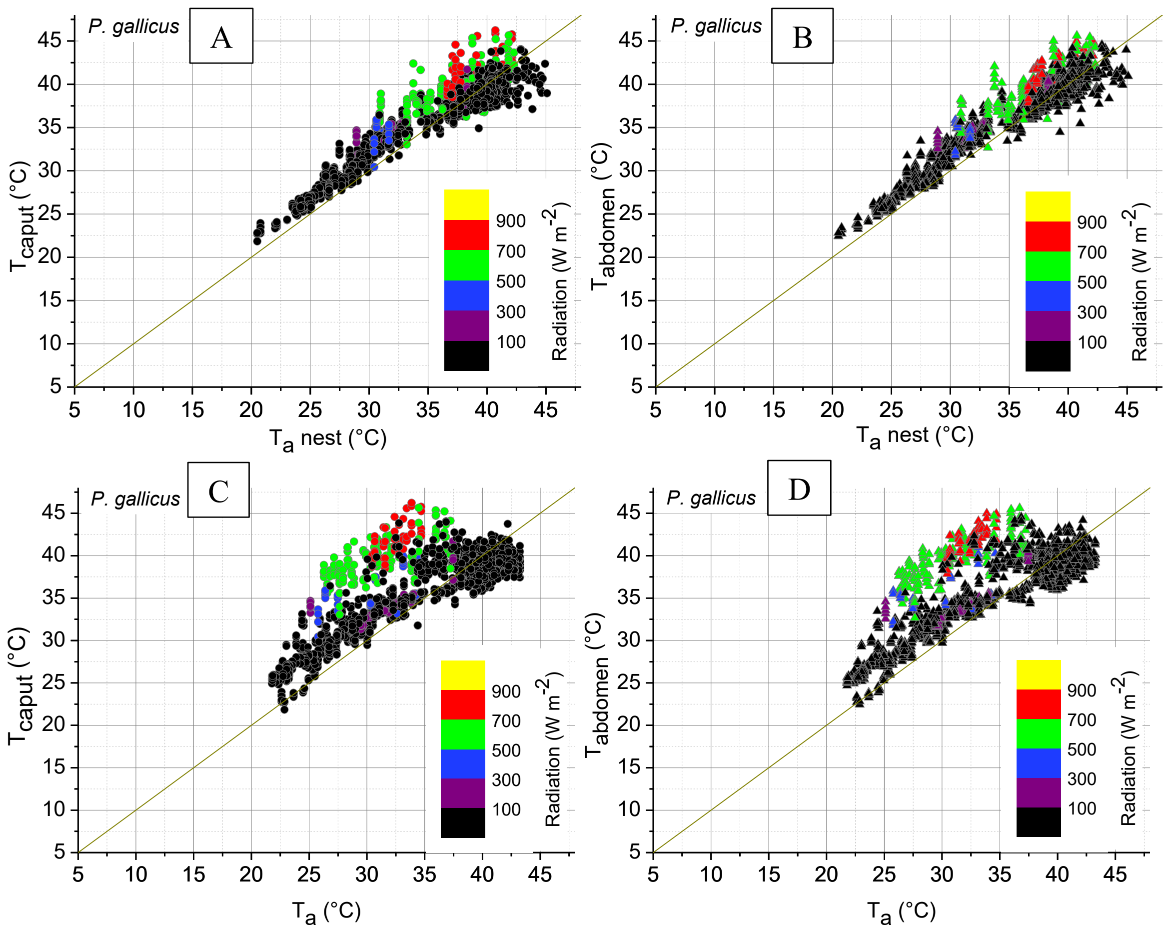

Figure 44 – ***Surface temperature of the caput (A, C) and abdomen (B, D) of adults of* P. biglumis *measured at the nest in dependence on ambient air temperature close to the nest (T_a nest) (A, B) and ambient temperature in shade a few meters away (T_a) (B, D).***

T_{thorax} = mean temperature of the thorax of all measured adult individuals; radiation = global radiation that hits the nest (sunrays); T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest; diagonal line = Isoline: Tth = Ta nest (A, C) / Tth = Ta (B, D)

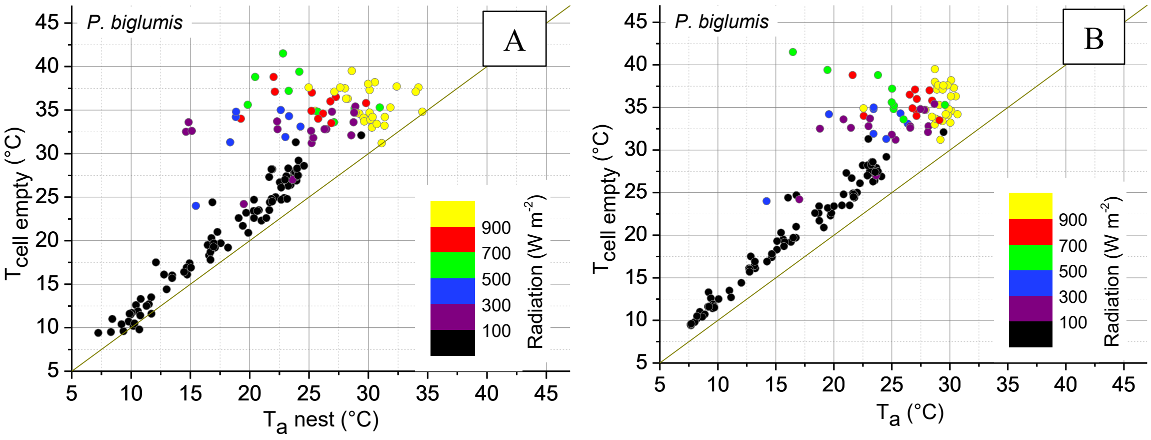

Figure 45 – Surface temperature of the empty cells of* P. biglumis *measured at the nest in dependence on ambient temperature close to the nest (T_a nest) (A) and some meters away (T_a) (B).
$T_{cell\ empty}$ = temperature of all measured empty cells; T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest; diagonal lines = Isoline: temperature of y-axis = x-axis.

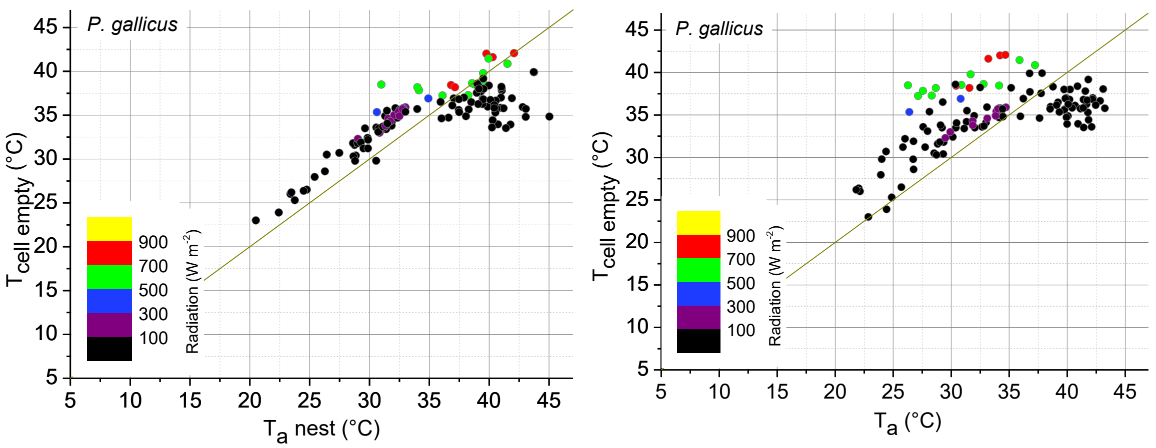

Figure 46 – Surface temperature of the empty cells of* P. gallicus *measured at the nest in dependence on ambient temperature close to the nest (T_a nest) (A) and some meters away (T_a) (B).
$T_{cell\ empty}$ = temperature of all measured empty cells; T_a = ambient temperature 1-3m away from nest; T_a nest = ambient temperature directly beside the nest; diagonal lines = Isoline: temperature of y-axis = x-axis.

Cell changes during fanning event

As the wasps were very active during a fanning event, the measured cells had to be changed sometimes. These modifications can be seen in Table 5 (page 71, Appendix). The shortcuts to the name of the moved tools are found in Table 2 (page 20).

Table 5 - Changed tools during fanning event

Nest/Fanner	Moved tool(s)	Time	Comment
W4/1	/	12:55:44.632	Start of fanning event
W4/1	W3z	12:55:46.299	
W4/1	W4r	12:55:47.299	
W4/1	W3r/z, W4r/z	12:55:47.632	
W4/1	W4r/z	12:55:48.632	
W4/1	W4r/z	12:55:49.299	
W4/1	W4r	12:55:49.632	
W4/1	W4r/z	12:55:53.666	
W4/1	W3r/z, W4r/z	12:55:55.301	
W4/1	W3r/z	12:55:55.699	
W4/1	W3r/z	12:55:56.033	
W4/1	W4r/z	12:55:57.033	
W4/1	W4r/z	12:55:57.366	
W4/1	/	12:55:57.699	Individual turns slightly
W4/1	/	12:55:58.933	End of fanning event
W4/1	W2r	12:55:59.599	
W4/2	WW	12:56:41.764	Individual is looking for starting position
W4/2	W4z/r, W1z/r, WW	12:56:42.098	
W4/2	W1r/z, WW	12:56:42.764	Start of fanning event
W4/2	W1r/z	12:56:42.764	
W4/2	W4r/z	12:56:43.464	
W4/2	W4r/z	12:56:46.131	
W4/2	W4r/z	12:56:47.131	
W4/2	W4r/z	12:56:47.465	
W4/2	/	12:56:53.800	Individual turns and stops fanning
W4/2	W1r/z, W2r/z, WW	12:56:54.332	Individual moved strongly
W4/2	W1r/z, W2r/z, WW	12:56:55.232	Individual moved strongly
W4/3	WW	12:57:02.065	Individual moved
W4/3	W4r/z, WW	12:57:02.399	Individual moved
W4/3	/	12:57:02.732	Start of fanning event

W4/3	W1r/z, W2r/z, WW	12:57:07.464	Individual turned
W4/3	W1r/z	12:57:07.798	Individual turned
W4/3	/	12:57:10:465	Individual turned slightly
W4/3	WW	12:57:10.799	Individual turned
W4/3	WW	12:57:11.465	
W4/3	/	12:57:12.799	Individual moved
W4/3	/	12:57:13.465	Individual moved
W4/3	W1r/z, W2r/z, WW	12:57:13.832	Individual turned
W4/3	W2r/z, WW	12:57:14.166	End of fanning event
W4/3	WW	12:57:14.399	
W4/3	WW	12:57:14.532	
W5/1	/	07:51:39.041	Start of fanning event
W5/1	W4r	07:51:42.940	
W5/1	W4r/z	07:51:44.607	
W5/1	W1r/z, W2r/z	07:52:07.040	End of fanning event
W5/1	W1r/z, WW	07:05:05.840	Individual moved strongly; other individual started as soon as W5/1 stopped with fanning event!
W5/2	WW	07:53:29.307	Individual turned
W5/2	WW	07:52:29.907	Start of fanning event
W5/2	/	07:52:35.575	Individual stopped shortly
W5/2	WW	07:53:00.174	End of fanning event
W5/2	W4r/z	07:53:01.541	
W5/2	W4r/z	07:53:01.874	
W5/3	W1r/z, WW	08:11:32.708	
W5/3	W2r/t, WW	08:11:33.675	Start of fanning event
W5/3	WW	08:11:56.442	
W5/3	W1r/z, W2r/z, WW	08:11:56.976	End of fanning event; individual moved
W5/3	W1r/z, W2r/z, WW	08:11:57.608	Individual moved
W5/3	W1r/z, WW	08:11:57.942	Individual moved