# Critical thermal maximum of three life stages of the paper wasp *Polistes dominula* Christ, 1791.

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**Zusammenfassung:** Thermische Grenzen, welche die lebensfähige thermische Nische wechselwarmer Organismen definieren, werden verwendet, um auf das Verbreitungsgebiet der Tiere zu schließen und Modelle für die aktuelle und zukünftige Ausbreitung unter sich ändernden Umweltbedingungen zu erstellen. Adulte *Polistes dominula* Christ, 1791 (Hymenoptera: Aculeata: Vespidae: Polistinae) sind ab ca. 18 – 20 °C aktiv und bemühen sich, die maximale Nest- (und damit Brut-) Temperatur unter etwa 42 °C zu halten.
Wir untersuchten das kritische thermische Maximum ($CT_{max}$) einer *P. dominula*-Population in Gschwendt (Steiermark, Österreich, Mitteleuropa) von drei Entwicklungsstadien (Larve, Puppe, Adult). Als Maß für das $CT_{max}$ wurde der Verlust der kontrollierten Atmung ermittelt. Einzelne Individuen wurden in eine Messkammer gesetzt und ihre $CO_2$-Abgabe mittels Durchfluss-Respirometrie gemessen, während die Temperatur von 25 °C auf 55 °C (Steigung 0,25 °C pro Minute) erhöht wurde. Die charakteristischen $CO_2$-Kurven wurden zur Bestimmung des Stillstands der kontrollierten Spirakelaktivität ausgewertet.
Larven, Puppen und Erwachsene zeigten unterschiedliche Muster in der $CO_2$-Freisetzung während des Experiments. Larven und Adulte produzierten deutliche $CO_2$-Spitzen und eine ausgeprägte postmortale Spitze; bei den Puppen fehlten während des gesamten Experiments eindeutige Muster, und auch der postmortale Peak war nicht so klar erkennbar. Das $CT_{max}$ unterschied sich signifikant zwischen den Entwicklungsstadien (Larven: 48,8 ± 0,8 °C, Puppen: 47,5 ± 0,6 °C, Erwachsene: 47,4 ± 1,1 °C; Kruskal-Wallis-Test $p < 0{,}01$). Ein paarweiser Vergleich mit Bonferroni-Korrektur zeigte einen signifikanten Unterschied nur zwischen Larven und Erwachsenen ($p < 0{,}05$).
Larven und Puppen fehlen die Möglichkeiten, hohen Umgebungstemperaturen entgegenzuwirken. Ihre Abhängigkeit von den thermoregulatorischen Maßnahmen der Adulten (z. B. Eintragen von Wassertröpfchen, Kühlung durch Fächeln), die möglicherweise nicht immer rechtzeitig erfolgen oder vollständig wirksam sind, könnte ihre erhöhte Hitzetoleranz erklären. Veränderte Umweltbedingungen wie allgemein erhöhte Umgebungstemperaturen, aber auch extreme Hitzewellen können eine Herausforderung für diese Wespenart darstellen.

**Abstract:** Thermal limits defining the viable thermal niche of ectothermic organisms are utilized to infer the animals' distribution area and to create models for current and future dispersal under changing environmental conditions. *Polistes dominula* Christ, 1791 (Hymenoptera: Aculeata: Vespidae: Polistinae) adults are active from about 18 – 20 °C up and strive to keep the maximum nest (and therefore brood-) temperature below about 42 °C.
We examined the critical thermal maximum ($CT_{max}$) of a *P. dominula* population in Gschwendt (Styria, Austria, Central Europe) at three stages of its life cycle (larva, pupa, adult). As a metric for the $CT_{max}$ the loss of controlled respiration was used. Single individuals were placed in a measuring chamber and their $CO_2$ emission measured by flow through respirometry while driving a temperature ramp from 25 °C to 55 °C at 0.25 °C per minute. The characteristic $CO_2$ curves were evaluated for the cease in spiracular activity.
$CO_2$ release patterns of larvae, pupae, and adults during the experimental procedure differed. Larvae and adults showed $CO_2$ spikes and a pronounced post-mortal peak; pupae lacked distinct patterns during the entire experiment, and also the post-mortal peak was not so clearly detectable. $CT_{max}$ differed significantly between life stages (larvae: 48.8 ± 0.8 °C, pupae: 47.5 ± 0.6 °C, adults: 47.4 ± 1.1 °C; Kruskal-Wallis-test $p<0.01$). Pairwise comparison with Bonferroni correction indicated a significant difference only between larvae and adults ($p < 0.05$).

Larvae and pupae mostly lack the possibilities to counteract high ambient temperatures. Their dependence on thermoregulatory measures of adult wasps (e.g., water droplets, evaporative cooling via fanning) that may not always be timely or fully effective could explain their increased heat tolerance. Changing environmental conditions like generally increased ambient temperature, and also extreme heat waves could pose a challenge for these paper wasps.

**Keywords:** *Polistes*, critical thermal maximum, thermal limits, thermal niche, respiration, behavior

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## Introduction

Thermal limits are routinely used to define the boundaries of the viable thermal niche of ectotherms. These data are utilized to infer biogeographic distribution and create models for current and future distribution under changing environmental conditions. The paper wasp *Polistes dominula* shows activity from an ambient temperature ($T_a$) of about 18 – 20 °C upwards. The colonies strive to keep the maximum nest (and thus brood) temperature below ~42 °C (Höcherl & al. 2016). Adult paper wasps have a behavioral repertoire to accomplish this: choice of nest site, entry of water droplets, and fanning behavior are most effective. The adults are mobile and, in extreme cases of hardship, will move to areas with less demanding thermal conditions. The brood, on the other hand, relies almost entirely on the adults regarding measures for survival at high $T_a$. Here, we shed light on possible physiological differences in the three life stages of *P. dominula* at their upper thermal limits to reveal an adaption on thermal exposure.

## Material and methods

We determined the $CT_{max}$ of a *P. dominula* population in Gschwendt (Styria, Austria, Central Europe) of three development stages during its life cycle; as (late) larvae, pupae, and adults. Single individuals were placed in a 3 ml respiratory measurement chamber submerged into a temperature-controlled water bath (Julabo F33HT), and their $CO_2$ emission was measured via flow through respirometry (ABB URAS14) while driving a temperature ramp from 25 °C to 55 °C at 0.25 °C per minute. Temperature was recorded at one second intervals via a data logger (Ahlborn ALMEMO 2890-9). Our measure for $CT_{max}$ was the cease of spiracular activity, i.e., the onset of respiratory failure. The respiratory $CO_2$ curves are characteristic for this type of heat stress and were evaluated via summation of the absolute changes in $CO_2$ output (ADS) and the subsequent regression line through the ADS curve (Lighton & Turner 2004). The point of inflection in the ADS is more clearly definable via the maximum ADS residual (distance of ADS to regression line). The $ADS_{residual}$ was used to determine the individuals‘ respiratory $CT_{max}$. For details in experimental setup and data evaluation see Käfer et al. (2012).

## Results

Larvae, pupae, and adults differed in their $CO_2$ release patterns. While larvae and adults showed $CO_2$ spikes during controlled respiration and a pronounced post-mortal peak, pupae lacked distinct patterns during the entire experiment, and the post-mortal peak was less clearly delimited (Figure 1). However, the change in $CO_2$ release patterns as a measure for the $CT_{max}$ was always detectable. Larvae had a higher $CT_{max}$ of 48.8 ± 0.8 °C than pupae (47.5 ± 0.6 °C) and adults (47.4 ± 1.1 °C). A Kruskal-Wallis-test showed a significant effect of life stage on $CT_{max}$ ($P < 0.01$). However, pairwise comparison with Bonferroni correction indicated a significant difference only between larvae and adults ($P < 0.05$).

| *P. dominula* | $CT_{max}$ | SD | max | min | N |
|---|---|---|---|---|---|
| larva | 48.8 | ±0.8 | 49.8 | 47.2 | 9 |
| pupa | 47.5 | ±0.6 | 48.3 | 46.9 | 4 |
| adult | 47.4 | ±1.1 | 49.6 | 45.6 | 20 |

**Table 1** Critical thermal maxima ($CT_{max}$) in *P. dominula* larvae, pupae and adults. All data in °C except number of individuals tested (N). Larvae differ significantly from adults ($p < 0.05$).

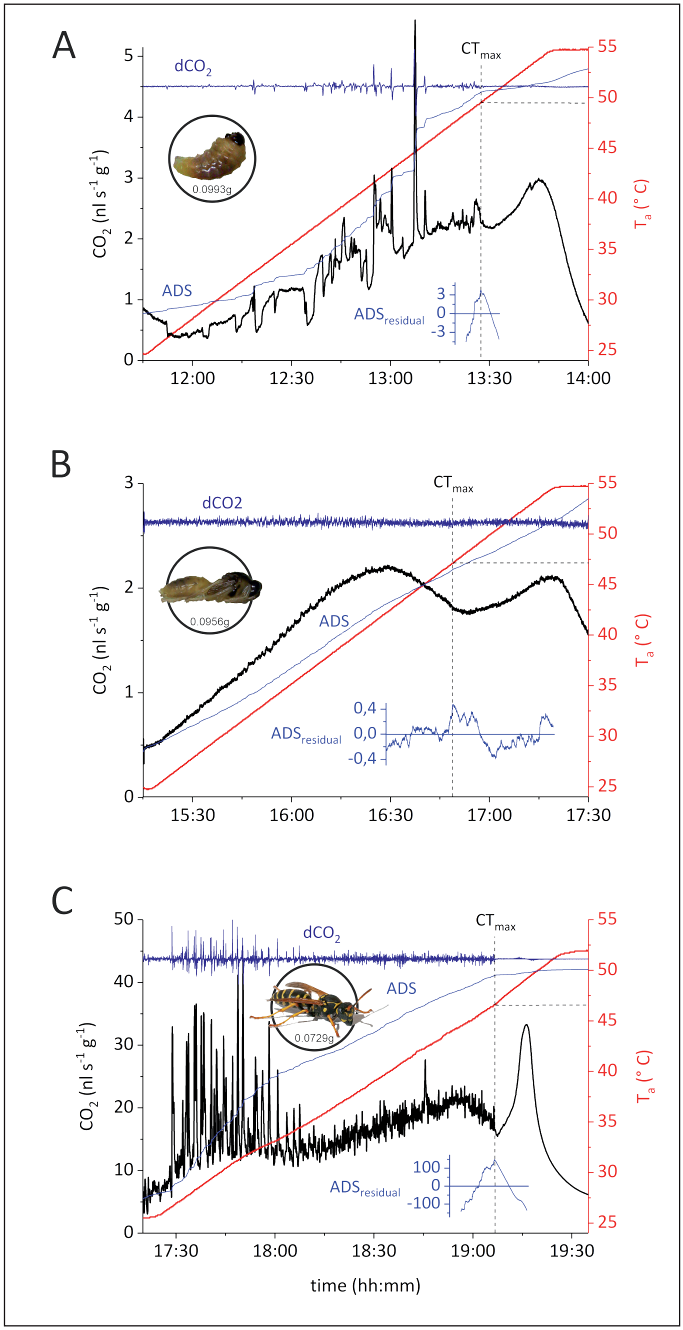


**Figure 1** Thermolimit experiments in a larva (A), a pupa (B), and an adult individual (C) of *Polistes dominula*. Respiratory $CT_{max}$ was defined as the point at which controlled respiration (i.e., $CO_2$ emission) ceased. The analytical method used to determine this point was the highest ADS residual ($ADS_{residual}$), derived from the absolute difference sum (ADS) via linear regression. The differential of the $CO_2$ data ($dCO_2$) is also shown as another way to indicate the end of spiracle activity.

**Discussion**

Larvae, pupae, and adults displayed different responses in their $CO_2$ release patterns during a defined heat stress experiment, which is also known from other holometabolic insect species (Vorhees & Bradley 2012; Bawa & al. 2021). Adults have the ability of behavioral measures to cope with high temperatures, among which fanning, evaporative cooling (see e.g. Stabentheiner & al. 2022), and ultimately moving to an area with a more favorable $T_a$ are the most effective. Larvae and pupae lack these opportunities. They depend on the interventions of adults, which may not always be timely or fully effective. The larvae's limited ability to respond to detrimental high temperatures could explain their need for increased heat tolerance. Why the similarly exposed pupae show a respiratory response to heat stress, but no significant difference to adults in $CT_{max}$ still needs to be clarified. Our results show that changing environmental conditions with increased ambient temperatures and extreme temperature events (i.e., heat waves) may pose a challenge for the survival of these paper wasps at their current breeding (nest) sites and could change their distribution area.

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