

Novel hatching cue in the neotropical damselfly *Megaloprepus caerulatus*: larval adaptation and maternal constraint

Arjèn E. van't Hof ^{a,b} & Ola M. Fincke ^{c*}

^a Department of Biology, University of Groningen, 9700 Groningen, The Netherlands

^b Biology Centre of the Czech Academy of Sciences, Institute of Entomology,
370 05 České Budějovice, Czech Republic; Email: arjenvant.hof@entu.cas.cz

^c Department of Biology, University of Oklahoma, Norman OK, USA

* Corresponding author: Email: fincke@ou.edu

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All relevant data not within the paper are available upon request.

Abstract. The evolution of sibling cannibalism as a maternal strategy is particularly challenging to explain when nurseries are shared among multiple females. Such is the case for the damselfly, *Megaloprepus caerulatus*, whose females lay eggs in bark above the water line in large, water-filled tree holes. Asynchronous egg hatching appears to be a maternal bet-hedging strategy to increase the chances that cannibalistic offspring hatch during windows of opportunity, which occur after the remaining large larvae emerge, having eaten all others. We investigated the proximate causes of asynchronous hatching. By monitoring the pattern of egg hatching under ambient temperature in an insectary, we found that egg hatching co-occurred with lower ambient temperatures, which decreased with increasing rainfall. Treating fully developed eggs to a lower temperature for two hours triggered increased hatching relative to controls at ambient temperature. Dissection of control clutches indicated that embryonic development of siblings was asynchronous. Results suggested that the hatching trigger is adaptive. Rainfall assures a recharge of the larval habitat with water and provides wet conditions essential for neonate mobility on bark. Only 40% of neonates in a 4-day drying treatment survived; none survived the 8- and 14-day treatments. This novel hatching trigger should increase the number of neonates entering the nursery after rains, constraining a mother's control over the timing of egg hatch, while increasing the competition among related and unrelated offspring for limited windows of opportunity in the shared nursery.

Key words. Dragonfly, cannibalism, embryonic development, maternal effect, phytotelmata, sibling cannibalism

Introduction

Sibling cannibalism most commonly occurs in species whose females oviposit in discrete patches such as nests, host plants or prey species (e.g., in vertebrates, Fitzgerald & Whoriskey, 1992; Kam et al., 1998; Stanback & König, 1992; in invertebrates, Baur, 1993; Kudo & Nakahira, 2004; Liebig et al., 2005; Osawa, 2002). Because mothers that minimize sibling cannibalism can increase their fitness (e.g., Iida, 2003; Mukai et al., 2017), determining any maternal selective advantage of such cannibalism can be challenging (Hamilton, 1964; Schausberger & Hoffman, 2008). Adaptive sibling cannibalism or siblicide is most apparent when maternal behavior promotes such behavior, as in ladybird beetles (e.g., Michaud & Grant, 2004) and some birds (e.g., Mock & Parker, 1997; Polis, 1981). When multiple females share the nursery, the adaptive advantage of sibling cannibalism becomes more difficult to explain. Then, cannibalism by unrelated individuals dilutes the advantage of sibling cannibalism. For example, when multiple female burying beetles are recruited to the same carcass, earlier hatching can give unrelated larvae an advantage (Eggert & Müller, 2000). Additionally, external, abiotic factors that trigger

egg hatching, as is commonly found in aquatic insects (e.g., Elliott, 1995; Livdahl, 1982), should further erode maternal control over hatching.

An extreme case of a shared nursery occurs in the neotropical helicopter damselfly, *Megaloprepus caerulatus* (Drury, 1782), whose females lay eggs in water-filled tree holes, where their larvae are top predators (Fincke, 1992a,b). These reproductive resources form in burls or branch break offs of upright, living trees or in fallen trees whose indentations in the bark fill with water. Mosquito larvae are the most common food in these nurseries (Fincke et al., 1997) but larger prey are preferred, and cannibalism is the main source of larval mortality (Fincke, 1994). Large holes of a liter or more in volume can produce several cohorts of a few adults over a wet season and are commonly controlled by territorial males that sequentially control access to these reproductive resources (Fincke, 1992a). Females come to defended holes to mate and lay their eggs, which they insert into moist tree hole bark about a cm above the water level (Fig. 1a–c). Note that before egg-laying, females and even males when inspecting a territory, often put their abdomens in the water as if testing for its presence. In contrast, Young (1980) assumed such females were laying eggs, and mistook as female a male *M. caerulatus* that exhibited the same behavior (Young, 1981). Newly hatched larvae, hereafter termed neonates, are confronted with variable conditions that neither they nor territorial males or egg-laying females can predict. Estimates of parental fitness under semi-natural conditions indicated that the number of offspring surviving in large holes was not correlated with the number of eggs laid by a female nor the number fertilized by a male (Fincke & Hadrys, 2001). Rather, the best predictor of parental fitness was the degree of hatching asynchrony, defined as the span of days over which eggs in a clutch hatched. Thus, asynchronous hatching in *M. caerulatus* is considered a maternal bet-hedging strategy to optimize offspring survivorship. After consuming the smaller larvae, the few remaining large larvae leave the nursery to emerge. Their absence creates a temporary window of opportunity during which time neonates entering the nursery have a chance of surviving to emergence. Such windows vary unpredictably across time, dependent as they are on the timing of previous ovipositions (Fincke & Hadrys, 2001) and the developmental rate of larvae (Fincke, 1992b). Somewhat analogous situations occur in more common aquatic systems with size-mediated priority effects (e.g., Raczyński et al., 2022; Sniegula et al., 2013).

Here, we investigate the heretofore unknown proximal causes of hatching asynchrony in *M. caerulatus* and its consequences for maternal egg-laying strategies. Previous work (Fincke, 2011; Fincke & Hadrys, 2001) revealed that eggs kept in an outdoor insectary often hatched after a heavy rainfall (O.M. Fincke, personal observation). Because those eggs were kept submerged in water, moistening by rain could not have been the proximate hatching cue. However, an obvious effect of

rainfall is a decrease in ambient temperature, which we here hypothesize is the proximate cue for egg hatching. We test this hypothesis in two ways: (1) indirectly, by quantifying hatching events along with the corresponding ambient temperature, and (2) directly, by experimentally lowering ambient temperature of embryos in the final stage of development.

To understand how a possible abiotic, proximal hatching stimulus interacts with patterns of embryonic development that are likely under female control as maternal effects (e.g., Bernardo, 1996; Mousseau

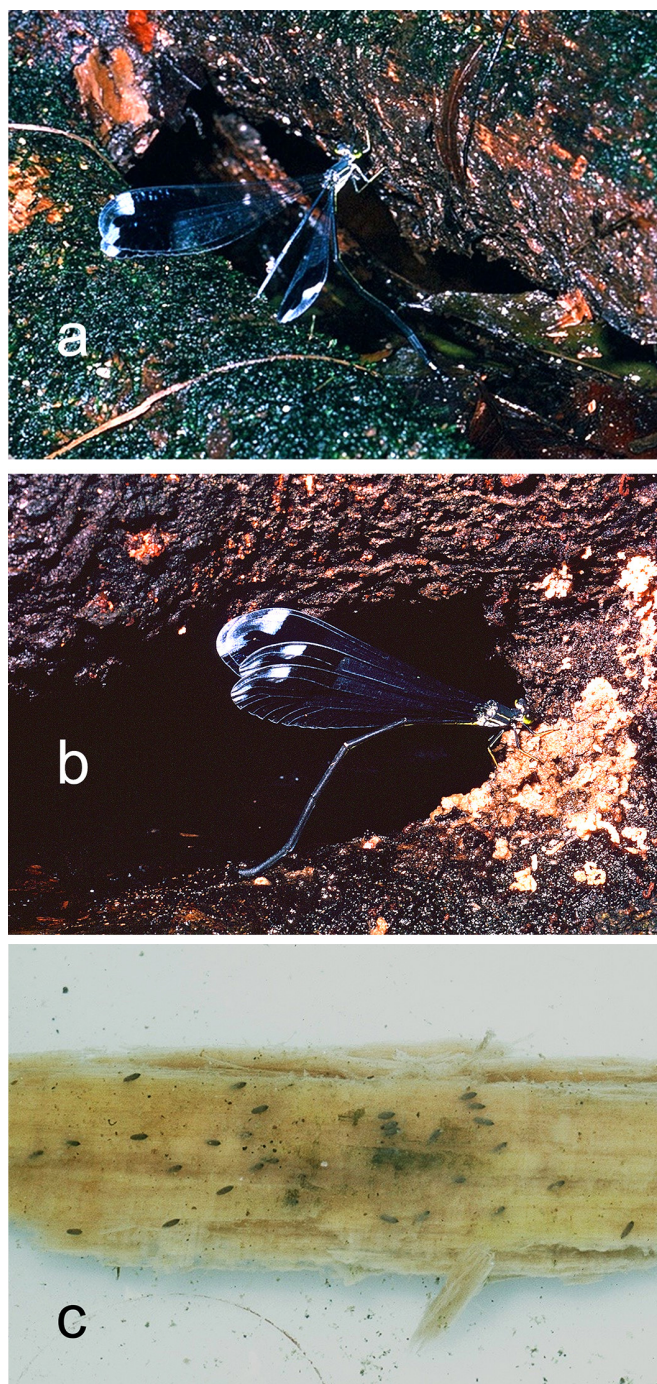


Figure 1. Before a female *Megaloprepus caerulatus* oviposits into the bark of a water-filled tree hole she typically: (a) first inserts her abdomen into the water before (b) laying her eggs about 2 cm above the water line, during which she inserts her ovipositor into the bark, expelling eggs (c), one at a time.

& Fox, 1999), we ask whether asynchronous hatching within a clutch is caused by asynchronous development of embryos that hatch upon completing development (Fig. 2a) or whether embryos develop synchronously, followed by variation in response to hatching stimuli (Fig. 2b). Finally, to determine the adaptive value of the hatching cue, we determine how long neonates can survive while moist, and test the hypothesis that wet bark increases the probability that a neonate reaches the water-filled nursery before dying. Because the number of neonates that compete for a given window of opportunity should depend on the number of developed eggs present along with any abiotic conditions that trigger the hatching eggs, we discuss the implications of the novel hatching trigger we found for the maternal strategy of egg-laying in this shared nursery.

Materials and methods

The research was conducted in the lowland tropical moist forest of Barro Colorado Island (hereafter, BCI),

which is located in Gatun Lake in the Panama Canal, part of Panama's Sbrania National Park. BCI's climate is characterized by a wet season from May to December with an average of 294 mm rain per month and 88.4 mm during dry season months, January–April. The incidence of daily rains typically begins to decrease in December, becoming lowest between January and April. The lowest maximum temperatures are in November, rising steadily over dry season until April. (Windsor, 1990). Whereas most of the current research was done from July–November 1999, we included unpublished relevant data generated from the same standardized methods in earlier years as noted.

Determining the pattern of egg hatching under ambient conditions

To quantify the effect of natural temperature variation on hatching patterns, we collected *M. caerulatus* females at territories where males defended large artificial tree holes (6–8 l. in volume, see Fincke, 1992a for details). We caught females that had not yet oviposited

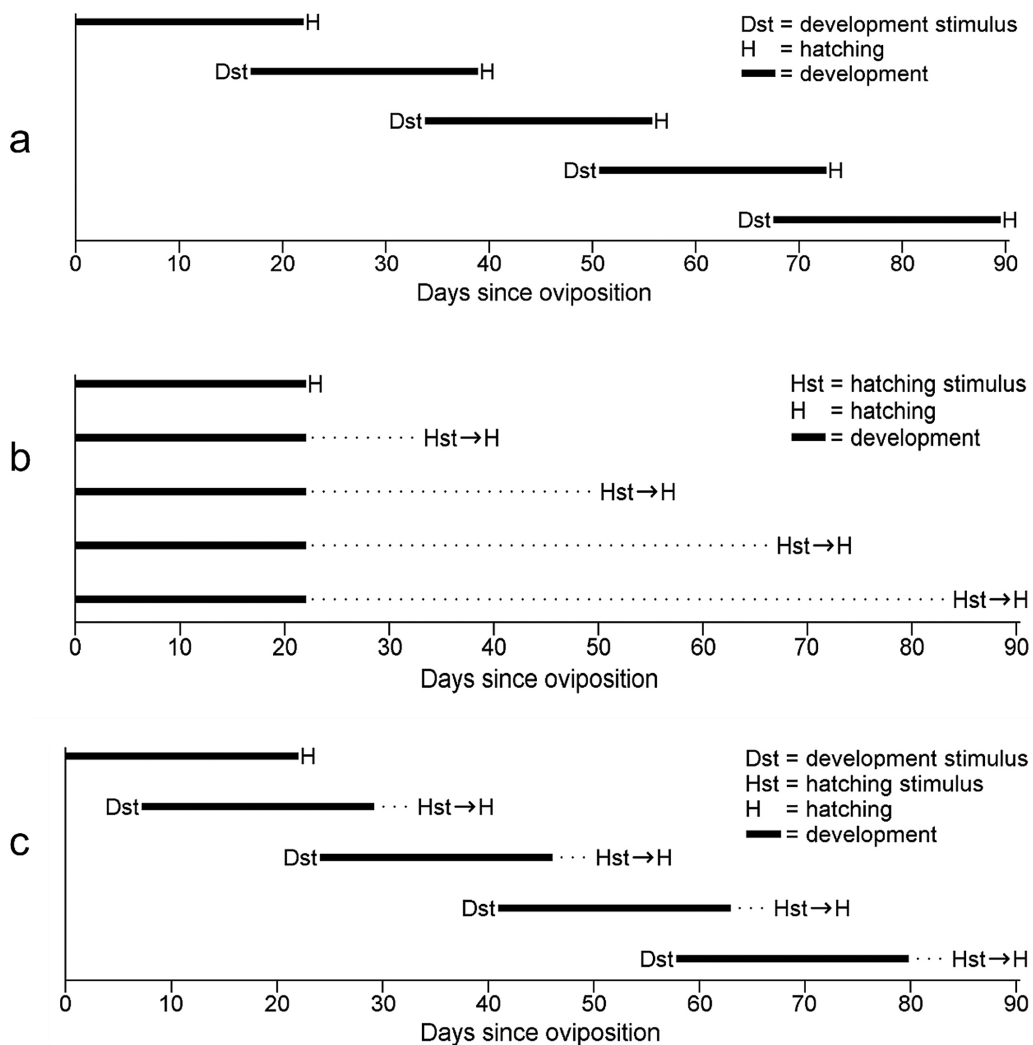


Figure 2. Hypothesized embryonic development for *Megaloprepus caerulatus* that could explain the observed, asynchronous hatching patterns: (a) staggered embryonic development after which eggs hatch when embryos are fully developed; (b) direct development of embryos after eggs are laid, but hatching is triggered by abiotic stimulus; (c) combination of staggered embryonic development coupled with a hatching stimulus that influences the hatching time of hatch-ready embryos.

at a site and individually marked them with a number on the fore wing and put each individually in a small cage with a moistened coffee filter as an oviposition substrate, on which had been noted female ID and date. Several hours later, we put the filter paper with eggs in its own plastic jar with 250 ml water and placed it in an outdoor insectary shielded from direct sunlight. Beginning 14–17 days after the oviposition date, typically each morning, but at times after 2–3 days, we checked jars for neonates, which were removed. We recorded the day of first hatch within each clutch and the number of neonates appearing on each day, typically for 60–67 days. In 1999, the clutches followed were manipulated as described below. Thus, to quantify the general pattern of egg hatch at the same study site, we used unpublished data on 61 unmanipulated egg clutches, each from a unique female, which had at least 75% hatching success collected via the above method during 1994–1998 (Fincke, 2011; Fincke & Hadrys, 2001). To quantify the extreme tail end of the hatching distribution, from July 1997 through February 1998, egg clutches were checked for neonates over a span of 185 days.

Tests of hatching trigger hypothesis

To test the probability that low natural temperatures were accompanied by rainfall, we measured daily minimum temperature from June–October, 1999 in the insectary with a $\pm 0.5^\circ\text{C}$ accuracy thermometer, which stored the extreme temperature values until it was reset daily between 8 am and 10 am. Temperature data earlier than 1999, and all rainfall data, monitored hourly and daily, respectively, are those recorded from the BCI weather station, about 150 m from the insectary. We accessed those data electronically.

We indirectly tested the hypothesis that lower ambient temperatures increase natural hatching using 19 clutches that were laid in the insectary between 9 October, 1996 and 15 February, 1997 and that had at least 75% hatching success. To be sure of the actual hatch day, we defined “solitary hatching days” as those on which eggs in a clutch hatched after at least four days of inactivity. We tested whether the mean minimum temperature on the days of solitary hatching differed from the mean minimum temperature of the overall period between the October and February dates.

We tested the temperature hatching stimulus directly using egg batches for testing that we removed randomly from clutches, keeping the remainder of the egg clutches in the insectary under ambient temperature to use as controls. We exposed a test batch to a decreased temperature of $22.3 \pm 0.5^\circ\text{C}$ for two hours, the temperature range that corresponded with the coldest days outdoors. Those eggs were not pre-selected for the final embryonic developmental stage, which was not visible through the chorion and could only be determined after dissection or inferred from hatching. However, the eggs were all at least 20 days old to assure that hatch-ready embryos were present. Furthermore, for analy-

sis we used only trials with at least five treatment and seven control eggs. Eggs that did not hatch after two hours of cold exposure were dissected to determine their embryonic stage. For subsequent cold treatment replicates we used the undisturbed control eggs from unique clutches that had a sufficient number of mature eggs remaining.

The number of fully developed embryos in a cold treatment batch is the number of hatched eggs plus the number of dissected, final-stage eggs (stage 6, fully developed; see below). We used the empirically established proportions of fully developed eggs in the treatment groups to predict the number of fully developed eggs in the control groups. Finally, we tested the null hypothesis that a temperature drop has no effect on hatching using Chi-Square based on hatched treatment eggs, unhatched but fully developed treatment eggs, hatched control eggs, and the expected number of fully developed control eggs, calculated using the known treatment variables.

Additionally, if a temperature decrease caused by rainfall is a proximal trigger for hatching, we expected that relative to the hatching spans in wet season, those in early dry season should be shorter, decreasing as the incidence of rains decreases, but before adults become non-reproductive in March. Using 61 clutches, each produced by one of 61 unique females (1995–1998 data), we compared hatching span and egg clutch size between wet season (May–November) and early dry season (December–February) with a generalized linear model (Genmod) in SAS v. 9.1 (SAS Institute Inc., Cary NC, USA) with a Poisson distribution.

Testing patterns of embryonic development

We opportunistically determined the timing of embryonic development within a given clutch across time using the eggs that did not hatch during the experimental cold treatments described above. We were unable to follow development within the first 10 days after oviposition because the vitelline envelopes of such eggs were so weak that they broke from the slightest contact, releasing a jelly-like substance. Thus, for older eggs, we removed the exo- and endochorion of the egg using dissecting needles and observed the embryo under a 40 \times magnification dissecting microscope, assigning it to one of six embryonic stages (after Siva-Jothy et al., 1995):

- 1: no development, egg transparent yellow
- 2: small embryo visible, no segmentation
- 3: embryo occupies $\frac{1}{3}$ – $\frac{1}{2}$ egg-space, beginning of segmentation
- 4: beginning of eyespot formation
- 5: medium eyespot visible
- 6: large eyespot / fully developed / final stage.

To test whether embryonic development was synchronized or asynchronous, we calculated the number of the above stages seen within the clutch of each unique female on each observation date. We assumed

that if embryo development within clutches was synchronized, there would be at most only a few developmental stages represented per check. In contrast, unsynchronized embryonic development should reveal persistent, multiple stages represented per check, resulting in a positive correlation between the number of development stages and numbers of checks for a given clutch.

Tests of drying on neonate survival and mobility

To examine the ability of neonates to withstand drying, three sets of 10, 0–1-day old larvae were individually placed in containers between two layers of six leaves that had just been soaked for 30 minutes and then drained. These were then placed in an outdoor insectary under natural photoperiod and temperature and shielded from rain. We scored the number of surviving larvae after 4, 8, and 14 days. Using the same design as that used for larger larvae in an earlier study (Fincke, 1994), we dried any 1-day-old larvae that were still alive after a check and repositioned them under leaves for further drying. This was not feasible with 0-day old neonates because larvae of that size can only be found after soaking the leaves in water, thereby disrupting the continuity of the drying process. Note that the above larvae became older but remained in their original developmental stage since they could not eat or molt.

To test the hypothesis that neonates are capable of reaching the water level in the absence of rain, we partly submerged a piece of bark by standing it in a tub of water for a week, under normal air humidity conditions but protected from rain and direct sunlight such that an undisturbed, vertical moisture gradient formed by upward capillary movement and evaporation. To measure the effect of this moisture gradient on mobility, we dried neonates on a coffee filter and positioned them individually on the piece of bark at 1, 2, 2.5, 3, 3.5 or 4 cm above the water level, using five replicates for each height. Height above two cm simulated cases when, between oviposition and hatching, tree hole volume is reduced, such as occurs with evaporation between rains, mammals drinking from the hole, and similar disturbances (e.g., De La Rosa & Ramirez, 1995), increasing the distance the neonate must traverse to reach the nursery. We measured the time required for a neonate to reach the water level from a given height, ending each run after a maximum of 15 minutes. Additionally, we explored how bark conditions, such as occurs during or just after a rain, affect the ability of neonates to reach the nursery. Using a 40× magnification dissecting microscope, we noted the mobility of neonates on: (1) dry, unsubmerged bark with no water added, (2) moist bark, made by pouring water on the bark and allowing it to dry briefly, and, (3) wet bark, made by pouring water on the bark immediately before releasing the neonate. Neonates used for this work were only seconds old, having just been hatched in the temperature-lowering experiment described above.

Unless specified otherwise, statistical tests were done in R, version 1.0 (R Core Team, 2000). Throughout, t-tests are two-tailed unless noted and means are presented with \pm s.e.

Results

Pattern of egg hatching under ambient conditions

For pooled clutches with at least 75% hatching success, the percentage of eggs that hatched (Y) per day after first hatch (X) under ambient temperature in the insectarium decreased exponentially as described by $Y = 9.2495 \times e^{-0.1060X}$, $p < 0.0001$ (Fig. 3). Day of first hatch varied between 14 and 30 days after oviposition, on average 20.69 ± 3.6 days post-oviposition. After the day of first hatch, on average, 10.06% of the remaining eggs hatched daily. Of the 61 total clutches, 36 (59%) were checked for 180 days. Six of these had hatching spans of over 90 days. The greatest span was 146 days; maximum hatching span in the total sample was 181 days but in a clutch with only 70% hatching (this was excluded from Fig. 3). Hatching span was not correlated with clutch size measured as eggs or neonates; for both, $r = 0.050$ ($p = 0.70$, $n = 61$).

Tests of hatching trigger and seasonal effects

There was a negative relationship between amount of daily rainfall (X) and temperature (Y) (linear regression $Y = -0.0080X + 24.17$, $p = 0.002$, Fig. 4). Similarly, the slope during the solitary hatching study below was -0.014 , $p < 0.05$.

The minimum temperature on the 30 solitary hatching days when neonates emerged after at least four days of hatching inactivity within a clutch (mean = $23.86 \pm 0.15^\circ\text{C}$; range, 22.4 – 25.6°C), was significantly lower than the minimum temperature over the entire 130-day span (mean = $24.13 \pm 0.00^\circ\text{C}$, one-tailed test, $t = 1.826$, $p < 0.05$).

In the temperature reduction experiment, only a single egg hatched in the control group, whereas 24 eggs hatched in the much smaller treatment group (Table 1). Adjusting the numbers to represent only fully developed embryos indicated that 24 out of 88 eggs hatched after cold treatments whereas only one out of the calculated expectancy of 588.5 control individuals hatched. Assuming that $1/588.5 = 0.0017$ (0.17%) was the normal hatching rate during these 2-hour experiment windows gives a Chi-Square p -value of < 0.0001 . When considering all eggs, including those that were not fully developed, the Chi-Square p -value remains < 0.0001 based on 24 out of 188 treatment hatchings and 1 out of 1276 control hatchings. Both calculations rejected the null hypothesis that temperature has no effect on egg hatch. Eggs hatched between 40 minutes and two hours after the end of the cold stimulus, whereas none of the control eggs hatched before day 21. Note that although a total of 132 eggs hatched dur-

ing the cold treatment, dissection of the unhatched control eggs revealed that not all were in the final development stage, thus reducing the hatched cold treatment eggs that could be used for analysis to 24.

In early dry season (December–February), the span over which eggs in a clutch hatched (mean = 41.3 ± 6.98 days, n = 17) was shorter than the hatching span in wet season (May–November, mean = 58.7 ± 4.90 days, n = 44; Wald $\chi^2 = 66.75$, $p < 0.001$). There was no difference in clutch size between early dry season (mean = 90.47 ±

1.0 eggs) and wet season (mean = 117.89 ± 14.11 eggs, Wald $\chi^2 = 0.20$, $p = 0.65$).

Embryonic development

There was a positive correlation between the check number (i.e., first, second, etc.) and the number of developmental stages present within eggs in a clutch on a given check ($r = 0.67$, $n = 16$, $p = 0.005$, Fig. 5). Whereas some embryos appeared synchronized up to stage 4,

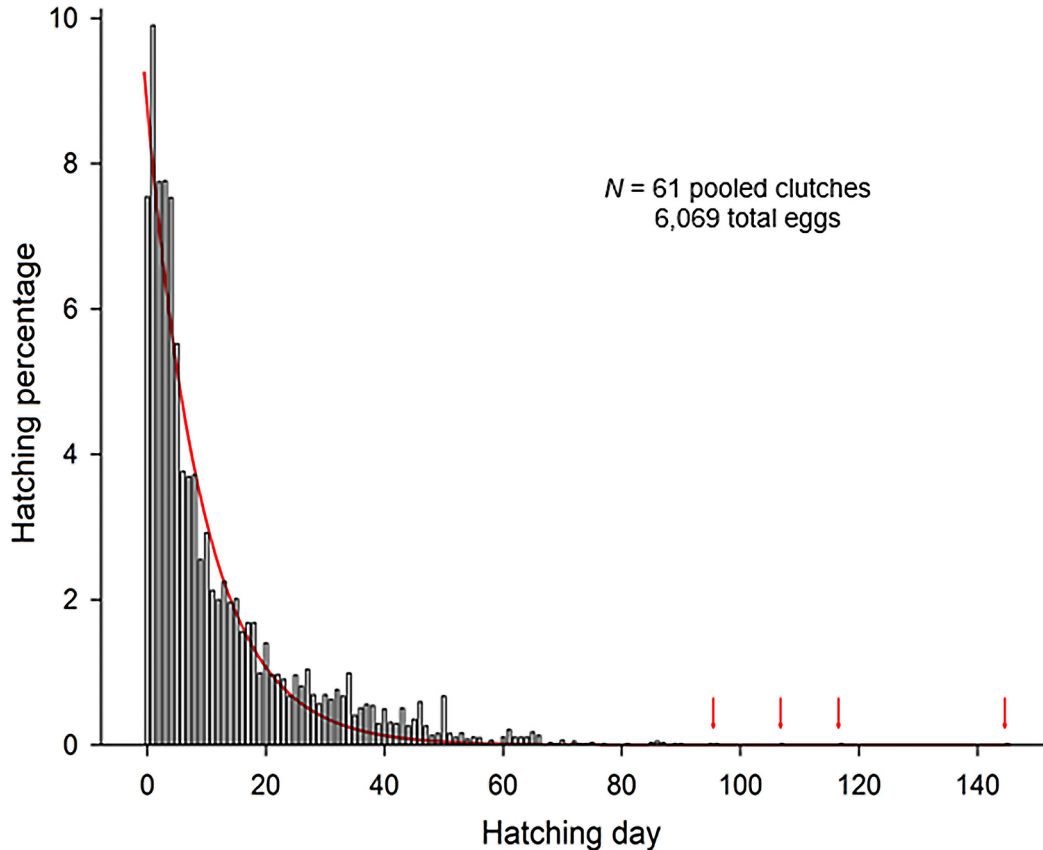


Figure 3. Hatching asynchrony of pooled, *Megaloprepus caerulatus* clutches with ≥ 75% hatching success, each laid by a unique female between May and February (1995–1998). Arrows indicate days of extreme hatches.

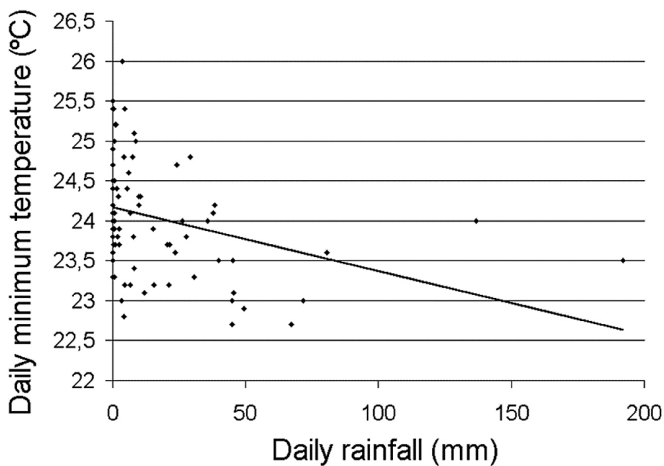


Figure 4. The relationship between rainfall and daily minimum temperature in the outdoor insectary between July 22 and October 31, 1999.

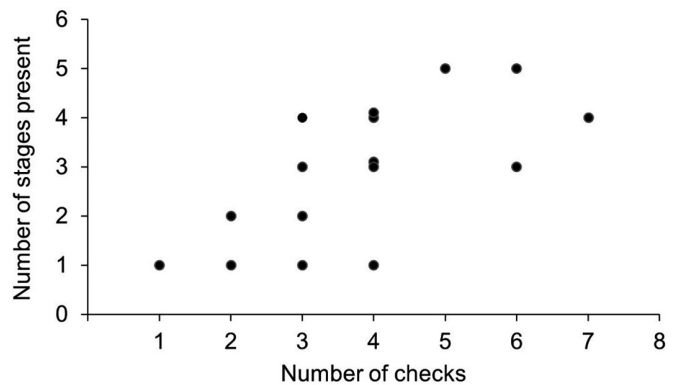


Figure 5. Maximum embryonic stages detected by dissection of unhatched control eggs on opportunistic checks of 16 *Megaloprepus caerulatus* clutches used in the lowered temperature experiment. Excluded were two clutches that had no embryos on any check because females likely lacked sperm.

Table 1. *Megaloprepus caerulatus* egg hatch of fully developed eggs during cold treatment versus controls. Clutch IDs correspond to ID number of egg-laying females. Egg age is days since oviposition. Cold treatment columns: Total = eggs exposed to 2-hour cold treatment; Hatched = number of cold-treated eggs that hatched; Unhatched fully dev. = unhatched eggs with fully developed embryos; Fully dev. total = sum of the two previous columns. Proportion fully dev. is 'fully dev. total' divided by 'total exposed eggs' (i.e., proportion of eggs that were fully developed at the time of the experiment, used to calculate the expected fully developed embryos in the control group). Control columns: Total = number of eggs kept at ambient temperature; Hatched (ambient) = number of control eggs that hatched; Estimated fully dev. controls = expected number of fully developed embryos present based on proportions in treatment groups. The most relevant values are in bold.

Clutch ID	Egg age (days)	Cold treatment					Control (ambient temperature)		
		Total eggs	Hatched eggs	Unhatched fully dev. eggs	Fully dev. total	Proportion fully dev.	Total eggs	Hatched eggs	Estimated fully dev. controls
63	20	10	0	2	2	0.200	134	0	26.800
62	20	13	0	7	7	0.538	181	0	97.462
32	21	7	0	5	5	0.714	163	0	116.429
67	23	5	5	0	5	1.000	7	0	7.000
62	23	9	0	2	2	0.222	136	0	30.222
69	23	12	3	4	7	0.583	80	0	46.667
49	24	5	0	4	4	0.800	24	0	19.200
65	24	6	0	3	3	0.500	98	0	49.000
72	27	13	3	3	6	0.462	57	0	26.308
62	28	16	0	6	6	0.375	87	0	32.625
63	28	33	3	10	13	0.394	94	0	37.030
53	29	11	2	5	7	0.636	84	0	53.455
39	31	5	1	1	2	0.400	9	0	3.600
53	35	14	0	2	2	0.143	54	0	7.714
69	37	5	1	2	3	0.600	9	0	5.400
62	37	14	5	2	7	0.500	18	1	9.000
53	40	5	0	2	2	0.400	34	0	13.600
53	60	5	1	4	5	1.000	7	0	7.000
SUM		188	24	64	88		1276	1	588.5

characterised by faint traces of pigmented ommatidia, others in the same clutch remained undeveloped. The first embryos to appear fully developed was on day 17 post-oviposition. Embryos in some eggs had yet to begin their development by day 53.

During hatching, as viewed under the dissection microscope, embryos appeared to increase their body volume by swallowing fluids. The outer egg layers split open at the side of the micropylar process by a strong increase in size of the elastic vitelline envelope. As soon as this stage was reached, the head, thorax, and part of the abdomen became visible; swallowing movements of the pharynx could be clearly seen. These movements continued until the vitelline membrane burst and the neonate appeared shortly thereafter. The prolarva moult occurred simultaneously with hatching; the first exuvia remained attached to the eggshell.

Effects of drying on neonate survival and mobility

In the drying experiment, only four out of ten neonates survived a drying period of four days. All 1-day old larvae were dead after eight days, with the exception of one that survived 14 days. Maximum lifespan of neo-

Table 2. Time required by *Megaloprepus caerulatus* neonates to reach the water level from different distances above the water line on bark with a vertical moist gradient. n = 5 replicates for each treatment. Dist. = distance from water level; Mean time = mean time (and range) to reach water level.

Dist.	Mean time	Failures
1 cm	0.57 min. (39–83 sec)	
2 cm	4.15 min. (169–424 sec)	
2.5 cm	5.57 min. (246–251 sec)	
3 cm	> 15 min. (> 446 sec)	3/5 never reached water
3.5 cm		5/5 were immobile
4 cm		5/5 were immobile

nates under dry conditions could have varied between five and eight days.

The naturally moist bark above the water line increased neonate mobility in reaching the water (Table 2). The water in the bark had reached a height of 3.5 cm above the water level by capillary dynamics. The maximum distance from which neonates were able

to reach the water surface was 3 cm, when two out of five larvae did not get stuck. On completely dry bark, the legs of neonates stuck to the body. These neonates made body motions that we interpreted as attempts to move towards the larval habitat, but they were immobile, remaining stuck in the same position. On moist bark, neonates moved only peristaltically whereas on wetted bark they walked in a layer of water, accompanied by peristaltic abdominal movements.

Discussion

Our results demonstrated a novel hatching stimulus within the order Odonata. As predicted if a drop in ambient temperature spurs egg hatch, both the lower temperatures on solitary hatching days and the more direct experimental lowering of ambient temperature (Table 1) indicated that embryos that had achieved hatching readiness delayed hatching until triggered by exposure to low temperatures. In his review of hatching cues for Odonata, Corbet (1999) found none that included a decrease in temperature. Known triggers include photoperiod, temperature increase, and water addition (Cham, 1992; Sawchyn & Gillot, 1974; Tennessen & Murray, 1978; Waringer & Humpesch, 1984; Wesenberg-Lund, 1913). Adaptive triggers for hatching appear to be the norm for Odonates. For example, for some dragonflies that lay eggs in riverbanks above the water level, hypoxia, the hatching trigger for eggs, signals that the eggs have become submerged (Miller, 1992; Punzo, 1988). The best-known adaptive trigger in Odonata is higher temperature that terminates egg diapause, primarily in temperate species (reviewed by Corbet, 1999).

Because drying had a severe negative effect on survival of neonates and 1-day old larvae, the hatching trigger for *M. caerulatus* also appears to be adaptive. Temperature decrease acts as a proxy for natural rainfall (Fig. 4). Synchronisation of hatching with rainfall offered two primary benefits for larval survivorship. First, it allows neonates to move to the aquatic habitat. No neonate in our natural bark experiment traversed more than 3 cm before dying (Table 2). Initially, the distance between eggs and water level is relatively small due to the oviposition behaviour of the female, although by the time the egg hatches that distance could be decreased by rainfall or increased by evaporation, coatis and monkeys drinking from the hole, and/or abiotic disturbances. Wetness of the bark, as occurs during rains, is essential for a neonate to reach the treehole because there is no extended prolarval stage such as in lepidoptera that lay eggs in plants far from water (e.g., Jones, Cham, 2021; 1978). Consistent with rainfall indirectly triggering egg hatch via lower temperatures is our finding that hatching spans were significantly shorter in early dry season. Then, fewer rains should result in fewer hatching triggers compared to wet season. Additionally, the higher temperatures of dry season (Windsor, 1990), should shorten development time

of embryos and larvae (e.g., Raczyński et al, 2022). This coupling of dry season effects, independent of any maternal effects, should promote the emergence of offspring before the nursery dries completely. In contrast with the co-occurring *Mecistogaster linearis*, which oviposits in tree holes typically at the end of the wet season, eggs of *Megaloprepus caerulatus* do not survive the dry season, during which nearly all tree holes dry out completely (Fincke, 1992b).

The second benefit of hatching after a rain is increased survivorship, as neonates are much more sensitive to dry conditions compared to larger larvae. The maximum lifespan of neonates in the drying experiment was between four and eight days. In contrast, larvae with a body length of 22.7 mm, which are about two months old, can survive drying under leaves for at least two weeks (mean survival time 23.9 ± 1.3 days, Fincke, 1994). An interesting contrast are the tree-hole-dwelling larvae of the libellulid *Lyriothemis cleis* in southeast Asia; half-grown individuals can survive complete drying in air for four days before being completely revived by water (Orr, 1994).

We found that both synchronous and asynchronous embryo development (Fig. 2c) explains the observed hatching pattern (Fig. 3). Once laid, a majority of eggs exhibited direct development, reaching the final stage of development and typically hatching within 18 to 25 days. This reflects the priority advantage in survivorship of being among the first neonates in the first clutch laid in the nursery after it fills with water, either early in the season or at the creation of a new habitat when a tree falls and its holes fill with water (Fincke & Hadrys, 2001). Deviation from the trend line on days 1–5 of the pooled clutches (Fig. 3) could reflect variation in maternal and seasonal effects, egg temperature or moisture in the bark, and/or degree of temperature drop if that alters the strength of the hatching cue.

As an adaptive maternal response to unpredictable windows of opportunity that occur in the larval habitats, the characteristic asynchrony of embryo development that we documented is likely under maternal control via laying order, egg size (e.g., Schenk & Sönderath, 2005), or other maternal contribution (e.g., vitellogenin, Hagedorn & Kunkel, 1979; Libbrecht et al., 2013). Preliminary data indicated that the large eggs of *M. caerulatus* varied little from the mean size of 1.25 mm, but suggested that moisture condition of the eggs or laying order may affect the initiation of development. Within a clutch, the longest hatching span is found in the final quartile of hatched eggs and is greater than the combined means of the first, second, and third quartile (Fincke & Hadrys, 2001), a result similar to that observed in our sample of pooled eggs (Fig. 3). As in that earlier study, we found no significant correlation between clutch size and hatching span.

In *M. caerulatus*, the abiotic trigger, over which mothers have no control, poses a maternal constraint on an otherwise adaptive bet-hedging strategy. Within the pattern of developmental asynchrony (Figs 2c, 5), the temperature trigger should increase the temporal clus-

tering of both siblings and unrelated neonates, thereby increasing competition among neonates for the unpredictable windows of opportunity. Indeed, such a maternal constraint may explain why females may lay as many as 200 eggs in a large nursery (Fincke, 1992a), when 50 eggs appear sufficient to maximize hatching span (Fincke & Hadrys, 2001) and 100 sufficient for maximizing size and number of surviving offspring of a single mother in a large nursery (Fincke, 2011). Importantly, in the Fincke & Hadrys (2001) experiment, one egg clutch was added to the nursery only once every two weeks, based on the average frequency that females were observed at defended tree holes. However, six females were seen to oviposit over a 4-week span at one defended territory (Fincke, 1992a) and multiple females have been found over the course of a day at very large territories (O.M. Fincke, unpublished data). Hence, clutch size may be more important to female fitness than it was thought to be earlier. To wit, very large clutches may help counter the additional competition for windows of opportunity that results from synchronous hatching of unrelated larvae due to the hatching trigger.

Our observations suggest that the first stage of the hatching mechanism described by Degrange (1974) for Zygoptera generally also applies to *M. caerulatus*. Before hatching, full-grown embryos swallowed water from their environment, after which they burst out of their eggshell, similar to the behavior of coenagrionid species (Degrange, 1961). The relatively opaque eggshell of *M. caerulatus* prevented us from accurately identifying hatch-ready embryos without dissecting the eggs. Thus, the percentage of egg-hatches in the treatment group (Table 1) underestimates the effectiveness of the hatch trigger as inferred from results of natural hatches in our analyses of the solitary hatch days and seasonal trend in hatching spans.

Logistical constraints imposed on our methods included: (1) difficulty in finding sufficient fecund, mature females before they began egg-laying, and (2) time required to check all egg clutches for neonates for longer than 65 days. Thus, we economized on information gleaned by using clutches in the temperature reduction experiment to additionally test between synchronous and asynchronous development, while underestimating maximum hatching spans.

On BCI, four additional odonates (the damselflies *Mecistogaster linearis*, *M. ornata*, and the aeshnid dragonflies, *Triacanthagyna dentata* and, rarely, *Gynacantha membranalis*) share larval habitats with *Megaloprepus* (Fincke, 1998). Here we ignored any effects of those species because the most common larval competitors for *M. caerulatus* in large tree holes are conspecifics (Fincke, 1992b, 1999). The genus *Megaloprepus* includes four distinct clades (Feindt et al., 2014; Selys, 1860), all which have been raised to species status (Feindt & Hadrys, 2022). Because all four species occur in tropical moist or rain forests (Fincke et al., 2018), we predict that the proximal hatching trigger of temperature reduction applies to the genus more generally.

Given that the egg is the least-studied stage of developing Odonata (reviewed by Stoks & Córdoba-Aguilar, 2012), promising future work includes identification of proximal egg variables under maternal control and parental effects more generally (e.g., Fincke, 1992a; Sniegula et al., 2016). Maternal strategies of most odonate genera that inhabit tropical phytotelmata remain unknown. Anthropogenic climate change will likely make their shared nurseries more vulnerable to drying and could potentially affect abiotic hatching cues. Given the critical role of gravid females in dispersal and the impact of their offspring on community structure of phytotelmata (e.g., Kitching, 2000; Petermann et al., 2015; Yanoviak, 2001), these female top predators merit more attention.

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References

- Baur, B. (1993). Intraclutch egg cannibalism by hatchlings of the land snail *Arianta arbustorum*: non-random consumption of eggs. *Ethology, Ecology and Evolution*, 5, 329–336. doi:10.1080/08927014.1993.9523020
- Bernardo, J. (1996). Maternal effects in animal ecology. *American Zoologist*, 36, 403–407. doi:10.1093/icb/36.2.83
- Cham, S. A. (1992). Ovipositing behaviour and observations on the eggs and prolarvae of *Ischnura pumilio* (Charpentier). *Journal of the British Dragonfly Society*, 8, 6–10.
- Cham, S. (2021) Egg hatching, prolarvae, and larval developmental time in *Chalocolestes viridis* (Vander Linden) (Willow Emerald Dragonfly) in Britain. *Journal of the British Dragonfly Society*, 37, 40–59.
- Corbet, P. S. (1999). *Dragonflies: Behaviour and Ecology of Odonata*. Ithaca, N.Y. Comstock Publishing Associates.
- Degrange, C. (1961). L'éclosion des Odonates zygoptères *Agrion puella* (L.) et *Enallagma cyathigerum* (Charp.). *Travaux du Laboratoire d'hydrobiologie et de pisciculture de l'Université de Grenoble*, 52–53, 69–76.
- Degrange, C. (1974). L'oeuf et l'éclosion de *Calopteryx virgo* L. (Odonata, Zygoptera, Calopterygidae). Considérations générales sur l'éclosion des larves des odonates. *Travaux du Laboratoire d'Hydrobiologie et de Pisciculture de l'Université de Grenoble*, 64–65, 269–286.
- De La Rosa, C. & Ramirez, A. (1995) A note on phototactic behavior and on phoretic associations in larvae of *Mecistogaster ornata* Rambur from northern Costa Rica (Zygoptera: Pseudostigmatidae). *Odonatologica*, 24, 219–224.
- Eggert, A.-K. & Müller, J. K. (2000). Timing of oviposition and reproductive skew in cobreeding female burying beetles (*Nicrophorus*

- vespilloides*). *Behavioral Ecology*, 11, 357–366. doi:10.1093/beheco/11.4.357
- Elliott, J. M. (1995). Egg hatching and ecological partitioning in carnivorous stoneflies (Plecoptera). *Comptes rendus de l'Academie des Sciences, Serie III, Sciences de la Vie*, 318, 237–243.
- Feindt, W., Fincke, O. & Hadrys, H. (2014). Still a one species genus? Strong genetic diversification in the world's largest living odonate, the Neotropical damselfly *Megaloprepus caerulatus*. *Conservation Genetics*, 15, 469–481. doi:10.1007/s10592-013-0554-z
- Feindt, W. & Hadrys, H. (2022). The damselfly genus *Megaloprepus* (Odonata: Pseudostigmatidae): Revalidation and delimitation of species-level taxa including the description of one new species. *Zootaxa*, 5115, 487–510. doi:10.11646/zootaxa.5115.4.2
- Fincke, O. M. (1992a). Consequences of larval ecology for territoriality and reproductive success of a Neotropical damselfly. *Ecology*, 73, 449–462. doi: 10.2307/1940752
- Fincke, O. M. (1992b). Interspecific competition for tree holes: consequences for mating systems and coexistence in neotropical damselflies. *The American Naturalist*, 139, 80–101. doi.org/10.1086/285314
- Fincke, O. M. (1994). Population regulation of a tropical damselfly in the larval stage by food limitation, cannibalism, intraguild predation and habitat drying. *Oecologia*, 100, 118–127. doi:10.1007/BF00317138
- Fincke, O. M. (1998). The population ecology of *Megaloprepus caerulatus* and its effect on species assemblages in water-filled tree holes. In J. P. Dempster & I. F. G. McLean (Eds.), *Insect Populations: In theory and in practice* (pp. 391–416). Dordrecht: Kluwer Academic Press. doi:10.1007/978-94-011-4914-3_17
- Fincke, O. M. (1999). Organization of predator assemblages in Neotropical tree holes: effects of abiotic factors and priority. *Ecological Entomology*, 24, 13–23. doi:10.1046/j.1365-2311.1999.00166.x
- Fincke, O. M. (2011). Excess offspring as a maternal strategy and constraints in the shared nursery of a giant damselfly. *Behavioral Ecology*, 22, 543–551. doi:10.1093/beheco/arr015
- Fincke, O. M. & Hadrys, H. (2001). Unpredictable offspring survivorship in the damselfly *Megaloprepus caerulatus* shapes parental strategies, constrains sexual selection, and challenges traditional fitness estimates. *Evolution*, 55, 653–664. doi:10.1554/0014-3820(2001)055[0762:UOSITD]2.0.CO;2
- Fincke, O. M., Xu, M., Khazan, E. S., Wilson, M. & Ware, J. L. (2018). Tests of hypotheses for morphological and genetic divergence in *Megaloprepus* damselflies across Neotropical forests. *Biological Journal of the Linnean Society*, 125, 844–861. doi:10.1093/biolinnean/bly148
- Fincke, O. M., Yanoviak, S. P. & Hanschu, R. D. (1997). Predation by odonates depresses mosquito abundance in water-filled tree holes in Panama. *Oecologia*, 112, 244–253. doi:10.1007/s004420050307
- Fitzgerald, G. J. & Whoriskey, F. G. (1992). Empirical studies of cannibalism in fish. In M. A. Elgar & B. J. Crespi (Eds.), *Cannibalism: Ecology and Evolution among Diverse Taxa* (pp. 238–255). Oxford: Oxford University Press.
- Hagedorn, H. H. & Kunkel, J. G. (1979). Vitellogenin and vitellin in insects. *Annual Review of Entomology*, 24, 475–505. doi:10.1146/annurev.en.24.010179.002355
- Hamilton, W. D. (1964). The genetical evolution of social behaviour I. *Journal of Theoretical Biology*, 7, 1–16. doi.org/10.106/022-5193(64)90038-4.
- Jones, J. C. (1978). A note on the use of the terms instar and stage. *Annals of the Entomological Society of America*, 71, 491–492. doi:10.1093/aesa/71.4.491
- Kam, Y. C., Lin, C. F., Lin, S. & Tsal, Y. F. (1998). Density effects of oophagous tadpoles of *Chirixalus eiffingeri* (Anura: Rhacophoridae): importance of maternal brood care. *Herpetologica*, 54, 435–433.
- Kitching, R. L. (2000). *Food Webs and Container Habitats: The natural history and ecology of phytotelmata*. Cambridge, U.K: Cambridge University Press. doi:10.1017/CBO9780511542107
- Kudo, S. & Nakahira, T. (2004). Effects of trophic eggs on offspring performance and rivalry in a sub-social bug. *Oikos*, 77, 28–35. doi:10.1111/j.0030-1299.2004.13169.x
- Libbrecht, R., Corona, M., Wende, F., Azevedo, D. O., Serrão, J. E. & Keller, L. (2013). Interplay between insulin signaling, juvenile hormone, and vitellogenin regulates maternal effects on polyphenism in ants. *Proceedings of the National Academy of Sciences*, 110, 11050–11055. doi/10.1073/pnas.1221781110
- lida, H. (2003). Small within-clutch variance in spiderling body size as mechanism for avoiding sibling cannibalism in the wolf spider *Pardosa pseudoannulata* (Araneae: Lycosidae). *Population Ecology*, 45, 1–6. doi:10.1007/s10144-002-0133-5
- Liebig, J., Monnin, T. & Turillazzi, S. (2005). Direct assessment of queen quality and lack of worker suppression in a paper wasp. *Proceedings of the Royal Society of London B: Biological Science*, 272, 1339–1344. doi.org/10.1098/rspb.2005.3073.
- Livdahl, T. (1982). Competition within and between hatching cohorts of a treehole mosquito. *Ecology*, 63, 1751–1760. doi:10.2307/1940117
- Michaud, J. P. & Grant, A. K. (2004). Adaptive significance of sibling egg cannibalism in Coccinellidae: comparative evidence from three species. *Annals of the Entomological Society of America*, 97, 710–719. doi:10.1603/0013-8746(2004)097[0710:ASOSEC]2.CO;2
- Miller, P. L. (1992). The effect of oxygen lack on egg hatching in an Indian dragonfly, *Potamarcha congener*. *Physiological Entomology*, 17, 68–72. doi:10.1111/j.1365-3032.1992.tb00991.x
- Mock, D. W. & Parker, G. A. (1997). *The Evolution of Sibling Rivalry*. Oxford: Oxford University Press. doi:10.1093/oso/9780198577430.001.0001
- Mousseau, T. A. & Fox, C. W. (1999). The adaptive significance of maternal effects. *Trends in Ecology and Evolution*, 13, 403–407. doi:10.1016/S0169-5347(98)01472-4
- Mukai, H., Hironaka M., Tojo, S. & Nomakuchi, S. (2018). Maternal hatching synchronization in a subsocial burrower bug mitigates the risk of future sibling cannibalism. *Ecology and Evolution*, 8, 3376–3381. doi.org/10.1002/ece3.3894
- Orr, A. G. (1994). Life histories and ecology of Odonata breeding in phytotelmata in Bornean rainforest. *Odonatologica*, 23, 365–377.
- Osawa, N. (2002). Sex-dependent effects of sibling cannibalism on the life history traits of the ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biological Journal of the Linnean Society of London*, 76, 349–360. doi.org/10.1046/j.1095-8312.2002.00070x.
- Petermann, J. S., Farjalla, V. F., Jocque, M., Kratina, P., MacDonald, A. A. M., Marino, N. A. C., de Omena, P. M., Piccoli, G. C. O., Richardson, B. A., Richardson, M. J., Romero, G. Q., Videla, M. & Srivastava, D. S. (2015). Dominant predators mediate the impact of habitat size on trophic structure in bromeliad invertebrate communities. *Ecology*, 96, 428–439. doi:10.1002/ece3.2797
- Polis, G. A. (1981). The evolution and dynamics of intraspecific predation. *Annual Review of Ecology and Systematics*, 12, 325–251. doi:10.1146/annurev.es.12.110181.001301.
- Punzo, F. (198 [Versio8]). Effects of low environmental pH and temperature on hatching and metabolic rates in embryos of *Anax junius* Drury (Odonata: Aeshnidae) and the role of hypoxia in the

- hatching process. *Comparative Biochemical Physiology (C)*, *91*, 333–336. doi:10.1016/0742-8413(88)90038-2
- R Core Team. (2000). *R: A language and environment for statistical computing (Version 1.0.0)*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Raczyński, M., Stoks, R. & Sniegula, S. (2022). Warming and predation risk only weakly shape size-mediated priority effects in a cannibalistic damselfly. *Scientific Reports*, *12*, 17324. doi.org/10.1038/s41598-022-22110-6.
- Sawchyn, W. W. & Gillot, C. (1974). The life history of *Lestes congener* (Odonata: Zygoptera) on the Canadian Prairies. *Canadian Entomologist*, *106*, 367–376. doi:10.4039/Ent106367-4
- Schausberger, S. & Hoffman, D. (2008). Maternal manipulation of hatching asynchrony limits sibling cannibalism in the predatory mite *Phytoseiulus persimilis*. *Journal of Animal Ecology*, *77*, 1109–1114. doi:10.1111/j.1365-2656.2008.01440x
- Schenk, K. & Sönderath, D. (2005). Influence of egg size differences within egg clutches on larval parameters in nine libellulid species (Odonata). *Ecological Entomology*, *30*, 456–463. doi:10.1111/j.0307-6946.2005.00707.x
- Selys Longchamps, E. de (1860) Synopsis des Agrionines. Première Légion—Pseudostigma. *Bulletins d'Académie royale des Sciences, des Lettres et des Beaux-Arts de Belgique*, *2*, 9–27.
- Siva-Jothy, M. T., Gibbons, D. W. & Pain, D. (1995). Female oviposition-site preference and egg hatching success in the damselfly *Calopteryx splendens xanthosoma*. *Behavioral Ecology and Sociobiology*, *37*, 39–44. doi:10.1007/BF00173897
- Sniegula, S., Golab, M. J., Drobnik, S. M. & Johansson, F. (2016). Seasonal time constraints reduce genetic variation in life-history traits along a latitudinal gradient. *Journal of Animal Ecology*, *85*, 187–198. doi:10.1111/1365-2656.12442.
- Sniegula, S., Golab, M. J., Johansson, F. (2018). Size-mediated priority and temperature effects on intra-cohort competition and cannibalism in a damselfly. *Journal of Animal Ecology*, *88*, 637–648. doi:10.1111/1365-2656.12947.
- Stanback, M. T. & König, W. D. (1992). Cannibalism in birds. In M. A. Elgar & B. J. Crespi (Eds.), *Cannibalism: Ecology and evolution among diverse taxa*. pp. 277–298. Oxford, UK: Oxford University Press.
- Stoks, R. & Córdoba-Aguilar, A. (2012). Evolutionary ecology of Odonata: a complex life cycle perspective. *Annual Review of Entomology*, *57*, 249–265. doi:10.1146/annurev-ento-120710-100557
- Tennessee, K. J. & Murray S. A. (1978). Diel periodicity in hatching of *Epitheca cynosura* (Say) eggs (Anisoptera: Corduliidae). *Odonatologica*, *7*, 59–65.
- Waringer, J. A. & Humpesch U. H. (1984). Embryonic development, larval growth and life cycle of *Coenagrion puella* (Odonata: Zygoptera) from an Austrian pond. *Freshwater Biology*, *14*, 385–399. doi:10.1111/j.1365-2427.1984.tb00162.x
- Wesenberg-Lund, C. (1913). Mitteilungen aus den biologischen Süßwasserlaboratorien Hilleröd u. Lyngby (Dänemark). Nr. XVI. Odonaten-Studien. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, *6*, 155–228. doi:10.1002/iroh.19130060204
- Windsor, D. M. (1990). Climate and moisture variability in a tropical forest: long-term records from Barro Colorado Island, Panamá. *Smithsonian Contributions to the Earth Sciences*, *29*, 1–145. doi:10.5479/si.00810274.29.1
- Yanoviak, S. P. (2001). Predation, resource availability, and community structure in Neotropical water-filled tree holes. *Oecologia*, *126*, 125–183. doi:10.1007/s004420000493
- Young, A. M. (1980). Feeding and oviposition in the giant tropical damselfly *Megaloprepus coerulatus* (Drury) in Costa Rica. *Biotropica*, *12*, 237–239. doi:10.2307/2387986
- Young, A. M. (1981). Notes on the oviposition microhabitat of the giant tropical damselfly *Megaloprepus coerulatus* (Drury) (Zygoptera: Pseudostigmatiade). *Tombo*, *23*, 1–4. doi:10.2307/2387697