Synchronization of Circadian Bioluminescence as a Group-Foraging Strategy in Cave Glowworms

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Synopsis Flies of the genus *Arachnocampa* are sit-and-lure predators that use bioluminescence to attract flying prey to their silk webs. Some species are most common in rainforest habitat and others inhabit both caves and rainforest. We have studied the circadian regulation of bioluminescence in two species: one found in subtropical rainforest with no known cave populations and the other found in temperate rainforest with large populations in limestone caves. The rainforest species is typical of most nocturnal animals in that individuals are entrained by the light:dark (LD) cycle to be active at night; in this case, their propensity to bioluminesce is greatest at night. The dual-habitat species shows an opposite phase response to the same entrainment; its bioluminescence propensity rhythm is entrained by LD exposure to peak during the day. Nevertheless, in LD environments, individuals do not bioluminesce during the day because ambient light inhibits their bioluminescence (negative masking), pushing bioluminescence into the dark period. This unusual and unexpected phenomenon could be related to their association with caves and has been suggested to be an adaptation of the circadian system that promotes synchronization of a colony's output of bioluminescence. Here, we use controlled laboratory experiments to show that individuals do synchronize their bioluminescence rhythms when in visual contact with each other. Entrainment of the bioluminescence rhythm to the biological photophase causes colony-wide synchronization, creating a daily sinusoidal rhythm of the intensity of bioluminescence in the many thousands of individuals making up a colony. This synchronization could provide a group-foraging advantage, allowing the colony to glow most brightly when the prey are most likely to be active.

Introduction

Circadian clocks allow animals to partition physiological and behavioral processes to an appropriate time of day. They have been investigated in detail using model species in controlled environments, but there has been less research on the plasticity of the circadian system in reaction to environmental extremes. Can organisms living in constant environments, such as those found in caves, do away with clock control of metabolism and behavior? Some evidence suggests that behaviors can be adaptively decoupled from clock control in constant environments; for example, under the constant conditions of the high-latitude arctic night or day, reindeer show minimal signs of circadian rhythmicity of locomotory rhythms (van Oort et al. 2007). Using the same reasoning, rhythms are expected to be repressed or eliminated in obligate cave-dwelling organisms due to constant environmental temperatures and a complete lack of daylight (Langecker 2000; Sharma 2003). Surprisingly, there have been few investigations of the circadian behavior of troglobophilic species, i.e., those that thrive both in caves and in epigean environments. They are the ideal target to tell us how organisms accommodate the transition between two extremes, one experiencing light:dark (LD) cycles and the other, constant darkness. Cave representatives of a troglobophilic cave cricket showed little sign of circadian rhythmicity in locomotion but comparison with epigean individuals was not made (Hoennen 2005). Some cave inver-
tebrates (Jegla and Poulson 1968; Koilraj et al. 2000) and vertebrates (Biswa and Ramteke 2008; Cavallari et al. 2011) have been shown to be capable of maintaining rhythmcity, but a reduction in the strength of rhythms associated with the degree of troglobitism is apparent in comparative studies (Trajano and Menn-Baretto 1995, 2000). A number of studies of trogloxenes—organisms such as bats, crickets or harvestmen that leave the cave each night—have shown that the experience of the external light regimen can entrain the behavior of exiting from the cave (Campbell 1976; Gnaspini et al. 2003). In turn, trogloxenes, especially bats, can entrain the rhythms of troglobites through their periodic locomotory activity or fecal deposition (Stringer and Meyer-Rochow 1997; Biswas and Ramteke 2008).

The larvae of the genus *Arachnocampa* both inhabit caves (hypogean) and conventional external (epigean) environments (Meyer-Rochow 2007). They are sessile, terrestrial insects that employ a sit-and-lure predatory strategy based on the emission of bioluminescence (Broadley and Stringer 2001; Willis et al. 2011). In epigean environments, they are found in areas of persistent high humidity, for example, in undergrowth or on embankments in forest-covered habitats, frequently near streams or waterfalls (Richards 1960; Meyer-Rochow 2007). Caves with streams provide high-humidity environments and some species reach very high numbers in caves, to the extent that some populations have become tourist attractions (Pugsley 1984; Baker and Merritt 2003; Merritt and Clarke 2012).

Nine species of *Arachnocampa* have been described (Baker 2010); one species is present in New Zealand, seven species are allopatrically distributed along the eastern coast of Australia and a single species is present on the southern Australian island of Tasmania. This research is concerned with the rainforest-associated southeastern Queensland species, *Arachnocampa flava*, that has no known cave populations, most likely due to the lack of appropriate geological features in its range (Baker et al. 2008), and the Tasmanian species, *Arachnocampa tasmnienisis*, that both has troglobitic and epigean populations (Merritt and Clarke 2011). Larvae and adults of Arachnocampa do not show obvious troglomorphic traits such as loss of eyes or reduction of pigmentation. In fact, the degree of larval pigmentation appears to be a polyphenic response to exposure to light during development because troglobitic individuals are less pigmented than epigean individuals of the same species (Baker 2010).

Larval behavior and snare construction have been well documented (Gatenby 1959; Meyer-Rochow 2007). The worm-like larvae fashion a horizontal mucus tube or runway along which they traverse backward and forward, holding on using anteriorly directed cuticular hooklets located ventrally on the abdominal segments (Gatenby 1960). The mucus tube is suspended from the substrate (rock, earth, or logs) with silk guy lines. Larvae spend a considerable portion of their time maintaining fishing lines—silk lines dotted with mucus droplets hung from the guy lines. Flying insects, disoriented or attracted to the light, are caught in the snares. As they struggle, the larva moves toward the prey, draws up the line with the prey attached and then covers it with more silk and mucus, and begins feeding (Stringer 1967; Meyer-Rochow 2007). Both in caves and forest, larvae tend to be concentrated in colonies rather than dispersed. However, within colonies, they are regularly spaced because they interact aggressively with one another, sometimes resulting in cannibalism (Meyer-Rochow 2007).

Light is produced in enlarged cells at the distal tips of the malpighian tubules (Wheeler and Williams 1915), an adaptation of the tubules that is unique to this insect genus. The light-producing cells lie within a slightly swollen, transparent structure at the end of the terminal segment. The large, mitochondria-rich cells have been studied at the ultrastructural level (Green 1979) and their innervation has been investigated (Rigby and Merritt 2011). They emit blue-green light (Lee 1976) that is diffused through a reflector made up of a mass of trachea. Emission of light comes under neural regulation (Gatenby 1959; Rigby and Merritt 2011). Experimental data indicate that bioluminescence is actively repressed when the insect is in the doused state (no expression of bioluminescence) and the repression is modulated at night and determines light intensity. Exposure to external light is a key stimulus that represses bioluminescence (Richards 1960). The dimming response is relatively slow, larvae taking about a minute to turn off completely and then, after a period, they begin to glow again. In forest environments, bioluminescence is initiated soon after dusk, reaches a peak a few hours later and dims through the night (Merritt and Aotani 2008; D. J. Merritt, unpublished data).
Because bioluminescence effectively attracts prey only under dark conditions and the period of darkness experienced by epigean and hypogean larvae differs, the circadian regulation of the bioluminescence is a subject of interest. To some authors, the dimming response to ambient light suggested circadian regulation to be unlikely because the dimming reflex would suffice to ensure that bioluminescence occurs only at night (Gatenby 1959; Stringer 1967); however, the first rigorous observations using timelapse photography showed that the southeastern Queensland species, _A. flava_, does indeed show circadian regulation (Merritt and Aotani 2008). Larvae placed in constant darkness and temperature showed a sustained sine wave-like pattern of bioluminescence. Individuals showed idiosyncratic free-running periods, mostly greater than 24h. The rhythm does not persist beyond several weeks in constant darkness (DD), becoming damped until larvae glow more or less continuously. As expected, light entrains this rhythm. When larvae were exposed to artificial LD cycles for several days before being returned to DD, the phase of the underlying bioluminescence propensity rhythm (BPR) was entrained, thereby promoting peak bioluminescence during the subjective dark period (scotophase). In addition, the amplitude of the rhythm was restored by exposure to light-dark conditions, perhaps reflecting the need to replace essential bioluminescence-related metabolites during the doused period (Willis et al. 2011).

What happens in caves where larvae are exposed to constant darkness? One would expect that, in the absence of entrainment cues from LD cycles, larval bioluminescence would free-run in the absence of light entrainment and in the presence of stable temperatures. Therefore, individual larvae might be expected to bioluminesce continuously at low levels because in _A. flava_ in DD in the laboratory, the amplitude of the daily cycle progressively damped over time (Merritt and Aotani 2008), or perhaps they would continue to cycle but out of phase with one another. In fact, cave larvae of the dual-habitat species, _A. tasmaniensis_ (there are no troglobitic colonies of _A. flava_ to allow a comparison between cave and forest populations of the species), were found to maintain high-amplitude, highly synchronized 24-h rhythms of bioluminescence (Merritt and Clarke 2011). A difficult-to-explain characteristic of the bioluminescence rhythm was that the rhythm of bioluminescence intensity peaked in the late afternoon. Bats’ activity cannot explain the phase because bats are not present in Tasmanian caves (see Merritt et al. 2012). Populations in different caves showed a similar afternoon acrophase. Looking more closely at different colonies within a single cave, some smaller colonies showed consistent within-group rhythms that were out of phase with the main colony on the ceiling (Merritt and Clarke 2011). In combination, these observations suggested that, within a colony, larvae were entraining to each other in a process of mutual synchronization. Support for this came from exposure of larvae in the dark zone to artificial LD for several days and recording the phase of the colony afterward. Light entrained the BPR of the cave colonies; however, the bioluminescence cycle shifted so that the time of the peak intensity (acrophase) shifted to match the time of exposure to light, despite the light itself causing the larvae to douse (Merritt et al. 2012). We thought that exposure to light might have a dual function, causing larvae of the cave-adapted species _A. tasmaniensis_ to douse and also positively entraining their rhythm.

Laboratory experiments in which larvae were taken from the dark zone of a cave and exposed to artificial point sources emulating larval lights provided further evidence for a capability to synchronize. The larvae did not douse in response to exposure to dim light and steadily shifted phase to match the photophase of the on–off cycle of light exposure (Merritt and Clarke 2011; Merritt et al. 2012). Exposure of the rainforest species, _A. flava_, to the same conditions produced the opposite phase response; they shifted phase to match the scotophase. The conclusion was that the two species have opposite entrainment responses when exposed to external light. The paradoxical entrainment of _A. tasmaniensis_’ BPR to match the photophase was considered to be an adaptation that, first, allowed larvae to adapt to the variable light regime within the twilight zone of caves and second, allowed larvae to synchronize their glowing cycles within the dark zone, perhaps increasing the likelihood of capturing prey (Merritt et al. 2012); however, no direct evidence has yet been obtained to show that detection of bioluminescence released by adjacent larvae is sufficient to entrain and synchronize bioluminescence.

In this study, we compare the two species’ ability to synchronize to conspecific light, using
a simple laboratory-based experimental setup in which three in-phase larvae are exposed to a single larva out of phase with them. We also conduct an in-cave experiment in which we track the time-course of the return to synchronization after light-induced disturbance of the natural bioluminescence cycle.

**Methods**

**Collection of larvae of A. tasmaniensis and A. flava**

Larvae of *A. tasmaniensis* were collected from colonies within the dark zone of Mystery Creek Cave, Southern Tasmania, a natural limestone cave that supports a large population of *A. tasmaniensis* (Driessen 2010). Larvae were collected from colonies within deeper regions of the cave that are infrequently visited by the public. Medium to large larvae (150–300 mm), corresponding to fourth and fifth instars (Merritt and Clarke 2011), were collected and transported to the laboratory in a cool (8–12°C), dark environment. In the laboratory, larvae were housed in individual halved, upturned, polypropylene containers that were clay-roofed to accommodate construction of webs by larvae (Fig. 1). The front of each container was covered with thin transparent plastic sheet to contain the larva. Containers were placed in aquaria filled with a low level of water to maintain a humid microclimate. Larvae of *A. tasmaniensis* were kept in a controlled temperature cabinet at 12°C (±1°C), chosen because it is close to the mean annual temperature of 8.2°C (±0.2°C) (Driessen 2010) in constant darkness. Larvae of *A. flava* were collected from rainforest at Springbrook National Park, Queensland, placed in individual tubes and returned to the laboratory. They were housed at 23°C (±1°C) on a 12:12 LD cycle. Larvae of both species were allowed a minimum of 2 weeks to settle and construct webs before experimentation. During non-experimental periods, larvae were fed one wild-type *Drosophila melanogaster* regularly (every 1–2 weeks) under dim red light.

**Recording bioluminescence**

A Sony XCD-X710 Firewire camera (connected to a computer) was used to record the bioluminescence of larvae. The camera was programmed to take a photograph every 10 min (BTV Pro Carbon v.5.4.1, BenSoftware, London); camera and program settings were similar between experiments. For some experiments, a Canon EOS 1000D digital SLR camera (Canon Australia, Sydney, Australia) was used with 18–50 mm lens at maximum aperture, F3.6, and 30 s exposure at ISO equivalent 1600, programmed to capture an image every 10 min using a PClix (Visual Effects Inc., www.pclix.com) intervalometer. ImageJ (v.1.45s; Rasband 1997–2008) was used to calculate the intensity of each bioluminescing larva by first converting colored images to grayscale, selecting the pixels constituting a single larva’s light and summing the pixel values (each ranging from between the threshold level and 255). Consequently, the units of light intensity are arbitrary and not calibrated against standard sources; however, they are consistent within an experiment.

**Assessing synchronization**

![Fig. 1](https://via.placeholder.com/150)

**Fig. 1** Arrangement of larvae for synchronization experiments. (A) Individual larvae were maintained in transparent plastic-fronted containers. (B) Four containers were arranged in a square so that each larva was in line of sight of the others but was separated from them by transparent plastic sheeting. (C) Containers were placed in a transparent aquarium, and groups of four were visually isolated from other groups by placing an opaque tube around each. Light emitted by larvae was imaged from below.
Larvae of *Arachnocampa tasmaniensis* housed in containers (Fig. 1A) were divided into two treatments and exposed to opposing LD cycles (using an LED lamp on a timer) so that larvae would exhibit divergent phases of their bioluminescence rhythms when placed together in DD. Treatment-1 (T1) larvae (*n* = 22) were exposed to light from 2200 to 0400h and those in treatment 2 (T2) from 1000 to 1600h (*n* = 8). After 4 days of LD, larvae were shifted into DD to monitor their bioluminescence rhythms for 2 days and confirm that the BPR of larvae from each treatment group was centered on the subjective photophase. Larvae from both treatments were then arranged to face each other in a square configuration consisting of three larvae from treatment 1 and one larva from treatment 2 (Fig. 1B). Seven such groups were arranged in an aquarium to maintain high humidity and the remaining larvae from T1 and T2 were placed separately as controls. Each group of four and the control larvae were covered with a light-blocking tube to eliminate the potential influence of other groups’ bioluminescence (Fig. 1C). The camera was located beneath the aquarium, directed upward, and the light intensity of the larvae recorded at 10-min intervals for 18 days. This experiment was repeated for larvae of *A. flava* but under conditions better suited to this species (temperature 23°C ± 1°C). As larvae of *A. flava* entrain to glow during the foregoing scotophase when placed in DD, treatment-1 larvae were exposed to light between 1800 and 0600h and treatment-2 larvae between 0600 and 1800h.

Of the 30 larvae of *A. tasmaniensis* used in this experiment, 7 pupated or permanently ceased glowing during the experimental period, 2 of them prior to the time of synchronization. Of the 30 larvae of *A. flava*, 3 pupated or ceased bioluminescing. The times at which peaks occurred prior to cessation of bioluminescence were included in the analysis as they could have influenced the group’s rhythmicity.

Determination of peaks of bioluminescence

To determine the time of daily peaks of bioluminescence, a low-pass Butterworth filter was applied to the time series of bioluminescence intensity using MATLAB v.7.13 (MathWorks Inc., MA). This filter reduces noise due to variations in light intensity between recording intervals (Levine et al. 2002), allowing calculation of the time of peak bioluminescence at a resolution of 10 min. The same approach was used previously in studies of the rhythmicity of bioluminescence in *Arachnocampa* (Merritt and Clarke 2011; Merritt et al. 2012) and in other analyses of circadian rhythms (Levine et al. 2002). Individuals whose peak values were widely divergent from expected circadian values were regarded as arrhythmic.

Statistical analysis and defining synchronization

Paired two-tailed t-tests were used to assess whether the average daily peak time of bioluminescence of larvae at each position (*n* = 7) was significant for each day. If peak times between larval positions were significantly different (*P* < 0.05), larvae were classified as unsynchronized. However, if peak times were similar (*P* ≥ 0.05) and larvae displayed similar free-running periods, they were classed as synchronized. To control for family-wise error rate in multiple comparisons between the average time of the bioluminescence peak for each larva, P-values derived from the t-tests were adjusted using a Holm algorithm (Holm 1979) in RStudio v.0.96.228 (RStudio, www.rstudio.org). The periodicity of larvae was derived from fitting linear regression models to the peak times of bioluminescence of individuals across days.

Phase-shift and synchronization within a troglobitic population

A beam of LED light was focused into the midst of a large colony of *A. tasmaniensis* on the ceiling of Mystery Creek Cave, Tasmania, and the bioluminescence of the colony recorded using the Canon EOS 1000D digital SLR and PClix intervalometer described above. The light-projection apparatus utilized a 12-V 20 white LED array in a MR16 reflector at one end of a plastic tube, 0.64-m long and 50-mm in inner diameter. Two Fresnel lenses were positioned inside the tube to produce a beam of light with limited divergence. The projector was tripod-mounted and the beam shone onto a portion of the cave’s ceiling 10m above the cave’s floor, producing a focal spot 1.6m in diameter. A 12-V timer was used to expose the focal population to LD 3:21 over 5 days with the photophase centered on 02:00h AEST (on at 00:30h and off at 03:30h). Light intensity was measured at 2 lux in the center of the beam.

In post-processing, the circular spot on the cave’s ceiling that was illuminated by the focal spot of light was selected as a region of interest in each image and an annulus of the same area (in pixels) of the focus spot and concentric with
it was delineated as a second region of interest for analysis (Fig. 3A). A maximum-intensity Z-projection of 47 consecutive images occurring prior to the first exposure to light was analyzed using particle analysis in ImageJ to provide an estimate of the number of individuals within the focal region (78 larvae) and the outer annulus (60 larvae).

**Results**

**Synchronization**

Seven sets of four larvae of *A. tasmaniensis* were arranged in a square, facing each other and given a positional identification, P1–P4 (Fig. 1). One larva of the four (P3) was pre-exposed to a different LD cycle to the other three so that initially the bioluminescence rhythm of this larva was out of phase with the other three. Over the first 2 days of recording, the larvae were visually isolated from one another so that each individual’s pre-set phase could be confirmed; then the four larvae were exposed to each other. As shown previously, under such DD conditions, the intensity of bioluminescence fluctuates in a sine-wave-like fashion at circadian frequencies (Merritt and Clarke 2011). For analysis and display of the results, the acrophase (time of occurrence of the peak) of the bioluminescence cycle was chosen as a reference point to indicate phase. The three larvae (P1, P2, and P4) that were initially in phase remained in phase throughout the 19-day mutual exposure with a periodicity of 424 h (Fig. 2A). Notably, in each replicate, the larva (P3) that initially was out-of-phase phase-advanced until it adopted a periodicity and time of peak that closely matched the other three larvae. All four larvae continue to display a similar average period (P>0.05, Holm adjusted) over the remaining days of the experiment. Two control larvae were monitored during the experiment. One was entrained to the same bioluminescence rhythm as the single larva in each set of four (P3) and the other to the same rhythm as the three in-phase larvae. Each control larva was visually isolated so that it would free-run

![Graph showing time-course of synchronization of bioluminescence in Arachnocampa tasmaniensis (top) and A. flava (bottom).](image)
without any entraining visual stimuli. Both control larvae showed a consistent free-running period of greater than 24 h throughout: 24.58 h for one and 24.78 h for the other (Fig. 2A).

The average daily times of acrophase of larvae P1–P4 were not statistically different from each other from day 6 onwards (P>0.05, Holm adjusted). Visual inspection of the curve indicates that synchronization is achieved around days 8–9 of the experiment, 6 days after the group’s mutual exposure, when the curve of time of peak changed from a negative slope to a positive one (Fig. 2A).

For a cross-species comparison, larvae of *A. flava* were exposed to the same experimental regime. The control larvae showed an ongoing free-running period of greater than 24h for each pre-treatment (24.25 h for one and 24.53 h for the other) (Fig. 2B). In direct contrast to *A. tasmaniensis*, the out-of-phase (P3) larvae of *A. flava* showed no signs of synchronization to the groups of three in-phase larvae (Fig. 2B). Student’s t-tests conducted on the average times of the bioluminescence peaks of larvae for all possible comparisons with position-3 larvae confirmed that they were significantly different (P<0.05, Holm adjusted). The plots of time of acrophase of larvae at each position were linear, indicative of free-running, and the single out-of-phase larva did not show the deviation in period displayed by *A. tasmaniensis* (Fig. 2A versus B). Larvae at positions 1, 2, and 4 showed greater divergence and more variation in their average periodicity when compared with the corresponding larvae of *A. tasmaniensis*. However, the average time of bioluminescence peaks of larvae at these positions was statistically similar throughout (P>0.05, Holm adjusted). The experiment using *A. flava* was terminated after 16 days as larvae still were not showing signs of synchronization.

Phase-shift in situ in a cave

To examine the time-course of synchronization in a natural habitat, a beam of LED light was focused into the midst of a large colony of *A. tasmaniensis* on the ceiling of Mystery Creek Cave, exposing a focal population of 78 larvae to LD 3:21 over 5 days, during which larvae in the spotlight showed a progressive phase shift (Fig. 3B and C). Bioluminescence in the spot-lit zone was compared with that of 60 larvae present within an annulus surrounding the spot-lit area (Fig. 3A), based on the assumption that larvae within the annulus would have received a consistent, but low, intensity of exposure to light.

On days 1 and 2 of observation, prior to exposure to light, the focal population and the comparison population showed a similar acrophase (focal population mean, 17:45h and comparison population mean, 17:45 h; n = 2). After the onset of daily light pulses, the acrophase of the focal population progressively phase-delayed to approach the time at which light-exposure was occurring. By comparison, the acrophase of the
comparison population remained steady (Fig. 3C). Notably, this group showed increased intensity of bioluminescence at the time of exposure over four of the five periods (Fig. 3B). It was observed that all larvae within the center of the beam of light did not cease to glow under light; however, their intensity could not be calculated due to interference from exposure to light. After 5 days of pulsed light, the focal population’s acrophase was measured at 00:40 h and the comparison population at 17:40 h. Over the 9 days after termination of exposure to light, the focal population phase-delayed at greater than 1 h per day so that its acrophase approached that of the comparison population (Fig. 3C). The comparison group showed a degree of phase-advance, especially over days 10–16. Due to the need to terminate fieldwork, recordings were terminated before the acrophase of the exposed colony could coincide with that of the rest of the colony.

Discussion

This is the first specific demonstration that larvae of *A. tasmaniensis* synchronize their bioluminescence rhythms through the detection of light cues from the bioluminescence of surrounding larvae. Experimental exposure of larvae to point sources of light in the laboratory (Merritt et al. 2012) or to LD cycles of artificial light within caves (Merritt and Clarke 2011) suggested a capability for synchronization but, until now, it had not been proven. A feature of both the preceding and current research is that two related species of *Arachnocampa* show different phase shifts in response to entrainment by light. One species is cave adapted; the bioluminescence rhythm is entrained by light to peak during the light-exposure phase (a photophasic BPR) and is capable of synchronization. The other is rainforest adapted, the bioluminescence rhythm is entrained by light to peak during the light-exposure phase (a photophagic BPR) and is capable of synchronization through the detection of cumulative light cues of the surrounding larvae. The phase matching was rapid and consistent across the seven replicates. From these laboratory experiments, it is apparent that, while larvae synchronize, the period of the synchronized group is the average of the free-running periods of the participating larvae.

In the rainforest-adapted species, *A. flava*, no sign of synchronization was evident using the same experimental design. The single out-of-phase larva continued to free-run and the phase of its BPR did not converge toward that of the other three larvae. In addition, the three larvae pre-set to a common phase tended to diverge, whereas in *A. tasmaniensis* their phase remained synchronized. The controls also free-ran. We conclude that the BPR of *A. flava* larvae is not influenced by bioluminescence emitted by nearby larvae, reinforcing the results of experiments in which larvae were exposed to lights emulating other larvae (Merritt et al. 2012).

Under the assumption that the intensity of light would affect the magnitude of the phase-shift—as suggested by in-cave experiments (Merritt and Clarke 2011)—exposure of one larva to the light of three other larvae was used in the expectation that the single larva would shift phase substantially to match that of the other three. We did not see any obvious sign of the three larvae phase-shifting to match phase with the single larva, although this could have occurred. In future, various experimental designs can be used to investigate whether phase change is mutual. Individuals are expected to possess windows of greater responsiveness to biological light, just as animals free-running in DD possess periods of higher and lower responsiveness to entrainment cues within each circadian cycle (Johnson et al. 2003). Construction of a phase–response curve would reveal the degree and direction of phase-shift in relation to the phase at the time of exposure to light.

In a foregoing experiment, the phase of a large troglobitic colony was manipulated by exposing it to periodic light (Merritt and Clarke 2011). In the current experiment, a tightly focused LED beam was trained onto the cave’s ceiling in a circle encompassing approximately 80 larvae. The intent was to induce a phase-shift in this population and to record the hypothesized return to synchronization with the surrounding unexposed larvae, presumably due
to the influence of the larger colony’s bioluminescence cycles on the smaller focal group. The focal population—as a group—phase-delayed until its acrophase approached that of the less-affected, surrounding population. The expectation was that the focal colony eventually would have locked into phase with the remaining population but field-recording was terminated before this took place.

Our preferred explanation for the ongoing phasesshift of the focal population after LD periods is that larvae synchronized to the light of surrounding larvae. This interpretation is in accord with the foregoing laboratory experiments. However, the uncontrolled factors associated with in-cave experimental manipulations mean that synchronization by entrainment to others’ light may not be solely responsible for the phase-shift; it remains possible that an unknown entraining stimulus was present. This possibility is reinforced by the observation that, in the laboratory, synchronized larvae continue to show a period of greater than 24h, whereas larvae from caves show a periodicity close to 24h over many months (D. J. Merritt, unpublished data). One hypothesis put forward in a foregoing study is that biotic interactions could indirectly entrain the BPR within troglobitic populations to a 24-h cycle (Merritt and Clarke 2011). It has been noted that larvae show a brightening response when they detect prey. Daily peaks of prey activity could shift the phase of the troglobitic population’s BPR so that its acrophase matched phase with the time of peak detection of prey. If the activity rhythm of flight of the prey is entrained by the external light regimen—many prey items are assumed to enter the cave through stream-drift rather than undergoing multiple generations in the cave—then the natural photoperiod could indirectly determine the phase of the populations from caves. This synchronization to each other and perhaps to the time of greatest availability of prey could provide a group-foraging advantage, allowing the colony to glow most brightly when the prey are most likely to be active. Another noteworthy point is that the in-cave exposures to light reveal signs that light intensity is competitively regulated; when larvae detect extraneous light they glow more brightly themselves. This is seen in Fig. 3C where the population within the comparison annulus brightens at the time the focal population is being exposed to light, but this does not appear to affect its phase. Similarly, some unexpected peaks in bioluminescence can be related to times of entry of the public or the experimenter into the cave when light from head-lamps seem to have “excited” the larvae, leading to an increase in their intensity of bioluminescence (D. J. Merritt, unpublished data). We noted the presence of clusters within larger colonies—groups within groups—that glow more brightly for longer than do the surrounding larvae (Merritt and Clarke 2011). It is possible that larvae brighten when they see others brighten, perhaps increasing their chances of capturing prey and at the same time mutually influencing the phase of others’ BPRs. This model is highly speculative but it does provide testable hypotheses.

The synchronization of bioluminescence seen in *A. tasmaniensis* is unlike the social synchronization seen in troglobites such as bats. Individuals practice “light sampling” whereby they fly close to the cave’s mouth to detect light intensity and, when it has reached a suitable threshold, communicate the fact to the rest of the population by flying back into the cave (Marimuthu et al. 1978). Invertebrate troglobites such as crickets and harvestmen leave the cave nightly, meaning that dawn or dusk can regularly entrain the activity cycle (Campbell 1976; Gnaspini et al. 2003). This is very different to *Arachnocampa* in which the immobility of larvae makes sampling of the external light regimen impossible. Further, bats are not present in Tasmanian caves (Doran et al. 1997). Consequently, synchronization in *Arachnocampa* occurs through mutual entrainment rather than through regular entrainment by direct transmission of external light cues.

A consequence of the phase relationship between light and bioluminescence in *A. tasmaniensis* is that it produces a paradoxical reaction to solar light. In epigeic environments, solar light entrains the BPR so that its acrophase occurs during daylight; however, bioluminescence is not effective during daylight. In fact, daylight masks bioluminescence, and the drive to bioluminesce appears to be released after dusk, producing nocturnal bioluminescence (Merritt et al. 2012). The divergent rhythmicity of the two closely related species raises questions as to what adaptive advantages the alternative modes may provide. As discussed by Merritt et al. (2012), the differences are probably related to their ecology; *A. tasmaniensis* is associated both with caves and with epigeic habitats while *A. flava* lives exclusively in epigeic environments. The photophasic BPR of *A. tasmaniensis*
is more suited to the variable light conditions that can be experienced in cave ecosystems—from constant darkness to regular solar light cycles—than a scotophasic BPR. The photophasic BPR and the resulting synchronization that populations adopt under regimes of low solar light could have facilitated the habitation of caves as well as of external environments. This circadian response would allow the optimization of their bioluminescence display (and thus attractiveness to phototactic prey) across both hypogean and epigean habitats. Given that caves offer ideal conditions and can provide a refuge against climatic extremes (Baker et al. 2008), it seems likely that cave-dwelling Arachnocampa evolved a circadian system optimized for both cave and rainforest ecosystems. Perhaps A. flava lost, or never gained, an underlying photophasic BPR because a scotophasic BPR response is more suitable for their ecology (Merritt et al. 2012).

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