

Same Temporal Niche, Opposite Rhythmicity: Two Closely Related Bioluminescent Insects With Opposite Bioluminescence Propensity Rhythms

David J. Merritt, Essie M. Rodgers, Ami F. Amir & Arthur K. Clarke

To cite this article: David J. Merritt, Essie M. Rodgers, Ami F. Amir & Arthur K. Clarke (2012) Same Temporal Niche, Opposite Rhythmicity: Two Closely Related Bioluminescent Insects With Opposite Bioluminescence Propensity Rhythms, *Chronobiology International*, 29:10, 1336-1344, DOI: [10.3109/07420528.2012.728549](https://doi.org/10.3109/07420528.2012.728549)

To link to this article: <https://doi.org/10.3109/07420528.2012.728549>



Published online: 06 Nov 2012.



Submit your article to this journal [↗](#)



Article views: 401



View related articles [↗](#)



Citing articles: 7 View citing articles [↗](#)

Same Temporal Niche, Opposite Rhythmicity: Two Closely Related Bioluminescent Insects With Opposite Bioluminescence Propensity Rhythms

David J. Merritt,¹ Essie M. Rodgers,¹ Ami F. Amir,¹ and Arthur K. Clarke²

¹School of Biological Sciences, The University of Queensland, Brisbane, Queensland, Australia, ²School of Zoology, University of Tasmania, Hobart, Tasmania, Australia

Archnocampa species, commonly called glowworms, are flies whose larvae use light to attract prey. Here we compare rhythmicity in two of the nine described species: the Tasmanian species, *Archnocampa tasmaniensis*, which inhabits caves and wet forest, and the eastern Australian mainland species, *A. flava*, primarily found in subtropical rainforest. Both species show the same nocturnal glowing pattern in external (epigeal) environments and the same inhibition of bioluminescence by light and both species show circadian regulation of bioluminescence. We find that the underlying circadian bioluminescence propensity rhythm (BPR) of the two species peaks at opposite phases of the day:night cycle. Larvae of *A. flava*, placed in constant darkness in the laboratory, bioluminesce during the subjective scotophase, typical of nocturnal animals, whereas *A. tasmaniensis* shows the opposite tendency, bioluminescing most intensely during the subjective photophase. In *A. tasmaniensis*, which are exposed to natural day:night cycles, light exposure during the day overrides the high bioluminescence propensity through negative masking and leads to a release of bioluminescence after dusk when the BPR is on the wane. A consequence is that *A. tasmaniensis* is able to start glowing at any phase of the light:dark cycle as soon as masking by light is released, whereas *A. flava* is locked into nocturnal bioluminescence. We suggest that the paradoxical BPR of *A. tasmaniensis* is an adaptation for living in the cave environment. Observations of bioluminescence in colonies of *A. tasmaniensis* located in the transition from a cave mouth to the dark zone show that glowing is inhibited by light exposure but a peak bioluminescence follows immediately after “dusk” at their location. The substantial difference in the circadian regulation of bioluminescence between the two species probably reflects adaptation to the cave (hypogean) habitat in *A. tasmaniensis* and the forest (epigeal) habitat in *A. flava*. (Author correspondence: d.merritt@uq.edu.au)

Keywords: *Archnocampa*, Cave, Circadian pacemaker, Collective behavior, Glowworms, Group foraging, Paradoxical timing, Phylogenetics, Synchronization

INTRODUCTION

For bioluminescent animals, the effectiveness of biological light as a signal is negligible during daylight, so all known terrestrial bioluminescent animals are either nocturnal or crepuscular in their light output. Some firefly species tend to select defined portions of the dusk and early evening for their circadian-regulated mate signaling (Dreisig, 1975; Lall et al., 1980; Lloyd, 1983). Here, we investigate the rhythmicity of bioluminescence in two species of *Archnocampa*, a genus of bioluminescent flies. *Archnocampa tasmaniensis* is found on the island of Tasmania south of the Australian mainland in forest environments, especially in rainforest gullies with an understory of tree ferns, as well as being found in very high numbers in caves (Driessen, 2010), so the species can be regarded as a troglophile (Hamilton-Smith, 1967). *Archnocampa flava*, from southeast

Queensland, is not known from caves, possibly due to a lack of such habitats in its distribution (Baker et al., 2008). Other species in the genus have significant cave populations (Baker, 2010). Cave populations are not cryptic species because mitochondrial DNA (MtDNA) studies have shown that cave and forest populations from any one geographic region are genetically similar (Baker et al., 2008).

Larvae of all species produce light to attract flying insects, usually small aquatic flies (Broadley & Stringer, 2001; Driessen, 2010) that are caught in a silk web spun by the larvae (Baker & Merritt, 2003; Meyer-Rochow, 2007). Larvae tend to be present in colonies and they do not move far from where they hatch. Cave colonies are concentrated near cave entrances because they prey upon flying insects washed into the cave as immature stages through stream drift (Pugsley, 1984). Within

Submitted April 30, 2012, Returned for revision June 4, 2012, Accepted August 13, 2012

Address correspondence to David J. Merritt, School of Biological Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia. Tel.: +61 7 33653478; Email: d.merritt@uq.edu.au

colonies, larvae are regularly spaced due to aggression between them if their snares make contact. In caves with fauna-rich in-flow streams, large populations can be present in the completely dark zone some distance from the entrance. The adults are slow-flying flies that live for only a week (Baker & Merritt, 2003).

Arachnocampa larvae have long been known to react to light exposure by ceasing to glow when exposed to artificial light and reactivating bioluminescence after the light is dimmed (Gatenby, 1959; Meyer-Rochow & Waldvogel, 1979). For this reason, bioluminescence was initially considered to be environmentally regulated: dawn and dusk could be the signals to initiate and terminate bioluminescence (Gatenby, 1959; Stringer, 1967). In fact, controlled exposure to light:dark (LD) cycles and constant darkness (DD) in the species, *A. flava*, showed that its bioluminescence is under circadian regulation as well as being directly inhibited by daylight (Merritt & Aotani, 2008). The circadian component was revealed in the form of a free-running rhythm under DD that becomes progressively damped with time, attributed to the maximum light output being reduced in compensation for a longer period of light production per day (Merritt & Aotani, 2008). Light exposure entrains the bioluminescence propensity rhythm (BPR) to peak during the subjective night (Merritt & Aotani, 2008). As well as entraining the circadian rhythm, light causes direct inhibition of bioluminescence, a process known as negative masking (Mrosovsky, 1999), by which light suppresses activity in a nocturnal species. In *A. flava*, masking and entrainment can be considered to act in concert to ensure nocturnal bioluminescence. The result led to the question of whether rhythmicity persists in the dark zone of cave populations of *Arachnocampa* because studies of cave organisms have shown a loss of responsiveness to light in some long-term cave isolated species (Cavallari et al., 2011), whereas others possess a capability of synchronizing their activity to the availability of food (Biswas & Ramteke, 2008). An in situ study of cave millipedes showed that approximately half of a sample showed circadian rhythms, and after entrainment to artificial light approximately two-thirds showed circadian rhythmicity in DD (Koilaraj et al., 2000). Regarding *Arachnocampa*, larvae in caves have been reported to glow continuously (Richards, 1960), but analysis of video recordings has indicated longer continuous bouts of glowing during the evening (Broadley & Stringer, 2009; Ohba & Meyer-Rochow, 2004). In an in situ study in caves, Merritt and Clarke (2011) assessed bioluminescence rhythmicity in the Tasmanian species, *A. tasmaniensis*, chosen because large cave populations were present. Time-lapse digital photography lasting several days showed that larvae outside the cave and in the cave mouth glow only during the night. In contrast, larvae in the dark zone showed strongly synchronized, rhythmic bioluminescence with a peak occurring in the afternoon. Observations in the dark zone of different caves has shown that, in the absence of solar light,

larvae show a sinusoidal daily cycle of bioluminescence and larvae within a colony are synchronized to show the same pattern (Merritt & Clarke, 2011, 2012). Two experimental approaches showed that the BPR of the day-peaking dark-zone populations entrains to match the time of light exposure (Merritt & Clarke, 2011). First, in situ exposure of a large population in the dark zone to artificial LD caused most larvae to cease glowing during light exposure, but on return to natural DD after several days of LD, the population's acrophase had shifted to match the time of light exposure (Merritt & Clarke, 2011). Second, larvae transferred from the dark zone to an incubator where they were exposed to point sources of light emulating other larvae shifted acrophase to match the cycle of the emulated larvae.

This unusual phenomenon—entrainment to promote peak bioluminescence at a time when bright light inhibits bioluminescence—was suggested to be an adaptation that allows larvae in the cave dark zone to synchronize to each others' glows. The advantage of population synchronization was suggested to be that simultaneous, colony-wide production of bioluminescence can produce a group-foraging advantage and that periods of low bioluminescence allow replenishment of the metabolic substrates required for bioluminescence (Merritt & Clarke, 2011; Willis et al., 2011). This synchronization hypothesis has not yet been directly tested. To explain the observation that cave-mouth and purely epigeal populations of the same species in the same locality bioluminesce only during the dark period, it was suggested that the species is plastic: those developmentally exposed to natural LD cycles entrain the BPR to peak during the scotophase, whereas those deep in the cave entrain the BPR to peak during the photophase, which could be more accurately called the "biophotophase" (Merritt & Clarke, 2011).

The first aim of this study was to determine whether the bioluminescence propensity rhythms of cave and cave-mouth larvae are in fact different, as suggested by Merritt and Clarke (2011). We find that both populations of *A. tasmaniensis* entrain in the same way, with the BPR peaking during the photophase. We then make specific comparisons between *A. tasmaniensis* and *A. flava*, experimentally confirming the opposite entrainment responses of the species to the same light exposure conditions.

METHODS

Exposure to Point-Source Light Under LD Cycles

Arachnocampa tasmaniensis larvae were collected from Mystery Creek Cave, Tasmania, or from the mouth of a nearby cave, Arthurs Folly (cave identification no. 101). *A. flava* were taken from a location in Springbrook National Park, Queensland. Larvae were transported to the laboratory in light-tight, insulated containers and then placed in individual, clay-roofed, transparent plastic-fronted containers in an aquarium. Individual containers were shielded so that larval glows were not visible to each

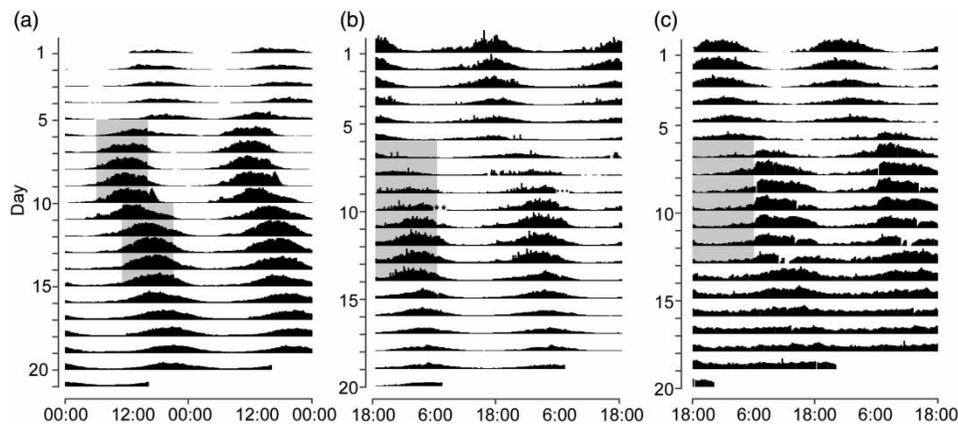


FIGURE 1. Double-plot of bioluminescence intensity of two species of *Arachnocampa* exposed to point sources of light in a controlled temperature cabinet. (A) *A. tasmaniensis* from deep in a cave exposed to DD, followed by LD 10:14, then phase-shifted LD 10:14 before DD. (B) *A. tasmaniensis* from a cave mouth exposed to LD 10:14 for 8 d (gray bars) matched phase with the light exposure period. Asterisks on day 10 indicate period of data loss due to equipment malfunction. (C) *A. flava* from a rainforest setting show decreased bioluminescence in response to the light exposure. After 7 d of exposure, the bioluminescence acrophase matched the photophase of the foregoing exposure. Light exposure shown as transparent gray bars in all panels.

other but visible to the camera. For maintenance, larvae were fed weekly by placing one or two anesthetized *Drosophila melanogaster* adults in their snares.

The aquarium was sealed and placed in an incubator at 12°C (*A. tasmaniensis*) or 23°C (*A. flava*). A camera was mounted in front of the aquarium and images of larval bioluminescence were taken at 10-min intervals. Fourteen *A. tasmaniensis* larvae originally taken from the dark zone of Mystery Creek Cave were used for the experiment illustrated in Figure 1A, 12 *A. tasmaniensis* from the cave mouth of Arthurs Folly were used for Figure 1B, and 12 *A. flava* were used for Figure 1C. Some experiments used a Canon digital SLR camera (EOS 1000D, 18-50 mm lens at aperture F3.5, 30-s exposure at ISO equivalent 1600, images 3872 × 2592 pixels) with exposure intervals regulated by a PClx intervalometer, others used a Sony XCD-X710 Firewire camera (images 1024 × 768 pixels) connected directly to a computer (Merritt & Aotani, 2008) and exposure intervals regulated using BTVPro (<http://www.bensoftware.com>). A fiber-optic light apparatus was used to expose larvae to point sources emulating larval bioluminescence (Merritt & Aotani, 2008). Blue-green light was distributed into nine individual 1-mm-diameter black-jacketed fiber-optic cables fixed at even intervals into a plastic strip. The fiber-optic tips were directed at the larvae by fixing the strip to the front of the aquarium so that each larva could see all nine of the point sources. Light intensity was modulated by eye so that each fiber-optic tip approximated the intensity of a bright larva. Exposure was regulated using a timer.

Exposure to Masking-Inducing LD Cycles

For exposure to LD cycles, *A. tasmaniensis* larvae from the mouth of Arthurs Folly Cave were maintained in individual containers in an aquarium as described above. A desk lamp comprising 12 white light-emitting diodes

(LEDs; 100 lux) was placed inside the incubator and operated under control of an electronic timer under 12:12 LD, with lights off at 18:00 h. Preliminary experiments showed that this light intensity caused larvae to cease bioluminescence within 10 min. For exposure to ultradian cycles, 30 *A. flava* larvae and 10 *A. tasmaniensis* larvae in aquaria were exposed to the following light:dark regimes over 10 d: days 1–3: LD 12:12 with dark onset at 18:00 h; days 4–5, LD 12:12 cycle with a 2-h dark pulse (onset at 11:00 h); days 6–7: ultradian cycles (LD 2:2); days 8–10, DD.

Recording Bioluminescence in Caves

The equipment and time-lapse method used for recording bioluminescence in caves has been described (Merritt & Clarke, 2011). Briefly, a digital SLR camera, either Pentax (K200D, 18-55 mm lens, aperture f4, 30-s exposure, ISO equivalent 1600) or Canon (EOS 1000D, 18-55 mm lens, aperture f4.5, 30-s exposure, ISO equivalent 1600), powered by an external battery, was mounted on a tripod and exposures taken at 10-min intervals controlled by a Harbortronics DigiSnap 2000 external programmable remote control unit. Field recordings were conducted in Mystery Creek Cave, Tasmania, through the summers of 2009 to 2011. Cameras were placed at different locations in the cave to record natural bioluminescence patterns of colonies on the cave ceiling in the transition from the cave mouth to the dark zone, a distance of approximately 70 m. Recording from each site lasted 48 h or longer. The approximate locations of the camera are shown in Figure 4.

Bioluminescence Intensity Analysis

The application “ImageJ” was used to analyze light output of individuals and groups of larvae. Images were converted to 8-bit grayscale and a threshold level was selected to separate the bioluminescence signals from

camera chip noise (a pixel value usually between 5 and 40). The intensity of each glow was calculated at each time point of the multiday series using the “integrated density” function to calculate the sum of pixel values above the threshold level within any selected area. The units for light output are arbitrary and were not calibrated against standards. Due to the use of different cameras and camera-to-subject differences, the absolute values of light intensity are not comparable between experiments; however, they are consistent within an experiment. For comparison of bioluminescence levels at different locations in Mystery Creek Cave, intensity curves from each site were normalized to the maximum intensity at the site.

Lomb-Scargle periodograms were calculated using the application LSP (created by R. Refinetti, available free via the Internet). To calculate the time of day at which bioluminescence peaks occur, the time series was first smoothed by applying a Butterworth filter (low-pass 12–16 h, high-pass 72 h) using MatLab functions to create a smooth sinusoidal curve eliminating frame-to-frame intensity variation. The occurrence of the peak of the smooth curve was calculated using peak analysis as described (Levine et al., 2002) to give a time—to the nearest 10 min—of acrophase for each bioluminescence cycle.

Procedures follow the ethical guidelines outlined by the journal editors (Portaluppi et al., 2010).

RESULTS

Exposure to Point Sources of Light

To confirm that *A. tasmaniensis* from the dark zone show light-entrained circadian control of bioluminescence, larvae were taken from a cave to the laboratory and exposed to DD, followed by LD, followed by a LD shift and exposed to DD again. The light source used in the experiment was dim point sources of light emulating other larvae (Merritt & Clarke, 2011). At the initiation of the experiment, all larvae showed approximately the same phase, as they had been kept in DD within visual contact and their bioluminescence was synchronized. During initial DD, the free-running period of the summed group bioluminescence was 24.65 h, calculated using Lomb-Scargle periodogram (LSP) analysis. Of the 14 individuals, 9 displayed significant rhythmicity, with tau ranging from 24.35 to 29.9 h, mean 25.46 h. Under LD, the bioluminescence acrophase shifted to occur during the light exposure period, both during the initial exposures period and in the subsequent LD shift (Figure 1A). The larvae free-ran upon return to DD. We conclude that the rhythm of deep cave larvae is circadian and entrains to LD cycles, confirming earlier findings (Merritt & Clarke, 2011).

The same lighting system was then applied to *A. tasmaniensis* larvae from the cave mouth and *A. flava* from rainforest. After DD for 7 d, they were exposed to point sources of light (12:12 LD) emulating other glow-worms (Figure 1B). In *A. tasmaniensis*, light exposure

did not inhibit larval bioluminescence and the larvae were entrained to reach bioluminescence acrophase near the midpoint of the light exposure regime (Figure 1B). In contrast, *A. flava* from forest showed partial masking in response to the point light exposure, as manifested by a slight increase in bioluminescence after lights-off (Figure 1C). On return to DD, the propensity to bioluminescence was highest at the scotophase of the exposure regime; the opposite to *A. tasmaniensis*. *A. flava* showed amplitude damping over time in DD, as noted previously (Merritt & Aotani, 2008). In that study, damping was attributed to compensation for the longer period of light production per day, leading to a reduction in the maximum daily light output.

The comparison of deep cave and cave mouth *A. tasmaniensis* shows that both populations have the

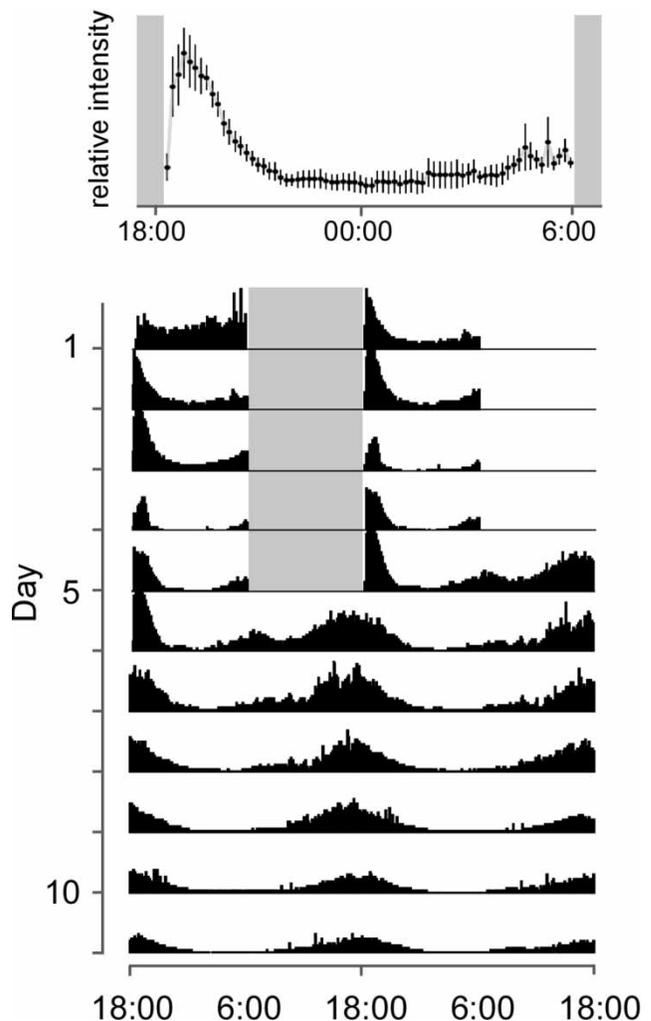


FIGURE 2. Bioluminescence intensity of *Arachnocampa tasmaniensis* exposed to artificial light in the laboratory. Larvae were taken from a cave-mouth setting where they experienced natural day:night cycles and were then exposed to 12:12 LD cycles in the laboratory (light exposure shown as transparent gray bar) for 5 d before being exposed to DD. Top: Bioluminescence intensity (mean and standard error) over the first 6 dark periods (days 1–6). Bottom: Circadian double-plot of intensity over 12 d.

same entrainment characteristics. The comparison of *A. tasmaniensis* with *A. flava* shows that the BPR of the two species takes on opposite phases under the same entraining conditions and that, in both species, dim point sources of light can entrain the rhythm.

Exposure of Cave-Mouth *A. tasmaniensis* Larvae to DD

Next, we exposed larvae of *A. tasmaniensis* from a cave mouth where they had developed under exposure to natural day:night cycles to 12:12 LD using light of high enough intensity (100 lux) to cause negative masking, followed by DD. Under light exposure during LD, they douse in response to light exposure (Figure 2, gray bar) and they bioluminesce most intensely soon after lights off. The removal of negative masking at lights off leads to a rapid ramp-up of bioluminescence, reaching a peak approximately 30–40 min later (Figure 2). Bioluminescence declined through the scotophase, followed by a secondary peak before lights-on (Figure 2). On transfer to DD, the larvae tended to bioluminesce most brightly around the subjective light period, initially with minor peaks at subjective dawn and dusk (Figure 2). Over the next 7 d, the minor peaks coalesced so that the bioluminescence output curve became sinusoidal, reaching an acrophase between 16:40 and 17:20 h, calculated by peak analysis after Butterworth band-pass filtering. The free-running period of the summed group bioluminescence over 7 d is 24.18 h (LSP analysis). Of the 10 individuals, 9 displayed significant rhythmicity, with tau ranging from 22.85 to 24.38 h, mean 23.81 h. The experiment shows that the BPR of cave-mouth *A. tasmaniensis* reaches acrophase during the photophase.

Exposure of *A. tasmaniensis* and *A. flava* Larvae to Ultradian LD Cycles

The different phases of the BPR in the two species suggested that *A. tasmaniensis* would initiate bioluminescence when exposed to a dark pulse during the photophase, whereas *A. flava* would not. In a controlled-temperature, light-tight cabinet, *A. tasmaniensis* larvae from the cave mouth and *A. flava* from forest were exposed to LD regimes for several days and then a 2-h dark period in the middle of the photophase for two consecutive days, followed by a 2:2 LD ultradian cycle. The light source used was sufficiently intense to cause larvae to cease bioluminescence. The expectation was that *A. tasmaniensis* would readily bioluminesce during the dark pulse because the underlying bioluminescence tendency is high, whereas *A. flava* would show the opposite reaction because it is entrained to bioluminesce most intensely during the night. Indeed, *A. tasmaniensis* emitted brightly during the 2-h dark pulse (Figure 3A), indicating that they were primed to bioluminesce as soon as the masking stimulus was removed. During the initial 2-h dark pulse, *A. flava* did not begin to bioluminesce at all (Figure 3B). When subsequently exposed to ultradian cycles of 2:2 LD for 2 d, the intensity of bioluminescence in each 2-h period matched the expected intensity curve if the larvae were in fact in DD: *A. tasmaniensis* showed maximum bioluminescence during the dark pulses that occurred during their subjective day, whereas *A. flava* showed maximum bioluminescence in the dark pulses during their subjective night (Figure 3B).

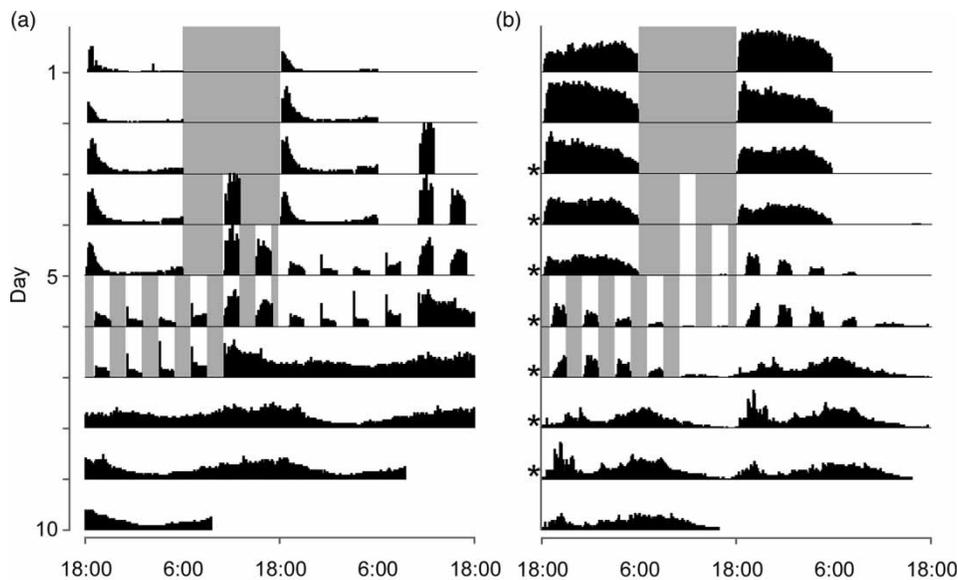


FIGURE 3. Double-plots of bioluminescence intensity of *Arachnocampa tasmaniensis* (A) and *Arachnocampa flava* (B) showing response to ultradian LD pulses after 4 d of 12:12 LD and finally in DD. On day 4, larvae were exposed to a 2-h dark period centered on midday. From day 5 they were exposed to LD 2:2 and returned to constant darkness on day 8. Light exposure is shown by the gray bars. In A, all 2-d plots are presented at the same scale. In B, intensity decreased day to day so the vertical scale was altered at the points indicated by asterisks.

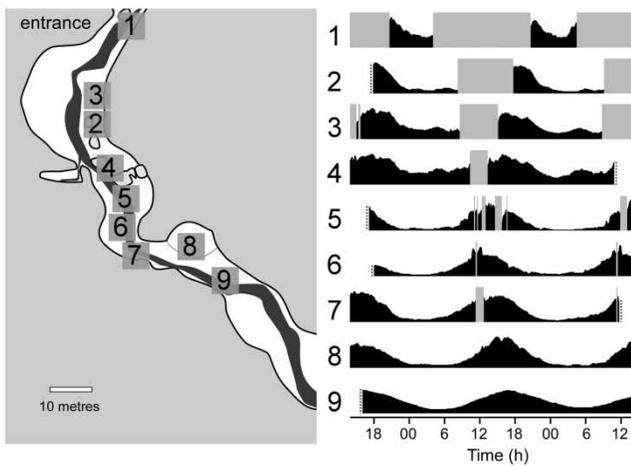


FIGURE 4. The time course of bioluminescence output of colonies of *Arachnocampa tasmaniensis* transitioning from the cave entrance to deeper in Mystery Creek Cave. Left: Diagram of cave showing approximate locations of colonies imaged using time-lapse photography. The course of the stream through the cave is shown in dark gray. Right: Bioluminescence output of each colony over 48 h, normalized to the maximum intensity. Gray bars indicate times at which solar light prevented detection and measurement of the larval bioluminescence. Dashed vertical line indicates onset or termination of recording.

Bioluminescence Patterns in the Twilight Zone

The laboratory experiments in which *A. tasmaniensis* were exposed to ultradian light cycles suggest that larvae of this species can initiate bioluminescence at any time upon exposure to darkness. A prediction is that in colonies located progressively deeper in a cave, the larval bioluminescence would be brightest after dusk at that location in the cave because the BPR would prime larvae to bioluminesce as soon as darkness falls. Cameras were placed at different locations in Mystery Creek Cave to record natural bioluminescence patterns along a distance of approximately 70 m from the cave mouth to the dark zone (Figure 4). The camera settings used to image bioluminescence produce an overexposed image during times of high light penetration into the cave. Consequently, we cannot be certain that all larvae stopped glowing during these periods, but observations have shown that light levels intense enough to saturate the photographic image invariably cause larvae to switch off their bioluminescence.

In the cave-mouth area (Figure 4, location 1), bioluminescence was highest soon after the onset of “dusk,” followed by a drop in intensity. The cave-mouth larvae experienced 16.5 h of photographically detectable daylight, with onset and offset corresponding relatively closely to the time of civil sunrise (04:23 h) and sunset (20:24 h) at the location and date. On one day, bioluminescence intensity rose as dawn approached, producing a U-shaped curve. Further from the cave mouth, the local topography shields the colony from direct light exposure for most of the day, so that larvae were exposed to photographically detectable light for approximately 9, 7, and 3 h per day in locations 2, 3, and 4,

respectively (Figure 4). Note that at each location the peak bioluminescence intensity occurred after dusk. Deeper into the cave, the local topography prevented light exposure for most of the day (locations 5, 6, 7). In these colonies, the maximum bioluminescence output is centered around the time of brief light exposure. The upswing preceding the acrophase occurred before light exposure. Deeper still (locations 8, 9), no detectable ambient light exposure was recorded and the acrophase occurred between 12:00 and 18:00 h, as determined in an earlier study (Merritt & Clarke, 2011).

DISCUSSION

We have shown that the two species react differently to entrainment by light: *A. flava* acts like a typical nocturnal animal, being entrained by daylight to bioluminesce during the night, whereas *A. tasmaniensis* is entrained by daylight to bioluminesce during the day, but the inhibition of bioluminescence by daylight negatively masks the propensity to glow so that bioluminescence occurs only at night. The obvious question arising from the comparison of species is why would the BPRs of *A. flava* and *A. tasmaniensis* oscillate in antiphase in response to the same stimuli? In particular, why should *A. tasmaniensis* show the seemingly paradoxical tendency of a highest propensity during the day? We consider that the answer lies in the link between *A. tasmaniensis* and the cave environment. We will discuss two, possibly linked, functions: (1) to enable larvae to respond facultatively to the extreme variability in the duration of light exposure in the twilight zone of caves, and (2) to enable adaptive, population-based synchronization of bioluminescence in caves.

First, we compare the two species in how masking and entrainment work together in different ways to determine the bioluminescence pattern. *A. flava*'s BPR is typical of a nocturnal animal in which the propensity for the activity peaks during the night, for example, wheel-running in rodents (Dardente et al., 2004; DeCoursey, 1972; Kas & Edgar, 1999) or nocturnal activity in insects such as crickets or cockroaches (Roberts, 1960). The BPR is sinusoidal and it damps over time, as revealed under DD in this study and by Merritt and Aotani (2008). Under natural LD exposure, this pattern is modulated by negative masking by daylight; the post-lights-off peak normally seen in the wild or under LD disappears under free-running conditions on transfer to DD and the bioluminescence curve becomes sinusoidal (Merritt & Aotani, 2008; Merritt & Clarke, 2009), indicating that this post-dusk peak is at least partly due to the release of accumulated “drive” at the removal of negative masking by light as darkness falls and the underlying propensity rhythm is on the upswing. In comparison, negative masking plays a much more significant role in the realized bioluminescence pattern of *A. tasmaniensis*. The underlying BPR is also sinusoidal, with less tendency to damp over time. Light exposure during the day negatively masks and

overrides the high bioluminescence propensity at that time and leads to a release of bioluminescence after dusk when the BPR is on the wane. A simple experimental light regime exhibits the fundamental differences between the two species: exposure to dark pulses during the photophase resulted in rapid onset of high levels of bioluminescence in *A. tasmaniensis*, whereas *A. flava* remained doused (Figure 2).

As a consequence, *A. tasmaniensis* is able to start glowing at any phase of the light:dark cycle, whereas *A. flava* is locked into nocturnal bioluminescence. At the latitude where this study was conducted, the extremes of the annual LD cycle are LD 14:10 in summer and LD 10:14 in winter (Merritt & Clarke, 2009), so epigeal larvae need only accommodate annual ± 2 h differences in the timing of dawn or dusk. Cave populations are exposed to much more variability due to the characteristics of light penetration into the cave. The precise light regime experienced by individuals depends on where the mother deposited them as eggs and varies depending on distance from the cave entrance, the topography of the cave in the immediate vicinity, and seasonal changes in the angle of the sun. In colonies imaged with time-lapse photography that are exposed to a period of daylight, the peak intensity occurred soon after daylight drops below detectable levels, i.e., at the onset of darkness at that location within the cave. This is precisely what would be expected in light of the masking and entrainment characteristics of this species: at the onset of darkness during the subjective day, negative masking is released and, combined with the elevated phase of the underlying BPR, would produce a peak of bioluminescence soon after lights-off at any site within the cave. A substantial period of light exposure, upwards of 3 h, appears to be required to see this pattern. Shorter exposures have less impact on the underlying sinusoidal curve, e.g., the shape of the bioluminescence output curve at locations 5–9 (Figure 4) is sinusoidal, whereas the curve in locations 1–4 shows an asymmetrical daily curve with an acrophase occurring soon after lights on.

In comparison, *A. flava* reacts very differently to short days. When larvae were exposed to progressively shorter artificial photophases (LD 12:12 to LD 4:20), akin to the LD cycles experienced by *A. tasmaniensis* in the twilight zone, the intensity of the postdusk peak progressively reduced and the delay between lights-off and the peak increased (Merritt & Clarke, 2009). The bioluminescence curve tends to peak at the middle of the extended dark period, due to their scotophase-centered BPR. Thus, if *A. flava* were located in the cave twilight zone, individuals would be predicted to show a flattened bioluminescence curve. Further, they would not synchronize to one another in cave chambers devoid of external light, given our experimental results indicating that they show partial negative masking in response to dim point sources of light.

It is possible that the postdusk peak seen in *A. tasmaniensis* is an adaptation to produce greatest

bioluminescence at a time when the larvae are most likely to attract prey, or at least to provide a mechanism for producing a short period of intense light rather than a continuous low-level emission. In the only survey of prey flight time, conducted in the dark zone of the same cave, flying insects show peak activity between 18:30 and 21:30 h (Merritt & Clarke, 2011). Surveys of prey flight times within the twilight zone of caves are needed to test whether potential prey insects are more likely to initiate flight and become attracted to bioluminescence at the onset of dusk at their particular location.

A second explanation is that the BPR of *A. tasmaniensis* is required for synchronization of light output by colonies deeper within caves where solar light is absent. A consequence of the paradoxical entrainment is that detection of the low-intensity, sub-masking-threshold light emitted by other larvae should cause colonies to become synchronized; however, we acknowledge that the required experimental confirmation of synchronization is not yet published (Maynard and Merritt, in preparation). Laboratory exposure to low-intensity light emulating other glowworms entrains larvae to the photophase of the 24-h LD cycle without any masking: it occurs in dark-zone larvae (Merritt & Clarke, 2011) as well as in those from the cave mouth (this study). On the other hand, *A. flava* do not entrain to match the photophase of low-intensity point-source exposure. The possible benefit of synchronization of rhythms in the dark zone is that it could provide a group-foraging mechanism that increases participants' likelihood of attracting prey. Theoretically, a transition point occurs within the cave where the intensity of solar light falls below the detection threshold and is replaced as an entraining stimulus by the detection of the lights of other larvae. Empirically, such a transition point occurs approximately 60 m from the cave entrance in Mystery Creek Cave where 1-min camera exposures detected no light other than bioluminescence (Figure 4, location 8). Here, the colony is synchronized, showing a sinusoidal curve of bioluminescence output, with an acrophase at approximately 16:00 h. The two explanations that we have proffered for the paradoxical BPR in *A. tasmaniensis*—postdusk peak and synchronization—could both be adaptive or one could be incidental to the other.

Evolution of Species Differences

Members of the genus *Arachnocampa* are not obligate cave dwellers, rather they are troglaphiles, capable of living and reproducing in either epigeal or hypogean habitats under appropriate conditions. The unusual bioluminescence regulatory mechanism seen in *A. tasmaniensis* could have evolved to maximize prey capture capability in the complete darkness or truncated light regimes experienced in caves or near cave mouths where larvae tend to be concentrated. Species with no current link to caves, such as *A. flava*, might show a BPR more typical of a nocturnally bioluminescent animal because they have lost—or have never gained—

the ability to synchronize and accommodate the extreme light regimes experienced by the cave-adapted species. A survey of the entrainment behavior of cave and forest populations of the nine species of *Arachnocampa* in the context of their phylogenetic relationship (Baker et al., 2008) should determine whether the bioluminescence rhythmicity type is constrained by phylogeny or by habitat and tell us which BPR condition is likely to be ancestral.

This is the first documented case of two closely related species showing different underlying propensity rhythms that ultimately produce the same outcome under exposure to LD cycles. An ability to facultatively switch temporal niche has been shown, for example, in response to food constraints or the availability of running wheels in captive rodents (Kronfeld-Schor & Dayan, 2008). Numerous examples exist where different individuals of a species can be either nocturnal or diurnal (Refinetti, 2006) and different developmental stages of a species can occupy different temporal niches, for example, nymphal crickets are diurnal and adults are nocturnal (Okada et al., 1991). Laboratory-based manipulative experiments are required to detect the cryptic divergent underlying rhythmicity of the type seen here. As an example of the approach, exposure to DD was needed to show that, whereas the active phase of mice shifts in response to food shortage, the phase of the underlying pacemaker remains relatively unchanged (Hut et al., 2011). The substantial difference in rhythmicity between the two closely related species considered here must be assumed to reflect some selective advantage, supporting arguments for an adaptive significance of circadian clocks (Johnson, 2005; Sharma, 2003; Yerushalmi & Green, 2009). Although we acknowledge that *Arachnocampa* are troglomorphic and, as such, would be less likely to lose rhythmicity than obligate cave dwellers, it is informative that the more cave-adapted of the two species maintains strong rhythmic control of its bioluminescence propensity, to the extent that it shifts from solar entrainment to mutual entrainment in the transition from the epigeal to hypogean environment.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- Baker CH. (2010). A new subgenus and five new species of Australian glow-worm (Diptera: Keroplatidae: *Arachnocampa* spp.). *Mem. Qld. Mus.* 55:149–177.
- Baker CH, Merritt DJ. (2003). Life cycle of an Australian glow-worm *Arachnocampa flava* Harrison (Diptera: Keroplatidae: Arachnocampinae: Campara). *Aust. Entomol.* 30:45–55.
- Baker CH, Graham GC, Scott KD, Cameron SL, Yeates DK, Merritt DJ. (2008). Distribution and phylogenetic relationships of Australian glow-worms *Arachnocampa* (Diptera, Keroplatidae). *Mol. Phylogenet. Evol.* 48:506–514.
- Biswas J, Ramteke AK. (2008). Timed feeding synchronizes circadian rhythm in vertical swimming activity in cave loach, *Nemacheilus evezardi*. *Biol. Rhythm Res.* 39:405–412.
- Broadley RA, Stringer IAN. (2001). Prey attraction by larvae of the New Zealand glowworm, *Arachnocampa luminosa* (Diptera: Mycetophilidae). *Invert. Biol.* 120:170–177.
- Broadley RA, Stringer IAN. (2009). Larval behaviour of the New Zealand glowworm, *Arachnocampa luminosa* (Diptera: Keroplatidae), in bush and caves. In Meyer-Rochow VB (ed.). *Bioluminescence in focus—a collection of illuminating essays*. Kerala: Research Signpost, 325–355.
- Cavallari N, Frigato E, Vallone D, Fröhlich N, Lopez-Olmeda JF, Foà A, Berti R, Sánchez-Vázquez FJ, Bertolucci C, Foulkes NS. (2011). A blind circadian clock in cavefish reveals that opsins mediate peripheral clock photoreception. *PLoS Biol.* 9:e1001142.
- Dardente H, Menet JS, Challet E, Tournier BB, Pevet P, Masson-Pevet M. (2004). Daily and circadian expression of neuropeptides in the suprachiasmatic nuclei of nocturnal and diurnal rodents. *Mol. Brain Res.* 124:143–151.
- DeCoursey PJ. (1972). LD ratios and entrainment of circadian activity in a nocturnal and a diurnal rodent. *J. Comp. Physiol.* 78:221–235.
- Dreisig H. (1975). Environmental control of daily onset of luminescent activity in glow-worms and fireflies (Coleoptera: Lampyridae). *Oecologia* 18:85–99.
- Driessen MM. (2010). Enhancing conservation of the Tasmanian glow-worm, *Arachnocampa tasmaniensis* Ferguson (Diptera: Keroplatidae) by monitoring seasonal changes in light displays and life stages. *J. Insect Conserv* 14:65–75
- Gatenby JB. (1959). Notes on the New Zealand glow-worm, *Bolitophila (Arachnocampa) luminosa*. *Trans. R. Soc. N. Z.* 87:291–314.
- Hamilton-Smith E. (1967). The Arthropoda of Australian caves. *J. Aust. Entomol. Soc.* 6:103–118.
- Hut RA, Pilon V, Boerema AS, Strijkstra AM, Daan S. (2011). Working for food shifts nocturnal mouse activity into the day. *PLoS ONE* 6: e17527.
- Johnson CH. (2005). Testing the adaptive value of circadian systems. *Methods Enzymol.* 393:818–837.
- Kas MJ, Edgar DM. (1999). A nonphotic stimulus inverts the diurnal-nocturnal phase preference in *Octodon degus*. *J. Neurosci.* 19:328–333.
- Koilraj AJ, Sharma VK, Marimuthu G, Chandrashekar MK. (2000). Presence of circadian rhythms in the locomotor activity of a cave-dwelling millipede *Glyphiulus cavernicolus sulu* (Cambalidae, Spirostreptida). *Chronobiol. Int.* 17:757–765.
- Kronfeld-Schor N, Dayan T. (2008). Activity patterns of rodents: the physiological ecology of biological rhythms. *Biol. Rhythm Res.* 39:193–211.
- Lall AB, Seliger HH, Biggley WH, Lloyd JE. (1980). Ecology of colors of firefly bioluminescence. *Science* 210:560–562.
- Levine JD, Funes P, Dowse HB, Hall JC. (2002). Signal analysis of behavioral and molecular cycles. *BMC Neurosci.* 3:1–25
- Lloyd JE. (1983). Bioluminescence and communication in insects. *Annu. Rev. Entomol.* 28:131–160.
- Merritt D, Clarke A. (2012). The impact of cave lighting on the bioluminescent display of the Tasmanian glow-worm *Arachnocampa tasmaniensis*. *J. Insect Conserv* Epub ahead of print. doi: 10.1007/s10841-012-9493-0.
- Merritt DJ, Aotani S. (2008). Circadian regulation of bioluminescence in the prey-luring glowworm, *Arachnocampa flava*. *J. Biol. Rhythms* 23:319–329.
- Merritt DJ, Clarke AK. (2009). Rhythmic regulation of bioluminescence in glow-worms, *Arachnocampa*. In Meyer-Rochow VB(ed.). *Bioluminescence in focus—a collection of illuminating essays*. Kerala: Research Signpost, 291–303.
- Merritt DJ, Clarke AK. (2011). Synchronized circadian bioluminescence in cave-dwelling *Arachnocampa tasmaniensis* (glowworms). *J. Biol. Rhythms* 26:34–43.

- Meyer-Rochow V. (2007). Glowworms: a review of *Arachnocampa* spp. and kin. *Luminescence* 22:251–265.
- Meyer-Rochow VB, Waldvogel H. (1979). Visual behaviour and the structure of dark and light-adapted larval and adult eyes of the New Zealand glowworm *Arachnocampa luminosa* (Mycetophilidae: Diptera). *J. Insect Physiol.* 25:601–613.
- Mrosovsky N. (1999). Masking: history, definitions, and measurement. *Chronobiol. Int.* 16:415–429.
- Ohba N, Meyer-Rochow VB. (2004). Luminescent behaviour in the New Zealand glowworm, *Arachnocampa luminosa* (Insecta; Diptera; Mycetophilidae). In Tsuji A (ed.). *Bioluminescence & chemiluminescence: progress and perspectives. Proceedings of the 13th International Symposium*, 2–6 August, Singapore: World Scientific, Yokohama, Japan, pp. 23–26
- Okada Y, Tomioka K, Chiba Y. (1991). Circadian phase response curves for light in nymphal and adult crickets, *Gryllus bimaculatus*. *J. Insect Physiol.* 37:583–590.
- Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. *Chronobiol. Int.* 27:1911–1929.
- Pugsley C. (1984). Ecology of the New Zealand glowworm, *Arachnocampa luminosa* (Diptera: Keroplatidae), in the glowworm cave, Waitomo. *J. R. Soc. N. Z.* 14:387–407.
- Refinetti R. (2006). Variability of diurnality in laboratory rodents. *J. Comp. Physiol. A* 192:701–714.
- Richards AM. (1960). Observations on the New Zealand glowworm *Arachnocampa luminosa* (Skuse) 1890. *Trans. R. Soc. N. Z.* 88:559–574.
- Roberts SKdF. (1960). Circadian activity rhythms in cockroaches. I. The free-running rhythm in steady-state. *J. Cell. Comp. Physiol.* 55:99–110.
- Sharma VK. (2003). Adaptive significance of circadian clocks. *Chronobiol. Int.* 20:901–919.
- Stringer IAN. (1967). The larval behaviour of the New Zealand glow-worm *Arachnocampa luminosa*. *Tane* 13:107–117
- Willis R, White C, Merritt D. (2011). Using light as a lure is an efficient predatory strategy in *Arachnocampa flava*; an Australian glowworm. *J. Comp. Physiol. B* 181:477–486.
- Yerushalmi S, Green RM. (2009). Evidence for the adaptive significance of circadian rhythms. *Ecol. Lett.* 12:970–981.