Laboratory observations reveal that mature *Vespa mandarinia* larvae continue calling at night

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Abstract

Vespa hornet larvae produce a rhythmic 'rasping' sound by rubbing their mandibles against the cell walls of their nests. The call is thought to be a larval provisioning cue. However, detailed observations of larval calls have been limited to a few species, and it is not known whether the call can be influenced by the external environment, such as light and time of day, or by internal larval states, such as feeding. We conducted laboratory observations of larval calls under workerless conditions to investigate the effects of 1) larval stage and size, 2) daily variation, 3) light conditions, and 4) feeding on sound production. *Vespa mandarinia* larvae produced sounds regardless of their status, such as position, light condition, time of day, and worker absence. During mastication, the larvae stopped calling. A key finding of this research is the novel discovery that larvae produce sounds at night, which is a previously undocumented behaviour.

INTRODUCTION

Animals produce and perceive vibroacoustic signals (Greenfield, 2016; Virant-Doberlet, Stritih-Peljhan, Žunič-Kosi, & Polajnar, 2023). Insects, which include more than 200,000 species, utilize vibrational communication produced through various means, such as body and wing movements, high-frequency muscle contractions, and scraping mandibles (Cocroft & Rodríguez, 2005; Virant-Doberlet et al., 2023). In social insects, vibrational signals play an important role in communication among nestmates. Vibrational communication has many forms and functions (Hunt and Richard 2013), such as mating (Conrad & Ayasse, 2019), foraging (Hrncir & Barth, 2014), alarm signalling (Bota, Schöner, Schöner, & Eberhard, 2022; Hager & Kirchner, 2013), adult–larva communication (Pepiciello, Cini, Nieri, Mazzoni, & Cervo, 2018) and larval development cues (Suryanarayanan, Hermanson, & Jeanne, 2011).

Vespidae, including hornets (Vespinae) and paper wasps (Polistinae), make nests from soft paper pulp with sturdy hexagonal cells. The material and shape are favourable for the transmission of vibrational signals (Hunt & Richard, 2013). *Polistes* wasps exhibit three types of body oscillation behaviours: antennal drumming, abdominal wagging, and lateral vibration. These types of signals are used for communication among adults and from adults to larvae (Pratte & Jeanne, 1984; Suryanarayanan et al., 2011). In *Vespa* hornets, larvae produce vibroacoustic signals using their mandibles (Edwards & Others, 1980; J. Ishay, 1975; J. Ishay & Brown, 1975; J. Ishay & Landau, 1972; J. Ishay, Motro, Gitter, & Brown, 1974; J. Ishay & Schwartz, 1973; Jacob Ishay & Schwartz, 1973; Makoto Matsuura & Yamane, 1990; Schaudinischky & Ishay, 1968; Yamane, 1976). *Vespa* larvae rub their mandibles against their cell walls and produce a rhythmic “rasping” sound. This rasping sound has been reported mainly in *Vespa crabro* (M. Matsuura, 1974), *Vespa orientalis* (J. Ishay, 1975; J. Ishay & Landau, 1972), and *Dolichovespula sylvestris* (Jeanne, 1980; Weyrauch, 1935). Yamane (1976) reported that “this behaviour has been confirmed in almost all the *Vespa* species observed by me”. *D. sylvestris* and *Vespula* wasps also produce scraping sounds, but not all *Vespula* species do (Jeanne, 1980; Makoto Matsuura & Yamane, 1990; Weyrauch, 1935). Fourth- and fifth-instar male, queen, and worker larvae of *V. orientalis* produce this sound (Schaudinischky & Ishay, 1968).

This sound is known to be a “hunger” cue to workers (Makoto Matsuura & Yamane, 1990; Schaudinischky & Ishay, 1968). In an experiment with *V. orientalis*, when a recorded vibration sound was played for workers, they performed brood-care-like behaviour (Schaudinischky & Ishay, 1968). *Vespa* workers forage during the daytime (Edwards & Others, 1980). If there is no new food for the larvae at night, do the larvae stop calling? In addition, a previous study reported for *V. crabo* that “only after receiving food does the larva stop emitting “hunger” signals, but it resumes them as soon as it has finished masticating” (Jacob Ishay & Schwartz, 1973). However, knowledge of larval calls is very concentrated in a few species, although it is known that mature larvae produce sounds (Yamane, 1976). Thus, this study aims to confirm the universality of the larval call and to determine when *V. mandarinia* larvae produce the sound. We offer the first detailed observation of this behaviour in *V. mandarinia*. Laboratory observations of larval calls in an experiment without workers were made to investigate the effects of 1) larval stage and body size, 2) daily variation, and 3) mastication on the production of the sound.

METHODS

Collection and laboratory setup

V. mandarinia is a common hornet in East Asia (Archer, 1995). These insects are commonly found from open fields to low mountains across Japan, excluding the area south of Amami Oshima. We collected a comb containing more than 25 larvae on 2023-10-20 [Day 0] in Toyota, Aichi Prefecture, Japan. The nest comb was placed upside down inside an incubator and kept at 25°C under dark

conditions during the non-experimental period. Food was not provided to the larvae until Day 6. The experimental schedule is shown in Figure 1. Adult workers were not included in this study for safety reasons. We labelled each larva by position and determined the larval stage (1st, 2nd, 3rd, 4th and 5th) on the basis of head width. Seventeen 5th-instar larvae (A01-A17) and eight 4th-instar larvae were used in our experiment (Figure 2). After observation, the body lengths of the 5th-instar larvae were measured using a digital camera (OLYMPUS, TG-3) and ImageJ software. The bodies of three larvae (A01, A02 and A17) collapsed and so there were no data on body length for these specimens.

Recording: Group-level calls

To investigate whether the light conditions affected the calling behaviour of the larvae, group-level recordings were made of calls for 30 minutes on Day 3 under different light conditions (for details on the timing of the observation, see Figure 1). The nest comb was placed upside down inside an incubator at 25°C. After the group-level calls were recorded under dark conditions for more than 30 minutes, the light (a white light) was turned on, and the group-level calls were again recorded for more than 30 minutes. Continuous group-level recordings under different light conditions (dark and light) were conducted twice between 2 pm and 5 pm (daytime) and between 8 pm and 10 pm (nighttime). A microphone (Yeti, BM400S Logicool) and Quick Time Player (version 10.5 on MacBook Pro, OS13.5) were used for recording. White noise was removed via the noise reduction function on Audacity (version 3.4.2). WaveSurfer (version 1.8.8p6) was used to plot a spectrogram. After recording, the number of group-level calls was counted every minute for 20 minutes (n = 20) by an observer who listened to the recording.

Observation: Individual-level calls

Larval calls were manually counted under light conditions from Day 3 to Day between 5 and 6 pm. The comb was placed facing upwards on a table. We used handheld counter and a digital timer to determine the total number of calls per minute. Each larva's calls were counted twice, and the average number of calls was used for data analysis. Manual observations were made for 30 min. The first observation was conducted on Day 3 at 6 pm. To investigate the daily variation in the number of calls, we conducted observations every six hours from 12 pm on Day 4 to 6 pm on Day 5 (six time points).

To investigate whether the larvae stopped calling while eating, we fed the larvae a piece of cricket and observed the number of calls at three time points on Day 6. After manually observing the calls per individual at 9 am (before feeding), we fed the larvae approximately 0.05 g of defrosted house cricket between 12 pm and 3 pm. We allowed the larvae to feed on the crickets ad libitum for 3 hours, and the amount consumed per individual was not controlled. Manual observations of calls were conducted at two additional times: at noon (while larvae were eating) and at 3pm (after the meal but within 5 minutes of removing the food).

Statistics

The relationship between body length and number of calls was plotted using the number of calls on Days 4 and 5 (data points = 5). Linear regression and R^2 values were calculated to elucidate the significance of the association between body length and the number of calls, with data separated by larval head colour. A t test was used to compare the number of calls between fifth-instar larvae and fourth-instar larvae. The differences in the number of calls with respect to daily variation and feeding treatment were compared using the Tukey HSD test.

Results

We observed that larvae produced a rasping sound when the comb was placed upside down (Figure 2a, Video S1). Even in the absence of workers and nurse hornets, larvae continued to call in this study.

Larvae continued to make sounds throughout the 30-minute recording regardless of the time of day and light conditions (Figure 3a-d). The number of group-level calls per minute at night was significantly greater than that during the day (Figure 4, Tukey-HSD test, $P < 0.05$). During the daytime, the frequency of calls under the light condition was significantly greater than that under the dark condition (Figure 4, Tukey-HSD test, $P < 0.05$).

Only fifth-instar larvae continuously produced the sound (Figure 5a and Video S1). The number of calls by fifth-instar larvae was significantly greater than that by fourth-instar larvae (Figure 5a, Wilcox test, $P < 0.05$). We confirmed the viability of fourth-instar larvae by observing their response when we provided food (Figure 2c) or to physical stimuli (tapping them with tweezers). A positive linear relationship existed between body length and the frequency of calls for fifth-instar larvae (Figure 5b, linear regression, $y = 0.57x - 6.5$, $P < 0.01$, $R2 = 0.089$). There was no significant effect for fourth-instar larvae (Figure 5b, linear regression, $p = 0.49$).

To examine the daily variation in larval calls, we conducted observations every 6 hours from 12 pm on Day 4 to 6 pm on Day 5. Although some fifth-instar larvae made fewer calls (ID: A01, A03, A09, and A16), there was no significant trend in the number of calls among the times of day at which the observations were made (Figure 6a, Tukey-HSD test). This result suggests that fifth-instar larvae produce sounds continuously throughout the day and night. The number of calls did not differ before and after feeding (Figure 6b, paired t test with Bonferroni correction). There was substantial variation among individuals in the number of calls immediately after the food was removed (Figure 6b). During mastication, the larvae stopped calling (Figure 3e). After removing the food, the larvae turned their bodies around, rapidly opened and closed their mandibles, and resumed calling.

DISCUSSION

V. mandarinia larvae produce sounds regardless of their status, including position, time of day, light conditions, and worker absence. We confirmed that *V. mandarinia* larvae continued calling even when their nest was turned upside down, which is inconsistent with the findings of a previous study of *V. orientalis* (Schaudinischky & Ishay, 1968). Sound production depended on the larval stage (Figure 5), which is consistent with the findings of previous studies (Ishay and Landau 1972, Yamane 1976, Edwards 1980, Matsuura and Yamane 1990). For early-stage larvae, prioritizing energy conservation for growth could be crucial. Some fifth-instar larvae did not produce the rasping sound. There are three possible reasons for this result. First, larvae have soft mandibles immediately after moulting, which may prevent them from producing the rasping sound. Second, under severe starvation, larvae may become immobilized. After the observation on Day 6, some larvae died and then turned black. Third, fifth-instar larvae, having reached a stage at which they are ready to pupate, cease to receive nourishment from workers. This cessation of feeding may result in a corresponding cessation of sound production. To reveal why young larvae do not produce calls, additional observations are needed of the amount of food larvae typically received and the brood care that workers provide to young larvae.

A key finding of this research is that larvae make sounds during the night (Figure 3, 4, 6a). *V. mandarinia* is known to be a diurnal hornet; only one species, *V. crabro*, forages after sunset in Japan (Makoto Matsuura & Yamane, 1990). It is not reasonable for larvae to continue making a “begging” call at night because new food is not available for larvae at night. In a future study, the times at which workers feed larvae should be investigated to determine why larvae beg for food at night. Compared with the number of daytime calls, there was an increase in the number of group-level larval calls at night; this likely holds significance, as it coincides with the return of most workers to the nest, thus ensuring that all workers are exposed to these larval sounds. These nighttime larval calls are likely used for information-sharing, informing workers about the current number of larvae and food requirements of the following day. The observation that larger larvae call more frequently supports this possibility, as larger larvae require more food to sustain their bodies from a metabolic standpoint

(Figure 5b). This hypothesis is further reinforced by the observation that the larvae examined in this study were new queens, for which body size is a critical determinant of successful overwintering (Saga, Kanai, Shimada, & Okada, 2024). Specifically, among social insects with queens that independently found new colonies, larger queens have a competitive advantage in founding new nests (Heinze & Tsuji, 1995; Keller, 1994; Wiernasz & Cole, 2003).

The decrease in the number of group-level larval calls observed during the daytime without artificial illumination can be attributed to the engagement of adult wasps in foraging activities, during which their ability to receive signals pertaining to nutritional needs is compromised. Conversely, the increase in call frequency during the daytime with artificial illumination could be due to the altered environmental conditions, deviating from the natural state of darkness. Further detailed observations under conditions closer to the natural conditions experienced by this species could validate these possibilities, enhancing our understanding of larval communication dynamics.

In summary, after more than 48 hours of starvation, fifth-instar *V. mandarinia* larvae exhibited a continuous calling behaviour. The novel finding of this research is that larvae produce sounds at night; this is a previously undocumented behaviour. When larvae eat, they stop calling, which is consistent with the findings of a previous study (Jacob Ishay & Schwartz, 1973). Although the production of provisioning cues can be costly, the only thing that larvae can do in the nest is beg for food.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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Figures

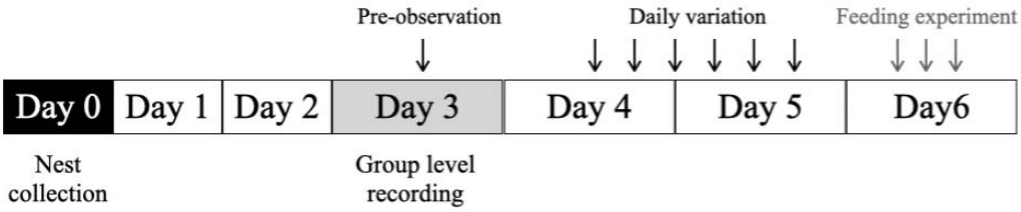

Figure 1. Experimental schedule.
The arrows indicate the number of larval calls per individual. Group-level recordings were conducted on Day 3.

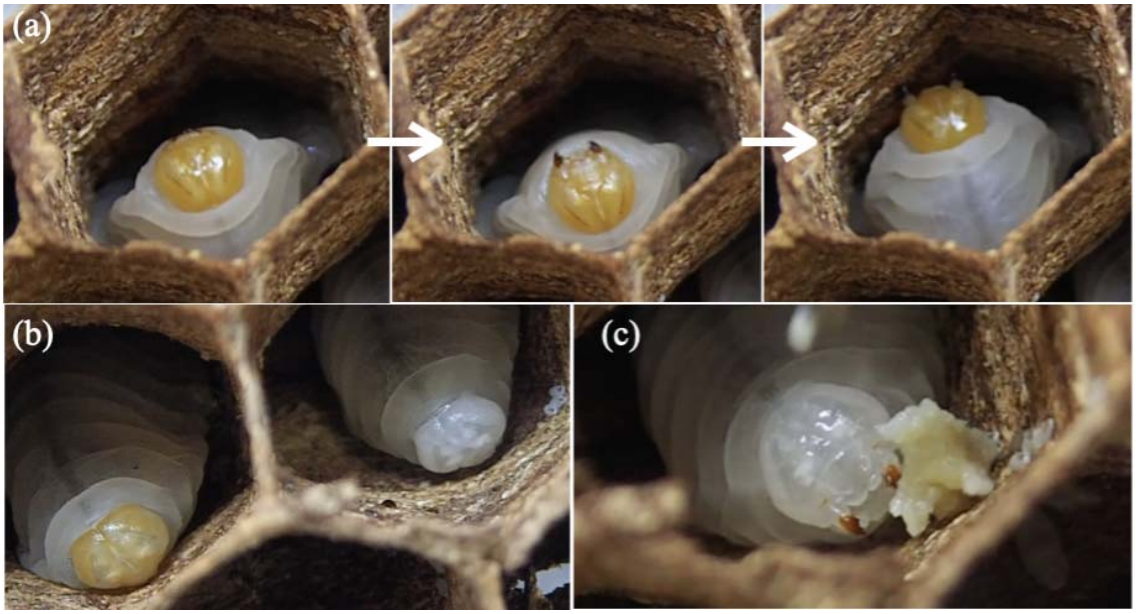

Figure 2. Larval call and stages of *Vespa mandarinia*.
Fifth-instar larvae rub their mandibles against their cell walls and produce a rhythmic ‘rasping’ sound, as recorded on October 23, 2023 (a). The number of calls per minute for fourth-instar and fifth-instar larvae. Larvae of *Vespa mandarinia* on October 24, 2023 (b-c). The larva on the left with a yellow head is a fifth-instar larva, and the larva on the right with a white head is a fourth-instar larva (b). A fourth-instar larva eating a piece of cricket (c). Photograph credit: Haruna Fujioka.

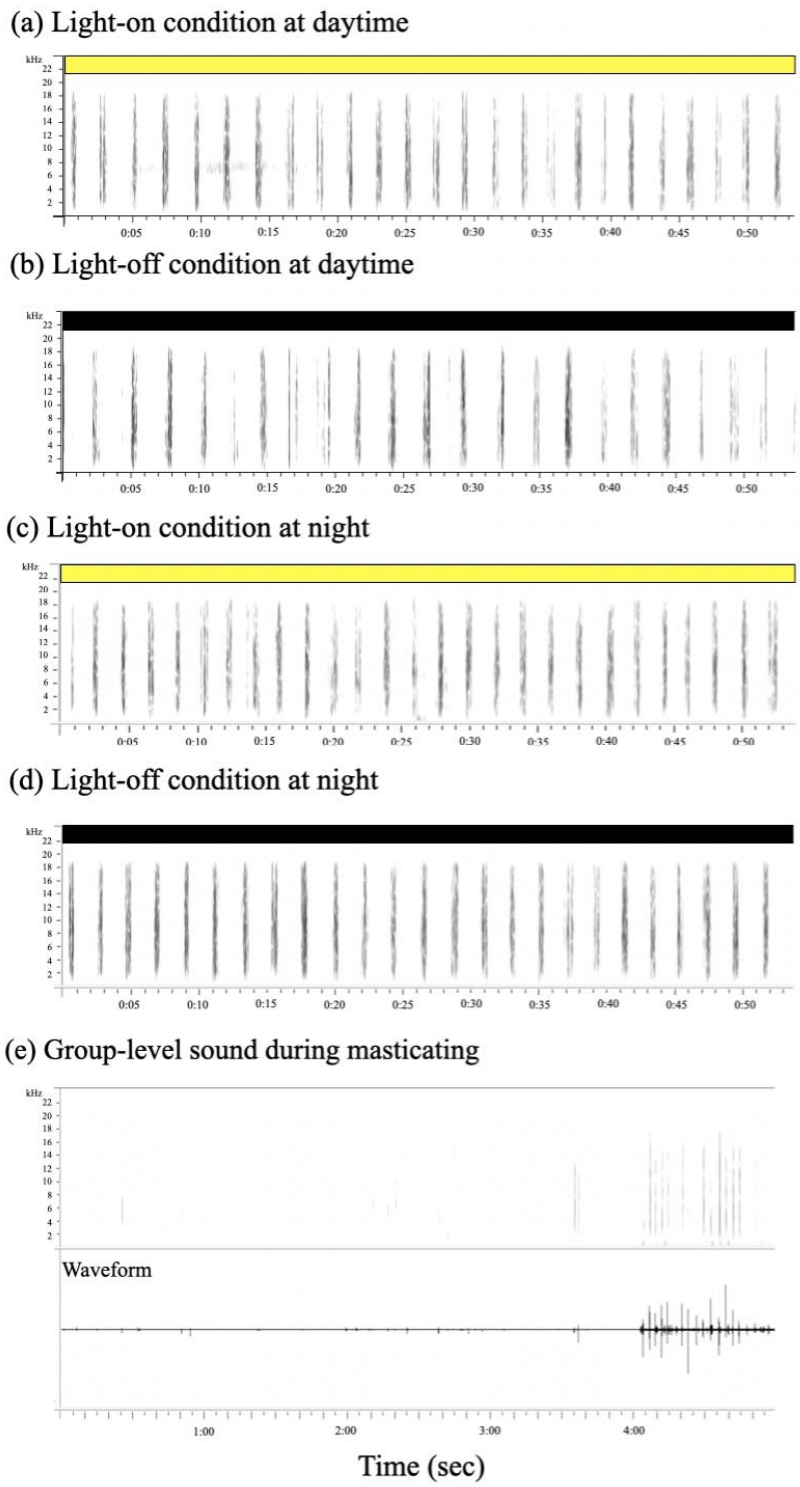

Figure 3. Spectrogram of group-level calls.

Spectrograms of group-level calls under light conditions (a, c) and dark conditions (b, d). Daytime observations were made between 2 pm and 5 pm, and nighttime observations were made between 8 pm and 10 pm. The y axis indicates the frequency (Hz). Spectrogram and waveform of group-level calls in the feeding experiment (e). Larvae stopped calling during mastication.

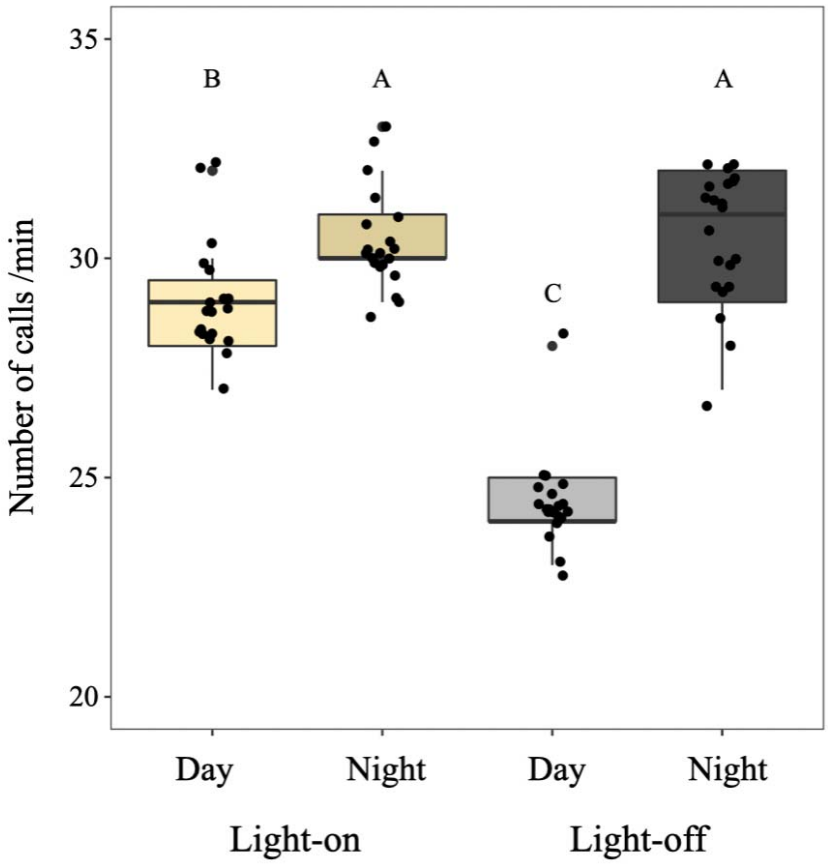

Figure 4. Number of group-level calls per minute under dark and light conditions on Day 3. The daytime observations were made between 2 pm and 5 pm, and the nighttime observations were made between 8 pm and 10 pm. The yellow and grey boxes indicate light and dark conditions, respectively. Different letters above the boxed indicate significant differences according to the Tukey-HSD test (A–C: $P < 0.05$).

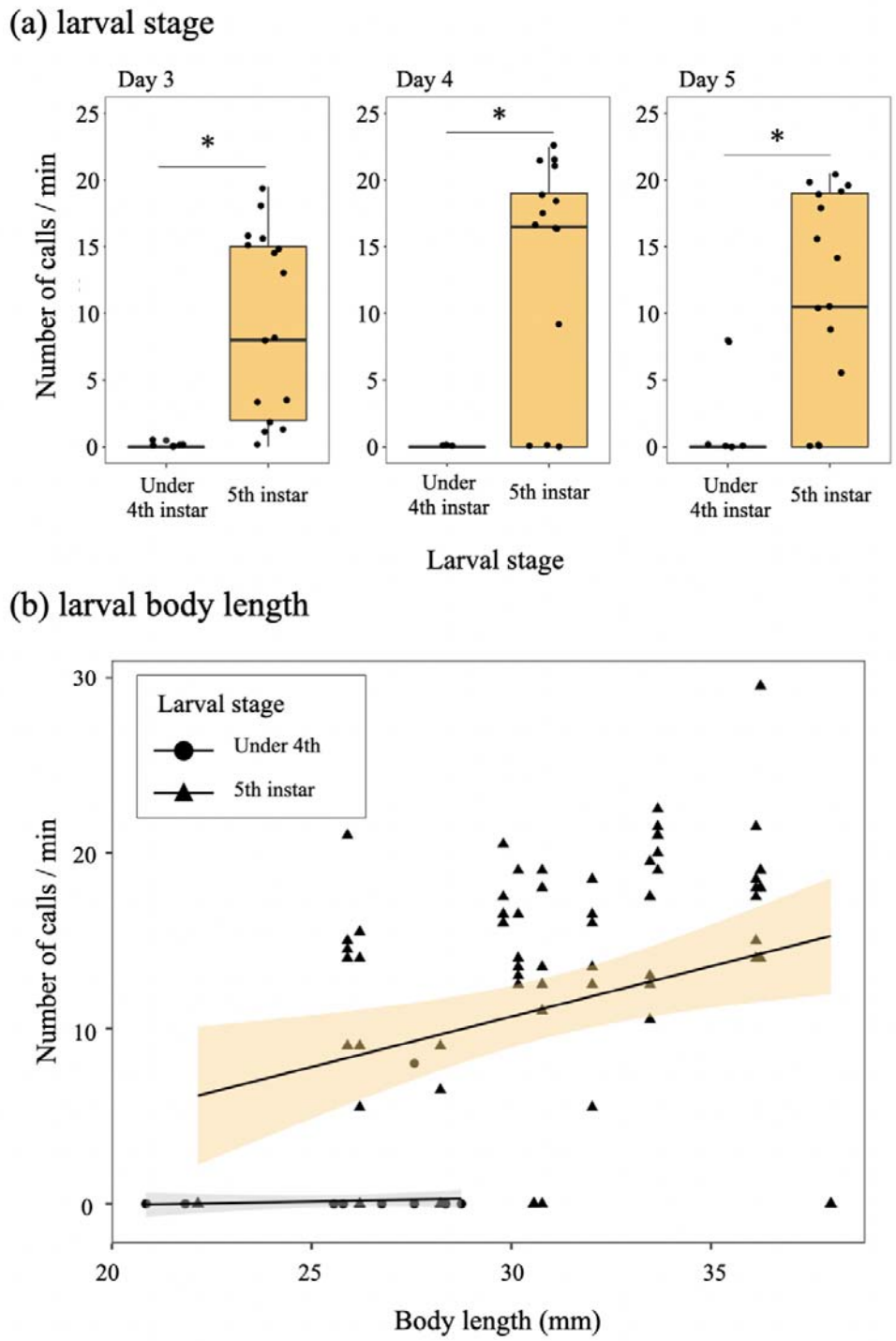

Figure 5. Effects of larval stage and body length on larval calls at the individual and group levels. The number of calls was observed at 6 pm under light conditions from Day 3 to Day 5 (a). The asterisks indicate significant differences according to the Wilcoxon test ($P < 0.05$). Larger larvae called more frequently than smaller larvae (b). The circles and triangles indicate fourth- and fifth-instar larvae, respectively. The number of calls by fifth-instar larvae was positively correlated with larval body length ($y = 0.57x - 6.5$, $p < 0.006$, $R^2 = 0.089$).

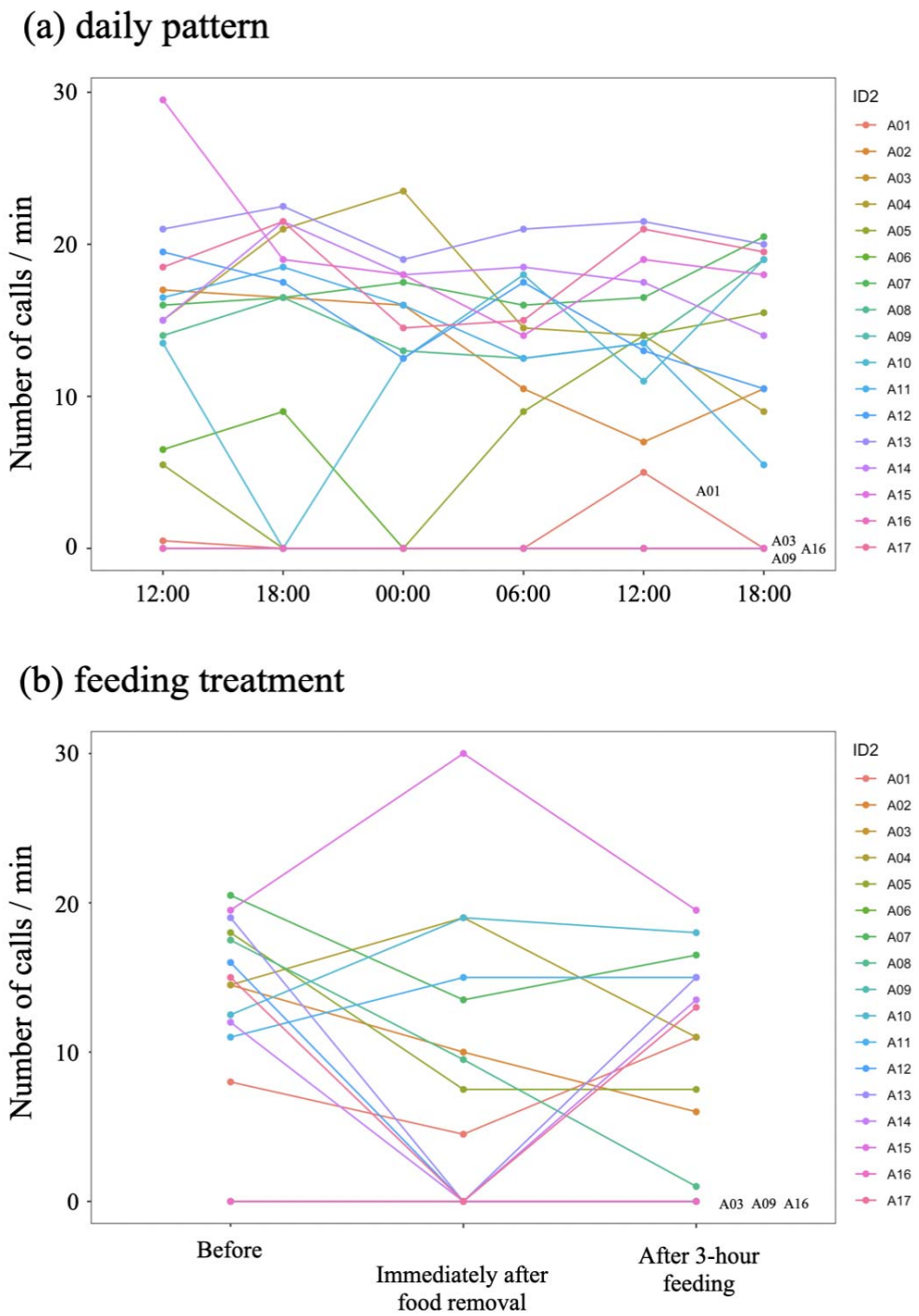

Figure 6. Larvae stopped calling during mastication.
Daily pattern of the number of calls at the individual level every 6 hours from 12 am on Day 4 to 6 pm on Day 5 (b). The number of calls at the individual level before feeding at 9 am, immediately after removing the food, and after 3 hours of feeding at 3 pm on Day 6 (c). The individual identities are sorted by larval body length.