Pupal Acoustic Behaviour of *Troides helena helena* (Linnaeus, 1758) (LEPIDOPTERA: PAPILIONIDAE) from West Java, Indonesia
(Tingkah laku Suara Pupa *Troides helena helena* (Linnaeus, 1758) (LEPIDOPTERA: PAPILIONIDAE) dari Jawa Barat, Indonesia)

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Received: November 2017, **Accepted**: January 2018

ABSTRACT

Troides helena helena (Linnaeus, 1758) can be found in Java and Bali. The larval and adult stages of this butterfly do not produce any sound, but the pupal stage produces sound in response to a touching stimulus. Sony PCM-M10 recorder and Pettersson M500 USB Ultrasound Microphone were used to record the sound. Adobe Audition 3.0 was used to visualize the oscillogram and audiospectrogram of the sound. Beyond our expectations, the pupae produce ultra sound hisses which are composed of many impulses. A one-day-old pupa has a frequency range between 0.2-40 kHz; however of 2-days to 18-days-old has a frequency range between 0.2-150 kHz. The sound wave consist of two sound elements, i.e. the audible sound element (0.2-20 kHz) and inaudible sound element (>20-150 kHz) and the bandwidth is between 200 Hz to around 150 kHz, with the strongest spectrum energy at around 2.6 kHz, 4 kHz, 6 kHz, 9 kHz and 23 kHz and some strong spectrum energy is also visible at around 40 kHz.

Key words: acoustic, behaviour, butterfly, pupa, *Troides helena helena*

ABSTRAK

Kupu *Troides helena helena* (Linnaeus, 1758) dijumpai di Jawa dan Bali. Stadium larva dan dewasa kupu ini tidak memproduksi suara, tetapi stadium larva akan memperoduksi suara sebagai reaksi sebuah stimulus berupa sentuhan. Alat perekam suara Sony PCM-M10 dan ultrasonik mikrofon Pettersson M500 USB digunakan untuk merekam suara pupa kupu ini. Perangkat lunak Adobe Audition 3.0 digunakan untuk memvisualisasi gelombang suara dan spektrum frekuensi suara. Hasil visualisasi gelombang suara pupa *T. h. helena* ternyata diluar dugaan kami, karena pupa memproduksi suara ultrasonik yang dibentuk dari banyak nada pulsa (*impulse*). Pupa berumur satu hari mempunyai kisaran frekuensi suara antara 0,2-40 kHz; sedangkan pupa berumur 2-18 hari mempunyai kisaran frekuensi antara 0,2-150 kHz. Suara pupa mempunyai dua elemen suara, yaitu elemen terdengar telinga manusia (*audible*) (0,2-20 kHz) dan elemen tidak terdengar telinga manusia (*inaudible*) (>20-150 kHz) dan lebar ban antara 200 Hz sampai sekitar 150 kHz, dengan spektrum energi kuat pada posisi sekitar 2,6 kHz, 4 kHz, 6 kHz, 9 kHz dan 23 kHz dan beberapa spektrum energi yang juga kuat terlihat pada posisi sekitar 40 kHz.

Kata kunci: akustik, perilaku, kupu, pupa, *Troides helena helena*

INTRODUCTION

Troides helena helena (Linnaeus, 1758) is one of *T. helena* sub-species which can be found in Java and Bali (Peggie 2011). *Troides helena* is included in the protected species in Indonesia and the trade is regulated by Convention on International Trade in Endangered Species of wild fauna and flora (CITES). Male and female of this butterfly can be easily distinguished by dark markings of the hindwings (Figure 1). Since November 2016, *Troides helena* has been reared in the butterfly garden of Museum Zoologicum Bogoriense (S 60^0 29' 40.2", E 106^0 51'06.3"; 165 m asl.) to reveal its ex-situ biology for conservation purpose. During the rearing activities, pupae of *T. h. helena* were observed to produce a very audible clicking sound at certain times, but larval and adult stages never produce any sound.

It has been known for over 200 years that pupal stage of butterflies can produce sounds (Hoegh-Guldberg 1972). Larval and pupal stages for many species of Lycaenidae produce sound and their sound were already documented by Downey (1966) and Hoegh-Guldberg (1972). Other publications include association between lycaenid and ants (Travassos & Pierce 2000), sound production of butterfly *Hamadryas feronia* of Nymphalidae (Yack *et al.* 2000). However, publications on larval and pupal stage on species member of Papilionidae are still lacking at this time.

Pupae of *T. h. helena* which were reared in the butterfly garden of MZB have average length of 40.66±4.56 mm (17 individuals). The pupa produced sound when it was disturbed by touching its body with hand or other objects, including other pupa. However the pupa will not produce sound by blowing the wind toward its body; in this case only a touch can make the pupa produce sound. This phenomenon has been confirmed by making a recording for two consecutive nights to get the natural sound of the pupa. This behavior may be similar to lycaenid-ants mutualism, in which some lycaenid larvae or pupae would produce sound after the ants touch the body of the larva or the pupa (Brown 2006; Devries 1991; Travassos & Pierce 2000).

During the study of pupal sound of *T. h. helena*, we did not conduct observations on sound producing organs. It is most likely that the sound was produced by the stridulatory organs, which are common in larvae and pupae of Lycaenidae group and the organs are present at segment 5 and 6 of the body (Downey 1966; Hoegh-Guldberg 1972).

In this paper, we provide spectral and temporal characteristics of sound that were produced by *T.h.helena*'s pupae; then discuss whether there is a relationship between the sounds of the pupa and the age of the pupa.

MATERIALS AND METHODS

Sounds of 17 individuals of pupae (11 males; 6 females) were recorded every other day, so we have sound data from one-day-old to 18-days-old pupae. After 18 days, the pupae are ready to emerge into adult butterflies. Sound recording was held in a soundproof room in MZB from 16 January to 1 February 2017.

Sony PCM-M10 recorder and Pettersson M500 USB Ultrasound Microphone were used to record pupal sound. The Sony recorder was set at a sampling frequency of 94 kHz and a bit rate of 24 bits, and the ultra sound microphone was set by connecting it to a PC tablet with trigger sensitivity level of 3% (wide bandwidth), db 0-100%. Room temperature during sound recording was around 27°C. The recording duration for each pupa was about one minute. To find out whether the pupa makes a natural sound at night without any touching activity on the pupa's body; 3 individual pupae were prepared for recording for two consecutive nights (25-26 January 2017) by using Pettersson M500 USB Ultrasound Microphone with recording time between 5.00 PM to 8.00 AM (total 30 hours of recording).

Pupa will produce sounds when the body of the pupa is in contact with other objects; this behavior was similar with several species of Lycaenidae (Downey 1966); Hoegh-Guldberg 1972). In this study, a brush was used to touch the abdomen (Figure 2); one touch usually produces one sound, but sometimes the pupa keeps silent even when its body was touched by a brush many times. To maximize the sound, the distance between the pupa and the Sony recorder was about 5 cm and the distance for Pettersson M500 USB Ultrasound Microphone was about 15 cm. To avoid fatigue condition, recording time for one individual pupa was about 1 minute. Sound characters that were used in this study are pulse train duration of audible and inaudible sound, energy of strong frequency,

Figure 1. Male individual (left), and pupa (right) of *Troides helena helena* from West Java. Horizontal scale bar is equal to 1 cm long (Photograph by D. Peggie).

minimum and maximum frequency. For modulated maximum frequencies that were generally present at the inaudible sound frequency, the maximum frequency that was used as sound character was the most dominant frequency which was generated by the spike row in a single pulse train.

Adobe Audition 3.0 software was used to visualize the oscillogram and audiospectrogram of the sound by taking FFT (Fast Fourier Transformation; 1024 points) at Hanning window. Statistic software *Past* software application (Hammer *et al.* 2001, http://palaeo-electronica.org/2001_1/past/issue1_01.htm) was used to analyze data of the sounds, including pulse train duration, strong frequencies on each pulse train, minimum and maximum frequency of each pulse train. The statistic software application was also used to construct data charts.

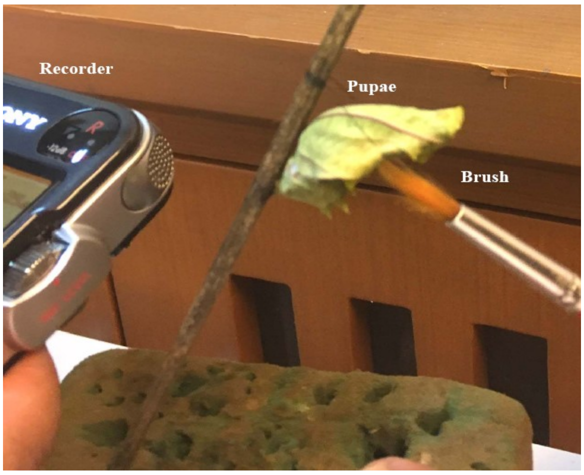


Figure 2. Sound provoked to the pupa of *Troides helena helena* by external agitation using a brush (photograph by D. Peggie).

RESULTS

Sounds of the pupa are ultrasound hisses, formed by pulse train which consist of a series of many spikes (Figure 3). The spikes are usually very dense in the initial to the middle row, and are usually not dense at the middle to the end of row, so gaps in between spikes can be seen clearly. Duration of these gaps was about 1-2 ms (mille second). Pulse train of sound that was formed by many spikes makes visualisation of the pulse train have amplitude and frequency modulation.

A total of 1785 sound was analyzed in this study. The visualization of audiospectrogram indicates a dependence on the age of the pupa; a one-day-old pupa has a range of frequencies between 0.2-40 kHz; however sound waves of pupae of 2-days to 18-days-old pupae consist of two sound elements the audible sound element (0.2-20 kHz) and inaudible sound element or ultrasound (>20-140 kHz) and the bandwidth is between 200 Hz to 140 kHz.

Most raw data of audible or inaudible sound durations are dynamic (CV>12%) (Gerhardt 1991); whereas all of raw data of the maximum frequency of inaudible sound is static. The sound of pupa is divided into two parts; they are the audible part and the inaudible part. The two parts are different in sound duration and sound frequency.

A. Audible sound

Audible pupal sound of *T. h. helena* starts from around 200 Hz. Sound frequency rises rapidly following the age of pupa. A one-day-old pupa has a maximum frequency of about 40 kHz (Figure 4); however, a two-days-old pupa has a maximum frequency of more than 100

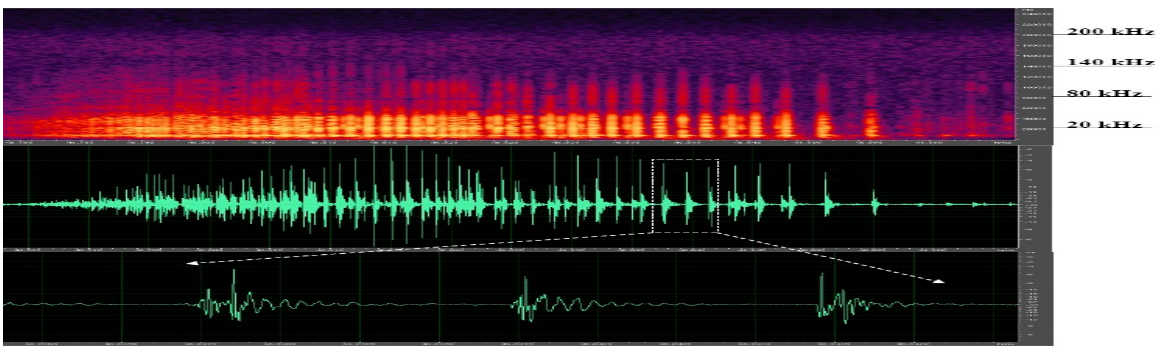


Figure 3. Audiospectrogram and oscillogram of one pulse train and tree spikes of seven days old pupa by using Pettersson M500 USB ultrasound microphone.

kHz. The relationship between duration of the sound and sound frequency in the one-day-old pupa is individualistic; of the four individuals, only two individuals make sounds that can be well visualized. A male pupa (code 3123) produced sound with a static frequency, it was 20 kHz with duration 51.5 ± 4.7 ms (n=34); in this case there is no correlation between sound duration and sound frequency. However, on a female pupa (code 3124), there was a significant relationship between sound duration (68.5±14.2 ms) and sound frequency (40.3±0.9 kHz) (Pearson Correlation=0.0463; 2 tailed; n=42; p=0.002), which shows the tendency of longer duration of the sound resulted in higher frequency. Most sounds that were produced by the spikes have strong energy; the energy of the audible sound was usually divided into four peaks (Figure. 8), the peaks were at 2.6 kHz, 4 kHz, 6 kHz and 9 kHz.

Results of trend line of linear regression analysis which was based on average value of audible sound durations each day of pupa age showed at Table 1. The duration of sounds that was released by the pupa was also individualistic behavior (Figure. 5); which was a recording time of about 1 minute generated the number of sound and its duration was likely to depend on the pupa's fitness; but generally, when the pupa gets older, the duration tended to be static, which was characterized by the R^2 value of less than 4; or longer which was characterized by the R^2 value of more than 4. Overall there was no decreasing trend of sound duration with age of pupa, which was this behavior can be proven from no negative trend line equation of any results of linear regression trend line that were shown at Table 1.

B. Inaudible sound

Elements of the ultrasonic sound produced various maximum frequencies, the lowest maximum frequency was around 100 kHz and the highest was about 140 kHz, but some reached 150 kHz (Figure 6 and 7). The maximum frequency in a single pulse train varied widely, some pulse trains had a constant frequency and some had a frequency modulation (Figure 7); one individual pupa could produce flat and modulated frequency in one minute touching. Constant frequency in a single pulse train could be at the level of 110 kHz, 130 kHz, 120 kHz and 140 kHz; flat frequency was never at 100 kHz or 150 kHz, although the 100 kHz frequency was the lower limit and 150 kHz was the upper limit. Pulse trains that had modulation frequency showed an undulation wave

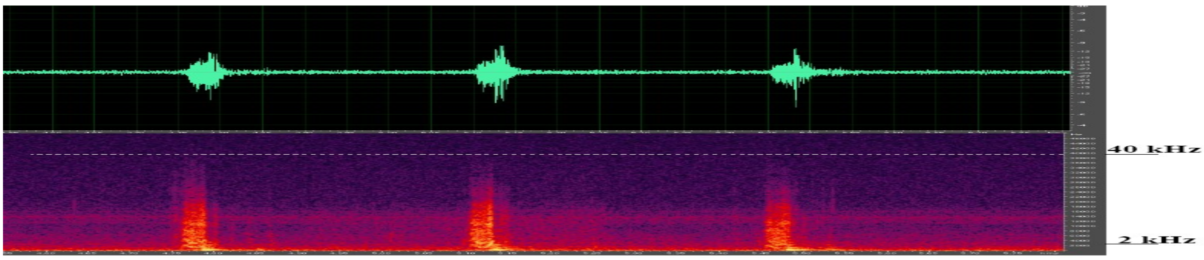
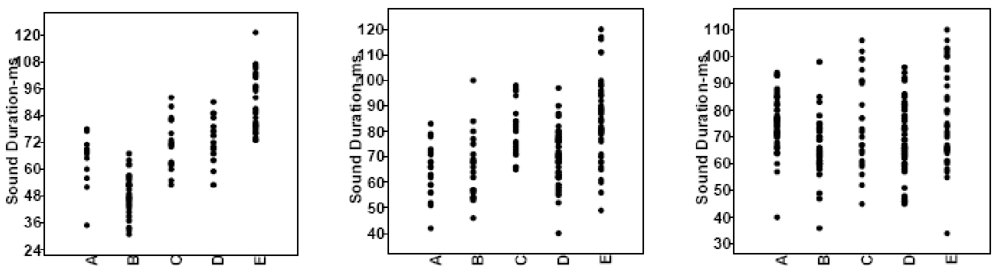


Figure 4. Oscillogram and audiospectrogram of sound (3 pulse train) of one-day-old pupa by using Sony PCM-M10 recorder. White line was frequency at 40 kHz.



3123-Male **3124-Female** **3116-Male**

Figure 5. Individual duration of sound that were produced by three individuals of pupae (individual code: 3123, 3124 and 3116) of *Troides helena helena*. Y axis is sound duration in mille second; X axis is day of observation (young to old age).

formation, the frequency could be high in the front row and then down in the middle row and up in the rear section row, or the undulation could be otherwise. Similar with the audible sound, most of inaudible sound also had strong energy; the spectrum energy of the inaudible sound was usually divided into two peaks (Fig. 8), the peaks were at 23 kHz and 40 kHz.

The number of sound and its duration of inaudible sound was also individualistic behavior, which was similar to the case of audible sound (Figure 5). Results of statistical analysis by using two tailed T-test showed that the duration of audible sound in general was significantly different from the duration of inaudible sound, but only three cases showed no significant difference (Table 2).

The results of this pupa sound research reveal dynamic data is on sound duration and sound frequency, but not on the data of energy which is constant; therefore only the data of sound duration and sound frequency were then analyzed. Results of correlation analysis between sound duration and maximum frequency showed that there was

Table 1. Results of linear regression which is taken from the average value of duration per day of nine individuals of pupae of *Troides helena helena* by using Sony PCM-M10 recorder. M: male; F: female. R^2: coefficient determination.

Tend Line of Linier Regression	Individual code								
	3114-M	**3115-F**	**3116-M**	**3122-M**	**3124-F**	**3123-M**	**3127-M**	**3132-M**	**3136-M**
Days of observation (young to old age)	8	8	8	6	8	7	7	6	2
Average value of duration	113.210; 73.428; 100.100; 63.222; 86.425; 103.400; 98.116; 102.059	94.167; 60.555; 85.871; 80.193; 77.538; 80.333; 81.423; 93.444	101.273; 61.118; 97.934; 102.256; 96.789; 103.935; 92.023; 100.763	31.407; 32.222; 36.343; 38.704; 28.400; 68.692	68.524; 58.461; 74.000; 72.167; 84.895; 89.318; 66.048; 91.930	51.759; 65.857; 83.428; 64.241; 85.182; 79.454; 100.429	61.643; 49.500; 57.385; 70.200; 88.480; 75.412; 82.488	63.000; 78.409; 77.704; 87.158; 87.954; 94.797	77.933; 90.250
Trend line Equation	y = 0.9343x + 88.291	y = 0.9524x + 77.405	y = 1.9464x + 85.753	y = 5.0662x + 21.563	y = 3.1007x + 61.715	y = 6.2485x + 50.7	y = 5.1948x + 48.522	y = 5.6307x + 61.796	y = 12.317x + 65.617
R^2	**0.018**	**0.049**	**0.116**	**0.407**	**0.408**	**0.693**	**0.644**	**0.899**	**1.000**

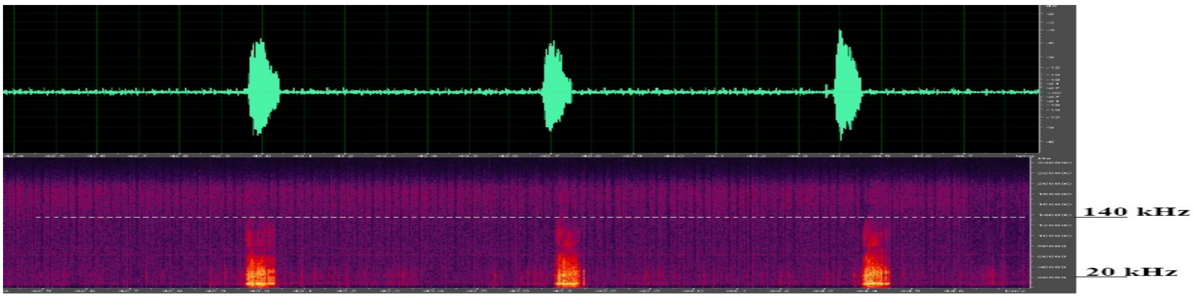


Figure 6. Oscillogram and audiospectrogram of sound (3 pulse train) of five-days-old pupa by using Pettersson M500 USB Ultrasound Microphone. White line is frequency at 140 kHz.

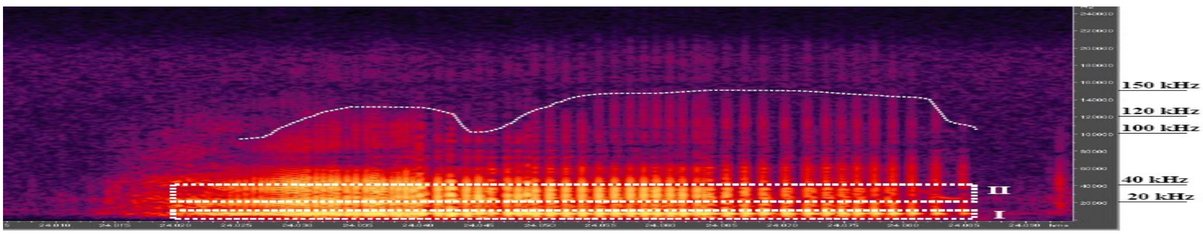


Figure 7. Visualisation of frequency modulation (upper white line) of one pulse train of *Troides helena helena*'s pupa; (I) energy of frequency of audible sound; (II) energy of frequency of inaudible sound.

a phenomenon that was also individualistic (Table 3); the significant correlation value of the results spread randomly from day 6 to day 18 of nine individual pupae, but most of the results of the analysis showed no correlation between the sound duration with the maximum frequency of sound.

Results of linear regression trend line analysis which was based on average value of inaudible sound durations and sound frequency each day of pupal age are shown at Table 4. Tendency of the inaudible duration with age was similar to tendency of audible sound (Table 1). However, results of linear regression trend line analysis which was based on average value of inaudible sound frequency showed a tendency to static or decrease with pupal age (Table 4), in which the decreasing tendency was shown by negative equation of individuals 3132 and 3115; in this case only individual 3124 had a strong rising tendency.

The results of the determination coefficient (R^2) of the audible sound duration with the inaudible sound frequency are shown in Table 4, and there was an inverse result. The sound duration tends to increase but the sound frequency tends to decrease as the pupae get older.

DISCUSSION

Inaudible sound in invertebrates that has frequency of more than 20 kHz is frequently registered in insects (Kohler *et al.* 2017). The organ that plays a role in sound production in insects is stridulatory organ, in which the presence of the organ on the body of the insect varies among species. The organ may be found at the

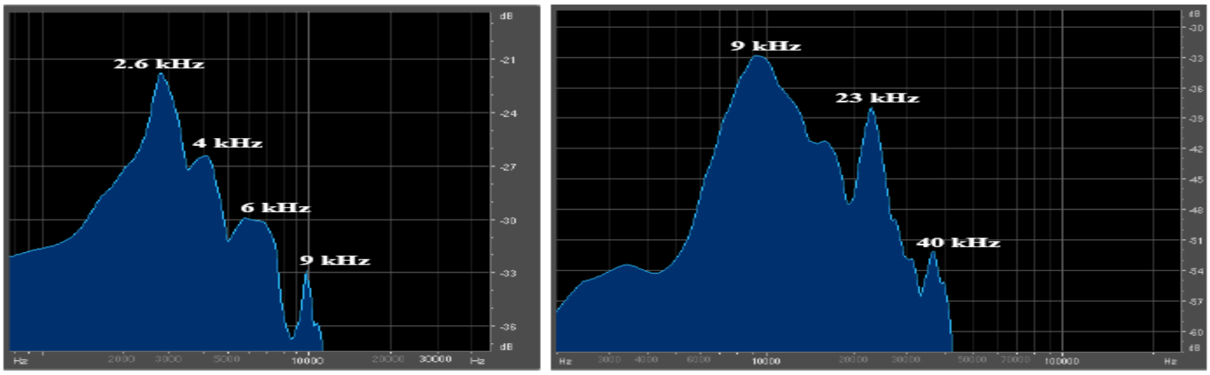


Figure 8. Spectrum Energy below 10 kHz of one pulse train of seven–days-old pupa by using Sony PCM-M10 recorder (left); and energy above 10 kHz of one pulse train of seven-days-old pupa by using Pettersson M500 USB Ultrasound Microphone (right).

Table 2. Results of Two tailed T-test analysis between audible sound duration and inaudible sound duration per day of nine individuals of *Troides helena helena* pupae. M: male; F: female; (*) not significantly different, p>0.05.

Two tail T-test: Audible Duration versus Inaudible Duration/age	Individual code								
	3114-M	3115-F	3116-M	3124-F	3123-M	3127-M	3132-M	3136-M	3117-M
p for day 6	-	-	-	-	-	0.000	-	-	-
p for day 7	-	-	-	-	-	-	0.000	-	-
p for day 8	-	-	-	0.082*	-	-	0.000	-	-
p for day 9	0.033	0.001	0.000	-	-	-	-	-	-
p for day 10	-	-	-	-	-	0.150*	-	-	-
p for day 11	0.000	0.029	0.000	-	-	-	0.000	-	-
p for day 12	-	-	-	0.001	0.031	0.000	-	-	-
p for day 13	0.000	0.008	0.000	-	-	-	-	0.000	0.000
p for day 14	-	-	-	-	-	-	0.000	-	-
p for day 15	-	-	-	0.005	0.074*	0.000	-	0.019	-
p for day 16	0.000	0.000	0.000		0.000		0.000	-	-
p for day 17	-	-	-	0.010	-	0.000	-	-	-
p for day 18	0.034	0.000	0.000	-	-	-	-	-	-

abdominal segment (Devries 1991; Downey 1966; Hoegh-Guldberg 1972), on legs (Lees 1992), at the reproductive organ (Gwynne 1986) or at the wings (Garzon-Orduña 2004; Garzon-Orduña 2012; Kane 1982; Mohl & Miller 1976; Yack *et al*. 2000). Pupa of *T. h. helena* appears to have stridulatory organ at the abdominal segment, because when its body was touched by using a brush (Figure 2), the abdominal part constricted to the distal part of the body. It seems that the development of the stridulatory organ occurs only at the pupal phase, because there is no sound produced by larvae and adults. The sound is very specific, because it has audible and inaudible components in one bandwidth frequency.

The sound that was produced by the pupa of *T. h. helena* was individualistic behavior (Table 2 and Table 3), because it does not depend on sex and the age of the pupa. It is most likely the fitness factor of the pupa affects the ability of the pupa to make a sound, because when the pupa was touched for more than a minute has a tendency of fatigue, which is indicated by the decreased number of sounds produced. This individualistic characteristic was also found in cherry leaf roller caterpillar (Fletcher *et al*. 2006).

The individualistic audible and inaudible sound characters of *T. h. helena* pupa had a positive tendency depending on the age for duration character (Table 1 and Table 4), but showed a negative trend on the sound frequency character (Table 4). Positive trends on sound duration are most likely influenced by the presence of teeth of the stridulatory organ, so when the pupa grew older, the number of new teeth increased. This possible addition of new teeth have not been able to produce ultra sonic sounds, consequently the duration of the audible sound was significantly different from the duration of the inaudible sound. On the contrary, when the pupa was getting older, the teeth that grew first would decrease its function, so increasing age made inaudible sound frequency tended to decrease, but inaudible sound duration tended to rise (Table 4). This phenomenon may be the cause that made that the pulse trains have a modulation maximum frequency (Figure 7).

Pupa of *T. h. helena* does not produce sound when its body is not in contact with other objects (in this study a brush was used). The pupa gave response to the touching by releasing ultrasonic sound. The ultrasonic was most likely related to its natural defensive mechanism to expel parasites or predators. In some species of pest-type moths on agricultural plants, the moths are highly sensitive to ultrasonic sounds thus can escape the predation (Payne & Shorey 1968; Salehi *et al*. 2016; Spangler & Takessian 1983). Unlike sounds that are produced by some groups of beetles, which use sound for communication among individuals of the same species (Castellanos & Barbosa 2006; Kojima *et al.* 2012a; Kojima *et al.* 2012b) or for communication with other species, as can be seen for the most popular cases of the symbiotic relation of lycaenid

Table 3. Results of correlation analysis between sound duration and sound frequency per day of nine individuals of Troides helena helena pupa by using Pettersson M500 USB Ultrasound Microphone. M: male; F: female; (*) significant correlation.

Correlation analysis: Duration versus Frequency/age	Individual code								
	3114-M	3115-F	3116-M	3124-F	3123-M	3127-M	3132-M	3136-M	3117-M
p for day 6	-	-	-	-	-	0.031*	-	-	-
p for day 7	-	-	-	-	-	-	0.779	-	-
p for day 8	-	-	-	0.096	0.025*	-	-	-	-
p for day 9	0.672	0.366	0.785	-	-	-	0.007*	-	-
p for day 10	-	-	-	0.548	0.936	0.651	-	-	-
p for day 11	0.442	0.839	0.588	-	-	-	0.794	-	-
p for day 12	-	-	-	0.043*	0.297	0.745	0.220	-	-
p for day 13	0.488	0.16	0.233	-	-	-	-	0.715	0.005*
p for day 14	-	-	-	-	-	-	0.958	-	-
p for day 15	-	-	-	0.577	0.152	0.005*		0.376	-
p for day 16	0.25	0.015*	0.001*	-	-	-	0.487	-	-
p for day 17	-	-	-	0.067	0.273	0.404	-	-	-
p for day 18	0.754	0.686	0.322	-	-	-	-	-	-

Table 4. Results of linear regression trend line which was taken from the average value of duration and frequency per day of six individuals of *Troides helena helena* pupae by using Pettersson M500 USB Ultrasound Microphone. M: male; F: female; (*) negative equation. R^2: coefficient determination

Individual code					
3116-M	**3132-M**	**3115-M**	**3127-F**	**3124-F**	**3123-M**
5	6	5	5	5	5
75 (9)	71.34 (7)	68.65385 (9)	58.625 (6)	64.42105 (8)	63.45455 (8)
66.15152 (11)	64.75 (9)	68.1 (11)	55.85714 (10)	66.36842 (10)	49.14815 (10)
75.83333 (13)	68.07895 (11)	71.28571 (13)	73.91667 (12)	80.5 (12)	71 (12)
69.13953 (16)	64.42857 (12)	65.5 (16)	64.64286 (15)	70.42857 (15)	73.42857 (15)
77.65789 (18)	78.45455 (14)	81 (18)	69.12195 (17)	84.93333 (17)	88.89286 (17)
	76.32727 (16)				
y = 0.8304x + 70.265	y = 1.7827x + 64.324	y = 2.2092x + 64.28	y = 2.978x + 55.499	y = 4.5085x + 59.805	y = 7.5157x + 46.638
0,072	**0,32**	**0,338**	**0,404**	**0,631**	**0,669**
127.5556 (9)	137.2727 (7)	120.7692 (9)	119.6875 (6)	120.5263 (8)	119.0909 (8)
138.7879 (11)	138.2143 (9)	127.5 (11)	120; (10)	116.3158 (10)	91.48148 (10)
114.5833 (13)	116.0526 (11)	113.2143 (13)	110.8333 (12)	115.9091 (12)	112.3529 (12)
120.6977 (16)	115 (12)	127.3684 (16)	123.5714 (15)	124.0476 (15)	116.4286 (15)
137,3684 (18)	130 (14)	117,381 (18)	126,0976 (17)	130,4444 (17)	115,3571 (17)
	131,0909 (16)				
y = 0.1535x + 127.34	y = -1.6173x + 133.6*	y = -0.6908x + 123.32*	y = 1.6392x + 115.1	y = 2.7568x + 113.18	y = 1.748x + 105.7
0,0005	**0,089**	**0,03**	**0,2**	**0,522**	**0,061**

butterflies with ants (Devries 1990; Devries 1991; Naomi *et al.* 2002; Travassos & Pierce 2000). In the case of *T. h. helena*'s pupa, the sound is most likely only to defend itself from disturbance of parasites or predators, but further study is needed to investigate this.

The types of sound that were produced by Papilionidae group have not been widely reported. There are very wide spectrums of sounds, which include audible and inaudible components that have not been reported elsewhere for butterflies. Based on phylogeny of the Papilionidae group, *Pachliopta* is also in the same clade of Troidini with *Troides* (Caterino *et al*. 2001). At the butterfly garden at Cibinong Science Center, LIPI, some other butterflies, including *Pachliopta adamas*, are raised. Brief observation on pupae of *P. adamas* indicated that this species also produced audible and inaudible sound by touching its body, and larval and adult stages do not produce sound. Pupa behaviour of *P. adamas* is very similar to *T. h. helena*. It is likely that this close relative of *T. h. helena* also indicates similar sound characters. However, further study is needed, also by adding more sound description of other closely related taxa as published by Caterino *et al*. (2001).

CONCLUSIONS

The sound that was produced by pupae of *T h. helena* is individualistic oriented and unaffected by the sex and the body length of the pupa. Sound frequency spectrum has a very wide bandwidth, which starts from 0.2 kHz up to a maximum of 150 kHz. Duration of the sound tends to rise but the sound frequency tends to decrease in accordance with the increase of pupal age.

ACKNOWLEDGMENTS

We are thankful to Pak Supadi who has helped us a lot in sound recording activities. Many thanks to be addressed to Dr. Arjan Boonman who has helped us analyse the sounds. Finally, many thanks are due to Biovillage Project that has financed the breeding, rearing and maintenance of the butterfly garden at Cibinong Science Center, LIPI.

REFERENCES

Brown, SG. 2006. Clicking caterpillars: Acoustic aposematism in *Antheraea polyphemus* and other Bombycoidea. A dissertation. Submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Master of Science in Biology. Carleton University, Ottawa, Ontario. ix+61 pp.

Castellanos, I. & P. Barbosa. 2006. Evaluation of predation risk by a caterpillar using substrate-borne vibrations. *Animal Behaviour* 72: 461-469.

Caterino, M.S., RD. Reed, MM. Kuo & FAH. Sperling. 2001. A partitioned likelihood analysis of swallowtail butterfly phylogeny (Lepidoptera: Papilionidae). *Systematic Biology* 50(1):106–127.

Devries, PJ. 1990. Enhancement of symbioses between butterfly caterpillars and ants by vibrational communication. *Science* 248 (4959): 1104-1106.

Devries, PJ. 1991. Call production by myrmecophilous riodinid and lycaenid butterfly caterpillars (Lepidoptera): morphological, acoustical, functional, and evolutionary patterns. *American Museum Novitates* (3025): 1-23.

Downey, JC. 1966. Sound production in pupae of Lycaenidae. *Journal of the Lepidopterists' Society* 20(3): 129-155.

Fletcher, LE., JE. Yack, TD. Fitzgerald & RR. Hoy. 2006. Vibrational communication in the cherry leaf roller caterpillar *Caloptilia serotinella* (Gracillarioidea: Gracillariidae). *Journal of Insect Behavior* 19(1): 1-18.

Garzon-Orduña, IJ. 2004. Phylogenetic relationships and character evolution of the neotropical butterfly genus *Hamadryas* (Nymphalidae: Biblidinae). A Dissertation. Submitted to the Graduate Faculty of the University of New Orleans in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Conservation Biology. 111 pp.

Garzon-Orduña, IJ. 2012. Phylogenetic evidence for loss of sound production and a shift in sexual recognition signals in *Hamadryas* butterflies (Nymphalidae: Biblidinae). *Systematic Entomology* 37: 84-101.

Gerhardt, HC. 1991. Female mate choice in treefrogs: static and dynamic criteria. *Animal Behaviour* 42: 615-635.

Gwynne, DT. 1986. Ultrasound production by genital stridulation in *Syntonarcha iriastis* Lepidoptera: Pyralidae): long-distance signalling by male moths? *Journal of the Linnean Society* 88: 363-376.

Hammer, Ø., DAT. Harper & PD. Ryan. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 9pp. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Hoegh-Guldberg, O. 1972. Pupal sound production of some Lycaenidae. *Journal of Research on the Lepidoptera* 10(2): 127-147.

Kane, S. 1982. Notes on the acoustic signals of a neotropical satyrid butterfly. *Journal of the Lepidopterists' Society* 36(3): 200-206.

Kohler, J., M. Jansen, A. Rodriguez, P.J.R. Kok, L.F. Toledo, M. Emmrich, F. Glaw, C.F.B. Haddad, M-O. Rodel & M. Vences. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa* 4251(1): 001-124.

Kojima, W., T. Takanashi & Y. Ishikawa. 2012a. Vibratory communication in the soil: pupal signals deter larval intrusion in a group-living beetle *Trypoxylus dichotoma*. *Behavioral Ecology and Sociobiology* 66:171-179.

Kojima, W., Y. Ishikawa & T. Takanashi. 2012b. Pupal vibratory signals of a group-living beetle that deter larvae. Are they mimics of predator cues? *Communicative and Integrative Biology* 5(3): 262-264.

Lees, DC. 1992. Foreleg stridulation in male *Urania* moths (Lepidoptera: Uraniidae). *Journal of the Linnean Society* 106: 163-170.

Mohl, B & LA. Miller. 1976. Ultrasonic clicks produced by the peacock butterfly: a possible bat repellent mechanism. *Journal of Experimental Biology* 64: 639-644.

Payne, TL. & HH. Shorey. 1968. Pulsed ultrasonic sound for control of oviposition by cabbage looper moths. *Journal of Economic Entomology* 61(1): 3-7.

Peggie, D. 2011. *Precious and Protected Indonesian Butterflies.* PT Binamitra Megawarna, Jakarta, 72 pp.

Salehi, SS., A. Rajabpour, A. Rasekh & M. Farkhar. 2016. Repellency and some biological effects of different ultrasonic waves on Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae). *Journal of Stored Products Research* 69: 14-21.

Spangler, HG & A. Takessian. 1983. Sound perception by two species of wax moths (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America* 76(1): 94-97.

Travassos, MA & NE. Pierce. 2000. Acoustics, context and function of vibrational signalling in a lycaenid butterfly–ant mutualism. *Animal Behaviour* 60: 13-26.

Yack, JE., LD. Otero, JW. Dawson, A. Surlykke & J.H. Fullard. 2000. Sound production and hearing in the blue cracker butterfly *Hamadryas feronia* (Lepidoptera, Nymphalidae) from Venezuela. *The Journal of Experimental Biology* 203: 3689 -3702.