การเปรียบเทียบวงจรชีวิตหนอนปลอกน้ำ *Himalopsyche acharai* (Trichoptera: Rhyacophilidae) ในภาคเหนือ ประเทศไทยกับหนอนปลอกน้ำ *H. japonica* จากประเทศญี่ปุ่น

The Comparison on Life Cycle of *Himalopsyche acharai* (Trichoptera: Rhyacophilidae) found in Northern Thailand and *H. japonica* from Japan

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บทคัดย่อ

การศึกษาวงจรชีวิตของแมลงน้ำอันดับไทรคอบเทอร่า (Carnivorous caddisfly) *Himalopsyche acharai* ในลำธารน้ำบนภูเขา ในภาคเหนือประเทศไทย ที่ระดับความสูง 900 เมตรจากระดับน้ำทะเล การเก็บข้อมูลทุกเดือน พบการ เจริญเติบโตตัวอ่อนของแมลง ตลอดทั้งปีภายใต้อุณหภูมิน้ำต่ำ และมีการพัฒนาวงจรชีวิตแบบหลายรุ่นต่อปีในทั้งสองแหล่ง และพบว่า *H. acharai* อาศัยอยู่ในน้ำที่มีอุณหภูมิต่ำกว่า *H. japonica* ที่อาศัยในประเทศญี่ปุ่น และ *H. acharai* มีขนาดเล็กกว่า *H. japonica* อย่างมีนัยสำคัญ ความกว้างของหัวกะโหลก ของแมลงหนอนปลอกน้ำ ในระยะตัวอ่อนที่ 5 ที่เก็บในช่วงฤดูร้อนมีขนาดเล็กกว่าตัวอ่อนในฤดูหนาว ดังนี้ อุณหภูมิของน้ำเย็นมีผลต่อขนาดของตัวอ่อน ในขณะเดียวกัน ความหนาแน่นของแมลงหนอนปลอกน้ำลดลงในช่วงฤดูน้ำหลาก ความหนาแน่นของแมลงชนิดนี้ลดลงหากกระแสน้ำแยกเป็นหลายสายในฤดูแล้ง และเพิ่มขึ้นเมื่อกระแสน้ำไหลรวมกัน ในฤดูแล้งตัวอ่อนจะเคลื่อนย้ายจากลำธารสาขาสู่ลำธารหลัก การลดลงของความหนาแน่นประชากร จะขึ้นอยู่กับน้ำหลากและฤดูแล้งที่ทดแทนระหว่างกันของประชากรในพื้นที่ลำธารหลักและลำธารสาขา

คำสำคัญ: แมลงหนอนปลอกน้ำ *Himalopsyche acharai H. japonica*

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ABSTRACT

The life cycle of the carnivorous caddisfly, *Himalopsyche acharai,* was studied in two mountain streams at about 900 m ASL in Northern Thailand and it was compared with *H. japonica* in Japan. From monthly field sampling, larvae developed continuously throughout the year under mild water temperature and the life cycles in both streams are estimated to be multivoltine. The water temperatures of the streams inhabited by *H. acharai* are clearly milder than the streams inhabited by *H. japonica* in Japan, *H. acharai* are significantly smaller in size than *H. japonica*. The head capsule widths of 5th instar larvae of *H. acharai* collected during rainy season in relatively warm water temperatures were significantly smaller than the larvae collected during dry season in relatively cool water temperatures. This observation suggests that relatively cool water temperature makes larvae large. While the insect density decreased in the main stream due to flooding in rainy season, the density increased in branch streams. In dry season, the density in branch streams decreased from drought but increased in the mainstream. It is suggested that the larvae are supplied from branch streams to the mainstream in dry season. The population density fluctuations caused by flood and drought are supposed to be compensated between local populations in the mainstream and branch streams.

Keywords: *Himalopsyche acharai*, *Himalopsyche japonica*

Introduction

Genus *Himalopsyche* belongs to the family Rhyacophilidae, order Trichoptera. There are numerous studies of life cycles of *Rhyacophila* which are common species in Rhyacophilidae (Elliott, 1968; Manuel and Folsom, 1982; Singh *et al.*, 1984; Martin, 1985; Irons, 1988; Dixon and Wrona, 1992; Lavandier and Cereghino, 1995). Although most *Himalopsyche* species are widely distributed in Himalayan, southeastern and eastern regions in Asia, including tropical zones (Schmid and Botosaneanu, 1966), the life cycle of only one temperate species has been reported, *viz. Himalopsyche japonica*, which has a univoltine cycle in cool mountain streams in central Japan (Tsuruishi, 2003). However, many other species of *Himalopsyche* are distributed in tropical regions in southern Asia and are assumed to have different life cycles. It is known that flexible voltinism occurs in *Rhyacophila* species due to water temperature differences between habitats at different latitudes or elevations (Gose, 1970; Mackay, 1984; Rutherford and Mackay, 1986; Lavandier and Cereghino, 1995). The life cycle of *H. japonica* is also affected by the water temperature of their habitats (Tsuruishi, 2006). At present global warming is an important concern for us. If we know the life cycle of species in tropical regions, we can compare it with that of *H. japonica*, and thereby supply information to help predict the effect of global warming on the ecosystem. Therefore, we studied the life cycle of the tropical species *H. acharai* in Thailand. *H. acharai* in northern Thailand was described in the adult stage in 1989 (Malicky and Chantaramongkol, 1989). The immature stages inhabit waterfalls in high mountain tropical streams. To estimate the life cycle, we collected *H. acharai* in Doi Inthanon National Park monthly, which includes the highest mountain in Thailand (2565m ASL). Water temperature was also recorded hourly and compared with the thermal condition of streams in Japan, where *H. japonica* is located.

Methods

Study sites

H. acharai was collected at Siritarn waterfall (SW) (836m ASL N18°32'34.3 E98°34'52.6) and Mae Klung Pat stream (MKP) (967m ASL N18°32'37.85 E98°34'20.37) in Doi Intanon National Park in northern Thailand (Fig. 1). The channel bed of both sites consists of bedrock. MKP is a branch of the main stream of SW and the amount of water is less than the SW site. The water temperature in these streams is quite close and mild throughout the year (Mean ± S.D.: 19.60 ± 2.65 ºC) (Fig. 2). The maximum water temperature was about 25 ºC in April and the minimum was about 13 ºC in December.

Figure legends

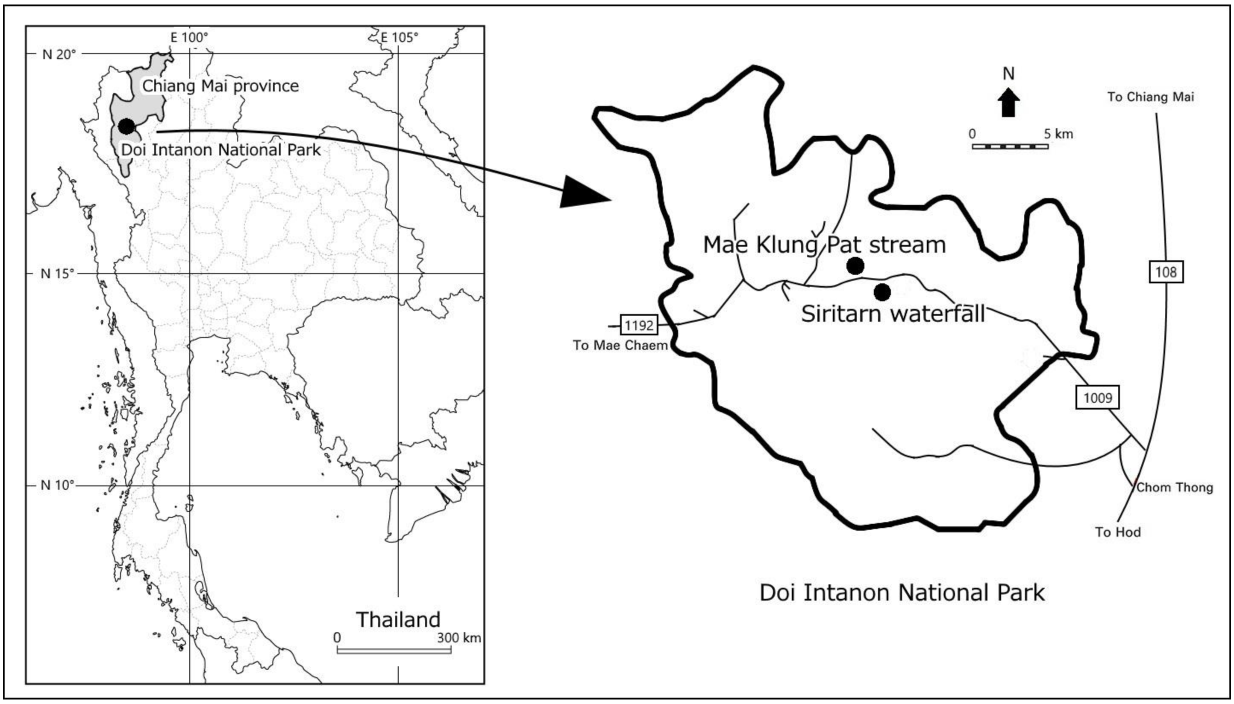

Figure 1 Map of Thailand showing the study site at Doi Intanon National Park.

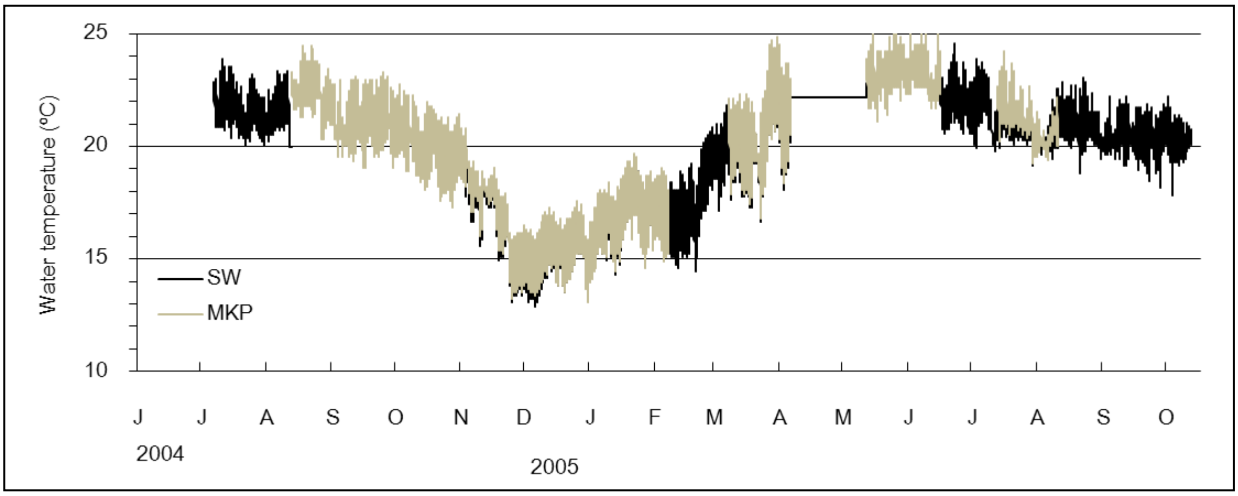

Figure 2 Water temperature at Siritarn waterfall (SW) and at Mae Klung Pat stream (MKP).

Field sampling

To estimate the life cycle of *H. acharai*, we conducted monthly field sampling at SW (June 2004 - October 2005) and at MKP (August 2004 - October 2005). The larvae of *H. acharai* were identified easily in the field by their unique morphological character. They have a large body with well-developed claws and characteristic filamentous gills on their dorsal body like hair. Only one species (*H. acharai)* in the genus *himalopsyche* was recorded at the sites. The immature stages of *H. acharai* were collected by scrubbing the surface of bedrock using an upper D-frame net. The specimens from the net were sorted by tweezers and preserved in 95% ethanol. The limitation of time for collection of insects is one hour. Egg masses of *H. acharai* which could be found on the surface of rocks in the splash zone of the water fall were counted. Big black circular eggs of *Himalopsyche* are distinguishable from ellipsoidal eggs of genus *Stenopsyche.* We also tried to find and carefully collect fresh pupal cast-off carapaces stuck on rocks emerging above the water surface. They were counted as substitutes for the number of adults. The larvae in cocoons not yet metamorphosing to pupae were counted as pre-pupae.

Size Measurement

In the laboratory, head capsule width (across widest spot) of all larvae was measured using slide calipers (DIGIMATIC CD-15C, Mitutoyo; Kawasaki, Japan) to the nearest 0.01 mm under a microscope. The wet weights of each individual were also measured to the nearest 1 mg with an electric balance (LIBROR EB-330H, Shimadzu, Tokyo). Larval instars were determined based on the results of head capsule width range of each instar (1st instar 0.34-0.40 mm, 2nd instar 0.48-0.59 mm, 3rd instar 0.72-0.95 mm, 4th instar 1.06-1.54 mm, 5th instar 1.62-2.39 mm).

Comparison of size of larvae and pupae between *H. acharai* and *H. japonica*

To reveal the effect of water temperature warming for insect size the head capsule width of the 5th instar larvae and the pupal wet weight were compared between *H. acharai* and *H. japonica.* The specimens of *H. japonica* collected from four mountain streams were supplied by the surveys in Tsuruishi (2006). The coolest streams were Takinoyu stream and Yana stream (about 1500m ASL). The annual average water temperature of these two high mountain streams was about 6 ºC and annual thermal volume (sum of water temperature above 0 ºC, Degree-days) is 2076 ºC-days at Takinoyu, 2326 ºC-days at Yana. The other two streams were Oizumi stream and Oguro stream (about 1000m ASL). The annual average water temperature was about 8 ºC and annual thermal volume is 3000 ºC-days at Oizumi and 3011 ºC-days at Oguro stream (Tsuruishi, 2003, 2006). The head capsule width of the 5th instar larvae from these four streams was measured using the same method of *H. acharai*. Then, the presence of significant difference among six sample groups (Takinoyu, Yana, Oizumi, Oguro, SW and MKP) was analyzed using One-way ANOVA. Next, Tukey's HSD test was conducted to consider all possible pairwise differences of means among six sample groups. The pupal wet weight of *H. japonica* at Oguro and Yana streams was also measured using the same method of *H. acharai* and was compared with those of *H. acharai* at SW. Statistical analysis software SPSS for Windows was used for all statistical analysis.

Comparison of larval size of *H. acharai* between rainy and dry season

To compare the size of larvae which grew in different water temperature conditions, rainy season (relatively warm) and dry season (relatively cool), the head capsule width of the 5th instar larvae were compared between the two sample groups from rainy season (August-November 2004 and June-October 2005) and one sample group from dry season (December 2004-April 2005). At first, presence of a significant difference among the three sample groups was analyzed using One-way ANOVA. And then, Tukey's HSD test was conducted to consider all possible pairwise differences of means among three sample groups.

Water temperature data collection

Water temperatures were measured every hour using self-recording thermometers (“Tidbit” Onset, Bourne, MA, USA) from 21st July 2004 to 27th Oct 2005 at SW and 27th August 2004 to 25th August 2005 at MKP. The amount of annual effective water temperature for development of *H. acharai* (annual available degree-days accumulation; sum of water temperature above the minimum threshold temperature for development of *H. acharai*) was determined for the SW by the summation of hourly water temperatures above 5 ºC, estimated as the developing threshold temperature of the species from egg to adult (Tsuruishi, 2010). The sum of hourly water temperature (degree-hours) was divided by 24 to convert into degree-days. Because the water temperatures between the two study sites were quite similar, we substituted the data at MKP for the lack of data at SW due to the thermometer being washed away by flood in rainy season at SW. The thermometers were washed away at both study sites between 20th April and 26th May in 2005. The water temperature was replaced at 22.19 ºC, which is the average temperature of 19th April and 27th May at SW (Fig. 2).

Results

Life cycle

We collected 266 individuals of *H. acharai* in the Siritarn waterfall and 749 in Mae Klung Pat stream. The results of larval head capsule width and wet weight is shown on Table 1. On the scatter diagram of the head capsule width there are 5 cohorts (Fig. 3), suggesting that the larva has 5th instar stages the same as *H. japonica* (Tsuruishi, 1999, 2003). Forty-seven egg masses were found in MKP, but only 3 egg masses were found in SW. All instar larvae, pupae, and adults were collected almost through a year (Fig. 4). There is no clear cohort of particular developmental stage.

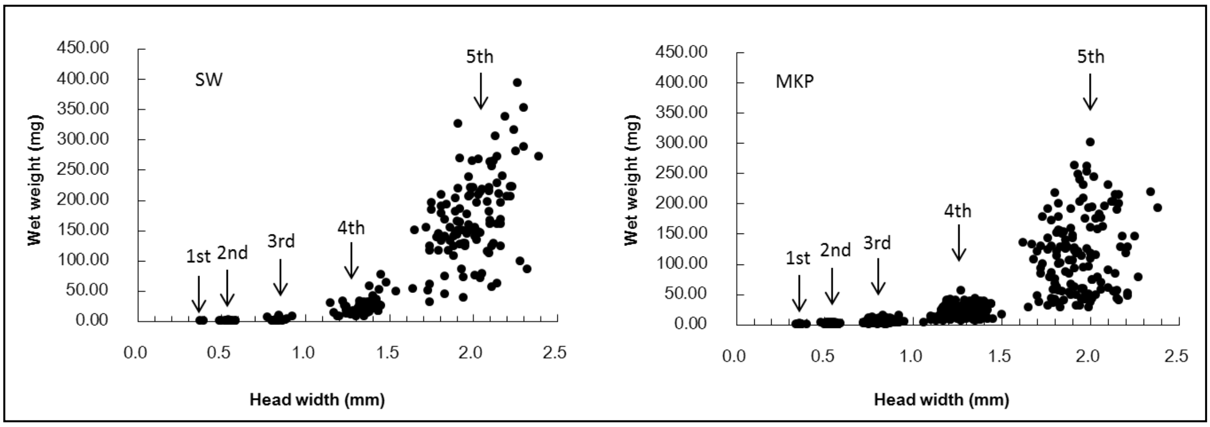

Figure 3 Head width and wet weight of larvae at Siritarn waterfall (SW) and at Mae Klung Pat stream (MKP).

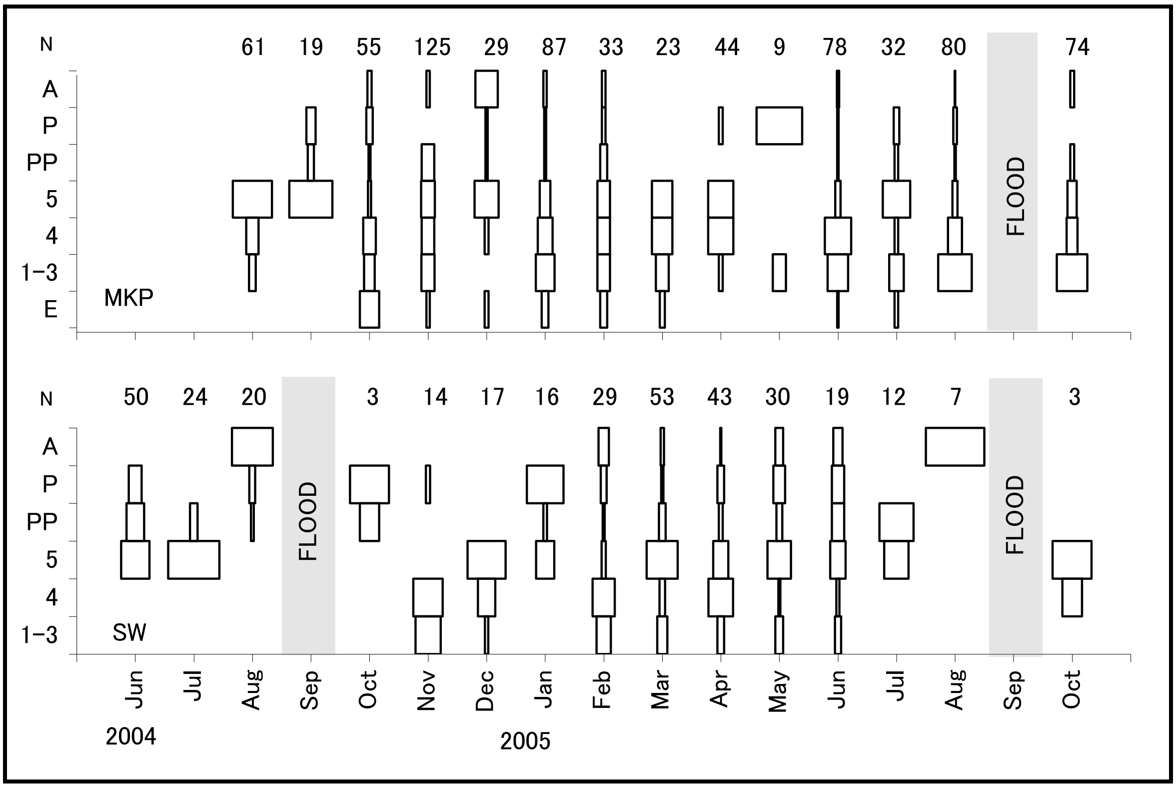

Figure 4 Seasonal changes in the developmental stage composition of *Himalopsyche acharai* at Siritarn waterfall (SW) and at Mae Klung Pat stream (MKP). Percentage constituents of each stage are shown by histograms. N: the number of specimens, A: adult, P: pupa, PP: prepupa, 5: 5^{th} instar larva, 4: 4^{th} instar larva, 1-3: 1^{st} to 3^{rd} instar larva, E: egg mass.

In rainy season, most insects which inhabit the surface of bedrock were flashed away by succession of violent freshets caused by tropical heavy rain at SW (Fig. 5). It was difficult to find any insects, including *H. acharai*, in the rainy season and the first to fourth instar larvae were not found in mid-late rainy season (from June to September) at SW. On the other hand, abundant larvae including first to fourth instar were found even in rainy season at MKP where water flow is not so violent such as SW. In contrast, in dry season, decrease of fast current habitat for *H. acharai* by water shortage was observed at MKP (Fig. 6). This impact by water shortage at MKP was dissolved in rainy season.

Figure 5 Succession of violent freshet caused by tropical heavy rain at Siritarn Waterfall.

Figure 6 Decrease of fast current habitat by water shortage was observed in dry season at Mae Klung Pat stream.

The seasonal fluctuation of numbers of *H. acharai* collected is different between the two sites (Fig. 7). The number of insects increased in dry season (from October to April) and decreased in rainy season (May to September) at SW. Reversely, the number decreased in dry season and increased in rainy season at MKP. At both sites few *H. acharai* were collected in May 2005 because of very heavy freshet at the beginning of May.

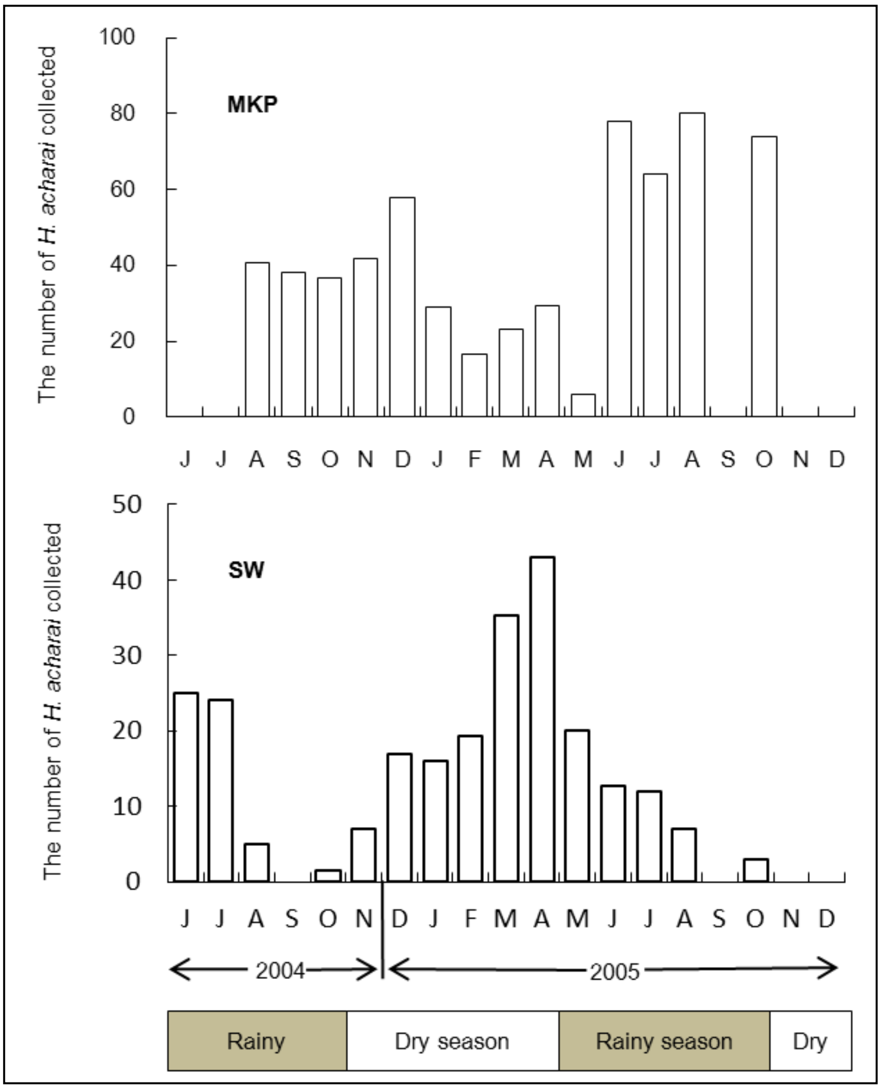

Figure 7 Seasonal changes of the number of *H. acharai* collected at Siritarn waterfall and at Mae Klung Pat stream.

Comparison of size of larvae and pupae between *H. acharai* and *H. japonica*

The head capsule width of 5th instar larvae was significantly different between *H. acharai* and *H. japonica* (Fig. 8; one-way ANOVA $p < 0.001$, Tukey HSD $p < 0.01$). *H. acharai* samples were significantly smaller than *H. japonica* and *H. japonica* samples in Takinoyu and Yana stream were larger than those in the Oizumi and Oguro streams (Tukey HSD $p < 0.01$). Pupal wet weight of *H. acharai* was also less than *H. japonica* (Fig. 9). Mean wet weight is 145 mg for *H. acharai* at SW, 307 mg at *H. japonica* in Oizumi stream, 309 mg in Oguro stream. The relationship between annual thermal volume (degree-days) at the 6 study sites and 5th instar head width shows negative correlation (Fig. 10).

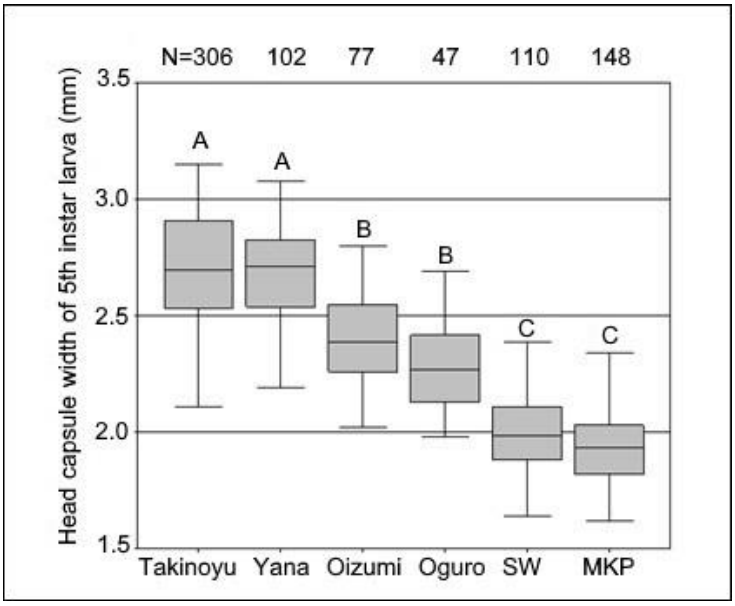

Figure 8 Comparison of head capsule width of 5th instar larvae of *H. acharai* and *H. japonica* (Tsuruishi 2006) between six streams. Significant differences were found among group A, B and C by Tukey's test (one-way ANOVA $p < 0.001$, Tukey HSD $p < 0.01$) (A: *H. japonica* in up streams (Takinoyu and Yana) B: *H japonica* in middle streams (Oizumi and Oguro) C: *H. achara*i in SW and MKP). Median line is shown in quartile range with minimum and maximum.

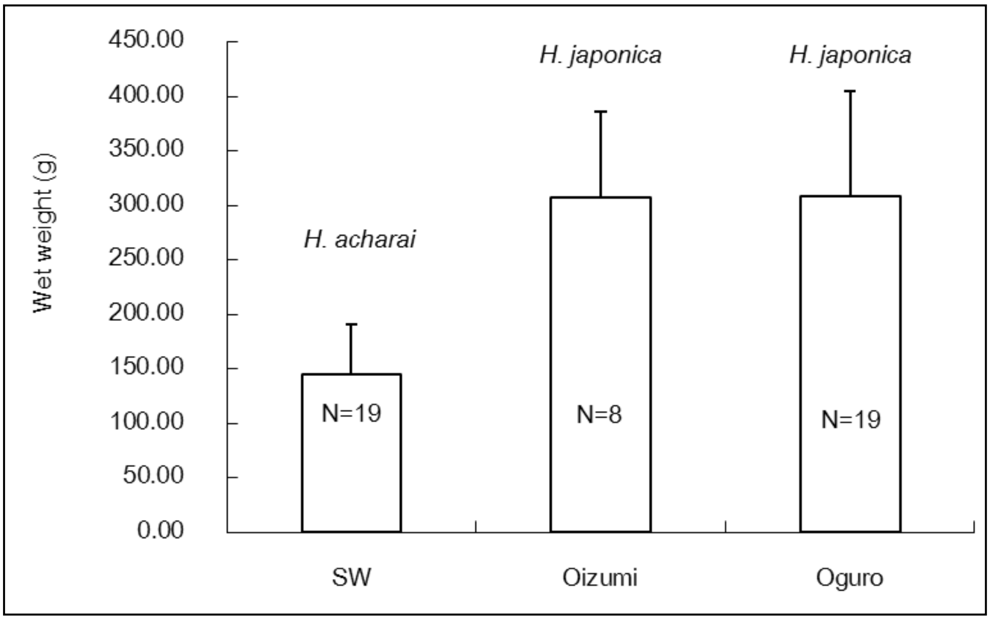

Figure 9 Comparison of pupal wet weight of *H. acharai* at Siritarn waterfall (SW) and *H. japonica* at Ogizumi stream and Oguro stream.

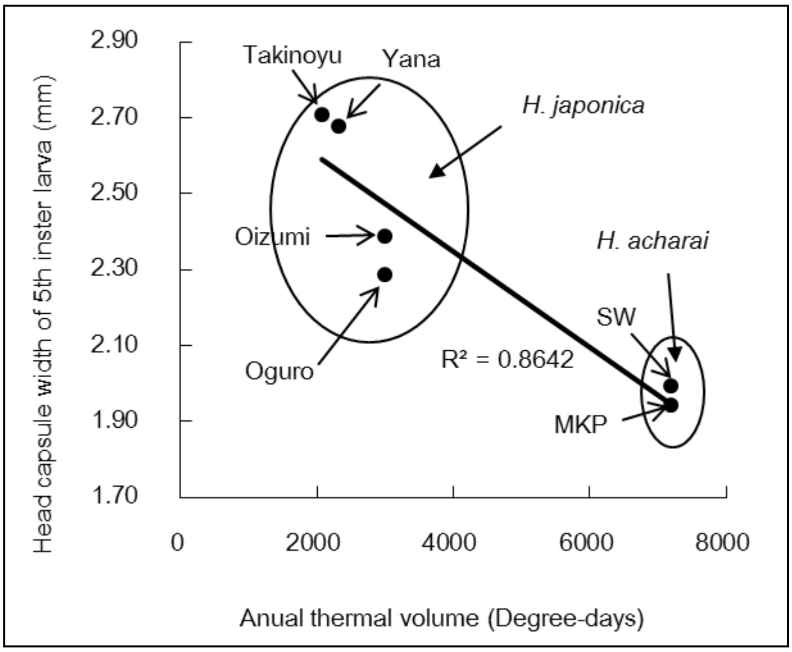

Figure 10 Mean head capsule width of 5th instar larvae and annual thermal volume of the streams of *H. acharai* and *H. japonica*.

Comparison of larval size *H. acharai* between rainy season and dry season

The head capsule width of 5th instar larvae of *H. acharai* in dry season (relatively cool, December 2004-April 2005) was significantly larger than those collected in rainy season (relatively warm, August-November 2004 and June-October 2005) at MKP (Fig. 11; one-way ANOVA $p < 0.001$, Tukey HSD $p < 0.01$).

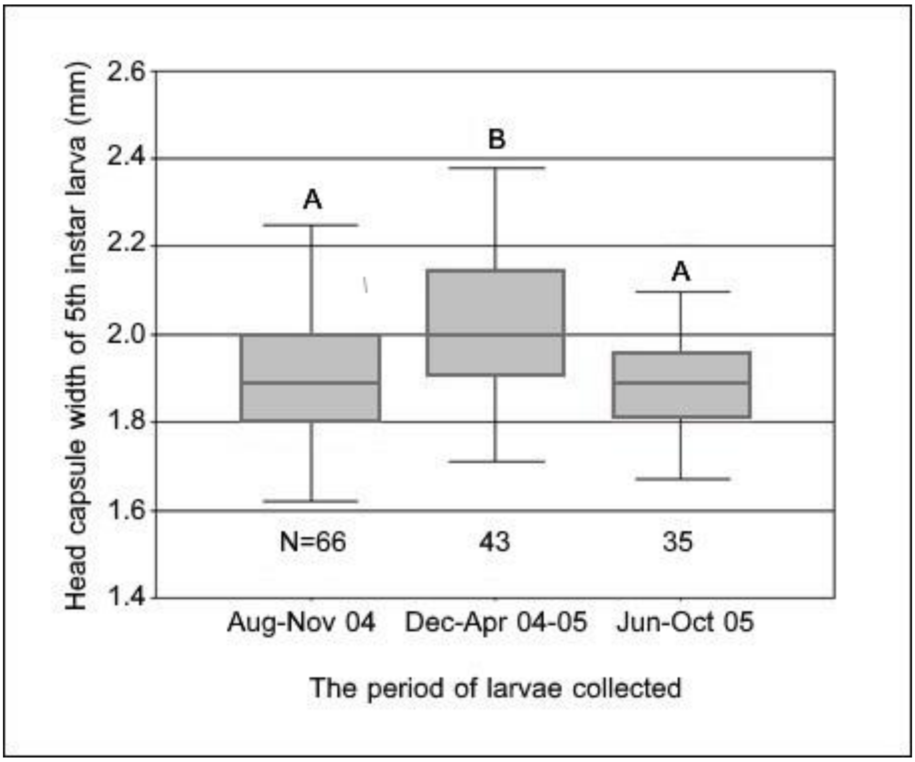

Figure 11 Comparison of head capsule width of 5th instar larvae of *H. acharai* among the larvae which were collected in rainy season (Aug-Nov 2004 and Jun-Oct 2005) and dry season (Dec 2004-Apr 2005) at MKP. Significant differences were found between **A** and **B** (one-way ANOVA $p < 0.001$, Tukey HSD $p < 0.01$). Median line is shown in quartile range with minimum and maximum.

Water temperature

The water temperature was similar at the two sites and fluctuated between 13 ºC in December and 25 ºC in April (Fig. 2). Annual thermal volume (sum of water temperature above 0 ºC) was calculated as about 7200 ºC-days and annual available degree-days (sum of water temperature above *5* ºC, the minimum threshold temperature for development of *H. acharai*) was about 5300 ºC-days.

Discussion

Life cycle

Seasonal changes in the developmental stage composition of *H. acharai* have no clear cohort at the same developmental stages. Adults seem to emerge without synchronicity from matured pupae one after another. As for *H. japonica* inhabiting streams in Japan in a temperate zone, it was suggested that fifth instar larvae and pupae ceased their development in cold winter and restarted the next spring synchronously. Therefore, some cohorts at the same developmental stage formed (Tsuruishi, 2003, 2006). However, because suitable thermal condition for their development is provided throughout the year for *H. acharai* it is unnecessary for them to cease development to adapt to cold winter in their life cycle in tropical mountain streams; matured ones develop to adults one after another. It was known by rearing experiments in the laboratory that the theoretical lower threshold temperature for development of *H. acharai* is around 5 ºC and the thermal constant for one generation egg to adult is around 1200 ºC-days (Tsuruishi, 2010). When annual available degree-days at SW and MKP are estimated by these physiological data, it is around

5300 ºC-days. The voltinism of *H. japonica* is estimatable using the thermal constant for one generation and annual available degree-days of the water temperature in the field (Tsuruishi and Arunrat, 2012). For example, when annual available degree-days in the field are equivalent to the thermal constant for one generation, the population will be univoltine. Now annual available degree-days at SW and MKP (5300 ºC-days) are more than 4 times the thermal constant for one generation of *H. acharai* (1200 ºC-days). Therefore, it is suggested that *H. acharai* has a multivoltine life cycle, 4 generations per year in thermal condition of 1200 ºC-days×4 generations=4800 ºC-days < 5300 ºC-days. In addition, our survey shows a lot of egg masses were found easily in MKP which is a branch, but few egg masses were found in SW in the main stream. This result may be caused by the upstream migration in females for oviposition, as reported in the *Rhyacophila* species (Svensson, 1974).

Size comparison

Tsuruishi (2006) described that larvae of *H. japonica* develop more slowly in cool streams than those in more mild streams, and so the larvae in cool streams grow larger than those in mild streams. The study sites of *H. acharai* are milder than the streams of *H. japonica* and *H. acharai* are significantly smaller than *H. japonica*. Comparison of annual thermal volume (ºC-days) and head capsule width of the 5th instar larvae between 6 study sites suggests that the cool thermal condition produces larger body size for *Himalopsyche*. It was reported that the threshold low water temperature for growth is lower than that for development to the next instar (Mochizuki et al., 2006). When water temperature in the stream decreases lower than the developmental threshold but still higher than the growth threshold, the larvae can grow without developing to the next instar. This growth period is supposed to be able to make larvae large. The water temperature at the study sites of *H. acharai* was higher than their developmental threshold throughout the year, because the developmental threshold of *H. acharai* is known to be around 5 ºC (Tsuruishi, 2010). Therefore, *H. acharai* is presumed to be able to develop to the next instar without the growth period and the body size becomes smaller than *H. japonica*. However, in this study, the head widths of 5th instar larvae which grow in the warm period were significantly smaller than the larvae that grow in the cool period. Thus, it is suggested that even though the water temperature is sufficiently higher than the developmental threshold and larvae did not have the growth period, relatively cool water temperature also may be able to make larvae large.

From the results of collected insect number, the insect population density was supposed to decrease in rainy season at SW and in dry season at MKP. The amount of water flow in SW is larger than MKP and the most insects seemed to be flushed away by violent freshet in rainy season at SW. On the other hand, marked shortage of flowing water was observed at MKP in dry season (Fig. 6). The current speed of the *H. acharai* habitat at MKP in dry season was about 100 cm/s lower than rainy season (Tsuruishi *et al*., 2008). Larvae of *H. japonica* prefer the microhabitat where current speed is very fast, more than 200 cm/s (Tsuruishi and Yoshida, 2002). *H. acharai* larvae, especially grown larvae, also prefer fast flow microhabitats (Tsuruishi *et al*., 2008). It is suggested that the population density of *H. acharai* decreased due to shortage of suitable microhabitat in dry season at MKP.

Conclusion

This study suggests that global warming makes the insect body small and causes multivoltine life cycle. The natural impacts by rain on *H. acharai* are different between the habitats with different water flows. While the insect density will decrease in the main stream in rainy season, a suitable microhabitat will be produced and the density will increase in branch streams. In the subsequent dry season, the suitable microhabitat will be produced in the main stream and the water shortage in branch streams will cause larval flowing. This larval flowing will supply larvae from branches to the main stream. The decrease of population density by natural impacts is supposed to be compensated between local populations in the mainstream and branch streams. This mutual supply system should be important to sustainable subsistence of *H. acharai* populations. Our results suggest the importance of the link between adjacent habitat and local population and it agrees with previous reports which insist that habitat fragmentation causes reduction or extinction of local population (Jansson and Angelstam, 1999; Naugle et al., 1999). Thus, dynamic seasonal change of flowing water amount between rainy season and dry season in tropical Asian mountain streams causes critical impacts for inhabiting insects such as drought and flood. However *H. acharai* is regarded as well adapted to these natural impacts. This study supplies the fact that natural environment changes drastically and seasonally but it controls annual dynamic fluctuation of aquatic organisms exquisitely in tropical Asian streams.

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Table 1 Size of *H. acharai* immature stages in the study sites.

Study site		Larval instar 1st	2nd	3rd	4th	5th	Pupa
Siritarn waterfall	Number	2	14	18	49	110	19
		Head width (mm)					
	Mean ± S.D.	0.38±0.01	0.54±0.03	0.84±0.04	1.34±0.09	1.99±0.16	-
	Range (min-max)	(0.37-0.38)	(0.49-0.58)	(0.77-0.92)	(1.15-1.54)	(1.64-2.39)	-
		Wet weight (mg)					
	Mean ± S.D.	1.00±0.00	1.14±0.53	4.00±3.09	25.90±14.65	171.96±71.54	144.68±46.41
	Range (min-max)	(1)	(1-3)	(1-10)	(8-78)	(32-394)	(88-256)
Mae Klung Pat stream	Number	11	60	138	179	148	-
		Head width (mm)					
	Mean ± S.D.	0.36±0.02	0.54±0.02	0.81±0.05	1.27±0.08	1.94±0.15	-
	Range (min-max)	(0.34-0.40)	(0.48-0.59)	(0.72-0.95)	(1.06-1.50)	(1.62-2.38)	
		Wet weight (mg)					
	Mean ± S.D.	1.09±0.30	1.65±0.73	5.71±3.08	21.13±9.35	121.97±66.55	-
	Range (min-max)	(1-2)	(1-4)	(1-16)	(3-56)	(28-330)	

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