

# LARVAL INSTARS DETERMINATION AND SECONDARY PRODUCTION OF THE MAYFLY *Baetis ideii* (EPHEMEROPTERA: BAETIDAE) FROM RIVERS IN THE BUKIT MERAH CATCHMENT AREA, PERAK

SITI HAMIDAH, I.<sup>1</sup>, SUHAILA, A.H.<sup>1\*</sup> and NUR AIDA, H.<sup>2</sup>

<sup>1</sup>School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

<sup>2</sup>School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

\*E-mail: ahsuhaila@usm.my

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## ABSTRACT

The study of an insect's life cycle is important to understand the abundance, diversity, development and the environment in which it lives. To date, only limited investigations have been conducted in Malaysia on the mayfly *Baetis*, a genus of ephemeropterans. Here, we report a study on the larval instar determination and secondary production of *Baetis ideii* in four rivers in the Bukit Merah catchment area, Perak. Samples were collected monthly from May 2014 until April 2015 using the kick-net and drag methods. *B. ideii* displayed nine instar stages on a discriminant function analysis (DFA) scatter plot. Based on an analysis of nymphal body length and the emergence time of early and final larva instar, *B. ideii* was found to be multivoltine in the Ara, the Jelai, Kurau and Ayer Itam rivers. The estimated annual production of *B. ideii* in the four rivers ranged from  $-3.64 \text{ mg m}^{-2} \text{ y}^{-1}$  to  $15.35 \text{ mg m}^{-2} \text{ y}^{-1}$  and the annual P/B ratio varied from  $-2.14$  to  $1.56$ . The highest turnover ratio for this species was recorded in Ara River. Different types of habitat and substrate at each river determined the abundance, voltinism, and secondary production of *B. ideii* in the four rivers.

**Key words:** Aquatic insects, immature larva, multivoltine, disturbed rivers, peninsular Malaysia

## INTRODUCTION

Mayflies or Ephemeroptera, are widely distributed group of aquatic insects worldwide. Ephemeropterans are highly sensitive (non-tolerant) towards changes in the aquatic environment where they spend most of their time (Merritt *et al.*, 2008). McShaffrey and McCafferty (1990) found that ephemeropteran larvae, especially of *Baetis* sp., are very selective in the choice of their surroundings. Hence, different species of *Baetis* may have dissimilar habitat requirements in terms of substrate and depth below the water surface.

The biology of aquatic insects, including their life histories, field development and growth, is an important aspect in understanding the dynamics of limnology (Che Salmah *et al.*, 2006). Detailed knowledge of the life history of *Baetis* sp. (Ephemeroptera: Baetidae) is critical for the

determination of its voltinism and the calculation of secondary production (Rosenberg & Resh, 1993). Peràn *et al.* (1999) emphasized the importance of life cycle information of aquatic insects in understanding seasonal variations in their presence and annual production. *Baetis* is the dominant group of Ephemeroptera, contributing largely to total production in rivers (Benke & Jacobi, 1986). However, difficulty in establishing the life history of *Baetis* and its production could arise from its asynchronous development and rapid growth (Benke & Jacobi, 1986). Some taxa in the Ephemeroptera have long life cycles and high levels of biomass production and accumulation (Siti Hamidah, 2017). The biomass of freshwater macroinvertebrates inside a given area upsurges if immigration or production exceeds losses (Huryñ & Wallace, 2000).

There have been previous studies conducted in the West on the life histories of ephemeropterans (Benke, 1993; Benke & Jacobi, 1986; Benke *et al.*, 1984), some focusing on *Baetis* sp. (Salas &

\* To whom correspondence should be addressed.

Dudgeon, 2003). However, similar studies in Malaysia are rather limited. Among the reports available are those by Hafezul *et al.* (2016) and Suhaila and Che Salmah (2010).

This study was done to understand the life history of *B. ideii* and to assess its secondary production in selected rivers from the Bukit Merah catchment area, Perak. Depending on land use activities nearby, different types of effluents have encroached into the rivers in the Bukit Merah catchment area. Hence, the different land use activities were expected to affect the population dynamics and secondary production of *B. ideii* in the sampled rivers.

## MATERIALS AND METHODS

### Sampling sites

This study was carried out in the Ara, Jelai, Kurau and Ayer Itam rivers in the Bukit Merah catchment area (BMCA) in Kerian District of the northern peninsular state of Perak (Fig. 1). The depth of the sampling area at each river ranged from 27.8 cm to 47.0 cm. The highest average water velocity encountered during sampling,  $0.70 \pm 0.09$  m/s, was encountered at the Ara River. This was followed by the Jelai River ( $0.65 \pm 0.05$ ), Ayer Itam River ( $0.53 \pm 0.06$ ) and Kurau River ( $0.45 \pm 0.05$ ). The rivers received different types of effluents according to human activities in the vicinity. There were rubber, oil palm, and maize plantations on both sides of the river banks of the Ara River ( $05^{\circ} 05. 428' N$  and  $100^{\circ} 51. 193' E$ ). Jelai River ( $05^{\circ} 00. 852' N$  and  $100^{\circ} 48. 604' E$ ) was surrounded by banana (*Musa paradisiaca*) plantations, with a small rubber plantation on the upper site of the river. Jelai River received sewage from the nearby village areas. In contrast, Kurau River ( $04^{\circ} 54. 213' N$  and  $100^{\circ} 49. 991' E$ ), situated in the virgin forest reserve of Hutan Simpan Bintang Hijau, was chosen as control site since there were no agricultural activities nearby. Lastly, Ayer Itam River ( $05^{\circ} 00. 888' N$  and  $100^{\circ} 49. 998' E$ ), a recreational river, was susceptible to slight organic pollution. All the selected rivers were categorized Class II in the Water Quality Index (WQI).

### Collection and measurement of *Baetis ideii*

Ten samples of *B. ideii* larvae were collected on monthly basis for a year (May 2014 till April 2015) from the Ara, Jelai, Kurau and Ayer Itam rivers using the kick-net and drag sampling techniques (Merritt *et al.*, 2008). D-frame aquatic nets (cone shaped nets of 300  $\mu$ m mesh, 40 cm wide, 30 cm high and 60 cm long) were fitted to 100 cm long handles used for sample collection. The sorted collected samples

were preserved in universal bottles containing 80% ethanol. *Baetis* spp. were identified following keys prepared by Morse *et al.* (1994), Muller-Liebenau (1984) and Yule and Yong (2004).

The body length (from the anterior margin of the head to the posterior edge of the last abdominal segment) of *B. ideii* larvae was measured using digital calipers (0-125 mm). Following Miyairi and Tojo, (2007), head width (HW) was measured across the widest portion of the head capsule from outer margins of left and right compound eyes to the nearest 0.1 mm under a dissecting microscope attached with Olympus Series Image Analyzer (Olympus Optical Co., Japan) and AnalySIS<sup>®</sup> LS Starter software. The measurement for the length of the wing pad, including the mesonota, was taken from the base according to Cayrou and Cereghino (2003). The frequency of each nymphal body length was determined for the analysis of size-frequency distribution (Fonseca Leal & Assis Esteves, 2000). The Hynes method was used to calculate secondary production of *B. ideii* (Hynes and Coleman, 1968). Dry mass (DM) value was obtained from the regression equation developed by Benke and Jacobi (1986) as shown below:

$$\log DM = 3.325 \log HW + 0.102$$

$$DM = \text{EXP}^{3.325 \log HW + 0.102}$$

where HW = head width

Dry mass obtained from the above regression was converted to ash-free dry mass (AFDM) by multiplying with 0.928.

### Measurements of physical parameters

The physical characteristics of each river (width, depth, pH, temperature and flow velocity) were recorded concurrently with the collection of *B. ideii* every month from May 2014 until April 2015. All the physical parameters were measured *in situ* using a measuring tape, Flow Probe 101 and YSI Model 550A (YSI Inc., Ohio, USA). The type of embeddedness and substrate were also visually observed over a stretch of 50 m along the river following Barbour *et al.* (1999).

### Data analysis

The abundance of *B. ideii* was not normally distributed ( $P < 0.05$ ) in the four sampling rivers. The intervals for each instar stages of *B. ideii* were determined using size frequency histograms and Canonical Discriminant Function (IBM Statistical Package for Social Science, SPSS Statistics version 20<sup>®</sup>). The Production/Biomass (P/B) ratio of *B. ideii* was calculated to estimate its growth rate.

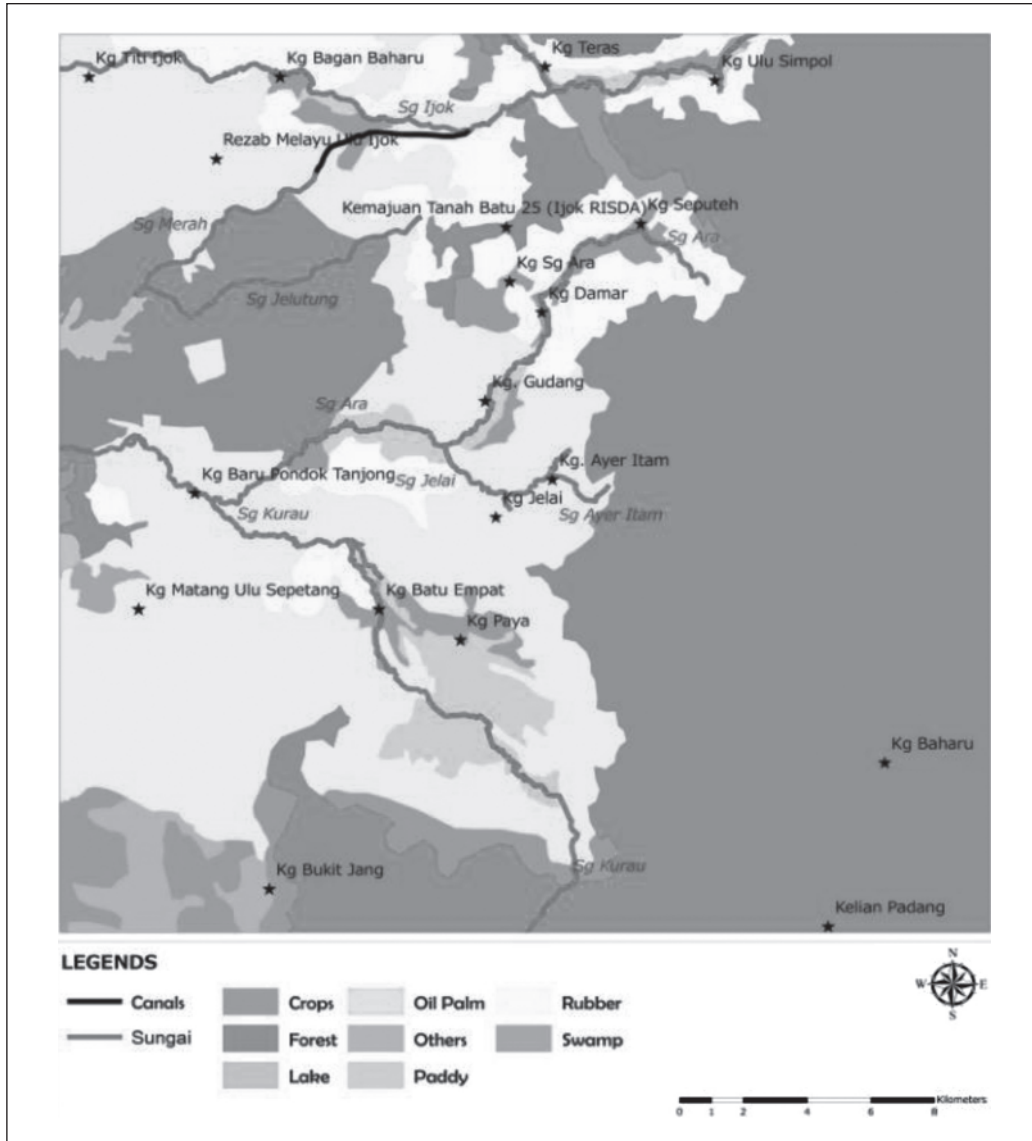


Fig. 1. Location of sampling areas: Ara, Jelai, Kurau and Ayer Itam rivers in Bukit Merah catchment area.

**RESULTS**

**Separation of *Baetis idei* into Instar Classes**

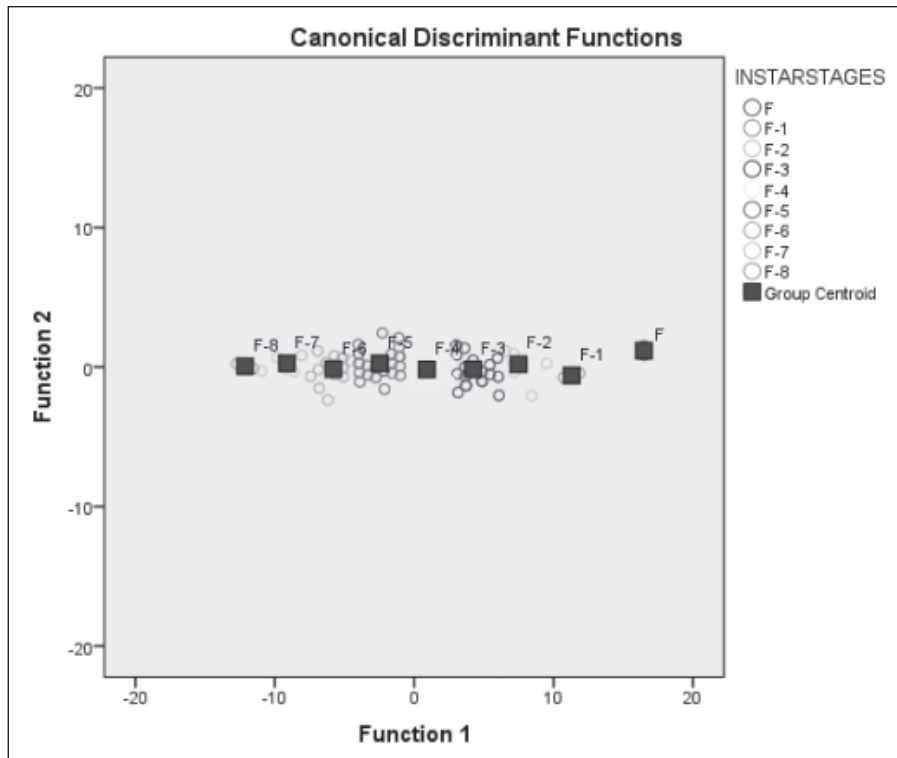
Overall, a total of 48 individuals from the Ara River, 50 individuals from the Jelai River, 11 individuals from the Kurau River, and 10 individuals from the Ayer Itam River were used to construct instar stages of *B. idei* larvae. There was a strong relationship between body length and wing pad length of *B. idei* larvae collected from the four rivers (Ara River,  $R^2 = 0.698$ , Jelai River,  $R^2 = 0.704$ , Kurau River,  $R^2 = 0.947$ , and Ayer Itam River,  $R^2 = 0.752$ ). Separation of the instar stages by the discriminant function analysis (DFA) revealed two discriminant functions that were statistically significant (Table 1). Based on the Wilks lambda calculation, nine instar stages (F-8 – F) were formed on the DFA scatter plot (Fig. 2), where F-8 represents the youngest instar while F class represents the late instar. A class interval

of 0.5 mm body length and 0.1 mm wing pad length was chosen to classify all the *B. idei* larvae in their stages (Table 2). The nymphal body length ranged from 1.0 to 6.3 mm and wing pad length ranged from 0.1 to 1.7 mm in the four rivers.

Instar F-4 was the dominant instar for *B. idei* in the Ara, Jelai, and Ayer Itam rivers (Fig. 3, 4 and 6) while instar F-5 and instar F-6 were the dominant instars in the Kurau River (Fig. 5). Early (F-8) and late instar (F) of *B. idei* were both found in the Kurau River (Fig. 5) but not in the Ayer Itam River (Fig. 6).

**Table 1.** Standard canonical discriminant function coefficients of body length (BL) and wing-pad length (WPL) of *Baetis idei*

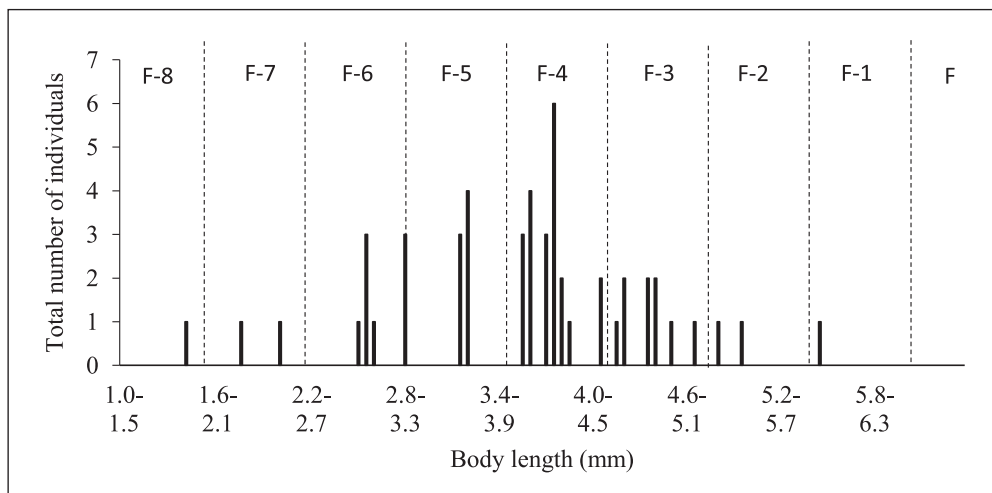
Parameter	Canonical discriminant function	
	Function 1	Function 2
BL	1.013	-0.301
WPL	-0.043	1.056



**Fig. 2.** Canonical discriminant function plot for instar stages of *Baetis idei*: pooled data of *B. idei* from Ara, Jelai, Kurau and Ayer Itam rivers.

**Table 2.** Ranges of body length and wingpad length of *Baetis idei* instar classes

Instar class	Body length (mm)	Wingpad length (mm)
F-8	1.0–1.5	0.0–0.1
F-7	1.6–2.1	0.2–0.3
F-6	2.2–2.7	0.4–0.5
F-5	2.8–3.3	0.6–0.7
F-4	3.4–3.9	0.8–0.9
F-3	4.0–4.5	1.0–1.1
F-2	4.6–5.1	1.2–1.3
F-1	5.2–5.7	1.4–1.5
F	5.8–6.3	1.6–1.7



**Fig. 3.** Separation of *Baetis idei* into instar stages based on body length of nymphs from the Ara River.

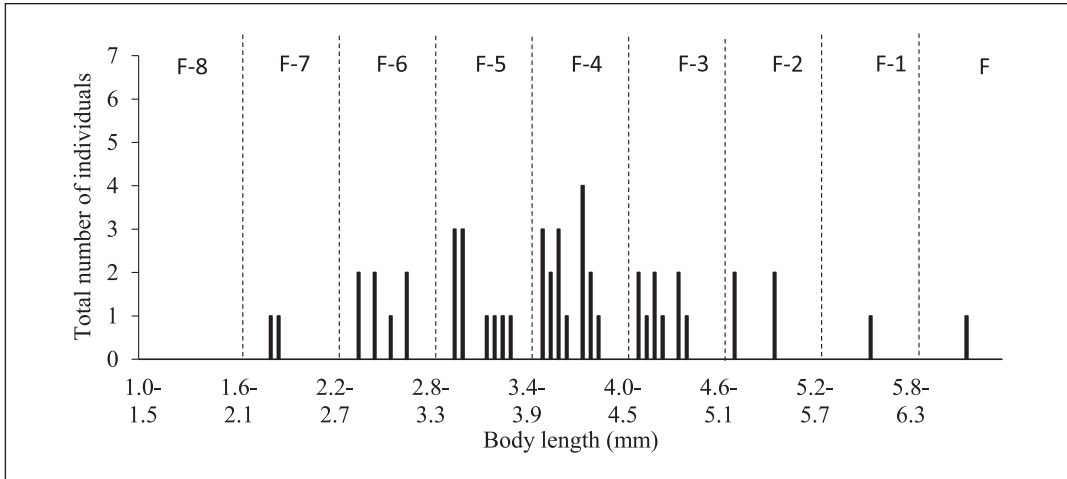


Fig. 4. Separation of *Baetis idei* into instar stages based on body length of nymphs from the Jelai River.

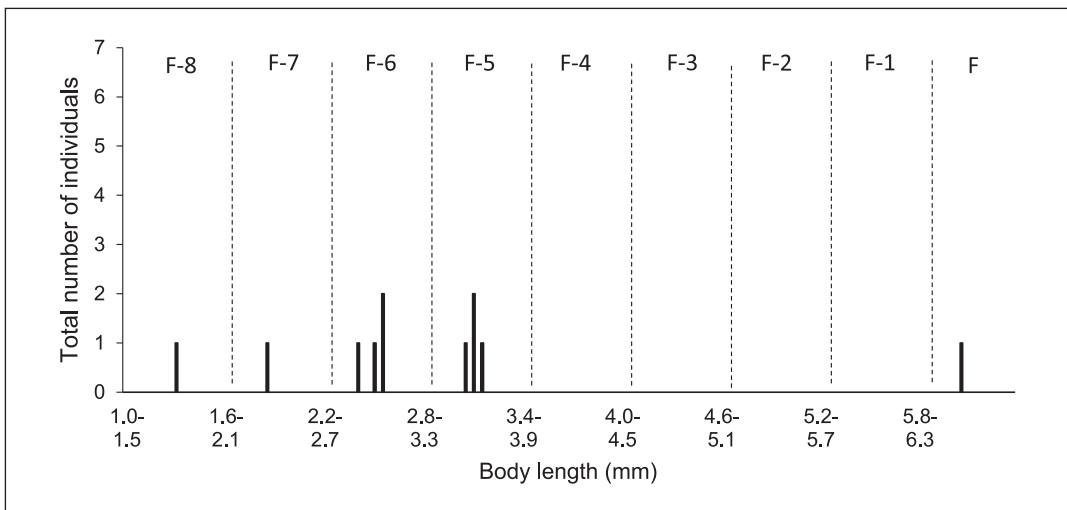


Fig. 5. Separation of *Baetis idei* into instar stages based on body length of nymphs. Data from the Kurau River collected over 12 months.

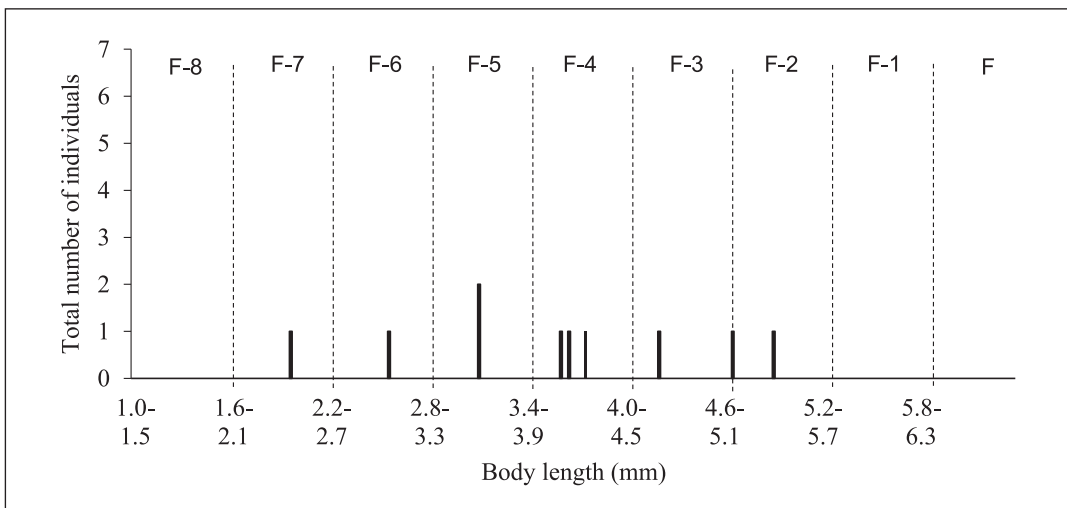


Fig. 6. Separation of *Baetis idei* into instar stages based on body length of nymphs from the Ayer Itam River.

### Development of *Baetis ideii* larvae in the sampled rivers

Throughout the one year of sampling, December 2014 had the highest mean abundance of *B. ideii* collected from the Ara and Jelai rivers (1.25 individuals/month in the Ara River; 1.00 individual/month in the Jelai River) (Fig. 7, 8). Instar 4 was the most abundant instar found in December 2014 in both the Ara and Jelai rivers. Early nymphs (F-8, F-7, and F-6) of *B. ideii* in the Ara River were encountered in August, November, December 2014 and January 2015 (Fig. 7), while in the Jelai River they were collected in August, September, October, December 2014 and February 2015 (Fig. 8). The highest mean of *B. ideii* from the Kurau River was recorded in November 2014 (0.33 individuals/month), with instar 5 as the dominant instar (Fig. 9). Both early (F-7) and final instars (F) were found in October 2014. Meanwhile, November and December 2014 were the months with the greatest abundance (0.25 individuals/month) of *B. ideii* in the Ayer Itam River (Fig. 10). In November 2014, Instar 5 was the dominant instar (0.2 individuals/month), while early instars of *B. ideii* in Ayer Itam River were recorded in December 2014 (Fig. 10).

Early (F-8, F-7, F-6) and final instars (F-2, F-1, F) of *B. ideii* appeared several times during the sampling months. Therefore, the number of generations of *B. ideii* in the Ara, Jelai, Kurau and Ayer Itam rivers were likely to be multivoltine. However, the type of voltinism for *B. ideii* in the four rivers could not be determined accurately because the insects were encountered in only a few of the sampling months. This was especially true for the Kurau and Ayer Itam rivers.

### Secondary production of *Baetis ideii*

The estimated secondary production values for *B. ideii* in the Ara, Jelai, Kurau and Ayer Itam rivers were calculated using the Hynes size frequency method. Among the four studied rivers, the Ara River had the highest annual P/B for *B. ideii* (P/B=1.56), followed by Jelai (1.33) and Ayer Itam (0.02) rivers (Table 3), while Kurau River had the lowest annual P/B for *B. ideii* (-2.14).

### Physical parameters at the four sampled rivers

At  $17.9 \pm 0.17$  m, the Jelai River was the widest of the four rivers (Table 4). The highest mean water temperature recorded was at the Ara River (26.3°C), while the lowest mean water temperature was 24.5°C at the Ayer Itam River. Water flow was relatively slow in all selected rivers, ranging from 0.45 m/s to 0.68 m/s. The Ara River provides a suitable physical habitat for aquatic insects, especially mayflies. This river consists of 20% cobble, 20% gravel, and 60% sand, with a partly shaded canopy cover.

### DISCUSSION

*B. ideii* was found abundantly in the Ara River due to its suitability as a preferred habitat characterized by the presence of aquatic plants (*Brachiaria* sp. and *Colocasia esculenta*) and a suitable mix of gravel and cobble. *Baetis* prefers such a habitat (Bunn *et al.*, 1999). Most of the *Baetis* spp. in the four sampling rivers had similarly small body size which allowed quick growth to accommodate three or more generations per year. Water temperatures in Ara, Jelai, Kurau, and Ayer Itam rivers were almost similar, ranging between 24°C to 27°C, an optimal range for multivoltine populations. Water temperature is the major growth regulator for most of the *Baetis* sp. (Brittain, 1982). According to a study by Bottova and Derka (2013), when water temperatures range from 0 to 10°C, *Baetis alpinus* have asynchronous bivoltine life cycles whereas *Baetis rhodani* have univoltine life cycles. Species compositions are highly restricted by the variability of water temperature (Dobrin & Giberson, 2003; Lehmkuhl, 1979). In addition, the underlying cause of variations in the number of generations per year are the thermal differences between habitats (Ward & Stanford, 1982) that caused by the different altitudes or latitudes.

A Cohort Production Interval (CPI) of 12 months was chosen in this study because, according to Gonzalez *et al.* (2003), this is the maximum interval for univoltine populations. Lee *et al.* (2008) also find a CPI value of 12 to be widely applicable to the whole population at all the developmental stages. The life cycles for most of the *Baetis* sp. in the rivers were typically continuous, with three or four generations recorded in a year. In the present study, *B. ideii* was found to have nine instar stages, in agreement with the findings of Hafezul (2016) on *Baetis* spp. Different numbers of instar stages have been reported for other families of the order Ephemeroptera. For example, Gonzalez *et al.* (2003) reported six instar stages for *Epeorus torrentium* (Ephemeroptera: Heptageniidae) while a study on *Thalerosphyrus* (Ephemeroptera: Heptageniidae) revealed nine instar stages (Suhaila *et al.*, 2016). Findings from Vega *et al.* (1998) showed that the recruitment period was short for most ephemeropterans and the immature had a small body size that delayed egg development. In the present study, early and final instars of *B. ideii* appeared several times during the one year collection period. Since F, F-1, F-7 and F-8 instars appeared several times throughout the sampling months in the Ara, Jelai, Kurau and Ayer Itam rivers, *B. ideii* is classified as having a multivoltine life cycle. The short development time of the *Baetis* spp. is due to their rapid growth rate and small terminal

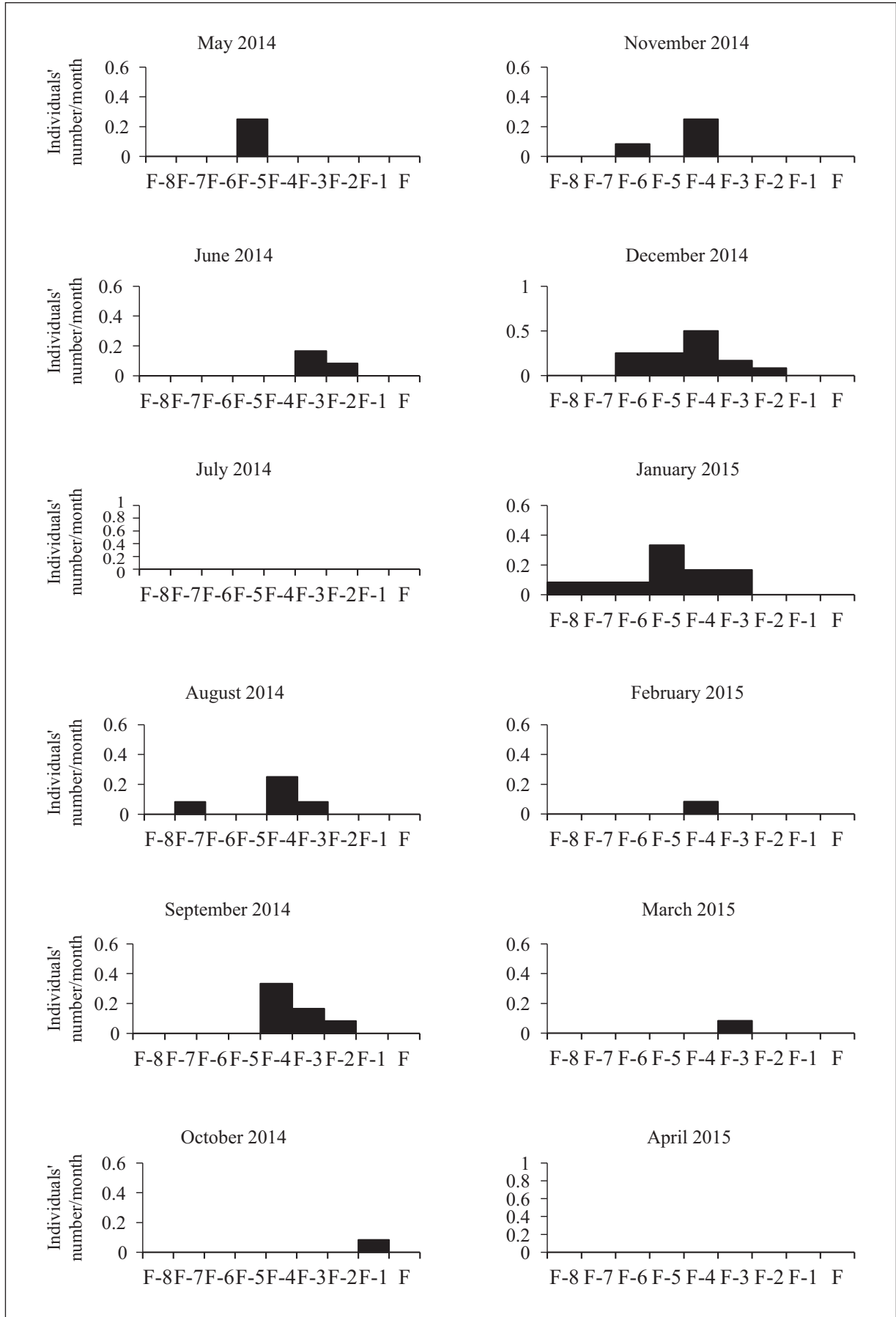


Fig. 7. Size-frequency distribution of *Baetis idei* in the Ara River. Data collected from May 2014 to April 2015.

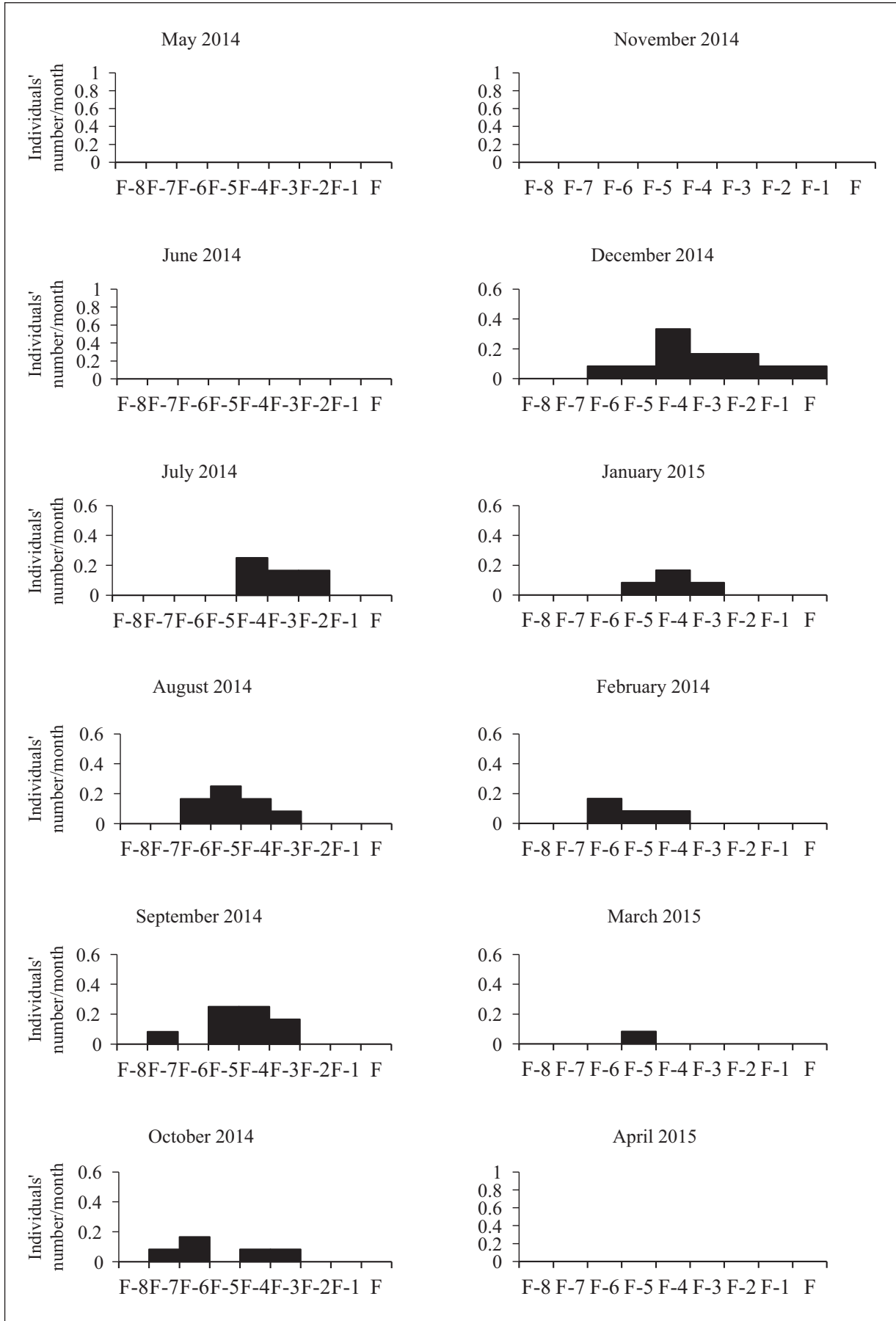


Fig. 8: Size-frequency distribution of *Baetis idei* in the Jelai River. Data collected from May 2014 to April 2015.



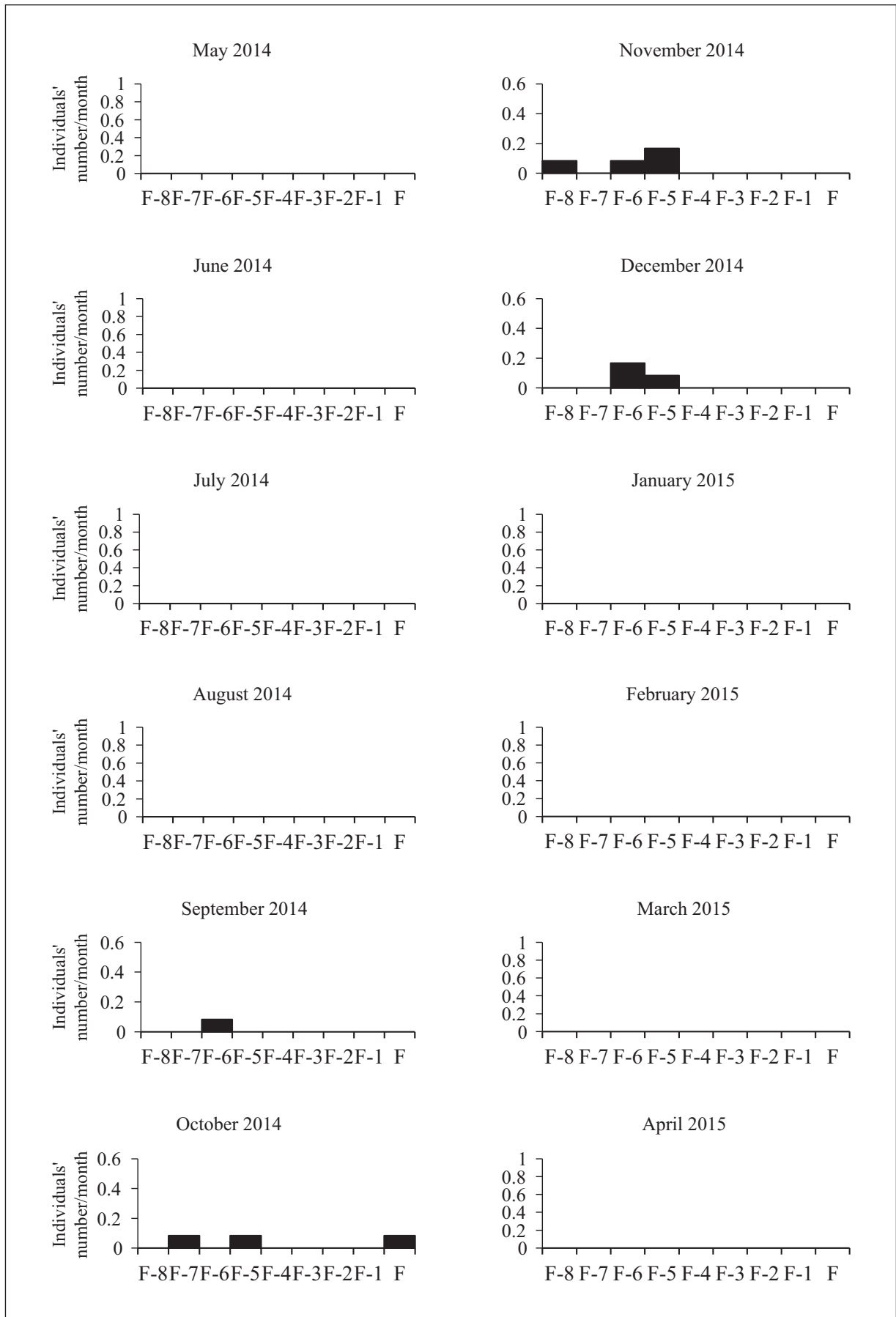


Fig. 9: Size-frequency distribution of *Baetis idei* in the Kurau River. Data collected from May 2014 to April 2015.

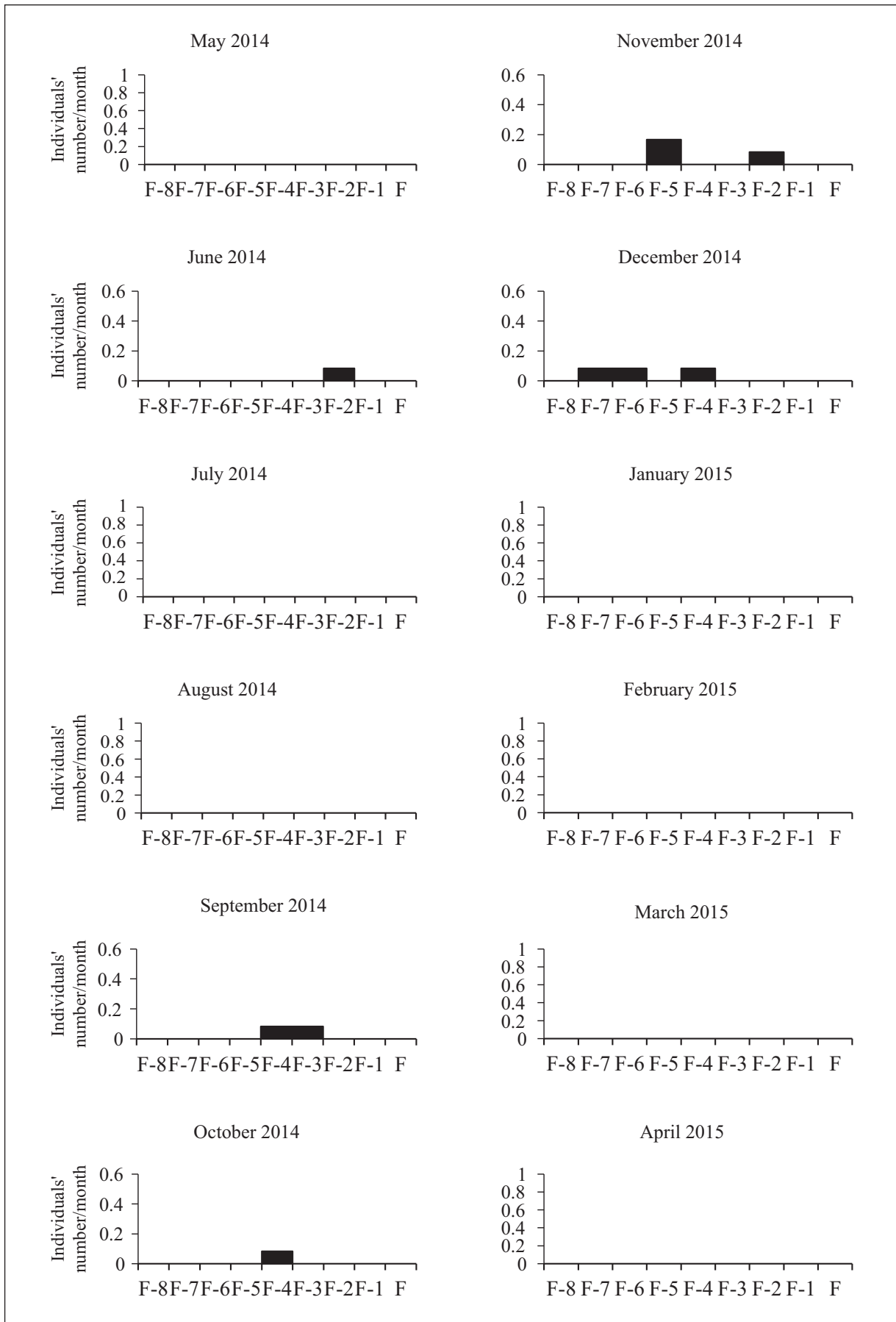


Fig. 10: Size-frequency distribution of *Baetis idei* in the Ayer Itam River. Data collected from May 2014 to April 2015.

**Table 3.** *Baetis ideii* secondary production, total annual production, and the annual production/biomass (P/B) (turnover ratio) at the Ara, Jelai, Kurau and Ayer Itam rivers determined by the Hynes size-frequency method

<i>Baetis ideii</i>	Ara	Jelai	Kurau	Ayer Itam
Total production, P	16.55 mg m <sup>-2</sup> y <sup>-1</sup>	14.61 mg m <sup>-2</sup> y <sup>-1</sup>	-3.92 mg m <sup>-2</sup> y <sup>-1</sup>	0.04 mg m <sup>-2</sup> y <sup>-1</sup>
Total production, P (AFDM)	15.35 mg m <sup>-2</sup> y <sup>-1</sup>	13.56 mg m <sup>-2</sup> y <sup>-1</sup>	-3.64 mg m <sup>-2</sup> y <sup>-1</sup>	0.04 mg m <sup>-2</sup> y <sup>-1</sup>
Total biomass, B	10.58 mg	10.96 mg	1.84 mg	1.58 mg
Total biomass, B (AFDM)	9.82 mg	10.17 mg	1.71 mg	1.46 mg
Annual P/B (AFDM)	1.56	1.33	-2.14	0.02

**Table 4.** Mean ( $\pm$  standard error) values of physical parameters of the Ara, Jelai, Kurau, and Ayer Itam rivers

Physical parameter	River			
	Ara	Jelai	Kurau	Ayer Itam
Location	N 05° 05. 428' E 100° 51. 193'	N 05° 00. 852' E 100° 48. 604'	N 04° 54. 213' E 100° 49. 991'	N 05° 00. 888' E 100° 49. 998'
Width (m)	9.77 $\pm$ 0.12	17.9 $\pm$ 0.17	13.35 $\pm$ 0.34	12.93 $\pm$ 0.39
Depth (cm)	31.47 $\pm$ 2.30	27.79 $\pm$ 2.30	36.61 $\pm$ 3.33	46.96 $\pm$ 4.12
Water pH	6.88 $\pm$ 0.19	7.28 $\pm$ 0.17	6.97 $\pm$ 0.18	7.14 $\pm$ 0.13
Water temperature (°C)	26.33 $\pm$ 0.38	25.45 $\pm$ 0.32	24.59 $\pm$ 0.38	24.45 $\pm$ 0.32
Water flow velocity (m/s)	0.70 $\pm$ 0.09	0.65 $\pm$ 0.05	0.45 $\pm$ 0.05	0.53 $\pm$ 0.06
Substrates	20% Cobble, 20% Gravel, 60% Sand	10% Gravel, 90% Sand	10% Bedrock, 20% Boulder, 30% Cobble, 20% Gravel, 20% Sand	10% Bedrock, 10% Boulder, 20% Cobble, 20% Gravel, 40% Sand
Embeddedness	Marginal  Cobble, gravel and sand particles were (25%–50%) exposed on the water	Poor  Gravel and sand particles were <25% exposed on the water	Optimal  Bedrock, boulder, cobble, gravel and sand particles were <75% exposed on the water	Suboptimal  Bedrock, boulder, cobble, gravel and particles were 50%–75% exposed sand on the water
Canopy cover (% shaded)	Partly shaded (40%)	Open (<30%)	Shaded (>70%)	Shaded (>70%)

size (Landis *et al.*, 2010). Furthermore, Benke and Jacobi (1986) note that the growth rate of *Baetis* is faster in tropical rivers owing to higher temperatures; its production is, therefore, lower. Previous studies on *Baetis* spp. have found the multivoltine life cycle to be common in many locations (Clifford, 1982; Wallace & Anderson, 1996). Another previous study undertaken by Benke and Jacobi (1994) documented univoltine, trivoltine and multivoltine life cycles among a wide variety of mayflies in a subtropical river. Huang *et al.* (2009) note that voltinism in ephemeropterans is flexible and depends on the habitat in which that the population is found.

Using Hynes method (Huryn, 1996), the growth rate of *B. ideii* was used to calculate its annual production in the rivers. Ara River appeared to provide the most suitable habitat for *B. ideii* compared to the Jelai, Kurau and Ayer Itam rivers. Higher production, biomass, and annual P/B ratio

of *B. ideii* in the Ara River indicated that *B. ideii* developed more rapidly at that location. Previous studies by Benke (1993) and Benke *et al.* (1984) showed that insects with high P/B values usually had rapid development and multiple cohorts.

Annual P/B ratio, which reflects the rapidity in which living organism can replace itself (Webster *et al.*, 1983), was very low for *B. ideii* in the four selected rivers in comparison with similar values obtained in previous investigations by Bottova and Derka (2013); Gaines *et al.* (1992); Gaines (1987); Jackson and Fisher (1986). Production of *B. ideii* in the Ara and Jelai rivers was greater compared to the biomass which was low due to the small body size of the insect. Nevertheless, a high rate of production was maintained because this species was able to reproduce quickly. In contrast, *B. ideii* in the Kurau and Ayer Itam rivers had greater biomass compared to their production. These findings showed that body size of *B. ideii* from these two rivers was bigger

than those from the Ara and Jelai rivers. In fact, annual P/B ratios for *Baetis* spp. have rarely been reported to be greater than 12 (Allan, 1985; Zelinka, 1980). Meanwhile, *B. ideii* in the Kurau River showed negative values for production (P) and annual P/B ratio. According to Benke (1970) a negative value for annual production implies that there is less addition of young individuals to the population compared to the previous instar as time passes.

The growth rates of *Baetis* sp. in the rivers also has a major impact on the production and biomass generated. Higher growth rates lead to higher annual productivity and biomass of *Baetis* spp. The main factors governing the growth rate of *Baetis* spp. are food quality and predation (Benke, 1998), population density (Feminella & Resh, 1990), body size (Huryň & Wallace, 1987), temperature (Fisher *et al.*, 1982), and available time for the development (Butler, 1982). The hydrological environment (water temperature, velocity, depth, and type of substrate) affects the abundance and growth rate of *Baetis* spp. Huryň and Wallace (2000) observe that under optimal temperature, secondary production is highest when high biomass combines with a rapid growth rate. In addition, a previous study by Peckarsky *et al.* (1993) showed that the presence of predators affected the abundance of *Baetis* sp. in the studied areas. For example, the larval mass of *Baetis* sp. increased 50 percent in the absence of predators. Overall, *Baetis* represents a major part of the area's biomass and production export (Jackson & Fischer, 1986).

## CONCLUSION

*Baetis ideii*, endemic in the Ara, Jelai, Kurau and Ayer Itam rivers of Perak, has nine instar stages with a multivoltine life cycle. Among the rivers surveyed, the Ara River provided the most favorable conditions for the growth of *B. ideii* in terms of hydrological characteristics, substrate type, and canopy cover. Overall, annual P/B of *B. ideii* was also highest in the Ara River. The production of *B. ideii* in the Ara and Jelai rivers was greater as compared to biomass because this species is able to reproduce quickly.

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