Mud-puddling behaviour of butterflies in the Soraya research station, district of Subulussalam, Aceh, Indonesia

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Abstract. Butterflies, mostly the male congregate and uptake the sodium and amino acids from the mud, dung, and urine of mammals or decaying flesh and then transferred to the female during mating. This behaviour is usually called as puddling. Hence, the objective of the present study was to identify the puddle butterflies in the Soraya river banks, their sex and age ratio. Five sampling points were observed for three hours (09:00-12:00 am) during 5 d each. The number of individual and number of species of butterflies that do puddlings were observed. A total of 25 species of butterflies belonging to four families were identified. Appias lyncida and Graphium sarpedon were the most active in puddling followed by Caleta elna, Pathysa antiphates and Appias lybithea. Generally, butterflies that do puddle were the male and the young individual.

1. Introduction

During the caterpillar stages, butterflies acquire a limited amount of minerals. This limited mineral will be sequestered and used in the next stage of life (adult). Adult butterflies feed on nectar flowers, moisture fruits and certain moist substance [1,2]. Many species of butterflies particularly the male seek out nutrients in certain moist substances such as mud, wet soil and sand, dung and urine of mammals, animal carcasses and decay materials, and they suck up the fluid [2,3]. This behaviour was called puddling. They obtain sodium and amino acid from the fluids [1,2]. The sodium and amino acid which uptake through puddling behaviour by males will be transfer to the females during matting [3,4]. The eggs sodium content of Gluphisia septentrionis sired by puddlers were higher than nonpuddler [5].

The mud-puddling behaviour is known from the temperate zone as well as tropical habitats. The phenomenon commonly was happened in the tropical region. Large number of butterfly individuals, mostly male, congregate and uptake the sodium and amino acid from the mud or wet soil [6]. Previous study reported that there were 21 species butterflies puddling in the Sungai Sarah, Aceh Besar, Indonesia. They more preferred the dung of carnivores, were 18 species than those of herbivores were only 8 species. [7]. At least 46 species of butterflies was recorded attracted to the baits for puddling in the rain forest of Kinabalu National Park (Sabah, Malaysia) and 54 species was recorded in the farmland outside the park [6].

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Leuser Ecosystem has varying carrying capacity and biodiversity. Four key species in Ecosystem Leuser are orang-utan, elephant, Sumateran rhinos and Sumateran tigers. Many studies of Leuser biodiversity have been done, particularly about four key species and their habitats, meanwhile study on insects still few. One of the research station located in the Leuser Ecosystem is Soraya Research Station. There are many types of habitats in this station. Insects, especially butterfly found in every habitat, including the bank of river. The area around the river is a favourite habitat for butterflies caused has a high humidity and there are different types of vegetation. Many species of butterflies also puddle on the river banks to suck sodium and amino acid from the moist mud. Until nowadays, there is no information on the puddling behaviour of butterflies at Alas River in the Leuser Ecosystem. Therefore, the objectives of the present study were to identify the puddle butterflies in the Soraya river banks and their sex and age ratio.

2. Material and methods

2.1. Study site
The study was conducted at Alas river in Soraya Research Station, District of Subulussalam, Aceh Province (Figure 1). Soraya Research Station was established by the Leuser Development Program (LDP) at the end of the 1994. The research area was originally a former forest conservation area of PT. Indonesian Industrial Development Company. The research station was closed in 2001 due to conflict in Aceh and reopened in 2014 with the collaboration of the Leuser Conservation Forum and the Forestry Services of Aceh Province. This station is one of the research stations on biodiversity and ecosystem in the Leuser Ecosystem, Aceh Province, Indonesia [8]. Soraya Research Station is geographically located between 2°55’25” NL and 97°55’43’ EL. The topography of the station's height ranges from 75-350 m above sea level [8].

![Figure 1. The location of study sites in the Alas River, Soraya Station Research.](image)

2.2. Data collection
The study was conducted at Alas River in Soraya Research Station, District of Subulussalam, Aceh Province, Indonesia from April to May 2019. Five study sites along the Alas River were used in this study (Figure 1). The flat and sandy river banks were chosen as the study sites. The minimum distance between each study site was 500 m. In each locations were plotted an area of 5 × 2 m for observing butterflies that do puddling for three hours, starting at 09.00 am to 12.00 am. Observation in each study sites were carried out for five days in sunny weather conditions.
The species and number of individual butterflies who do puddling and the frequency of puddling of each individual are recorded and counted. Observations on puddling behaviour were carried out at a distance of 3 - 4 m from the study site (5 × 2 m). Every individual who puddles is photographed and recorded. Based on the photographs or recordings, further identification of the butterflies’ species, sex, wing wear is then carried out [9]. The wing condition was used to approximate the age of butterfly, therefore the age of butterflies were grouped into young (rating 1.5 - 2.0) and old (2.0 - 5.0) follow Boggs [9]. Some butterflies that are not known of species are captured using the insect nets measuring 40 cm in diameter and length stick is 2 m and then identified. After identifying the butterflies were released again.

2.3. Data analyses
Base on the data were obtain and then the sex ratio and age ratio were calculated. Furthermore, the Shannon’s diversity index, Margalef’s species richness, and Sorensen’s evenness index were used to describe the butterflies that puddling in five study site of Alas River [10, 11].

3. Results and discussion
The results showed that there were 25 butterfly species belonging to four families (Lycaenidae, Nymphalidae, Papilionidae and Pieridae) that did puddling in all study sites (SS) (Table 1). All of four families also found in earlier studies [1,7,12,13]. The butterflies species that belong to Papilionidae and Pieridae found in all study sites, excepted Trogonoptera brookiana. In general, butterfly species in both families are widespread in various types of habitat and have a variety of host plants. Papilionidae also known as a stronger flyer and can fly quickly.

The number of species recorded in each study site varied from 17 to 22 species and the number of individual varied also, from 58 to 80 individuals (Table 1). The difference in number of species and number of individual, probably due to diversity and density of vegetation around in each study site and the type of sedimentation on the blanks of the river. The diversity of butterfly species tends to be higher in habitats where there are many vegetation species. Furthermore, the amount of organic material that is buried in the river bank due to river water flow will be increasing the mineral content in the mud. As a result, many butterflies come to puddling in that location. In the SS-1 and SS-3 the vegetation more abundance than those are SS-2, SS-4 and SS-5.

Butterflies are very interested in puddling on mammalian dung. In SS-1 and SS-3 there are traces of mammalian dung’s, because those locations were place for animals, especially pigs, looking for food, drink and done wallow. Butterflies puddling in dung carnivore more than those in dung herbivore. This due to the high sodium content in that dung [2,7]. The number of species recorded in this study as similar as reported on dung traps in the Sarah River district of Aceh Besar, Indonesia [7] and on elephant dung in Wasgamura, Sri Lanka [12], but less than in the rain forest of Kinabalu National Park (Sabah, Malaysia) [6]. Only 50% of the butterfly species in this study are the same as previous studies on the Sarah River [7].

Appias lyncida (Pieridae) and Graphium sarpedon were the highest number of individual recorded in this research, followed by Caleta elna and Appias lybithea; contrarily Trogonoptera brookiana was the lowest. Two species of Appias (A. lyncida and A. lybithea), G. sarpedon and C. elna that found were cosmopolitan and gregarious species. They always puddle in large numbers in groups, especially the male. The same phenomenon also occurs in the Sarah River where A. lyncida, A. lybithea and G.sarpedon dominates in puddling frequency on the bait that used [7] and also in around the Sarah River area [13]. Trogonoptera brookiana is one species whose its population has been reduces and is rarely found. This species is one of the butterflies protected by the Indonesian government.

Most of butterflies that do puddling are the young males. Population of male in all study sites was around 87.50 to 91.38% and 84.42 to 86.57% are young individual (Table 1). Mud-puddling is the behaviour of butterflies in obtaining minerals, especially sodium and amino acids. This behaviour mostly carried out by young males because the sodium and amino acids will be transfer to the females during matting and the young males more active flyers. Sodium and calcium phosphate play an
important role in the process of hardening egg shells and for flight activity [14]. The bait traps from dung carnivore were more preferred by butterflies in puddling than the herbivore dung [7].

Table 1. The butterflies species that puddle on the Alas River Ecosystem Leuser Aceh, Indonesia.

| No | Family / Species         | SS-1 | SS-2 | SS-3 | SS-4 | SS-5 | Total |
|----|--------------------------|------|------|------|------|------|-------|
| 1  | Lycenidae                |      |      |      |      |      |       |
|    | Caleta elna              | 4    | 5    | 5    | 5    | 6    | 25    |
| 2  | Caleta roxus             | 2    | 2    | 2    | 3    | 11   |       |
| 3  | Jamides parasaturatus    | 2    | 1    | -    | -    | -    | 3     |
| 4  | Jamides zebra            | 1    | -    | 2    | 2    | 6    |       |
| 5  | Nymphaidae               |      |      |      |      |      |       |
| 6  | Athyma reta              | 2    | 3    | 1    | -    | 6    |       |
| 7  | Euthalia aconthea        | -    | 1    | 1    | -    | 2    | 4     |
| 8  | Junonia orithya          | -    | 3    | -    | 1    | -    | 4     |
| 9  | Neorina lowii            | 4    | 1    | -    | -    | 5    |       |
| 10 | Polyura athamas          | 3    | 3    | 2    | 5    | -    | 13    |
| 11 | Polyura delphis          | 3    | 2    | 1    | 3    | 2    | 11    |
| 12 | Vindula dejone           | 1    | 3    | 5    | 2    | 2    | 13    |
| 13 | Yiptima fasciata         | 2    | -    | 2    | -    | 3    | 7     |
| 14 | Papilionidae             |      |      |      |      |      |       |
| 15 | Graphium agamemnon       | 2    | 2    | 3    | 1    | 3    | 11    |
| 16 | Graphium doson           | 5    | -    | 3    | -    | 3    | 11    |
| 17 | Graphium euryphilus      | 7    | -    | 2    | 3    | -    | 12    |
| 18 | Graphium evemon          | 2    | 4    | 3    | 2    | 4    | 15    |
| 19 | Graphium sarpedon        | 10   | 5    | 7    | 8    | 3    | 33    |
| 20 | Papilio polytes          | 2    | -    | 3    | 5    | -    | 9     |
| 21 | Pathysa antiphates       | 7    | 4    | 4    | 4    | 2    | 21    |
| 22 | Trogonoptera brookiana   | -    | -    | -    | 1    | 1    |       |
| 23 | Pieridae                 |      |      |      |      |      |       |
| 24 | Appias lyncida           | 10   | 15   | 14   | 12   | 7    | 58    |
| 25 | Appias libythea          | 3    | 7    | 7    | 3    | 7    | 27    |
| 26 | Catpsilia pomona         | 3    | 6    | 1    | 4    | 14   |       |
| 27 | Eurema hecabe            | 3    | 4    | 4    | 3    | 2    | 16    |
| 28 | Eurema sari              | 2    | 2    | 1    | 4    | 2    | 11    |
| Number of species | 22  | 17  | 20  | 19  | 17  | 25 |
| Number of individual | 80 | 64  | 77  | 67  | 58  | 346 |
| Sex Ratio (Male : Female) | 88.75 | 87.50 | 90.91 | 89.55 | 91.38 | 89.60 |
| Age Ratio (Young : Old) | 85.00 | 85.94 | 84.42 | 86.57 | 84.48 | 85.26 |

Note: SS = Study Site

Old butterflies also do puddling. Table 1 showed that 13.43% to 15.58% of old individuals sucked the moist mud and sand in the blanks of Alas River. More than 75% of old butterflies that do puddling were the females. The old butterflies have a puddling in order to replace the declining concentration of sodium and calcium phosphate in their bodies. Especially for the older females the puddling behaviour increases in the post reproductive period [2,3,4,14].

The results showed that the value of species richness (Margalef) butterfly doing puddling on the five stations classified as moderate to high, diversity index (Shannon-Wiener) were moderate and with a high evenness index (Sorensen's) (Table 2). These results suggest that environmental conditions around the river support for the life of a butterfly and allegedly the mud on the riverbank contains
minerals needed by the butterfly. The diversity index value in a habitat is influenced by the number of species and number of individuals found in the habitat. Along the high value of species richness and evenness, then the value of diversity index was also high \cite{10, 11}.

**Table 2.** The diversity index (H’), species richness index (R), and evenness index (E) of the puddle butterflies in the Alas River, Ecosystem Leuser Aceh, Indonesia.

| Study Site (SS) | R  | H’  | E   |
|----------------|----|-----|-----|
| SS-1           | 4.792 | 2.882 | 0.932 |
| SS-2           | 3.847 | 2.559 | 0.903 |
| SS-3           | 4.374 | 2.756 | 0.920 |
| SS-4           | 4.281 | 2.706 | 0.919 |
| SS-5           | 3.940 | 2.646 | 0.934 |

4. Conclusion

The number of 25 species belonging to four families butterflies were observed do puddlings on the mud of Alas River banks. *Appias lyncida* and *Graphium sarpedon* were the most active in puddling. The most of individuals that do the puddling were the young male butterflies.

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