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Notes on the final emergence and moulting pattern of *Ischnura senegalensis* Rambur, 1842 (Zygoptera, Coenagrionidae) and *Anax immaculifrons* Rambur, 1842 (Anisoptera, Aeshnidae)

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ABSTRACT: The complete emergence of two odonate species *Ischnura senegalensis* (Rambur, 1842) (Zygoptera, Coenagrionidae) and *Anax immaculifrons* (Anisoptera, Aeshnidae) were studied at Wardha district of Maharashtra, India. The average time to complete emergence in *Ischnura senegalensis* was 92.2 minutes and in the case of *Anax immaculifrons* the average time for the (F-0) stage larvae for emergence it into fully flying adult was 40 days. *I. senegalensis* emerges in a vertical posture and emerges between 8 00h and 16 00h during the day. Emergence of the *A. immaculifrons* was observed at night, with times ranging from 21h to 01h and it emerges in a vertical posture. The (F-1) stage larva of *A. immaculifrons* was cannibalised by the (F-0) stage larva by only cutting the thorax and separating the head and abdomen. One unsuccessful emergence was observed in *A. immaculifrons*, where one of the wings remain wrinkled and unstretched.

KEY WORDS: Odonata, emergence, exuvia, instar, moulting, pharate

INTRODUCTION

The life cycle of the amphibiotic insect order Odonata, which includes dragonflies and damselflies, comprises three stages: eggs, larvae, and adults. The eggs and larvae are aquatic, while the adults are terrestrial and aerial. When the aquatic larvae mature, they leave the water to undergo ecdysis, the final molt, which is a pivotal event in their life cycle. This process, where the adult emerges from the aquatic exuvia, involves various rhythmic movements. (Thaokar *et al.,* 2019). In contrast to Corbet (1999) who postulated four stages for the full emergence of odonata, Andrew and Patankar (2010) mentioned involvement of only three stages of odonata emergence.

The period of odonata emergence is very vulnerable, as the immobile and pregnable stage is exposed to a variety of adverse biotic and abiotic environmental factors like wind, rain, temperature, oxygen level, lack of suitable emergence support and predation

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(Boda *et al.,* 2015; Thaokar *et al.,* 2019). Odonates usually exhibits two types of emergence patterns, horizontal pattern which is commonly seen in Zygoptera and Anisoptera, and the vertical emergence which is common in the remaining groups. The two emergence patterns in odonates, horizontal and vertical, have different effects on the insects' transition from the larval to the adult stage. (Eda, 1963). The primary factor influencing the emergence patterns in odonates is the type of available substratum in their environment.However inverted emergence has also been observed in some species of Zygoptera (Rowe, 1987). The emergence posture is generally depending on the availability of substratum; availability of flat surface such as leaves, etc. influence horizontal emergence, whereas availability of vertical substratum (wooden sticks, small twigs, aquatic plant etc.) helps to emerge in vertical posture (Inoue, 1964). However, *Ceriagrion coromandelianum* can moult in both horizontal as well as vertical position (Thaokar *et al.,* 2019).

Several authors have studied the final emergence of odonates, focusing primarily on subtropical and temperate regions. Early research by Trotter (1966) and Ubukata (1981) explored emergence patterns, while Banks and Thompson (1985) and Gribbin and Thompson (1990, 1991) examined environmental factors. Corbet (1999) provided a comprehensive review, and more recent work by Haslam (2004), Baird and Burgin (2013), and Boda *et al.* (2015) continued to refine these findings. However, studies from the Indian region are limited, with contributions from Mathavan and Pandian (1977), Andrew (2010, 2012), and Andrew and Patankar (2010), along with Thaokar *et al.* (2019). Studying the final emergence of odonates is important to understand regional differences, fill knowledge gaps in underrepresented areas like India, and contribute to broader ecological insights. *Ischnura senegalensis* and *Anax immaculifrons*, both described by Rambur in 1842, are common odonates in Nagpur, central India. *I. senegalensis* is a widespread Coenagrionidae damselfly found in Africa and South-East Asia, while *A. immaculifrons* is a large Aeshnidae dragonfly ranging from Europe to Japan. Both species breed in stagnant water bodies, streams, and rivers (Andrew *et al.*, 2008).The life history and larval morphology of *Ischnura senegalensis* and *Anax immaculifrons* have been thoroughly studied, as evidenced by the works of Sangal and Kumar (1970), Kumar (1973, 1984), and Okude *et al.* (2017). The present paper describes the pattern and process of emergence of the damselfly *I. senegalensis* and dragonfly *A. immaculifrons* with a note on the mortality and larval cannibalism.

MATERIALS AND METHODS

Three larvae of *A. immaculifrons* were collected on 19 February 2023, from Kakaddara (20°52'48"N; 78°23'23"E) of Wardha district, Maharashtra. Out of three larvae, two were in (F-1) stage; one was in (F-0) stage. The six larvae of *I. senegalensis* were collected from Dham river of Pawnar (20°47'08"N; 78°39'56"E), Wardha district, Maharashtra, on 18 March 2023. All the collected larvae of *Ischnura senegalensis* were of (F-0) stage. The observations on emergence and moulting pattern were made at the Post Graduate Department of Zoology, Dr. R. G. Bhoyar Arts, Science, Commorce College, Seloo, Wardha (20°49'41"N; 78°41'58"E), Maharashtra, India. The collection of larvae was done with the help of D loop larvae collecting net and some are hand-picked. Dragonfly larvae were kept in large bottles (8cm diameter, 25cm height), while damselfly larvae were kept in small bottles (6cm diameter, 20cm height). In addition, (F-0) larvae were kept in an aquarium (26cm length, 10cm width, 20cm height) until they emerged. As emergence supports, wooden sticks and small twigs were placed in the containers. Feeding materials for *Anax immaculifrons* were prawns, water beetles, and fly maggots and for *Ischnura senegalensis* were may fly larvae, mosquito larvae. Various stages of wing development and moulting during metamorphosis were photographed using a Nikon D5300 DSLR equipped with a Nikkor Af-p 70-300 mm lens. The emergence time was recorded using an electronic stop watch. The F-0 (= Final) and F-1 (= Final minus 1) stages were determined using keys and data from (Okude *et al.,* 2021).

RESULTS AND DISCUSSION

Emergence of *Ischnura senegalensis*

Five of the six collected larvae were the emerged individuals were all female. The final emergence of the damselfly *I. senegalensis* was observed on March 20, 2023. It began at 11.27 am. and ended at 12.48 pm. Based on Andrew and Patankar (2010), the process has been divided into three observable stages, which are described below.

Stage I: At 11.27 h, the F-0 larva climbed the stick after coming out from the water. On the dry stick, it moved 14 cm prior to stopping. Larva started shaking its abdomen horizontally. After resting on the stick for ten minutes, the abdominal and head movements began. These movements started out very slowly and later moved vertically. The head and thorax were then pushed up against the stick. The abdomen's back was firmly pressed up against the base while the legs were spread out. The larva shifted the head to the side and curled up the tip of the abdomen. The head and thorax were raised, and the fore and hind legs' grip was reset. This movement continued interspaced with long intervals of motionless rest. It raised the anterior region of the body by flexing the legs, and a split appeared along the dorsal region of the thorax's cuticle. It took about 31 minutes for stage-I of moulting.

Stage - II: At 11.58h within one minute the head and thorax just elevated from the split exuvia without

Fig. 1 Image a Stage I, Image b-e Stage II and Image f-l Stage III of the final emergence of *Ischnura senegalensis*.

Fig. 2 Image a-b Stage I, Image c-f Stage II and Image g-h Stage III of the final emergence of *Anax immaculifrons*

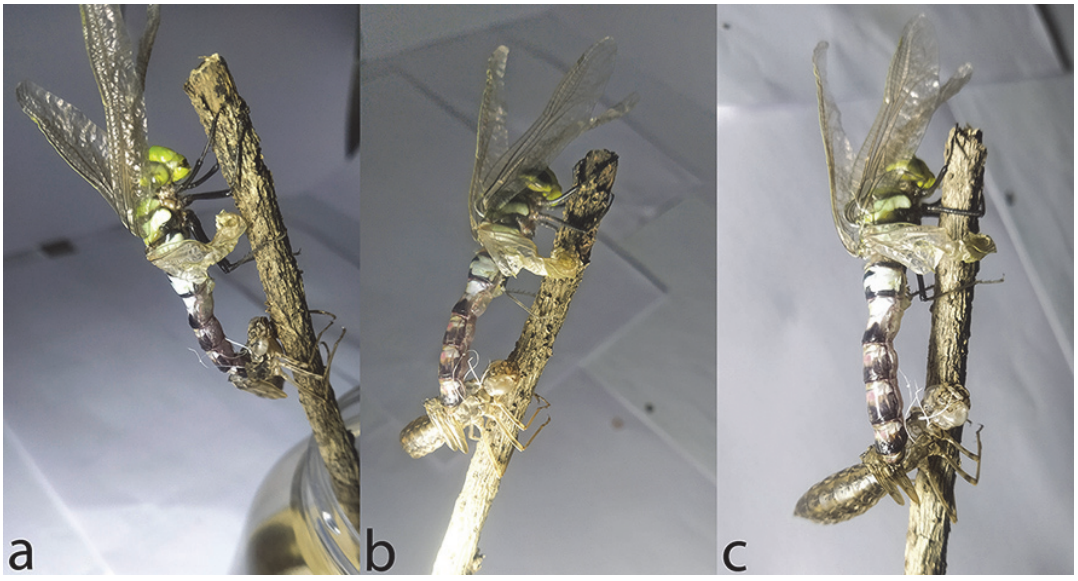

Fig. 3 Image a-c Incomplete metamorphosis during final emergence of *Anax immaculifrons*.

wriggling leaving the exuvia on the stick. The legs were straight sticking along the dorsal part of body and half of the abdomen along with head and thorax was outside the exuvia at 12.01h. The legs started flexing slowly. Initially only the forelegs exhibited movement and after few seconds all legs started moving and pawning the air and in search of substratum. The body of the pharate was still supported by the trapped abdomen. The thorax and abdomen formed an angle of 90 degree. Forelegs hold the wall of aquarium as it found suitable grip for all legs, the pharate smoothly extracted the remaining part of the abdomen from the exuvia without wriggling at 12.02h. The pharate move about 5 cm from the emergence site and hold the wall of aquarium at 12.04h for the movement all the legs played crucial role. For holding the forelegs fixed at the border wall of aquarium. The tiny compact wings lay parallel to the abdomen. It was 12.05h and the end the stage-II. This stage took only 8 minutes.

Stage -III: Fore, mid and hind legs of pharate rested on the wall of aquarium. Then telescoped abdomen and wings were expanding simultaneously, the rate of expansion of wings are faster than the abdominal expansion at 12.06h. The length of wings and abdomen same at 12.09h and continued stretching of wings at larger length as compared to abdomen. Wings were completely stretched and of greenish yellow in colour still stuck to each other at 12.10h. The pharate maintained its position and doesn't show the subject moving their legs or tightening their grasp on the tank wall. Pharate's abdomen kept growing gradually until it was fully extended at 12.20h. As the abdomen grew, the pharate forced water (15 times) out of the rectum at regular intervals to empty the gut. The thorax was pale golden in tone, and the eyes were pale brownish. The genital segments are a light golden-brown tone, and the centre of the abdomen was a soft yellow colour. In addition to the transparency of the wings, the body's pigmentation occurred concurrently on its whole surface at 12.45h, making the newly emerging imago flight-ready. The imago now displayed its distinctive species-specific colour patterning, which took 1.5 hours to complete on the adult body. Stage-III took 39 minutes.

A comparative account of five complete metamorphosis shows that, on an average the duration of Stage-I was 33.6 minutes (36.44 %), Stage-II was 14.6 minutes (15.85 %) and Stage-III was 44 minutes (47.73 %). The average time to complete emergence was 92.2 minutes (Table 1).

Emergence of *Anax immaculifrons*

Out of three captured larvae two were emerged, of which one successfully emerged and another was unsuccessful emergence. The first (F-0) stage larva collected on 19 February 2023, emerged on 2 April 2023 took 42 days for emergence. And the second (F-0) stage larva which undergoes moulting on 27 February 2023 from (F-1) stage to (F-0) stage took 38 days for emergence and emerged on 6 April 2023. The average time for the (F-0) stage larvae for emerged it into fully flying adult was 40 days. The documentation of final emergence of *Anax immaculifrons* and complete session of moulting was observed on the night of 2 April 2023. As the availability of substratum was stick so the emergence is vertical at an angle of 40- 50 degree. Some behavior of dragonfly is very prominent that they required complete darkness and zero disturbance for emergence if they feel any threatening signal then they just quickly go inside the water. The emergence time of *Anax immaculifrons* ranges from 9pm to 1am

Stage I: The underwater larva waited for sunset and no interruptions before attaching to the wooden

Table 1. *Ischnura senegalensis* duration and average timing (in minutes) of the three stages of the final emergence recorded in the laboratory

Time (h)	I	II	III	Total
11.27 - 12.45	31	8	39	78
08.36 -10.16	41	12	47	100
14.23 – 16.06	35	18	50	103
13.02 - 14.35	29	20	44	93
14.42 - 16.09	32	15	40	87
Total	168	73	220	461
Average	33.6 (36.44 %)	14.6 (15.83 %)	44 (47.73 %)	92.2

pole. At 19.15h, the larva was resting with its head and mid-thorax out of water. 2 days before emergence, the larva has only taken its head out of the water. The climbing began at 20.30h and was staggered approximately 13 inches from the water level, as the larva climb routinely on stick from 2 days before emergence, so it was ignored. Stage I was noticed only until the larva was attached to the wooden rod, and then Stage II was observed at 23.47h.

Stage II: The adult's head and thorax emerged through the split. The thorax appeared first, followed by the head. It didn't wiggle, but it did move up and down. The head and thorax hung out and jerked once by 23.55h. The abdomen was still inside the exuvia when they emerged, and pigmentation began slowly in the head and thorax while the abdomen was still inside the exuvia. The legs that were folded against the thorax began to twitch. The adult hanging out breathed heavily, expanding and relaxing his body. It has begun a prominent antero-posterior humping action in an attempt to remove its skin from larval exuviae. The pharate's body was filled with haemolymph and appeared green. The legs were entirely stretched. Pharate wings entirely extended in Stage-II at 00.06h on April 3, 2023, however transparency was present in Stage-III. The pharate grips the stick hard and forces the abdomen out of exuvia. At 00.25h, Stage II was finished.

Stage III: The pharate held the stick tightly with all three pairs of legs. The abdomen gently stretched, and finally the wings became transparent. While the abdomen was still bent, by 1.02h it had completely extended, straightened, and began to sclerotize. The pharate cleansed the gut by forcefully releasing water from the rectum at regular intervals while the abdomen expanded. The eyes and exposed mouthparts were a pale green colour. The thorax and abdomen are black and white in coloration. At 01.39h, Stage III was finished. The imago now displayed its species-specific colour patterning on the adult body, which took 4 hours.

Case of an unsuccessful emergence and larval cannibalism

On April 6, 2023, one *Anax immaculifrons* larva was seen crawling out of the water, in preparation for its eventual emergence. After the thorax had fully emerged, the notches were visible. However, the right hind wing was not quite stretched and remaining three were stretched. The pharate's abdomen became wedged inside the exuvia, was ultimately pulled out at the same time as it emerged, and continued to tilt.

Among the two *Anax immaculifrons* (F-1) larvae collected from Kakaddara on February 19, 2023, one of the (F-1) larvae had a moult to reach the (F-0) stage on February 27, 2023, eight days after the collection. By merely severing the thorax and dividing the head and abdomen, the (F-1) stage larva was cannibalised by the (F-0) stage larva on March 15, 2023. According to the observation, the (F-1) stage larva's fleshy abdomen was not fully consumed by (F-0) stage larva.

Moulting stages of the *I. senegalensis* is similar to that of the *Ceriagrion coromandelianum* as both the species belongs to same family. The average time to complete the emergence was about 92.2 minutes. After emergence, *C. coromandelianum* stand on the exuvia but *I. senegalensis* move from the place of emergence and undergoes stretching and pigmentation (Thaokar *et al.,* 2019). Due to the firmness of the substratum, the emergence is vertical and at an angle of 40 to 50 degrees. One larva of *I. senegalensis* emerged even there is absence of gill at the end of abdomen. The emergence time of *I. senegalensis* ranges from 8h to 16h and its diurnal like other Zygopterans. Out of 2 larvae of *A. immaculifrons* one larva moulted completely and another with unsuccessful emergence because of lack of hardening of wing. In Stage -II of *A. immaculifrons* some amount of wing stretching and hardening has started and abdomen of pharate protrude late from the exuvia. In both *I. senegalensis* and *A. immaculifrons* the posture of emergence was tilted vertical since substrates were sticks. In (Thaokar *et al.,* 2019) *C. coromandelianum* appears to be an opportunistic species and can moult in both horizontal as well as vertical position. The maximum time for the stretching and spreading of the body and wings

in both the groups in Stage-III were observed in both the anisopteran *A. immaculifrons* and zygopteran *I. senegalensis*. In *A. immaculifrons* some time of Stage-III reduce due to sclerotization and spreading of wing started at ending of Stage-II.

During emergence of adult odonata, mortality usually occur for three major reasons, these are failure to moult, failure to expand and harden the wings, and predation (Thaokar *et al.,* 2019). In *A. immaculifrons* one of the above-mentioned types, failure in expansion of one wing was observed. It is important to note that, during larval collection, one *A. immaculifrons* larva of (F-1) stage was collected from a waterbody of drying River bed. It was found crawling at the pond's edge about 1 m from the water. Corbet (1999) mentioned that larvae of odonates performed terrestrial locomotion unrelated to emergence, and according to him, this phenomenon most likely occurs due to stress comes from water deprivation. (F-1) stage of larvae of *Libellula depressa* has also been observed moving towards water sources from drying water body (Piersanti *et al.,* 2007).

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