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Delvinder Kaur

Singapore Zoo, Wildlife Reserves
Singapore 80 Mandai Lake Road,
Singapore

Luis Carlos Neves

Singapore Zoo, Wildlife Reserves
Singapore 80 Mandai Lake Road,
Singapore

Francis Cabana

Singapore Zoo, Wildlife Reserves
Singapore 80 Mandai Lake Road,
Singapore

Rearing unlikely dragons: Breeding of the dragon headed katydids, *Eumegalodon blanchardi* (Orthoptera: Tettigoniidae)

Delvinder Kaur, Luis Carlos Neves and Francis Cabana

Abstract

The Dragon headed katydid (*Eumegalodon blanchardi*), is a long-horned grasshopper. They have proven to be a difficult species to breed under human care. One of the major challenges in the breeding process is a low egg hatch rate. In this study we trialled three different incubation methods to determine which has a higher hatchability and monitored hatchling growth. Method A was a typical incubation technique used for other katydid species, Method B left the eggs in the banana pseudostem longer before placing in incubator and Method C only used the pseudostem. Method C had a significantly higher hatching success rate of 96.3% versus Method A's 38.1%. Perhaps the banana pseudostems' antimicrobial properties protected the eggs against microbial attacks and provided adequate insulation and humidity. Humidity in particular is an important factor in other katydid species' hatch success. Optimising growth rates based on diet is a logical next step.

Keywords: Orthoptera, Tettigoniidae, incubation, invertebrates, grasshopper, mandibles

Introduction

The Tettigoniidae (Orthoptera, Ensifera, Tettigoniidae) are commonly referred to as the Long-horned grasshoppers. They are described this way because their long slender antennae are known to exceed their body length ^[1]. There are 19 subfamilies currently recognized ^[2]. The Dragon-headed katydids (*E. blanchardi*) originate from the primordial forests of Borneo. They are omnivorous, consuming a variety of reproductive and vegetative plant parts as well as other insects.

Males and females can easily be distinguished. The main difference is the presence of an ovipositor in the females, which protrudes out of the abdomen. It is short, thick and curved upwards, adapted to make a slit in the stem of the plant used as substrate for oviposition. After insertion, the eggs are carefully arranged inside ^[2]. As a group, the katydids are known to oviposit into plant parts such as leaves and into soil substrate. In some cases, oviposition takes place into the plant stems and branches. The Malaysian leaf katydid (*Ancylecha fenestrata*) is another large species which is found in Peninsular Malaysia. Although often bred in captivity, very little is known of its existing biology. The female *A. fenestrata* only lays their eggs in certain plants which are also the food plants for the respective species' adults and nymphs ^[3]. Oviposition habits are based on the shape of the ovipositor and their eggs ^[4]. Female *E. blanchardi* possess a long narrow ovipositor, which is slightly curved towards the end. Other species with similar ovipositor morphology, like *Conocephalus dorsalis* and *Leioblastes laevis beier*, are known to deposit their eggs in plant stems ^[5, 6]. Katydids are known to be rather diversified across the world, having different adaptations which can easily be distinguished based on their morphology.

Specific information on *E. blanchardi* is scarce as compared to other species. A publication in 1983, Bushcrickets – Singers in the Night, described the *Eumegalodon* genus as 'conspicuous' but yet nothing was known about their natural living conditions and morphology ^[7]. Unfortunately, very little has since been recorded about their behaviours and biology in the wild. The San Diego Zoo's breeding of *Lesina intermedia* is often used as a model for breeding under human care ^[8]. Breeding *E. blanchardi* has proven to be challenging under human care with little to no records of eggs hatching. Female katydids can produce hundreds of eggs ^[5] and our population at Singapore Zoo (Singapore) is fertile and mating, however the hatching rates have historically been low and survival to adulthood lower still.

Correspondence

Francis Cabana

Singapore Zoo, Wildlife Reserves
Singapore 80 Mandai Lake Road,
Singapore

We would propose that the incubation methods described in Chang ^[8] may not be appropriate for *E. blanchardi*, and perhaps it is too unique within the region to use *L. intermedia* as a model species. Our study aims to measure the hatching and survivability rates of *E. blanchardi* under three different incubation and rearing methods and to track this amongst individuals. We also aimed to record the lifecycle of *E. blanchardi* to suggest further enhancements in their daily husbandry to increase survivability, notably diet.

Materials & Methods

Experimental Set-up

At Singapore Zoo (Singapore), *E. blanchardi* were kept in two different settings: the main exhibit (135 cm x 57 cm x 76 cm) where they can be viewed by visitors and the other in the back of house (140 cm x 60 cm x 78 cm). Both holding tanks were similar in size, providing the katydids ample climbing space and leaves for locomotion. We housed them in groups with more males than females (average 5.2), in order to stimulate male competition. The exhibit had a temperature range of 25 to 26 °C and relative humidity of 75%, while the holding had a temperature range of 28 to 32 °C with a relative humidity of 85%. The katydids seemed to do well in both settings as they were seen engaging in their normal behaviours: consuming food, mating & oviposition. The katydids were also fed daily with feeder invertebrates such as crickets to meet their protein requirements.

Banana pseudostems were placed in the exhibit for a period of 16 to 21 days, depending on the rate of deterioration of the stem. The banana pseudostems were at least 12 cm in thickness to ensure sufficient lifespan for egg development before deterioration of the pseudostem. Pre-soaked superabsorbent polymers in bags were attached to the ends of the stem to also increase longevity.

Incubation Methods

When the katydids started showing signs of breeding, we randomly placed them within one of three incubation methods (Methods A, B or C). Each differed in how long the eggs were left in the banana pseudostem and incubation temperature and humidity. Method A: We removed the eggs from the banana pseudostem immediately, and placed in an incubator. The incubator was set at 28 °C, with a humidity level of 70%. The setting of the incubator was replicated following San Diego Zoo's protocol when breeding another species of dragon headed katydid, *L. intermedia* ^[8]. The eggs were transferred to a small plastic container, with moist vermiculite (1:1) in the base. The eggs were placed onto a piece of window screen to prevent them from getting too wet. The pseudostems were placed in the tanks with the breeding katydids for 12 days. Method B: We left the eggs in the banana pseudostem for 22 days at room temperature (30 °C), with a humidity range of 89 to 99%, before removing it to be placed in the incubator. The pseudostems were placed in the tanks with the breeding katydids for 12 days. Method C: The eggs were left to incubate and hatch in the original banana pseudostem. Two banana pseudostems were placed in the exhibit with the adults for a period of 16 days. Unlike Method A or B, it was left in the exhibit for a longer period of time as the adults in tank were still in the process of biting holes in the banana pseudostem. The banana pseudostem was transferred to a plastic tank for a period of 26 days. Since the moisture trapped in the plastic container was accelerating the deterioration of the banana pseudostem, it was transferred to a fine mesh box.

We conducted a Pearson's Chi Square test to determine if there was an association between the frequency of successful hatchlings and incubation method: A, B or C.

Development of Hatchlings

To understand the lifecycle and the requirements of this species, we recorded the development process of the species. We noted the measurements (in mm) of the total length of the specimen's body (total head, thorax and abdomen length), antenna and ovipositor every three days. Throughout the data collection, one standard Vernier calliper tool was used to record the mandible lengths. Sample mandibles were removed from dead specimens of different instars and measured using a compound microscope.

Results

Hatching Success

There was a significant association between hatching success and incubation method ($\chi^2=42.693$, $P\leq 0.0001$). Leaving the banana pseudostem inside the enclosure (Method C) led to the highest hatchability of 96.3% and a survivability of 45.3% (Table 1). We also observed a difference in the appearance of the eggs. On day 34, despite both methods A and B allowing the eggs to be in the exhibit for the same period of time. The eggs varied between 1.0 and 1.2 cm in length (Figure 1) and those of Method B were on the larger end (Figure 2).



Fig 1: Eggs oviposited into banana pseudostem (sectional view)



Fig 2: Comparison of Method A and Method B's eggs on day 34

Another observation was that eggs processed according to method A were more prone to fungal infection. The total incubation period lasted between 56 and 68 days. There was no difference in incubation length for methods A or B.

Table 1: Three different methods for incubating the eggs of the dragon-headed katydid (*Eumegalodon blanchardi*) and its effect on hatchability and nymph survivability.

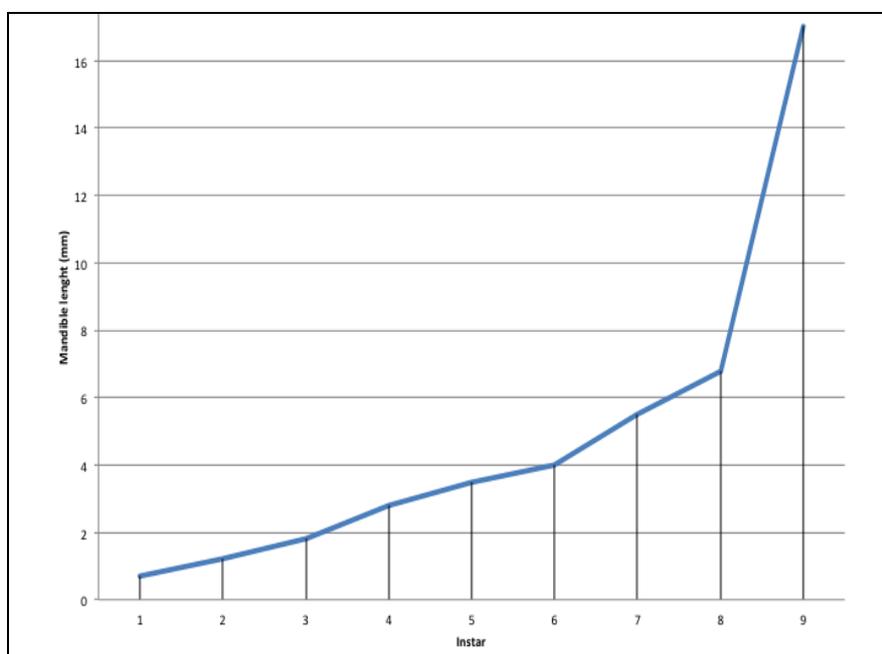
Method	# of days eggs in banana trunk	Hatchability (hatched/total eggs)	Survivability (survived/total)	Comments
A	12	38.1% (21/55)	4.8% (1/21)	27% of eggs lost to fungus
B	24	57.8% (33/57)	15.2% (5/33)	-
C	59	96.3% (53/55)	45.3% (24/53)	20 nymphs were unaccounted for

As for Method C, a total of 33 nymphs came out of the banana pseudostem on the first day, through a hole bitten in stem by the adults. This method allowed the eggs to develop faster as compared to A or B. Eleven days after the nymphs came out of the banana pseudostem, we dissected the banana pseudostem to find 53 empty egg casings and 2 unhatched eggs. In terms of hatchability, most of the eggs survived this method of breeding, with a 96.3% hatch rate. However, after hatching 20 nymphs were unaccounted for; as the banana pseudostem was deteriorating, a number of parasitic insects were observed in the banana pseudostem and it is possible the unaccounted nymphs were predated. This may also have been

a stocking issue rather than a problem with the banana pseudostem.

Development

The mortality rate of the nymphs was highest within the first eight days. On the fourth day, the nymphs varied in weight from 0.02 to 0.05 grams. By the fifth day, those individuals that weighed 0.02 grams had all died. The length of the mandibles also varied across all instars. First instar nymphs had mandible length of 0.7mm while the ninth instar (adults) measured up to 17mm (Figure 3).

**Fig 3:** Growth rate of mandibles of the dragon-headed katydid (*Eumegalodon blanchardi*) at different instar stages

Development of wings was first recorded at sixth instar (Figure 4). The wing development did not vary between male and female instars. Although the development of male and female *E. blanchardi* were similar in the early stages, females started taking longer time to develop in the later stages (Table 2 & Figure 5, 6, 7). Additionally, females were also undergoing ovipositor development which was first recorded at their fifth instar (Table 3). Similar to many other orthoptera, females were heavier as compared to males. Figure 4. Wing development of the dragon-headed katydid (*Eumegalodon blanchardi*) of the 4th (photo A), 5th (photo B), 6th (photo C) and 9th (photo D) instar stage. Photograph: WRS/David Tan

**Fig 4**

Table 2: Average growth of 24 female and 21 male dragon-headed katydids (*Eumegalodon blanchardi*) throughout their different instar growths, at the Singapore Zoo.

Moult	Female			Male		
	Day	Length (cm)	Weight (g)	Day	Length (cm)	Weight (g)
1 st	11.7±2.9	1.3±0.1	0.1±0.0	11.6±1.0	1.3±0.1	0.1±0.0
2 nd	24.3±2.3	1.4±0.1	0.2±0.1	23.6±1.3	1.4±0.1	0.2±0.1
3 rd	37.7±4.7	1.6±0.1	0.3±0.1	35.4±2.9	1.8±0.4	0.4±0.1
4 th	54.0±7.8	1.9±0.2	0.7±0.0	51.6±4.9	2.0±0.2	0.7±0.2
5 th	68.3±8.5	2.3±0.1	0.9±0.0	66.1±5.5	2.4±0.3	1.1±0.2
6 th	95.0±4.6	3.1±0.3	2.1±0.7	81.9±7.8	3.0±0.3	1.9±0.4
7 th	123.7±4.0	3.9±0.4	4.3±0.5	106.7±13.4	3.8±0.3	3.6±0.8
8 th	162.7±4.7	5.0±0.2	6.6±0.3	141.9±13.5	4.9±0.7	5.2±0.6

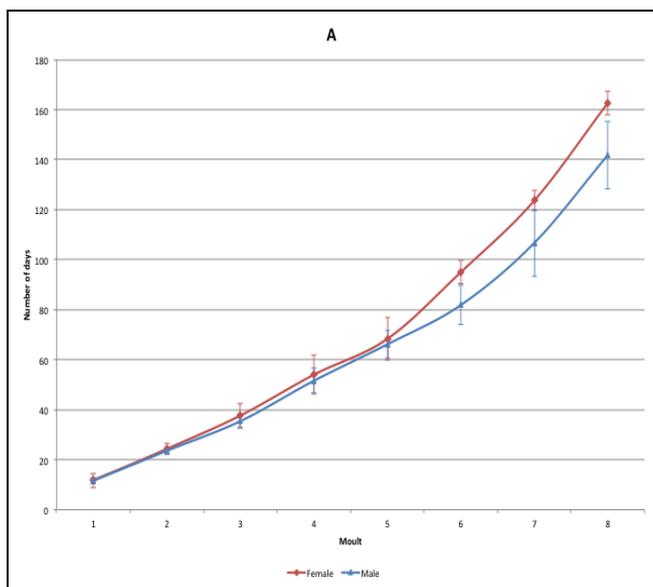


Fig 5: Average length of female and male dragon headed katydids (*Eumegalodon blanchardi*) at different moults

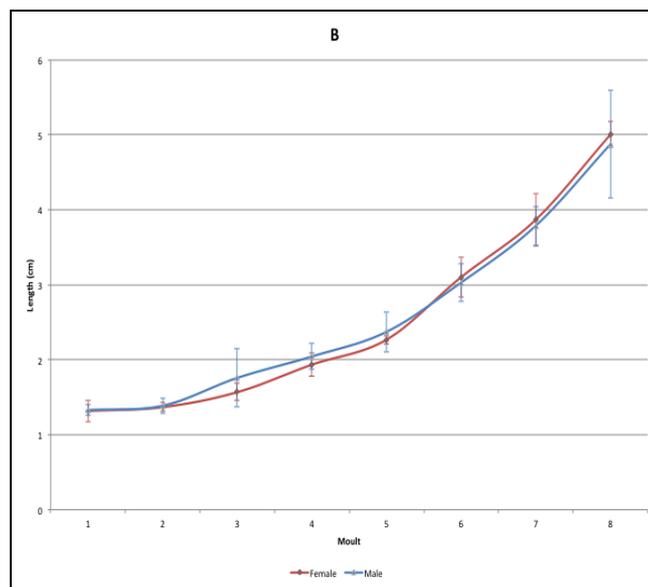


Fig 6: Average weight of female and male dragon headed katydids (*Eumegalodon blanchardi*) at different moults

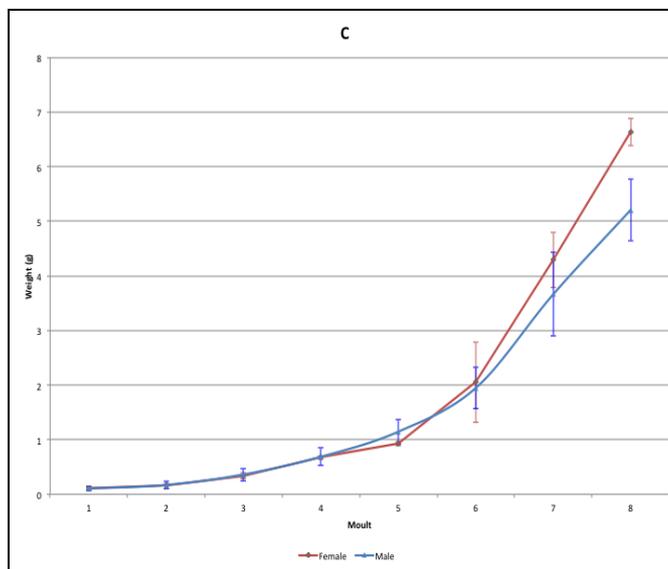


Fig 7: Average number of days taken to undergo moulting for female and male dragon-headed katydids (*E. blanchardi*)

Table 3: Development of ovipositor in female *Eumegalodon Blanchardi*

Instar	Approximate length of ovipositor (mm)
5 th (A)	2
6 th (B)	5
7 th (C)	13
8 th (D)	38
9 th (E)	45

Discussion
Incubation Success

After historical low hatching success of *E. blanchardi*, our trials revealed that allowing the eggs to remain in the banana pseudostem led to significantly higher hatching rates. Over the years we have seen an increasing number of studies which evaluate the effects and oviposition site preference of soil substrate moisture of selected orthoptera species. Crickets

may prefer higher soil moisture for oviposition as it is suspected to play a crucial role in the development of their eggs as well^[9, 10]. Therefore, as the banana pseudostems contain high moisture levels, the eggs were kept constantly hydrated. This is also believed to be beneficial for Tettigoniidea eggs, as they are known to absorb moisture through contact water and the absence of it could lead to dormancy^[11]. Banana pseudostems are also known to possess pathogenesis-proteins with antimicrobial properties^[12, 13] which could have played active roles in protecting eggs from fungus during incubation. Experiments done with *Scapteriscus borellii* and *Melanoplus sanguinipes* agree that females are known to refrain from ovipositing when they do not have access to suitable substrates for their eggs^[10, 14]. Our *E. blanchardii* would not even mate if we did not include the pseudostem which supports this notion. Evaluation of suitable substrate sites has even allowed researchers to analyse more efficient ways to manage Tettigoniidae outbreaks in Italy^[15].

There is still limited literature on oviposition site studies of stem substrates, making comparisons between our study and others difficult. A study done on artificial substrate for egg laying and embryonic development for the predatory bug, *Macrolophus caliginosus*, evaluated the effectiveness of several artificial substrate mediums to plant oviposition substrates. Comparisons were done based on the number of eggs oviposited on the selected substrates and survivability of offsprings^[16]. Results showed that although egg development was possible, adult yield was better when maintained in their host plant which also agrees with our results. Similarly, this study aims to derive the necessary requirements of a suitable substrate. However, while the aforementioned study compares several mediums as substrate sites, this study aims to study the plant-egg relationship and its effect on incubation of the *E. blanchardi*. According to the preference-performance hypothesis coined by Jaenike^[17], there is a direct correlation between oviposition preference and host suitability for nymphal development^[18]. Females are thus assumed to select high-quality hosts to oviposit their eggs. Therefore, it is important to understand substrate preference due to its vital role in insect fitness. By removing the eggs from the banana pseudostem, at different time periods and doing a comparison of hatchability and survival rate of offsprings left to incubate naturally in the banana stem with no interference, allows us to measure the effectiveness of the pseudostem as an egg-laying medium. Based on the study, the banana pseudostem which was naturally selected by the *E. blanchardi* as an oviposition substrate was evaluated to be a beneficial medium for egg development, producing the highest adult yield.

Katydid Reproduction

The male katydids produce mating calls by rubbing their wings against each other, with the assistance of specialized structures found in their forewings^[19], where else the female produces a chirping sound. In various publications, the call of the male has been presumed to be a tool to serenade the females. A significant and interesting characteristic of the male dragon headed katydids would be their mating calls which sound very similar to the rattle of the Rattlesnake. Mating calls by the males were often heard in the mornings and the evenings, and mating was observed shortly after they were set up. Often, upon arrival of animal care staff in the morning, the katydids were already engaging in mating behaviour. This happened in both settings. At times we observed mating to last for more than an hour. Interestingly,

as the male excretes spermatophore, it glows a fluorescent green before turning a milky white. This was observed with four different mating sessions. The remains of the spermatophore of katydids in general, are left attached to the external areas of the cloaca, allowing the female to consume it. This is also vital to the development of the fertilization^[20]. The females bit holes in the pseudostem, exposing several layers of collenchyma. We observed the females were not the only ones biting a hole in the banana pseudostem; males also participated in this activity. Within three to five days after mating, the females started depositing eggs into the banana pseudostem by inserting her strong blade-like ovipositor, into the holes created. Egg laying was accompanied by a rhythmic wave-like motion along the ovipositor. Mating behaviour was observed as early as 48 h after oviposition.

Katydid Husbandry Based on Life Stage

Through the trials held in Singapore Zoo, we determined that the nymphs required a modified captive diet which differed from their adult counterparts. It has been established for orthopterans, such as grasshoppers, that the relationship between diets and mandibular morphology can be analysed by looking at size, as well as shape component^[21]. While studies have made comparisons of mandibular morphology across species, research of the development of mandibles for specific orthopteran species is scarce. However, for lepidopteran larvae, it is largely accepted that mandibular morphology plays a vital role in nutrient acquisition^[22], which explains younger instars preference to leaf portions which contain lesser fibre making it easier to consume as compared to adults whom are not as selective^[23]. Similarly during the upkeep of the *E. blanchardi*, younger instars were presented with food with fleshy bits exposed (e.g. corn kernels were sliced open) for easy consumption. This is attributed to the size of their mandibles. The mandibles of the first instar accounted for only approximately 4% the size of adult mandibles. While the first full consumption of cricket (*Achetes* spp.) nymph was recorded in the second instar, we first observed them biting through corn kernels only in their fourth instar. Their mandibles are very slow to develop and appear to only be large and strong enough to self-feed by the fourth instar. The diet provided beforehand must be soft and not covered by chitin, cellulose or a cuticle. By investigating the mandible development, it was possible to cater a specialised diet for different stages. Based on observation, *E. blanchardi* undergo a total of eight moults. Most of the katydids which successfully achieved full development were able to attain a lifespan of twelve to fifteen months.

Younger instars had smaller antennae diameters which were prone to breakage. Therefore, one of the main indicators of the specimen undergoing successful moulting was the growth of their antennae. The katydids developed full wings at their ninth instar, similar to *Tegmina* spp., which are seen in many insects as a predator avoidance mechanism. This may possibly be associated with their dry leaf- cryptic roles^[24]. However, there is no literature depicting the strategies used by the immature stages. Once this instar is reached, we assume they are sexually mature and then mix them with similar sized adults for breeding to commence.

Conclusion

Throughout the incubation trials, we determined that certain conditions are more favourable for hatchability, nymph development and survival rate. Using solely banana

pseudostem as incubation substrate has led to a high hatch rate, which carried on through to reach full nymph development. This may be a model for other species which oviposit into plant vegetative parts. Further studies on the development of the nymphs should be conducted to understand their life cycle, which would be beneficial in establishing self-sustaining viable ex-situ populations and guidelines for potential reintroduction of *E. blanchardi* or closely related species, if necessary.

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