Application of a video technique to study the time budget of mosquito larvae behaviour

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Abstract

Constructing a time budget is an integral part of data collection when studying animal behaviour. Advancement in information communication technologies offers new ways of recording animal behaviour and obtaining data from digital images. We video taped mosquito larva behaviour and digitalized the images. Time budgets were then constructed from the digital images and analyzed. Digitalizing the tape recordings facilitated data collection for making the time budget. The observation experiments and the video technique are presented and discussed.

Introduction

Constructing a time budget when studying the behaviours of animals in nature is essential in understanding the animal's social structure, physiology and how it interacts with its environment. Mosquito larvae behaviours have been traditionally studied by direct observational methods (Duhrkopf and Benny 1990; Walker and Merritt 1991; Clements 1999; Workman and Walton 2003; Yee et al. 2004; Tuno et al. 2004). This way of observing and studying the behaviour of larvae is limited by the ability of the observer to watch and record carefully their behaviour. The density and tempo of the larval movements also makes it difficult for an observer to track each individual larva. Sometimes if the observer is not careful, slight variations in larval behaviour can be easily missed. Furthermore, this way of observing can be very tiresome for the observer and can take a long time to gather data.

The rapid expansion of information communication technologies (ICT) offers many opportunities to re-examine larval behaviours more closely. Video recording and the digitalization of images is one such technology available that can be used to study larval behaviour (Brackenbury 2001). Indeed some studies (Megan 2006; Workman and Walton 2003) video taped larval behaviours as part of their experiments in one form or another. However, constructing and analyzing the time budget was not the focus of these studies.

The digitalization of images allows data to be studied in detail using appropriate software that is available. The images can also be archived in electronic format for teaching, demonstrations or for further studies. For example, multiple data can be obtained from an achieved Digital Versatile Disc (DVD) format of a video recording of larval behaviour for various studies. These technological advances are also making it possible to publish video images of animal behaviour in scientific literature.

I describe here an application of a video technique used to study the behavioural time budgets of the larvae of three mosquito species, namely *Aedes albopictus*, *Culex pipiens* and *Anopheles stephensi*. The conversion of the video tape recordings to digital images made it easier to construct a time budget for the behaviours observed. Statistical analysis on the time budgets by ANOVA was done using computational tools from a website (Lowry 2006). Significant differences were compared by pair-wise comparison using Tukey HSD test (Atil and Unver 2001; Sokal and Rohlf 1995). The video technique including the results of the observation experiments are presented and discussed.

Materials and methods

Mosquitoes

Adults and larvae were maintained in an insectaria following standard protocols. The temperature was set at 26 degree Celsius and the relative humidity was maintained at 65 %. The insectaria had a 15 hours 9 hours day night cycle. Light was provided by four 40 watt fluorescent light bulbs.

To obtain eggs, adult mosquitoes were allowed to have a blood meal from a restrained mouse. Eggs were then collected on filter papers on the third day post-blood meal and were hatched in 250 ml of de-chlorinated plastic cups. (Surface area = 95cm-blood meal and were hatched in 250 ml of de-chlorinated plastic cups. (Surface area = 95cm2).

Newly hatched larvae were fed ®Tetramin (www.tetrajp.com) baby fish food and at the late second to early third instar stage were transferred to 33x24x7 cm pans. Water was changed every other day. Only fourth instar larvae were used for our experiments.

Observation experiment set up

The experiments were done in a 59-(length) x 28-(depth) x 35-(height) cm glass aquarium. The aquarium was filled with de-chlorinated tap water and incubated for one week to permit growth of micro-organisms on the walls including the floor, in the water column and at the air-water interface (Walker and Merritt 1991). A few pebbles were also put in the aquarium to allow micro-organisms to grow on their surface. Larvae at the fourth instar stage were individually pipetted into the aquarium and allowed to acclimate for one hour before larval behaviours were recorded. We used a total of 120 larvae for the observation experiments. Each experiment was replicated with 40 fourth instar larvae for each of the three species studied.

Definition of larval behaviours

We used established definitions of larval behaviours (Walker and Merritt 1991; Clements 1999) with minor modifications to suit our experiment design and set

up. Some behaviours that were observed by Walker and Merritt (Walker and Merritt 1991) were not included in our study. Other behaviours were combined.

Briefly, the definitions of behaviours used in our study were as follows:

1. Float/suspension feed – the larva is attached to the water's surface via its respiratory siphon with the body hanging obliquely into the water column. Anopheles larva lies horizontally in line with the air-water interface. The larva may be still or move slowly as a result of brush movements.

2. Float/interfacial feed – the larva is attached to water's surface and its body bent into a U shape so that its mouth brushes makes contact with the air-water interface. Anopheles larva attaches itself in parallel with the water's surface and rotates its head 180 degrees to make contact with the air-water interface.

3. Autogroom – At either at the surface or underwater, a larva bends its body into a U shape and works its mouthparts against its own body.

4. Dive – A larva spontaneously descends from a position near the water surface using a wriggling, swimming motion.

5. Brushwall – A larva that is underwater and its siphon detached from the air-water interface brushes the wall of the observation chamber with its mouthparts. The larva may be still or moving.

6. Wriggle-swim – A larva moves through the water column by flexing and un-flexing movements of its entire body forming a wriggling motion.

7. Underwater/mouth swim – A larva moves forward in the water column as a result of its suspension feeding movements, not by flexing its body. The larva is not attached to the water's surface.

8. Allogroom/feed – A larva directs its mouthparts against a nearby larva.

9. Underwater/still – A larva remains motionless while underwater, usually at the bottom of the water column.

10. Rise – A larva, when underwater, ascends through the water column to the surface.

11. Float/brushwall – the larva is positioned at the surface while attached to the air-water interface via its siphon and brushes the wall of the observation chamber with its mouth parts. The larva may be still or moving.

12. Bottom feed – A larva after diving and reaching the bottom of the water column brushes the floor, pebbles or chews a substrate. A larvae

brushing the wall of the observation chamber approximately 1-2cm from the floor was also regarded as bottom feed. This behaviour combines the float/substrate brush and chew substrate behaviours that were observed by Walker and Merritt (Walker and Merritt 1991).

Video taping of larvae behaviour

A larva in the aquarium in any behavioural state was chosen at random and filmed for five minutes using a handy-cam (Sony Co. Japan) video recorder. To enhance image contrast, recordings were done with a white card placed behind the aquarium in daylight conditions.

Twenty fourth instar larvae were placed in the aquarium and allowed to acclimatize for one hour prior to filming. A larva was than chosen at random and filmed for five minutes. After videotaping 10 larvae, all 20 larvae in the aquarium were removed and replaced with a new group of 20 fourth instar larvae. This was done to ensure a larva was not filmed twice. Again one hour of acclimatization time was allowed and 10 larvae were videotaped at random, each larva being recorded for five minutes. This procedure was followed for all three species of mosquitoes studied.

The focal-individual sampling method was possible because the tempo of larval behaviour and the low density of larvae in the aquarium allowed the observer to track an individual larva (Walker and Merritt 1991) and videotape its behaviour. Care was taken not to videotape a larva more than once. The observer became familiar with larval behaviours through preliminary observation of more than 300 larvae.

Results

Time budget for individual behaviours

The total video time was 320 minutes (Table 1). The individual time budgets for each of the three species studied, *Anopheles stephensi* (Table 2), *Aedes albopictus* (Table 3) and *Culex pipiens* (Table 4) showed significant differences in the mean time spent in some behavioural states but not in others. *Anopheles stephensi* spent majority of their time in the float/suspension feed (32.88%) and bottom feed states (29.43%) while *Culex pipiens* spent nearly 80% of the time float/suspend feeding. *Aedes albopictus* used the entire aquarium to move and feed. There were also great variations in the duration as reflected in the wide range in some behaviours.

Table 1. Total observation time for *Aedes albopictus*, *Culex pipiens* and *Anopheles stephensi* 4th larvae

Species	Total video taped time (minutes)
Aedes albopictus (n=22)	110
Culex pipiens (n=20)	100
Anopheles stephensi (n=22)	110

Table 2. Time budget for *Anopheles stephensi* 4th instar larvae behaviour (n=22)

Behaviour	Duration (mean ± SD)	Range	% Total time (total time = 2892 sec)	Frequency
Float/suspension feed	5.71 ± 5.03	80	11.08	56
Dive	23.78 ± 9.54	33	10.68	11
Wriggle-swim	4.2 ± 7.79	32	2.9	20
Brushwall	10.50 ± 8.73	26	2.9	8
Bottom feed	40.52 ± 35.36	147	29.43	21
Rise	15.0 ± 3.67	10	7.78	15
Float/brushwall	1,44 ± 1,5	4	0.45	9
Float/interfacial feed	2.68 ± 27.79	126	32.88	44
Underwater/still	11.33 ± 9.61	19	1.18	3
Underwater/mouth swim	-	-	-	-
Allogroom/feed	1.33 ± 0.87	3	0.55	12
Autogroom	1.67 ± 0.58	1	0.17	3

Table 3. Time budget for *Aedes albopictus* 4th instar larvae behaviour (n=22)

Behaviour	Duration (mean ± SD)	Range	% Total time (total time = 2892 sec)	Frequency
Float/suspension feed	19.15 ± 19.08	133	29.02	83
Dive	11,63 ± 7.55	62	14.44	68
Wriggle-swim	2.67 ± 4.45	9	4.25	87
Brushwall	14.17 ± 17.22	62	16.56	63
Bottom feed	17.68 ± 12.79	44	12.91	40
Rise	16.17 ± 9.05	36	17.13	58
Float/brushwall	9.0 ± 8.4	30	2.47	15
Float/interfacial feed	3.28 ± 6,43	29	2.35	39
Underwater/still	-	-	-	-
Underwater/mouth swim	-	-	-	-
Allogroom/feed	-	-	-	-
Autogroom	1.84 ± 1.67	5	0.86	26

Table 4. Time budget for *Culex pipiens* 4th instar larvae behaviour (n=20)

Behaviour	Duration (mean ± SD)	Range	% Total time (total time = 2892 sec)	Frequency
Float/suspension feed	117 ± 113.99	297	80.82	34
Dive	23.33 ± 14.04	53	5.64	12
Wriggle-swim	2.08 ± 0.9	3	0.5	12
Brushwall	9	-	0.18	1
Bottom feed	73.14 ± 14.78	43	10.32	7
Rise	8.1 ± 4.75	13	1.79	11
Float/brushwall	5.75 ± 5.62	11	0.46	4
Float/interfacial feed	1.08 ± 0.23	1	0.26	12
Underwater/still	-	-	-	-

Underwater/mouth swim	-	-	-	-
Allogroom/feed	1	-	0.02	1
Autogroom	-	-	-	-

Float/suspension feed

This was a common behaviour among the three species studied with significant difference in the time spent performing this behaviour (F=55.94, df=2, P<0.0001). *Culex pipiens* larvae spent a longer time (117±113.99 sec) in this behavioural state compared to *Anopheles stephensi* (5.71±5.03 sec) or Aedes albopictus (19.15±19.08 sec). The difference was significant between Culex pipiens and both *Aedes albopictus* and *Anopheles stephensi* (P <0.01, HSD [0.01] = 30.59) but not significant between *Aedes albopictus* and *Anopheles stephensi*.

Float/interfacial feed

All three threes species studied commonly performed this behaviour with significant differences in the mean time (F=11.16, df=2, P<0.0001). *Anopheles stephensi* larvae spent the longest time (21.61±27.79 sec) compared to both *Aedes albopictus* (3.2±6.43 sec) and *Culex pipiens* (1.08±0.23 sec). The difference in the mean time was significant between *Anopheles stephensi* and *Aedes albopictus* as well as between *Anopheles stephensi* and *Culex pipiens* (P <0.01, HSD [0.01] = 17.24). The difference was not significant between *Aedes albopictus* and *Culex pipiens*.

Dive

Diving was commonly observed in *Aedes albopictus*, *Culex species* and *Anopheles stephensi*. The mean time it took to dive for a larva was also significantly different among the three species (F=16.54, df=2, P<0.0001). *Aedes albopictus* larvae generally dived faster (11.63±7.55 sec) than both *Culex pipiens* (23.33±14.04 sec) and *Anopheles Stephensi* (23.78±9.54 sec) larvae. Pair-wise comparison showed *Aedes albopictus* to be significantly different to both *Culex pipiens* and *Anopheles stephensi* (P <0.01, HSD [0.01] = 9.06) but *Culex pipiens* and *Anopheles stephensi* were not significantly different from each other.

Brushwall

This was a common behaviour in *Aedes albopictus* (14.17±17.22 sec) and Anopheles stephensi (10.50±8.73 sec) species but was not common in *Culex pipiens*. Whereas *Aedes albopictus* brushed the wall while at the surface of the water as well after diving, *Anopheles* brushed the wall of the aquarium mostly after diving. In addition, *Aedes albopictus* frequently used this behaviour to feed underwater by moving away from the water's surface. There was no difference in the mean time spent performing this behaviour between the three species (F=0.22, df=2, P=0.81).

Float/brushwall

All three species studied commonly brushed the walls of the container while attached to the water's surface (Aedes albopictus = 9.0±8.4 sec; Culex pipiens = 5.75±5.62 sec; Anopheles stephensi = 2.6±27.79 sec). There was no significant difference in the mean time (F=1.56, df=2, P=0.23). Anopheles stephensi = 2.6±27.79 sec). There was no significant difference in the mean time (F=1.56, df=2, P=0.23).

Wriggle swim

Wriggle swim was a transition behaviour connecting one behaviour with another and was common among all three species. While *Culex pipiens* performed this behaviour near the water's surface most of the time, Anopheles stephensi used this behaviour mostly at the bottom of the water column. *Aedes albopictus* performed this behaviour both near the water's surface and at the bottom of the water column. There was no difference in the amount of time spent performing this behaviour between the three species (Aedes albopictus = 2.67±4.45 sec; *Culex pipiens* = 2.08±0.9 sec; *Anopheles stephensi* = 4.2±7.79 sec; F=0.93, df=2, P=0.40).

Bottom feed

All three species commonly fed at the bottom of the water column. However, the amount of time spent feeding at the bottom was significantly different between the species (F=21.28, df=2, P<0.0001). *Culex pipiens* spent the longest time feeding at the bottom of the water column (73.14±14.78 sec) followed by *Anopheles stephensi* (40.52±35.36 sec) and *Aedes albopictus* (17.68±12.79 sec). Interestingly, *Culex pipiens* spent nearly twice as long as *Anopheles stephensi* and four times as long as *Aedes albopictus*. Pair-wise comparison showed *Culex pipiens* to be significantly different to both *Aedes albopictus* and *Anopheles stephensi* (P<0.01, HSD [0.01] = 25.67). There was also significant difference between *Anopheles* and *Aedes* species (P<0.05, HSD [0.05] =20.42). (P<0.01, HSD [0.01] = 25.67). There was also significant difference between *Anopheles* and *Aedes* species (P<0.05, HSD [0.05] =20.42).

Autogroom

This behaviour was only observed in *Aedes albopictus* (1.84±1.67 sec) and *Anopheles stephansi* (1.67±0.58 sec) species. *Aedes albopictus* performed this behaviour more frequently compared to *Anopheles stephensi* but the mean times were not significantly different (F=0.06, df=2, P=0.81). Autogroom was not seen in *Culex pipiens* larvae.

Allogroom/feed

Allogroom/feed was commonly seen in *Anopheles stephensi* (1.33±0.87 sec) but was a rare behaviour in *Aedes albopictus* and *Culex pipiens*. Allogroom/feed in Anopheles stephensi commonly commenced when larvae

bumped into each other while in the float/suspension feed state. However, upon contact with another larva, they immediately moved away from each other. In *Aedes* and *Culex* species allogroom/feed commenced smoothly, albeit rarely.

Rise

This was a common behaviour observed in all three species. There was significant differences in the average time it took for a larva to reach the surface (F=4.38, df=2, P=0.02). *Aedes albopictus* (16.17±9.05 sec) and *Anopheles stephensi* (15.0±3.67 sec) took significantly longer time to get to surface compared to *Culex pipiens* (8.1±4.75 sec; P <0.05, HSD [0.05] = 6.68). *Culex pipiens* (8.1±4.75 sec; P <0.05, HSD [0.05] = 6.68).

Underwater/still & underwater/mouth swim

Underwater/still was the only seen being in *Anopheles stephensi*. Mouth swim was not seen in all the three mosquito species we studied.

Discussion

When studying the behaviour of an organism, constructing the time budget is an integral part of the data collection process. In field studies, time budget data can be recorded by just a pencil and paper. However, sometimes, it is essential to observe an organism's behaviour in a laboratory. The rapid expansion of ICT provides new ways to build a time budget when studying an organism's behaviour in the laboratory. Converting video tape recordings to a DVD is one such technology that is readily available. A DVD format of behaviour recording can be viewed on a personal computer using the appropriate software and a time budget can easily be constructed. Furthermore, the digital images can be archived for demonstrations and teaching. Multiple data can also be obtained from a single recording in future studies.

In this study, we recorded 12 well known larval behaviours using a handy-cam video recorder (Sony Co. Japan). The images were converted to DVD and viewed on a personal computer (Mac Os X version 9.3) using the inbuilt DVD viewing software. We used the timer feature to construct a time budget for the larval behaviours. This method of constructing a time budget was easier for the observer to gather data. For example, if the observer missed a behaviour, the rewind feature was used to go back to the section where the lapse occurred and the behaviour recorded. Furthermore, if the observer was interrupted in the middle of a data recording session, the DVD player simply had to be paused using the 'pause' feature or stop the session and after returning, fast forward to the session where last viewed and the data recording session can recommence.

The slow motion feature was also an advantage. This feature allowed the exact times of the beginning and ending of a behaviour to be recorded. viewing software. We used the timer feature to construct a time budget for the larval behaviours. This method of constructing a time budget was easier for the observer to gather data. For example, if the observer missed a behaviour, the

rewind feature was used to go back to the section where the lapse occurred and the behaviour recorded. Furthermore, if the observer was interrupted in the middle of a data recording session, the DVD player simply had to be paused using the 'pause' feature or stop the session and after returning, fast forward to the session where last viewed and the data recording session can recommence. The slow motion feature was also an advantage. This feature allowed the exact times of the beginning and ending of a behaviour to be recorded.

The three larval species used in our study belong to different mosquito genera and the structures of their larval stages are very different. *Anopheles* mosquitoes do not have siphon and they lie parallel to the water's surface (Clements 1999). They have abdominal palmate hairs and tergal plates. The two culicine species have no palmate hairs or tergal plates and they posses siphons of different lengths that allow them to suspend at an angle from the water surface (Clements 1999). Because of these different characteristics the three species behave differently in the water. Recording their behaviour on video tape and digitalizing the images made it easier to observe these differences. The construction of their time budgets was also easier. The significant differences noted in the statistical analysis in the time budget of some of the behaviours confirmed the observed behavioural differences.

In conclusion, converting the video tape recordings to DVD format made it easier to construct a time budget. The differences in the time budget of the mosquito species studied can be explained by the differences in the anatomy and physiology of the larval stages of these species.

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