1 The relationship between wing length, blood meal volume, and fecundity for seven
2 colonies of *Anopheles* species housed at the Armed Forces Research Institute of
3 Medical Sciences, Bangkok, Thailand

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Abstract

Established colonies of *Anopheles campestris*, *An. cracens*, *An. dirus*, *An. kleini*, *An. minimus*, *An. sawadwongporni*, and *An. sinensis* are maintained at the Armed Forces Research Institute of Medical Sciences (AFRIMS). Females were provided blood meals on human blood containing citrate as an anticoagulant using an artificial membrane feeder. The mean wing length, used as an estimate of body size, for each species was compared to blood-feeding duration (time), blood meal volume, and numbers of eggs oviposited. Except for *An. campestris* and *An. cracens*, there were significant interspecies differences in wing length. The mean blood meal volumes (mm^3) of *An. kleini* and *An. sinensis* were significantly higher than the other 5 species. For all species, the ratios of unfed females weights/blood meal volumes were similar (range: 0.76-0.88), except for *An. kleini* (1.08) and *An. cracens* (0.52), that were significantly higher and lower, respectively. Adult females were allowed to feed undisturbed for 1, 3, and 5 minute intervals before blood feeding was interrupted. Except for *An. campestris* and *An. sawadwongporni*, the number of eggs oviposited were significantly higher for females that fed for 3 minutes when compared to those that only fed for 1 min. This information is critical to better understand the biology of colonized *Anopheles* spp. and their role in the transmission of malaria parasites as they relate to the relative size of adult females, mean volumes of blood of engorged females for each of the anopheline species, and the effect of blood feeding duration on specific blood meal volumes and fecundity.

Keywords: *Anopheles*, blood meal volume, wing length, feeding duration, oviposition

1. Introduction

Malaria is the number one arthropod-borne disease worldwide (Molyneux and Fox, 1993). Female mosquitoes blood feed on warm blooded (mammals and birds) and cold-blooded (reptiles and amphibians) hosts to obtain protein and other nutrients for egg production, while males feed on plant nectar and are not involved in the direct transmission of mosquito-borne pathogens, including malaria (Garnham, 1966, Klein et al. 1987). Blood feeding on human hosts is the link between human – mosquito – *Plasmodium* spp. parasite life cycle. Anautogenous female mosquitoes commonly ingest from 2-4 times their mean weights of nutrient starved mosquitoes when fully engorged (Nayar and Sauerman, 1975). In nature, blood feeding is often interrupted by host defensive behaviors, resulting in multiple blood meals on the same or different hosts in order to complete egg development, while at the same time increasing opportunities for infection and multiple transmissions of pathogens (Edman and Kale, 1971; Edman et al., 1974). Blood meal volume also influences mosquito fecundity, as well as the acquisition of pathogens from infected animal and bird hosts to mosquitoes (Kershaw et al., 1955; Woke et al., 1956). Blood meal volume is affected by a range of factors including ambient temperatures, mosquito age, mating status, parity, gonotrophic cycle, blood feeding history (e.g., blood meal host and number of multiple feeds), and infection status (Lehane, 2005). The maturation and number of eggs oviposited is dependent upon the quality (host) and quantity of blood ingested that exceeds the minimum threshold level (Dodd and Burges, 1995). Several authors demonstrated a positive correlation between the numbers of eggs oviposited and mean wing length of females for several *Anopheles* species (Shannon and Hadjinicalao, 1941; Briegel, 1990b; Kelly and Edman, 1992; Kitthawee et al., 1992; Hogg et al., 1996; Renshaw et al., 1994). The Armed Forces

Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand, maintains seven colonies of *Anopheles* spp. in a state of the art insectary. Mosquito colonies are maintained and used for conducting malaria vector competence, malaria transmission blocking, insecticide efficacy and resistance, malaria vaccine trials, and other basic biological studies. These data can be used to better understand the epidemiology of malaria by identifying vector species and potential effects of control measures that target vector populations that more effectively reduce malaria transmission in Thailand and other malaria endemic regions. Six species, *Anopheles dirus*, *An. minimus*, *An. campestris*, *An. cracens*, *An. sawadwongporni*, and *An. sinensis* are endemic to Thailand and other Southeast Asian countries and associated Pacific Islands (Lee et al., 2007; Obsomer et al., 2007; Foley et al., 2008, 2009; Muenworn et al., 2009; Sinka et al., 2011; Amir et al., 2013). The first established colony of *An. kleini*, recently identified from the Republic of Korea, is a cryptic species (including *An. sinensis* and 3 other *Anopheles* spp.) (Rueda, 2005), and has been implicated as a more competent vector of *Plasmodium vivax* than *An. sinensis* sensu stricto in Korea (Lee et al., 2007; Phasomkusolsil et al., 2014a). *Anopheles sinensis* is commonly collected in Korea, accounting for >90% of all *Anopheles* spp. collected south of Seoul, but also extends to northern Thailand and the Pacific Islands where it is considered a poor malaria vector (Lee et al., 2007; Foley et al., 2009). *Anopheles kleini* has not been detected in Thailand.

Uniform rearing methods are used, based on species requirements, to ensure that colonies approximate reproduction, vector competence, and susceptibility to insecticides in nature. While multiple publications document the relationship of body size, feeding duration, blood meal volume, and egg production for many of the

malara vectors, there is a paucity of information involving the seven species housed at AFRIMS.

The aim of the present study was to determine and compare (1) the relative size of adult females maintained in colony at AFRIMS, (2) the mean volumes of blood consumed by each of the anopheline species, and (3) the effect of blood feeding duration on specific blood meal volumes and fecundity.

2. Materials and methods

2.1. Mosquito colonies and rearing conditions

Female mosquitoes were allowed to feed to repletion for oviposition as part of normal colony maintenance. Approximately 200 eggs were placed on the water surface in plastic trays (size 30 x 35 x 5 cm) containing 1.5 L of distilled water and reared at low larval densities (approximately 150 larvae/pan) and high diet according to methods described by Kitthawee et al. (1992) to produce synchronized emergence and uniformly large adults. Pupae were removed daily, counted, and placed in screen-topped plastic cups (16 cm dia and 16.5 cm high) with 600 ml filtered water and 2-3 cotton balls saturated with a 5% multivitamin solution (Multilim[®] syrup) placed on the screen top as a food source for newly emerged adults and changed daily. After 2 days, most of the adult mosquitoes had emerged and the water was gently drained from the mosquito cup. Five to seven day-old mosquitoes were provided blood meals via an artificial membrane feeding method as described by Phasomkusolsil et al. (2014b). The vitamin-saturated cotton balls were removed from the holding containers 12 hours prior to blood feeding and replaced with water-saturated cotton balls. Six hours prior to blood feeding the water soaked cotton balls were removed to increase blood feeding. Colonies were maintained and all studies

conducted at $25\pm 2^{\circ}\text{C}$, $80\pm 10\%$ relative humidity, and 12 h light:12 h dark photoperiod.

2.2. Artificial blood-feeding technique

A circulating water bath LAUDA[®] (LAUDA DR.R.WOBSE GMBH & CO.KG, Germany), filled and connected to feeding cups via flexible tubing was operated at 37°C . Sausage casing membranes (pig intestine) were stretched across the bottom of the feeder cups (surface area: 2.54 cm^2), secured with a rubber band, and then the feeder placed in contact with the mesh netting (screened) top of the plastic container holding 5-7 day-old female mosquitoes. Human blood containing citrate as an anticoagulant (obtained from the Thai Red Cross Society) was added (1.5 ml) to the glass feeder well and mosquitoes allowed to blood feed for 30 min. The numbers of fully engorged females were recorded and then provided cotton balls saturated with 5% multivitamin syrup solution as described above. The number of partially blood fed and unfed females were recorded and discarded in accordance with standard protocol.

2.3. Comparison of blood meal volume

Female mosquito body sizes were estimated by measuring their wing length (a standard indicator of body size) (Koella and Lyimo, 1996). One wing was dissected from the body and placed in a drop of distilled water on a microscope slide and, using an eyepiece micrometer, the wing length determined as the distance from the axillary incision to the apical margin (excluding fringes) as described by Nasci (1986).

A total of 15 female mosquitoes of each species that were provided only a 5% multivitamin solution were included as the control group. Cotton balls saturated with

5% multivitamin solution were provided to the control group as previously described and removed 1-hour prior to weighing them individually on an OHAUS® scale (Switzerland). A total of 20 female mosquitoes were allowed to feed uninterrupted for 5 minutes, after which the blood source was removed. All unfed and partially fed females were discarded in accordance with standard practice and 15 females selected as the “blood fed” test group. One hour after blood feeding, the females were anesthetized with ether and weight determined as described above. Blood meal weights were calculated as the difference between mean weights of unfed (control) females and individual weights of blood fed females.

2.4. Blood feeding duration

Human blood meal weights and volumes were obtained by varying the lengths of blood feeding time. Females were allowed to feed individually for 1, 3, and 5 minutes on a membrane feeder as described above. The duration of blood feeding was measured using a stop watch from when the female was observed inserting her mouthparts into the sausage casing membrane and terminated when the female withdrew her proboscis. Females that didn’t initiate blood feeding within five minutes or completed blood feeding before the predetermined feeding period were discarded and data were excluded from the study. Ten females that fed for the allotted times were anesthetized with ether and weighed 1-hour after blood feeding and the quantity (weight) of blood ingested by each individual was calculated by subtracting the mean weights of the unfed (control group) from individual blood fed females as described above. Blood volume was determined as: $V = M/D$, where V = blood volume (mm^3), M = blood weight (mg), and D = density (1.05 mg/mm^3) (Jeffery, 1956). Since the anopheline species used in this study did not mate in the cages under laboratory conditions, they were mated manually as described by Ow Yang et

al. (1963). A total of 12-15 engorged females from each feeding period were anesthetized with ether, mated manually, placed individually in a glass vial (2 cm dia, 6 cm high) with water to a depth of 1 cm containing a piece of filter paper (3.5 cm in diameter) as an oviposition substrate, and then provided a cotton ball saturated with 5% multivitamin solution placed on top of the screen-topped glass vial. After 72 hours post-feed, females were removed, discarded in accordance with standard practices, and the number of eggs for 10 females counted under a stereomicroscope. To ensure that sufficient numbers of females oviposited for a better analysis of mean numbers and range of eggs oviposited, more than 10 females were placed in oviposition vials. To eliminate bias, the first 10 females that oviposited were selected. There was no attempt to determine if females oviposit more or fewer eggs if oviposition was delayed, since only up to 15 females were placed in oviposition vials.

2.5. Statistical Analysis

The relationships between wing length and blood meal volume for each species were evaluated and analyzed by one-way analysis of variance (ANOVA), with differences among groups determined by Duncan's multiple range test (DMRT). The relationship between wing length and blood meal volume, aggregated over all 7 *Anopheles* species was analyzed using simple linear regression. Relationships between blood-feeding times, blood meal volumes, and numbers of eggs oviposited were evaluated and analyzed by one-way ANOVA, followed by a multiple means comparison with DMRT. All data were analyzed using SPSS for Windows version 16.0.

3. Results

The mean blood meal weights (mg) and volumes (mm³) and wing lengths (mm) for each of the seven species of anophelines are presented in Table 1. The mean blood meal volumes for *An. kleini* and *An. sinensis* were similar (2.5 mm³), and significantly higher than the other species. The mean wing length of *An. kleini* (4.4 mm) was significantly longer than *An. sinensis* (4.2), followed by *An. campestris* (3.4), *An. cracens* (3.3), *An. dirus* (3.2), *An. sawadwongporni* (2.9), and *An. minimus* (2.9). Overall, there were significant differences for wing lengths between the *Anopheles* spp., except for *An. campestris* and *An. cracens* ($P < 0.05$). The ratio of the mean blood meal volumes for all *Anopheles* spp. was 0.83 and similar (range 0.76-0.88) for five species, but was significantly lower (0.52) and higher for *An. cracens* and *An. kleini* (1.08), respectively. The overall ratio of mean wing lengths (as an estimate of body size)/mean blood meal volumes was 1.68 and varied from a low of 1.33 (*An. dirus*) to a high of 2.88 (*An. minimus*), while the ratios of the other five species were similar (range 1.43-1.93). When data were pooled for the seven species, a positive relationship between blood meal volume (mm³) (0.7557) and wing length (-0.5413) was significant ($r^2 = 0.7327$) (Figure 1).

One-way ANOVA results showed that as blood feeding duration increased, blood meal volumes increased as well as the numbers of eggs oviposited for all seven of *Anopheles* spp. (Table 2). In general, for each species, the numbers of eggs oviposited increased significantly as blood feeding duration increased. However, there was no significant differences observed in the number of eggs oviposited and the duration of feeding time of 1 and 3 min for *An. campestris* (Figure 2) and *An. sawadwongporni* (Figure 7) and duration of blood feeding time of 3 and 5 min for *An. campestris*, *An. cracens* (Figure 3), *An. kleini* (Figure 5), and *An. minimus* (Figure 6),

while the number of eggs oviposited were significantly higher for *An. dirus* (Figure 4) and *An. sinensis* (Figure 8) that fed for 1, 3, and 5 min. Thus, blood meal volume and feeding duration was a determining factor of fecundity (numbers of eggs oviposited).

4. Discussion

The overall purpose for the colonization of selected mosquito species is to rear uniform broods similar to nature that produce repeatable results when used in studies (e.g., vector competence to selected pathogens of human and veterinary importance and susceptibility to insecticides). Differences may occur as the result of colonization e.g., length of time the species has been colonized, natural habitat (forest versus open areas), geographic distributions (tropical versus subtropical/temperate climates), rearing temperatures, food availability in various natural habitats compared to lab-reared diets, blood feeding behavior, or other unknown factors (Nasci, 1990; Stickman and Kittayapong, 2003; Bevens, 2007). For example, the mean wing length of *An. kleini* collected at Warrior Base, a US military training site near the demilitarized zone in the Republic of Korea was 5.0 mm compared to a mean of 4.4 mm for the AFRIMS colony (D. Foley, personal communication). Similarly, *An. sinensis* sensu stricto from Gangwha Island and Warrior Base were both 4.8 mm compared to 4.2 mm for the AFRIMS colony. Kitthawee et al. (1992) reported that wing length of *An. dirus* from larvae reared at low densities of 100 larvae/pan and high densities of 400 larvae/ pan were 3.4 mm and 2.8 mm compared to a mean of 3.2 mm (150 larvae/pan) for the AFRIMS colony. Wing length of colonized species should be compared with wild caught specimens collected over a period of time that identify seasonal changes that impact on vector competence and insecticide susceptibility, e.g., period of larval

development (temperature) and size (nutrition). Moreover, adult size was related to larval density and protein accumulation during immature life.

Wing length is frequently used as an indicator of size, and the confidence in the relationship between wing length, body size, and blood fed weights/volumes is crucial to using this information in estimating aspects of adult biology (e.g., mortality and fecundity) (Nasci, 1990; Hogg et al., 1996; Koella et al., 1996; Stickman and Kittayapong, 2003; Bevins, 2007). Species differences in the correlation of wing lengths and blood meal weights were variable and should be determined to better understand the relationship of wing length variation as an estimate of body size, blood meal weights/volumes, and the numbers of eggs oviposited in the first gonotrophic cycle. In general, within each species larger females produced more offspring and survived longer than smaller ones (Packer and Corbet, 1989), were more successful in obtaining blood meals in field environments (Haramis, 1983; Nasci, 1986), and took larger blood meals and had greater fertility (Briegel, 1990a). For *Aedes aegypti*, the metabolic relationships between blood meal utilization and fecundity have been quantitatively determined, in relation to within species wing length as an estimate of body size, blood meal source, and age, and can be similarly used for comparative analyses for *Anopheles* spp. (Briegel, 1990a, b).

Based on the study by Chadee et al. (2002), mosquitoes that completed the blood feeding process were simply divided into two groups: fast feeding (blood feeding complete in <2 min) and slow feeding (blood feeding requiring $\geq$ 2 min to complete). In this study, females that completed their blood meal at 1 and 3 min were classified as fast feeders, while females that took 5 min to complete blood feeding were classified as slow feeders. Results showed that the quantity of blood ingested by slow-feeding females was significantly greater than for fast-feeding

females, which also resulted in increased fecundity as feeding durations increased from 1 to 3 and 5 min. This trend confirmed that within *Anopheles* spp., significantly more eggs were produced as increased feeding duration and blood meal volumes increased, similar to Colless and Chellapah (1960) and Edman et al. (1975) who showed that fecundity increased as blood meal volumes increased. In addition to increased fecundity as a function of blood meal volumes (Briegel, 1990b), mortality decreased after blood feeding.

For mosquitoes, blood feeding has associated risks and mortality increases as a function of the blood volume and blood feeding times. Thus, the primary advantage for “fast feeding” is the reduced risk of being swatted, which goes with visiting the host as few times and as short a time as possible. Female mosquitoes demonstrate a relatively fast-feeding and flight response behavior in order to escape attempts by people and animals to swat them, which in some cases may result in incomplete blood meals. Blood-engorged mosquitoes experience impaired flight ability due to increased blood meal weights and are at increased risk (e.g., pecking, tail swishing, or swatting) while on the host and as a result of slower flight and avoidance response mechanisms (Edman et al., 1984), increased resting times and predation (e.g., spiders and rodents), and predation due to increased mass (e.g., birds and bats) (Guinan and Sealy, 1986; Roitberg et al., 2003). Thus, while increased feeding times and larger blood meals are positively correlated with increased reproduction (numbers of eggs), this behavior may negatively impact on overall longevity and number of reproductive cycles. Roitberg et al. (2003) stated that mosquitoes should be able to accurately assess blood volumes, whereas the likelihood of escape is a complex variable that depends on a number of variables, e.g., host species, reaction speed, blood feeding site, persistence, and physiological conditions. In *Culex*

nigripalpus, females that were interrupted before they were <50% engorged usually attempted to refeed on a human forearm when it was offered within 6 hr after the first blood meal, while fewer refeed after a 12 or 24 hr period, a physiological result of initiation of egg development and decline in feeding activities. The feeding response in partially fed individuals seems to be all or none (Edman et al., 1975). Therefore, colonized mosquitoes over an extended period of time may be larger (due to the high nutrition larval diets, low larval populations, and good environmental rearing conditions) and blood feed for longer periods, in part due to little or no host defense behavior since hosts are often anesthetized or secured, and creating a larger proportion of slow-feeders over time. Absolute blood meal volume and proportional volume changes were good predictors of fecundity. Moreover, application of the data on blood meal volumes are of interest in the interpretation of findings on infectivity to mosquitoes based of gametocyte levels not detectable by ordinary microscopic examination of blood films (Jeffery, 1956). However, these data must be used with caution since established colonies may be based on elective genetic populations that as a result of “bottlenecks” in the initial colonization attempt that affect vector competence and insecticide resistance when compared to field populations. While these data can be used to provide estimates for establishing vector competence and insecticide susceptibility, they must be confirmed by follow-up field investigations.

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References

- Amir, A., Sum, J.S., Lau, Y.L., Vythilingam, I., Fong, M.Y., 2013. Colonization of *Anopheles cracens*: a malaria vector of emerging importance. *Parasit. Vectors.* 6, 81.
- Bevins, S.N., 2007. Establishment and abundance of a recently introduced mosquito species *Ochlerotatus japonicus* (Diptera: Culicidae) in the southern Appalachians, USA. *J. Med. Entomol.* 44, 945-952.
- Briegel, H., 1990a. Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. *J. Insect Physiol.* 36, 165-172.
- Briegel, H., 1990b. Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *J. Med. Entomol.* 27, 839-850.
- Chadee, D.D., Beier, J.C., Mohammed, R.T., 2002. Fast and slow blood-feeding durations of *Aedes aegypti* mosquitoes in Trinidad. *J. Vector Ecol.* 27, 172-177.
- Colless, D.H., Chellapah, W.T., 1960. Effects of body weight and size of blood-meal upon egg production in *Aedes aegypti* (Linnaeus) (Diptera, Culicidae). *Ann. Trop. Med. Parasitol.* 54, 475-482.
- Dodd, C.S., Burges, N.R.H., 1995. Why do insects bite? A review of blood sucking behaviour. *J. Royal Army Med. Corps.* 141, 151-156.
- Edman, J.D., Cody, E.F., Lynn, H.C., 1975. Blood feeding activity of partially engorged *Culex nigripalpus* (Diptera: Culicidae). *Entomol. Exp. Appl.* 18, 261-268.
- Edman, J.D., Day, J.F., Walker E.D., 1984. Field confirmation of laboratory observations on the differential antimosquito behaviour of herons. *Condor.* 86, 91-92.

350 Edman, J.D., Kale, H.W., 1971. Host Behavior: Its Influence on the feeding success
351 of mosquitoes. Ann. Entomol. Soc. Am. 64, 513-516.

352 Edman, J.D., Webber L.A., Schmid, A.A., 1974. Effect of host defenses on the
353 feeding pattern of *Culex nigripalpus* when offered a choice of blood sources.
354 J. Parasitol. 60, 874-883.

355 Foley, D.H., Rueda, L.M., Peterson, A.T., Wilkerson, R.C., 2008. Potential
356 distribution of two species in the medically important *An. minimus* complex
357 (Diptera: Culicidae). J. Med. Entomol. 45, 852-860.

358 Foley, D.H., Klein, T.A., Kim, H.C., Sames, W.J., Wilkerson, R.C., Rueda, L.M.,
359 2009. Geographic distribution and ecology of potential malaria vectors in the
360 Republic of Korea. J. Med. Entomol. 46, 680-692.

361 Garnham, P.C.C., 1966. Malaria parasites and othe Haemosproidia, Blackwell
362 Scienfic Pulblications, Oxford, 1114 pgs.

363 Guinan, D.M., Sealy, S.G., 1986. Diet of house wrens (*Troglodytes aedon*) and the
364 abundance of the invertebrate prey in the dune-ridge forest, Delta Marsh,
365 Manitoba. Can. J. Zool. 65, 1587-1596.

366 Haramis, L.D., 1983. Increased adult size correlated with parity in *Aedes triseriatus*.
367 Mosq. News 43, 77-79.

368 Hogg, J.C., Thomson, M.C., Hurd, H., 1996. Comparative fecundity and associated
369 factors for two sibling species of *Anopheles gambiae* complex occurring
370 sympatrically in the Gambia. Med. Vet. Entomol. 10, 385-391.

371 Jeffery, G.M., 1956. Blood meal volume in *Anopheles quadrimaculatus*, *A. albimanus*
372 and *Aedes aegypti*. Exp. Parasitol. 5, 371-375.

373 Kelly, R., Edman J.D., 1992. Mosquito size and multiple transmission of avian
374 malaria in the laboratory. J. Am. Mosq. Control Assoc. 8, 386-388.

375 Kershaw, W.E., Lavoipierre., J.M.M., Beesley, W.N., 1955. Studies on the intake of
 376 microfilariae by their insect vectors, their survival, and their effect on the
 377 survival of their vectors. VII. Further observations on the intake of the
 378 microfilariae of *Dirofilaria immitis* by *Aedes aegypti* in laboratory conditions:
 379 The pattern of the intake of a group of flies. Ann. Trop. Med. Parasitol. 49,
 380 203-211.

381 Kitthawee, S., Edman, J.D., Upatham E.S., 1992. *Anopheles dirus* size and
 382 fecundity: relationship to larval density and protein accumulation. Southeast
 383 Asian J. Trop. Med. Public. Health 23, 128-131.

384 Klein, T.A., Young, D.G., Telford, S.R. Jr., 1987. Vector incrimination and
 385 experimental transmission of *Plasmodium floridense* by bites of infected *Culex*
 386 (Melanoconion) erraticus. J. Am. Mosq. Control Assoc. 3: 165-175.

387 Koella, J.C., Lyimo, E.O., 1996. Variability in the relationship between weight and
 388 wing length of *Anopheles gambiae* (Diptera: Culicidae). J. Med. Entomol. 33,
 389 261-264.

390 Lee, W.J., Klein, T.A., Kim, H.C., Choi, Y.M., Yoon, S.H., Chang, K.S., Chong, S.T.,
 391 Lee, I.Y., Jones, J.W., Jacobs, J.S., Sattabongkot, J., Park, J.S., 2007.
 392 *Anopheles kleini*, *Anopheles pullus*, and *Anopheles sinensis*: potential vectors of
 393 *Plasmodium vivax* in the Republic of Korea. J. Med. Entomol. 44, 1086-1090.

394 Lehane, M.J., 2005. Managing the blood meal. In The Biology of Blood-Sucking in
 395 Insects, Cambridge, 2nd Edition, Cambridge University Press, New York, NY. pp
 396 84-115.

397 Molyneux, M., Fox, R. 1993. Diagnosis and treatment of malaria in Britain. British
 398 Med. J. 306: 1175-1180.

399 Muenworn, V., Sungvornyothin, S., Kongmee, M., Polsomboon, S., Bangs, M.J.,
 400 Akathanakul, P., Tanasinchayakul, S., Prabaripai, A., Chareonviriyaphap, T.,
 401 2009. Biting activity and host preference of the malaria vectors *Anopheles*
 402 *maculatus* and *Anopheles sawadwongporni* (Diptera: Culicidae) in Thailand. J.
 403 Vect. Ecol. 34, 62-69.

404 Nasci, R.S., 1986. The size of emerging and host-seeking *Aedes aegypti* and the
 405 relation of size to blood-feeding success in the field. J. Am. Mosq. Control
 406 Assoc. 2, 61-62.

407 Nasci, R.S., 1990. Relationship of wing length to adult dry weight in several mosquito
 408 species (Diptera: Culicidae). J. Med., Entomol. 27, 716-719.

409 Nayar, J.K., Sauerman, D.M. Jr., 1975. The effects of nutrition on survival and
 410 fecundity in Florida mosquitoes Part 2. Utilization of a blood meal for survival.
 411 J. Med. Entomol. 12, 99-103.

412 Obsomer, V., Defouny, P., Coosemans M., 2007. The *Anopheles dirus* complex:
 413 spatial distribution and environmental drivers. Malaria J. 6, 26.

414 Ow Yang, C.K., Sta Maria, F.L., Wharton, R.H., 1963. Maintenance of a laboratory
 415 colony of *Anopheles maculatus* Theobald by artificial mating. Mosq. News 23,
 416 34-35.

417 Packer, M.J., Corbet P.S., 1989. Size variation and reproductive success of female
 418 *Aedes punctator* (Diptera: Culicidae). Ecol. Entomol. 14, 297-309.

419 Phasomkusolsil, S., Kim, H.C., Pantuwattana, K., Tawong, J., Khongtak, W.
 420 Schuster, A.L., Klein, T.A., 2014a. Colonization and maintenance of *An. kleini*
 421 and *An. sinensis* from the Republic of Korea. J. Am. Mosq. Control Assoc. Hyg.
 422 30, 1-6.

423 Phasomkusolsil, S., Pantuwatana, K., Tawong, J., Khongtak, W., Monkanna, N.,
 424 Kertmanee, Y., Damdangdee, N., McCardle, P. W. , Schuster, A. L., 2014b.
 425 Factors influencing the feeding response of laboratory-reared *Aedes aegypti*. .
 426 Southeast Asian J. Trop. Med. Public. Health. 45, 40-46.

427 Renshaw, M., Service, M.W., Birley, M.H., 1994. Size variation and reproductive
 428 success in the mosquito *Aedes cantans*. Med. Vet. Entomol. 8, 179-186.

429 Roitberg, B.D., Mondor, E.B., Tyerman, J.G.A., 2003. Pouncing spider, flying
 430 mosquito: blood acquisition increases predation risk in mosquitoes. Behav.
 431 Ecol. 5, 736-740.

432 Rueda, L.M., 2005. Two new species of *Anopheles* (*Anopheles*) Hyrcanus Group
 433 (Diptera: Culicidae) from the Republic of South Korea. Zootaxa. 941: 1-26.

434 Shannon, R.C., Hadjinicalao, J., 1941. Egg production of Greek anophelines in
 435 nature. J. Econ. Entomol. 34, 300-305.

436 Sinka, M.E., Bangs, M.J., Manguin, S., Chareonviriyaphap, T., Patil, A.P.,
 437 Temperley, W.H., Gething, P.W., Elyazar, I.R.F, Kabaria, C.W., Harbach, R.E.,
 438 Hay, S.I., 2011. The dominant *Anopheles* vectors of human malaria in the Asia-
 439 Pacific region: occurrence data, distribution maps and bionomic precis. Parasit.
 440 Vectors. 4, 89

441 Strickman, D., Kittayapong, P., 2003. Dengue and its vectors in Thailand: calculated
 442 transmission risk from total pupal counts of *Aedes aegypti* and association of
 443 wing-length measurements with aspects of the larval habitat. Am. J. Trop. Med.
 444 Hyg. 68, 209-217.

445 Woke, P.A., Ally, M.S., Rosenberger, C.R., 1956. The number of eggs developed
 446 related to the quantities of human blood ingested in *Aedes aegypti* (L.). Ann.
 447 Entomol. Soc. Am. 49, 435-441.

Table 1. Unfed and blood fed weights and weight and volume of blood meals for each *Anopheles* spp. in relationship to body size (wing length) and blood meal weights/ volumes.

<i>Anopheles</i> Species (N = 15)	Weight of unfed female $\pm$ SD (mg) ^{1/}	Weight of engorged female $\pm$ SD (mg) ^{1/}	Volume of blood meal $\pm$ SD (mm ³) ^{1/}	Ratio of unfed weight/ blood meal volume	Wing length (mm) $\pm$ SD ^{1/}	Ratio of wing length/ blood meal volume
<i>campestris</i>	1.6 $\pm$ 0.2 ^c	3.8 $\pm$ 0.2 ^d	2.1 $\pm$ 0.0 ^d	0.76	3.4 $\pm$ 0.1 ^c	1.62
<i>cracens</i>	1.2 $\pm$ 0.1 ^d	3.7 $\pm$ 0.1 ^d	2.3 $\pm$ 0.1 ^c	0.52	3.3 $\pm$ 0.1 ^c	1.43
<i>dirus</i>	2.1 $\pm$ 0.2 ^b	4.6 $\pm$ 0.2 ^c	2.4 $\pm$ 0.0 ^b	0.88	3.2 $\pm$ 0.1 ^d	1.33
<i>kleini</i>	2.7 $\pm$ 0.4 ^a	5.3 $\pm$ 0.4 ^a	2.5 $\pm$ 0.1 ^a	1.08	4.4 $\pm$ 0.1 ^a	1.76
<i>minimus</i>	0.7 $\pm$ 0.1 ^e	1.5 $\pm$ 0.1 ^f	0.8 $\pm$ 0.0 ^f	0.88	2.3 $\pm$ 0.1 ^f	2.88
<i>sawadwongporni</i>	1.2 $\pm$ 0.1 ^d	2.8 $\pm$ 0.1 ^e	1.5 $\pm$ 0.0 ^e	0.80	2.9 $\pm$ 0.1 ^e	1.93
<i>sinensis</i>	2.2 $\pm$ 0.4 ^b	4.8 $\pm$ 0.4 ^b	2.5 $\pm$ 0.1 ^a	0.84	4.2 $\pm$ 0.1 ^b	1.68
Mean	1.67	3.79	2.01	0.83	3.39	1.68

^{1/} Means in each column followed by the different letters are significantly different ($P < 0.05$, by one-way ANOVA and Duncan's Multiple Range Test).

Table 2. Number of eggs oviposited for each *Anopheles* spp. in relationship to blood feeding time and volume of blood ingested. Blood serum excreted during or shortly thereafter blood feeding was not included.

Species of mosquito (N = 10)	Time	Volume of blood meal $\pm$ SD (mm ³) *	No. of eggs per female $\pm$ SD [†]
<i>An. campestris</i>	1 min	1.1 $\pm$ 0.1	90.8 $\pm$ 28.7 ^a
	3 min	1.7 $\pm$ 0.3	142.5 $\pm$ 61.0 ^{ab}
	5 min	2.2 $\pm$ 0.3	160.5 $\pm$ 41.4 ^b
<i>An. cracens</i>	1 min	1.4 $\pm$ 0.2	86.0 $\pm$ 24.5 ^a
	3 min	2.3 $\pm$ 0.3	129.7 $\pm$ 14.8 ^b
	5 min	3.0 $\pm$ 0.2	143.9 $\pm$ 17.5 ^b
<i>An. dirus</i>	1 min	0.6 $\pm$ 0.3	58.4 $\pm$ 9.9 ^a
	3 min	1.1 $\pm$ 0.2	101.0 $\pm$ 11.9 ^b
	5 min	1.6 $\pm$ 0.3	132.7 $\pm$ 13.1 ^c
<i>An. kleini</i>	1 min	1.2 $\pm$ 0.4	81.6 $\pm$ 12.3 ^a
	3 min	1.9 $\pm$ 0.2	132.8 $\pm$ 16.4 ^b
	5 min	2.8 $\pm$ 0.3	144.8 $\pm$ 12.1 ^b
<i>An. minimus</i>	1 min	0.5 $\pm$ 0.1	36.3 $\pm$ 14.7 ^a
	3 min	0.9 $\pm$ 0.2	71.1 $\pm$ 14.3 ^b
	5 min	1.1 $\pm$ 0.1	71.1 $\pm$ 7.2 ^b
<i>An. sawadwongporni</i>	1 min	0.8 $\pm$ 0.3	90.0 $\pm$ 15.8 ^a
	3 min	1.3 $\pm$ 0.2	107.4 $\pm$ 42.3 ^a
	5 min	1.7 $\pm$ 0.2	150.3 $\pm$ 10.6 ^b
<i>An. sinensis</i>	1 min	0.4 $\pm$ 0.2	59.4 $\pm$ 8.5 ^a
	3 min	1.5 $\pm$ 0.5	104.5 $\pm$ 17.7 ^b
	5 min	2.0 $\pm$ 0.3	149.4 $\pm$ 37.4 ^c

* For all *An.* species , volume of blood meal are significantly different between times within the same species

[†] Number of eggs, data followed by the different superscripted letters are significantly different between times within the same species.

Figure 1. Linear regression between blood meal volume (mm³) and wing length (mm) for seven colonies of *Anopheles* spp. females that blood fed on human blood by membrane feeder.

Figure 2. Relationships between blood-feeding times, volume of blood meal, and number of eggs oviposited for *Anopheles campestris*.

Figure 3. Relationships between blood-feeding times, volume of blood meal, and number of eggs oviposited for *Anopheles cracens*.

Figure 4. Relationships between blood-feeding times, volume of blood meal, and number of eggs oviposited for *Anopheles dirus*.

Figure 5. Relationships between blood-feeding times, volume of blood meal, and number of eggs oviposited for *Anopheles kleini*.

Figure 6. Relationships between blood-feeding times, volume of blood meal, and number of eggs oviposited for *Anopheles minimus*.

Figure 7. Relationships between blood-feeding times, volume of blood meal, and number of eggs oviposited for *Anopheles sawadwongporni*.

Figure 8. Relationships between blood-feeding times, volume of blood meal, and number of eggs oviposited for *Anopheles sinensis*.