Wing size and parity as markers of cohort demography for potential *Anopheles* (Culicidae: Diptera) malaria vectors in the Republic of Korea

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ABSTRACT: Wing lengths of parous (P) and nulliparous (NP) PCR-identified female *Anopheles belenrae*, *An. kleini*, *An. pullus*, and *An. sinensis* were determined from weekly trap collections at Camp Humphreys (CH), Ganghwa Island (GH), and Warrior Base (WB), Republic of Korea (ROK) during Jun-Oct, 2009. Wing length was greatest at the beginning and end of the study period. Wing length of NPs tended to be less than that of Ps before the period of maximum greening (Jul-Aug) but greater thereafter. Larger specimens tended to be Ps, and weekly wing length of Ps appeared less variable than NPs, possibly due to selection. A bimodal wing length frequency distribution of *An. sinensis* suggested two forms comprising small- (≤4.5 mm, SW) and large-winged females (>4.5 mm, LW). LW comprised the majority of peaks in abundance, however %SW, while still a minority, often increased during these times suggesting a density-dependent effect. At WB and GH, a two to three-week periodicity in %SW was obvious for *An. sinensis* and *An. kleini*. Analyses of weather station and satellite data showed that smaller-winged *An. sinensis* were associated with warmer, more humid, and greener times of the year. SW and LW specimens possibly result from agricultural practices that are common across large areas; regular synchronous peaks of SW and LW were observed from different sites. Peaks in SW Ps followed peaks in NPs in a 'ripple effect' one to two weeks apart, suggesting that wing length combined with parity could be used to follow the emergence and survival of mosquito cohorts. *Journal of Vector Ecology* **45 (2): 366-379. 2020.**

Keyword Index: Anopheles sinensis, seasonality, malaria, parity, mortality, wing length, population dynamics, Korea.

INTRODUCTION

Wing length is an important indicator of the life history of vector populations. Mosquito wing lengths are correlated with adult dry weights and have been used as an index of body size (Fish 1985, Lounibos et al. 1995, Takken et al. 1998, Charlwood 2003). Reproductive success of a number of mosquito species was positively correlated with female size (Lyimo and Takken 1993, Ameneshewa and Service 1996, Takken et al. 1998), as was adult survival (Takken et al. 1998) and parasite and symbiont infection rates (Dutra et al. 2016). Hassan et al. (1996) found that wing length of a parous population of *Aedes albopictus* (Skuse) in Penang Malaysia was significantly longer than the wing length of the nulliparous population. Cooler larval temperature conditions result in larger adult *Ae. albopictus* that are more likely to become infected with chikungunya virus (Westbrook et al. 2010). Charlwood (2003) recommended that wing length measurements be included as a co-variate in any analysis for determining age-specific adult mortality.

Geographic variation in size distribution within mosquito species were noted by Fish (1985) and interpreted in terms of variation in larval habitat conditions, especially levels of larval stress and competition for resources. A paucity of food, the presence or absence of predators, and internal parasites or pathogens may affect size variability or skew the size-frequency distribution of a mosquito population (Dutra et al. 2016, Fish 1985, Renshaw et al. 1994). For example, during periods of stress, smaller larvae may not survive and thus adult sizes at emergence will be skewed (Charlwood 2003, Renshaw et al. 1994). Fish (1985) compared the coefficient of variation (CV) and skewness (G1 statistic) of mosquito wing length distributions, and Lounibos et al. (1995) included kurtosis (a measure of the degree to which a data set is peaked) in an analysis of wing length variation. Fish (1985) concluded that populations of *Aedes triseriatus* (Say) showed high CV and a mean size of less than half the maximum attainable by this species, indicating that larval environments were not conducive for maximum growth for most specimens. By contrast, he observed that the permanent wooded swamp-breeding species, *Culiseta morsitans* (Theobald), had a low CV, with most individuals approaching maximum size, which may have been a result of the action of predators reducing larval population densities, thereby increasing available food resources for survivors.

Costanzo et al. (2018) found positive relationships between size and fecundity when *Ae. albopictus* larvae were reared at high temperatures and low resource levels but the

relationship disappeared when mosquitoes were reared at a low temperature or with high levels of resources. Barreaux et al. (2018) found that the combination of temperature and nutrient levels of the larval environment of *Anopheles gambiae* Giles affected the relationship between body size and longevity, with larger mosquitoes living longer under some conditions but not others. Additionally, radiant heat can substantially increase surface water temperatures in rice fields (Mogi and Okazawa 1996), and drainage, evaporation, oviposition site selection, or movement of larvae could concentrate larvae in shallow or deeper water with different thermal regimes affecting larval developmental rates and adult size.

Wing length may be related to epidemiologically important aspects of adult mosquito life history, particularly survival and fecundity. For adult females, an oviposition cycle involves locating a host, blood-feeding, rest, locating an oviposition site, and oviposition, each of which involves different levels of risk affecting survival rates (Charlwood 2003). Examination of the ovaries of mosquitoes allows the separation of parous (P) females that have oviposited at least once from nulliparous (NP) females that have never oviposited (Detinova 1962). Determination of the P rate (parity) provides an insight into the adult population age structure and vector potential, since increased parity is related to increased adult survival and the potential to transmit parasites.

Plasmodium vivax malaria poses a continuing health problem in the Republic of Korea (ROK) for those living or traveling near the demilitarized zone (DMZ), particularly in northern Gangwon and Gyeonggi provinces, as well as the northern Incheon Metropolitan City. Members of the *Anopheles* (*Hyrcanus*) Group, commonly associated with widespread wetland rice agriculture, are possible vectors, and this study utilized specimens of that Group that were reported in Foley et al. (2017). Those authors explored seasonal variation in parity and abundance at three geographically separated sites with rice growing activities that were in or bordered malaria endemic areas of the ROK. The present study uses wing length as a measure of vector fitness and is, to our knowledge, the first published exploration of *Anopheles* spp. wing length in field-collected specimens in the ROK. Seasonal variation in wing length, parity, and environmental metrics across these three sites were analyzed to gauge whether seasonality is localized or subject to area-wide factors. Seasonal variation of adult female size and demographic estimates were also analyzed to gain an insight into the larval and adult ecological conditions that affect the dynamics of competent malaria vectors and malaria transmission in the ROK.

MATERIALS AND METHODS

Mosquito collection

Adult mosquitoes were collected weekly during 2009 at three ROK sites, 30-110 km apart, i.e. Warrior Base training area (WB; 37.9191°N, 126.7416°E), Camp Humphreys U.S. Army Garrison (CH; 36.9580°N, 127.0303°E), and a cowshed at Soljeong-ri, Ganghwa Island (GH; 37.7765°N, 126.4497°E).

Sites were located in or near vivax malaria endemic areas near the DMZ and all had rice growing in the vicinity but were not environmentally identical (e.g., compare EVI values in Figure 1 of Foley et al. 2017). Sampling was conducted during June-Oct, i.e., weeks 24-41 at WB and weeks 27-42 at CH and GH (calendar weeks follow the ISO 8601 standard), which comprised the majority of the mosquito season. Adult mosquitoes were collected at WB and CH using Mosquito Magnet® traps (Pro Model, Woodstream Corp., Lititz, PA), while adults were collected at GH using CDC black-light traps. For logistical reasons, a black-light trap (model 'Black Hole' by BioTrap, http://www.bio-trap.com) was used at GH. Blacklight trap catches may be biased towards older individuals and those resting after a blood meal; the Mosquito Magnet used at WB and CH collects females seeking a blood meal (Foley et al. 2017). Comparison of mosquito data from different trap types requires caution but does allow results to be compared to other studies using those trap types and can strengthen conclusions about observed area-wide effects when these are seen regardless of trap type (Foley et al. 2017). Blood-fed and gravid anophelines and the remaining anophelines were counted. Dead and dried specimens were not dissected, which introduces the possibility of bias, e.g., if smaller or NP specimens were more likely to die overnight in the trap. However, as the majority of specimens were live or recently deceased and able to be dissected, this is not seen as a major potential source for error. Since members of the *Anopheles* (*Hyrcanus*) Group cannot be identified morphologically, they were identified to species by polymerase chain reaction (PCR) methods based on variation in the internal transcribed spacer-2 (ITS-2) gene region (Wilkerson et al. 2003, Li et al. 2005). This study of wing length utilized the same specimens identified and dissected for parity in Foley et al. (2017).

Parity determination and demographic parameters

Parity was determined from a sample of anophelines from each collection site and period. Of 2,107 *Anopheles* from WB, 1,633 were dissected for parity; of 1,365 from CH, 1,042 were dissected, and of 16,874 for GH, 1,989 were dissected. Parity was determined by dissecting ovaries under a stereomicroscope (x100) and observing the coiling pattern of tracheolar skeins (Detinova 1962).

Seasonal variations in longevity and birth rates for each mosquito species at each site were calculated as in Foley et al. (2017), using weekly parity rates, trap catch numbers (as an estimate of recruitment), and degree-day estimates of the gonotrophic cycle. It has come to our attention that the calculations of mortality in Foley et al. (2017, Figure 8), using the methods of Briet (2002), are incorrect, in that the slope between succeeding smoothed mosquito densities was used as a weekly estimate of birthrate minus mortality (ß - μ) rather than as a daily estimate (i.e., divided by 7). Thus, the method in Foley et al. (2017) should have read: "data on weekly parity rates and abundance were smoothed over three collections [e.g., for Wk 30 in the formula notation of Excel this was = AVERAGE(Wk 29:Wk 31)], the natural logarithm of abundance was calculated [e.g., for Wk 30 it was = LN(Wk 30)], the daily log slope between succeeding smoothed

abundance points (ß - μ)/7 calculated [e.g., for Wk 30 it was = (LN(Wk 31) - LN(Wk 30))/7], the birthrate (ß), assuming a two-day gonotrophic cycle (g = 2), for Wk 30 it was = -1/2*LN (smoothed parity for Wk 30), the mortality rate (μ) would be the birthrate minus (ß - μ)/7, the daily survival for Wk 30, for example with g = 2, it was the square root of the parity of Wk 30 [i.e., = POWER(parity of Wk 30,0.5)] and the longevity = 1/(-LN(daily survival Wk 30)).” Consequently, mortality estimates in Foley et al. (2017) should be disregarded and those given here used instead.

Wing length

Wing length measurements were determined by measuring from the wing tip (excluding fringe) to the base of the wing as described by Belkin (1962), using a stereomicroscope and graticule. As wing tips were sometimes damaged or missing, the crossvein *m-cu* to the wing base was measured to estimate the total wing length, according to a regression of both measurements. One wing/female was measured without removing the specimen from the Eppendorf tube housing the specimen; wings could be viewed and measured under the microscope directly through the transparent tubing.

Wing lengths were analyzed in MS-Excel® (Microsoft® Corp, Seattle, WA) where measures of wing length variation were calculated, i.e., mean, skewness, kurtosis, and CV (CV = SD x 100/mean). Skewness is the degree of asymmetry of a distribution around its mean or departure from a normal distribution; positive values indicate a distribution with lower means and an asymmetric tail extending toward greater wing lengths, whereas negative values indicate a distribution with higher means and an asymmetric tail extending toward smaller wing lengths. A limitation of skewness is that it is dependent on sample size, so an attempt was made to standardize this by calculating skewness on a subsample of 50 data points. Kurtosis is the relative peakedness or flatness of a distribution compared with the normal distribution; positive values indicate a relatively peaked distribution (leptokurtic), whereas negative values indicate a relatively flat distribution (platykurtic) of wing lengths. Low values of CV indicate more homogeneity and high values more diversity of wing lengths.

Astronomical, satellite, and weather data

Potential covariates of parity and wing length were investigated from astronomical, satellite, and weather data. We obtained 2009 weather data for Munsan (ID 47099099999, 37.883°N, 126.767°E, 4.5 km south of WB), and for Pyeongtaek (ID 47127099999, 36.967°N, 127.033°E, 1 km from CH), using the National Climatic Data Center website (http://www.ncdc. noaa.gov/oa/ncdc.html). Data were “Surface Data Global Summary of the day” and “Surface data hourly global,” e.g., mean dew point, mean sea level pressure, sky cover, visibility, wind speed, wind direction, total precipitation, and daily temperature (minimum, mean, and maximum). Missing data were identified and then hourly data were averaged to give daily averages and summed in the case of precipitation. We ranked Sky cover as: clear (0), scattered cloud (1), broken cloud (2), overcast (3), and obscured or partial obscured (nil).

Weather data were also obtained for Ganghwa Island (GH) via the Korean Meteorological Administration (http://www. kma.go.kr/weather/observation/past_table.jsp). However, only average wind speed, temperature (mean, minimum, and maximum), precipitation, and relative humidity were used. Other weather and astronomical data from Munsan were applied to GH mosquito data.

Data on fraction of the moon illuminated, daylight duration, moonrise/set, and sunrise/set (for location 37° 55’N, 126° 45’E, zone 9 h E of Greenwich) were obtained from the U.S. Naval Observatory (https://www.usno.navy.mil/USNO/ astronomical-applications). From these data, the number of minutes of moonlight before sunrise and after sunset on each date were calculated. The resulting length of moonlight was multiplied by the fraction of moon illuminated on that day to give a more accurate estimate of the strength and duration of moonlight each day. This metric was further refined by multiplying moonlight by the average daily amount of cloud cover, average daily sky cover, and by the average daily visibility.

A time series of Normalized Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI) data for 2009 were obtained from the USGS MODIS Reprojection Tool Web Interface (MRTWeb, (http://mrtweb.cr.usgs.gov/). Data (h27v05 and h28v05) were 16-day 500 m resolution products from the Terra (MOD13A1) and Aqua (MYD13A1) satellites giving an eight-day phased dataset. Crop phenology data were obtained as detailed by Foley et al. (2017), with the exception that only 1 km radius polygons centred on the CH, GH, and WB sites were used to extract pixels using ESRI®Arc Map 9.2 Spatial Analyst Tools. NDVI and EVI values at 250 m resolution were also obtained from the MODIS products MOD13Q1 and MYD13Q1.

Night- and day-time Land Surface Temperatures (LST) (MOD11A2) averaged over an eight-day period (1 km resolution) were obtained for the study period. Pixel values within a 1 km radius of the study sites was extracted, and the average daily LSTs were then averaged over calendar weeks. LST values over the study period varied in the order: CH>WB>GH (day-time), and GH>CH>WB (night-time). However, due to the amount of built area, especially at CH, another data set was calculated using a mask of agricultural area within a 2 km radius. An exploration of the impact on satellite data of different radius polygon (buffer) sizes, as conducted by Foley et al. (2017), was beyond the scope of the present study.

Linear regression analysis of potential covariates with wing length was performed in MS-Excel with statistical significance of the R² calculated using http://vassarstats.net/ corr_big.html.

RESULTS

The wings of 4,032 specimens were measured for this study. Full (wing tip to base) wing length to partial (*m-cu* to base) wing length was highly correlated: *An. belenrae* (y = 1.402x + 0.889, R² = 0.928, df = 176, P < 0.0001), *An. kleini* (y = 1.369x + 0.990, R² = 0.914, df = 295, P < 0.0001), *An.*

pullus (y = 1.387x + 0.935, R^2 = 0.910, df = 173, P < 0.0001), and *An. sinensis* (y = 1.445x + 0.758, R^2 = 0.902, df = 3042, P < 0.0001). Although only a small minority of wings were incomplete, these results indicated that full wing length could be reliably estimated using measurements of partial wing lengths if needed.

For *An. sinensis*, wing lengths ranged from 3.70-5.50 mm, *An. kleini* 3.90-5.70 mm, *An. pullus* 3.90-5.50 mm, and *An. belenrae* 4.00-5.50 mm. A summary of the wing metrics for each of the four species collected at the three study sites is shown in Table 1. Although wing length differences for each of the four species were not marked, they tended to be in the following order: *An. sinensis<An. belenrae<An. pullus<An. kleini*.

Parity and wing length

The weekly mean wing length of parous and nulliparous *An. sinensis*, *An. belenrae*, *An. kleini*, and *An. pullus* for the three study sites is shown in Figures 1a and 1b, along with abundance per trap from Foley et al. (2017). The data shows extensive variation by parous state, site, species, and week collected. Wing lengths tended to be higher at the beginning and/or the end of the mosquito season. For all three sites, the wing lengths of NP *An. sinensis* appeared to approximate P *An. sinensis*, until the second half of the mosquito season when NP wing length appeared to be greater than that of P. Wing lengths of NPs appeared more variable over the course of the mosquito season than for Ps, possibly due to selection. A decrease in the mean wing length for *An. sinensis* was observed during week 29, followed by a peak during week 32, and then followed by a decrease in week 33. To some degree, a steady increase was observed during weeks 34-37 for *An. sinensis* at all sites, suggesting a possible regional effect, and coinciding with the start of crop senescence (Foley et al. 2017). A decline in wing length was also observed for *An. belenrae* and *An. kleini* during weeks 32-33 (Figure 1b). A peak in mean wing lengths of Ps for week 31 was observed for

An. belenrae from GH and WB, suggesting a possible regional effect.

For the most abundant species, *An. sinensis*, regression of mean wing lengths of NP vs P over week 29-39 for CH (lagged one week) and GH and WB (lagged two weeks) showed significant positive relationships, i.e., CH: y = 0.273x + 3.416, R^2 = 0.856, df = 9, P < 0.001; GH: y = 0.408x + 2.760, R^2 = 0.743, df = 6, P < 0.050; WB: y = 0.976x + 0.095, R^2 = 0.627, df = 8, P < 0.050. This observation, and the fact that despite extensive testing no other lag settings resulted in a significant correlation, was in accordance with the hypothesis that newly emerged (NP) cohorts identifiable by mean wing lengths are being resampled as Ps up to two trapping periods (weeks) later.

Mean wing lengths of *An. sinensis* also appeared to be positively correlated with parity (larger individuals were more likely to be P), but only during specific trapping periods, and often only when parity was lagged in relation to wing length. For CH, parity (weeks 32-40) lagged one week in relation to mean wing length of Ps and one to two weeks for NPs (weeks 30-38) was significantly correlated (y = 0.826x - 4.287, R^2 = 0.800, df = 7, P<0.005). Potentially, knowing the wing length of NPs at CH was indicative of parous rates in the following two weeks. Interestingly, mean NP wing lengths lagged two weeks in relation to parity for weeks 31-40 were also significantly correlated (y = 0.659x - 2.784, R^2 = 0.857, df = 8, P < 0.0005). Potentially, knowing the parous rate indicates what the mean wing length would be for NPs two weeks ahead.

For *An. sinensis* at WB, mean wing lengths of Ps and parity (not lagged) over weeks 30-38 were described by the relationship: y = 1.011x - 4.040, R^2 = 0.794, df = 7, P < 0.005, while mean wing lengths of NPs and parity lagged two-weeks over the same period were described by the relationship: y = 1.479x - 6.293, R^2 = 0.838, df = 7, P < 0.0005.

For *An. sinensis* at GH, mean wing lengths of Ps and parity (not lagged) over weeks 33-38 gave the regression: y = 2.027x - 8.765, R^2 = 0.962, df = 4, P < 0.005, while mean

Table 1. Wing length metrics for *Anopheles belenrae, An. kleini, An. pullus*, and *An. sinensis* females at Camp Humphreys (CH), Ganghwa Island (GH), and Warrior Base (WB), Republic of Korea in 2009.

Species	Location	Mean	SD	Skewness	Kurtosis	CV	N
An. belenrae	GH	4.849	0.323	-0.435*	-0.245	6.655	133
An. belenrae	WB	4.853	0.325	-0.312	0.466	6.707	63
An. kleini	CH	4.720	0.428	-1.442	1.439	9.077	7
An. kleini	GH	4.947	0.116	0.041	-0.227	2.341	12
An. kleini	WB	5.020	0.269	-0.603*	0.885	5.353	421
An. pullus	CH	4.879	0.211	0.119	-0.435	4.320	51
An. pullus	GH	4.916	0.341	-0.993	0.780	6.937	49
An. pullus	WB	4.885	0.340	-0.619*	-0.162	6.969	105
An. sinensis	CH	4.726	0.356	-0.557*	-0.451	7.540	858
An. sinensis	GH	4.820	0.323	-0.690*	-0.148	6.706	1,518
An. sinensis	WB	4.756	0.331	-0.339*	-0.508	6.966	815

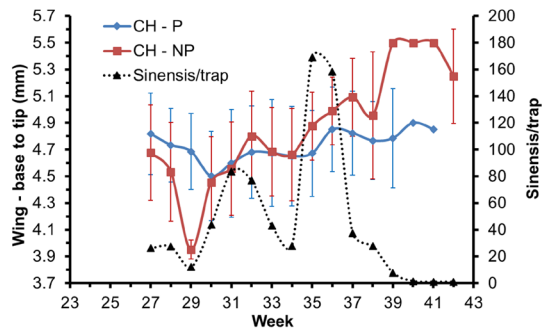*Based on a subsample of up to 100 specimens.

Figure 1a. Weekly mean wing lengths of parous (P) and nulliparous (NP) *Anopheles sinensis* and estimated abundance per trap at Camp Humphreys (CH), Ganghwa Island (GH), and Warrior Base (WB), Republic of Korea in 2009. Vertical bars are ± 1 SD.
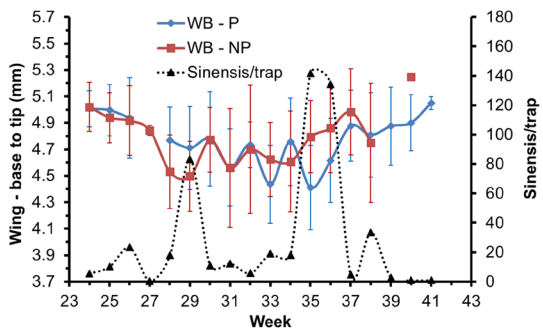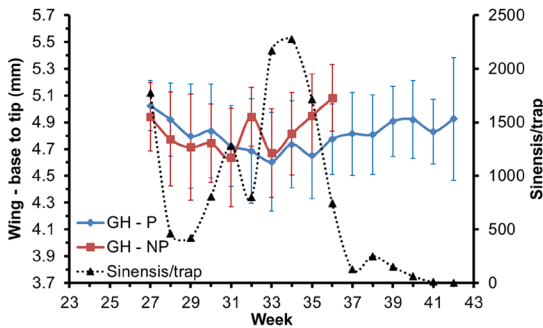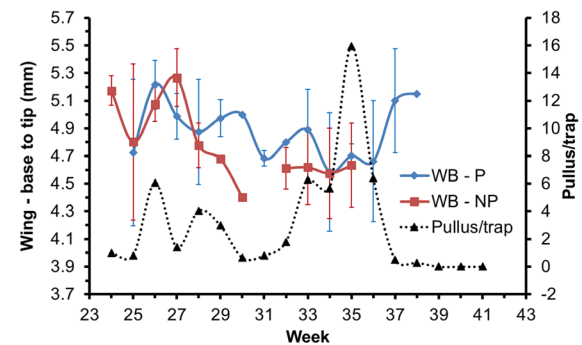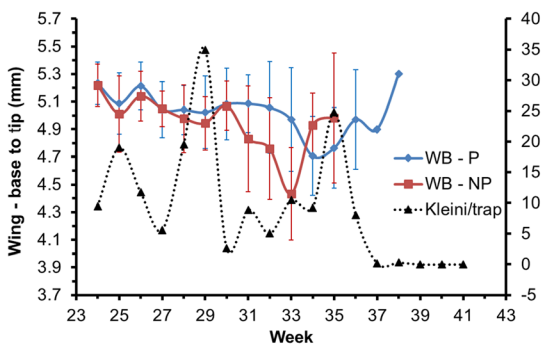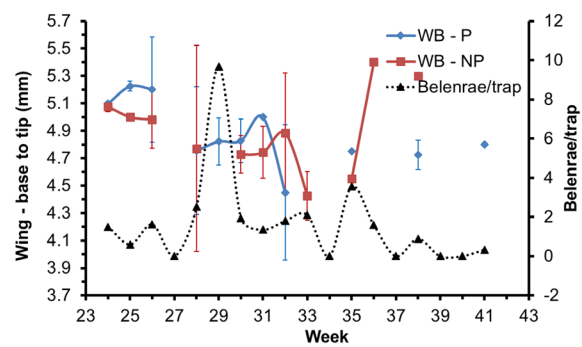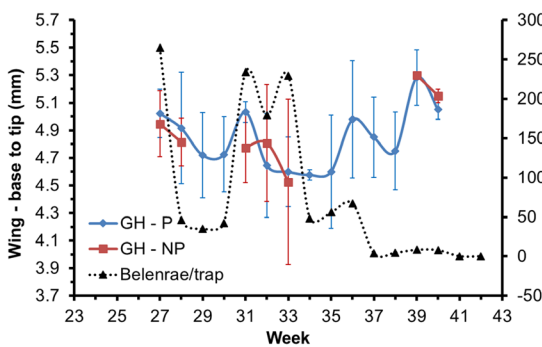
Figure 1b. Weekly mean wing lengths of parous (P) and nulliparous (NP) *Anopheles belenrae, An. kleini,* and *An. pullus*, and estimated abundance per trap at Camp Humphreys (CH), Ganghwa Island (GH), and Warrior Base (WB), Republic of Korea in 2009. Vertical bars are ± 1 SD.

lengths of NPs and parity lagged two-weeks over weeks 31-36 gave the regression: y = 0.924x - 3.655, R² = 0.822, df = 4, P < 0.050. For certain time periods, mean wing lengths potentially predict parity up to two weeks in advance, with longer mean wing lengths indicating a higher parous rate.

Mortality and wing length

Mortality estimates are given in Figure 2. Over weeks 24-41, average daily mortality rates were highest for *An. belenrae*, *An. kleini,* and *An. pullus* at WB (0.324, 0.306, and 0.343, respectively). The highest average mortality rate for *An. sinensis* was at GH (0.269). The lowest average mortality was for *An. belenrae* at CH (0.002) and *An. kleini* at GH (-0.001). *Anopheles belenrae* exhibited the lowest mortality at week 29 and the highest at week 31 for both WB and GH, which coincided with the peaks in parity noted above. Mortality rates for *An. kleini* showed a seasonal decline at WB, and rates were generally higher at WB than at GH, possibly related to a milder environment and proximity to hosts at the latter trap location. *Anopheles pullus* and *An. sinensis* showed variation that differed for each location. Weak evidence for a linear relationship between mortality and wing length existed, e.g., larger-winged P females were associated with lower mortality for *An. kleini* at GH over weeks 27-41 (y = -1.388x + 4.971, R² = 0.775, df = 5, P < 0.009).

Frequency distribution of wing lengths

The frequency distribution of NP *An. sinensis* collected from GH (n=455) demonstrated a bimodal distribution rather than a normal distribution for total wing length (Figure 3). Females were subsequently classified as 'small-winged' (<= 4.5 mm) and 'large-winged' (> 4.5 mm). Wings of 'small-winged' (SW) females were on average 16% shorter than 'large-winged' (LW) females, suggesting a significant difference in body mass. *Anopheles sinensis*, confirmed to species using the ITS2 gene that were SW and LW, showed no difference in their DNA sequence for the cytochrome oxidase subunit I (COI, results not shown), reinforcing the hypothesis that the differences are intraspecific in nature.

Abundance, parity, and wing length

LW females were almost always more abundant than SW, and LW comprised the majority of specimens collected during the largest peaks in abundance (Figure 4). For *An. sinensis*, at WB and to a certain extent at GH, SW numbers increased or decreased in line with LW numbers, but at CH, SW numbers declined steadily to zero from week 30. This suggests very different ecological conditions at CH. Peaks in abundance of *An. kleini* were also comprised mainly of LW females (Figure 4).

SW as a percentage of total catch (%SW) showed a regular two to three-week oscillation for *An. sinensis* at WB, and at GH to a certain extent, but not for *An. sinensis* at CH (Figure 4). This periodicity was also evident for *An. kleini* at WB, although SW were less common up to week 30 compared to weeks 31-36. Higher %SW at WB for *An. sinensis* and *An. kleini* coincided with peaks in abundance regardless of the size of the peaks, suggesting a density dependent effect. The

oscillation in %SW was largely concordant between the more northerly sites (WB and GH) and between *An. sinensis* and *An. kleini*, particularly during weeks 29, 31, 33, and 35.

The percentages of Ps and NPs that are SW for each week and location are shown in Figure 5. Peak trap collection numbers for *An. sinensis* collected at WB (weeks 35-36, see Figure 4) coincided with a high percentage of Ps that are SW than at most other times. However, early (week 29) and late (week 38) abundance peaks at WB coincided with high percentage of NPs that are SW than at most other times, suggesting that peaks in abundance at those times are largely the result of newly emerged rather than older mosquitoes. Peaks in %SW P and NP appear temporally related, as they showed regular synchronicity or a 'ripple effect'; a one-week delay between peaks was noted for *An. sinensis* collected at CH, while there was a two-week delay at GH, and one to two-week delay at WB. *Anopheles kleini* %SW P and NP at WB similarly showed a one-week delay for weeks 28-32 and a two-week delay for weeks 33-35.

Astronomical, satellite, weather data

We have data on survival, mortality, weather, astronomical, and satellite data for each time period, site, and species, and these data are available from the corresponding author upon request. In the interest of space, only a selection for *An. sinensis* from WB is shown here (Table 2). Redundancy was apparent, as some variables were highly correlated, e.g., temperature and LST, relative humidity and dew point, and EVI and NDVI. Most variables did not result in a significant linear relationship with wing length, or did at one location but not another, or for certain periods but not others, or for one species but not another, or for NPs but not Ps, and vice versa. The following is a selection of variables that resulted in a significant linear relationship for *An. sinensis*.

Mean wing length for weekly trap catches across the three sites was negatively correlated with Night-time LST (y = -16.145x + 95.195, R² = 0.558, df = 47, P < 0.0001), i.e., cooler evening conditions resulted in larger-winged specimens. Dividing the data into early (to week 34) and late (from week 35) resulted in higher R² values in the case of CH and WB, with the regression lines for the later (cooler) period having the larger slopes. For WB: the size of NPs was negatively correlated with Dew point (av/day) (y = -20.208x + 161.13, R² = 0.685, df = 14, P < 0.0001), i.e., smaller-winged *An. sinensis* NPs were associated with times of higher humidity. The wing length of NPs was negatively correlated with Night-time LST (y = -10.278x + 67.275, R² = 0.720, df = 14, P < 0.0001), i.e., smaller NPs were associated with warmer nights. The size of Ps was negatively correlated with the EVI (y = -0.301x + 1.896, R² = 0.713, df = 10, P < 0.0006), i.e., smaller-winged Ps corresponded to higher values of EVI (greenness).

At CH, NDVI showed a stronger negative correlation (R²) than EVI, for *An. sinensis* Ps as well as NPs. NP mean wing length was also positively correlated with the number of daylight hours (y = -0.005x + 9.170, R² = 0.778, df = 14, P < 0.0001); daylight hours and the size of NPs increased steadily throughout the mosquito season (weeks 27-42). Also, at CH there was a significant positive correlation of mean wing
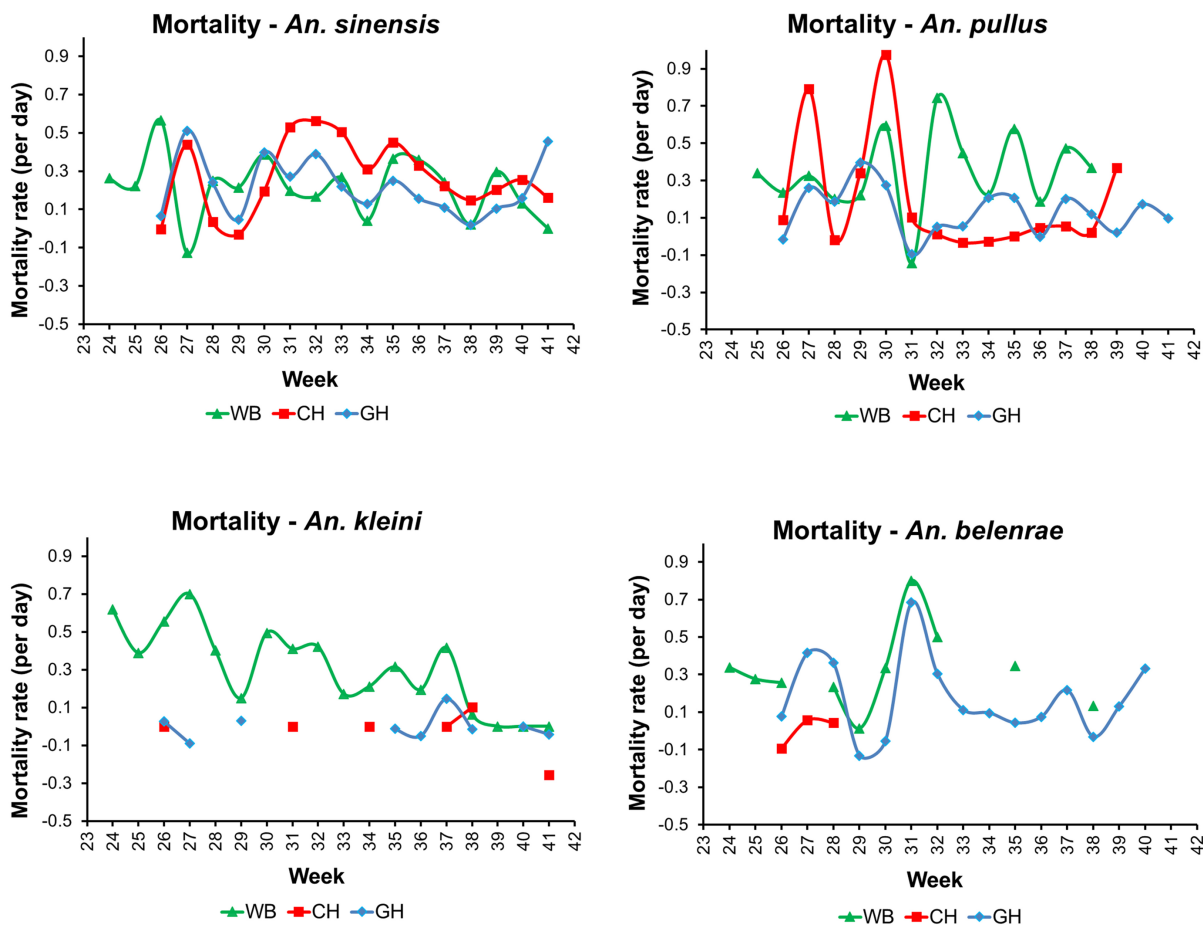
Figure 2. Weekly changes in estimated mortality for *Anopheles belenrae, An. kleini*, *An. pullus,* and *An. sinensis* at Camp Humphreys (CH), Ganghwa Island (GH), and Warrior Base (WB), Republic of Korea in 2009.
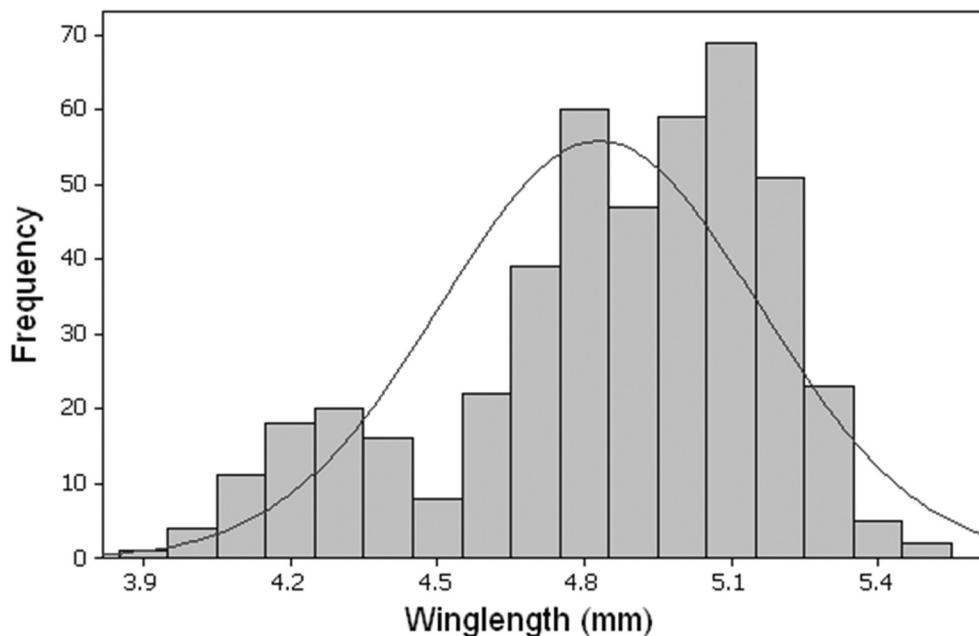

Figure 3. Frequency distribution of wing lengths (N = 455) showing apparent bimodality for *Anopheles sinensis* nulliparous females collected from Ganghwa Island, Republic of Korea in 2009. A normal distribution curve is given for comparison.

length for NPs with average daily air pressure (y = 9.703x + 963, R^2 = 0.791, df = 14, P<0.0001). Air pressure at CH was higher at the end of the mosquito season, in line with increasing wing sizes.

Wing length for GH did not show clear trends with climate data, which may be related to the trap type and location in a cow shed. *Anopheles belenrae*, *Ae. kleini,* and *An. pullus* showed similar patterns to *An. sinensis* at the three sites, but lower numbers of these species limit conclusions.

DISCUSSION

This study showed that wing length was generally greatest at the beginning and end of the study period and appeared to be related to temperature (see LST and T-MIN values in Table 2), with cooler conditions resulting in larger specimens. Wing length of NPs tended to be less than or equal to wing lengths of Ps before the period of maximum greening (Jul-Aug) but were often greater than Ps after that period. Larger specimens tended to be Ps, and weekly wing length of Ps appeared less variable than NPs, possibly due to selection against smaller, less fit adults. Peaks in adult mosquito abundance during weeks 35-36 (CH and WB) and 33-34 (GH) reported by Foley et al. (2017) are due to Ps. A bimodal wing length frequency distribution of the most frequently collected species, *An. sinensis,* suggested two forms comprising small- (≤4.5 mm, SW) and large-winged specimens (>4.5 mm, LW). Smaller-winged *An. sinensis* were associated with warmer, more humid, greener times of the year. LW mosquitoes comprised the majority of peaks in abundance, however, %SW, while still a minority, often increased during these times, suggesting a density dependent effect. The two most northerly sites (WB and GH) showed a two to three-week periodicity in %SW for *An. sinensis* and *An. kleini*, such as that resulting from synchronous crop phenology and agricultural practices. Peaks in %SW Ps followed peaks in NPs in a 'ripple effect' one to two weeks apart, suggesting that wing length combined with parity could be used to follow the emergence and survival of mosquito cohorts.

Using the formula developed for *Ae. triseriatus* weight (McCombs, 1980. Master's thesis, University of Notre Dame) (dry weight = 0.009 wing length^3 – 0.017), the dry weight for *An. sinensis* ranged from 0.44-1.48 mg, a three-fold difference. In the ROK, anopheline larvae inhabit a diverse range of habitats, including flooded rice fields, drainage ditches, ponds, stream margins, and swamps (Rueda et al. 2006, Kim et al. 2007, 2009, Sames et al. 2008). However, Claborn et al. (2002) could find no environmental factors that were predictive of high larval densities of *An. sinensis* s.l.

Barr (1985) found that high mortality of larvae is common in a wide variety of mosquito species. For *Culiseta incidens* (Thomson), he found that mortality was density-dependent and was due mainly to lack of available nutrients. High larval density can delay development, and in the case of the bromeliad breeding *Wyeomyia vanduzeei* Dyar and Knab, lead to bimodality in pupation in laboratory experiments, with the first pupae weighing more than later pupae (Frank et al. 1985).

Larval species composition can have an asymmetric impact on adult size of co-habitating species, e.g., *Culex quinquefasciatus* Say impacts negatively on the body size of *An. gambiae* (Kweka et al. 2012). Transplant experiments have shown that species respond differently to different types of larval habitat; *An. coluzzii* Coetzee and Wilkerson larvae survive better than those of *An. gambiae* s.s. in habitats such as rice fields that are larger, more permanent, and rich in aquatic predators (e.g., backswimmers, water boatman, dragonfly, and beetles), while the reverse is true in temporary water bodies (Diabaté et al. 2008, Minakawa et al. 2004, 2005, Sunahara et al. 2002). Munga et al. (2013) found that development time and wing length for *An. gambiae s.l.* was not related to habitat disturbance or predation. However, Ong'wen et al. (2020) found that the presence of a predator (dragonfly nymphs) decreases development time of *An. gambiae* larvae, as well as increasing the susceptibility of emerging adults to a parasite (fungus). Early and late egg hatching has been observed even within egg batches for various mosquito species, possibly reflecting a mixed strategy to respond to larval habitat variability. For example, Yaro et al. (2006) speculated that larger adults can be produced as a result of longer developmental times in larval sites with good nutrition and low risk of desiccation or predation, and smaller adults produced as a result of shorter developmental time in sites with poor nutrition and high risk of predation.

For the situation in the ROK, mean size, as estimated by wing length, could be related to such factors as the nutrient levels or temporary nature of larval habitats, the water temperatures affecting development rate, predation, or larval competition influenced by the levels of intra- and interspecific crowding. For example, intermittent flooding of rice followed by evaporation or drainage, or the movement of larvae to the periphery of crops, could concentrate larvae, and possibly predators, in shallow water where mid-day temperatures and development rates are higher. This could result in the early emergence of smaller adults, aided by the death of late or slow developing larvae if water dries up. Alternatively, stressors such as crowding or predation could favor the survival of larger individuals. Can wing length metrics shed light on larval conditions in the ROK?

Fish (1985) hypothesized that there are characteristic wing size-frequency distribution patterns that reflect larval habitat conditions. These signature patterns could include: 1) populations with low CV, high mean values and zero to negative skewness that indicate few if any environmental constraints as larvae, and 2) high CV, low mean wing length, and positive skewness that indicate poor larval conditions such as limited larval food. Fish (1985) classified CV values as: >25 (high), 15-25 (moderate), and <15 (low). High CV was seen as a result of temporary habitats, low CV of more permanent habitats. High predation is thought to reduce larval density, resulting in low to moderate CV and low or negative skewness in the more permanent habitats. The highest CV in the present study was ~9 which corresponded to more permanent habitats with higher predation, as may occur with rice paddies. However, Fish (1985) warned that light trap catches could result from a mix of specimens from different

Table 2. Mean wing lengths (mm) and associated metrics (standard deviation, skewness, kurtosis, coefficient of variation) for *Anopheles sinensis* females, and a selection of climate/weather data, for calendar weeks of 2009 at Warrior Base, Republic of Korea.

Week-End date	Length (Mean)	Length (SD)	Length (N)	Skew	Kurtosis	CV	T-MIN[1]	LST[2]	DEWP[3]	EVI[4]
20-20090517							8.33	13.31	50.05	0.295
21-20090524							9.44	13.19	54.70	0.359
22-20090531							8.89	14.63	49.73	0.352
23-20090607							10.56	15.64	56.41	0.380
24-20090614	5.02	0.16	11	0.77	-0.59	3.23	11.11	15.91	57.82	0.408
25-20090621	4.97	0.20	49	-0.12	-0.51	4.07	15.56	17.05	63.45	
26-20090628	4.92	0.27	103	-1.13	1.21	5.59	15.56	18.23	63.59	0.381
27-20090705	4.87	0.06	4	0.62	0.88	1.26	17.78	18.90	66.07	0.518
28-20090712	4.69	0.28	57	-0.49	-0.30	6.03	16.11	19.57	66.59	0.478
29-20090719	4.64	0.31	108	-0.23	-0.78	6.78	20.00	20.71	70.39	
30-20090726	4.79	0.33	53	-0.63	-0.18	6.91	15.00	19.10	66.40	0.524
31-20090802	4.57	0.34	45	-0.16	-0.76	7.41	18.89	19.98	68.80	
32-20090809	4.70	0.28	18	0.04	0.29	5.98	20.00	20.97	69.43	0.531
33-20090816	4.55	0.29	17	-0.18	-1.01	6.44	18.89	21.00	69.88	0.594
34-20090823	4.73	0.34	35	-0.04	-1.44	7.21	17.22	19.70	68.84	
35-20090830	4.60	0.36	77	-0.25	-0.94	7.72	14.44	17.90	64.86	0.511
36-20090906	4.73	0.35	82	-0.06	-1.13	7.33	14.44	16.79	62.63	0.481
37-20090913	4.89	0.27	28	0.33	-0.22	5.53	12.22	15.58	59.00	0.441
38-20090920	4.81	0.32	114	-0.40	-0.12	6.67	11.67	15.08	56.91	0.441
39-20090927	4.88	0.30	8	-1.20	0.91	6.06	10.56	13.90	57.29	0.407
40-20091004	5.02	0.25	3	-0.59		5.02	6.11	12.15	54.50	
41-20091011	5.05	0.05	3	0.00		0.99	6.11	8.97	45.79	0.340
42-20091018							3.89	8.10	46.64	0.281

[1]Mean minimum temperature (°C); [2]Night-time Mean Land surface temperature (°C); [3]Mean daily dew point; [4]Enhanced Vegetation Index.

larval habitats that yield adults of different sizes, so CV could be higher and kurtosis lower than if specimens came from the same or similar larval habitats. Weekly variations in wing metrics (CV, kurtosis, mean, skewness) were highly variable in our study, suggesting a high level of variation in conditions for larval growth. Skewness was generally negative with distributions having a tail of smaller individuals, suggesting that most survivors were larger than would be expected from a normal distribution and that many individuals experienced sub-optimal larval conditions and did not survive to adult. Kurtosis was also often negative indicating a flatter-than-normal distribution but showed oscillations on a one to three-week basis suggesting pulses of more homogeneous wing lengths followed by periods of more heterogeneous wing lengths. Data on the types of and changes in larval habitats within the vicinity of each study site would assist in the interpretation of wing metrics variation, but only some data are available, for WB (Kim et al. 2011). These authors noted that rice paddies produced the most anopheline larvae (in July), with the other habitat categories generally peaking

in August, while for rice paddies, the "Young-Mature rice" stage produced the most larvae for the majority of *Anopheles* spp. identified.

Parity rates

We assume that nulliparous mosquitoes are generally newly emerged, and abundance and size of individuals should reflect larval conditions in the previous week or so. Parous mosquitoes are older, and in general numbers should partly reflect adult conditions affecting survival and reproduction in the previous week or so.

Mean wing lengths of Ps and NPs were generally greater at the beginning and end of the study period, possibly due to seasonal changes in temperature or vegetation phenology affecting development rates. Within *An. sinensis,* NPs were generally smaller than Ps at the beginning of the study period (weeks 24-31) but were larger after than time, possibly related to the period of maximum greening (Foley et al. 2017) or agricultural practices accompanying crop phenology. NPs were not observed at GH after week 36 but Ps were collected
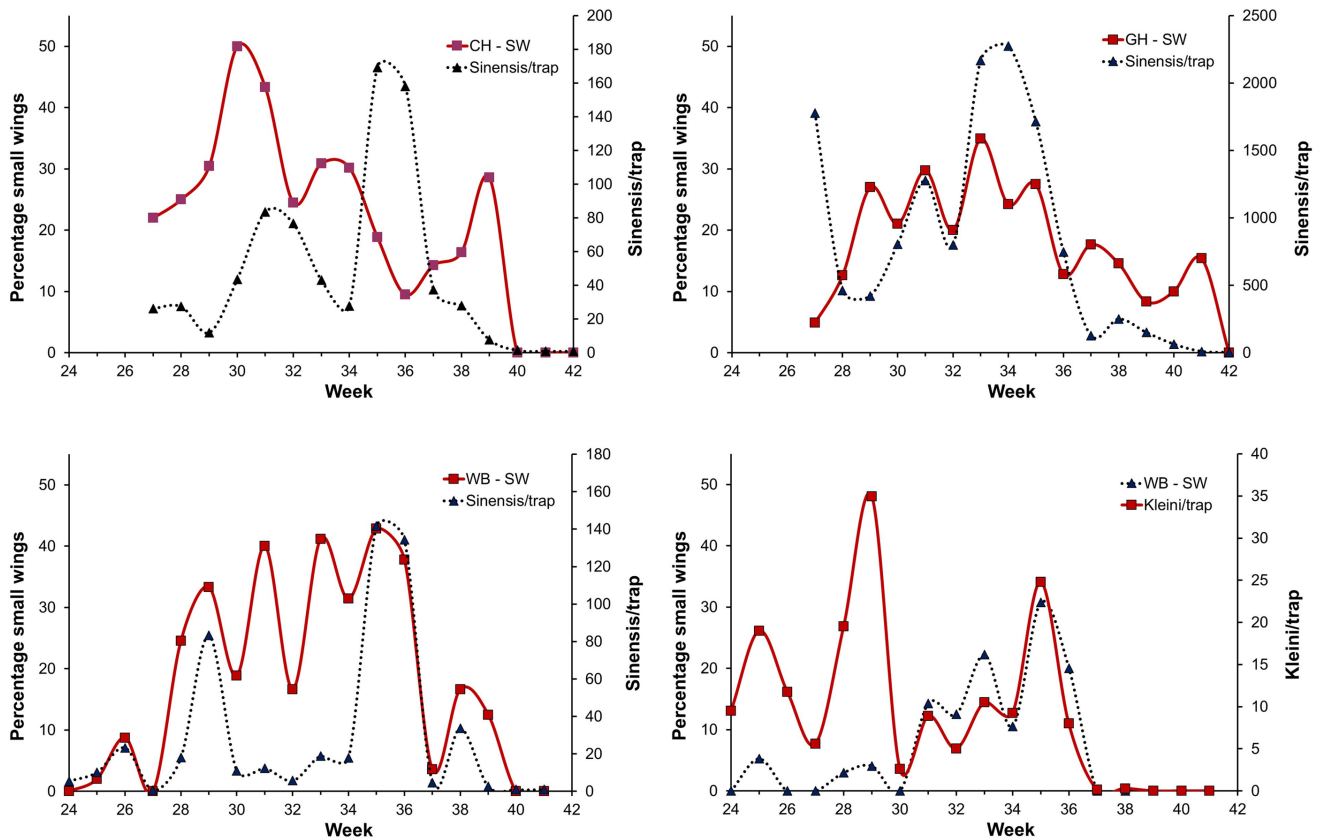
Figure 4. Weekly percentage of specimens of *Anopheles sinensis* and *An. kleini* that were small-winged (SW, <= 4.50 mm), and estimated total abundance per trap at Camp Humphreys (CH), Ganghwa Island (GH), and Warrior Base (WB), Republic of Korea in 2009.

through week 42 when the collection period was terminated. If this represents the decline of natural populations when recruitment has ceased, then adult females can survive at least six weeks based on trapping in a cow barn at GH. By this logic, adults survive upwards of three weeks at the end of the mosquito season for *An. sinensis*, *An. kleini,* and *An. pullus* at WB (Figures 1a, 1b). The concentration of potential hosts (cows) and milder climate likely inside a cow barn at GH could result in higher parity and lower mortality estimates for *Anopheles* trapped there. Some support for this contention can be seen from Figure 2 for the later part of the mosquito season. GH appeared to have consistently lower mortality than CH for *An. sinensis*, and lower mortality than WB for *An. belenrae*, *An. kleini,* and *An. pullus*.

Variation in mean wing length was most extreme for NPs at CH (Figure 1a) with lows of 3.9 mm and highs of 5.5 mm, suggesting that conditions for larvae varied more at this site than at the more northerly sites (GH and WB). The divergence between mean wing lengths of *An. sinensis* Ps and NPs was particularly noticeable at CH during the end of the mosquito season, suggesting that despite larger newly emerged NPs, those that survived at least one gonotrophic cycle tended to be smaller.

The 'Ripple effect'

When parity was ignored, as in Figure 4, peaks in the %SW showed a remarkable periodicity (about two weeks),

with much synchronicity evident between collection locations and species. This oscillatory phenomenon suggests cohorts of mosquitoes are entrained, either by the onset of suitable climatic conditions at the start of the mosquito season, or by area-wide synchronized agricultural practices affecting larval habitats.

The trapping signature over time of an isolated mosquito cohort should show up as a peak in NPs followed by a series of ever diminishing peaks in corresponding Ps as the cohort ages. The %SW in Figure 5 appear to show ageing of mosquito cohorts, with peaks in NPs followed by a lag of one to two trapping intervals (weeks) before a smaller matching peak in Ps is evident. This pulsed phenomenon occurred regardless of the location or species (*An. sinensis* and *An. kleini*), but the timing of these peaks was not always synchronous between locations. One apparent departure from prediction is that later P peaks are sometimes greater than the NP peaks but this may be an artefact of sampling or of using percent as the axis of ordinates. It is not known whether changing the temporal granularity of our trapping regime from weekly to sub-weekly intervals would retain or drown out this signature, but it is likely that the gonotrophic cycle is shorter than our weekly trapping interval; degree-day estimates of the gonotrophic cycle varied from two to seven days (Foley et al. 2017). If the trapping interval was sub-weekly then it might be possible, using %SW and parous state, to more accurately measure the interval from one feeding attempt to another for
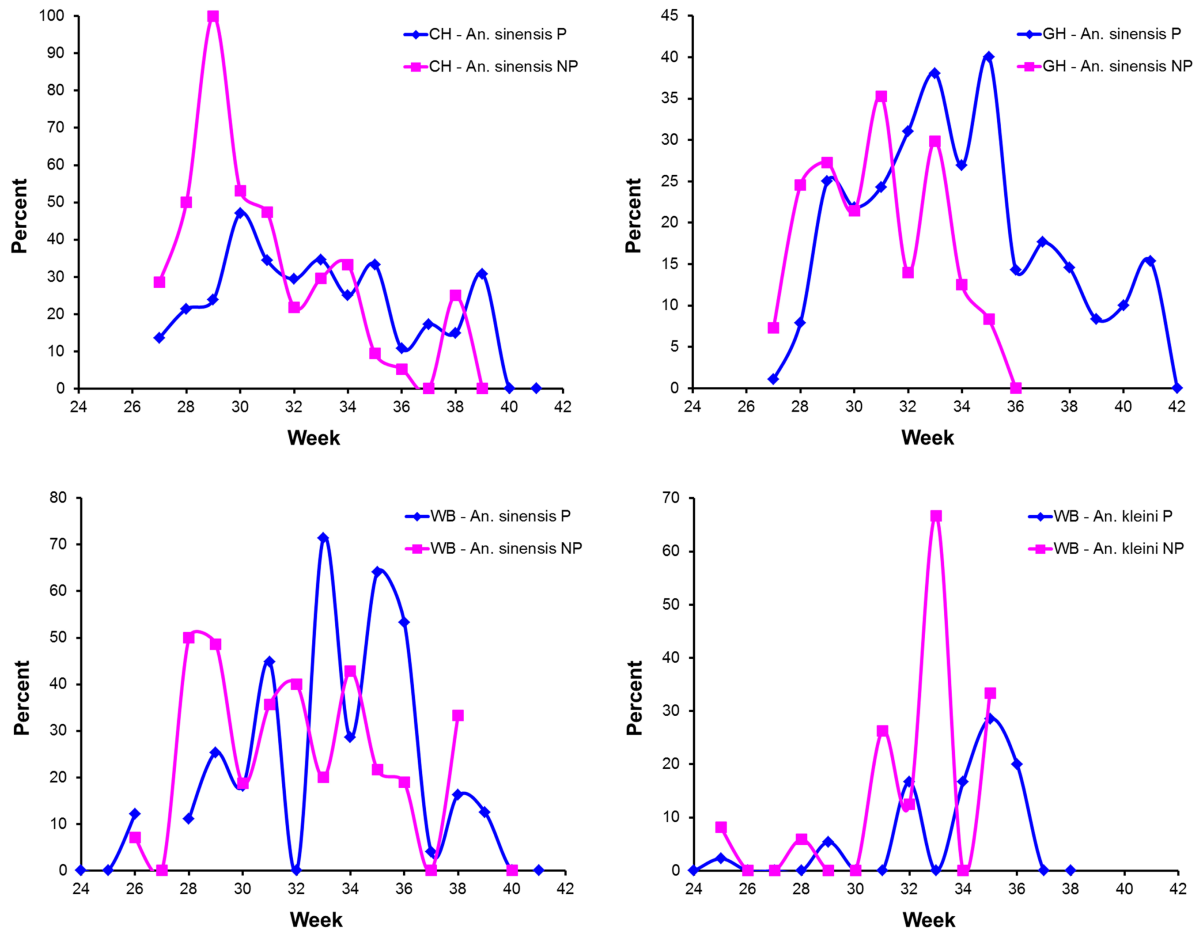
Figure 5. Percentage of parous (P) and nulliparous (NP) *Anopheles sinensis* and *An. kleini* that were small-winged (<= 4.50 mm) from weekly collections at Camp Humphreys (CH), Ganghwa Island (GH), and Warrior Base (WB), Republic of Korea in 2009.

these mosquito cohorts. Interestingly, Charlwood (2003) lists five methods for the determination of mosquito longevity, including studies of the rate of disappearance of marked populations. Wing length combined with parity appears to be a novel additional method for longevity studies and could serves as a natural marker of the emergence and survival of a particular adult mosquito cohort in the ROK.

Vectorial capacity

What importance is a study of mosquito wing length and how does it relate to medically important characteristics such as vectorial capacity? Bevins (2008) showed that interspecific larval competition between *Ae. albopictus* and *Ae. triseriatus* reduced survival and increased the adult size of the latter species, possibly due to reduced intraspecific competition that resulted in more food being available. The resulting larger adults were more likely to develop La Crosse virus than specimens from mono-specific habitats (Bevins 2008). For adult *An. gambiae*, smaller females take longer to reproduce and contribute fewer offspring to the next generation and have a greater risk, through more frequent blood feeding, of being infected with malaria parasites at an early stage (Lyimo and Takken 1993). However, Manoukis et al. (2006) found no effect of size on survivorship of malaria vectors in Mali

and concluded that reduced transmission was unlikely to be caused by higher larval densities causing smaller body size and lower survivorship.

In studies of the age structure of natural populations of anopheline mosquitoes, a common assumption is that females are gonotrophically concordant, i.e., once the females are inseminated, they produce a batch of eggs after each blood meal. However, Briegel (1990) and Lyimo and Takken (1993) observed that for *An. gambiae s.l.,* the critical size permitting oviposition from the first blood meal was a wing-length of 3 mm. Below this threshold, females require multiple blood meals to develop eggs. The presence of small adult females could lead to an underestimate of longevity based on parity measurements, since longevity estimates assume a constant number of days between adult emergence and first oviposition that could be violated by different feeding outcomes of large and small mosquitoes.

The current study did not directly measure the relationship between wing length and vectorial capacity. Mosquito size does appear to relate to survival as estimated from lagged parity rates, but this relationship could vary depending on temperature and nutrients levels of the larval environment (Barreaux et al. 2018). Knowing the wing length of NPs at CH, for example, was indicative of parous rates in

the following two weeks and could potentially be used in surveillance to predict mosquito longevity and the suitability of mosquitoes to develop malaria parasites.

Assumptions and limitations

An assumption in our study is that dispersal of mosquitoes into or out of the catchment area of the traps is minimal. According to Shim and Kim (1999), the flight range of *An. sinensis* s.l. was shorter than 5 km and rarely over 10 km with a mean of 6 km and a maximum of 16 km (Moon and Cho 2001), or 20 km in the case of Cho et al. (2002). Reductions in the availability of larval habitat and/or overcrowding of immature stages could encourage greater dispersal of mosquitoes. However, it is difficult to see why there would be a need for directed mosquito dispersal given the widespread co-occurrence of water and agriculture in the Korean peninsula.

According to Bruce-Chwatt (1980), in subtropical areas such as Syria some species (e.g., *An. sacharovi* Favre) may undertake a long pre-hibernation flight (up to 19 km). Foley et al. (2012) speculated about synchronized movement of adult mosquitoes in Korea, and recently, it was found that blood-fed mosquitoes avoid the dry season around the Sahel of Africa by migrating long distances at high altitudes on prevailing winds (Huestis et al. 2019). One possibility is that the drop in %SW at the end of the mosquito season coincides with a dispersal phase, but lower temperatures and reduced larval density may also explain this phenomenon.

Future studies

This wing length study was part of a more ambitious exploration of possible drivers, such as weather and climate, on weekly changes in adult mosquito abundance and parity, as well as on the levels of *Plasmodium* infections in medically important anopheline species in the ROK. Technical issues that resulted in the possibility of false *Plasmodium* positives (Harrison et al. 2013) led us to believe that these data were unreliable, and so we did not report them. However, exploring the relationship of vector infection and infectivity rates with mosquito trap catch numbers, parity, and wing length metrics remain a worthy goal of future ecology research.

Our reliance on means in regression analyses may not account for many situations where heterogenous variances and complex interactions between ecological factors occur (Cade and Noon 2003). For example, Dunham et al. (2002) found that quantile regression estimates indicated a nonlinear, negative relationship with the upper 30%, vs no relationship with mean regression estimates of cut-throat trout densities across stream widths and depths. Density-dependent processes and intraspecific competition for resources may occur with water management practices that result in mosquito larval crowding, for example, which may best be revealed by only considering mosquitoes at the lower range of wing lengths. Analyzing %SW may reveal patterns and possible relationships with response variables that would not be obvious using means of total mosquito wing lengths. Quantile regression of wing lengths may be useful for investigating relationships between variables.

The mosquito season (approximately weeks 24-42 or Jun-Oct) appeared to be comprised of different phases that varied for each location; unknown seasonal effects could confound regression analyses of wing length vs weather and satellite data. Obtaining data from multiple locations at single time points may be needed to confirm the effect of potential drivers of mosquito abundance, parity, and wing length. Future studies may also benefit from an exploration of buffer size for satellite-derived data and the use of portable weather stations to more accurately measure local conditions. Finally, future studies would benefit greatly from field studies to determine the conditions of larval habitats, and the timing of local and regional agricultural practices affecting the seasonality of mosquito demographic characteristics.

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