

Density Dependence, Landscape, and Weather Impacts on Aquatic *Aedes japonicus japonicus* (Diptera: Culicidae) Abundance Along an Urban Altitudinal Gradient

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Abstract

The Asian Bush Mosquito, *Aedes (Finlaya) japonicus japonicus* (Theobald) is an important globally invasive mosquito species. In comparison with other major invasive mosquitoes, relatively little is known about *Ae. j. japonicus* population dynamics in the field. Here, we present results from a 54-biweek long study of *Ae. j. japonicus* abundance in ovitraps set across the altitudinal gradient of Mt. Konpira, Nagasaki, Japan. Spatially, we found that *Ae. j. japonicus* fourth instar larvae (*Aj4il*) were more abundant at the base and top of Mt. Konpira and in ovitraps with more platykurtic water temperature (WT) distributions. In contrast, we found that temporally *Aj4il* were more abundant when ovitrap WT was more leptokurtic with 2 weeks of lag, and with high relative humidity SD with 2 months of lag. We also found that *Aj4il* were unlikely present when ovitrap WT was below 12.41°C. Parameter estimates for the Ricker model suggested that *Ae. j. japonicus* population growth was under density-dependence regulation, with a stable population dynamics whose fluctuations were associated with changes in ovitrap WT kurtosis and demographic stochasticity. Our results suggest that *Aj4il* abundance is more sensitive to temperature changes in kurtosis than mean values, potentially limiting the predictive ability of *Ae. j. japonicus* niche models based on the increase of average temperatures with global warming, and suggesting this mosquito species has a relatively coarse-grained response to temperature changes.

Key words: Schmalhausen's law, population regulation, environmental kurtosis, stochastic Ricker model, zero-inflated model

The Asian bush mosquito, *Aedes japonicus* (Theobald), is a species complex comprising *Ae. j. shintienensis* Tsai & Lien, *Aedes japonicus amamiensis* Tanaka et al., *Aedes japonicus yaeyamensis* Tanaka et al., and *Aedes japonicus japonicus* of which the last one has as native range the Japanese archipelago and the Korean peninsula (Tanaka et al. 1979). In recent years, *Ae. j. japonicus* has also become a major globally invasive species, being now established in North America, Europe, and Hawaii (Kampen and Werner 2014, Kaufman and Fonseca 2014). Moreover, *Ae. j. japonicus* has also been found in used tires imported from Japan into New Zealand, and could potentially also colonize temperate and subtropical areas in the southern hemisphere (Laird et al. 1994). Medical importance for the Asian bush mosquito is particularly interesting, given its adaptability to transmit different pathogens over its native and invasive range. Although *Ae. j. japonicus* has been documented as an important Japanese Encephalitis virus vector in Japan (Kitaoka et al. 1950, Takashima et al. 1988, Takashima and Rosen 1989), it is a La Crosse virus vector in North America (Harris et al. 2015,

Westby et al. 2015), and experimental studies have shown its vectorial competence for West Nile virus (Sardelis and Turell 2001), Eastern Equine Encephalitis (Sardelis et al. 2002), and St. Louis Encephalitis virus (Sardelis et al. 2003) transmission.

In Japan, *Ae. j. japonicus* has a wide latitudinal distribution, being present in all the main islands of the Japanese archipelago, from temperate Hokkaido Island to subtropical southern Kyushu Island, and smaller outlying islands north of the Ryukyu archipelago (Kamimura 1968, Mogi 1996, Maekawa et al. 2016). The Asian bush mosquito has a similar latitudinal distribution in North America, where it has been observed from temperate Ontario and Quebec, in Canada, to subtropical Alabama and Mississippi in southern United States (Kaufman and Fonseca 2014), while in Europe it has been found at temperate latitudes (Kampen and Werner 2014). This geographic distribution, coupled with a phenology where Asian bush mosquito adults emerge in March, and pupation is still common by mid-October at temperate latitudes (Matuo 1961) and observations about a negative correlation between *Ae. j. japonicus*

immature abundance and temperature (Bartlett-Healy et al. 2012) has led to the suggestion that its niche might be constrained to relatively low temperatures (Kaufman and Fonseca 2014). However, it is also known that Asian Bush mosquitoes likely have a year-round pupation and adult emergence at subtropical latitudes (Mogi 1996), raising questions about the ecological relationship of immature Asian bush mosquitoes with temperature, especially whether patterns of temperature variability might play a role on the ecology of *Ae. j. japonicus*, as expected under Schmalhausen's law, which states that organisms are sensitive not only to average environments but also to environmental patterns of variability, and that organisms become more sensitive to small changes in environmental factors when pushed toward their tolerance limits from a different environmental factor (Chaves and Koenraadt 2010).

Ecological studies of *Ae. j. japonicus* in Japan have shown its frequent occurrence at bamboo stumps (Kurihara 1958, Kurashige 1961), treeholes (Sakakibara 1960, Tsuda et al. 1994), rock pools (Nakata et al. 1953, Matuo 1961), and artificial containers (Omori et al. 1952, Sota et al. 1994, Yamauchi 2010, Shiraishi 2011). In North America, field studies have found the Asian bush mosquito in artificial containers, tires, and treeholes (Andreadis et al. 2001, Bartlett-Healy et al. 2012, Kaufman et al. 2012) and are suggestive of major negative impacts on the native mosquito fauna (Andreadis and Wolfe 2010), although laboratory studies suggest interspecific interactions might be weak when compared with intraspecific interactions (Hardstone and Andreadis 2012), but competition impacts on fitness might become evident in the adult stage (Alto 2011). Laboratory and mesocosm studies have also suggested population abundance regulation via the impact of predation by other mosquito larvae (Freed et al. 2014). Special attention has been given to the interactions between *Ae. j. japonicus* and the Asian tiger mosquito, *Aedes albopictus* (Skuse) another major invasive species, where it has been suggested that *Ae. albopictus* could outcompete *Ae. j. japonicus* based on mesocosm studies (Armistead et al. 2008), which made the implicit assumption that these two species only co-occur in the same type of habitats. Nevertheless, field studies in both Japan (Tsuda et al. 1994) and the United States (Bartlett-Healy et al. 2012) have shown that these two mosquito species might colonize different habitats, with *Ae. j. japonicus* preferring larger-sized treeholes (Tsuda et al. 1994) and larger artificial containers (Bartlett-Healy et al. 2012). However, both species often co-occur when habitat diversity is constrained, e.g., in bamboo stumps at bamboo groves (Kurihara 1958, Kurashige 1961) or in ovitraps (Zea Iriarte et al. 1991). At the larger landscape level, it has been observed that *Ae. j. japonicus* predominates over *Ae. albopictus* in rural environments, whereas the opposite holds in urban environments, where *Ae. albopictus* is more common (Bartlett-Healy et al. 2012). It has also been observed that *Ae. j. japonicus* has a wider altitudinal range than *Ae. albopictus* in landscapes where both species co-occur along altitudinal gradients (Shiraishi 2011). Surprisingly, none of these studies has looked at the temporal association of *Ae. j. japonicus* abundance with weather factors nor to the impacts of density dependence in field population dynamics, despite the central role that mosquito abundance has for pathogen transmission as presented in the mathematical expression for vectorial capacity (Garrett-Jones 1964) or mathematical models of mosquito-borne disease transmission (Smith et al. 2014), where, overall, disease transmission increases with vector abundance. Thus, here we present results from a 54-biweek-long study where we followed *Ae. j. japonicus* fourth instar larvae (*Aj4il*) and pupae population dynamics sampled in ovitraps at Mt. Konpira, Nagasaki, Japan, a place where this mosquito species colonizes ovitraps and co-occurs with *Ae. albopictus* (Zea Iriarte et al. 1991, Chaves 2017a) and

where our previous results showed the imprint of density dependence in a time series of *Ae. j. japonicus* adults (Chaves 2016). We specifically asked which weather and landscape factors were associated with the abundance of this mosquito along the altitudinal gradient of Mt. Konpira and whether density dependence could be detected in the time series formed by our observations.

Materials and Methods

Study Site

Our study was done in Nagasaki city, the capital of Nagasaki prefecture, which according to the 2009 Japanese National Census has over 440,000 residents and a population density of 1,100 people/km² (Statistics Bureau, Ministry of Internal Affairs and Communications of Japan, <http://www.stat.go.jp/index.htm>). Nagasaki City is located in Kyushu Island, western Japan (Fig. 1A), near the southwestern tip of Nagasaki prefecture (Fig. 1B). Many mountains are present within the city limits, which host several primary and secondary forests, among which Mt. Konpira, east of the Atomic Bomb detonation site, is primarily covered by natural vegetation (Fig. 1C). In Mt. Konpira, we sampled immature mosquitoes across the altitudinal gradient from its base at 109 m and up to 320 m (Fig. 1D). For the study, we placed 27 ovitraps along three transects, where ovitraps were placed at the same coordinates of ovitraps from a study performed in 1989 (Zea Iriarte et al. 1991), thus allowing the evaluation of changes in the altitudinal distribution of this species.

Mosquito Sampling

Immature mosquitoes were sampled using ovitraps, which were made with 350-ml Coca-Cola cans painted black, inside and outside, with an acrylic spray paint (Kanpe Hapio Co., Ltd., Osaka, Japan). We made a 5 mm opening which not only served as drainage when liquid contents went over 280 ml but also used to hold the ovitrap to a tree using a black 1-mm diameter nylon cord. All traps were uniformly set at 1.2 m above ground level, because this height was used in a previous study where *Ae. j. japonicus* was sampled at our study site using similar ovitraps (Zea Iriarte et al. 1991). Ovitrap were filled with 280 ml of rain water collected during April 2014 and were set on 18 May 2014, a time when most mosquito species in the study area start their adult activity (Chaves et al. 2015, Chaves 2016, Hoshi et al. 2017). Like in natural treeholes we let leaves naturally fall in ovitraps (Zea Iriarte et al. 1991, Tsuda et al. 1994). Ovitrap were surveyed every 2 weeks from 1 June 2014 until 23 June 2016. During each survey, we looked for the presence of small mosquito larvae, i.e., first to third instar, and we counted the number of fourth instar larvae of *Tripteroides bambusa* (Yamada), which can be easily identified in the field (Tanaka et al. 1979). We did not count small mosquito larvae given the unreliability of counts for these stages (Sota et al. 1994) and to also keep undisturbed the recruitment dynamics of fourth instar larvae and pupae. We chose a biweekly sampling frequency, given that immature development for most mosquito species is around 2 weeks, including the Asian bush mosquito raised in laboratory colonies over the temperature range at our study site (Williges et al. 2008, Hoshino et al. 2010). Then we removed all other fourth instar larvae and all pupae for identification of emerged adults after their rearing in an insectary (Hoshi et al. 2014b). For mosquito identification, we used the taxonomic key by Tanaka et al. (1979) and vouchers were deposited in the Entomological Collection at the Institute of Tropical Medicine of Nagasaki University, Japan. In a few cases, mosquitoes were identified by sequencing the mitochondrial cytochrome c oxidase I region following the protocol presented by Taira et al. (2012).

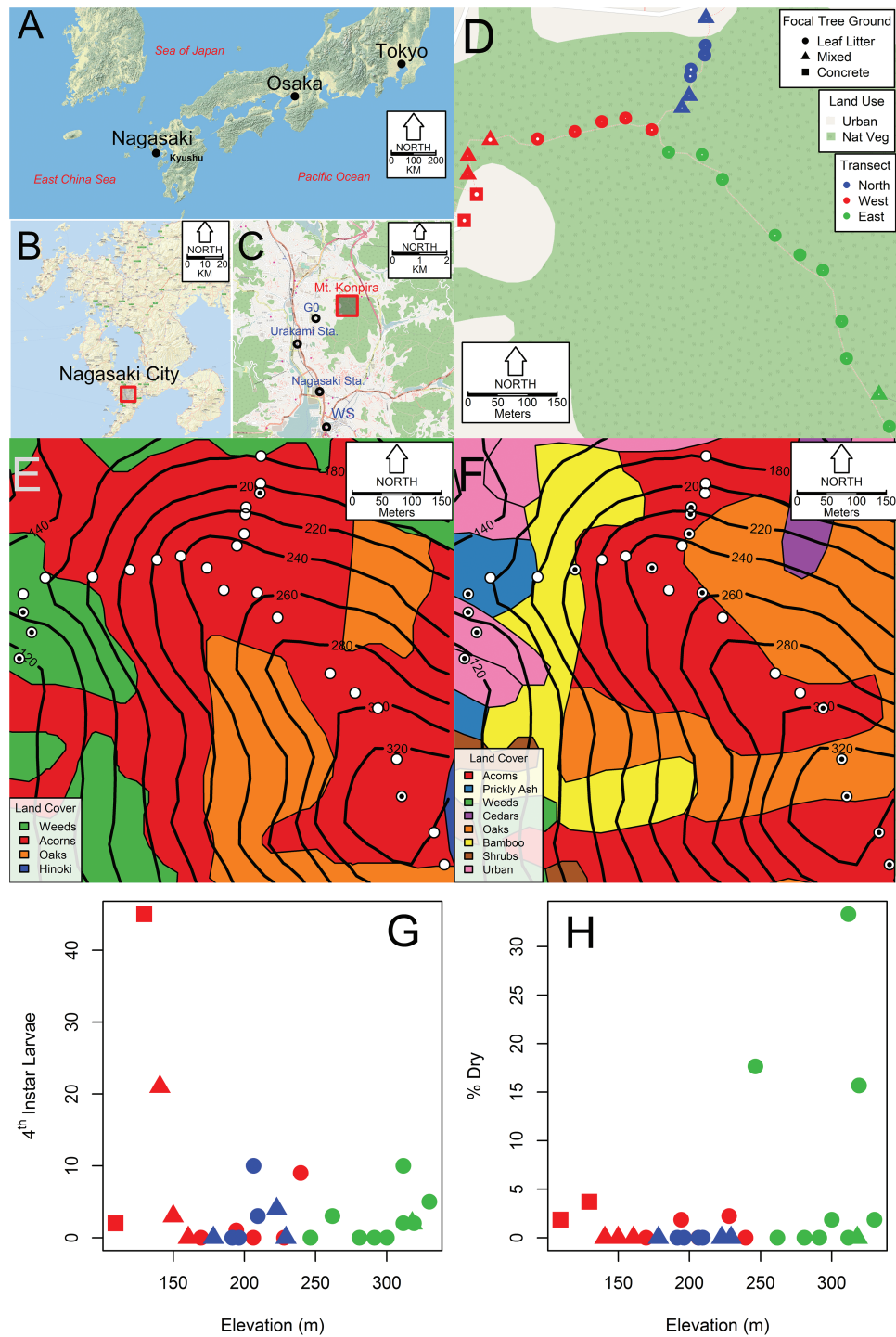


Fig. 1. Study location (A) Nagasaki is located in Kyushu island, western Japan; (B) Nagasaki City, highlighted in the map, is located in southwest Nagasaki Prefecture; (C) Zoom up of Nagasaki City, showing the location of Mt. Konpira highlighted in the map, the Atomic Bomb detonation point (GO), Nagasaki City Weather Station (WS) and the two main train stations in Nagasaki (Urakami Sta and Nagasaki Sta); and (D) Zoom up of Mt. Konpira, indicating land use as urban or Natural Vegetation (Nat Veg). Contour lines indicate the height in meter. Dominant ground cover at each tree where ovitraps were located is indicated by symbol type (See inset legend), while the white dot in the center of each point is proportional to the canopy openness. Transects are identified by color (See Inset legend). (E) Land cover in 1984 and sampling locations by (Zea Iriarte et al. 1991), indicated by white dots, and *Aedes japonicus japonicus* presence in 1989, indicated by black dots; (F) Land Cover in 2012, sampling locations, indicated by white dots, and *Ae. j. japonicus* presence in 2014–2016, indicated by black dots; (G) Total number of *Aj4il* sampled at each ovitraps in 2014–2016; (H) Percent of times an ovitraps got dry. In panels G and H, symbols correspond to those presented in panel D.

Weather Data

During each sampling session we measured WT in ovitraps using an AD-5617WP infrared thermometer (A&D Co., Tokyo, Japan). Temperature records were processed and we generated the mean, median, SD, and kurtosis for each ovitrap during the duration of our study. We generated time series (mean, SD, and kurtosis) using data from all ovitraps for each sampling session. We also recorded whether traps were dry or had some water content, provided previous research has indicated that these qualitative categories show the best association with immature treehole mosquito abundance at our study site (Zea Iriarte et al. 1991, Tsuda et al. 1994), but also in artificial containers elsewhere (Makiya 1974, Sota et al. 1994). Here, we want to highlight that kurtosis was selected as a measure of environmental variability given its relevance for testing Schmalhausen's law (Chaves et al. 2012). For example, while SD or variance measure the dispersion of a variable around its mean, there is no information on how narrow or wide are the conditions around the mean of the variable. However, this component of variability can be assessed by looking at kurtosis, which can tell whether the 50% of states around the mean (second and third quartiles) have low or large variability when compared with the 50% of extreme states around the mean (first and fourth quartiles). Thus information from kurtosis allows to define a variable (or environment) as platykurtic, i.e., with low kurtosis and relatively highly variable around the mean, or leptokurtic, with a high kurtosis and having relatively low variability around the mean (Ross 2014). Data on daily temperature (including the average, maximum, and minimum), relative humidity, and rainfall records from the Nagasaki city meteorological station (WMO Station ID: 47817, Fig. 1C) were obtained from 1 May 2014 to 23 June 2016 from the website of the Japanese Meteorological Agency (<http://www.data.jma.go.jp/obd/stats/etrn/index.php>). These data were then processed by estimating the average (in the case of Rainfall the sum), SD and kurtosis of each weather variable for the 14 d before the mosquito sampling day.

Landscape Data

Land use was broadly classified as urban or natural vegetation (Fig. 1D), based on the 2016 classification by Open Street Map (available at <http://www.openstreetmap.org/relation/382313>). Meanwhile, land cover, i.e., a more detailed classification that considered the specific type of vegetation, was obtained from the Japanese Ministry of the Environment for 1984 and 2012 (available at <http://gis.biodic.go.jp/webgis/>). The 1984 land cover at our study site (Fig. 1E) included the following categories: weeds, hinoki (Japanese Cypress: *Cryptomeria japonica* and *Chamaecyparis* spp.), oaks (referring to deciduous *Quercus* spp. plantations), and acorns (referring to a coppice forest of *Quercus* spp. and *Castenopsis* spp. trees). By 2012, land cover diversified (Fig. 1F), with the emergence of urban areas (including housing and roads), the replacement of acorns by madake bamboo (*Phyllostachys bambusoides*), and prickly ash (a secondary forest of broad-leaved pioneer deciduous tree spp., which includes spurge, *Mallotus* spp. and the ailanthus-like prickly ash, *Zanthoxylum ailanthoides*). In the western side of our study site also some areas dominated by weeds were also replaced by planted shrubs, while in the northwest some of the oaks were replaced by a secondary forest of planted cedars (mainly evergreen *Quercus* spp.). For further details about the landcover classification, please refer to <http://gis.biodic.go.jp/webgis/sc-016.html>.

We also characterized the ground and quantified canopy openness at the locations where traps were deployed (Chaves 2017b). For the ground, we estimated a ground cover index, whose high values

were associated with grounds dominated by concrete and low values associated with leaf litter. Meanwhile, canopy openness (mean and SD) was estimated using a standard method based on digital photographs taken with a 180° fish eye ball lens (Frazer et al. 2001) with photographs taken on 25 May 2014. For further details about the procedures for ground cover index estimation and canopy openness quantification, please refer to the study by Chaves et al. (2015). We also employed an ASTER digital elevation model, with a 10 m resolution (available at <http://gdem.ersdac.jspacesystems.or.jp/>), to estimate the elevation, slope, aspect, flow direction, roughness, and terrain roughness index at each ovitrap location (Brunsdon and Comber 2015). More specifically, elevation was measured in meters and slope in degrees at each ovitrap location. Aspect is the direction of the slope (where 0° is north, 90° east, 180° south, and 270° west) and flow direction gives the direction of the largest drop (or smallest rise if the focal cell is lowest) in elevation considering the eight neighboring cells in relation to the focal cell where an ovitrap was located. For flow direction, values were assigned as increasing powers of two in clockwise direction, 2⁰ was east, 2¹ southeast, 2² south, 2³ southwest, 2⁴ west, 2⁵ northwest, 2⁶ north, and 2⁷ northeast. Roughness was the maximum elevation difference between the set of nine cells composed by the focal cell containing an ovitrap and its eight surrounding neighbors, while terrain roughness index was the average of the difference between the focal cell and its neighbors (Wilson et al. 2007).

Spatial Analysis

We evaluated changes in presence/absence of *Ae. j. japonicus* along the altitudinal gradient of Mt. Konpira by comparing our records from 2014 to 2016 with data from 1989 (Zea Iriarte et al. 1991) using a logistic regression (Faraway 2006) where presence in 2014–2016 was studied as function of presence at the same site in 1989. Given the spatial nature of this analysis, we estimated Moran's I spatial autocorrelation index on model residuals, a statistic that has the null hypothesis of spatial independence (Brunsdon and Comber 2015) in addition to testing other assumptions of logistic regression. To generate the spatial weights matrix for the estimation of Moran's I index, we identified all sampling locations within a distance of 0–64 m for each of the 27 sampling locations in our study. The 64-m distance radius was chosen because this was the largest minimum distance between any two sampling locations. Once neighbors were identified, weights were made proportional to the number of neighbors for each sampling location (Brunsdon and Comber 2015).

Spatial abundance patterns of *Ae. j. japonicus* in 2014–2016 were studied using NB-generalized linear models (NB-GLM). These models were chosen given the overdispersed count nature of the total abundance per ovitrap during the study period (Venables and Ripley 2002). Briefly, these models can be described by the next general equation:

$$\text{link}(Y_i) = \mu + \sum_j \rho_j X_j + \varepsilon_i \quad (1)$$

where Y_i is the total cumulative *Ae. j. japonicus* abundance at a given ovitrap i , $\text{link}()$ is the link function, a natural logarithm for a NB-GLM, μ the model intercept, ρ_j 's are coefficients for the covariates (X_j), and ε_i the error. As some of the covariates were highly correlated (Pearson's $\hat{r} > 0.75$), the process of covariate selection was done in stages. In a first stage, we considered a series of models that have the following common set of covariates: temperature SD and

Kurtosis, ground cover index, mean, and SD canopy openness, but where variables that were highly correlated were alternatively tested. More specifically, models contained either flow direction or aspect; a second-degree polynomial of elevation, mean, or median temperature and roughness, slope, or terrain roughness index. These models were compared through their Akaike Information Criterion (AIC), a metric that trade-offs the number of parameters in a model versus the model goodness of fit and suggest the best model based on the minimization of the AIC value (Kuhn and Johnson 2013). We found that the best model from this stage was the one using the common set of covariates and flow direction, a second-degree polynomial of elevation and the slope (Supp Table 1 [online only]). This model was then used as a 'full' model that was then simplified by a process of backward elimination, i.e., where models with the same number of parameters are compared and the model minimizing AIC is kept through several rounds until there are no further decrease in AIC (Faraway 2004). The 'full' model went through five rounds of simplification (Supp Table 2 [online only]) after which the best model had ground cover index, SD of canopy openness, a second-degree polynomial of elevation and the kurtosis of ovitrap WT as covariates. For this 'best' model, we tested model assumptions, including the assumption of spatial independence by estimating Moran's I spatial autocorrelation index on the residuals, where the spatial autocorrelation weight matrix was generated following the same procedure described for the logistic model to study changes in *Ae. j. japonicus* distribution between 1989 and 2014–2016. The spatial models did not include the abundance of the two other common *Aedes* spp. mosquitoes *Aedes flavopictus* Yamada (Supp Fig. 1A [online only]) and *Aedes albopictus* (Supp Fig. 1B [online only]) given the lack of a significant correlation with the former ($\hat{r} = -0.152$, $t = -0.770$, d.f. = 25, $P = 0.45$) and a high positive correlation with the latter ($\hat{r} = 0.724$, $t = 5.251$, d.f. = 25, $P = 1.9e-05$), which could obscure any association with landscape and weather variables if considered in the process of model selection. *Tr bambusa* (Supp Fig. 1C [online only]), the dominant species in the community, was also not included in the models given its abundance was not significantly correlated with *Ae. j. japonicus* ($\hat{r} = -0.250$, $t = -1.319$, d.f. = 25, $P = 0.199$) and that no correlation with *Ae. j. japonicus* was found when studying adult abundance at the study site (Chaves 2016, Hoshi et al. 2017). For the model selected as best, we also performed a jackknife test, a test where parameters are estimated by leaving one observation out at a time, building a distribution of these estimates for each parameter and furtherly estimating the mean (Rizzo 2007). The mean from the distribution of each parameter is then expected to be identical or nearly identical to the GLM parameter estimates when an appropriate distribution is employed for the data modeled.

Temporal Analysis

Temporal patterns were studied using methods for time series analysis (Shumway and Stoffer 2011). We first started by estimating the autocorrelation function (ACF) and partial autocorrelation function (PACF), which, respectively, show the correlation of observations for the whole time series at different time lags and only considering consecutive lags. On the basis of the information from this analysis, we fitted a first-order autoregressive model that was then used to prewhiten time series of the weather variables mentioned earlier, i.e., cumulative rainfall, mean relative humidity, and temperature (including maximum, average, and minimum), to estimate cross-correlation functions (CCFs) between these variables and the abundance of *Ae. j. japonicus*, and to determine the significance of their correlation at different time lags. Prewhitening was employed

to ensure that significant correlations observed in the CCF were not an artifact of the two time series having a similar autocorrelation structure (Hoshi et al. 2014a). The information from the correlation functions was then used to fit zero-inflated count models that accounted for the excess abundance of zeroes that do not correspond to a Poisson or NB distribution (Korner-Nievergelt et al. 2015). Zero-inflated models assume that observed zeroes emerge from the mixture of zeroes that are expected from a count distribution, e.g., Poisson or NB, and structural zeroes that could reflect unsuitable conditions for the dynamics of a process following a count distribution (MacDonald and Zucchini 1997). Zero-inflated models have the following general form:

$$\text{link}(N_t) = \pi_0 + \pi_1 \left(\mu + \sum_j \phi_{t-j} N_{t-j} + \sum_k \sum_j \gamma(k)_{t-j} \text{Cov}(k)_{t-j} + \varepsilon_t \right) \quad (2)$$

Where $\text{link}()$ is a link function, in the models we fitted a natural logarithm given the count nature of our data, π_0 is the Bernoulli probability that an observation is a structural zero, i.e., a zero that can be interpreted, in our study, as the product of unsuitable conditions for the presence of immature mosquitoes in the ovitraps, or a number following a count distribution ($\pi_1 = 1 - \pi_0$); μ is an intercept, ϕ_{t-j} are autoregressive coefficients and $\gamma(k)_{t-j}$ are coefficients for the k^{th} covariate at time lags $j > 0$; and ε_t is the error term, which we assumed to be either Poisson or NB. The probability of a structural zero was estimated using a logit function:

$$\pi_0 = \frac{e^{(\mu_x + \beta \text{Cov}_t)}}{1 + e^{(\mu_x + \beta \text{Cov}_t)}} \quad (3)$$

Where μ_x is an intercept, β is a coefficient for a covariate (Cov) at time t . Zero-inflated models were fitted using maximum likelihood (Zeileis et al. 2008). The process of model selection for the best zero-inflated model also followed a two stage process. In the first stage we compared zero-inflated Poisson and NB models which considered the probability of structural zeroes was constant (equation 3 being a function only of μ_x), or that considered the abundance of larval mosquitoes in the previous time step (AR(1)) or the presence/absence of larval mosquitoes in the previous time step or the square root of the ovitrap WT without a time lag ($\sqrt{\text{WT}(0)}$). In this first stage, we also compared models which alternatively considered the SD of the average, maximum, and minimum temperature, with 5 biweeks of lag (Supp Table 3 [online only]) because these measurements were highly correlated (Pearson's $\hat{r} > 0.75$). The second stage for model selection started with the 'Full' model selected through the minimization of AIC in the first stage, which was then furtherly simplified following a process of backward elimination (Supp Table 4 [online only]) similar to the one previously described for the selection of the best spatial model. For the model selected as best, we also performed a jackknife test (Rizzo 2007), whose implementation is already described in the spatial analysis section.

Density-Dependence Modeling

Density dependence was studied using the Ricker model. In its simplest form, the Ricker model describes population abundance at time t (N_t) with the following equation:

$$N_t = \lambda_0 N_{t-1} e^{(bN_{t-1})} \quad (4)$$

where b , is a density-dependence parameter that implies density dependence when $b < 0$ (Turchin 2003). The parameter λ_0 is the

intrinsic population growth rate, which allows to predict whether a population is stable, i.e., approximately constant around a fixed value and without regular cycles, when $\ln(\lambda_0) < 2$, meaning also that fluctuations are likely due to the impact of environmental changes (Mangel 2006). To account for this last possibility, the Ricker model can accommodate covariates that can account for fluctuations when estimated parameters suggest the dynamics of a focal population is stable:

$$N_t = \lambda_0 N_{t-1} e^{(bN_{t-1} + \alpha Cov_{t-j})} \quad (5)$$

where α is the coefficient for a given covariate (Cov_{t-j}) with j biweeks of lag. To fit the Ricker model, we made several considerations. The first one was that we did not consider observations of zero in N_{t-1} because our zero-inflated model analysis showed that most zeroes were structural. Then we considered whether counts followed a Poisson distribution or were overdispersed following a NB distribution with environmental stochasticity, i.e., with a constant overdispersion parameter (κ_E) or NB with demographic stochasticity, where the overdispersion parameter ($k_D N_{t-1}$) is a function of population size (Melbourne and Hastings 2008). To fit the models, we used a maximum likelihood procedure we described in detail elsewhere (Chaves et al. 2015, Chaves et al. 2012) and compared the models using AIC.

Software

All statistical analyses, maps, and Geographic Information Systems procedures were made using the language R version 3.4.0.

Results

Figure 1F shows the location of the 15 ovitraps where *Ae. j. japonicus* was found during our study period, which included four of the five locations where *Ae. j. japonicus* was caught in 1989 (Fig. 1E) by Zea Iriarte et al. (1991). Indeed, *Ae. j. japonicus*-positive locations in 1989 were four times (confidence limits: 0.49–85.15) more likely to be occupied in 2014–2016, yet these increased odds were not statistically significant ($P > 0.24$), while errors from this model were spatially independent (Moran's $I = 0.0108$, $P > 0.41$), ensuring a sound inference. Figure 1F also shows that *Ae. j. japonicus* was mainly present in areas whose landcover was urban, or dominated by bamboo, acorns, and/or oaks. Figure 1G shows the cumulative abundance of *Ae. j. japonicus* through our study period and as function of altitude. Supp Fig. 2 (online only) is a video showing the number of fourth instar larvae at each ovitrap, and their cumulative number across all sites, showing that number of larvae per ovitrap

ranged between 0 and 17. We collected a total of 122 *Aj4il*, the average by ovitrap (\pm SD) being 4.52 ± 9.37 individuals. It is clear that *Ae. j. japonicus*, although present in all transects, was more abundant in the west transect, mainly at sites whose ground was dominated by concrete. Figure 1H shows that traps at low altitude in the eastern transect and high in the mountain were more likely to become dry, but even traps that were dry around 30% of the time had *Aj4il* at some point during the study (Fig. 1G). We also collected a couple of pupae, in the ovitrap with the highest cumulative abundance of *Aj4il* (Fig. 1G), which due to their low number were not considered in the subsequent analyses. Around 5% ($n = 5$) of the larvae were identified by DNA barcoding.

The statistical analysis of *Ae. j. japonicus* spatial abundance patterns (Table 1) showed a significant association ($P < 0.05$) with a second-degree polynomial of elevation, where parameters indicate a u-shaped relationship with a minimum around 250 m, and the kurtosis of ovitrap WT, where the relationship was negative indicating that *Ae. j. japonicus* was more likely to thrive in ovitraps where the temperature was more platykurtic (or proportionally more variable around the mean). This *Ae. j. japonicus* potential to thrive in variable environments might also reflect the nearly significant ($P \cong 0.05$) positive association with the ground index and the SD of canopy openness, which also suggest a preference for variable environments. All other variables considered in the spatial models were discarded by the process of model selection (Supp Tables 1 and 2 [online only]), which also suggested that elevation was a covariate with a more clear association with *Ae. j. japonicus* abundance than the median and mean WT of the ovitraps (Supp Table 1 [online only]). Also, it is worth noting the Moran's I index supported the lack of spatial autocorrelation in the model residuals (Table 1) and this in conjunction with the fulfillment of other NB-GLM assumptions ($\chi^2 = 27.25$, d.f. = 21, $P = 0.1627$), ensures the validity of these statistical inferences. Moreover, parameter estimates from the spatial NB-GLM of Table 1 were nearly identical to those obtained using a jackknife (Supp Fig. 3 [online only]).

Figure 2 shows the time series of collected data. Figure 2A shows the total abundance, i.e., including data from all ovitraps, of *Aj4il*, where clear troughs with no larvae are seen during the two winters that spanned the study. *Aj4il* were present in at least one ovitrap during 27 of the 54 biweeks that lasted our study. More specifically, we did not observe any *Aj4il* and pupae between 18 October 2014 and 21 March 2015, and then between 29 October 2015 and 16 March 2016. During our study period, the average biweekly number (\pm SD) of *Aj4il* was 2.26 ± 5.45 . It can also be seen that during 2015, a season we studied completely, the population peaked at the start and end of the season, being the spring peak larger. In Fig. 2A, *Ae. j. japonicus* abundance was weighted

Table 1. Parameter estimates for the best NB model explaining the cumulative spatial abundance of *Aj4il* in Mt. Konpira, Nagasaki, Japan

Parameter	Estimate	SE	Z	$P(> z)$
Intercept	31.81321	12.05539	2.639	0.00832*
Ground index	0.223828	0.123476	1.813	0.06987
SD of canopy openness	0.112404	0.061491	1.828	0.06755
Elevation	-0.1488	0.058788	-2.531	0.01137*
Elevation ²	0.000296	0.000117	2.536	0.01122*
Kurtosis of WT	-7.41186	3.702981	-2.002	0.04533*
Overdispersion	0.523	0.210	—	—
Moran's I^a	0.194	—	—	0.132

*Statistically significant ($P < 0.05$).

^aInference for Moran's I spatial autocorrelation index is based on 1,000 Monte Carlo randomizations.

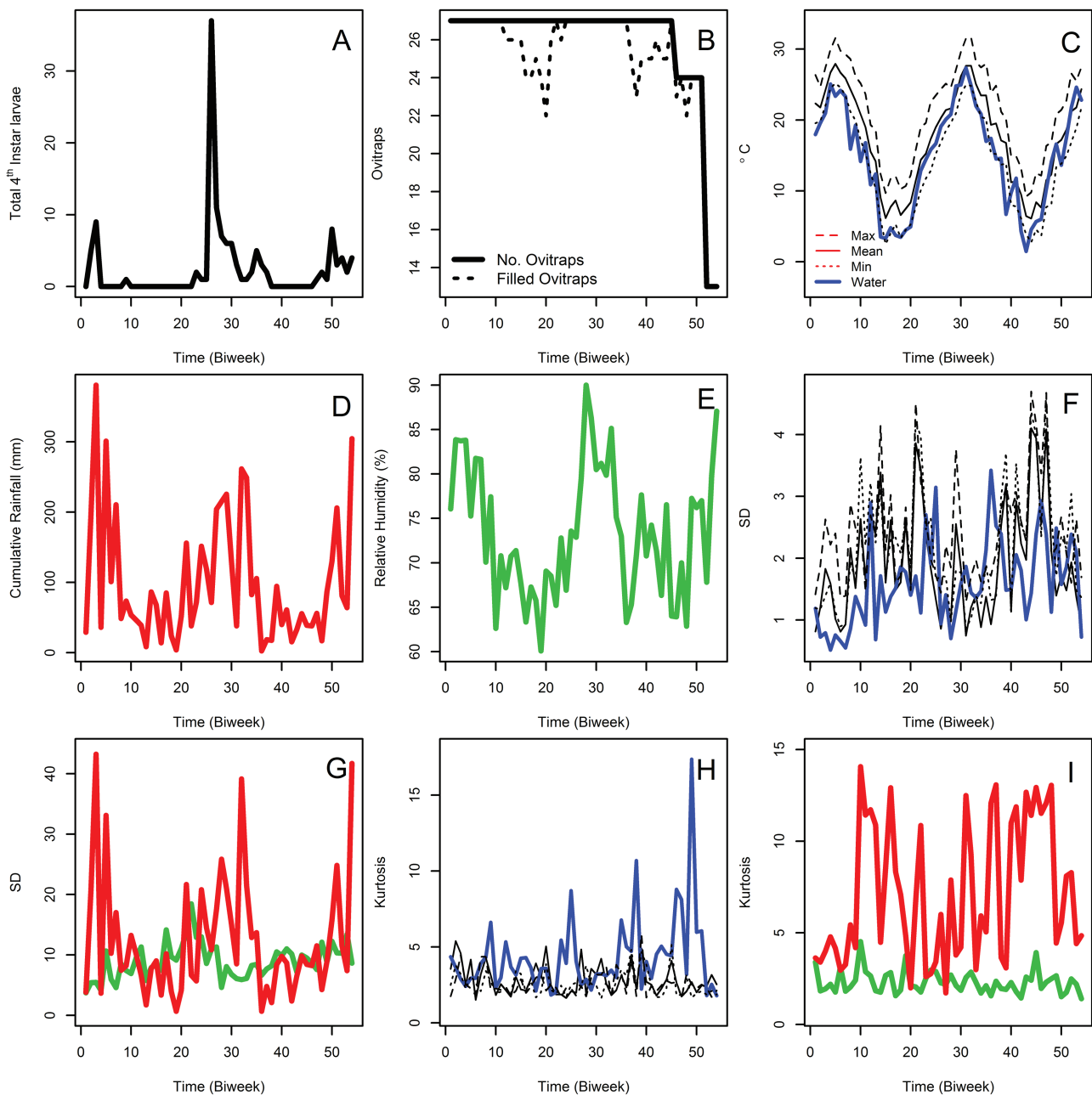


Fig. 2. Time series (A) *Aj4il* abundance, data from all ovttraps; (B) Number of sampled ovttraps and ovttraps with water, i.e., Filled Ovttraps; (C) Biweekly temperature including: water in the ovttraps (Water), air maximum (Max), air minimum (Min), and Air average (Mean); (D) Biweekly cumulative rainfall; (E) Biweekly average relative humidity; (F) SD of the temperature measurements, please refer to the inset legend of panel C for details; (G) SD of rainfall and relative humidity, for line guidance please refer to panels D and E; (H) Kurtosis of the temperature measurements, please refer to the inset legend of panel C for details; (I) Kurtosis of rainfall and relative humidity, for line guidance please refer to panels D and E. SD and kurtosis were based on daily averages for air temperature measurements, rainfall, and relative humidity recorded by the Nagasaki Weather Station, while for ovttrap water temperature estimates were based on 'in situ' measurements from the ovttraps.

from week 51 to 54 given changes in the number of surveyed ovttraps. Figure 2B shows the number of ovttraps that were followed during the study, highlighting that by the 46th biweek three traps were lost following a major snowstorm and its associated cold spell and then by the 51st biweek around half of the remaining traps (11 of 24) were destroyed in an act of vandalism. Supp Fig. 2 (online only) is a video showing the spatial distribution of the collected larvae through time, showing that larvae were clustered in a few ovttraps, as well as the location and time of traps that got dry, or were broken during the snowstorm before the 46th biweek

or vandalized before the 51st biweek. Figure 2C shows records of air temperature from the Nagasaki weather station and WT when the larvae were sampled in the ovttraps. Figure 2D shows the cumulative biweekly rainfall records of Nagasaki and Fig. 2E shows the relative humidity. Here it is worth highlighting that ovttraps got mostly dry during the winter (Fig. 2B and Supp Fig. 2 [online only]), the dry season of Nagasaki (i.e., with low rainfall, Fig. 2D) and when temperature and relative humidity (Fig. 2E) also decrease. Figure 2F shows the SD of the temperature measurements, while Fig. 2G does so for rainfall and relative humidity.

Figure 2H shows the Kurtosis of the temperature records, while Fig. 2I does so for rainfall and relative humidity. Unlike, average measurements, SD and kurtosis measurements were variable during the winter, and in the case of WT kurtosis (Fig. 2H), and relative humidity SD (Fig. 2G), these time series seemed to lead variability in *Aj4il* abundance. Nevertheless, these potential associations were systematically studied using tools from time series analysis whose results are described below.

A preliminary analysis the *Aj4il* abundance time series is presented in Fig. 3. The autocorrelation function (ACF, Fig. 3A) and the partial ACF (PACF, Fig. 3B) suggest that the *Ae. j. japonicus* larvae time series was a first-order autoregressive process, indicating that observations were significantly autocorrelated up to 1 biweek lag. The CCF between *Aj4il* abundance and ovitrap WT average (Fig. 3C), SD (Fig. 3D) and kurtosis (Fig. 3E) suggests that only the SD (Fig. 3D) of WT (1 and 3 biweek lags), relative humidity (4 biweek lag) minimum and maximum temperature (5 biweek lag), and the kurtosis of WT (1 biweek lag), were correlated with changes in *Ae. j. japonicus* abundance, being the association positive in all cases.

The information from the cross-correlation analysis was then used to fit a series of ‘full’ zero-inflated count models to choose the best count distribution and covariates for the inflation part of the model (Supp Table 3 [online only]), where we fitted models with the SD of mean, maximum, and minimum temperature given the high correlation between these time series (Pearson’s $\hat{r} > 0.75$). We

found that the best distribution for the count part of the model was a Poisson one, which clearly outperformed an NB distribution (Supp Table 3 [online only]), and that the best covariate explaining the presence of structural zeroes was the square root of WT. We choose WT as a covariate given that it had a threshold-like relationship with *Aj4il* abundance, where no fourth instar larvae were observed when temperatures were below 10°C (Fig. 4A) and where the observed relationship could be linearized by square root transforming the WT. Then we proceeded with a process of model selection starting with the best ‘full’ model, which was then simplified (Supp Table 4 [online only]) until finding the ‘best’ model. Table 2 shows parameter estimates for the best zero-inflated Poisson time series model, indicating that counts of *Aj4il* were increased with the number of fourth instar larvae the previous biweek (about 2%, i.e., $\exp(0.02691)$), 11.5% for each unit increment in WT kurtosis the previous biweek, meaning that increases in population size were associated with more leptokurtic environments (i.e., with low variability around the mean) and 27% increment for each unit increment in the SD of relative humidity. Meanwhile, the probability of structural zeroes (Table 2) in the abundance of *Aj4il* is a sigmoid function of WT (Fig. 4B). From the parameter estimates, a threshold temperature value (WT), below which $P > 0.5$ indicates that a zero is structural that can be obtained by solving the equation:

$$\mu_x + \beta\sqrt{WT} = 0 \quad (6)$$

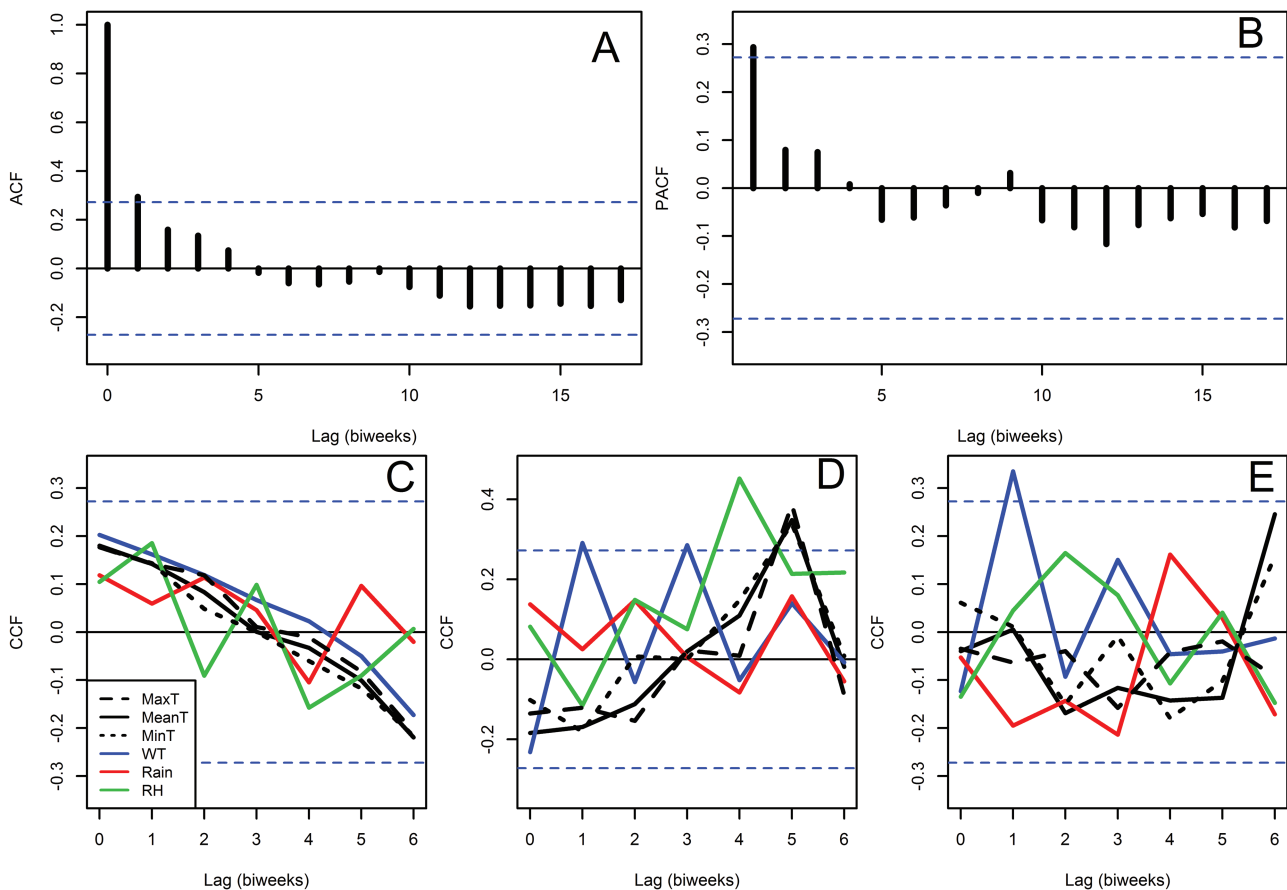


Fig. 3. Correlation functions (A) *Aj4il* biweekly abundance ACF; (B) *Aj4il* biweekly abundance PACF; (C) CCF between *Aj4il* biweekly abundance and maximum air temperature (MaxT), average air temperature (MeanT), minimum air temperature (MinT), ovitrap average WT, rainfall (Rain), and relative humidity (RH); (D) CCF between *Aj4il* biweekly abundance and the SD of MaxT, MeanT, MinT, WT, Rain, and RH; (E) CCF between *Aj4il* biweekly abundance and the kurtosis of MaxT, MeanT, MinT, WT, Rain, and RH. In all plots horizontal dashed lines indicate the 95% confidence intervals inside which correlations are expected by random.

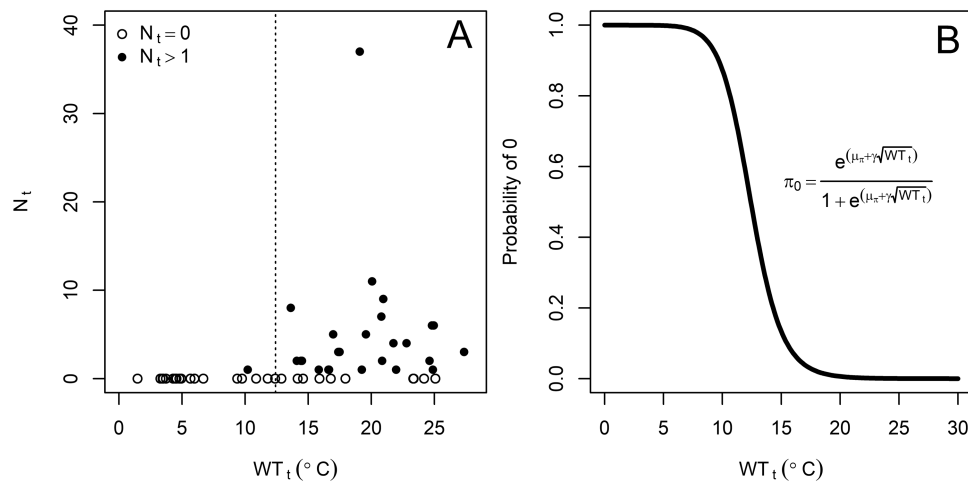


Fig. 4. Zero inflation (A) *Aj4il* abundance as function of ovitrap water average temperature, the dashed line indicates a temperature of 12.41°C, the threshold temperature below which a zero mosquito abundance can be considered structural; (B) Probability of structural zeroes in the *Aj4il* abundance time series as function of WT. The equation in this plot shows the logit function generating the structural zeroes. Parameter estimates are presented in Table 2 (where Intercept = μ and $\sqrt[3]{WT(0)} = \beta$).

Table 2. Parameter estimates for the best zero-inflated Poisson model explaining the temporal abundance of *Aj4il* in Mt. Konpira, Nagasaki, Japan

Model component	Parameter (lag)	Estimate	SE	Z	P(> z)
Counts	Intercept	0.6884	0.1569	4.388	<7E-05*
	AR(1)	0.02572	0.00882	2.918	0.0035*
	KWT(1)	0.1065	0.0265	4.026	<5E-05*
	SDRH(4)	0.2473	0.0238	10.128	<2E-16*
Zero inflation	Intercept	18.836	8.548	2.203	0.0276*
	$\sqrt[3]{WT(0)}$	-5.346	2.364	-2.262	0.0237*

Model components indicate whether parameters are used for modeling the counts or the structural zeroes in the model (Zero inflation). For the covariates, time lags are indicated within parenthesis, i.e., lag. Covariates included AR (autoregressive component); KWT (kurtosis of the WT); SDRH (SD of relative humidity); WT (average water temperature). For the analysis, the mean of the KWT and SDRH time series were removed to ease parameter interpretation.

*Statistically significant ($P < 0.05$).

which gives a temperature threshold of 12.41°C below which a zero count of *Aj4il* larvae is likely (with $\pi_0 > 0.5$) structural (Figs 4A and B). It is worth highlighting that the model presented in Table 2 also had an appropriate goodness of fit ($\chi^2 = 50.877$, d.f. = 43, $P = 0.191$). Moreover, parameter estimates from the zero-inflated Poisson model of Table 2 were nearly identical to those obtained using a jackknife (Supp Fig. 4 [online only]).

Regarding density dependence in the counts of *Aj4il*, Fig. 5 shows that *Ae. j. japonicus* per capita growth rate decreased with density, as expected when a population is regulated by density dependence. It is worth noting that such a decrease is not observed when the per capita growth rate of *Aj4il* is plotted as function of fourth instar larvae abundance of *Ae. flavopictus* (Supp Fig. 5A [online only]), *Ae. albopictus* (Supp Fig. 5B [online only]) and *Tr bambusa* (Supp Fig. 5C [online only]), indicating that these species, which were also common at our study site, unlikely impact the population growth of *Ae. j. japonicus*. Then, to more formally test for density dependence, we fitted the Ricker model to the data removing values of zero in N_{t+1} , which were mainly structural. As a first step we chose an appropriate distribution for the observed counts (Supp Table 5 [online only]) where we considered the Poisson and NB distributions. For the latter, we tested cases where we assumed environmental and demographic stochasticity. When fitting these models, we also considered the forcing by ovitrap WT kurtosis (1 biweek lag), which was positively and

significantly ($P < 0.05$) associated with the *Ae. j. japonicus* population growth rate (Fig. 6), unlike SD of WT (1 biweek lag, Supp Fig. 6 [online only]). We found that demographic stochasticity NB showed the best fit in the autonomous, i.e., without covariates, and forced cases (Supp Table 5 [online only]).

Table 3 shows parameter estimates for the autonomous (Fig. 7A) and forced (Fig. 7B) demographic stochasticity NB Ricker model. When comparing parameter estimates from both models, the growth rate and the density-dependence parameters are slightly smaller in the forced model, which has a slightly higher overdispersion parameter (i.e., the data are less disperse). This result implies a slightly higher carrying capacity in the forced model, yet with a slower population growth rate, abundance also increasing with the ovitrap WT kurtosis. In any case, the negative coefficients for both models robustly indicate that *Aj4il* were regulated by density dependence at our study site, a result also suggested by the curve fitted for the autonomous model (Fig. 7A) and the surface fitted for the forced model (Fig. 7B).

Discussion

Understanding the population dynamics of disease vectors is fundamental for managing vector-borne disease transmission, given the positive association between vector-borne disease transmission and

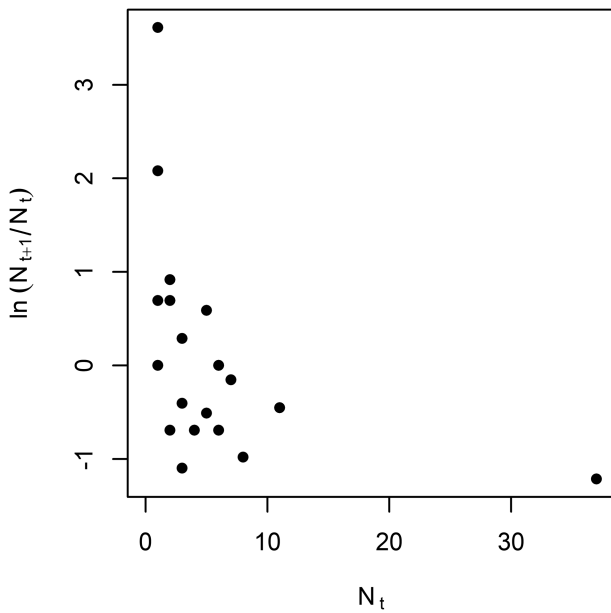


Fig. 5. Per capita population growth rate (r) of *Ae. j. japonicus* as function of its density, (N_t), in Mt. Konpira, Nagasaki, Japan.

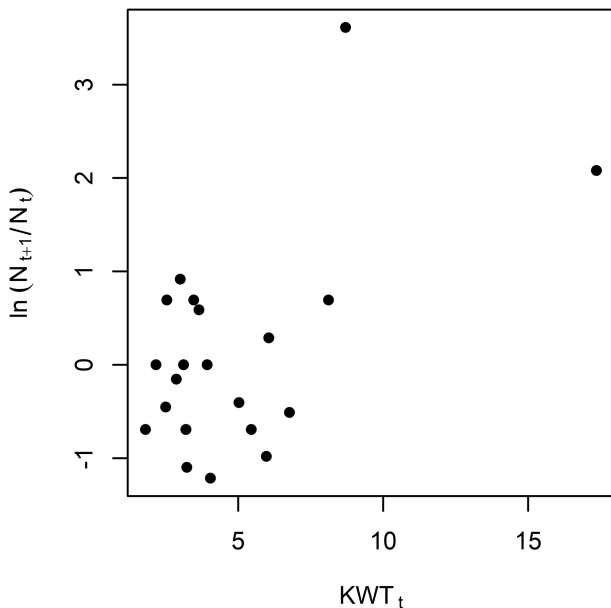


Fig. 6. Per capita population growth rate (r) of *Ae. j. japonicus* as function of ovitrap WT kurtosis (KWT_t), in Mt. Konpira, Nagasaki, Japan.

vector abundance (Smith et al. 2014). Moreover, knowledge about the natural history and ecology of vectors can help to understand spatial and temporal transmission patterns of major vector-borne diseases, and even help to predict the potential emergence of new ones, yet there are major knowledge gaps about the basic ecology of dominant vector species (Reisen 2014, Weaver and Reisen 2010), including *Ae. j. japonicus* in its native range (Kaufman and Fonseca 2014). Most of our results help to fill a few of those gaps, confirming previous observations about the natural history of *Ae. j. japonicus* while also providing new insights about the ecology of this mosquito. Regarding the natural history of *Ae. j. japonicus*, we saw a phenology of fourth instar larvae similar to that reported by Matuo (1961)

for Kyoto, Japan (north of Nagasaki) where these larvae are present from March until mid-late October. Qualitatively, we also observed that abundance seemed bimodal, with peaks early and late during the mosquito season, as observed elsewhere (Kaufman and Fonseca 2014). Going one step ahead, our zero-inflated Poisson modeling strategy even allowed to predict that fourth instar larvae are unlikely to be present when temperature in their larval habitats is maintained below 12.41°C, using a novel method that might be useful to make quantitative predictions about the phenology of different insect species, at any ontogenetic stage, in seasonal environments. Meanwhile, our low success sampling *Ae. j. japonicus* likely reflects the low pupation rates of this species in small treeholes (Tsuda et al. 1994) and artificial containers (Bartlett-Healy et al. 2012). However, it can also be a byproduct of *Ae. j. japonicus* relatively long developmental time (Hoshino et al. 2010), which could also explain why we only collected pupae from one of the hottest ovitraps in August 2015.

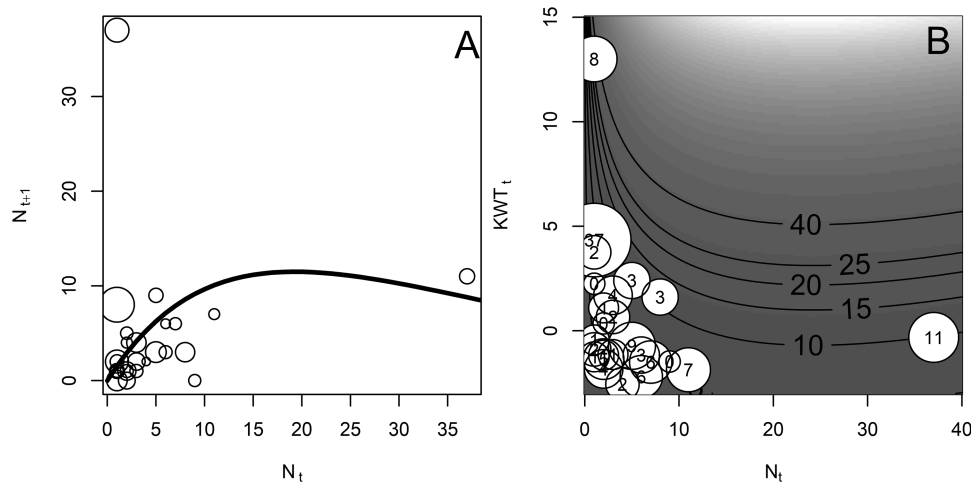
The altitudinal range of *Aj4il* at Mt. Konpira did not change between 1989 and 2016, despite major landcover changes at our study site (Fig. 1E and F) and a 2.5°C increase in the mean temperature at Nagasaki city (Chaves 2017a). We believe that we found *Aj4il* present at more ovitraps than Zea Iriarte et al. (1991) in 1989 because of our longer and more frequent sampling. Our previous data also suggest that *Aj4il* ovitrap absence is unlikely related to adult dispersal ability, given that *Ae. j. japonicus* adults were present all over the mountain (Chaves 2016, Chaves 2017b). Similarly, it is unlikely this pattern was due to the presence of (or potential interactions with) other mosquito species present across the ovitraps, where the mosquito community was dominated by the bamboo mosquito (*Tr bambusa*), which accounted for over 80% of the larvae that we found (over 10,000 individuals), followed by *Ae. flavopictus* (over 400 individuals) and then by the more clustered *Ae. albopictus* (slightly over 100 individuals), whose abundance was even positively associated with that of *Ae. j. japonicus*. Only *Armigeres subalbatus* (Coquillet) larvae (~40 individuals) had a distribution (Chaves et al. 2015) that did not overlap with that of *Ae. j. japonicus*. Nevertheless, for the potential interspecific interaction between *Ar subalbatus* and *Ae. j. japonicus*, previous studies in Japan have suggested the interactions might be modulated by differences in the microbial community, which does not allow the survival of *Ae. j. japonicus* in nutrient-rich water (Kurihara 1958, Kurihara 1960). It is an open question if *Ae. j. japonicus* did not oviposit in the ovitraps where it was absent, which can be assessed by using the ovitraps to collect eggs, a potential hypothesis being that it preferred to colonize nearby rock pools and/or large treeholes, something whose testing was beyond the goals of this study. Our spatial analysis showed that environmental factors might, at least partially, explain the distribution and abundance of *Ae. j. japonicus* in Mt. Konpira, where we found that *Ae. j. japonicus* preferred high variability in light incidence, with grounds dominated by concrete and with relatively large fluctuations in WT (more platykurtic distributions, or where kurtosis is small), which suggests that this species might select the larval habitats it colonizes, a common trait in mosquitoes (Day et al. 1990, Harrington et al. 2008, Nguyen et al. 2012).

In contrast with the negative impact of ovitrap WT kurtosis for the cumulative spatial abundance of *Ae. j. japonicus*, we found that temporally fourth instar larvae were more abundant following periods when ovitrap WT was more leptokurtic (high kurtosis values, which means with relatively low variability around the mean), which makes sense given that in such periods insect growth might be optimal (Colinet et al. 2015). We also found that *Ae. j. japonicus* larval abundance was positively correlated with the SD of relative humidity, which might be related with the recruitment of new individuals

Table 3. Parameter estimates for the NB Ricker models (autonomous and forced) with demographic stochasticity applied to the temporal abundance of *Aj4il* in Mt. Konpira, Nagasaki, Japan

Parameter	Forced (AIC = 135.8)				Autonomous (AIC = 143.5)			
	Estimate	SE	Z	$P(> z)$	Estimate	SE	Z	$P(> z)$
Population growth intrinsic rate ($\hat{\lambda}$)	1.328	0.307	4.320	<0.00001*	1.621	0.419	3.869	<0.0001*
Density dependence (\hat{b})	-0.0413	0.0135	-3.063	<0.003*	-0.0519	0.0145	-3.574	<0.0005*
Overdispersion (\hat{k})	0.460	0.169	2.714	<0.007*	0.2881	0.0929	3.101	<0.005*
Forcing coefficient for WT kurtosis ($\hat{\alpha}$)	0.2401	0.0816	2.942	<0.003*	-	-	-	-

When fitting the models zeroes in N_{t+1} were removed. AIC indicates the Akaike Information Criterion for each model. *Statistically significant ($P < 0.05$).

**Fig. 7.** Fits for the demographic stochasticity Ricker model applied to the *Aj4il* biweekly abundance time series with zeroes removed (A) Autonomous, circle size is proportional to the kurtosis of ovitrap WT; (B) Forced by the kurtosis of ovitrap WT (KWT_t). In this panel contour lines indicate the expected *Aj4il* biweekly abundance and circles are proportional to N_{t+1} , which is indicated by a number at the center of each circle.

via oviposition, given both the lag of two months and the positive association between changes in relative humidity and oviposition (Chaves and Kitron 2011) as well as mosquito movement (Dow and Gerrish 1970, Day and Curtis 1994, Harrington et al. 2005).

Parameter estimates for the Ricker model suggest that fourth instar *Ae. j. japonicus* larvae are under density-dependence regulation, that their population dynamics are stable, resembling what we observed in adults of the Asian bush mosquito (Chaves 2016), a common pattern that has been observed in other mosquito species either as larvae (Hoshi et al. 2014a) or as adults (Yang et al. 2008a, b, Chaves et al. 2015, Chaves 2016, Hoshi et al. 2017), and that fluctuations might be generated by: 1) demographic stochasticity, where at low population abundance changes in population size are more unpredictable (Levins 1969), and 2) changes in WT kurtosis, where population size increases when larval habitat temperature becomes more leptokurtic.

Finally, the dual relationship of *Ae. j. japonicus* with WT kurtosis offers new insights into the niche relationship of this species with temperature, which unlikely is merely related with mean values, but with the overall pattern of temperature variability, something that can only be unveiled in longitudinal studies like ours. On one hand, we have a situation where habitats that potentially maximize the fitness of *Ae. j. japonicus* immature stages are those with a relative high temperature variability around the mean (or platykurtic, with a low kurtosis and relatively highly variable around the mean), which might confer advantages over species more sensitive to temperature changes, but on the other hand, the abundance in those habitats is maximized when that variability is decreased (and temperature

becomes more leptokurtic, with a high kurtosis and relatively low variability around the mean). Therefore, *Ae. j. japonicus* spatial abundance patterns follow predictions for species with a coarse environmental grain living over environments different enough to have a concave fitness set where genetic/phenotypic polymorphisms are favored (Levins 1968). Nevertheless, when variability is reduced in the environment, like when the environment temporally becomes more leptokurtic, the fitness set is forced into becoming convex and it is expected that more fit genotypes/phenotypes on this new environment become predominant in a population that was more diverse in a more platykurtic environment, thus reducing the overall genetic diversity in a species, like what has been observed for *Ae. j. japonicus* whose genetic diversity decreases in hotter, also more leptokurtic, environments (Egizi et al. 2015). This ecological pattern might also lead to a counterintuitive pattern of decreased genetic diversity when a population outbreak happens, i.e., a sudden unexpected abundance increase (Chaves et al. 2014), although the population size is larger than when genetic diversity is higher, a hypothesis that can be readily tested with available markers for the genetic study of *Ae. j. japonicus* (Kaufman and Fonseca 2014, Egizi et al. 2015). The patterns of *Ae. j. japonicus* association with temperature that we observed also suggest that forecasts for expansion/contraction of the geographical range of this species could be more accurate if they consider temperature variability because the colonizing success and coexistence of *Ae. j. japonicus* with other species when they colonize the same habitat, e.g., *Ae. albopictus*, might be ultimately shaped by their different strategy to cope with changing environments, something that cannot

be evaluated when these inter-specific interactions are forced into an environment described by a Dirac delta function probability, where kurtosis become infinite like the case for mesocosm experiments done at fixed temperatures (Alto 2011). Moreover, field studies have suggested that co-existence of *Ae. j. japonicus* with other mosquito species, especially *Ae. albopictus*, might be primarily shaped by the selection of different larval habitats (Tsuda et al. 1994, Bartlett-Healy et al. 2012), and certainly our results suggest that at least in small artificial containers, like our ovitraps, these two species not only co-occur but also spatially and temporally positively correlated, without impacting each other growth.

Acknowledgments

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Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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