# Appropriate Larval Food Quality and Quantity for *Aedes albopictus* (Diptera: Culicidae)

Author(s): Ruth Müller , Timm Knautz , Johannes Völker , Aljoscha Kreß , Ulrich Kuch , and Jörg Oehlmann

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SHORT COMMUNICATION

# Appropriate Larval Food Quality and Quantity for *Aedes albopictus* (Diptera: Culicidae)

RUTH MÜLLER,[1,2,3] TIMM KNAUTZ,[2] JOHANNES VÖLKER,[2] ALJOSCHA KREß,[1,2] ULRICH KUCH,[1] AND JÖRG OEHLMANN[1,2]

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**ABSTRACT** The Asian tiger mosquito *Aedes albopictus* (Skuse, 1894) is a globally invasive prominent vector of viral and parasitic pathogens. To soundly guide insecticide use in control programs it is crucial to use standardized test systems under rigorously controlled environmental conditions that allow for comparisons across laboratories. An acute standard test procedure (24 h) for insecticide resistance monitoring of mosquitoes has been published by the World Health Organization in 1998, but a standardized chronic test to monitor sublethal insecticide effects on the life cycle of mosquitoes does not yet exist. As a first step toward a standardized chronic bioassay (half-life-cycle-test), the exclusion of qualitative and quantitative food effects by means of standardized, optimal larval feeding would greatly facilitate inter-laboratory comparisons. Against this background we evaluated food qualities and quantities for the aquatic part of the *A. albopictus* life cycle under different thermal conditions. Five mg TetraMin (Tetra, Melle, Germany) larva$^{-1}$ at 25°C rendered the lowest mortality and large pupae. Our fundamental data on *A. albopictus* feeding provide an opportunity to standardize experiments and thus support inter-laboratory comparisons of studies on the ecotoxicology of this dangerous vector mosquito.

**KEY WORDS** larval diet, half-life-cycle-test, temperature, *Aedes albopictus*

The Asian tiger mosquito *Aedes albopictus* (Skuse) is one of the most invasive species worldwide and rapidly spreading within and between continents (Benedict et al. 2007). It is a significant risk to human and animal health because it can function as a vector for at least seven alpha-, eight bunya-, and three flaviviruses (Shroyer 1986, Mitchell 1995, Gratz 2004) as well as parasites (Genchi et al. 2009), and plays an increasingly recognized role in the transmission of dengue and chikungunya viruses.

To rationally guide insecticide use in control programs, standardized test systems are crucial because they allow for the collection of data under rigorously controlled environmental conditions and comparisons across different laboratories (WHO 1998). An acute standard test procedure (24 h) for insecticide resistance monitoring in malaria vectors has been published (WHO 1998, Liu et al. 2004), but a standardized test system that allows the monitoring of chronic and sublethal insecticide effects on the life cycle of mosquitoes does not yet exist.

As larval nutrition is an important prerequisite for the standardization of a chronic test, we investigated the appropriate food quality and quantity for the aquatic development stages of *A. albopictus* under controlled environmental conditions. We examined the feeding value of plant, animal, and artificially composed diets, and we determined three quantities of the best diet at three different temperatures (20, 25, and 30°C) to develop an optimal feeding protocol in a harmonized test system. The framework for the tested food types follows Timmermann and Briegel (1996), whereas the range of food quantities is derived from Yee et al. (2007a) and Carrieri et al. (2003) as well as Organisation for Economic Co-operation and Development (OECD 2004) instructions for the midge *Chironomus riparius* (Meigen, 1804).

## Materials and Methods

**Material Examined.** *Ae. albopictus* mosquitoes were sourced from an in-house culture of a laboratory strain donated by Biogents AG (Regensburg, Germany). Its geographical origin is Singapore, but its breeding history could not be determined. Twenty-four to 48 h old first instar larvae were transferred to dishes filled with 600 ml of a 1:1 mixture of de-ionized and tap water. Experimental units containing 30 larvae per replicate were distributed to environmental chambers (MKKL 1200, Flohrs Instruments, Utrecht, The Netherlands) keeping a photoperiod of 16:8 (L:D) h and either 25 ± 1°C (experiment on food quality; $n = 5$) or 20, 25, and

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[1] Biodiversity and Climate Research Centre (BiK$^F$), Senckenberganlage 25, 60325 Frankfurt am Main, Germany.

[2] Goethe University Frankfurt am Main, Department of Aquatic Ecotoxicology, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany.

[3] Corresponding author, e-mail: ruthmueller@bio.uni-frankfurt.de.

30 ± 1°C (experiment on food quantity; $n = 3$). Water levels were held constant by refilling with deionized water. No intraspecific competition was observed under these larval densities which are representative for natural breeding sites of water volumes ≤1 liter (Carrieri et al. 2003).

**Food Quality Experiment.** *A. albopictus* larvae were fed either European beech (*Fagus sylvatica*) (Linnaeus, 1753) leaf litter (fallen leaves, collected in spring, stored in darkness for 1.5 yr), the fish foods TetraPhyll and TetraMin (Tetra, Melle, Germany), or lyophilized larvae of *Chironomus* sp. (FD Blood Worms, Tropical, Ruhmannsfelden, Germany). Food portions were standardized based on calorimetric values measured in triplicate using a C 200 calorimeter (IKA Werke, Staufen, Germany). In total, each larva was fed equicalorically 101.72 ± 0.14 J. Food portions were adjusted to larval age to prevent putrefaction (10 ± 2% on day 0, 2, 4, and 5; 20 ± 0.1% on day 7, 8, and 9) and offered as 250–630 μm particles dissolved in medium (7.5 g/L).

To analyze the number of microorganisms, 2 ml test media sampled after the last pupation and stored at −20°C were filtered through a 0.22 μm polycarbonate filter (Isopore membrane filter, Millipore, Billerica, MA). The filters were stained with 0.05% acridine orange (3 min), incubated with 1% sodium pyrophosphate (30 s), washed with deionized water, and dried in darkness overnight. The number of bacteria was counted in three replicates (9 to 10 spots replicate$^{-1}$) using fluorescence microscopy (Zeiss, Oberkochen, Germany). Fungal tubes were qualitatively assessed. The total estrogenic activity of diets was determined using the yeast estrogen screen (Wagner and Oehlmann 2009) ($n = 8$). For this assay, 15, 30 and 150 mg of the different food types were extracted in 5 ml methanol using ultrasonic sound (20 min), and the extracts were filtered using 0.2 μm PTFE filters (Neo-Lab, Heidelberg, Germany).

**Food Quantity/Temperature Experiment.** In total, each larva was fed either 2.5 mg (low food regime, LF, 50.9 ± 0.7 J), 5 mg (medium food regime, MF, 101.72 ± 0.14 J), or 10 mg (high food regime, HF, 203.4 ± 0.28 J) of TetraMin (250–630 μm particle size). To prevent putrefaction, feeding was scheduled differently according to larval development under the three test temperatures (Delatte et al. 2009): At 20°C 10 ± 2.0% of the total food was provided on day 0, 2, 4, 6, and 20 ± 0.8% on day 8, 10, and 12; at 25°C 10 ± 2.0% on day 0, 2, 4, 5, and 20 ± 0.1% on day 7, 8, and 9; at 30°C 10 ± 1.5% on day 0, 2, 3, 4, and 20 ± 1.5% on day 5, 6, and 7.

**Data Collection and Analyses.** The experimental units were checked at least daily for pupae that were removed and fixed in 80% ethanol. After removal of all pupae, water quality was determined using colorimetric tests for nitrite, ammonium, and phosphate (Merck, Darmstadt, Germany) and nitrate (Hach-Lange, Düsseldorf, Germany), and WTW sensors for pH and conductivity measurements (TetraCon 325 and SenTix, Weilheim, Germany).

After assessing mortality, the number and mean pupation time ($PT_{50}$, the day when 50% of larvae had pupated) were calculated separately for males and females (Motulsky 2007). Only $PT_{50}$ values obtained from regression with a coefficient of $R^2 > 0.99$ were included in statistical analysis. The sexed pupae (gonocoxopodites: large and partially bilobed in males; small and spiculate in females) were measured using a stereo microscope and the software DISKUS (Carl H. Hilgers, Königswinter, Germany): abdominal length of pupae (AL) from the third to the eighth segment, abdominal width (AW) at the fifth segment, and the area of the cephalothorax (CT) using a lateral view.

Data were tested by analysis of variance (ANOVA) ($P < 0.05$) and Tukey post hoc test (honest significant difference for unequal sample sizes). The homogeneity of variances was tested with Cochran's test at the 1% level. In case of heterogeneity of variances, data were transformed (food quality experiment: arcsine transformation of mortality and sex ratio, food quantity/temperature experiment: arcsine/square-root transformation of mortality). In addition, the integrative dependence of AL, AW, and CT on the variables sex, food, and temperature was tested by MANOVA (arcsine/square-root transformation of CT).

## Results

**Effects of Food Quality.** The calorimetric values of the four diets differed significantly, decreasing from TetraMin (20.34 ± 0.03 kJ/g) to TetraPhyll (19.99 ± 0.04 kJ/g), leaf litter (18.14 ± 0.06 kJ/g), and lyophilized chironomid larvae (17.48 ± 0.12 kJ/g; $F = 1{,}087$; df = 3; $P < 0.01$). The amount of calories was converted into diet-specific weights to equicalorically feed the larvae. Bacteria as potential indirect food source increased from TetraPhyll ($1.41 \times 10^6 \pm 0.05 \times 10^6$ cells/ml, mean ± SEM) to leaf litter ($2.35 \times 10^6 \pm 0.05 \times 10^6$ cells/ml), TetraMin ($2.71 \times 10^6 \pm 0.13 \times 10^6$ cells/ml), and lyophilized chironomid larvae ($3.10 \times 10^7 \pm 0.69 \times 10^6$ cells/ml), but did not differ significantly among food types ($F = 0.85$; df = 3; $P = 0.53$). Fungal growth was infrequently observed only in the leaf litter treatment. Slight estrogenic activity was detected in leaf litter and TetraMin, but far below the limit of quantification (=5.8 ng EEQ mg/diet).

The surveillance of the leaf litter fed larvae was terminated at day 35 (24 d after the last food supply) when a mean mortality of 70.0 ± 17.1% was observed, although very few individuals had not pupated by this time. If qualitatively compared with other diets, however, a high concentration of potentially toxic nitrite (competing with oxygen at hemoglobin binding positions) was measured in the leaf litter vessels (Table 1). The high concentration of nitrite in the leaf litter treatment coincided with the longest time needed for larval development (more than twofold $PT_{50}$ of males [14.9 ± 2.3 d] and females [22.1 ± 4.4 d]; Table 1). Similarly, the morphometric values of both sexes were lowest if larvae were fed with leaf litter.

**Table 1. Chemical and physical parameters (mean ± SD) and standing time (d) of test vessels during the half-life-cycle-tests with *Aedes albopictus* in the food quality exp (leaf litter, TetraPhyll, TetraMin, lyophilized chironomid larvae) and in the food quantity/temp exp (quantity: LF, MF, HF, temp: 20, 25, 30°C)**

| | $NO_2^-$ | $NO_3^-$ | $NH_4^+$ | $PO_4^{2-}$ | pH | Con | $PT_{max}$ |
|---|---|---|---|---|---|---|---|
| Leaf litter[a] | 1.00 ± 0.00 | 2.86 ± 0.15 | 0.32 ± 0.03 | 0.05 ± 0.00 | 7.31 ± 0.09 | 361 ± 45 | >35 |
| TetraPhyll | 0.22 ± 0.13 | 2.90 ± 0.10 | $5.65^a$ ± 0.79 | $2.29^a$ ± 0.47 | $7.52^{ab}$ ± 0.06 | 404 ± 91 | 10 ± 1 |
| TetraMin | 0.44 ± 0.66 | 2.77 ± 0.26 | $5.84^a$ ± 2.41 | $1.77^a$ ± 1.27 | $7.33^a$ ± 011 | 391 ± 58 | 10 ± 1 |
| Chironomid larvae | 0.19 ± 0.26 | 2.67 ± 0.29 | $11.63^b$ ± 1.25 | $5.00^b$ ± 0.00 | $7.70^b$ ± 0.21 | 496 ± 105 | 11 ± 1 |
| 20°C | | | | | | | |
| LF | $0.34^a$ ± 0.23 | 0.39 ± 0.26 | $1.33^a$ ± 0.58 | 2.50 ± 0.87 | 8.17 ± 0.29 | 405 ± 106 | $26^a$ ± 6 |
| MF | $0.07^b$ ± 0.04 | 0.29 ± 0.14 | $6.33^b$ ± 1.16 | 2.67 ± 0.58 | 7.97 ± 0.27 | 366 ± 46 | $16^b$ ± 2 |
| HF | $0.09^{ab}$ ± 0.02 | 0.72 ± 0.46 | $6.00^b$ ± 1.73 | 4.33 ± 1.16 | 8.10 ± 0.42 | 432 ± 70 | $23^{ab}$ ± 4 |
| 25°C | | | | | | | |
| LF | 0.03 ± 0.01 | 0.22 ± 0.08 | 0.83 ± 0.29 | 1.17 ± 0.58 | 7.47 ± 0.08 | 281 ± 8 | 14 ± 3 |
| MF | 0.01 ± 0.01 | 0.12 ± 0.06 | 2.08 ± 1.88 | 1.17 ± 0.72 | 7.21 ± 0.34 | 303 ± 10 | 11 ± 2 |
| HF | 0.02 ± 0.01 | 0.20 ± 0.05 | 5.17 ± 4.75 | 3.50 ± 2.60 | 7.10 ± 0.16 | 368 ± 55 | 9 ± 1 |
| 30°C | | | | | | | |
| LF | 0.29 ± 0.22 | 0.31 ± 0.25 | 2.01 ± 1.99 | $1.83^a$ ± 0.29 | 7.90 ± 0.08 | 359 ± 23 | 12 ± 4 |
| MF | 0.04 ± 0.02 | 0.18 ± 0.06 | 3.67 ± 1.16 | $3.33^b$ ± 1.53 | 7.95 ± 0.30 | 445 ± 75 | 13 ± 5 |
| HF | 0.05 ± 0.05 | 0.26 ± 0.06 | 5.67 ± 1.16 | $5.00^{ab}$ ± 0.00 | 8.01 ± 0.03 | 382 ± 23 | 7 ± 1 |

$NO_2^-$, nitrite (mg/L); $NO_3^-$, nitrate (mg/L); $NH_4^+$, ammonium (mg/L); $PO_4^{2-}$, phosphate (mg/L); pH, acidic or basic character; Con, conductivity ($\mu$S cm$^{-2}$); $PT_{max}$, max pupation time (d); LF, low food regime with 2.5 mg/larva; MF, medium food regime with 5 mg/larva; HF, high food regime with 10 mg/larva.

[a] Leaf litter treatment was excluded from statistical analysis because its surveillance was terminated at day 35 (24 d after last food supply) before pupation was fully completed.

Different letters indicate significant differences within the specific food treatments at specific temp with $P < 0.05$ at $n = 3$.

TetraPhyll, TetraMin, and chironomid larvae as food caused fast pupation times and high morphometric values (Fig. 1E and F). Although feeding with chironomid larvae resulted in increased concentrations of ammonium, phosphate, high pH, and conductivity (Table 1), the average mortality was lowest with chironomid food (Fig. 1A). Conversely, low levels of these physico-chemical parameters did not prevent the TetraPhyll fed animals from a significant increase in mortality if compared with animal diets ($P < 0.05$; c.f. Table 1; Fig. 1A).

These three diets had no effect on the sex ratio ($F = 1.47$; df = 2; $P = 0.27$) and only slight effects on the $PT_{50}$ (diet: $F = 1.74$, df = 2, $P = 0.20$, but diet x sex: $F = 6.63$, df = 2, $P = 0.01$) and abdominal length ($F = 6.23$; df = 2; $P = 0.007$) of *A. albopictus* pupae (Fig. 1B–D). Under a sex-specific point of view, the TetraMin fed female larvae developed most rapidly into the largest pupae and differed significantly from chironomid-fed females ($PT_{50}$, AL; $P < 0.05$), but there were no significant differences between females fed with TetraMin and TetraPhyll and among males.

**Interactive Effects of Food Quantity and Temperature.** The water physico-chemistry during the bifactorial half-life-cycle-test with *A. albopictus* (Table 1) was partly influenced by temperature (nitrite: $F =$

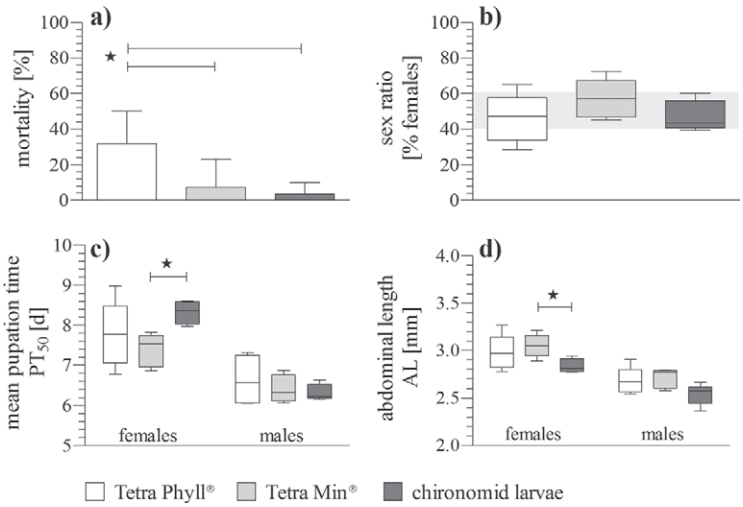


**Fig. 1.** Food quality experiment. Effects of differing food qualities on (a) mortality (%, mean ± SD), (b) sex ratio (%, ± 95% CI), (c) mean pupation time $PT_{50}$ (d, ± 95% CI), and (d) abdominal length (mm, ± 95% CI) of *Aedes albopictus* pupae. * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

**Table 2. Food quantity/temp exp.** ANOVA/ MANOVA for mortality, sex ratio and morphometric endpoints as assessed during a half-life-cycle-test with ***Aedes albopictus***, where variation was sourced by the independent variables food quantity, temp, (sex), and their interactions

| Source of variation | df | *F* | *P* |
|---|---|---|---|
| Mortality | | | |
| Food (F) | 2 | 3.90 | **0.04** |
| Temperature (T) | 2 | 2.04 | 0.16 |
| F × T | 4 | 1.30 | 0.31 |
| Sex ratio | | | |
| Food (F) | 2 | 1.74 | 0.20 |
| Temperature (T) | 2 | 0.54 | 0.59 |
| F × T | 4 | 2.06 | 0.13 |
| Morphometry | | | |
| Sex (S) | 3 | 22003.2 | **<0.001** |
| Food (F) | 6 | 473.26 | **<0.001** |
| Temperature (T) | 6 | 19.53 | **<0.001** |
| S × F | 6 | 103.18 | **<0.001** |
| S × T | 6 | 1.49 | 0.18 |
| F × T | 12 | 6.26 | **<0.001** |
| S × F × T | 12 | 7.39 | **<0.001** |

AL, abdominal length; AW, abdominal width; CT, area of the cephalothorax; *P*, significance level; df, no. of independent degrees of freedom; *F*, critical value from *F* distribution; bold numbers indicate significant effects.

6.24, df = 2, $P = 0.009$; ammonium: $F = 9.82$, df = 2, $P = 0.001$; phosphate: $F = 10.69$, df = 2, $P = 0.001$). Food regime had a significant impact on pH ($F = 27.16$; df = 2; $P < 0.001$), nitrite ($F = 3.65$; df = 2; $P = 0.047$), nitrate ($F = 4.75$; df = 2; $P = 0.022$), and phosphate ($F = 3.94$; df = 2; $P = 0.038$) concentrations, and on conductivity ($F = 6.32$; df = 2; $P = 0.009$). Interactive effects of temperature and food on chemical and physical water parameters could not be detected.

Although squared linear regression coefficients were low, maximal time to pupation was significantly correlated to pH ($R^2 = 0.32$; $P = 0.002$), and pupation success was negatively correlated to nitrate ($R^2 = 0.38$; $P < 0.001$) and phosphate ($R^2 = 0.16$; $P = 0.04$) concentrations and pH ($R^2 = 0.15$; $P < 0.04$). Larval mortality was significantly determined by food regime (Table 2; Fig. 2). The temperature of 20°C was suboptimal for larval survival as indicated by higher mortality (40.4 ± 34.7%) compared with 25°C (16.7 ± 10.4%) and 30°C (23.0 ± 13.2%; Fig. 3). Regarding only the food regimes at 20°C, LF and HF significantly differed from MF ($P = 0.009$).

The sex ratio of larvae with 51.6 ± 11.4% females was affected neither by temperature nor food (Table 2), in marked difference to the maximal time to pupation (food: $F = 30.76$, df = 2, $P < 0.001$; temperature: $F = 4.05$, df = 2, $P = 0.035$; interaction: $F = 3.23$, df = 4, $P = 0.037$). At 20°C, the mean time to pupation of both sexes was significantly prolonged at LF ($PT_{50\ (♀+♂)} = 17.2$d; $P < 0.001$) compared with higher food quantities (MF: $PT_{50\ (♀+♂)} = 12.8$d; HF: $PT_{50\ (♀+♂)} = 12.0$d), but it did not differ significantly among food regimes at 25 and 30°C. At 20°C the larvae mainly pupated after day 12 feeding while pupation at 25 and 30°C had nearly been completed before this final food supply (Fig. 2).

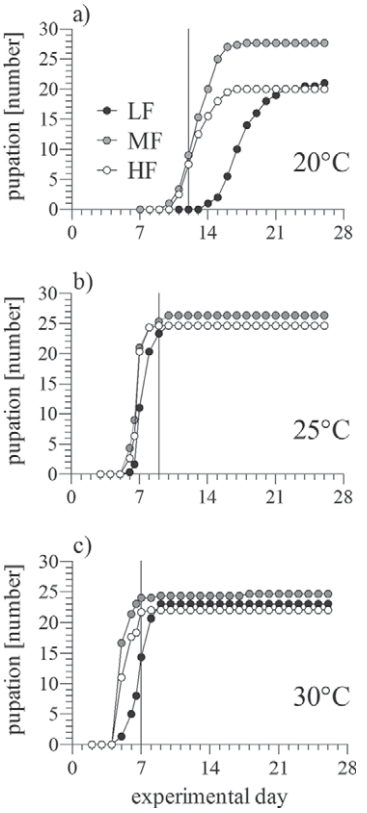


**Fig. 2.** Food quantity/temperature experiment. Effects of food quantity on pupation success of *Aedes albopictus* at three test temperatures. Cumulative pupation (mean number of both sexes) in dependence of experimental period (d) and food quantity of TetraMin (LF, MF, and HF) at 20°C (a), 25°C (b), and 30°C (c). The last day of feeding is marked by vertical lines. LF, low food regime with 2.5 mg/larva (black circles); MF, medium food regime with 5 mg/larva (gray circles), HF, high food regime with 10 mg/larva (open circles); $n = 3$ with 30 larvae per replicate, but $n = 2$ in the case of HF 20°C and LF 20°C.

The morphometric variables of pupae were highly influenced by food quantity and temperature and their interactions (except Sex × Temperature; see Table 2), but in different ways depending on sex. The HF regime was most beneficial for the size of *A. albopictus* pupae as indicated by usually maximal morphometric values, in particular at 25°C and in the case of females. The impact of different food quantities on morphometry was most pronounced at 20°C and in females, and least pronounced at 30°C and in males (Fig. 3). The CT of males was most responsive to food treatments if compared with the respective endpoints AL and AW.

## Discussion

The quality testing of the different larval diets revealed that animal diet is highly advantageous for the aquatic development stages of *A. albopictus*, most likely because of the easily assimilable protein and

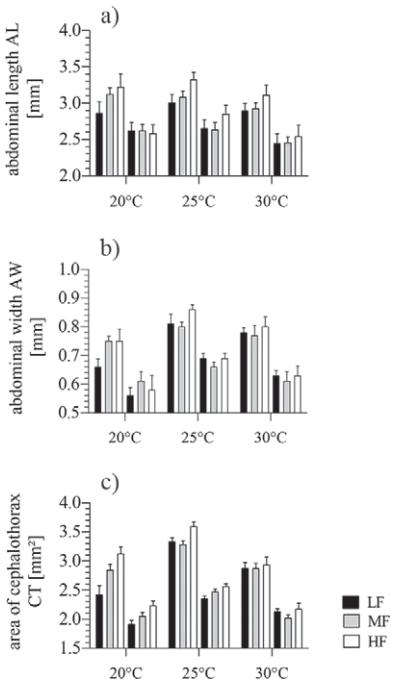


**Fig. 3.** Food quantity/temperature experiment. Effects of food quantity of TetraMin (LF, MF, and HF) and temperature on three morphometric endpoints of *Aedes albopictus* pupae during the half-life-cycle-test. Sex-specific (a) abdominal length (mm), (b) abdominal width (mm), and (c) area of the cephalothorax ($mm^2$) are plotted as weighted mean ± SEM. Left, females; right, males; LF, low food regime with 2.5 mg/larva (black bars); MF, medium food regime with 5 mg/larva (gray bars); HF, high food regime with 10 mg/larva (white bars).

high nitrogen content (Yee et al. 2007a, Kaufman et al. 2010). Timmermann and Briegel (1996) also observed maximum larval survival, fastest $PT_{50}$, and high protein and lipid contents of adults if animal diet (liver powder and TetraMin) was offered to the larvae of three mosquito species. It is noteworthy that commercial fish food seems to be more valuable regarding the pupation time and size of females than natural animal detritus (Fig. 1A, C, and D). TetraMin diet entailed the largest body sizes and caloric contents of adults (Timmermann and Briegel 1996). Moreover, the commercial ornamental fish food resulted in lower ammonium and phosphate concentrations, pH and conductivity than natural animal food (Table 1). Thus, we highly recommend the supply of animal diet in standardized feeding protocols for *A. albopictus* larvae, in particular animal-based ornamental fish food such as TetraMin.

Limitations of our study include the use of an older laboratory colony of *A. albopictus*, which may have resulted in less variation compared with wild individuals, and the choice and treatment of leaves used for leave litter feeding, which may not represent natural leaves found in phytotelmata. However, plant material overall is certainly not an appropriate food for *A. albopictus* larvae (Fig. 1; Timmermann and Briegel 1996; Yee et al. 2007a,b; Kaufman et al. 2010). Although TetraPhyll food led to large-sized survivors, mortality was consistently increased if plant materials were used as food (Fig. 1A and D). Although the particularly strong inhibition of the larvae by leaf litter diet in our study should be viewed with caution given that nutrient and tannin contents of leaves strongly depend on the drying method, species, litter age, and season or climate of the sampling locality (David et al. 2002, Dieng et al. 2002, Lorenz et al. 2004, Reiskind et al. 2009, Acero et al. 2010, Coq et al. 2010, Komes et al. 2011, Tharayil et al. 2011). Other authors also found that plant (nettle) feeding caused delayed eclosion and smaller sizes of adult mosquitoes than animal-based diets (Timmermann and Briegel 1996).

Beyond food quality, the interplay of food quantity and temperature should be considered to provide adequate feeding conditions for the aquatic life stages of *A. albopictus* in a standardized bioassay (Anderson and Cummins 1979). Our data show that the utilization of TetraMin is maximal at 25–30°C, although 25°C caused somewhat lower mortality and higher CT than 30°C (Figs. 2 and 3). At 25 and 30°C, all tested food quantities seemed to be optimal for survival (Fig. 2B–C). In contrast, adverse effects of low and high TetraMin quantities appeared at 20°C, a suboptimal temperature for *A. albopictus* (Delatte et al. 2009). The negative effects under LF at 20°C might be related to increased energy demand for larval metabolism (Hawley 1988, Timmermann and Briegel 1999), while an excess of animal detritus (HF) affected development time and mortality as also observed by Winters and Yee (2012). However, the morphometry of pupae followed almost always a positive food quantity-response-relationship. For specific experiments at suboptimal temperatures; therefore, it has to be decided whether a low mortality along with smaller individuals (on account of MF) or an increased mortality along with larger individuals (on account of HF) is more acceptable.

For an ecotoxicological chronic bioassay at optimal temperature, however, we highly recommend the supply of animal diet for *A. albopictus* larvae, in particular animal-based ornamental fish food such as TetraMin, using a medium food regime of 5 mg/larva offered at 25°C. Adherence to this feeding protocol may help to standardize experiments under rigorously controlled environmental conditions, thereby supporting interlaboratory comparisons to gain deeper insights into the ecotoxicology of this prominent mosquito vector of disease.

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