Suitability of monotypic and mixed diets for *Anopheles hermsi* larval development

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ABSTRACT: The developmental time and survival to eclosion of *Anopheles hermsi* Barr & Guptavanij fed monotypic and mixed diets of ten food types were examined in laboratory studies. Larvae fed monotypic diets containing animal detritus (freeze-dried rotifers, freeze-dried *Daphnia pulicaria*, and TetraMin® fish food flakes) and the mixotrophic protistan *Cryptomonas ovata* developed faster and survived better than larvae that were fed other monotypic diets. Survival to adulthood of larvae fed several concentrations of the diatom *Planothidium* (=*Achnanthes*) *lanceolatum* was poor (<13%) and larval development time was approximately twice that of larvae fed TetraMin® fish food flakes, the standard laboratory diet. Larvae fed monotypic diets containing prokaryotes (bacteria [*Bacillus cereus*] and cyanobacteria [*Oscillatoria prolifera*]) and brewer's yeast (*Saccharomyces cerevisiae*) failed to survive beyond the 1st and 2nd instar, respectively. Larvae fed only chlorophytes, single-celled *Chlamydomonas reinhardtii* and filamentous *Spirogyra communis,* failed to complete larval development, regardless of the concentration tested. Cohorts fed a combination of food types (mixed diets) usually developed better than cohorts fed monotypic diets. Food types that failed to support complete development when fed alone often facilitated development to adulthood when fed in combination with food types containing >1% C_{20} polyunsaturated fatty acids as total fat, but regardless of essential fatty acid content, algae that produced mucilage and filaments that sank out of the feeding zone were poor quality diets. ***Journal of Vector Ecology* 41 (1): 80-89. 2016.**

Keyword Index: Arachidonic acid, eicosapentaenoic acid, *Anopheles*, larval diets, nutrition, essential fatty acids.

INTRODUCTION

The family Culicidae is diverse taxonomically, with 113 genera and more than 3,500 species (Gaffigan et al. 2013), so it is not surprising ecologically that mosquito larvae feed on a wide variety of food types in a multitude of environments (Laird 1988). Food particles can differ greatly in size and the size range of food consumed differs depending on species, larval instar, and physical state of the food (living vs detritus). Even if the potential food is ingestible, it may be indigestible, of poor nutritional quality, or even toxic to the larva (Marten 2007). Food types ingested more readily may not be nutritionally more important than food types ingested less frequently. For example, the gut contents of mosquito larvae are often dominated numerically by bacteria (Walker et al. 1988), but the biovolume of bacteria in the gut contents might be equivalent to that of the small number of comparatively large algae (Merritt et al. 1990) and, moreover, heterotrophic bacteria can be a poor quality food for many aquatic consumers (Ahlgren et al. 1992, Taipale et al. 2014).

Clements (2000) concluded that if there are any important food types for mosquito larvae, then they are those that provide larvae with twenty-carbon polyunsaturated fatty acids (C_{20} PUFAs), specifically arachidonic acid [AA; 20:4,ω-**6**] and eicosapentaenoic acid [EPA; 20:5,ω-3]. These fatty acids are a major component of cellular and subcellular membranes (Stanley-Samuelson et al. 1988) and are precursors to eicosanoids, bioactive lipids that play many important physiological roles in insect immunity, reproduction, and ion transport (Stanley 2000, Stanley and Miller 2008). In addition to their critical physiological roles, stores of AA and EPA accumulated during larval development are transferred from triacylglycerols to functional polar lipids important for flight activity in adults (Sushchik et al. 2013). Constituents of the larval diet must provide these essential fatty acids because mosquitoes lack the enzymes needed to convert unsaturated fatty acids into C_{20} PUFAs (Dadd and Kleinjan 1978, Dadd 1983).

Not all food types contain C_{20} PUFAs, and animal-like protists were first postulated as the primary dietary source of C_{20} PUFAs for larval mosquitoes (Dadd and Kleinjan 1979). Later, Dadd et al. (1988) concluded that detritus composed of dead zooplankton was most likely the primary dietary source of C_{20} PUFAs for *Culex tarsalis* Coquillett. Although C_{20} PUFAs were considered to be "animal fatty acids," essential fatty acids occur infrequently in particular species of non-animal organisms, such as some diatoms (Dunstan et al. 1994), other protistans (Uttaro 2006), autotrophic bacteria (Gill and Valivety 1997), gram-negative bacteria (Valentine and Valentine 2004), some fungi (Kajikawa et al. 2004, Uttaro 2006), and some true plants (Napolitano 1994).

A greater understanding of diet quality, including the source(s) of essential fatty acids, might provide novel approaches for reducing mosquito production and could enhance the quality of laboratory-reared mosquitoes. The fitness of adult *Culex pipiens* L. is correlated with a larval diet high in C_{20} PUFAs (Dadd 1981, Dadd et al. 1989). Food quality (albeit digestibility or toxicity) influences adult mosquito production from developmental sites in nature but the selective removal or enhancement of particular algae for mosquito control will be difficult to achieve (Marten 2007). The fatty acid composition of natural populations of seven steppe mosquito species in three genera (*Ochlerotatus, Aedes,* and *Anopheles*) generally differed between larvae and adults more than among taxa (Sushchik et al. 2013). Sushchik et al. (2013) speculated that essential C_{20} PUFAs were redistributed between lipid fractions and tissues during metamorphosis and that EPA

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was obtained primarily from cryptophytes and dinoflagellates, but not diatoms, in the larval diet. The fatty acid composition of larvae also suggested that chlorophytes were an important source of C_{16} and C_{18}PUFAs. Biomass growth rate is directly related to the ratio of essential fatty acids to carbon in non-culicine aquatic consumers; PUFAs and sterols from phytoplankton were required for reproduction (Taipale et al. 2014). Supplementing standard laboratory larval mosquito diets with microorganisms containing these essential fatty acids might improve immature survival and enhance the quality of adults produced for inundative release programs.

Anopheles hermsi Barr & Guptavanij occurs south of the Tehachapi Mountains in California and sporadically in Arizona, Colorado, and New Mexico (Darsie and Ward 2005). This species is a potential vector of several pathogens and was responsible for two epidemics of *vivax* malaria in San Diego County, CA in the late 1980s (Barr and Guptavanij 1988).

The objective of this study was to examine the immature development of *An. hermsi* larvae being fed monotypic and mixed diets of a variety of food types known to differ in quality.

MATERIALS AND METHODS

Mosquito colony

A colony of *An. hermsi* was established by rearing larvae collected from two Riverside County, CA sites (33°43′10.20″N, 117°21′54.72″W and 33°52′24.24″N, 117°23′30.84″W) between 10 February and 8 March 2007. Larvae were reared in white enameled pans (22 x 33 x 5 cm) filled with ca. 2 liters of deionized (DI) water and fed ground TetraMin® Tropical Flakes (Tetra Werke; Melle, Germany) ad libitum daily. Pupae were transferred to DI water-filled 6-oz waxed paper cups and placed inside a nylon-covered cage to eclose. Adult females were blood-fed weekly on a Swiss-Webster mouse for 1 h in order to produce eggs. Eggs were oviposited in DI water-filled 6-oz waxed paper cups and transferred into new white enameled trays for rearing. The rearing room was held at 29 ± 3° C with a 16:8 (L:D) cycle.

Monotypic diets

Ten food types were used in this study. Brewer's yeast (*Saccharomyces cerevisiae*, Saccharomycetales: Saccharomycetaceae) was purchased from MP Biomedicals, LLC (Solon, OH). Cultures of *Planothidium* (=*Achnanthes*) *lanceolatum* (Achnanthales: Achnanthidiaceae), *Oscillatoria prolifera* (Oscillatoriales: Oscillatoriaceae), *Cryptomonas ovata* (Cryptomonadales: Cryptomonadaceae), *Chlamydomonas reinhardtii* (Volvocales: Chlamydomonadaceae), and *Spirogyra communis* (Zygnematales: Zygnemataceae) were obtained from the University of Texas Culture Collection of Algae (Austin, TX). Spores of *Bacillus cereus* (Bacillales: Bacillaceae) were obtained from Dr. Brian Federici, University of California-Riverside. All live organisms, except *B. cereus*, were cultured in DYIII liquid media (Lehman 1976) that had been sterilized by autoclaving. *Bacillus cereus* was cultured in nutrient broth (BD 234000; Becton Dickinson and Company, Franklin Lakes, NJ). Freeze-dried rotifers (unknown taxonomy) and freeze-dried *Daphnia pulicaria* (Cladocera: Daphniidae) were obtained from Brine Shrimp Direct, Inc. (Ogden, UT). The rearing success of larvae raised on each diet was compared with the standard rearing diet, TetraMin® fish flakes (FF).

For each food treatment, two 12-well tissue culture plates were filled with 3 ml of DI water per well and seeded with 72 eggs, three in each well, to start each treatment. Excess larvae were removed after they eclosed so that an individual larva remained in each of the 24 replicate wells. Treatments were housed in the colony rearing room. Larvae were fed every other day. Wells were emptied to ca. 0.5 ml with a disposable plastic dropper before every feeding.

Planothidium lanceolatum, *B. cereus, C. ovata*, and *C. reinhardtii* are single-celled organisms and thus were used directly from batch cultures. The filamentous *O. prolifera* and *S. communis* were pulverized using a glass rod pestle before measuring optical density of the culture. A subsample from each 50-ml culture sample was placed in a spectrophotometer to measure its absorbance at 550 nm. Culture samples were either diluted with DI water or concentrated via centrifugation as needed to obtain an absorbance of 0.2 ± 0.02 (mean ± SD). The wet weight of the unicellular algae (*C. ovata*) and filamentous algae (*S. communis*) at this optical density was approximately 0.30 g/ml and 0.01 g/ml, respectively. The amount of live food delivered was dependent on instar: 1:3 (ml food: ml DI water), 2:2, 3:1, and 4:0 for the 1st (L1), 2nd (L2), 3rd (L3), and 4th (L4) instars, respectively.

TetraMin® Fish Flakes, freeze-dried *D. pulicaria*, freeze-dried rotifers, and *S. cerevisiae* were fed to larvae in the following mass increments: 0.3:1 (mg food: instar), 0.6:2, 0.9:3, and 1.2:4. The dried foods were ground to the same consistency with mortar and pestle and fed as a surface food with 4 ml DI water. Wells were emptied to ca. 0.5 ml with a disposable plastic dropper before every feeding.

Individuals were observed every 12 h in order to monitor hatching, molting, pupation, and adult eclosion. Pupae were transferred to 1 oz plastic cups filled with 5 ml of DI water to eclose. These cups were covered with Parafilm™, which was punctured several times to allow gas exchange.

Mixed diets

Eight mixtures of food types and additional concentrations of the four algae/protists were fed to *An. hermsi* larvae. Combinations of two live food types (*C. reinhardtii* + *P. lanceolatum*, *C. reinhardtii* + *S. communis*, *C. reinhardtii* + *S. cerevisiae,* or *C. reinhardtii* + *O. prolifera*) were fed a double concentration (absorbance at 550 nm: 0.4 ± 0.04) of each food type, however, at half the volume for each food type that is listed above for the original treatments in order to keep the same ml food: ml DI water ratio used for the monotypic diet treatments. Combinations of one live food type and one ground dried food type (*C. reinhardtii* + FF, *O. prolifera* + FF, *S. cerevisiae* + FF, and *B. cereus* + FF) were fed in the same way as the monoculture treatments at the increments stated above. Larvae were also fed monocultures of *C. reinhardtii*, *C. ovata*, *P. lanceolatum,* and *S. communis* at double the concentration (absorbance at 550 nm: 0.4 ± 0.04) that was used for the aforementioned monoculture experiments.

In addition, the suitability of *B. cereus, C. reinhardtii,* and *S. cerevisiae* as a supplement to larval diets was investigated by rearing individuals on FF for different time periods and switching the diet to each food type after the first, second, and third stadia.

Treatments of larvae were fed FF for the same three periods after which food was withheld, serving as a negative control. The feeding regimes used in the monotypic diet treatments were followed. Potential toxicity of the algal medium and nutrient broth to *An. hermsi* was investigated by rearing individuals on a FF diet, replacing DI water with the DYIII medium and BD 234000 broth, respectively.

Statistical analyses

Statistical Analysis System (SAS) 9.1.3 SP4 (SAS Institute Inc., Cary, NC) was utilized to carry out ANOVAs of development time of each instar and total development time across the treatments. Statistically significant ANOVAs were followed by Tukey-Kramer post-hoc pairwise comparisons against the standard laboratory larval diet (FF).

Survivor functions for mosquitoes fed monotypic diets were calculated using Kaplan-Meier estimation (SURVIVAL module; SYSTAT ver. 9.0, Chicago, IL). Statistical significance of differences of mosquito survival from hatching to adult eclosion among diets was compared using log-rank tests; α was set at 0.005 to control for multiple comparisons.

RESULTS

Monotypic diets

The mean developmental time, total developmental times, and standard deviations for the individuals completing each instar for all monotypic diets are shown in Table 1. First instar larvae fed *P. lanceolatum* developed significantly slower than larvae fed FF. However, mean duration of L1 development (range: 64.0-84.8 h) did not differ significantly among larvae fed *S. cerevisiae, C. reinhardtii*, *C. ovata*, freeze-dried *D. pulicaria*, freeze-dried rotifers, and *S. communis*. The majority (92%) of larvae fed *S. cerevisiae* and all larvae fed *B. cereus* and *O. prolifera* died during the first stadium.

Second instar larvae fed *P. lanceolatum* and *S. communis* developed significantly slower than larvae fed FF (Table 1). Compared with the FF diet, mean duration of L2 development (range: 43.0-69.0 h) did not differ significantly among larvae fed *C. ovata*, *C. reinhardtii*, freeze-dried *D. pulicaria*, and freeze-dried rotifers. The remaining larvae fed *S. cerevisiae* died during the 2nd instar (Figure 1).

Third instars fed *P. lanceolatum* and *S. communis* developed significantly slower than larvae fed FF, requiring about twice as long until molting into 4th instars (Table 1). Development times of the 3rd instar (range: 61.2-78.8 h) did not differ significantly among larvae fed *C. ovata*, freeze-dried *D. pulicaria*, and freeze-dried rotifers. The remaining larvae fed *C. reinhardtii* died as 3rd instars.

Fourth instars fed *P. lanceolatum*, *C. ovata*, freeze-dried *D. pulicaria*, and freeze-dried rotifers developed significantly slower than larvae fed FF (88.5 vs > 132 h; Table 1). The remaining larvae fed *S. communis* died as 4th instars.

Among the food treatments (*P. lanceolatum*, *C. ovata*, freeze-dried *D. pulicaria*, and freeze-dried rotifers) in which pupae were present, the duration of the pupal stage did not differ significantly from the FF treatment. The mean duration (± SD) of the pupal stage across treatments was 53.6 (± 8.0) h.

Table 1. Mean duration (h ± SD) of *An. hermsi* stadia and total development by food type at 29° C.

	Instar					
Diet	L1	L2	L3	L4	P	Total
Fish food (FF) + DI water	70.5 ± 16.7[a]	59.5 ± 7.7[a]	69.0 ± 8.6[a]	88.5 ± 15.1[a]	52.8 ± 12.7[a]	340.0 ± 24.3[a]
FF + DYIII medium	67.6 ± 7.9[a]	62.2 ± 19.8[a]	57.2 ± 14.8[a]	93.0 ± 8.6[a]	52.4 ± 5.9[a]	331.6 ± 19.3[a]
Planothidium lanceolatum	138.5 ± 38.8[b]	115.6 ± 31.9[b]	144.0 ± 31.5[b]	270.0 ± 52.1[c]	54.0 ± 8.5[a]	690.0 ±110.3[c]
Bacillus cinereus	-*					
Chlamydomonas reinhardtii	56.5 ± 5.6[a]	69.0 ± 14.0[a]	-			
Cryptomonas ovata	64.0 ± 9.1[a]	43.0 ± 6.0[a]	71.7 ± 5.6[a]	188.7 ± 21.5[b]	54.0 ± 8.5[a]	402.0 ± 42.4[b]
Daphnia pulicaria	65.6 ± 7.9[a]	53.7 ± 7.1[a]	78.8 ± 15.3[a]	135.43 ± 33.3[b]	60.0 ± 10.9[a]	389.3 ± 43.0[b]
Oscillatoria prolifera	-					
Rotifera	74.5 ± 14.2[a]	51.4 ± 6.7[a]	61.2 ± 17.4[a]	132.9 ± 29.6[b]	55.6 ± 9.7[a]	373.1 ± 24.9[b]
Saccharomyces cerevisiae	78.0 ± 8.5[a]	-				
Spirogyra communis	84.8 ± 24.2[a]	172.8 ± 30.0[b]	148.5 ± 36.8[b]	-		

[a-c]For each column, different letters differ from the fish food diet by $P < 0.05$ (Tukey-Kramer test).

⋆ - Indicates that no individuals survived to molt pass/through this instar.

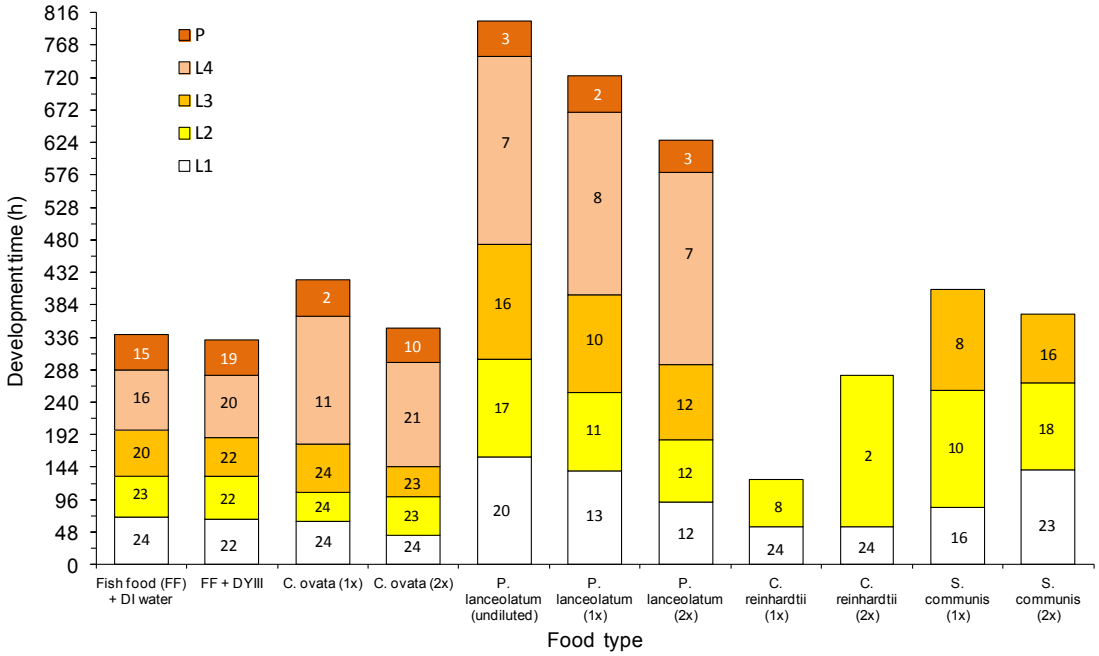

Figure 1. Mean duration of *An. hermsi* instars fed five monotypic diets (TetraMin® fish food, *Cryptomonas ovata*, *Planothidium lanceolatum*, *Chlamydomonas reinhardtii,* or *Spirogyra communis*) and different concentrations (1x vs 2x) of eukaryotic algae or protists. Mosquito development in deionized water (DI) did not differ from that in algal medium (DYIII), indicating that the latter was not toxic to *An. hermsi*. The numbers of individuals that successfully completed each instar are indicated for each diet. Twenty-four 1st instars were reared individually on each diet.

The total immature development time for larvae fed monotypic diets was shortest in the FF treatments. As compared to larvae fed FF and reared in DI water, development time was not affected significantly when individuals fed FF were reared in DYIII media (340.0 ± 24.3 h vs 331.6 ± 19.3 h, respectively). Development time for *An. hermsi* reared on freeze-dried rotifers, freeze-dried *D. pulicaria,* and *C. ovata* was 10, 15, and 18%, respectively, longer than the FF treatment (Table 1). The mean total development time for *An. hermsi* reared on the diatom *P. lanceolatum* was twice that of individuals reared on the FF diet ($P < 0.05$). The number of individuals eclosing successfully did not differ appreciably among the treatments containing animal-derived detritus but was reduced markedly (92%) for the algal diets that supported complete development. Larvae fed either FF or *C. ovata* and reared in BD 234000 broth did not survive beyond the 1st instar.

Survival to the adult stage improved five-fold and development time decreased by 17% when larvae were fed double concentrations of *C. ovata* (Figure 1). Immature development time was inversely related to the concentration of *P. lanceolatum* in the diet, but survival to adulthood was comparably low (two to three individuals) across the *P. lanceolatum* treatments. Relative to the results for the monoculture diet using *C. reinhardtii*, survival through L2 declined when larvae were fed a double concentration of *C. reinhardtii*; no individuals developed beyond L3. Survival increased and development time decreased when larvae were fed a double concentration of *S. communis* and when larvae were fed a combination of *S. communis* and *C. reinhardtii*. However, larvae did not develop past the 4th instar in both treatments.

Larvae fed individual food types known to contain little (< 1% of total fat) to no AA and EPA (Table 2) did not develop past the 3rd instar. Conversely, larvae fed individual food types known to contain AA (> 1% of total fat) and EPA (Table 2) developed beyond the 3rd instar. Survival differed significantly between the two diet groups (Figure 2; log-rank tests: animal detritus and mixotrophic *C. ovata* > chlorophytes, diatoms, fungi, yeast) and also among the diets known to contain AA (> 1% of total fat) and EPA (*D. pulicaria* = FF; FF = rotifers, *C. ovata*).

Figure 2. Kaplan-Meier survival functions for (1x) monotypic diets. The treatments that did not reach zero survival resulted in pupation.

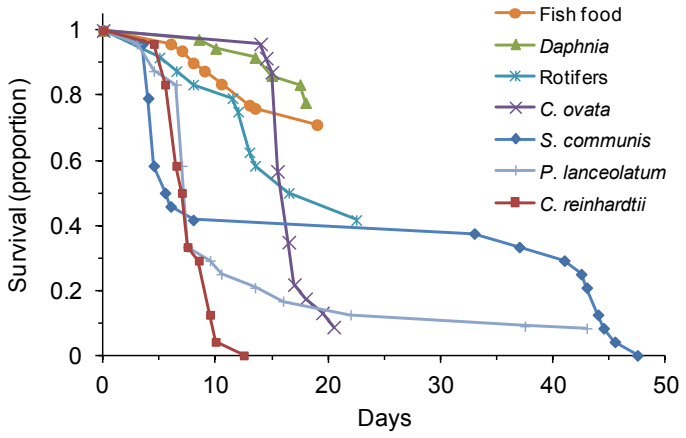

Table 2. Arachidonic and eicosapentaenoic acid content by food type. Fatty acid content is expressed as greater than 1% of total lipid profile (+), trace, or less than 1% of total lipid profile (T), lacking any C_{20} PUFAs (-), and unknown (?).

Food type	AA [20:4, w-6]	EPA [20:5, w-3]	Source
Fish food	?	+	Tetra, personal communication
Planothidium lanceolatum[1]	+	+	Caramujo et al. 2008
Bacillus cereus	-	-	Kämpfer 1994
Chlamydomonas reinhardtii	-	-	Kajikawa et al. 2006
Cryptomonas ovata	+	+	Beach et al. 2006
Daphnia pulicaria[2]	+	+	Brett et al. 2006
Oscillatoria prolifera[3]	-	-	Jahnke et al. 1989
Rotifers[4]	+	+	Kennari et al. 2008
Saccharomyces cerevisiae	T	T	Chakaorty et al. 2007
Spirogyra communis[5]	+	+	Stefanov et al. 1996

[1] Listed as the synonymn *Acananthes lanceolatum.*
[2]Data are for the closely related *Daphnia pulex.*
[3]Data are for the closely related *O. limnetica.*
[4]Data are for *Brachionus calyciflorus.*
[5]Data are for *Spirogyra crassa.*

Mixed diets

Relative to each food type alone, development time decreased and survival improved for larvae fed the following mixed diets: *C. reinhardtii* plus FF, *C. ovata* plus FF, *C. reinhardtii* plus *S. cerevisiae,* and *C. reinhardtii* plus *P. lanceolatum* (cf. Figures 1 and 3). Larvae fed *C. ovata* plus FF developed the fastest of any diet studied and larvae fed the four aforementioned mixed diets, as well as *S. cerevisiae* plus FF and *O. prolifera* plus FF, developed significantly faster than larvae fed FF alone ($P < 0.05$). Relative to *S. cerevisiae* alone, development time for L1 decreased and ≥ 50% of individuals completed immature development in the mixed diet treatments of *S. cerevisiae* plus FF and *S. cerevisiae* plus *C. reinhardtii* (Figure 3). Survival increased and development time decreased in the mixed diet treatments of *O. prolifera* plus FF and *O. prolifera* plus *C. reinhardtii*. Larvae fed *B. cereus* plus FF failed to survive past L1.

Larvae often completed development when they were provided FF through the 3rd instar and then switched to a food type that, when provided as a monotypic diet beginning in the 1st instar, failed to provide adequate nutrition to allow larvae to complete development; albeit, survival was very poor. *Anopheles hermsi* larvae fed FF through L3 did not pupate if food was withheld during the L4 (Figure 4). Larvae fed FF during the 1st and 2nd instars and then switched to *S. cerevisiae* alone at L2 or L3 also did not develop past the L4. *Anopheles hermsi* larvae switched from FF to *S. cersvisiae* at L4 completed development. *Anopheles hermsi* larvae fed FF during L1 then switched to *C. reinhardtii* at L2 failed to pupate; whereas, larvae fed FF through the 2nd or 3rd instar and then switched to *C. reinhardtii* acquired sufficient nutritional reserves from FF to complete development.

DISCUSSION

The suitability for *An. hermsi* development differed among the monotypic diets: animal detritus ≥ mixotrophic eukaryotes > obligatory photosynthetic eukaryotes > fungi > prokaryotes. Animal detritus is an important component of the larval diets of container-dwelling mosquitoes such as *Aedes aegypti* (L.), *Ae. albopictus* (Skuse), and *Ae. triseriatus* (Say) (Daugherty et al. 2000, Yee and Juliano 2006, Yee et al. 2007, Kaufman et al. 2010). *Anopheles quadrimaculatus* Say larvae routinely consume cladocerans, copepods, water mites, rotifers, and protistans (Wallace and Merritt 2004). Animal tissues are a potential source of AA and EPA for larval mosquitoes in nature (Merritt et al. 1992). Aquatic invertebrates, in general, are known to contain high concentrations of AA and EPA (Hanson et al. 1985) and the fatty acid profiles of daphniid and rotiferan species indicate high levels of C_{20} PUFAs (Oltra et al. 2000, Brett et al. 2006, Kennari et al. 2008). TetraMin® flakes include animal remains and are a high quality food used extensively for larval mosquito diets in the laboratory (Gerberg et al. 1994).

Cohorts of *An. hermsi* fed *C. ovata* developed more quickly and completely than cohorts fed any of the other live culture food types. Other protistans, such as euglenids, have been fed to a variety of mosquito species with similar results (Avissar et al. 1994). *Cryptomonas ovata* is mixotrophic, capable of both photosynthesis and hetertrophic consumption of bacteria (Porter et al. 1985). Some cryptomonads are also osmotrophic, capable of absorbing dissolved organic compounds across the cell surface (Reynolds 2006). Photosynthetic eukaryotes such as *C. ovata* and *P. lanceolatum* are known to produce essential ω-3 PUFAs (i.e., α-linolenic acid (18:3, ω-3): Sushchik et al. 2013), including C_{20}

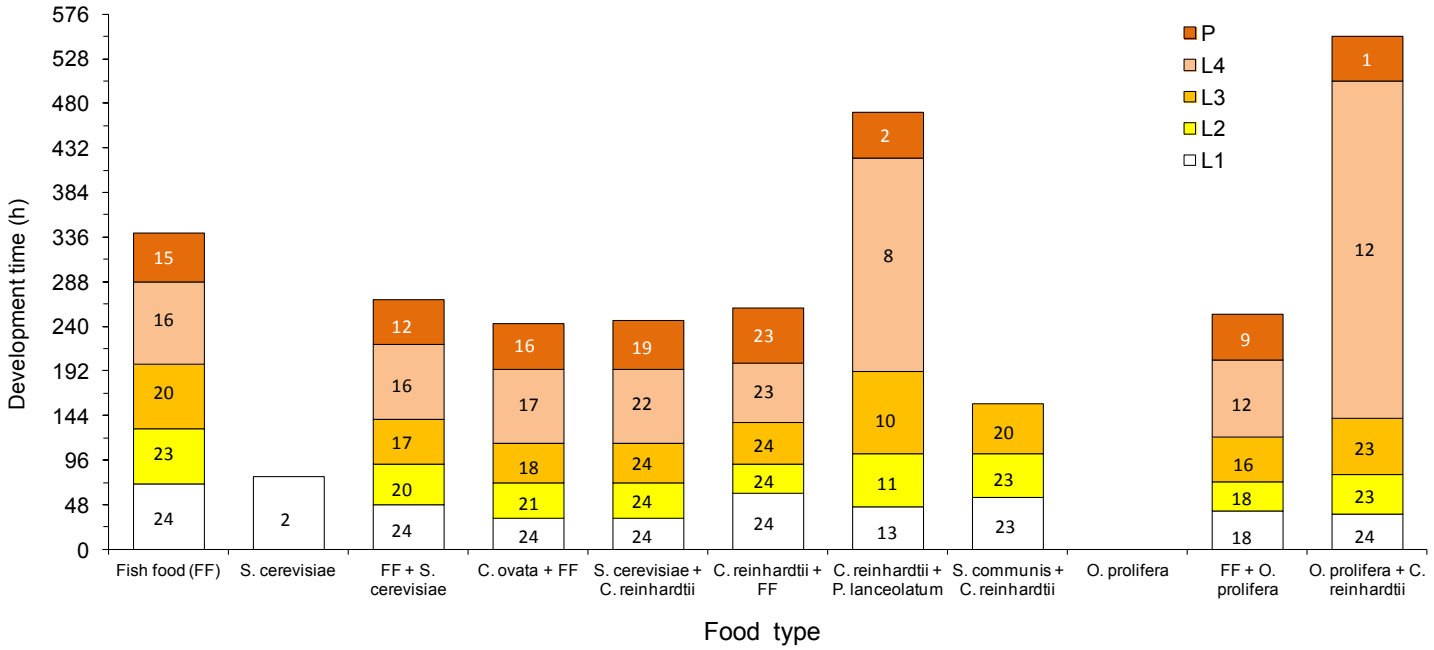

Figure 3. Mean duration of *An. hermsi* instars fed mixed diets. Development times of larvae fed three monotypic diets (fish food flakes, *Saccharomyces cerevisiae,* or *Oscillatoria prolifera*) are illustrated for comparison. The numbers of individuals that successfully completed each instar are indicated for each diet. Twenty-four 1st instars were reared individually on each diet.

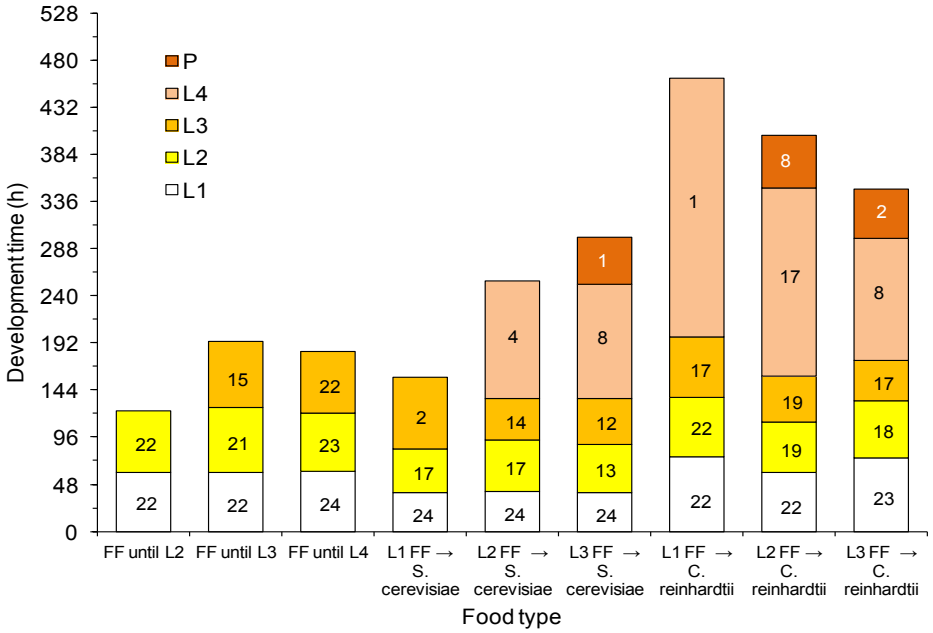

Figure 4. Mean duration of *An. hermsi* instars fed a high-quality diet (fish food flakes: FF) for three periods (through L1, L2, or L3) and then starved (negative control) or fed a poor-quality monotypic diet (*Saccharomyces cerevisiae* or *Chlamydomonas reinhardtii*). The numbers within each instar are the number of individuals that survived to complete the instar of the 24 1st instars reared individually on each diet.

PUFAs (Beach et al. 1970, Caramujo et al. 2008). Cryptomonads and diatoms are high-quality resources for metazoan consumers (Brett et al. 2006, Taipale et al. 2014).

Whereas diatoms can be an important source of essential fatty acids for aquatic consumers (Caramujo et al. 2008, Sushchik et al. 2013, Taipale et al. 2014), both reduced digestibility and lower availability in the feeding zone of *An. hermsi* larvae probably contributed to the lower diet quality of *P. lanceolatum*. Larvae fed *P. lanceolatum* took more than twice as long to develop than cohorts fed FF, *C. ovata*, freeze-dried rotifers, and freeze-dried *D. pulicaria*, yet the duration of the pupal stage was unaffected by larval diet. Larval development time decreased by 23% when *P. lanceolatum* concentration was increased about three-fold, but overall cohort survival was unchanged across the three food densities in the monotypic diets examined. This adnate diatom forms a mucilaginous mat in culture and, despite agitation during food additions, sank to the bottom of the tissue wells. Mucilage reduces digestibility of algae by mosquito larvae (Marten 2007). *Anopheles* larvae often concentrate feeding near the water surface (Fritz et al. 1989, Merritt et al. 1992), so a diet that sinks is expected to be less available than are diets that occur near the air-water interface.

The potential importance of essential fatty acids provided by diatoms in *An. hermsi* diets is suggested by the marked improvement of larval survival and a reduction of development time observed using a mixed diet containing the diatom and a poor quality diet (*Chlamydomonas*; cf. Figures 1 and 3) Rey et al. (2009) reared *Ae. aegypti* on mixed diets of a single species of non-adnate diatoms (freshwater: *Nitzschia kuetzingiana* or *Nitzschia palea;* marine: *Entomoneis* cf. *delicatula*, *Melosira lineata*, *Skeletonema costatum*, or *Thalassiosira weissflogii*), along with lab dietary medium (50:50 egg albumin/brewer's yeast). There were no significant differences in *Ae. aegypti* development time among the treatments and no observable allelopathic activity against *Ae. aegypti*.

Regardless of food density, chlorophytes were a comparatively poor quality diet for *An. hermsi*. Cohorts of larvae fed monocultures of *S. communis* or *C. reinhardtii* did not develop into adults. Differences in digestibility and toxicity among this diverse group of algae are associated with the diverse range of diet quality for immature mosquitoes (Marten 2007). Kajikawa et al. (2006) could not detect C_{20} PUFAs in *C. reinhardtii*; however, chlorophytes that contain high concentrations FAs without EPA are intermediate quality diets for aquatic microcrustaceans (Brett et al. 2006, Taipale et al. 2014). Green algae are generally an important source of 18:3, ω-3 (α-linolenic acid) and 18:2, ω-6 (linoleic acid) PUFAs in aquatic food webs (Sushchik et al. 2004). Dadd and Kleinjan (1979) postulated that the special dietary fatty acid requirement of mosquitoes evolved in a group of insects which normally has access to plentiful arachidonic acid during adult blood feeding and from animal-like protists as larvae, and mosquitoes have lost the enzymatic capability of converting di- and tri-enoic C_{18} fatty acids to the more unsaturated and longer chain compounds. It is unlikely that insufficient biomass of *C. reinhardtii* limited *An. hermsi* development because larvae fed a double concentration of *C. reinhardtii* developed more poorly than did larvae fed at the lower concentration. All cohorts fed a double concentration of other food types (i.e., *C. ovata, P. lanceolatum, S. communis*) developed better than their respective cohorts fed at the lower concentration. The deficiencies of the *Chlamydomonas* diet were overcome when *C. reinhardtii* was fed in combination with *P. lanceolatum* or FF.

The quality of *Spirogyra* as a diet for *An. hermsi* larvae was low even though this alga contains C_{20}PUFAs. Bond et al. (2005) found that *Anopheles pseudopunctipennis* Theobald could develop on *Spirogyra* spp. collected from the field. Stefanov et al. (1996) detected small amounts of AA and EPA in field-collected *Spirogyra crassa*; however, field-collected specimens could have been contaminated with PUFA-containing epiphytes. Diatoms were commonly found attached to filamentous algae at our collection sites of *An. hermsi*. Recommended daily rations of powdered dry foods (i.e., ground dog chow, guinea pig chow, hog chow, liver powder, yeast) range between 0.5 and 1.5 mg per 4th instar *Anopheles* larva (Fritz et al. 1989, Gerberg et al. 1994). The concentration of *Spirogyra* (wet weight: ~5 mg/ml/d) should have provided adequate food biomass for each *An. hermsi* larva, but the size of the filaments and settling might have limited availability of this diet. Moreover, the poor quality of *Spirogyra* as a larval food was observed for mixed diets: larvae fed *C. reinhardtii* in combination with *S. communis* developed faster than larvae fed *S. communis* alone, but no larva completed the 4th stadium. Factors, such as temperature, light intensity, nutrition, and growth phase (De Pauw et al.1984) can influence fatty acid composition of algae. We do not know whether our culture conditions or pulverization of *S. communis* reduced the rearing success of *An. hermsi.*

Of the dried powders (*S. cerevisiae*, FF, freeze-dried rotifers, and freeze-dried *D. pulicaria*) fed as surface food, *S. cerevisiae* was the only food that resulted in poor larval development. Timmermann and Briegel (1996) recorded similar results when feeding yeast to *An. albimanus* Wiedemann. Brewer's yeast is either a component of laboratory diets fed daily (Fritz et al. 1989) or is added once during *Anopheles* larval development (Gerberg et al. 1994). Brewer's yeast has been added in solution as a phagostimulant to standard lab diet (i.e., ground rodent food) for culicine larvae (Dadd 1970). Marten (2007) concluded that mortality of mosquito larvae fed species of Chlorococcales is usually due to indigestibility alone, since mortality declines and larval development time shortens when brewer's yeast is included in the diet. *Anopheles hermsi* completed immature development when brewer's yeast was fed in combination with FF or *C. reinhardtii*. The phagostimulatory effect of brewer's yeast coupled with the complement of nutrients from both organisms may have caused this result. *Saccharomyces cerevisiae* is thought to contain trace amounts of AA and EPA (Chakraborty et al. 2007), although other nutrients, such as particular amino acids and/or other fatty acids, may be lacking. Further research should address what nutrients mosquito larvae acquire from this widely used component of laboratory diets.

Prokaryotic food sources such as *B. cereus* and *O. prolifera* lack essential lipids [long-chain PUFAs (Jahnke et al. 1989, Kämpfer 1994) and sterols (Martin-Creuzburg et al. 2008, Taipale et al. 2014)]; all *An. hermsi* larvae reared on both diets died as 1st instars. Survival of *Culex* spp. was likewise poor when larvae were fed bacteria-laden nutrient broths (Hinman 1930). Larvae of *Cx. quinquefasciatus* and *Cx. tarsalis* fed a monoculture diet of phosphorus-enriched bacteria survived better than did *An. hermsi*

larvae; however, no adults developed (Peck and Walton 2006). Walker et al. (1988) and Merritt et al. (1990) found that larval mosquito gut contents (quantified as cells or particles) could contain over 90% bacteria. Therefore, bacteria presumably must play a nutritional role for mosquito larvae; however, heterotrophic bacteria that lack sterols are poor quality food for metazoan grazers (Martin-Creuzburg et al. 2011). Unlike other food types (i.e., *C. reinhardtii*, *S. cerevisiae*) for which a monotypic diet that did not support complete immature development facilitated adult eclosion when combined with FF, larval survival remained very poor when *B. cereus* was combined with FF. Toxicity of BD 234000 broth was suggested by poor survival of larvae fed FF in the bacterial growth medium.

Although many species of cyanobacteria are known to produce toxins (Berry et al. 2008) and *An. hermsi* failed to attain adulthood when fed a monotypic diet of cyanobacteria, when *O. prolifera* was fed in combination with FF or *C. reinhardtii*, adults developed in both treatments. Survival to adulthood (4%) was poor nevertheless.

A combination of factors contributes to the quality of diets of immature mosquitoes. Morphology, digestibility, and toxicity of food items influence the suitability of particular foods. Food types containing high concentrations of C_{20} PUFAs (i.e., animal detritus and mixotrophic protists) were often better monotypic diets for *An. hermsi* than were food types lacking these fatty acids, but availability and digestibility of the diet were important determinants of diet quality. Cohorts fed a combination of food types usually developed better than cohorts fed a single food type. Complex diets are indeed best for an omnivore. Mosquitoes might overcome the detrimental effects of diets lacking essential fatty acids. Dadd (1983) postulated that healthy female mosquitoes could lay eggs with adequate amounts of fatty acids for the full development of their offspring despite poor larval feeding conditions for the offspring. Understanding larval mosquito nutrient requirements will not only provide important insights into the ecology of particular mosquito species living in different environments, differences in the biochemical makeup and dietary requirements of aquatic invertebrates potentially influence the outcomes of higher order ecological interactions between species (Daugherty et al. 2000, Yee et al. 2007) and across food webs (Fink 2013, Sushchik et al. 2013).

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