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Evaluation of Critical Larval Habitat Physico-chemical Factors on Embryonic Development and Adult Fitness of *Culex quinquefasciatus* mosquitoes (Diptera: Culicidae)

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ABSTRACT

This study was carried out to evaluate the influence of selected physico-chemical factors (water temperature, pH and hardness) on duration of embryogeny, egg hatchability, and adult fitness (Wing Length and Fluctuating Asymmetry) of *Culex quinquefasciatus* mosquitoes under laboratory conditions. Freshly laid egg rafts of this mosquito species were exposed to different levels of these factors. Four temperature regimens (i.e., 28, 30, 32 and 34°C), seven pH levels (i.e., 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) and seven water hardness levels (10, 50, 100, 150, 200, 250, and 300 mg/L of $CaCO_3$) were independently tested on the egg rafts. The temperatures were regulated using thermostat, while pH and water hardness levels were maintained following standard procedures. The results revealed significant effects ($p<0.05$) of these physico-chemical factors on the entomological parameters measured. The duration of embryogeny varied significantly with values ranging from 19±1 to 24±4, 20±1 to 26±3 and 18±3 to 37.00±9.00 hours, respectively, for temperature, pH and Hardness. Egg hatchability rates fluctuated among the different treatments of a factor: temperature (77.13±48.46 to 204.75±20.27 larvae/egg raft), pH (113.25±13.92 to 218.75±22.34 larvae/egg raft) and water Hardness (98±12.13 to 214±10.22 larvae/egg raft). Wing length and fluctuating asymmetry also showed significant variation among the factors tested and between treatments of each factor. This study revealed profound effects of the physiochemical factors tested on the development and fitness of *Cx. quinquefasciatus* under laboratory conditions and could be employed in mosquito vectors control strategy.

Keywords: Hatchability, Embryogeny, Wing length, Fluctuating Asymmetry, Integrated Mosquito Management

1. INTRODUCTION

Mosquito transmitted diseases (e.g., filariasis, malaria, yellow fever and dengue) are the major causes of morbidity and mortality in sub-Sahara Africa [1]. Globally, over 500 million clinical cases and about one million deaths occur annually due to malaria: with sub-Saharan Africa accounting for over 90% of these statistics, while filariasis affects over 120 million people [2]. Yellow fever epidemic, on the other hand, has had debilitating effects on human populations in tropics [3]. In Nigeria, these diseases constitute the number one public health challenge, affecting negatively on the country's economic development to the tune of more than 1% growth penalty per year. The heavy burdens, exerted by mosquito-transmitted diseases informed the implementation of aggressive control interventions, using drugs and insecticides, particularly against the parasites and vectors, respectively [4, 5]. However, the wide spread incidence of drug-resistance in parasites and insecticide-resistance in vectors [6] has shifted attention to more critical control protocols that will target the vectors, especially, during pre-imaginal development [7]; thus reducing human-vector-parasite contact.

Mosquito larval occurrence and abundance is closely associated with availability and suitability of water bodies for breeding, and largely influenced by physico-chemical conditions of the habitation [8]. According to Adimi *et al.* [9], and Clement [10], water temperature, pH and hardness are among the most important determinants of the suitability of aquatic habitats for mosquito breeding. Temperature, for example, influence rates of embryonic and immature development in aquatic organism as well as determines the amount of dissolved oxygen for respiration [11], while, pH and water hardness determines ionic balance in aquatic fauna including mosquitoes [12, 13].

There is dearth of information on the relationships between physico-chemical factors of mosquito development in breeding sites in the tropics as a whole and Nigeria in particular. Most studies in the regions have concentrated on quantifying the prevailing physico-chemical factors with mere assumptions on their impacts on mosquito immature development [8, 14]. This study, therefore, was aimed at elucidating the direct effects of the identified critical physico-chemical parameters namely, temperature, pH, and water hardness on embryonic development and vectorial fitness attributes of adult mosquitoes using *Culex quinquefasciatus* as the model mosquitoes.

2. MATERIALS AND METHODS

2.1 Sources and Handling of Culex quinquefasciatus Mosquito Egg Rafts

Clay pots and plastic troughs (400 ml capacity), half-filled with distilled water, were set up as ovitraps for collection of mosquito egg rafts from the wild. Oviposited egg rafts were retrieved within 12 hours of ovitrap placement, and transferred to the Insectary Unit of the Department of Biological Sciences, Federal University of Technology, Minna. The techniques described by Weber and Weber [15] were used to screen and identify those belonging to *Culex quinquefasciatus.* The identified egg rafts were exposed to different regimen of the selected physico-chemical factors namely, water temperature, pH and hardness.

2.2 Simulation of Temperature Regimens and Exposure to Culex quinquefasciatus Egg Rafts

Four constant temperature treatments of 30, 32 and 34^{o}C and a Control experiment (Ambient, 28.00±0.02 ^{o}C), were set up with each having eight replicates. These temperatures were maintained constant using thermostats (Model: 300W, LifeTech Aquarium® GB4706.67-2003). An egg raft was introduced into each replicate trough (25 ml capacity) and monitored until hatching. After hatching, the total number of larvae hatched from each egg raft was counted, and all larvae from each egg raft were shared into rearing trough in batches of 50 larvae and fed with yeast everyday other day, until pupation and subsequent eclosion [16].

2.3. Preparation of pH Regimens and Exposure to Culex quinquefasciatus Egg Rafts

Various pH values of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 were obtained by diluting appropriate quantities of acid and base as described by Ivoke *et al.* [17]. Stock solutions of the acid and base were prepared For the Acid, 2% hydrochloric acid (HCl) was prepared by measuring 2 ml of concentrated HCl and 98 ml of distilled water into a beaker; and mixed thoroughly with a clean glass rod and stored in a reagent bottle. For the base, 4% sodium hydroxide (NaOH) was prepared by weighing 4 g of NaOH granules and dissolving in 96 ml of distilled water. To get acidic media, drops of prepared stock solution of acid (2%) were added to get the most stable reading. To get pH greater than 7.0, drops of prepared stock solution of base were added to obtain the most stable reading as displayed by pH meter

(Model: Jenway 3305) [17]. Single egg raft was, subsequently, introduced into the already prepared acidic or basic solution, as appropriate, in eight replicates and monitored for entomological indices.

2.4 Preparation of Water Hardness Regimens and Exposure to Culex quinquefasciatus Egg Rafts

Various water hardness levels (10, 50, 100, 150, 200, 250, and 300 mg/L $CaCO_3$) were prepared by dissolving the respective weights (mg) of Calcium Trioxocarbonate, $CaCO_3$, (Chemetrics, Inc., Calverton, VA) in a Litre of distilled water [18]. The regimens were tested against the egg rafts in replicates of eight (8) and monitored for entomological parameters.

2.5. Determination of Duration of Embryogeny and Hatchability of Culex quinquefasciatus Egg Rafts

Duration of embryogeny, expressed as time spent between incubation and hatching of the eggs, was determined by allowing incubated egg rafts to hatch completely after incubation. While hatchability of the egg was determined by counting the number of first instar larvae that hatch from each raft in any physico-chemical parameter [16]. All eggs were allowed for 72 hours post-incubating, to provide for late eclosion, and ensure total hatching.

2.6. Determination of Wing Length (WL) and Fluctuation Asymmetry (FA)

The wings are carefully detached from the thorax of emerged adult mosquitoes, with the aid of entomological pins. The left and wings were preserved in separate envelopes for further analyses, and WL determined by measurement using ocular micrometer (graticle) mounted on binocular Microscope. The values were expressed in millimeters (mm) according to the techniques of Gafur [19]. Wing FA was expressed as a difference between the length of Left and Right wings [16].

2.7. Data Analysis

Statistical analyses of data collected were carried out using Statistical Package for Social Scientists (SPSS) computer software for windows version 21.0. The mean values of duration of embryogeny and hatchability of egg rafts raised in the different physicochemical treatments were compared for significant differences using one way Analyses of Variance (ANOVA), at P=0.05.

3. RESULTS

3.1. Effects of Temperature on Duration of Embryogeny, Egg Hatchability, and Adult Fitness

There was a significant ($p<0.05$) reduction in duration of egg hatching (embryogeny) as temperature increased, and ranged from 19.00±1.00 hours (at 34ºC) to 24.00±4.00 hours (at ambient) (Table 1). Conversely, significant ($p<0.05$) decrease in mean number of larvae hatched per egg raft was observed with progressive increase in temperature. This varied significantly ($p<0.05$) among the temperature regimens; ranging from 77.13±48.46 (at 34ºC) to 204.75±20.27 larvae/egg raft (at 28.00±0.02 ºC). Generally, increase in temperature resulted in reduction in WL and increase in FA; with mosquitoes reared at ambient (Control) temperature having the longest wings (3.37±0.02 mm) and smallest FA (0.00±0.00 mm), while the mosquitoes cultured at 34ºC were the smallest (2.99±0.55 mm), and had the highest FA (0.04±0.02 mm) (Table 1).

3.2 Effects of Water pH on Duration of Embryogeny, Egg Hatchability, and Adult Fitness

These parameters were significantly ($p<0.05$) affected by water pH, especially at the extreme acid and alkaline conditions (Table 2). The duration of embryogeny was significantly ($p<0.05$) increased from 20.00±1.00 hours (at pH 7.0) to 26.00±3.00 hours (pH 10.0). Similarly, mean number of hatched larvae decreased significantly from 218.75±22.34 larvae/ egg raft (at pH 7.0) to 113.25±13.95 larvae/ egg raft (at pH 10.0). Mean WL also reduced significantly from 3.29±0.66 mm (at pH 7.0) to 3.04±0.75 at (pH 10.0), and FA increased from 0.00±0.00 (at pH 7.0) to 0.04±0.02 mm (at pH 10.0).

3.3 Effects of Water Hardness on Duration of Embryogeny, Egg Hatchability, and Adult Fitness

Analyses revealed that water hardness had significant ($p<0.05$) effects on embryonic duration, egg hatchability, wing length and fluctuation asymmetry of *Cx. quinquefasciatus* mosquitoes (Table 3). There was a significant ($p<0.05$) increase in the duration of incubation till hatching (embryogeny) as the degree of water hardness increased, with values ranging from 20.00±4.00 hours (at 10 mg/ $CaCO_3$) to 37.00±9.00 hours (at 300 mg/$CaCO_3$). There was, also, a significant ($p<0.05$) reduction in number of larvae hatched per raft as water hardness level increased; with the highest hatching success recorded as 213.50±23.41 larvae/ raft (at 10 mg/L $CaCO_3$), and

Table 1. Influence of water Temperature on Duration of Embryonic development, Egg Hatchability, and Adult Fitness Attributes of *Culex quinquefasciatus* Mosquitoes

Temperature (ºC)	Duration of Egg incubation till Hatching (Hrs)	Mean Number of Larvae Hatched (per Egg Raft)	Mean Wing Length (mm)	Fluctuation Asymmetry (mm)
Control (28.00±0.02, Ambient)	24.00±4.00^{c*}	204.7±20.27^{c}	3.37±0.02^{c}	0.00±0.00^{a}
30	23.50±3.00^{c}	172.88±38.59^{b}	3.29±0.12^{b}	0.00±0.01^{a}
32	20.50±2.00^{b}	152.88±21.76^{ab}	2.96±0.08^{b}	0.01±0.02^{b}
34	19.00±1.00^{a}	77.13±48.46^{a}	2.99±0.55^{a}	0.04±0.02^{c}

*Values followed by same superscript alphabet in a column are not significant different at $p<0.05$
Values are expressed as Mean±SD of mean

Table 2: Effects of Water pH on Duration of Embryonic development, Egg Hatchability, and Adult Fitness Attributes of *Culex quinquefasciatus* Mosquitoes

pH Level	Duration of Egg incubation till Hatching (Hrs)	Mean Number of Larvae Hatched (per Egg Raft)	Mean Wing Length (mm)	Fluctuation Asymmetry (mm)
4.0	-*	-	-	-
5.0	24.00±3.00^{b**}	154.00±8.36^{c}	3.17±0.13^{c}	0.02±0.01^{b}
6.0	21.00±2.00^{a}	195.50±37.59^{e}	3.17±0.67^{c}	0.01±0.01^{a}
7.0	20.00±1.00^{a}	218.75±22.34^{f}	3.29±0.66^{d}	0.00±0.00^{a}
8.0	21.00±1.00^{a}	173.25±14.22^{d}	3.19±0.87^{c}	0.00±0.01^{a}
9.0	25.00±3.00^{b}	120.50±25.02^{b}	3.14±0.01^{b}	0.02±0.02^{b}
10.0	26.00±3.00^{b}	113.25±13.92^{a}	3.04±0.75^{a}	0.04±0.02^{c}

*No Hatching of Egg Raft, **Values followed by same superscript alphabet in a column are not significant different at $p<0.05$. Values are expressed as Mean±SD of mean

Table 3: Effect of Water Hardness on Duration of Embryonic development, Egg Hatchability, and Adult Fitness Attributes of *Culex quinquefasciatus* Mosquitoes

Water Hardness Regimen (mg/L $CaCO_3$)	Duration of Egg incubation till Hatching (Hrs)	Mean Number of Larvae Hatched (per Egg Raft)	Mean Wing Length (mm)	Fluctuation Asymmetry (mm)
10	18.00±3.00^{a*}	213.50±23.41^{c}	2.95±0.42^{d}	0.00±0.00^{a}
50	19.00±4.00^{a}	214.00±10.22^{c}	2.93±0.23^{d}	0.00±0.00^{a}
100	20.00±4.00^{a}	211.75±12.14^{c}	2.84±0.14^{d}	0.01±0.01^{b}
150	24.00±4.00^{b}	201.00±13.28^{b}	2.57±0.13^{c}	0.02±0.02^{c}
200	24.00±6.00^{b}	184.25±18.02^{b}	2.35±0.18^{b}	0.03±0.01^{c}
250	25.00±6.00^{b}	103.00±24.32^{a}	2.15±0.30^{a}	0.03±0.02^{c}
300	37.00±9.00^{c}	98.00±12.13^{a}	2.10±0.33^{a}	0.03±0.02^{c}

*Values followed by same superscript alphabet in a column are not significant different at $p<0.05$; Values are expressed as Mean±SD of mean the lowest value of 98.00±12.13 larvae/ raft (at 300 mg/L $CaCO_3$). Mean WL also followed the same trend as the number of larvae hatched per egg raft, with values ranging from 2.15±0.33 to 2.95±0.42 mm at 300 and 10 mg/L $CaCO_3$, respectively. However, FA, like duration of embryogeny, varied inversely with water hardness; and were significantly ($p<0.05$) reduced with increase in water hardness levels. It has values ranging from 0.00±0.00 mm (at 10 and 50 mg/L $CaCO_3$) to 0.03±0.02 mm (at 250 and 300 mg/L) (Table 3).

4. DISCUSSION

4.1 Effect of Water Temperature on Entomological Parameters

The temperature at which an insect develops is critical in determining most immature and adult life traits [7]. More so, for optimal growth and development, a temperature range is required [20]; and this relationship is species-specific. In the present study, using *Culex quinquefasciatus* as a model vector, temperature exhibited various relationships with the entomological parameters measured. For example, it had an inverse relationship with duration of embryonic development, hatchability and wing (WL) length, while exhibiting a direct relationship with fluctuating asymmetry (FA).

In the present study, increasing breeding water temperature, significantly, reduced the duration of incubation and number of hatched larvae from egg rafts of *Cx. quinquefasciatus* mosquitoes. It also reduced general biological fitness indices by reducing the size of emergent imagines, using WL as proxy, and vectorial fitness, using FA as proxy. This implies that despite the ability of temperature to increase mosquito proliferation, through its ability to reduce duration of incubation, it significantly reduced other biological fitness indicators.

The reduced duration of embryogeny could be attributed to accelerated growth and development occasioned by increased metabolic rates due to increasing temperature [21]. It seemed, however, that increased temperature became deleterious beyond a threshold and could have led to development of thermal stress [22, 23], which resulted in reduction in hatchability, adult size and FA. Earlier study by Olayemi *et al.* [7] has reported that temperature beyond 30°C, significantly reduced fitness of mosquito. Tun-lin *et al.* [24] and Schneider *et al.* [25] had reported similar reduction in biological fitness of mosquitoes with increasing water temperature.

In the present study, the mosquito's egg rafts hatched at all temperatures tested (from 28 to 34°C), however, earlier studies by Azad and Dave [26] reported a temperature range of 26.6 to 28°C, as being optimal and suitable for hatching of mosquito eggs. Further, WL decreased with increase in temperature, while FA significantly increased. The mean WL (mm) of *Culex quinquefasciatus* mosquitoes decreased, significantly, with increase in temperature, with the highest fluctuating asymmetry at 34°C. Olayemi *et al.* [7], Loetti *et al.* [20], and Alto and Juliano [27] had observed similar observations in fluctuation asymmetry in laboratory reared-mosquito due to varying temperatures. The mosquitoes in this study were generally composed of relatively large individuals as their wing lengths were above 3.5 mm. Wing length of 3 mm is regarded, generally, as threshold requirement for significant vectorial capacity in *Culex* species [28] and bigger mosquitoes have been reported to live longer and lay more eggs than their smaller counterparts [29].

This information is critical in the development of strategic mosquito control interventions, bearing in mind the crucial role temperature play in the bio-ecophysiology of mosquitoes. More so, based on the ongoing global temperature increase due to climate change, mosquitoes in the Tropics may suffer casualty due to possibilities of reduction in mosquito egg hatching rates and eventual reduction in their population density with the resultant reduction in mosquito-vectored diseases.

4.2. Effect of Water pH Entomological Parameters

The study on effects of water pH on duration of embryonic development, egg hatchability, WL and FA of *Culex quinquefasciatus* revealed significant effects of this factor on all parameters studied. There was a general trend of occurrence in this laboratory investigation. There was a significant reduction in the performance of the mosquito as pH tilted towards acidity and alkalinity from neutrality (pH 7.0). Among the pH values tested, extreme acidic condition (pH 4.0) did not support embryology and eclosion, while, extreme alkaline (pH 10.0) did; though, with a significant increase in duration of embryogeny. Similarly, mean number of hatched larvae and WL decreased significantly as pH conditions shifted from neutrality to extremes. However, FA increased, significantly, as pH conditions tilted to extremities from neutrality.

The non-hatchability of egg rafts at pH 4.0 could be due to ovicidal potential of acidic conditions, which may not favor egg hatching. The decreased mean number of hatched larvae at extreme pH conditions could have resulted from the fatality of these conditions: as highly acidic conditions are lethal to early stages of mosquitoes [30]. The pH of neutrality (7.0) supported highest performance of the mosquito species (in terms of duration of embryogeny, hatchability rates, WL and FA) and may be the average tolerable pH condition for the species. This pH value has, also been reported to be the most preferable condition for breeding of many species of mosquitoes [31].

Gaunder [32] reported acid and alkaline death points for aquatic organism (fish) as about pH 4.0, and 6.8, respectively, with reproduction and growth diminishing with increasing acidity or alkalinity. Willkie and Wood [33] and Patz *et al.* [34] also observed that if pH falls below the tolerance range, death would ensue as result of disturbance of the acid and base of the hemolymph. Although, analyses revealed no significant ($p>0.05$) effect of water pH on body size of *Culex quinquefasciatus* mosquitoes (using wing length as proxy) at pH 5.0, 6.0, and 8.0, mosquitoes raised on pH 10 were the smallest, while those on pH 7 were the biggest. The latter mosquitoes would live longer, mate successfully [35, 36], and lay more eggs [29] and, may perhaps, be better vectors of disease.

This information may provide insight into the role played by pH conditions in the control of mosquito proliferation in the wild. Such information could be handy in the distribution of scarce economic resources in vector control interventions; such resources could, perhaps, be channeled to controlling vectors in habitats with most suitable favorable-breeding pH values, while allowing 'nature' to control those at deleterious pH conditions.

4.3. Effect of Water Hardness on Entomological Parameters

The presence of Calcium ion (Ca^{2+}) in water in form of $CaCO_3$ is responsible for its hardness properties [37]. Calcium is also important for the normal growth, development and physiology of insects, especially, mosquitoes [38]. It is present in cuticles, where it reinforces the exoskeleton [39], thus contributing insects' first line of defense. However, just as the quantities of $CaCO_3$ present in water determines its hardness properties, it also determine the productivity of mosquito larval habitat [40, 41]. It therefore, connotes that as other physicochemical parameters, necessary for the normal physiology, there must be a range of water hardness levels that would support the proliferation of mosquitoes, outside which, development is impaired or totally halted.

In the present study, water hardness significantly ($p<0.05$) affected duration of embryogeny, hatchability of egg-rafts, WL and FA of *Culex quinquefasciatus* mosquito. The mosquitoes performed, significantly, best at hardness range of 10 – 100 mg/L $CaCO_3$, and worst at 300 mg/L $CaCO_3$. For example, while water hardness range of 10 – 100 mg/L $CaCO_3$ elicited shortest incubation periods, highest numbers of hatched larvae, bigger mosquitoes and less stressed mosquitoes, the reverse were observed at hardness range of 200 to 300 mg/L $CaCO_3$. This implies that mosquitoes reared in the former range of water hardness levels, perhaps, may be better vectors of disease than those from the latter. Earlier study by Abernathy [42] reported that very soft waters (6.4 - 7.0 mg/L $CaCO_3$) produced the highest egg hatched and survivorship of Rainbow shark minnow fish, while Milad *et al*. [18] reported lowest egg hatchability rate at water hardness level of 300 mg/L $CaCO_3$. Mwangangi *et al.* [43] reported that wells having hard waters does not favor the proliferations of mosquitoes. Laboratory studies by Molokwu and Okpokwasili [44] and Blanksma *et al.* [45] has also shown that soft waters within the range found in this present study favors the hatching and development of *Clarias gariepinus* and development of Juvenile fathead minnows, respectively. While field studies by Oyewole *et al.* [46] and Olayemi *et al.* [47] showed that mosquitoes thrive best within the range of 10 - 100 mg/ $CaCO_3$. From the foregoing, it implies that a sound knowledge of water hardness levels regulate the growth and development of mosquitoes is pertinent in the development of cost-effective control strategies. For example, mosquito larval habitats having very high water hardness level may not support proliferation of mosquitoes and/ or may produce mosquitoes less fit to transmit disease-causing pathogens.

5. CONCLUSION

This research work has established that, water temperature, pH and hardness have affected the egg hatchability rate of *Culex quinquefasciatus* mosquitoes in Minna. More so, water temperature of 28 ºC, pH of 7.0 and hardness level ≤50 mg/L favors mosquito breeding dynamics. Therefore, the results of this present study could serve as vital information in developing mosquito vector control by manipulating environmental factors (temperature, pH and water hardness) in the microhabitat of a species.

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Conflicts of Interest

There are no conflicts of interest.

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