Ukubuiwe et al. The Journal of Basic and Applied Zoology (2020) 81:5
https://doi.org/10.1186/s41936-020-0139-6

The Journal of Basic and Applied Zoology

RESEARCH

Open Access

Quantifying the roles of water pH and hardness levels in development and biological fitness indices of *Culex quinquefasciatus* Say (Diptera: Culicidae)

Azubuike Christian Ukubuiwe[1*], Chioma Cynthia Ojianwuna[2], Israel Kayode Olayemi[3], Francis Ofurum Arimoro[4] and Chinenye Catherine Ukubuiwe[5]

Abstract

Background: The present study was designed to quantify the contributions of water pH and hardness required for development and adult fitness indices of *Culex quinquefasciatus* (*Cx. quinquefasciatus*) mosquitoes. To this end, seven water pH conditions (pH 4.0–10.0) and five hardness levels (0, 30, 90, 150 and 210 mg/L $CaCO_3$) were simulated following standard protocols. Day-old larvae of *Culex quinquefasciatus* were reared in these simulated water-media conditions till emergence. Entomological indices for immature developmental success and adult biological fitness parameters were measured.

Result: The results revealed significant effects of pH and water levels on the entomological parameters measured for the species. pH values of 4 and 10 and high hardness values ≥ 150 mg/L $CaCO_3$ reduced immature developmental successes and adult biological fitness indices. The optimum range of values for the development of the species is respectively pH 5–8 and 0–90 mg/L $CaCO_3$.

Conclusion: The present study reveals the significant negative influence of extreme pH and hardness levels on mosquito development and fitness indices; thus, it may be providing baseline information for developing sustainable robust vector control strategies for disease reduction through habitat manipulation.

Keywords: *Culex quinquefasciatus*, pH and hardness levels, Biological fitness indices, Minna

Background

Culex quinquefasciatus Say, 1823 mosquito is the common southern house mosquito. It is the principal vector of *Wuchereria bancrofti*, responsible for lymphatic filariasis, a debilitating public health disease (Elkanah et al., 2017; World Health Organization, WHO, 2017). The physico-chemical properties of the mosquito larval habitats determine its quality, in terms of attractiveness to gravid female mosquitoes for oviposition (Braks, Leal, & Cardé, 2007; Eneanya et al., 2018), developmental success of immature mosquitoes (Oyewole et al., 2009), species distribution (Minakawa, Mutero, Githure, Beier, & Yan, 1999) and adult life traits (Mwangangi et al., 2007). Among these physico-chemical components are water pH and hardness levels.

These components are affected by various factors such as the nature of underlying rock, agricultural inputs and anthropogenic activities (Rim-Rukeh, 2013; Robert, Awono-Ambene, & Thiolouse, 1998; Salihu et al., 2017; Singare, Lokhande, & Pathak, 2010). Having quantitative data on the influence of these two parameters on the development and fitness of mosquito vectors is important. The information will update, firstly, the current literature on biology of the vector and, secondly, the role the parameters play in biological fitness for disease transmission and, thirdly, provide information on the potential use of such parameters in vector control via environmental manipulation.

* Correspondence: a.ukubuiwe@futminna.edu.ng
[1]Applied Entomology Unit, Department of Animal Biology Sciences, Federal University of Technology, Minna, Nigeria
Full list of author information is available at the end of the article

For aquatic insects, ambient pH levels influence balance in body fluid ionic composition, which is chief in maintaining homeostasis and development (Daily, Hitt, Smith, & Snyder, 2012; Gillott, 2005; Patrick & Bradley, 2000). The pH of an aquatic habitat is also a major physical factor that limits survivorship and, hence, distribution of mosquito species (Yee, Kneitel, & Juliano, 2010), affecting availability of essential mineral and food elements for the development of mosquitoes (Clements, 2000). Studies have shown that mosquito larvae are capable of adapting to and tolerating fluctuations in ionic levels in these habitats (Nadia, Thamer, & Saad, 2005; Patrick & Bradley, 2000; Wigglesworth, 1933). Even though different ranges of pH values have been reported to support development and survivorship of mosquitoes in larval habitats in the wild, there is dearth of information on systematic studies on the influence of these ranges of pH on life history traits and/or contribution to biological fitness of mosquitoes.

Water hardness, on the other hand, is produced by the presence of cations of manganese, magnesium, calcium and ferrous iron. The relative quantities of these ions in water determine its level of hardness (Regional Aquatics Monitoring Program, RAMP, 2015). These ions, depending on concentration, play physiologic roles in structural formation, protection, metabolism, developmental and survivorship in aquatic organisms (Blanksma et al., 2009; Leschen & Cutler, 1994; Molokwu & Okpokwasili, 2004; Pelizza, Lopez-Lastra, Becnel, Bisaro, & Garcia, 2007; Poteat & Buchwalter, 2014). In nature, water hardness levels determine the quality of most aquatic habitat (Milad, Mohammed, & Seyed, 2011) and productivity of mosquito habitats, especially, domestic wells (Robert et al., 1998).

Studies have shown that despite the importance of physico-chemical factors in the development of aquatic organisms, especially mosquitoes, there are permissible levels for optimal immature development, which in turn determine the quality of adult life traits. These permissibility levels are usually species- and life stage-specific; outside these limits, stress-like conditions set in, affecting growth negatively by eliciting mortality (Mgbemena & Ebe, 2012; Mpho, Callaghan, & Holloway, 2002; Ukubuiwe et al., 2018a; Ukubuiwe, Olayemi, Omalu, Arimoro, & Ukubuiwe, 2018b). Further, environmental stress conditions greatly affect feeding rates in aquatic organisms (Merritt, Dadd, & Walker, 1992; Timmermann & Briegel, 1993), with its attendant reduction in metabolic energy and reserves (Timmermann & Briegel, 1999).

Unfortunately, in mosquito bioecology, data gathered from field observations on water pH and hardness levels usually form the basis for extrapolating their effects on bionomics of mosquitoes (Garba & Olayemi, 2015; Mwangangi et al., 2007). These data, though important, are not sufficient in understanding the actual contributions of these critical larval breeding factors to mosquito growth, hence the need for this study.

In the present study, a systematic report on the influence of varying levels of water pH and hardness levels (separately) on developmental successes and adult fitness attributes in *Cx. quinquefasciatus* mosquito was investigated. This is to provide first-hand quantitative information on the contribution of these critical micro-environmental larval habitat factors to vectorial success of mosquito species.

Materials and methods

Preparation of water media

Seven water pH levels and five hardness levels were prepared following standard procedures. The pH values (4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) were prepared according to techniques of Pelizza et al. (2007) and Ivoke, Mgbenka, and Okeke (2007), with slight modification. The levels were measured using a digital pH meter (model: Jenway 3305).

The water hardness regimens (0 (soft), 30 (slightly hard water), 90 (moderately hard water), 150 (hard water) and 210 mg/L $CaCO_3$ (very hard water)) were prepared based on standard classification of water hardness as described by Milad et al. (2011) and reported by Ukubuiwe et al. (2018c).

Source, rearing and maintenance of experimental insects

Culex quinquefasciatus mosquitoes were collected from a colony of the species maintained in the Insectary Unit of the Department of Animal Biology, Federal University of Technology, Minna. First larval instars of the mosquito species were reared in the prepared artificial (pH and hardness) water media according to the methods of Ukubuiwe et al. (2012). The pupae on pupating were placed in plastic bowls half-filled with the artificial media and kept in adult-holding cages until emergence. The adults were maintained on 10% sucrose solution (Ukubuiwe, Olayemi, Omalu, Arimoro, Baba, et al., 2018).

Entomological parameters measured

The effects of water pH and hardness levels on the immature survivorship, duration of development and larval growth rates of the mosquitoes were recorded daily at a 12-h interval (Timmermann & Briegel, 1999; Ukubuiwe, Olayemi, & Jibrin, 2016). After emergence, the numbers of emergent male and female mosquitoes were counted (while fed 10% sucrose solution). The longevity and daily mortality of the adult mosquitoes were recorded (Ukubuiwe, Olayemi, Omalu, Arimoro, Baba, et al., 2018). Adult fitness attributes of the mosquitoes were estimated from the wing lengths (Ukubuiwe, Olayemi, Omalu, Jibrin, & Oyibo-Usman, 2013).

Data analysis

Data generated from the independent studies were processed into means and standard deviation (mean ± SD) using Microsoft Office Excel 2016 and Statistical Package for Social Scientists (SPSS) version 21. Differences between means of any two entomologic variables (e.g. between the wing lengths of male and female mosquitoes from regimens of a physico-chemical factor) were compared using Student t test, while those among entomologic variables (e.g. among sexes from the various regimens of a physico-chemical factor) were compared for significant difference using one- or two-way analysis of variance (ANOVA), as appropriate. The differences in means were separated using Duncan multiple range test (DMRT). All decisions on the level of significance for statistical comparison of means were assumed at $p = 0.05$.

Results

Effects of rearing-water pH and hardness levels on the duration of development and larval growth of *Culex quinquefasciatus*

Analyses revealed that extreme pH and hardness conditions had significant negative effects on developmental times and larval growth rates of the species (Table 1 and Fig. 1a, b). Larvae reared at pH 7.0 had the shortest duration of development (i.e. fastest development), while those at extreme acidic (4.0) or alkaline (10.0) pH conditions had, significantly (df = 6, $p < 0.05$), increased duration of development (Fig. 1a). As water hardness level increased, the duration of the development of the species gradually increases, with cohorts reared at the highest concentration (210 mg/L $CaCO_3$ spending the longest time as immature (Fig. 1b). Unlike the larvae, developmental times of pupae were not affected at all pH (df = 6, $p = 0.204$) and hardness (df = 4, $p = 0.181$) conditions tested (Table 1).

Developmental time (i.e. total larval + pupal durations) also followed similar pattern as the larval life stages, i.e. delayed development at extreme water conditions. At pH 10.0 and 210 mg/L $CaCO_3$, the species had the longest duration of development. On the other hand, immature mosquitoes reared at pH 7.0 and 0 mg/L $CaCO_3$ had the shortest developmental times (Table 1).

Larval growth rate (LGR) of the species was also significantly affected by both water conditions (i.e. pH and hardness). The larval size increased as pH tilted towards neutrality. However, with increasing alkaline conditions, larval size started reducing and was lowest at pH 10.0 (Table 2). Similarly, water hardness condition had an inverse relationship with LGR, i.e. increase in hardness resulted in reduced LGR, although at lower hardness levels (≤ 30 mg/L $CaCO_3$) analyses revealed no significant difference (df = 4, $p = 8.14$) in LGR.

Effects of rearing-water pH and water hardness level on life-stage survivorship of *Culex quinquefasciatus*

Analyses revealed significant effects of increasing pH and hardness conditions on survivorship of all life stages of the species (Fig. 2a, b and Table 3). Larval instar survivorship was the highest at pH levels of 5.0–8.0, and the lowest at pH 4.0 and 10.0 (Fig. 2a). Similarly, hardness levels ranging from 0 to 90 mg/L $CaCO_3$ (i.e. ≤ moderately hard water) supported the highest survivorship (Fig. 2b). Among larval instars, LI was the most affected, especially at the highest concentration tested (i.e. 210 mg/L $CaCO_3$). Pupal survivorship, on the other hand, was the highest at pH 8.0 and the lowest at pH 10.0 (Table 3).

Table 1 Effects of water pH and hardness levels on the duration of the development and growth rate of *Culex quinquefasciatus*

Physico-chemical parameter	Concentration	Stage duration (days)		
		Larvae	Pupae	Total
pH levels	4.0	11.69 ± 0.04^{e*}	0.76 ± 0.16^{a}	12.46 ± 0.50^{f}
	5.0	8.76 ± 1.97^{c}	0.82 ± 0.09^{a}	9.58 ± 1.92^{d}
	6.0	8.19 ± 0.93^{b}	0.82 ± 0.06^{a}	9.01 ± 0.94^{c}
	7.0	6.68 ± 0.75^{a}	0.83 ± 0.11^{a}	7.51 ± 0.81^{a}
	8.0	7.83 ± 0.74^{ab}	0.79 ± 0.06^{a}	8.62 ± 0.74^{b}
	9.0	9.81 ± 0.94^{d}	0.84 ± 0.08^{a}	10.65 ± 0.96^{e}
	10.0	12.54 ± 0.34^{f}	0.87 ± 0.29^{a}	13.41 ± 0.46^{g}
Hardness regimen (mg/L $CaCO_3$)	0	6.96 ± 0.30^{a}	1.17 ± 0.01^{a}	8.13 ± 0.30^{a}
	30	8.33 ± 0.19^{b}	1.16 ± 0.07^{a}	9.46 ± 0.16^{b}
	90	9.20 ± 0.14^{c}	1.25 ± 0.01^{ab}	10.45 ± 0.13^{c}
	150	10.87 ± 0.18^{d}	1.33 ± 0.11^{b}	12.20 ± 0.24^{d}
	210	15.85 ± 0.40^{e}	1.48 ± 0.05^{c}	17.33 ± 0.45^{e}

* Within a column for a physico-chemical parameter, means (± SD) followed by the same letter are not significantly different at $p < 0.05$ according to Duncan multiple range test following analysis of variance (ANOVA)

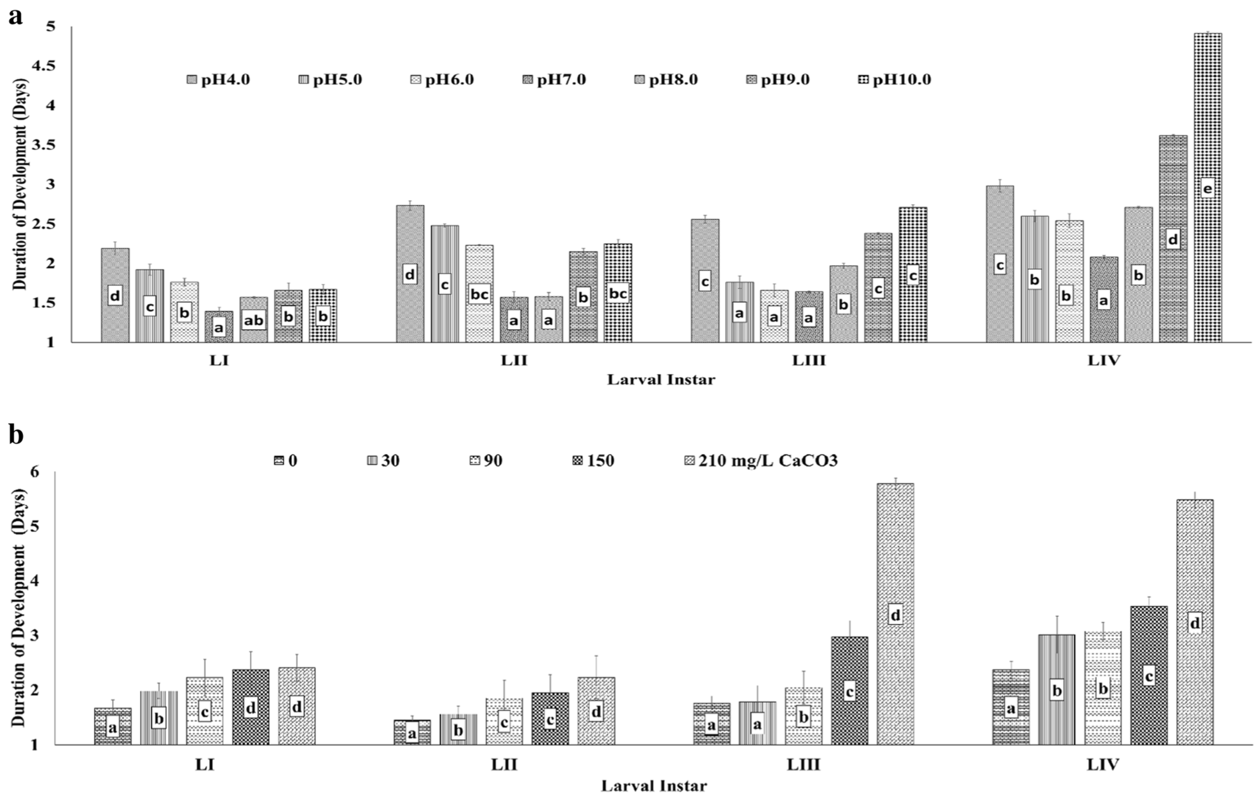

Fig. 1 Bars followed with the same letter are not significantly different at $p < 0.05$ according to Duncan multiple range test following ANOVA and represent mean (± SD). **a** Effects of water pH on developmental times of larval instars (I–IV) of *Culex quinquefasciatus*. **b** Effects of water hardness on developmental times of larval instars (I–IV) of *Culex quinquefasciatus*

Daily adult survivorship of the species reduced significantly with the increase in physico-chemical conditions. At extreme acidic (pH 4.0) and alkaline (pH 10.0) conditions, survivorship was the lowest and was respectively 51.93 ± 16.30 and 61.13 ± 7.24%; meanwhile, at pH range of 5.0–9.0 (range = 76.16 ± 2.29–82.43 ± 1.52%), adult survivorship was the highest (Table 3). Likewise, adult mosquitoes reared at 0 to 90 mg/$CaCO_3$ (i.e. ≤ moderately hard water) had the highest survivorship (range = 74.48 ± 6.20 to 78.16 ± 7.34%), while those reared in 'very

Table 2 Effects of water pH and hardness levels on growth rate of *Culex quinquefasciatus*

Physico-chemical parameter	Concentration	Larval growth rate (mg/day)
pH levels	4.0	0.0468 ± 0.02514[a]
	5.0	0.0571 ± 0.0165[c]
	6.0	0.0611 ± 0.0067[c]
	7.0	0.0749 ± 0.0080[d]
	8.0	0.0639 ± 0.0065[c]
	9.0	0.0509 ± 0.0049[b]
	10.0	0.0403 ± 0.0474[a]
Hardness regimen (mg/L $CaCO_3$)	0	0.0599 ± 0.0022[d]
	30	0.0586 ± 0.0014[d]
	90	0.0561 ± 0.0009[c]
	150	0.0526 ± 0.0010[b]
	210	0.0316 ± 0.0011[a]

* Within a column for a physico-chemical parameter, means (± SD) followed by the same letter are not significantly different at $p < 0.05$ according to Duncan multiple range test following analysis of variance (ANOVA)

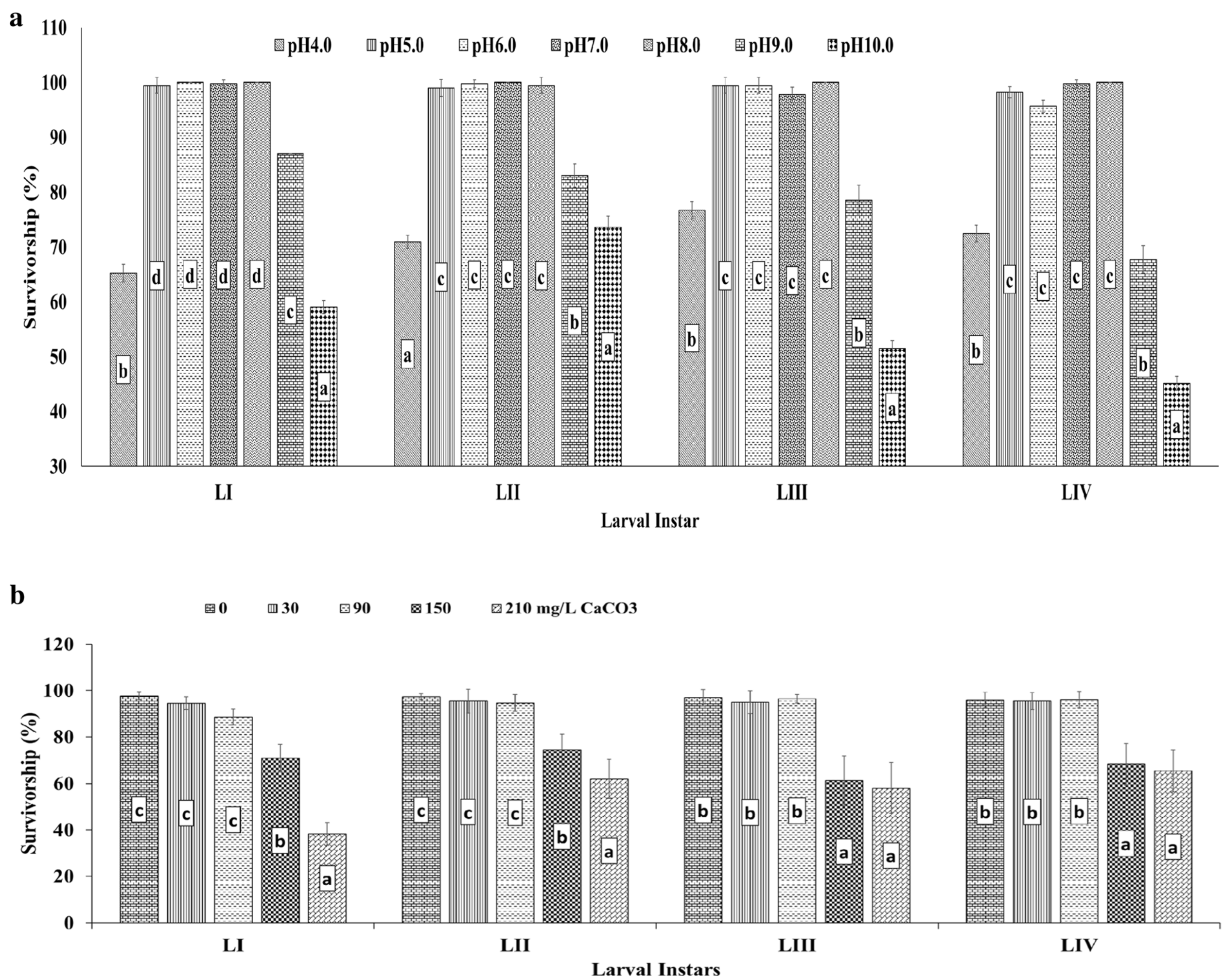

Fig. 2 Bars followed with the same letter are not significantly different at $p < 0.05$ according to Duncan multiple range test following ANOVA and represent mean (± SD). **a** Effects of water pH on daily survivorship of larval instars of *Culex quinquefasciatus*. **b** Effects of water hardness on daily survivorship of larval instars of *Culex quinquefasciatus*

hard' water condition (210 mg/L $CaCO_3$) had the lowest (44.93 ± 8.41%) (Table 3).

Generally, survivorship among sexes decreased with the increase in water conditions (Fig. 3). However, between sexes in a factor, male (♂) mosquitoes were more affected than their female (♀) counterparts. In water pH treatments, ♂ mosquitoes (range = 33.99 ± 39.39 to 80.92 ± 3.50%) were more affected than the ♀ mosquitoes (range = 57.86 ± 13.08 to 84.35 ± 1.70%). Similar trends were seen among water hardness treatments. The range for ♂ mosquitoes is 69.78 ± 7.20 to 73.94 ± 4.08% and for ♀ mosquitoes, 51.80 ± 22.74 to 80.72 ± 8.01%.

Effects of rearing-water pH and hardness on emergence success and longevity of *Culex quinquefasciatus*

Analyses showed significant ($p < 0.05$) negative effects of extremes of pH on emergence success and longevity of the species, as the least numbers of adult emerged at pH 4.0 and 10.0. On the other hand, the highest emergence success rates were obtained from pH range of 5.0 to 9.0 (range = 46.75 ± 2.05 to 49.63 ± 0.52 adults/50 larvae) (Table 4). Water hardness showed similar inverse relationship with these parameters, with increased water hardness conditions, significantly, reducing emergence success (range = 10.38 ± 3.16 to 88.00 ± 4.11 adults/100 larvae) (Table 4).

Post-emergence longevity (PEL) of the adult mosquitoes was also affected by pH regimens. Adult mosquitoes from water media with pH range of 6.0–8.0 lived the longest (> 18 days), whereas pH conditions below or above this range (i.e. < pH 6.0 or > pH 8.0) reduced adult longevity. The shortest longevity was observed in mosquito cohorts from pH 4.0 and 10.0 (Table 3). Mosquito cohorts from hardness regimen of 0–90 mg/L $CaCO_3$ lived longer (range = 15.07 ± 1.17 to 16.16 ± 0.80 days) than those from higher hardness levels (< 10 days) (Table 4).

Table 3 Effects of water pH and hardness level on life stages' survivorship of *Culex quinquefasciatus*

Physico-chemical condition	Concentration	Survivorship (%)			
		Larvae	Pupae	Average immature	Adult
pH levels	4.0	63.51 ± 12.33[a]	84.73 ± 2.364[b]	59.28 ± 18.33[a]	61.13 ± 7.24[b]
	5.0	99.05 ± 0.64[b]	98.96 ± 1.57[c]	99.04 ± 0.49[b]	79.04 ± 3.56[c]
	6.0	98.73 ± 1.53[b]	98.20 ± 2.32[c]	98.65 ± 1.17[b]	81.01 ± 3.70[c]
	7.0	99.31 ± 1.56[b]	96.57 ± 4.55[c]	98.76 ± 1.79[b]	80.67 ± 2.42[c]
	8.0	99.88 ± 0.35[b]	99.25 ± 1.04[c]	99.75 ± 0.43[b]	82.43 ± 1.52[c]
	9.0	98.80 ± 0.77[b]	97.63 ± 2.89[c]	98.57 ± 0.89[b]	76.16 ± 2.29[c]
	10.0	57.31 ± 21.85[a]	55.27 ± 46.33[a]	50.27 ± 23.35[a]	51.93 ± 16.30[a]
Hardness regimen (mg/L $CaCO_3$)	0	96.93 ± 1.16[c]	99.73 ± 0.77[c]	97.49 ± 0.88[c]	74.48 ± 6.20[c]
	30	95.06 ± 1.50[c]	94.81 ± 5.79[c]	95.01 ± 1.60[c]	76.92 ± 6.89[c]
	90	93.94 ± 1.15[c]	98.32 ± 2.18[c]	94.82 ± 1.28[c]	78.16 ± 7.34[c]
	150	81.69 ± 3.06[b]	85.80 ± 9.88[b]	82.51 ± 3.00[b]	61.36 ± 5.48[b]
	210	55.90 ± 11.32[a]	58.89 ± 15.89[a]	56.50 ± 11.05[a]	44.93 ± 8.41[a]

Within a column for a physico-chemical parameter, means (± SD) followed by the same letter are not significantly different at $p < 0.05$ according to Duncan multiple range test following analysis of variance (ANOVA)

Discussion

Effects of rearing-water pH and hardness conditions on duration of development and larval growth rate of *Culex quinquefasciatus*

In the present study, rearing-water with pH values ranging from 4.0 to 10.0 was used to rear immature *Cx. quinquefasciatus* mosquitoes to determine the effects on the duration of the development and larval growth rate of the species. The result showed a growth regulating effect of pH on the species, with extreme pH values eliciting, significantly (df = 6, $p < 0.05$), longer durations of development of the species. Those reared at pH 7.0 were the fastest developer, while those reared at pH values of 4.0, 9.0 and 10.0 were the slowest. Earlier, Woodhill (1942) and Tanimura and Sonoji (1952) reported that pH ranges of 3.6–4.2 increased the development times in *Aedes aegypti*).

This ability of *Cx. quinquefasciatus* to develop in the pH ranges tested, though with decreased survivorship at extreme pH conditions, may suggest possible tolerance of the species to a wide range of pH conditions. Although there is little or no field information to support the development of the species at all the water pH ranges studied, field reports from the study area (Minna) and areas with similar ecotype suggest that pH range of 6.0 to 8.0 is ideal for the development of the species in nature (Garba & Olayemi, 2015; Ikeh et al., 2017; Olayemi, Omalu, Famotele, Shegna, & Idris, 2010; Salihu

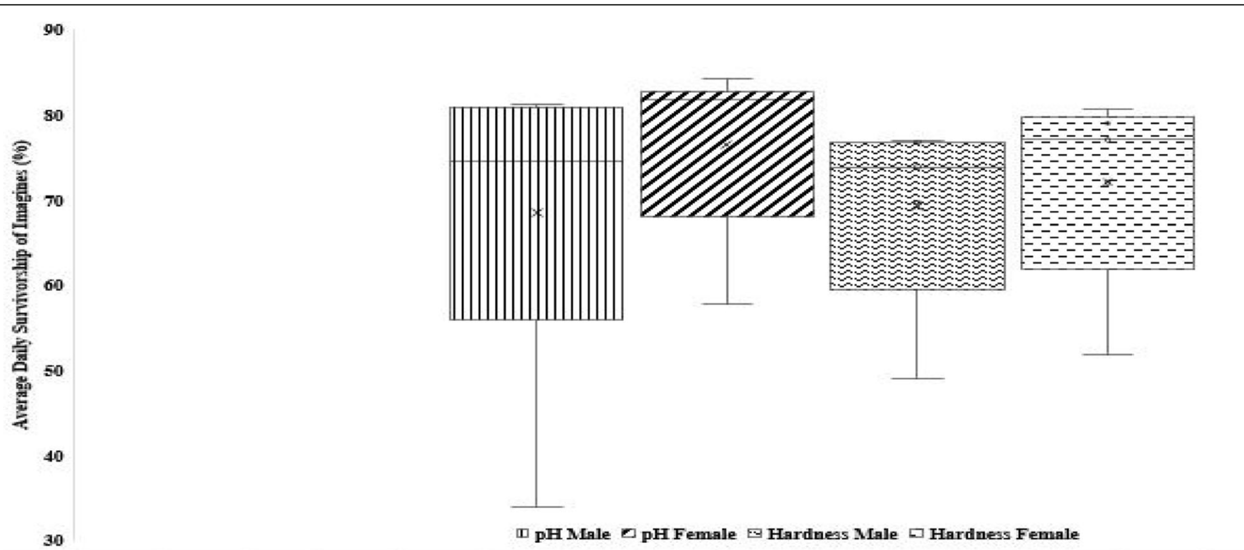

Fig. 3 Biplots of effects of water pH and hardness on daily survivorship of male and female adult life stages of *Culex quinquefasciatus*

Table 4 Effects of water pH and hardness levels on emergence rates and longevity of adult life stage of *Culex quinquefasciatus*

Physico-chemical parameter	Concentration	Number of emergent imagoes	Average post-emergence longevity (days)****
pH levels	4.0	12.75** ± 6.85[b]*	9.73 ± 2.52[a]
	5.0	47.13 ± 1.13[c]	14.01 ± 1.79[b]
	6.0	46.87 ± 2.75[c]	20.59 ± 2.26[c]
	7.0	47.00 ± 4.28[c]	20.11 ± 1.84[c]
	8.0	49.63 ± 0.52[c]	20.04 ± 1.20[c]
	9.0	46.75 ± 2.05[c]	13.07 ± 0.83[b]
	10.0	8.75 ± 8.26[a]	9.53 ± 1.09[a]
Hardness regimen (mg/L $CaCO_3$)	0	88.00*** ± 4.11[d]	15.07 ± 1.17[c]
	30	77.38 ± 7.05[c]	16.16 ± 0.80[c]
	90	76.38 ± 5.10[c]	15.63 ± 2.30[c]
	150	37.25 ± 6.61[b]	8.20 ± 1.34[b]
	210	10.38 ± 3.16[a]	6.18 ± 1.27[a]

*Within a column, means (± SD) followed by the same letter are not significantly different at $p < 0.05$ according to Duncan multiple range test following analysis of variance (ANOVA). **Per 50 larvae. ***Per 100 larvae. ****Adult was sugar fed only

et al., 2017). This study, thus, peeks into resultant effects of these pH ranges on the species (*Cx. quinquefasciatus*) in conditions, where the habitats have been altered, passively, by anthropogenic activities or, actively, as a vector control strategy.

Species-specific range of pH conditions for the development of other mosquito species has been reported and included 3.3 to 8.1 for *Ochlerotatus taeniorhynchus*, 4.4 to 9.3 for *Aedes geniculatus* and *Anopheles plumbeus*, 3.3 to 9.2 for *Psorophora confinnis*, 2 to 9 for *Aedes flavopictus* and 2 to 10 for *Armigeres subalbatus* (Christophers, 1960). In the present study, the duration of the development of all immature life instars and stages (i.e. larval instars and pupae) of *Cx. quinquefasciatus* was significantly affected by water hardness. As water hardness level increased progressively, from 0 to 210 mg/L $CaCO_3$, there was a significant delay in the development. The species spent about twice the developmental time at 0 mg/L $CaCO_3$ to complete the development at 210 mg/L $CaCO_3$. This shows that the quantity of $CaCO_3$ present in a mosquito larval breeding habitat, though necessary for normal development, can determine the time taken to reach adulthood.

In this study, moderately 'hard water' supported the fastest development of the species. Similar water conditions have been reported for optimum development in laboratory studies for other aquatic organisms (Abernathy, 2004; Blanksma et al., 2009; Milad et al., 2011; Molokwu & Okpokwasili, 2004), and in field studies for mosquitoes (Kant, Pandey, & Sharma, 1996; Mgbemena, Opara, Osuala, & Iwuala, 2009; Mwangangi et al., 2007; Olayemi et al., 2010; Piyaratne, Amerasinghe, Amerasinghe, & Konradsen, 2005).

In the present study, larval growth rate, LGR, an indicator of daily weight accumulation, was significantly affected by pH and hardness levels. For example, LGR was the highest at pH value of 7.0 and the lowest at pH 10.0. The low LGR at pH 10 may indicate impaired feeding and/or growth processes during the phagoperiods. This impairment could probably be stress-related and/or disruption of physiological process, which affects the feeding process and accumulation of tenerals (Lazareviæ, Periæ-Mataruga, Vlahoviæ, Mrdakoviæ, & Cvetanoviæ, 2004). More so, neutral pH has been reported as ideal (in nature) for breeding of *Culex* mosquitoes (Sattler et al., 2005).

Effects of rearing-water pH and hardness levels on life-stage survivorship of life stages of *Culex quinquefasciatus*

One of the most important factors that serve as an index for pollution and survivorship of an organism is pH (Singare et al., 2010). In the present study, pH values ranging from 4.0 to 10.0 supported the survivorship of *Cx. quinquefasciatus* mosquitoes though affecting the life stages in various ways. This effect was, however, pH level- and life-stage-dependent. Though there are no field investigations or reports suggesting that the species thrive in this range of pH values (i.e. 4.0 to 10.0), generally, in this study, extreme values of pH (i.e. either too acidic or basic) significantly reduced survivorship, with the species exhibiting some degree of adaptation/tolerance to the extreme conditions by surviving till adulthood.

Further, even though the mosquito species showed some element of survivorship in all pH regimen investigated, it had higher survivorship at pH values ranging from 5 to 9; outside these, survivorship significantly reduced. These ranges may, perhaps, indicate the survival pH range for the species. Buchman (1931) had earlier reported that larvae of *Culex pipiens* lived in water at pH from 4.4 to 8.5, but died beyond these values.

Although little or no reports have shown that *Cx.* mosquitoes thrive in habitats with pH values of 4.0 and 10.0, the present study reveals that survivorship at these values may be low. It should be noted that in controlled experimentations (laboratory conditions), mosquito survivorship is usually high, when compared with field population. However, despite these expectations, survivorship at pH 4 and 10 was still low. It, therefore, means that, perhaps, where these pH conditions occur (either naturally or induced artificially as a vector control strategy) may elicit greater mortality in the wild (i.e. field/uncontrolled condition).

The survivorship recorded for this species (in this study) at extreme pH conditions could be attributed to tolerance, acclimation and regulatory abilities of the species to these extreme conditions. This is important as several coping capabilities which play vital role in species distribution under field conditions have been reported (Bayly, 1972; Bradley, 1994; Wigglesworth, 1933) Similar abilities have been reported for two members of the mosquito tribe Aedini, *Aedes aegypti* and *Ochlerotatus taeniorhynchus* (Clark, Flis, & Remold, 2004), and *Anopheles plumbeus* (Christophers, 1960), with remarkably different regulatory mechanisms for dealing with ionic and stressful environment (Patrick & Bradley, 2000).

In the present study, there was a significant reduction in larval and pupal survivorship at very high hardness level (above 90 mg/L $CaCO_3$). However, survivorship of both immature life stages was not affected by hardness level at and below 90 mg/L $CaCO_3$ (i.e. moderately 'hard water').

Despite the relatively high adult daily survivorship (> 50%) recorded for the species at the pH levels tested, extreme pH conditions (i.e. pH 4.0 and 10.0) elicited lower adult survivorship. Meanwhile, pH range of 5.0–9.0 supported very high survivorship. Furthermore, survivorship between the male and female of a regimen was not significantly affected. Water hardness also significantly affected daily survivorship of adult mosquito of the species, as hardness conditions above 90 mg/L $CaCO_3$ (i.e. moderately 'hard water') significantly lowered survivorship. This is interesting, as it may explain the variation in productivity of domestic and market wells, where the water was reported to be 'very hard' (Robert et al., 1998), and the reason why mosquito actively avoids well with such conditions (Rim-Rukeh, 2013). The present study also provides explanation for an earlier observation (100% mortality) made in an attempt to rear mosquitoes using very hard water (unpublished data).

Effects of rearing-water pH and hardness level on emergence and longevity of *Culex quinquefasciatus*

The quality of a mosquito larval habitat determines the productivity of the habitat, in terms of number at emergence, post-emergence survivorship and longevity (Minakawa, Sonye, & Yan, 2005). Furthermore, the quality of the habitat is greatly affected by the availability of mineral element and food elements important for the development, which is chiefly determined by the pH and hardness conditions of the water habitat (Clark et al., 2004).

In the present study, pH values greatly affected the number of emergent adult mosquito, with significantly higher number of emerging female than male mosquitoes at all pH levels investigated. Significantly lower numbers of emergent were observed at pH values of 4.0 and 10.0. Post-emergence longevity was significantly affected by the pH value of water used for rearing. Those reared at pH 6.0 to 8.0 lived the longest than those from other regimens; the shortest-lived were from pH 4.0 and 10.0. Woodhill (1942) noted that at pH 3.6 to 4.2, the percentage of emergence was considerably reduced in *Aedes aegypti*.

Similarly, water hardness also affected the number of emergent imagoes, percentage emergence and post-emergence longevity of the species. For example, higher numbers of adult mosquitoes emerged from the soft water (0 mg/L $CaCO_3$) regimen; beyond 90 mg/L $CaCO_3$, the numbers of emergent significantly reduced, being the lowest at 210 mg/L $CaCO_3$ ('very hard' water).

Generally, there were significantly higher numbers of female than male mosquitoes in all hardness regimens, with decreasing numbers of male and female as hardness condition increased. Similarly, hardness levels above 90 mg/L $CaCO_,$ significantly reduced adult longevity; mosquitoes reared at 210 mg/L $CaCO_3$ lived the shortest.

The findings of this study are epidemiologically important as water pH and hardness conditions have played significant roles in determining the bionomics and population density of mosquitoes and the resultant frequency of human-mosquito contacts. This information, although subject to field experimentation and trials, can be applied in the development of cost-effective tactics towards the reduction of mosquito population density and hence degree of human-vector contact. For example, natural mosquito breeding habitats with an average of pH 7.0 may be more productive with mosquitoes than others; such habitats should be prioritized in terms of allocation of scarce resource for control. The reverse could be obtainable for mosquito habitats with extreme (acidic and alkaline) pH values. More so, if the hardness of a water body is known, it can give an indication of the level of productivity of that habitat, or perhaps be manipulated by the application of hardness-causing agents like lime water to it. Further, mosquito habitat with water hardness within the range of 'very hard water' may not support the proliferation of mosquitoes; therefore, effort should be channelled to other productive habitats with mild hardness condition.

Conclusion

The present study revealed significant effects of water pH and hardness conditions on developmental and vectorial attributes of *Culex quinquefasciatus*. Extreme pH values and increasing water hardness delayed development. While other entomological variables such as immature and adult survivorship, adult emergence success and post-emergence longevity were variously affected by the water conditions tested. The information generated in this study can also be incorporated in the development of cost-effective integrated mosquito management (IMM) protocol. However, field evaluation of the influence of prevailing physico-chemical parameters on biological fitness is advocated to verify these laboratory outcomes.

Abbreviations

ANOVA: Analysis of variance; $CaCO_3$: Calcium trioxocarbonate; *Cx. quinquefasciatus*: *Culex quinquefasciatus*; DMRT: Duncan multiple range test; EVM: Environmental vector management (EVM); FA: Fluctuating asymmetry; HCl: Hydrochloric acid; HEP/UM: Higher education partnership/University of Mississippi; IMM: Integrated mosquito management; LGR: Larval growth rate; LI–IV: Mosquito larval instars one to four; Mean ± SD: Mean ± standard deviation; NaOH: Sodium hydroxide; SPSS: Statistical Package for Social Scientists; USAID: United State Agency for International Development; WHO: World Health Organization; WL: Wing length

Acknowledgements

Our deepest thanks go to the Management of Federal University of Technology, Minna, for facilitating the USAID/HEP/UM grant (UM SUB-AWARD NO. 15-12-024). We also appreciate the staff members of the Department of Animal Biology, Federal University of Technology, Minna, for providing a conducive environment for the study.

Authors' contributions

ACU, IKO and FOA conceived and designed the experiments. ACU, CCO and CCU performed the experiments. ACU, IKO, FOA and CCO analysed the data. UAC and CCU wrote the first draft of the manuscript. IKO and FOA corrected the draft copy. All authors agreed to the final state of the manuscript.

Funding

The funding sponsors (United State Agency for International Development (USAID), Higher Education Partnership/University of Mississippi (HEP/UM)) had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript; and in the decision to publish the results.

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Applied Entomology Unit, Department of Animal Biology Sciences, Federal University of Technology, Minna, Nigeria. [2]Department of Animal and Environmental Biology, Delta State University, Abraka, Delta State, Nigeria. [3]Applied Entomology Unit, Department of Animal Biology, Federal University of Technology, Minna, Nigeria. [4]Applied Hydrobiology Unit, Department of Animal Biology, Federal University of Technology, Minna, Nigeria. [5]Department of Microbiology, Federal University of Technology, Minna, Nigeria.

Received: 22 March 2019 Accepted: 20 January 2020
Published online: 10 February 2020

References

Abernathy, M. A. (2004). *Effect of water hardness on the survival of rainbow shark minnow (Epalzeorhynchos frenatum) eggs and larvae*. Unpublished M.Sc. Thesis Submitted to Department of Fisheries and Aquatic Sciences University of Florida, (p. 63).

Bayly, I. A. E. (1972). Salinity tolerance and osmotic behavior of animals in athalassic saline and marine hypersaline waters. *Annual Review of Ecology and Systematics*, *3*(1), 233–268.

Blanksma, C. B., Eguia, K. L., Lazorchak, J. M., Smith, M. E., Wratschko, M., & Schoenfuss, H. L. (2009). Effects of water hardness on skeletal development and growth in juvenile fathead minnows. *Aquaculture*, *286*, 226–232.

Bradley, T. J. (1994). The role of physiological capacity, morphology, and phylogeny in determining habitat use in mosquitoes. In *Ecological morphology: integrative organismal biology*, (pp. 303–318).

Braks, M., Leal, W., & Cardé, R. (2007). Oviposition responses of gravid female *Culex quinquefasciatus* to egg rafts and low doses of oviposition pheromone under semifield conditions. *Journal of Chemical Ecology*, *33*, 567–578.

Buchman, N. W. (1931). Untersuchungenti ber die Bedeutungd er Wasserstoffionenkonzentration fur die Entwicklung der Mtickenlarven. 2. *Angew*. *Entomology*, *18*, 404–416.

Christophers, S. R. (1960). *Aedes aegypti (L.) the yellow fever mosquito: Its life history, bionomics and structure*, (p. 750). Cambridge university press.

Clark, T. M., Flis, B. J., & Remold, S. K. (2004). pH tolerances and regulatory abilities of freshwater and euryhaline Aedine mosquito larvae. *Journal of Experimental Biology*, *207*, 2297–2304.

Clements, A. N. (2000). *The biology of mosquitoes: Development, nutrition and reproduction*, (p. 675). London: Chapman & Hall.

Daily, J. P., Hitt, N. P., Smith, D. R., & Snyder, C. D. (2012). Experimental and environmental factors affect spurious detection of ecological thresholds. *Ecology*, *93*, 17–23.

Elkanah, O. S., Elkanah, D. S., Wahedi, J. A., Samaila, A. B., Kela, S. L., & Anyanwu, G. I. (2017). Lymphatic filariasis in Muri Emirate: clinical and parasitological studies in Jalingo LGA, Taraba state, Nigeria. *Asian Journal of Medicine and Health*, *6*(1), 1–7.

Eneanya, O. A., Cano, J., Dorigatti, I., Anagbogu, I., Okoronkwo, C., Garske, T., & Donnelly, C. A. (2018). Environmental suitability for lymphatic filariasis in Nigeria. *Parasites & Vectors*, *11*, 513.

Garba, Y., & Olayemi, I. K. (2015). *Spatial variation in physicochemical characteristics of wetland rice-field mosquito larval habitats in Minna, north Central Nigeria*. Penang: International conference on agriculture, ecological and medical sciences (AEMS-2015).

Gillott, C. (2005). *Entomology*, (3rd ed., pp. 500–511). Springer publishing.

Ikeh, G. N., Olayemi, I. K., Ukubuiwe, A. C., Jibrin, A. I., Shehu, I., & Adeniyi, K. A. (2017). Contributions of commercial water-based human activity ventures to mosquito (Diptera: Culicidae) in Minna Metropolis, Nigeria. *International Journal of Applied Biological Research*, *8*(1), 174–185.

Ivoke, N., Mgbenka, B. O., & Okeke, O. (2007). Effects of pH on the growth performance of *Heterobranchus bidorsalis*, *Clarias gariepinus* hybrid juvenile. *Animal Research International*, *4*(1), 639–642.

Kant, R., Pandey, S. D., & Sharma, S. K. (1996). Mosquito breeding in relation to aquatic vegetation and some physico-chemical parameters in rice fields of central Gujarat. *Indian Journal of Malariology*, *33*, 30–40.

Lazareviæ, L., Periæ-Mataruga, V., Vlahoviæ, M., Mrdakoviæ, M., & Cvetanoviæ, D. (2004). Effects of rearing density on larval growth and activity of digestive enzymes in *Lymantria dispar* L. (Lepidoptera: Lymantriidae). *Folia biologica (Kraków)*, *52*(1–2), 105–112.

Leschen, R. A. B., & Cutler, B. (1994). Cuticular calcium in beetles (Coleoptera: Tenebrionidae: Phrenapetinae). *Annals of Entomology Society of American*, *87*, 918–921.

Merritt, R. W., Dadd, R. H., & Walker, E. D. (1992). Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology*, *37*, 349–376.

Mgbemena, I. C., & Ebe, T. (2012). Distribution and occurrence of mosquito species in the municipal areas of Imo State, Nigeria. *Analele UniversităŃii din Oradea - Fascicula Biologie*, *19*(2), 93–100.

Mgbemena, I. C., Opara, F. N., Osuala, F. O. U., & Iwuala, M. O. E. (2009). The physico-chemical characteristics of drainage breeding sites of mosquitoes in parts of Imo State, South Eastern, Nigeria. *International Journal of Environmental Health and Human Development*, *10*(2), 1–13.

Milad, K., Mohammed, S., & Seyed, A. H. (2011). Effects of water hardness on egg hatchability and larval viability of angelfish (*Pterophyllum scalare*). *International Journal of Research in Fisheries and Aquaculture*, *1*, 6–10.

Minakawa, N., Mutero, C. M., Githure, J. I., Beier, J. C., & Yan, G. (1999). Spatial distribution and habitat characterization of anopheline mosquito larvae in western Kenya. *American Journal of Tropical Medicine and Hygiene*, *61*, 1010–1016.

Minakawa, N., Sonye, G., & Yan, G. (2005). Relationships between occurrence of *Anopheles gambiae* s. l. (Diptera: Culicidae) and size and stability of larval habitats. *Journal of Medical Entomology*, *42*(3), 295–300.

Molokwu, C. N., & Okpokwasili, G. C. (2004). The effect of water hardness on egg hatchability and larval viability of *Clarias gariepinus*. *Aquaculture International*, *10*, 57–64.

Mpho, M., Callaghan, A., & Holloway, G. J. (2002). Effects of temperature and genetic stress on life history and fluctuating wing asymmetry in *Culex pipiens* mosquitoes. *European Journal of Entomology*, *99*, 405–412.

Mwangangi, J. M., Mbogo, C. M., Muturi, E. J., Nzovu, J. G., Kabiru, E. W., Githure, J. I., … Beier, J. C. (2007). Influence of biological and physicochemical characteristics of larval habitats on the body size of *Anopheles gambiae* mosquitoes (Diptera: Culicidae) along the Kenyan coast. *Journal of Vector Borne Disease*, *44*, 122–127.

Nadia, K., Thamer, M., & Saad, S. A. (2005). The effect of different NaCl, pH level of survival of *Culex* species (Diptera: Culicidae) larvae in Basrah. *Journal of Basrah Research (Science)*, *3*(2), 31–36.

Olayemi, I. K., Omalu, I. C. J., Famotele, O. I., Shegna, S. P., & Idris, B. (2010). Distribution of mosquito larvae in relation to physico-chemical characteristics of breeding habitats in Minna, north central Nigeria. *Review in Infection*, *1*(1), 49–53.

Oyewole, I. O., Momoh, O. O., Anyasor, G. N., Ogunnowo, A. A., Ibidapo, C. A., Oduola, O. A., … Awolola, T. S. (2009). Physico-chemical characteristics of *Anopheles* breeding sites: impact on fecundity and progeny development. *African Journal of Environment, Science and Technology*, *3*(12), 447–452.

Patrick, M. L., & Bradley, T. J. (2000). The physiology of salinity tolerance in larvae of two species of *Culex* mosquitoes: the role of compatible solutes. *Journal of Experimental Biology*, *203*(4), 821–830.

Pelizza, S. A., Lopez-Lastra, C. C., Becnel, J. J., Bisaro, V., & Garcia, J. J. (2007). Effects of temperature, pH and salinity on the infection of *Leptolegnia chapmanii Seymour* (Peronosporomycetes) in mosquito larvae. *Journal of Invertebrate Pathology*, *96*, 133–137.

Piyaratne, M. K., Amerasinghe, F. P., Amerasinghe, P. H., & Konradsen, F. (2005). Physico-chemical characteristics of *Anopheles culicifacies* and *Anopheles varuna* breeding water in a dry zone stream in Sri Lanka. *Journal of Vector Borne Diseases*, *42*, 61–67.

Poteat, M. D., & Buchwalter, D. B. (2014). Calcium uptake in aquatic insects: influences of phylogeny and metals (Cd and Zn). *Journal of Experimental Biology*, *217*, 1180–1186. https://doi.org/10.1242/jeb.097261.

Regional Aquatics Monitoring Program, RAMP (2015). Water quality. www.ramp-alberta.org/ramp/design+and+monitoring/components/water+quality.aspx. Retrieved on 03/08/2015.

Rim-Rukeh, A. (2013). Physico-chemical and biological characteristics of stagnant surface water bodies (ponds and lakes) used for drinking and domestic purposes in Niger Delta, Nigeria. *Journal of Environmental Protection*, *4*, 920–928.

Robert, V., Awono-Ambene, H. P., & Thiolouse, J. (1998). Ecology of larval mosquitoes (Diptera: Culicidae) in market-garden wells in urban Dakar, Senegal. *Journal of Medical Entomology*, *35*(6), 948–955.

Salihu, I. M., Olayemi, I. K., Ukubuiwe, A. C., Garba, Y., Gusau, A. Y., Nma-Etsu, M., & Usman, M. D. (2017). Influence of agro-chemical inputs on suitability of physicochemical conditions of rice-fields for mosquito breeding in Minna, Nigeria. *Journal of Mosquito Research*, *7*(17), 134–141. https://doi.org/10.5376/jmr.2017.07.0017.

Sattler, M. A., Mtasiwa, D., Kiama, M., Premji, Z., Tanner, M., & Lengeler, C. (2005). Habitat characterization and spatial distribution of *Anopheles* sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. *Malaria Journal*, *4*, 4 http://www.malariajournal.com/content/4/1/4.

Singare, P. U., Lokhande, R. S., & Pathak, P. P. (2010). Soil pollution along Kalwa Bridge at Thane Creek of Maharashtra, India. *Journal of Environmental Protection*, *1*, 121–128.

Tanimura, E., & Sonoji, I. N. E. (1952). An experimental study on the function of dilute solutions of strong inorganic acids upon larval mosquitoes etc. *Shikoku Acta Medica*, *3*, 36–40.

Timmermann, S. E., & Briegel, H. (1993). Water depth and larval density affect development and accumulation of reserves in laboratory populations of mosquitoes. *Bulletin of the Society of Vector Ecology*, *18*, 174–187.

Timmermann, S. E., & Briegel, H. (1999). Larval growth and biosynthesis of reserves in mosquitoes. *Journal of Insect Physiology*, *45*, 461–470.

Ukubuiwe, A. C., Olayemi, I. K., & Jibrin, A. I. (2016). Genetic variations in bionomics of *Culex quinquefasciatus* (Diptera: Culicidae) mosquito population in Minna, north-Central Nigeria. *International Journal of Insect Science*, *8*, 9–15. https://doi.org/10.4137/IJIS.S32516.

Ukubuiwe, A. C., Olayemi, I. K., Omalu, I. C. J., Arimoro, F. O., Baba, B. M., Ukubuiwe, C. C., Odeyemi, M. O., & Adeniyi, K. A. (2018a). Influence of Rearing-water Temperature on Life Stages' Vectorial Attributes, Distribution and Utilization of Metabolic Reserves in *Culex quinquefasciatus* (Diptera: Culicidae): Implications for Disease Transmission and Vector Control. *The Journal of Basic and Applied Zoology*, 79(32), 1-15 https://doi.org/10.1186/s41936-018-0045-3.

Ukubuiwe, A. C., Olayemi, I. K., Omalu, I. C. J., Arimoro, F. O., & Ukubuiwe, C. C. (2018b). Morphometric Diagnosis of the Effects of Water Hardness on Development of Immature Life Stages and Adult Vectorial Fitness of *Culex quinquefasciatus* (Diptera: Culicidae) Mosquito. *Zoomorphology*. https://doi.org/10.1007/s00435-018-0415-x.

Ukubuiwe, A. C., Olayemi I. K., Omalu, I. C. J., Arimoro, F. O., and Ukubuiwe, C. C. (2018c). Morphometric Diagnosis of the Effects of Water Hardness on Development of Immature Life Stages and Adult Vectorial Fitness of Culex quinquefasciatus (Diptera: Culicidae) Mosquito. Zoomorphology, 137(4), 511-518. https://doi.org/10.1007/s00435-018-0415-x.

Ukubuiwe, A. C., Olayemi, I. K., Omalu, I. C. J., Jibrin, A. I., & Oyibo-Usman, K. A. (2013). Molecular bases of reproductive and vectorial fitness of *Culex pipiens pipiens* (Diptera: Culicidae) mosquito populations, for the transmission of filariasis in north Central Nigeria. *Journal of Medical Science*, 1–7. JMS (ISSN 1682-4474)/. https://doi.org/10.3923/jms.2013.

Ukubuiwe, A. C., Olayemi, I. K., Omalu, I. C. J., Odeyemi, M. O., Jibrin, A. I., & Oyibo-Usman, K. A. (2012). Comparative assessment of immature survivorship and developmental duration of *Culex pipiens pipiens* (Diptera: Culicidae) populations in north central Nigeria. *Biomed Central Epidemiology*, *3*(10), WMC003753.

Wigglesworth, V. B. (1933). The adaptation of mosquito larvae to salt water. *Journal of Experimental Biology*, *10*(1), 27–36.

Woodhill, A. R. (1942). A comparison of factors affecting the development of three species of mosquitoes, etc. *Proceedings of Limnological Society. N. S. W*, *67*, 95–97.

World Health Organization, WHO (2017). *Annual report on lymphatic filariasis 2017*. Geneva: WHO www.who.int/mediacentre/factsheets/fs102/en Retrieved 27/01/2018.

Yee, D. A., Kneitel, J. M., & Juliano, S. A. (2010). Environmental correlates of abundances of mosquito species and stages in discarded vehicle tires. *Journal of Medical Entomology*, *47*(1), 53–62.

Publisher's Note