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ORIGINAL PAPER

Morphometric diagnosis of the effects of water hardness on development of immature life stages and adult vectorial fitness of *Culex quinquefasciatus* (Diptera: Culicidae) mosquito

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Abstract

In the present study, we elucidated the effects of different water hardness regimens on life stages of *Culex quinquefasciatus* mosquito using the techniques of morphometry. Five water hardness regimens (0, 30, 90, 150 and 210 mg/L $CaCO_3$) were prepared following standard protocols, and freshly hatched larvae reared in them until adulthood. Measurements were made for the lengths and widths of larval head capsule, thorax, abdomen, siphon, antennae, and anal papillae. Other parts measured include pupal trumpet, cephalothorax, and total immature body lengths, adult abdominal and wing parameters, volumes of fourth instar larvae and adult mosquitoes. Analyses revealed significant effects of water hardness on all body parts measured. The present study shows that water hardness levels significantly affect the morphometrics of *Cx. quinquefasciatus* mosquito, and could affect immature growth and development, and adult fitness attributes. It also reveals that moderately hard water are the best for overall fitness and performance of the species. The information generated from this study is important in understanding the interplay between water hardness and mosquito development.

Keywords Wing length · Fluctuating asymmetry · Physico-chemical · Morphometry

Background

The *Culex quinquefasciatus* mosquito is one of the foremost medical insects, with great public health importance in Tropical Africa and Nigeria inclusive. Its close habitation to human dwellings (Muturi et al. 2008), coupled with its ubiquitous breeding tendencies (Mwangangi et al. 2008) has provided an excellent ground for the spread of important debilitating human and animal diseases (Adeleke 2010). The success of this mosquito species is closely associated with its ability to identify breeding habitats with suitable physico-chemical conditions (Purcell and Almeida 2005) which maximize immature developmental successes (Braks et al. 2007) and, hence, adult fitness (Olayemi 2008; Olayemi et al. 2014a).

Mosquito-breeding habitats contain organic and inorganic salts, which determines suitability as an excellent oviposition site, for gravid female mosquitoes (Olayemi et al. 2010, 2014b). Among these salts is calcium trioxocarbonate IV, $CaCO_3$, which is responsible for temporary hardness experienced in water bodies (World Health Organisation, WHO 2011), and its relative presence determines

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the hardness category of an aquatic body (Water Quality Association, WQA 2018). Ecologically, five categories of water hardness are considered, namely, soft (< 17.0 mg/L $CaCO_3$), slightly hard (17.0–59 mg/L $CaCO_3$), moderately hard (60–119 mg/L $CaCO_3$), hard (120–180 mg/L $CaCO_3$), and very hard water (> 180 mg/L $CaCO_3$) (WQA 2018), and these have been adopted in the present study.

Physiologically, Ca^{2+} ion is important for normal development of insects (Auerswald and Gäde 2001), playing crucial role in most regulatory processes (Poteat and Buchwalter 2014) and anatomy of insects, especially in cuticle formation (Leschen and Cutler 1994). However, excessive presence of these inorganic salts can become toxic and sometimes lethal (Paulraj et al. 2011), thereby affecting growth and development, especially cuticle formation (Blanksma et al. 2009), which provides for increase in bodily size. Cuticles cover the external surface and lines some internal body structure of insects; their rigidity and integrity provide a first line of defence against insecticides and pathogens (Hopkins and Kramer 1992).

The degrees of hardness of water bodies are strong determinants of occurrence and distribution of aquatic organisms, especially mosquitoes (Robert et al. 1998; Oyewole et al. 2009; Tiimub et al. 2012). More so, distribution and occurrence of mosquitoes, especially, mosquito vectors play crucial role in disease pathogen transmission. For examples, studies have revealed that domestic wells (an important habitat type of mosquitoes) plays key roles in mosquito distribution, as these water bodies have different hardness levels, which in turn affect the survivorship of mosquitoes. Presently, there is little or no scientific report on the influence of water hardness levels on morphometrics of these organisms, especially, elucidating the effects on vectorial fitness. It is hoped that the present study will fill this gap of knowledge.

Further, data collected from field studies form the bases of most inference of physico-chemical quality on development and distribution of mosquitoes (Mwangangi et al. 2006, 2007; Oyewole et al. 2009; Mgbemena et al. 2009; Olayemi et al. 2010; Tiimub et al. 2012; Garba and Olayemi 2015). These reports are not only deficient; they do not provide holistic information of morphologic changes during preimaginal life as predicated by any physico-chemical parameters. Moreover, information generated during immature life stages are critical in understanding the biology of important insect pests, and are the basis for development of larval-based control protocols.

Although, relatively new, techniques in the field of morphometry (i.e., measurements of body parts for scientific procedures) can be employed to provide information on the physiology of growth and development in insects (Mondal et al. 2015; Tyagi et al. 2016). Hence, the present study involved the use of this technique to elucidate the effects of water hardness on life stages (larval instars I–IV, pupal and adult) of *Cx. quinquefasciatus* mosquito, to provide ample evidences on developmental changes that occur at immature life stages, in relation to vectorial fitness of adult mosquitoes.

Materials and methods

Preparation of stock and working solutions for water hardness regimens

The methods of Milad et al. (2011) for preparation of water hardness regimens were adopted for the stock and working solutions. Briefly, 1 g of calcium carbonate, $CaCO_3$ (Chemetrics, Inc., Calverton, VA) was weighed and dissolved in 1000 mL (1 L) of distilled water in a beaker, mixed thoroughly with a clean glass rod and stored in a reagent bottle. Five water hardness regimens based on standard classification of water hardness to mimic varying hardness conditions were generated. These are 0 (soft), 30 (slightly hard water), 90 (Moderately Hard water), 150 (Hard water), and 210 mg/L $CaCO_3$ (very hard water). The working solutions were prepared using the dilution formula of Milad et al. (2011). Each hardness regimen had four replicates and the whole study duplicated immediately after the first study (i.e., repeated) to minimize error due to sample size.

Source, rearing, and maintenance of experimental insects

From a colony of *Cx. quinquefasciatus* in the Insectary Unit of the Department of Animal Biology, Federal University of Technology, Minna, freshly laid eggs were collected. These were incubated in plastic hatching trays. On hatching, the approximately day-old larvae were reared at the rate of 1 larva/4 mL (i.e., 100 larvae/400 mL) of the artificial hardness media prepared. Day-old larvae were reared in plastic trays (30 cm × 25 cm × 5 cm), following standard techniques (Ukubuiwe et al. 2016). The larvae were fed fish feed (Coppens®), sprayed on the water surface at the rate of 0.32 mg/100 larvae every other day. To avoid scum, the rearing water was changed daily. On pupation, the mosquitoes were placed in well-labelled plastic bowls, (5 cm × 20 cm). The bowls were half-filled with the prepared media, and placed in adult-holding cages for emergence. Clean cotton pad, soaked in 10% sucrose solution, was used to maintain the emerged adults (Ukubuiwe et al. 2017).

Sample size of measured mosquitoes

At every life stage and larval instar, ten (10) mosquitoes were selected randomly for each development stage, and from each replicate (4 replicates per treatment) of a water hardness regimen (resulting in a total of 40 mosquitoes per treatment) for morphometric growth parameters and analysis. Thus, for the two rounds of study, a total of 2000 mosquitoes (i.e., 1000 × 2 repeats) were studied [comprising of 40 individuals per treatment × 5 life stages (4 larval instars + pupa + adult) × 5 concentrations].

Measurement of morphometric parameters

Larval body parts were measured according to modified methods of Bar and Andrew (2013) with the aid of an ocular micrometer mounted on a binocular microscope at magnification 4× (with a conversion factor of 0.263). Briefly, for larval body measurements: the lengths of head, thorax, abdomen, antennae, siphon, anal papillae and total body length (lengths of head + thorax + abdomen) were determined. Others include widths of head, thorax, abdomen and siphon. Pupal body parts measured include length of trumpet, cephalothorax, abdomen and total pupal length (sum of the lengths of cephalothorax and abdomen), while adult parameters measured include wing lengths, fluctuating asymmetry, FA (i.e., difference between right and left wings of the mosquito, and proxy for ontogenic stress), and surface area (a product of length and width of each wing), length and width of abdomen (Abrams et al. 1996).

Vectorial attribute parameters

The volume of fourth instar larvae, a proxy for biomass accumulation, was estimated as cube of the width of the thorax, and volume of adult, an index for blood meal ingestion, was determined as cube of length of the wing (Timmermann and Briegel 1993).

Data analysis

Data generated were processed into means and standard deviation using Microsoft Office Excel 2016. Differences in means of entomologic variables among treatments were compared using one-way and two-way Analysis of Variance (ANOVA) as appropriate. All decisions on statistical comparison of means was taken at $p = 0.05$ level of significance. The means were separated using Duncan multiple range test (DMRT).

Results

Effects of water hardness on body parts of larval instars of *Culex quinquefasciatus* mosquito

The influence of water hardness on the length and width of the body parts of larval instars of *Cx. quinquefasciatus* mosquito is shown in Fig. 1a, b. The principal components analysis (PCA) biplots showed that hard water (210 mg/$CaCO_3$) and moderately hard water (90 mg/$CaCO_3$), respectively, had the most significant effects on lengths and widths of the body parts of the mosquito species (Fig. 1a, b). Most of the body parts were mostly affected by the hardness regimens apart from the total body lengths of LIII and IV, and abdominal length of LIV. Meanwhile, all widths of the body parts were also affected by hardness regimen except width of head capsule and thorax of LIV.

Effects of water hardness on body parts of pupal stage of *Culex quinquefasciatus* mosquito

Analyses showed that water hardness had significant effects on the body lengths of this life stage (Fig. 2). The length of the cephalothorax ranged from 1.68 ± 0.13 to 1.91 ± 0.07 mm, trumpet, 0.46 ± 0.07 to 0.56 ± 0.05 mm and abdomen, 3.61 ± 1.47 to 4.26 ± 0.19 mm. Total pupal body length ranged from 5.75 ± 0.69 to 6.73 ± 0.31 mm, respectively, at 210 and 90 mg/L $CaCO_3$ (Fig. 2).

Effects of water hardness on adult morphometrics and fitness attributes of *Culex quinquefasciatus* mosquito

Abdominal lengths of adult *Cx. quinquefasciatus* was, significantly, affected by water hardness; the shortest lengths were obtained in mosquitoes reared at 210 mg/L $CaCO_3$, while longest from those reared at 30 and 90 mg/L $CaCO_3$. The wing lengths of the species followed similar trend as abdominal lengths. However, mosquitoes reared at water hardness ranging from 0 to 90 mg/L $CaCO_3$ had the widest wings. These resulted in variation in wing surface area with those reared at 90 mg/L $CaCO_3$ having the widest and those at 210 mg/L $CaCO_3$, the narrowest (Fig. 3).

Effects of water hardness on ptero-fitness of *Culex quinquefasciatus* mosquito

Fluctuating asymmetry (FA) was also affected significantly ($p < 0.05$) by the water hardness regimen. The male and female FA ranged, respectively, from 0.01 ± 0.01 to 0.03 ± 0.01 to 0.01 ± 0.01 to 0.02 ± 0.03 mm (Table 1).

Effects of water hardness on body sizes of fourth instar larvae and adult *Culex quinquefasciatus* mosquito

Analyses revealed significant ($p < 0.05$) effects of water hardness levels on these parameters. The body sizes of the fourth instar larvae ranged from 2.26 ± 1.04 to 4.76 ± 0.48 mm^3. The sizes of the male and female insects also varied significantly,

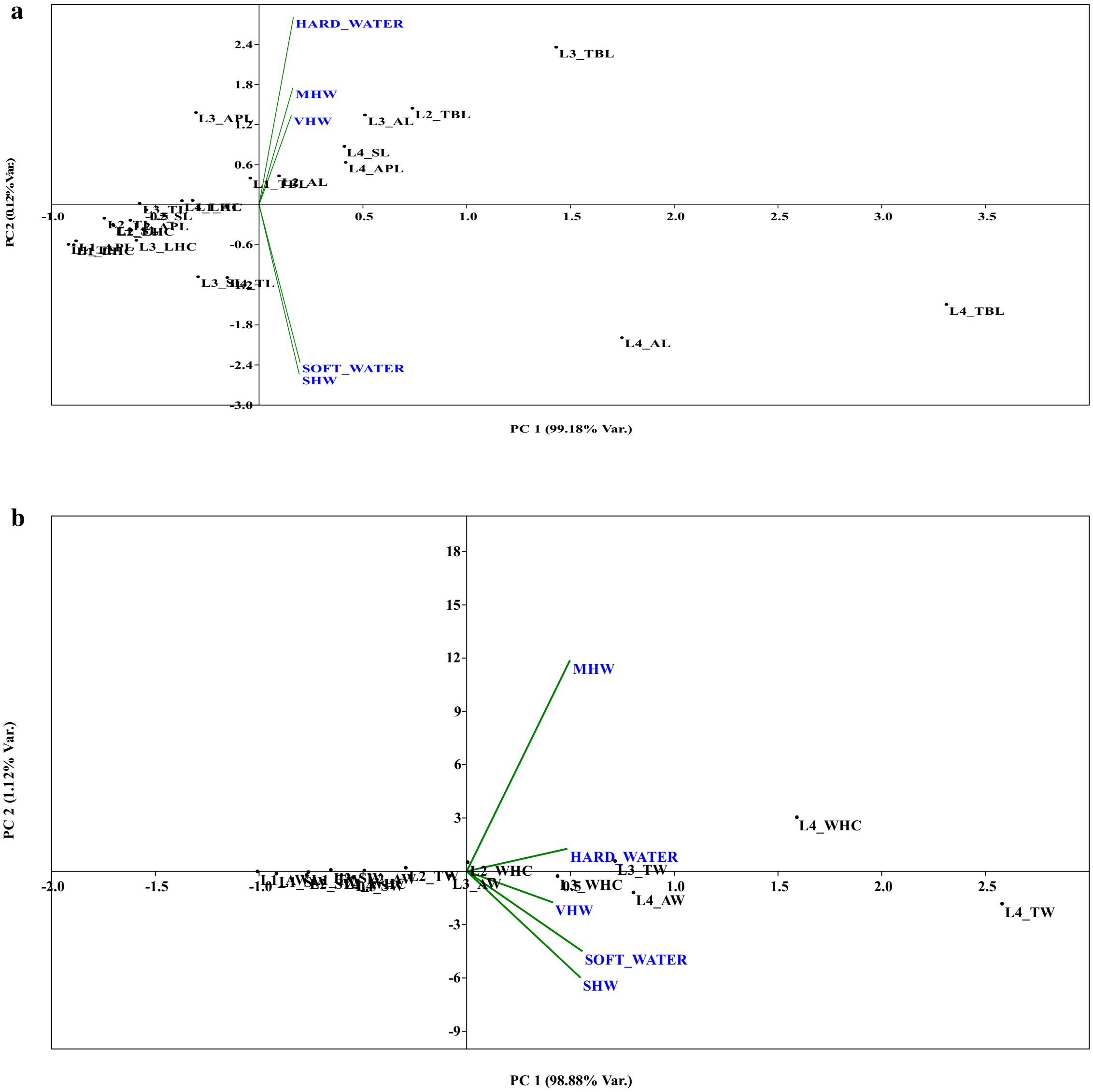

Fig. 1 **a** Principal components analysis (PCA) biplots for influence of hardness regimens on length of body parts of *Cx. quinquefasciatus* mosquito species (*n* = 2000). For each axis, the amount of variation explained by each axis is shown. Arrows indicate the direction and relative importance of the water hardness levels. Hardness regimens: soft water (0), SHW-slightly hard water (30), MHW-moderately hard water (90), hard water (150), and VHW-very hard water (210 mg/L $CaCO_3$). L1–L4 (larval stages 1–4). Body parts: *HCL* length of Head capsule, *TL* thoracic length, *AL* abdominal length, *SL* siphonal length, *APL* anal papillar length, *TBL* total body length. **b** PCA biplots for influence of hardness regimens on widths of body parts of *Cx. quinquefasciatus* mosquito species (*n* = 2000). For each axis, the amount of variation explained by each axis is shown. Arrows indicate the direction and relative importance of the water hardness levels. Hardness regimens: soft water (0), SHW-slightly hard water (30), MHW-moderately hard water (90), hard water (150), and VHW-very hard water (210 mg/L $CaCO_3$). L1–L4 (larval stages 1–4). Body parts: *WHC* width of head capsule length, *TW* thoracic width, *AW* abdominal width, *SW* siphonal width

and ranged from 19.66 ± 1.02 to 31.41 ± 2.54 mm^3 and 21.41 ± 2.06 to 37.99 ± 3.71 mm^3, respectively, at 210 and 90 mg/L $CaCO_3$ (Table 2).

Discussion

Although, calcium forms an integral part of the exoskeleton of insects (Leschen and Cutler 1994), and physiologically involved in many metabolic processes (Heming 1993). To ensure proper growth and efficient regulation of physiological processes in aquatic insects, development

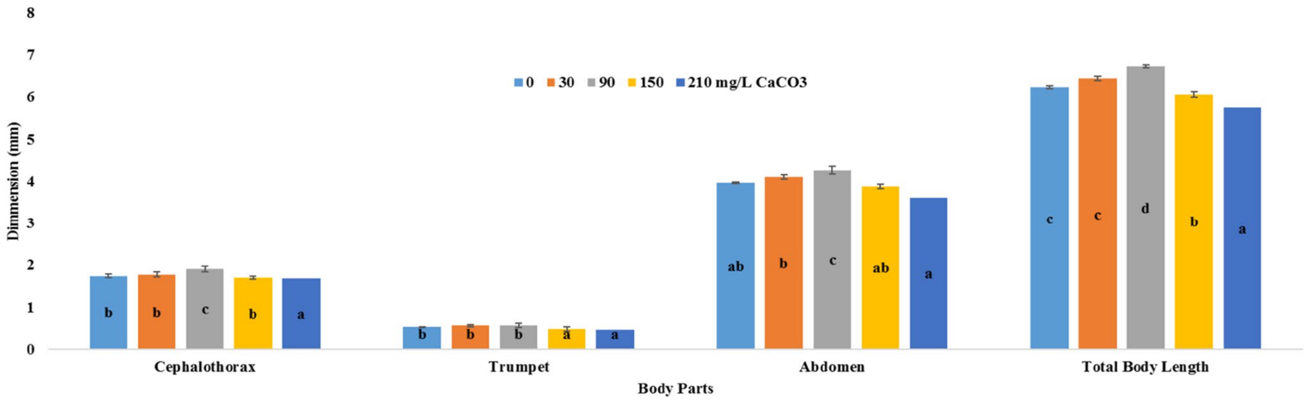

Fig. 2 Effects of water hardness on morphometrics of pupal life stage of *Culex quinquefasciatus* mosquito. Bars with same letter for a body part are not significantly different at $p < 0.05$ according to analysis of variance. Values expressed as Mean ± SD

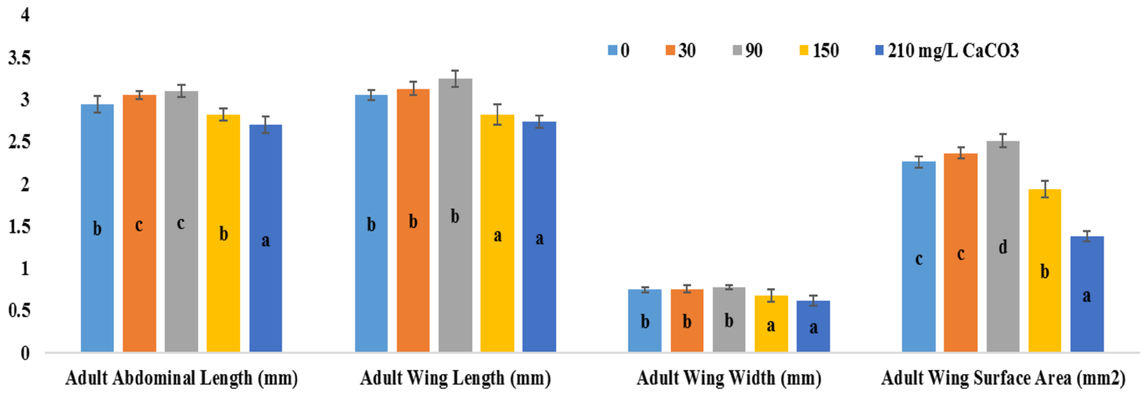

Fig. 3 Effects of water hardness regimens on morphometrics and adult fitness attributes of *Culex quinquefasciatus* mosquito. Bars with same letter for a body part are not significantly different at $p < 0.05$ according to analysis of variance. Values expressed as Mean ± SD

Table 1 Effects of water hardness on wing length and fluctuating asymmetry of *Culex quinquefasciatus* mosquito

Water hardness (mg/L $CaCO_3$)	Fluctuation asymmetry (mm)**	
	Male	Female
Soft (0)	0.01 ± 0.01^{a}*	0.01 ± 0.01^{a}
Slightly hard (30)	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
Moderately hard (90)	0.00 ± 0.01^{a}	0.00 ± 0.01^{a}
Hard (150)	0.02 ± 0.01^{b}	0.01 ± 0.02^{b}
Very hard (210)	0.03 ± 0.01^{c}	0.02 ± 0.03^{c}

*Values followed by same letter in a column, are not significantly different (at $p < 0.05$) according to analysis of variance

**Difference between left and right wings lengths. Values are expressed as mean ± SD

must take place within permissible limits. Values below or exceeding these limits creates pathologic conditions (Locke 2001). Being an integral part of the exoskeleton, excess deposition of the mineral salt (Ca^{2+}), may result in greater accumulation of calcium ions in cuticles: which can affect the ecdysal process of apolysis (Hopkins and Kramer 1992) and, hence, dimension of body parts.

In the present study, using morphometry, analyses showed significant negative effects of water hardness on the growth and development of *Cx. quinquefasciatus*; especially, at concentration above 90 mg/L $CaCO_3$ (i.e., moderately hard water). For example, gradual increase in length of the body parts were observed as the level of hardness increased from 0 to 90 mg/L $CaCO_3$, however, beyond this, the length of the body parts started decreasing. The reason for this was unclear, but could depict, perhaps, gradual deposition of excessive calcium within the cuticle of the insect, which restricts expansion, or may perhaps, represent expression of some form of toxicity of the chemical ($CaCO_3$).

Furthermore, the abdominal length (a proxy for volume of blood meals), width and surface area of adult mosquito wings (a proxy for fitness for flight) were significantly affected negatively, with increase in water hardness level.

Table 2 Effects of water hardness on body sizes of fourth instar larvae and adult *Culex quinquefasciatus* mosquito

Hardness (mg/L $CaCO_3$)	Larval size*** (mm^3)	Adult size**** (mm^3)	
		Male	Female
Soft (0)	$3.10 \pm 1.75^{ab*}$	$25.88 \pm 2.11_{a}^{b**}$	$29.85 \pm 1.98_{b}^{b}$
Slightly Hard (30)	4.59 ± 0.61^{b}	$31.41 \pm 2.54_{a}^{c}$	$31.37 \pm 1.26_{a}^{b}$
Moderately Hard (90)	4.76 ± 0.48^{b}	$30.80 \pm 2.74_{a}^{c}$	$37.99 \pm 3.71_{b}^{c}$
Hard (150)	2.68 ± 0.97^{a}	$22.16 \pm 2.31_{a}^{a}$	$22.80 \pm 3.47_{a}^{a}$
Very Hard (210)	2.26 ± 1.04^{a}	$19.66 \pm 1.02_{a}^{a}$	$21.41 \pm 2.06_{b}^{a}$

*Values followed by same letter in a column are not significantly different at $p < 0.05$ according to analyses of variance

**Values followed by same letter in a row for male and female are not significantly different at $p < 0.05$ according to analyses of variance

***Cube of diameter of fourth larval instar

****Cube of wing length of adult

For example, mosquitoes reared in water hardness levels above 90 mg/L $CaCO_3$ had relatively shorter abdominal length, smaller wing widths and surface areas. These are indication that, perhaps, the concentration of 90 mg/L $CaCO_3$ (i.e., moderately hard water), seems to be the optimum for normal physiologic processes of the mosquito species. It therefore means that these mosquitoes may have higher propensities for higher volumes of blood and further ranges of flight that other mosquitoes.

The present study also revealed significant effects of water hardness on the wing length (WL) and fluctuating asymmetry (FA) of *Cx. quinquefasciatus* mosquito. The wing length of a mosquito species is an index of quality of the mosquito (Mpho et al. 2002), while, FA is an indication of level of stress during ontogeny and degree of vectorial fitness. As regards size, the mosquitoes reared at 30 mg/L $CaCO_3$ (i.e., soft water) and 90 mg/L $CaCO_3$ (i.e., moderately hard) were the largest, and best fit (i.e., low FA), while individuals reared at 210 mg/L $CaCO_3$ (i.e., very hard water) were the smallest, and less-fit (i.e., high FA). These results imply, perhaps, that the mosquitoes from the earlier categories (i.e., from soft and moderately hard water) may be better quality and best fit for the transmission of disease pathogens.

The body size of the last larval instar is an index of biomass accumulation (Timmermann and Briegel 1993). In addition, the body size of adult mosquito indicates the volume of ingestible blood per bite of the mosquito species (Akoh et al. 1992). These indices are critical for survivorship and adult success. The present results showed significant effects of water hardness on these indices. The larvae reared at moderately hard water (i.e., 90 mg/L $CaCO_3$) were the largest by volume and could have accumulated the most tenerals. Those raised in soft water (i.e., 30 mg/L CaCO3) followed these, closely. The smallest larvae were those reared at very hard water (i.e., 210 mg/L $CaCO_3$). This may indicate that given the same opportunity, those reared at very hard water regimen, if they live long enough, may ingest less blood per blood meal time, develop less eggs, and constitute less nuisance to man, although, further study in this respect is advocated.

It is interesting to note that mosquitoes reared at 0 mg/L $CaCO_3$ were bigger than those raised at 150 and 210 mg/L $CaCO_3$, but smaller than individuals from soft (30 mg/L $CaCO_3$) and moderately hard water (90 mg/L $CaCO_3$). This may, perhaps, indicate that despite the toxicity of excess calcium expressed as shortening of lengths of body parts, it is still necessary for the normal functioning of the larvae (Li et al. 2002), and a threshold level is vital for this (Leschen and Cutler 1994). This result may also suggest that *Cx. quinquefasciatus* mosquito species' larvae develop optimally at 'moderately hard water' than at 'very hard water'; this information could be incorporated in manipulating the larval environment towards reducing, drastically, the population density and vectorial fitness of the mosquito species.

From the result obtained, there is an indication that an optimum water hardness of 90 mg/L $CaCO_3$ (i.e., moderate hardness) favours the growth and development of this species of mosquitoes, and at hardness levels above this value, development is severely affected, and less-fit mosquitoes were produced.

Conclusion

The present study on morphometrics of life stages of *Cx. quinquefasciatus* mosquito reveals that water hardness levels significantly affect immature growth and development, and adult fitness attributes. More so, species of the mosquito reared at moderately hard water (90 mg/L $CaCO_3$) performed best, as bigger mosquitoes and vectorial-fit mosquito populations were produced from this regimen.

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Author contributions UAC, OIK, OICJ, and AFO conceived and designed the experiments; UAC and UCC performed the experiments; UAC, OIK, OICJ and AFO analyzed the data; OIK and AFO contributed tools; UAC and UCC wrote the first draft of the manuscript; OIK, OICJ and AFO corrected the draft copy. All AUTHORS agreed to the final state of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

Abrams PA, Leimar O, Nylin S, Wiklund C (1996) The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. Am Nat 147:381–395

Adeleke MA (2010) Population dynamics of indoor sampled mosquitoes and their implication in disease transmission in Abeokuta, south-western Nigeria. J Vector Borne Dis 47:33–38

Akoh JI, Aigbodion FI, Kumbak D (1992) Studies on the effect of larval diet, adult body weight, size of blood-meal and age on the fecundity of *Culex quinquefasciatus* (Diptera: Culicidae). Insect Sci Appl 13:177–181

Auerswald L, Gäde G (2001) The role of calcium in the activation of glycogen phosphorylase in the fat body of the fruit beetle, *Pachnoda sinuate*, by hypertrehalosaemic hormone. Biochim Biophys Acta 1499(3):199–208

Bar A, Andrew J (2013) Morphology and morphometry of *Aedes aegypti* Larvae. Annu Res Rev Biol 3(1):1–21

Blanksma CB, Eguia KL, Lazorchak JM, Smith ME, Wratschko M, Schoenfuss HL (2009) Effects of water hardness on skeletal development and growth in juvenile fathead minnows. Aquaculture 286:226–232

Braks M, Leal W, Cardé R (2007) Oviposition responses of gravid female *Culex quinquefasciatus* to egg rafts and low doses of oviposition pheromone under semi-field conditions. J Chem Ecol 33:567–578

Garba Y, Olayemi IK (2015) Spatial variation in physico-chemical characteristics of wetland rice-field mosquito larval habitats in Minna, North-central Nigeria. In: Proceedings of the international conference on agriculture, ecological and medical sciences (AEMS-2015), Penang, Malaysia

Heming BS (1993) Structure, function, ontogeny, and evolution of feeding in thrips. In: Schaefer CW, Leschen RAB, (eds) Functional morphology of insect feeding. Thomas Say Publications in Entomology: Proceedings, Entomological Society of America, Lanham

Hopkins TL, Kramer KJ (1992) Insect cuticle sclerotization. Annu Rev Entomol 37:273–302

Leschen RAB, Cutler B (1994) Cuticular calcium in beetles (Coleoptera: Tenebrionidae: Phrenapetinae). Ann Entomol Soc Am 87:918–921

Li D, Scherfer C, Korayem AM, Zhao Z, Schmidt O, Theopold U (2002) Insect hemolymph clotting: evidence for interaction between the coagulation system and the prophenoloxidase cascade. Insect Biochem Mol Biol 32:919–928

Locke M (2001) The Wigglesworth lecture: Insects for studying fundamental problems in biology. J Insect Physiol 47:495–507

Mgbemena IC, Opara FN, Osuala FOU, Iwuala MOE (2009) The Physico-chemical characteristics of drainage breeding sites of mosquitoes in parts of Imo State, South Eastern, Nigeria. Int J Environ Health Hum Dev 10(2):1–13

Milad K, Mohammed S, Seyed AH (2011) Effects of Water hardness on egg hatchability and larval viability of angelfish (*Pterophyllum scalare*). Int J Res Fish Aquac 1:6–10

Mondal R, Devi NP, Jauhari RK (2015) Landmark-based geometric morphometric analysis of wing shape among certain species of *Aedes* mosquitoes in District Dehradun (Uttarakhand), India. J Vector Borne Dis 52:122–128

Mpho M, Callaghan A, Holloway GJ (2002) Effects of temperature and genetic stress on life history and fluctuating wing asymmetry in *Culex pipiens* mosquitoes. Eur J Entomol 99:405–412

Muturi EJ, Mwangangi J, Shililu J, Jacob BG, Mbogo C, Githure J, Novak RJ (2008) Environmental factors associated with the distribution of *Anopheles arabiensis* and *Culex quinquefasciatus* in a rice agro-ecosystem in Mwea, Kenya. J Vector Ecol 33(1):56–63

Mwangangi JM, Muturi EJ, Shililu J, Muriu SM, Jacob B, Kabiru EM, Mbogo CM, Githure J, Novak R (2006) Survival of immature *Anopheles arabiensis* (Diptera: Culicidae) in aquatic habitats in Mwea rice irrigation scheme, Central Kenya. Malar J 5:114

Mwangangi JM, Mbogo CM, Muturi EJ, Nzovu JG, Kabiru EW, Githure JI, Robert J, Novak RJ, Beier JC (2007) Influence of biological and physicochemical characteristics of larval habitats on the body size of *Anopheles gambiae* mosquitoes (Diptera: Culicidae) along the Kenyan coast. J Vector Borne Dis 44:122–127

Mwangangi JM, Muturi EJ, Shililu J, Muriu SM, Jacob B, Kabiru EW, Mbogo CM, Githure J, Novak R (2008) Contribution of different aquatic habitats to adult *Anopheles arabiensis* and *Culex quinquefasciatus* (Diptera: Culicidae) production in a rice agroecosystem in Mwea, Kenya. J Vector Ecol 33(1):129–138

Olayemi IK (2008) Influence of land-use on the fitness of *Anopheles gambiae*, the principal vector of malaria in Nigeria. Online J Health Allied Sci 7(4):3

Olayemi IK, Omalu ICJ, Famotele OI, Shegna SP, Idris B (2010) Distribution of mosquito larvae in relation to physico-chemical characteristics of breeding habitats in Minna, North Central Nigeria. Rev Infect 1(1):49–53

Olayemi IK, Olupinla T, Ukubuiwe AC, Odeyemi MO, Salihu IM (2014a) Distribution and oviposition dynamics of mosquito (Diptera: Culicidae) in response to ovitrap substratal material in Minna, Nigeria. Malaya J Biosci 1(2):117–125

Olayemi IK, Abdullahi-Sani H, Ukubuiwe AC, Adeniyi KA, Jibrin AI (2014b) Influence of ecological setting on occurrence of artificial container-breeding vector mosquito species (Diptera: Culicidae) and oviposition attraction to mineral salts in larval habitats, in Minna, Northcentral Nigeria. Int J Innov Sci Res 9(1):94–99

Oyewole IO, Momoh OO, Anyasor GN, Ogunnowo AA, Ibidapo CA, Oduola OA, Obansa JB, Awolola TS (2009) Physico-chemical characteristics of *Anopheles* breeding sites: Impact on fecundity and progeny development. Afr J Environ Sci Technol 3(12):447–452

Paulraj MG, Reegan AD, Ignacimuthu S (2011) Toxicity of benzaldehyde and propionic acid against immature and adult stages of *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say) (Diptera: Culicidae). J Entomol 8:539–547

Poteat MD, Buchwalter DB (2014) Calcium uptake in aquatic insects: influences of phylogeny and metals (Cd and Zn). J Exp Biol 217:1180–1186. https://doi.org/10.1242/jeb.097261

Purcell AH, Almeida RPP (2005) Insects as vectors of disease agents. http://www.dekkr.com/sdek/abstract-db=enc-content=a713596587. Accessed 12 Feb 2018

Robert V, Awono-Ambene HP, Thiolouse J (1998) Ecology of larval mosquitoes (Diptera: Culicidae) in market-garden wells in urban Dakar, Senegal. J Med Entomol 35(6):948–955

Tiimub BM, Adu BK, Obiri-Danso K (2012) Physico-chemical assessment of mosquito breeding sites from selected mining communities at the Obuasi municipality in Ghana. J Environ Earth Sci 2(10):123–129

Timmermann SE, Briegel H (1993) Water depth and larval density affect development and accumulation of reserves in laboratory populations of mosquitoes. Bull Soc Vector Ecol 18:174–187

Tyagi VAK, Sharma AK, Dhiman S, Srivastava AR, Yadav R, Sukumaran D, Agrawal OP, Veer V (2016) Malaria vector *Anopheles culicifacies* sibling species differentiation using egg morphometry and morphology. Parasit Vectors 9:202. https://doi.org/10.1186/s13071-016-1478-5

Ukubuiwe AC, Olayemi IK, Jibrin AI (2016) Genetic variations in bionomics of *Culex quinquefasciatus* (Diptera: Culicidae) mosquito population in Minna, North Central Nigeria. Int J Insect Sci 8:1–7

Ukubuiwe AC, Olayemi IK, Omalu ICJ, Arimoro FO, Salihu IM, Jibrin AI, Ukubuiwe CC, Yunusa RY (2017) Influence of photoperiod on larval growth indices and energy budget for metamorphosis in *Culex quinquefasciatus* mosquito (Diptera: Culicidae): its implication in integrated vector management. In: Proceedings from the 30th international conference of the Biotechnology Society of Nigeria (BSN), held at the Federal University of Technology, Minna from 27th to 30th August 2017, pp 167–176

Water Quality Association, WQA (2018) Scale deposits are a typical indicator of hard water. http://www.wqa.org. Accessed 14 Jan 2018

World Health Organisation, WHO (2011) Hardness in Drinking-water: background document for development of WHO guidelines for drinking water quality. WHO/HSE/WSH/10.01/10/Rev/1, p 19