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Research article

Gamete Quality and Reproductive Performance in *Clarias gariepinus* Chronically Exposed to Industrial Effluent Mixtures

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ABSTRACT

Clarias gariepinus fingerlings were raised to adulthood for nine months in sublethal concentrations (7.67%, 3.83%, 2.56% and 1.92%) of binary mixtures (75:25%) food:beverage effluents and an unexposed control group in a static/renewal bioassay to investigate the effects of industrial effluent on gamete quality and progeny survival. Body weight and GSI of female broodstock were determined. Gamete qualities were also evaluated. To determine reproductive outcomes and larval survival, half of the stripped eggs of female broodstock from all exposure groups were fertilized with milt of males from control exposures in the first experimental setup while for the second experimental setup, the other half of exposed female eggs were fertilized with milt from male broodstock from the same exposure concentrations. Significant concentration dependent decreases were recorded in broodstock body weight, sperm motility and milt volume, egg weight and diameter in all experimental exposures compared with control. Significant concentration dependent decreases were also recorded in hatching period, total number of hatched eggs, % egg viability and larval survival at 21 days. The observed decreases in gamete indices are consistent with lower reproductive outcomes and poor larval survival in higher exposure concentrations, and may influence recruitment and population growth in natural environments.

Keywords: *gamete quality, industrial effluents, hatching success, Clarias gariepinus*

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INTRODUCTION

The continuous discharge of contaminants from anthropogenic activities into surface waters is of major concern globally because it portends increased risks of uptake and toxicity to wildlife and humans (Adeogun *et al.*, 2013). As such the chemical burden of the environment is associated with the discharge of industrial waste water and has the potential to affect critical biological processes of resident species including reproductive success (Alquezar *et al.*, 2006). One of the most sensitive endpoints for evaluating reproductive fitness of fish population in any environment is the measure of gamete quality, described as the ability of sperm to fertilize an egg and produce viable larva (Wang et al. 2014). Common measures of gamete quality include milt volume, viability of spermatozoa, egg morphology and chemical profile of gametes and seminal fluid, hatchability of eggs and malformation rate of embryos and fry survival (Brooks *et al*., 1997; Rurawanga *et al*., 2004; Ciereszko 2008; Hallare *et al*., 2004).

Gamete quality is critical for fry integrity and larval survival in a number of species (Babin *et al*., 2007). Indices of reproductive failure such as decreased sperm count, acrosome integrity, delayed hatching of fish eggs and yolk-sac abnormalities have been attributed to contaminant exposure and uptake (Nguyen and Jansen 2001; Mathur *et al*., 2010) including heavy metals (Zhou *et al*., 2014). A number of chemicals from various point source discharges enter the aquatic environment from urban sources of which heavy metals have continued to be the focus of eco-toxicological studies because metals in their ionic form can traverse blood tissue barriers, interact with cellular ligands and impair the function of biomolecules (Alquezar *et al.*, 2006).

The direct implication of egg and sperm quality (Alavi *et al*., 2008; Hajirezaee *et al*., 2010; Wang *et al*, 2012) have been documented for a number of species including *Clarias gariepinus* - a freshwater species of commercial importance as

an affordable source of protein and available in most tropical inland water bodies (Adeogun and Chukwuka, 2012; Oguntuase and Adebayo 2014). As such evaluating the relationship between industrial discharge and reproductive outcomes will provide information necessary for assessing and establishing effects on other fish species exposed to environmental pollutants.

While a previous study demonstrated the alterations in reproductive success of pre-spawning adult female *C. gariepinus* brooders exposed to sub lethal concentrations industrial effluents (Adeogun and Chukwuka, 2012), the study did not take into account the reproductive effects of long term exposure of early life stage fish (male and female) to environmental contaminants. Hence this study was aimed at evaluating the effects of exposure to chronic exposures of binary mixtures of food and beverage industry effluent on gamete quality and survival of progeny of *Clarias gariepinus*.

MATERIALS AND METHODS

Acclimatization of *Clarias gariepinus* fingerlings

Laboratory-reared fingerlings of *C. gariepinus* ranging between 11.85 – 12.21 g (12. 03±0.18 g) were acclimatized in 250L-capacity outdoor concrete holding tanks filled with de-chlorinated tap water for seven days in a 12hr light: dark photoperiod. Fish were fed with 40% crude protein diet at 5% body weight and uneaten food was siphoned out daily to avoid pollution during the acclimatization period (OECD, 2002).

Collection of Effluents

Whole unfiltered effluents were collected from points of discharge of a food and beverage industry in Oluyole Industrial Estate, Ibadan and combined as binary mixtures in the ratio of (75: 25%; food:beverage). This gave a fraction of 3:1 v/v and was taken as the effluent stock solution. Required volumes (fractions) which were serial dilutions of the stock solution were measured into graduated plastic containers and made up to the required concentration with water according to the methods described by for toxicity evaluation (OECD, 2002; Reish and Oshida 1987).

Chronic Exposures

Nominal fractions of a predetermined 96 hr LC_{50} from mixture toxicities of 75:25% food and beverage effluents (Adeogun and Chukwuka, 2012) which gave concentrations of 7.67, 3.83, 2.56 and 1.92% respectively and control exposures (0.00%) devoid of effluent mixtures with two replicates each were used in a 9-month static/renewal bioassay protocol. Ten outdoor concrete tanks measuring 1.14 x 0.8 x 0.3 m (Length x Breadth x Height) were used for the chronic exposure and each tank was stocked with 350 fingerlings from February – October 2011. Exposure media was renewed every 72 hrs to maintain a constant chronic concentration.

Biometric indices

Subsamples of 20 fish were randomly selected weekly and body weight and length were measured with an Ohaun Compact digital weighing balance (Mettler Instruments) and a digital Vernier Calliper (Topac Instruments) respectively. Condition factor (k) was calculated using the equation

$$K = W/L^3 \times 100$$

where W= wet weight and L = total length (Fulton, 1902).

Induced Reproduction

Selection of brooders: After the nine-month exposure period, two experimental setups were used to assess gamete quality in male and female *C. gariepinus* broodstock. In the first experimental setup, a total 100 males (351.02±57.11g; 31.95±2.23cm) per replicate were randomly selected from the control exposures and subdivided into 10 batches of ten males (n=10) and placed individually in holding bowls of 30L capacity in two replicates for gonadal stimulation. Five female fish were also randomly selected from each exposure concentration including control exposures and randomly placed in individual holding tanks (30L) in two replicates per exposure group for gonadal stimulation.

In a second experimental setup, male and female broodstock (191.56±41.68 – 432.77±62.27 g), 20 (n=10 for male; n=10 for female fish) from each exposure concentration and control were selected and randomly placed separately in 30L holding bowls in two replicates for gonadal stimulation.

Injection of brooders: Fish gonadal stimulation entailed intramuscular administration of Ovaprim™ which contained fish gonadotropin releasing hormone (GnRH) and dopamine blocker receptor at the rate of 0.5mL Kg^{-1} (female) and 0.25mL Kg^{-1} (male) of body weight (Sahoo *et al*., 2005).

Collection of milt and evaluation of sperm quality: After a latency period of 12hrs, the selected male brooders in the first and second experimental setups were subsequently sacrificed by medullar transection and their testis was collected. Incisions were made on testicular lobes and fresh milt was squeezed into a petri dish and transferred into a 2 mL graduating cylinder to estimate milt volume (mL). A drop of milt from individual male fish was placed on a clean slide and an equal drop of distilled water was added to activate the spermatozoa and viewed under X40 magnification with an Olympus Micronal microscope. Motile spermatozoa were evaluated as a percentage of total sperm and sperm motility (%) was estimated using methods described by Lamai (1996).

Egg quality indices and fertilization of eggs: Eggs were stripped from female brooders (n=10 per exposure concentration) 12 hrs after inducing ovulation by applying gentle pressure to the abdominal area. Eggs were weighed with an Ohaun digital weighing balance and 1g of egg was collected per female and counted to determine total fecundity i.e. total number of eggs. Fifty eggs per female were subsequently measured individually for egg diameter using an ocular micrometer.

The remaining eggs were divided into two batches. The first batch of eggs were fertilized with milt from control male exposures while the second batch were fertilized with milt of male from the same exposure concentration as the female brooders by gentle mixing in plastic bowls and spread in hatching bowls (Huismann and Ritcher 1987).

Incubation of eggs and estimation of hatching success: Fertilized eggs of female fish (n=10 per exposure concentration) from all experimental setups were incubated for 48hrs and egg viability indices such as total number of hatched eggs/ hatching success and egg viability were determined based on the percentage number of hatched eggs (Aluko and Ali 2001).

Egg viability rate =
No. of hatched eggs / total no. of eggs in a batch x 100

Hatching success was estimated 48 hrs after incubation by counting the number of opaque and whitish (non-fertile) embryos. Larval mortalities were recorded on a daily basis and larval survival in each experimental setup was estimated after 21days post-fertilization. After 3–4 days the yolk sac was absorbed and the hatchlings were fed with Artemia®.

Estimation of gonadosomatic index
After stripping of female brooders (n=10 per exposure concentration) for egg collection, they were sacrificed by medullar transection and their gonads were extracted. The weight of gonads was determined and added to the weight of stripped eggs to obtain actual gonad weight. Gonado-somatic index (GSI) was calculated according to the equation
GSI= GW/ (BW-GW) x 100 (Van Aerle *et al*., 2001).
where GW=Gonad weight and BW=Body weight

Physicochemical parameters
Physicochemical parameters such as pH, temperature and dissolved oxygen (DO) were measured bi-weekly with a hand-held Electrochemistry multimeter (Topac Instruments). Some abiotic parameters measured during the study period were: temperature (27.52 ± 0.80ºC), pH (6.89±0.07), and dissolved oxygen (4.53 ±0.57 mg/L).

Statistical analysis
Data were presented as mean ± SD and variances between exposure groups were subjected to one-way analysis of variance (ANOVA). Duncan multiple range test was used to test significant differences across exposure concentrations. Data sets were analyzed with Origin version 8 software (Originlab USA) and differences between exposure concentrations were considered significant at $p<0.05$.
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RESULTS

Behavioural response of fish to effluents exposure
Fish exposed to varying sublethal concentrations of binary mixtures of food and beverage effluents exhibited occasional darting up and down the water column and spiral movement, especially at the highest concentration. Feeding activity was poor in exposed fish with the highest concentration having the lowest feeding activity. Fish population in the control (0.00%) exposures, on the other hand promptly accepted feed. This slow response to feeding was however not apparent by the 8th week, as the fish in the 1.92% exposure group responded actively to feeding. By the 12th week of exposure, fish in the highest concentration exposure accepted feed more readily but response to feeding was slower than those of fish in the control exposure groups.

Effects on somatic indices, gonad weight, GSI and condition factor of female *Clarias gariepinus* brooders
Female brooders in the control group (0.0%) had a significantly higher 2.4-fold increase in body weight compared to fish in the highest (7.67%) exposure concentration. There was a significant concentration dependent decrease in body weight across all exposure concentrations. Fish in the highest exposure concentration were also shorter in length but this decrease did not differ significantly from the control exposures. Condition factor of female brooders in the highest exposure concentration was lower than fish in the control group (Table 1).

Gonad weight showed a concentration dependent decrease with the gonadal weight of fish in the control and 1.92% exposures being significantly ($p<0.001$) higher than the 7.67% exposure concentrations (10.40±1.60 g) (Fig. 1a). Gonadosomatic index (GSI) showed a distinct concentration dependent decrease; however significant decreases were only recorded between fish in the highest exposure concentration and the control group (Fig. 1b).

Table 1:
Body weight, total length and condition factor of *Clarias gariepinus* female broodfish exposed to industrial effluents

Exposure conc. (%)	Wet weight (g)	Total length (cm)	Condition factor (CF)
0.00	531.90 ±44.85^{a}	36.84 ±1.18^{a}	1.06 ±0.53^{a}
1.92	317.20 ±30.84^{b}	35.63 ±1.08^{a}	0.70 ±0.31^{a}
2.56	289.80 ±29.78^{b}	35.49 ±1.85^{a}	0.64 ±0.47^{b}
3.83	259.20 ±27.97^{bc}	31.71 ±1.25^{a}	0.75 ±0.39^{a}
7.67	221.30 ±16.38^{c}	32.43 ±0.69^{a}	0.64 ±0.28^{b}

Values are given as mean ± standard deviation of mean (SD; n=10). Different alphabets along the same column indicates significant difference ($p<0.05$).

Effects on gamete quality

Sperm quality: Variations in sperm indices of *C. gariepinus* male broodstock exposed to binary mixtures of food and beverage effluents are shown in Figure 2. Concentration dependent decreases were recorded in sperm motility and milt volume of male broodstock in all exposure concentrations compared with control exposures. Significant ($p<0.001$) 2-fold decrease was recorded in sperm motility between the highest exposure concentration (39.15±1.26 %) and control (80.35±2.61 %) (Fig. 2a)
Milt volume was not significantly different between control exposure fish (1.31±0.04 mL) and the lowest exposure concentration (1.24±0.02 mL). However, significant decreases in milt volume were recorded between male brooders in the control group and the 2.56 (0.89±0.04 mL), 3.83 (0.86±0.04 mL) and 7.67 % (0.48±0.28mL) exposure concentrations respectively (Fig. 2b).

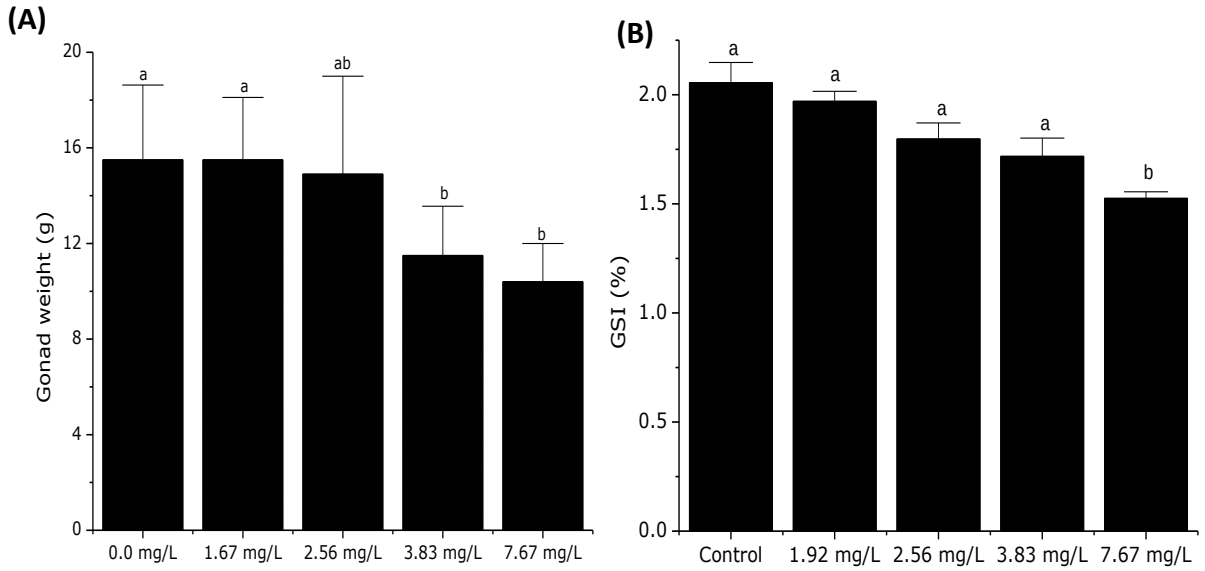

Figure 1:
Changes in gonad weight and gonadosomatic index of *Clarias gariepinus* female broodfish exposed to industrial effluents. Different alphabets along the charts indicate significant difference ($p<0.05$).

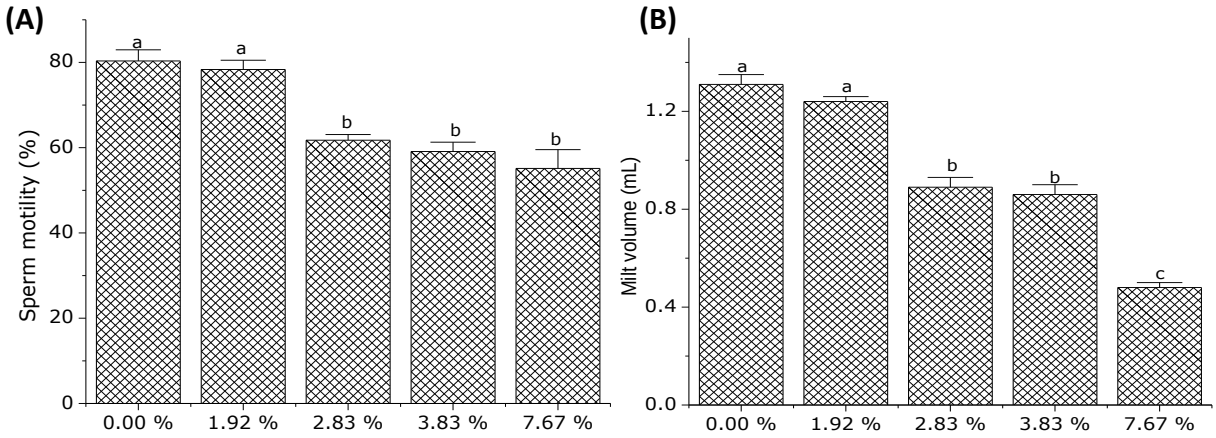

Figure 2:
Variations in sperm quality of *Clarias gariepinus* exposed to industrial effluents. Different alphabets along the charts indicates significant difference ($p<0.05$).

Egg quality: Concentration dependent decreases in egg weight, egg diameter and total number of eggs were recorded across all exposure concentrations compared with control exposures (Fig. 3). Mean egg weight ranged between 7.30 – 16.30 g and a significantly ($p<0.003$) lower egg weight was recorded in female brooders in the highest exposure concentration (7.30±1.27 g) compared with control (16.30±0.56 g) (Fig. 3a). Mean egg diameter ranged between 1.06 – 1.78 mm and eggs with significantly smaller diameter were recorded in fish from the highest exposure concentration (1.06±0.02 mm) compared with the control (1.78±0.04 mm) (Fig. 3b). There was also a significantly ($p<0.001$) lower total number of eggs in the highest exposure concentration (4205.05±170.77) compared with control 8706.00±180.48) (Fig. 3c).

Effects on reproduction: Variations in egg hatching time was recorded across exposure concentrations with eggs from the highest exposure concentrations showing a prolonged hatching period (26.08±1.72 hrs) compared with the control group (20.21±0.78 hrs) (Fig.4). In the first experimental setup, mean number of hatched eggs ranged between 92.00 – 3066.75 and a significantly lower number of hatched eggs was recorded in the highest exposure concentration (92.00±14.14) compared with control (3066.75±73.89) (Table 2). Percentage egg viability also decreased significantly in the highest exposure concentration (18.42±0.32 %) compared with control (77.32±0.32 %) (Table 2). Larval survival at 21 days ranged between 33.00 -2057.00 and there were significant decreases in % larval survival in all exposure concentrations with an 8.32 fold increase in larval survival between the highest concentration and control exposures (Table 2).

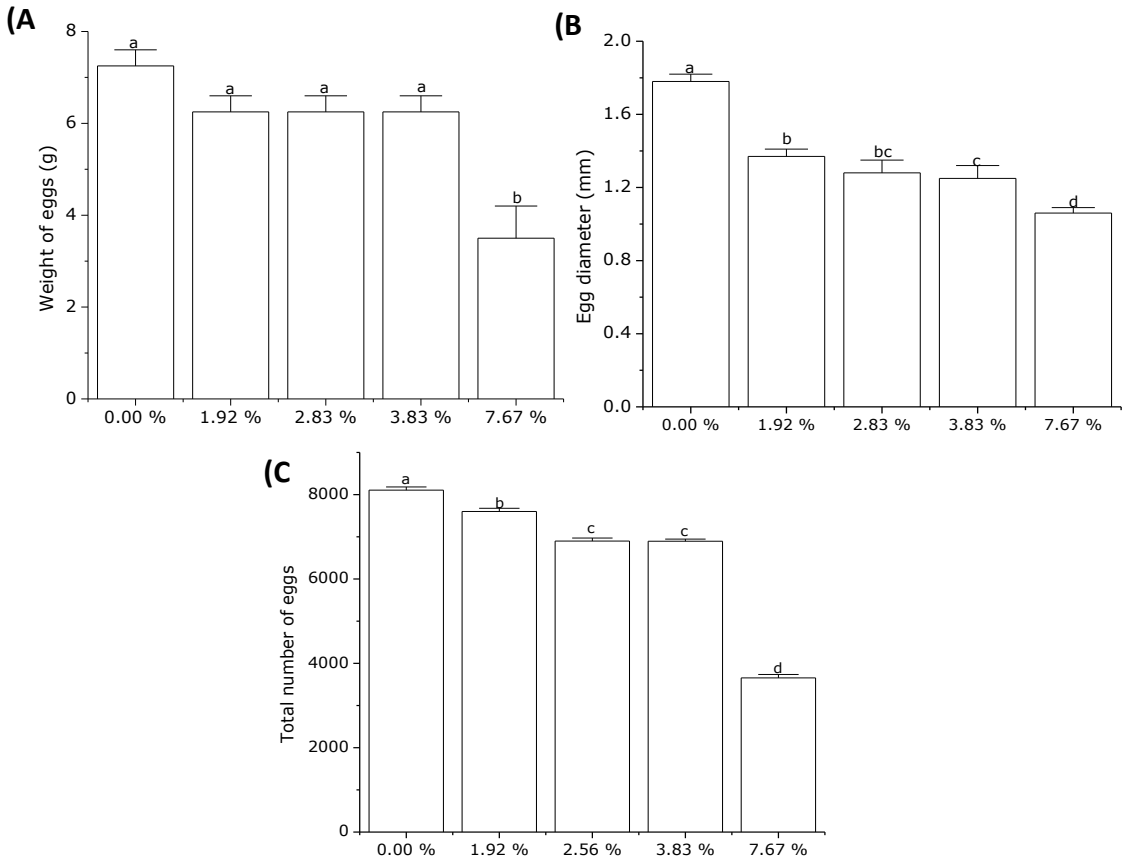

Figure 3:
Variations in egg quality of *Clarias gariepinus* exposed to industrial effluents. Different alphabets along the charts indicate significant difference ($p<0.05$).

Table 2:
Variations in hatched eggs, percentage egg viability and larval survival of *Clarias gariepinus* broodfish (exposed female and control male) exposed to industrial effluents

Exposure conc. (%)	No. of eggs	No. of hatched eggs	% egg viability	Larva survival at 21 days	% survival
0.00	4053.00±4.24^{a}	3066.75±73.89^{a}	77.32±0.32^{a}	2057.00±52.32^{a}	70.00±0.01^{a}
1.92	3800.00±0.05^{b}	2301.00±43.84^{b}	63.44±1.07^{b}	1227.00±25.69^{b}	58.70±0.01^{b}
2.56	3450.00±35.35^{b}	1786.00±60.81^{c}	55.61±1.09^{c}	706.00±29.69^{c}	48.22±0.01^{c}
3.83	3448.00±35.35^{b}	1110.25±49.85^{d}	37.62±0.94^{d}	119.00±18.38^{d}	36.80±0.02^{d}
7.67	1827.50±38.18^{c}	92.00±14.14^{e}	18.42±0.32^{e}	33.00±2.82^{e}	8.41±0.41^{e}

Note: Different alphabets along the same column indicates significant difference ($p<0.05$).
Values are given as mean ± standard deviation of the mean (SD; n=10)

In the second experimental setup where male and female brooders from the same exposure concentrations were cross fertilized, significant decreases were recorded in number of hatched eggs, egg viability and larval survival of progenies (Table 3). Eggs in the highest exposure concentration (7.67%) failed to hatch (0.00 %). As such, there were no survivors at 21 days post-hatch and although 10.00 % survival was recorded in the 3.83% concentration, there were no survivors (0.00 %) by the 21 day post-hatch period. 1.59 and 2.29 fold increases were recorded for % egg viability and larval survival at 21 days between the lowest exposure concentration (48.00; 29.97 %) and control (76.34; 68.78 %) exposures respectively (Table 3).

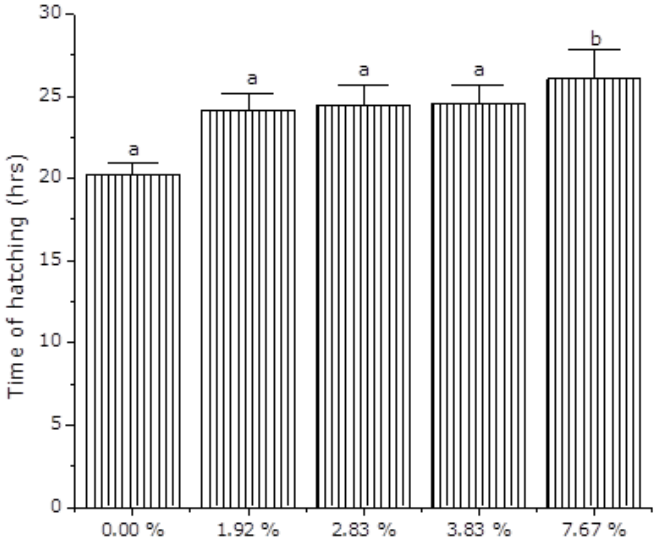

Figure 4
Changes in hatching period of *Clarias gariepinus* exposed to industrial effluents. Different alphabets along the charts indicate significant difference ($p<0.05$).

DISCUSSION

The ability of fish to produce high quality gametes is critical for successful and optimal reproductive outcomes and this is largely dependent on the quality of the rearing environment. As such high quality gametes may reflect the state of health or fitness of fish population in aquatic ecosystems (Hajirezaee et al., 2010).

The poor feeding activity recorded for fish in the highest exposure concentration implies reduced feeding activity which could affect growth rate, reproductive potential and population dynamics in the wild. Foraging behaviour has been documented to be highly vulnerable to chemical pollutants because they may have direct adverse effects on the central nervous system of fish, which coordinates these behaviours, or disturb the function of sensory systems receiving and processing biological information (Blaxter and Hempel, 1963). Furthermore the erratic swimming patterns recorded in higher concentrations implies that aquatic species exposed to contaminants in the wild may experience reduced stamina, inability to school, altered activity patterns and increased conspicuousness (hyperactivity), all leading to increased predator vulnerability, increased mortality rates with negative consequences for population size and structure (Mesa et al., 1994; Scott and Sloman 2004).

The significant decrease in fish body weight and condition factor with increasing effluent concentrations implies reduced food intake or altered metabolic processes resulting in low energy storage in tissues which can affect the reproductive outcome of fishes. Condition index is a function of lipid storage in tissues which in turn constitutes an important attribute of fish health and reproductive fitness. This is because lipids are used for energetic mechanisms associated with growth and survival as well as reproductive development (Weiss et al., 2001). As such reduced condition of fish may result in production of eggs with poor quality. The physiological state of female broodstock has been reported to affect the complements of nutrients deposited into the oocyte which in turn affects egg quality (Kjørsvik et al., 2003). In addition, the quantity and quality of broodstock diets is believed to have a great influence on egg quality and reproductive outcomes of several fish species (Brooks et al., 1997; Ling et al., 2006). Murawski et al., (2001) demonstrated the relationship between maternal size and egg size showing that declining average maternal size resulted in lower egg size and poor larval viability. A positive correlation between reduced weight of prespawning female *C. gariepinus* brooders, egg viability and offspring survival has also been documented (Adeogun and Chukwuka 2012).

The decrease in the gonadosomatic index (GSI) with increasing toxicant exposure may be indicative of delayed onset of gametogenesis in exposed broodstock and this in turn may be attributed to altered hormonal profile of the broodstock. Adeogun and Chukwuka (2012) had earlier reported elevated levels of lead, chromium and iron in the industrial effluents that fish were exposed to in this study.

Table 3:
Variations in hatched eggs, percentage egg viability and larval survival of *Clarias gariepinus* male and female broodfish exposed to industrial effluents

Exposure conc. (%)	No. of eggs	No. of hatched eggs	% egg viability	Larva survival at 21 days	% survival
0.00	4053.00±4.24^{a}	3023.50±6.36^{a}	76.34±0.22^{a}	1986.00±16.97^{a}	68.78±0.37^{a}
1.92	3800.00±0.05^{b}	1668.00±0.00^{b}	48.00±0.00^{b}	290.00±0.00^{b}	29.97±0.02^{b}
2.56	3450.00±35.35^{b}	825.00±9.89^{c}	30.00±0.01^{c}	112.50±0.70^{c}	10.00±0.25^{c}
3.83	3448.00±38.18^{b}	75.00±4.24^{d}	10.00±0.01^{d}	0.00±0.00^{d}	0.00±0.00^{d}
7.67	1827.50±38.18^{d}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{d}

Values are given as mean ± standard deviation of the mean (SD; n=10) Note: Different alphabets along the same column indicate significant difference ($p<0.05$).

These metal levels were higher than the permissible limit recommended by the National Environmental Standards and Regulatory Agency (NESREA, 2009) for industrial effluents discharged into surface waters. Such elevated levels may result in delayed gametogenesis leading to lower GSI in female broodstock from higher exposure concentrations. Van Duyn et al., (2010) documented that exposure to organometallic cations may cause delayed gametogenesis via dopamine neuron dysfunction which in turn may be linked to systemic failure of dopamine regulation and impairment of cellular proliferation and migration resulting in delayed embryonic development (Limke et al., 2004; Van Duyn et al., 2010). Contaminants with lipophilic properties, for example metals, have been implicated in such effects because of their ease of bioaccumulation through transcutaneous and transcellular mobility (Paran et al., 2001). Such delayed onset of gametogenesis may also be connected to the nutritional state and energy store of the mature fish at the time gametogenesis was due. Lipids are critical for energetic mechanisms associated with reproductive activity (Weis et al., 2001); as such insufficient lipid store may stall the progression of gametogenesis. Delayed gametogenesis in the higher exposure concentrations may not be unrelated to the energy allocation that entails adaptive responses to toxicity. Xenobiotic regulation across cell membranes involves both passive and active mechanisms and therefore requires energy. Since lipids constitute the main energy source for fish (Heath 1995), a decrease in lipid storage necessary for gametogenesis may be one of the natural outcomes in fish exposed to anthropogenic substances; hence the amount of available lipid energy store allocated to reproduction is dependent on biochemical and physiological responses of an organism to its environment (Munkittrick and McCarty 1995). A number of authors have also reported a reduction in GSI of fishes from sewage treatment sites (Jobling et al., 1998; Levitt et al., 2001). A significant reduction in reproductive efficiency of parent broodstock, fry growth and percentage survival of fries of the largemouth bass, *Micropterus salmoides*, treated with pulp and paper mill effluent has been reported (Sepúlveda et al., 2003) and authors attributed these changes to alterations in egg quality which is also connected with poor condition of the parent fish.

The vulnerability of sperm cells to xenobiotic compounds compared to other germ cells has been attributed to its smaller cytoplasmic space (Muira and Muira 2011). The significant decreases recorded in sperm motility and milt volume of male broodstock in all exposure concentrations compared with control exposures could be attributed to altered morphology and reduced energetic reserves and quality of spermatozoa. Among other features of sperm physiology that are considered crucial to reproductive success, sperm motility and volume are two of the most important factors for determining embryonic hatching success. Reduced sperm quality may explain the appreciable reduction in number of hatched eggs recorded between the gametes of unexposed female and exposed male brooders and exposed male and female brooders from the same exposure concentrations in the two experimental setups. Furthermore, reproductive failure (unhatched eggs and larvae) recorded in the eggs of unexposed females fertilized with exposed male is a direct reflection of poor sperm quality as a consequence of exposure of male brooders to industrial effluents. Lead and chromium are priority toxic metals and have been reported to be present at higher levels than allowable limits in an earlier study (Adeogun and Chukwuka 2012) resulting in synergistic toxic effects of the 75:25% food beverage mixture used in this study. Hernandez-Ochoa et al. (2005) reported that low levels of Pb in spermatozoa and seminal fluid resulted in decreased semen quality in Mexican human male population. Other reports have shown that metals like chromium have been associated with reduced semen quality in rodents and humans (Kumar et al., 2005; Wirth et al., 2007).

The reduced egg quality as reflected by lower egg weight, egg diameter, total number of eggs and number of hatched eggs with chronic exposure of brood-stock to increasing effluent concentration may be a direct effect of increased contaminant uptake particularly metals i.e. Pb, Fe and Cr which were higher than acceptable NESREA limits as reported in a previous study (Adeogun and Chukwuka 2012). Increased levels of Pb in gonads have been associated with decreased oocyte diameter and density which may be as a result of transfer of contaminant from parent to eggs (Alquezar et al., 2006). A significant uptake of Cd in fish ovary highlights the possibility of maternal transfer of metals to eggs and potential decrease in hatching success (Wang et al., 2014). Metals, as well as other pollutants, have the ability to reduce fish oocyte quantity and quality and may result in malformations and impaired development (Brooks et al., 1997). Studies relating egg size and reproductive success have implicitly inferred that a larger egg size is correlated to higher biochemical and nutritional constituents of eggs (Murry et al., 2008, Jastrebski and Morbey 2009) which in turn is a strong determinant of offspring size and survival. Larger larval size is also expected to provide benefits to offspring through reduced susceptibility to size-selective mortality (Donelson et al., 2008). Reports on walleye eggs showed that larger eggs contained greater yolk and oil volumes and greater total lipid content (Czesny et al., 2005). Fries from these relatively larger eggs were reported to be larger with a greater likelihood of early life survival and increased chances of surviving to maturity (Johnston et al., 2007).

The delayed time in hatching observed in this study may have resulted from hormonal modulation due to the presence of possible endocrine disruptors. The higher concentrations of endocrine disrupting chemicals than permissible limits for Pb and Cr in the effluents used for this study may have resulted in an impairment of gonadotropin releasing hormones (GnRH) that regulate reproduction through cell signalling and neuro-modulatory activity (Wen et al., (2010). In the teleost fish brain, dopamine is a neurotransmitter that regulates the release of a gonadotropin (GtH) from the pituitary under natural conditions via a feedback mechanism and exposure to heavy metals have been implicated in enhanced dopaminergic activity (Weltzien et al., 2006). As such sublethal exposures to chemical pollutants can impair hormonal regulation of reproduction, affect oocyte and ovarian growth, which in turn significantly affects the quality of eggs produced (Soso et al., 2007).

Hatching and survival outcomes are two of the most commonly used gamete quality criteria and have been correlated with morphological and biochemical parameters of gametes from different species (Bobe et al., 2010; Kjørsvik et al., 2003). As such the lower survival recorded for eggs of female broodstock exposed to higher effluent concentrations and hatching failure in the exposed male and female population may imply poor reproductive health of both male and female brood fishes due to higher contaminant uptake and these may play a significant role in the quality of gametes produced. Significant uptake of metals by fish ovary has been reported, highlighting the possibility of maternal transfer of metals to eggs and potential decrease in hatching success (Wang et al., 2014). Other reports have also indicated that the quality of fish sperm may be as important as the quality of fish eggs to achieve viable progenies and subsequent larval survival (Alavi et al., 2008; Oguntuase and Adebayo 2014). The ecological implication of small sized larva is that smaller size at hatching may increase vulnerability to predation and reduce hardiness to environmental conditions. Population recruitment success is often related to cumulative growth and survival rates of individual larvae which tend to be positively correlated with egg size (Berkeley et al., 2004; Bergenius et al., 2002).

In conclusion, our findings demonstrate that chronic exposure to binary mixtures of food and beverage effluents resulted in lower male and female fish weight and condition through alteration of feeding behaviour in *Clarias gariepinus* exposed to these effluents. This led to a concentration-dependent reduction in sperm and egg quality; hatching period and success and larval survival. The poor condition of fish and gamete quality indicates hormonal dysfunction and altered biochemical and cellular events in fish exposed to industrial effluents. The ecological consequences of these findings were reflected in reduced larval survival and may imply increased susceptibility to mortality in natural environments.

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