American Journal of Agricultural and Biological Sciences 7 (1): 61-70, 2012
ISSN 1557-4989

Altered Reproduction in *Clarias gariepinus* Exposed to Industrial Effluents

Adeogun Aina Olubukola and Chukwuka Azubuike Victor
Department of Zoology,
Faculty of Science, University of Ibadan, Ibadan, Oyo State, Nigeria

Abstract: Problem statement: Reproductive success is a key factor in determining species survival and the unregulated discharge of industrial effluents into rivers and streams in a developing country like Nigeria, can impair reproductive ability in non-target organisms like fish. Fish, generally accumulate contaminants from their environment in their tissues and these can be transmitted through the yolk lipids to the eggs. **Approach:** Prespawning *Clarias gariepinus* female brooders were exposed to composite effluent mixtures from a food and beverage industry (discharged into Ona river) for a period of thirty days to determine its effect on growth and fertility/reproductive success (viability of eggs and survival of the F_1 generation fries). Physico-chemical parameters of effluent samples and exposure concentrations were analyzed and effluent sample was further analyzed for heavy metals (Fe, Pb, Cd, Ni, Zn, Cu, Mn). **Results:** Exposure to the effluents caused decreased weight (-7.33%) and egg production (198 eggs) in the highest exposure concentration. Significant decreases ($p<0.05$) in the percentage of viable eggs (71.25, 62.48 and 30.12%) of exposed females and percentage survival of F_1 generation fries (68.68, 46.42 and 22.33%) with increasing effluent concentration was also observed in the order 0.00%> 4.40%> 6.60% and there were no viable eggs and fingerlings in the highest exposure concentration(13.20%). Effluent sample and exposure concentrations had acidic pH and low DO values while effluent sample contained Fe and Pb in concentrations above the specified standard by the National Environment Standards and Regulation Agency (NESREA) for food and beverage industry effluent discharged into surface waters. **Conclusion:** The toxic nature of these effluents on the reproductive success of *C. gariepinus* (a commercially important freshwater fish in Nigeria) is of great concern to sustainable fisheries if stringent measures to monitor effluent quality discharge into this river are not implemented.

Key words: Reproductive success, food and beverage industry, viable eggs, F_1 generation fries, *Clarias gariepinus*

INTRODUCTION

Release of complex mixtures of contaminants into natural habitats or the waters used for culture has led to a wide range of effects on natural biota (Hopkins *et al*., 2004; Evans and Nipper, 2007; Chukwu and Okhumale, 2009; Chukwu and Lawal, 2010) including reduced reproductive success in fish (Kime, 1995; Hugla and Thome, 1999). Reproductive impairment in fish by a variety of xenobiotics has been well reported (Sol *et al*., 1995; Shatunovskii *et al*., 1996; Lye *et al*., 1997; Ankley *et al*., 2001; Rickwood *et al*., 2006a; 2006b; Wu *et al*., 2007; Jezierska *et al*., 2000) and may manifest in adults in form of decreasing quality and/or quantity of gametes, which in turn may affect fertilization success, embryonic development, larval viability and subsequently species fitness and survival.

Studies on the effect of pollution incidence on reproductive success of commercial fisheries does not only provide information for managing fisheries and optimizing hatchery production, but can be used to extrapolate the effects of environmental pollution on reproductive functions of other vertebrates including humans. In view of this, several indices of egg quality (i.e., parameters which can predict the success rate of developing embryos or larvae) for a wide range of fish species in different environments have been extensively studied (Kjorsvik *et al*., 1990; Shields *et al*., 1997; Lahnsteiner and Patarnello, 2005; Thorsen *et al*., 2003; Bell and Sargent, 2003; Maeland *et al*., 2003; Tveiten *et al*., 2004).

Clarias gariepinus is a tropical species of commercial importance. It is abundant in the wild and is the most widely cultured fish in aquaculture and homestead fisheries in Nigeria because of its hardy

Corresponding Author: Adeogun Aina Olubukola, Department of Zoology, Faculty of Science, University of Ibadan, Ibadan, Oyo State, Nigeria Tel: +2348055261105

nature and ease of culture. It constitutes a major source of fish protein and is in high demand in Nigeria . Thus considering the world-wide decline in stocks of important wild fish species and the unending incidence of pollution in aquatic systems, investigations into the relationship between water quality and reproductive success of commercially important fish species is expedient, hence this study.

MATERIALS AND METHODS

Collection of effluent: Whole unfiltered effluent samples were collected from the points of discharge of a food and beverage industry in Oluyole industrial estate, Ibadan, Nigeria in March 2010 and stored at 4°C prior to usage.

Test animals: Seven-month-old pre-spawning brood stocks of *C.gariepinus* with an average weight of 208.00±5.20g were procured from a commercial fish farm in Ibadan, transported to the laboratory in oxygenated containers and kept in holding tanks (250L capacity).The fish were acclimated to laboratory conditions (temperature, 27.80±1.40°C; pH 7.05±0.40; DO 6.05±0.38 mg L^{-1}) for a period of two weeks. Fish were fed twice daily with a commercial fish food (Coppens® fish feed, 40% crude protein) at 3% body weight and uneaten food was siphoned out to prevent accumulation of metabolites.

Acute toxicological experiments: Whole unfiltered effluents were taken as stock solution and required volumes of each effluent were mixed together in the ratio 1:1 (v/v) to obtain equal concentrations of 50:50% (food: beverage) concentrations. This was taken as stock solution. Batches of 10 female brood stocks were exposed to exposure concentrations of 0.00, 40.00, 60.00 and 100.00% in a static bioassay for 96 h in two replicates while male brooders were maintained in the laboratory for the period of the experiment. Standard procedures for bioassay as described by (Reish and Oshida, 1986) and OECD, 2002 were used with slight modifications (12 g of fish to 1L of water). The experimental setup was observed for 96 h and the 96 h LC_{50} values were computed by probit analysis (Finney, 2009).

Sub-acute experiments: Nominal fractions of the LC_{50} values (4.40, 6.60 and 13.20%) and control exposures (0.00%) with no effluent added were used in a static/renewal bioassay in two replicates for a period of 30 days. Ten fishes (239.75±5.83 g) were introduced into each concentration and the exposure concentration was renewed every 72hr for the duration of the experiment.

Induced reproduction: On the 30th day, three fish were randomly selected from each exposure concentration, their wet weights were recorded and their gonads were stimulated. Male brooders were also randomly selected from holding tanks and their gonads stimulated. Gonadal stimulation entailed the use of Ovaprim^{RM} at the rate of 0.5 mL kg^{-1} of body weight. This was administered intramuscularly at an angle of 30-45° of the dorsal fin in the direction of the tail. Injected fish were kept in separate tanks and a latency period of eleven h was allowed. After 11 h, male brooders were sacrificed and their testes collected individually in 100 mL conical flasks and stored in the refridgerator as it had been observed that the life-span of fish sperm vary with the substrate within which the testes are kept and sperm also live longer periods in lower temperatures (Huisman and Richter, 1987). Stripping of eggs for female brooders was done by applying gentle pressure to the abdominal area (Huisman and Richter, 1987). Eggs were stripped into dry plastic bowls, weighed and fertilized with the milt of male by gentle mixing in a plastic bowl containing 9% saline solution to ensure longer sperm viability. Fertilized eggs were evenly spread into nursery bowls containing cacabands to simulate nursery grounds.

Estimation of broodstock fertility: Total numbers of eggs for each setup was counted and egg viability rates were determined based on the percentage number of hatched eggs (Aluko and Ali, 2001) where:

$$\text{Egg viability rate} = \frac{\text{no. of hatched eggs}}{\text{total no. of eggs in a batch}} \times 100$$

Fries were fed with Artemia 3 days post-hatch for 10 days and subsequently with Coppens (0.5 and 1.0 mm) for the duration of the experiment. The survival rate of F_1 generation fingerlings after 42 days was determined by the methods of Jensen (1996) where:

$$\text{Survival rate} = \frac{\text{no. of fries hatched alive}}{\text{total no. of hatchlings}} \times 100$$

Physico-chemical parameters: Physico-chemical parameters like temperature, pH and DO were measured for exposure concentrations and effluent sample with CS 933T Electrochemistry multimeter (Topac Instruments Inc.,). Effluent sample was further analyzed for alkalinity, hardness, chlorides, phosphates and sulphates (Clesceri, 1998). Heavy metal content of effluent sample (Fe, Pb, Ni, Zn, Cu and Mn) was analyzed with a HACH DR 890 Atomic Absorption

Spectrophotometer (AAS). Values were compared with acceptable limits for effluents discharged into surface waters NESREA, 2009.

Statistical analysis: Toxicological dose response data for quantal (mortality) response were analyzed by the probit method (Finney, 2009). Data on fish wet weight, egg count and number of hatchlings across exposure concentrations were analysed by one-way ANOVA (differences between means were considered significant when $p<0.05$) and Pearson's correlation coefficient. Correlations between parameters were considered significant at $p<0.05$.

RESULTS

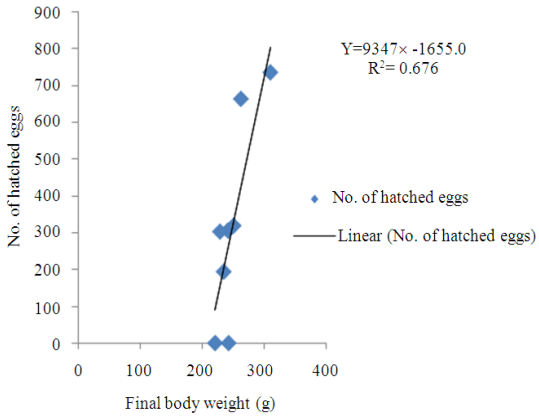
Fish in all exposure concentrations (except control) were observed to be stunned for about 30 sec when they were introduced into the test media. Uncoordinated swimming movements, hyperventilation, coughing and occasional darting upward to gasp for air were observed after one h. Fish movement gradually became sluggish especially in the highest exposure concentrations after 24 h, accompanied by occasional spasms. These behavioral responses were absent in the control exposures where no effluent was added. Dead fish were covered in thick mucus. Prior to exposure and in control exposures, fish were observed to have a black coloration on the dorsal surface while the ventral surface had a white coloration. The skin of fish in exposure concentrations became mottled and a grayish color was observed on the dorsal surface. The 96 h LC_{50} values were 53.14 and 52.47% for Replicates1 and 2 respectively with an average mean value of 52.81% (Table 1). 100% mortality was recorded in the 100.00, 80.00 and 60.00% exposures while there were no mortalities the control (0.00%) exposures.

Fish in exposure concentrations lost weight and the biggest weight loss (-5.33%) was recorded in the highest concentration (13.20%) while fish in control exposures gained weight (+1.56%). Significant decreases ($p<0.05$) in weight were also observed between fish in unexposed (control) concentrations and the 6.60 and 13.20% exposures (Table 2). The weights of stripped eggs were significantly smaller in the highest concentration than that of the control (unexposed) fish.

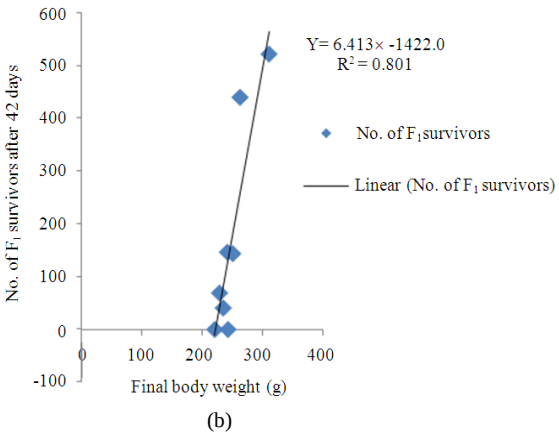
Table 1: Acute toxicity of food and beverage industry effluent on C. *gariepinus* female brood stock after 96 h

Mortality interval	R1; y = 1.193× + 113.4	R2; y = 1.188×+112.1
LC_{10}	86.67%	85.94%
LC_{50}	53.14%	52.27%
LC_{90}	19.61%	18.60%

Prolonged periods of hatching were recorded and this increased with increase in exposure concentrations (14-20 h in the higher concentrations as opposed to 10-12 h in control fish). Significantly ($p<0.05$) lower values were recorded for the total number of eggs that hatched from control exposures to the highest exposure. The highest exposure concentrations of 13.20% prevented egg viability in exposed fish as there were no hatchlings (0.00%) in this exposure concentration (Table 2). In other words the quality of eggs produced also determined the survival of fries. As depicted in Table 2, 70% survival was recorded from eggs derived from the control (unexposed) fish while in exposure concentrations, 0-46% of fries survived after 42 days. The survival profile for F_1 generation fries of exposed fish revealed a proportional decrease in survival of fingerlings with increase in concentration while there were no survivors in the highest exposure concentration (Table 2).



(a)



(b)

Fig 1: (a) Final body weight of *C. gariepinus* and no. of hatched eggs (b) Final body weight of *C. gariepinus* and no. of F_1 generation fries

Table 2: Change in wet weight, egg production, percentage viability of eggs of *C. gariepinus* and survival of F_1 generation fingerlings

Conc (%)	Initial weight (g)	Final weight (g)	% Change in weight	Weight of stripped eggs (g)	Total no. of eggs	No. of hatched eggs	Total no. of unhatched eggs	% Viability	No. of F_1 generation fries (at 42 days)	% Survival of F_1 generation fries (at 42 days)
0	283±32.53^a	286±33.94^a	0.87±0.69^a	1.51± 0.85^a	982±55.15^a	700±50.91^a	282± 4.24^a	71.25± 1.18^a	481± 41.01^a	68.68± 0.86^a
4.4	247 ± 6.63^a	245 ±6.36^{ab}	-0.81±0.21^{ab}	0.77± 0.57^b	501±36.77^b	312± 9.19^b	188± 27.58^a	62.48± 2.75^b	145± 1.41^b	46.42± 0.91^b
6.6	235 ± 2.83^a	231± 4.24^b	-1.71±0.62^{ab}	1.26 ±0.33^a	819±211.42^a	248± 7.07^b	571± 134.35^b	30.12± 1.63^c	55± 14.14^c	22.33± 1.24^c
13.2	239 ±11.31^a	231±15.56^b	-3.39±1.94^b	0.31 ±0.01^b	198±4.95^b	0.0±0.00^c	198 ±4.95^a	0.0±0.00^d	0.00±0.00^d	0.0±0.00^d

Values are means of triplicate readings±standard deviation means with same superscript along the same column are not significantly different (p>0.05)

Table 3: Pearson's correlation (R) values for fish weight, egg production and larval viability

Variables	No of hatched eggs	No of survivors	% survival	Initial weight	Final weight	Change in weight (%)	Weight of stripped eggs	Total no of eggs	Total no of unhatched eggs	% Viability
no of hatched eggs	1	0.963*	0.951	0.781*	0.823*	0.856*	0.854*	0.854*	0.077	0.887*
no of survivors		1.000	0.913*	0.858*	0.885*	0.816*	0.716*	0.716*	-0.129	0.810*
% survival			1.000	0.718*	0.769*	0.881*	0.716*	0.716*	-0.109	0.977*
Initial weight				1.000	0.995*	0.790*	0.518	0.518	-0.225	0.613
Final weight					1.000	0.844*	0.565	0.565	-0.199	0.671
Percentage change in weight						1.000	0.684	0.684	-0.023	0.853*
Weight of stripped eggs							1.000	1.000*	0.585	0.674
Total no of eggs								1.000	0.585	0.674
Total no of unhatched eggs									1.000	-0.090
% Viability										1.000

* = Correlation coefficient significant at p<0.05

Table 4: The values of physico-chemical characteristics of industrial effluent, exposure groups and NESREA acceptable limits

Parameters	Control	Exposures	Food and beverage Industry effluent	NESREA (2009)
Temperature (°C)	26.06±1.04	27.68±1.52	28.20±0.70	<40°C
pH	7.06±0.04	5.68±0.50	5.20±0.20	6.50-8.50
Dissolved Oxygen (DO) (mg L^{-1})	5.02±0.38	3.20±1.40	0.20±0.05	6.00
Biochemical Oxygen Demand (BOD) (mg L^{-1})	2.06±0.03	27.70±3.58	59.72±6.36	50.00
Chemical Oxygen Demand (COD) (mg L^{-1})	5.30±0.18	105.60±18.24	378.80±15.40	90.00
Alkalinity (mg L^{-1})	18.06±1.20	32.06±4.30	558.50±32.28	150.00
Hardness (mg L^{-1})	4.00±0.20	6.06±0.38	30.06±5.18	50.00
Total solids (TS) (mg L^{-1})	8.56±0.85	345.58±34.80	1650±32.50	2000.00
Total dissolved solids (TDS) (mg L^{-1})	2.75±0.30	103.28±11.30	685.20±33.20	--
Chloride (mg L^{-1})	2.30±0.03	35.80±4.50	215±18.50	300.00
Phosphate PO_4^{3-}(mg L^{-1})	--	--	3.46±0.14	5.00
Sulphate SO_4^{2-}(mg L^{-1})	--	--	37.06±3.04	250.00
Iron (Fe) (mg L^{-1})	--	--	3.06±0.05	0.30
Lead (Pb) (mg L^{-1})	--	--	4.02±0.06	0.05
Zinc (Zn) (mg L^{-1})	--	--	0.86±0.12	3.00-5.00
Nickel (Ni) (mg L^{-1})	--	--	0.02±0.01	0.05
Copper (Cu) (mg L^{-1})	--	--	0.03±0.01	2.00
Manganese (Mn) (mg L^{-1})	--	--	0.14±0.02	0.20

X ± SD represents means + Standard Deviation of values; NESREA = National Environmental Standards and Regulations Enforcement Agency

Test for relationship between the independent variable (fish body weight) and the dependent variables (number of stripped eggs and viability of eggs) were carried out using Pearson correlation statistic. Significant (p<0.05) positive correlations were observed between final weight of fish and weight of stripped eggs and total number of hatched eggs and percentage viability of eggs (Table 3). Figure 1 and 2 shows a scatter plot of the number of hatched eggs and fingerlings against the final body weight of parent fish (exposed female brooders). A linear relationship was recorded between fish body weight and number of hatched eggs and survival of fries. The coefficient of determination (R^2) indicated that about 0.676 (67%) and 0.801 (80%) of the trends observed in number of hatched eggs and F_1 generation fries after 42 days respectively was due to the changes in body weight of the exposed fish.

The values of physico-chemical parameters are presented in Table 4.Temperature values ranged from 26.40-29.20°C and were within normal ambient temperature for tropical waters. Acidic pH (5.60- 6.18) were recorded in all exposure concentrations except control exposures (7.06) with effluent sample being more acidic (5.40) than exposure concentrations. Dissolved Oxygen Concentration (DO) of effluent sample was very low (0.25 mg L^{-1}) but increased in all exposure concentrations (0.80-5.40 gm L^{-1}). Other

parameters like Alkanility and COD had values higher than the National Environmental Standards and Regulation Agency (NESREA) effluent limitation standard for food, beverages and tobacco industries. Concentrations of metals analyzed were not detected in exposure concentrations but effluent sample contained Pb and Fe in concentrations that exceeded NESREA's permissible limit for effluents discharged into surface waters and protection of the aquatic environment by these industries.

DISCUSSION

The reproductive output of a species is an integral endpoint in fish toxicology studies and an important aspect of risk assessment for aquatic ecosystems (Ankley *et al*., 2001). A wide array of chemicals enters the aquatic ecosystem from various sources (including industrial wastewater) and elicits responses that impair reproduction in fishes (Ankley *et al*., 2001). Survival of a species is highly dependent on the reproductive success of the species in question and for economically relevant species, water quality monitoring is necessary to aid evaluation of challenges posed by incidence of pollution (Adeogun, 2004). Behavioural reponses (uncoordinated swimming movement, hyperventilation, coughing and occasional darting movement) to poor water quality in exposed *C. gariepinus* broodstock, is an indication of the toxic effect of the food and beverage industry effluent eliciting behavioural changes in fish. These deviation from normal behavior in fish was further confirmed when such responses were not observed in the control setup with no effluent. Although the physiological effects of numerous chemicals have been evaluated, fish behavior is an endpoint that is not often used in toxicological assessment in spite of the fact that this parameter has the potential for use in toxicological studies as a sensitive indicator of reproductive impairment or other expressions of toxicity (Matthiessen and Logan, 1984; Scott and Crossman, 1985). Little *et al*. (1993) observed that behavioural changes in fish occur 75% earlier than the onset of significant mortality in fish. Some other authors have reported similar observations in different fish species. For example, Srivastava *et al*. (2007) reported hyper excitation, convulsions and rapid opercula movement in *Labeo rohita* and *Channa punctatus* exposed to paper mill effluent Pathan *et al*. (2009) observed hyperexcitation, erratic swimming, convulsions and jerky movement in *Rasbora daniconius* exposed to paper mill effluent. Consistent with these reports are temporary loss of equilibrium, hyperventilation and hyperactivity in juvenile members of the same species (*C. gariepinus*) exposed to textile industry effluent (Adeogun and Chukwuka, 2012).

The 96 h LC_{50} value of 52.81% reported in this study is higher than that of Vanerker *et al*. (2004) for physico-chemically treated herbal pharmaceutical effluent (96 h LC_{50}; 35.00-41.00%) on *Lebistes reticulata* and 20.06% for juvenile *C. gariepinus* exposed to textile industry effluent. A higher LC_{50} value is expected in adult *C. gariepinus* because the magnitude of toxic effect is directly proportional to corporal surface/ body weight of organisms and an advancement in physiology may confer better adaptation/coping strategies to environmental stressors in adult fish than juvenile/ fingerlings fish (Murthy, 1986).

The mottled skin colour and poor outlook of *C.gariepinus* may be as a result of depressed feeding or starvation in fish during the exposure period. According to McCue (2010), starvation can be referred to as a biological condition wherein a post-absorptive animal, otherwise willing or able to eat, is unable to do so as a result of some external influence such as anthropogenic disturbance. Severe anthropogenic disturbance among other factors like long-term ecological change can lead to long and frequent bouts of starvation. Despite the continuous use of energy for the purpose of survival, most animals do not process food all the time, hence leaving them dependent on endogenous physiological fuels for important processes such as survival and reproduction (Karasov, 1986; Dunham *et al*., 1989; Nagy *et al*., 1999; Whittow, 2000). In other words, situations such as a sustained change in water quality can induce starvation leading to reliance on endogenous fuel, hence disrupting the balance between the body mass and energy flux within the organism. Exposures of fish to industrial effluent resulted in poor water quality as evidenced by acidic pH and low DO in exposure concentrations with the exception of untreated control. These values were below the acceptable limits for maintenance of aquatic life NESREA, 2009. The tendency towards slight acidity in exposure concentrations could result in inhibition of growth leading to significant weight loss in fish in the highest concentration. Ikuta and Kutamara (1995) observed that the stress of acidification induced various physiological and ecological problems in fish. The low level of dissolved oxygen in effluent sample and exposure concentrations (in spite of artificial respiration) may be due to the high level of organic material in effluent that requires high levels of oxygen for chemical oxidation and decomposition. This may explain the high level of COD in effluent sample and may have grave implications for the survival of aquatic organisms that require a DO range of 5.40-8.50 mgL^{-1} for survival (Fakayode, 2005). Acidic pH coupled with low DO

may have a negative impact on the ability of fish to feed resulting in starvation and subsequent weight loss in parent fish. Sub-lethal acid stress affects reproduction in fish and Ikuta and Kitamura (1995) reported that when mature rainbow trout were reared in pH 4.5 just prior to spawning, the eyeing rate (index indicating normal development) of embryos from females exposed to acidic waters decreased drastically even when embryos were cultured in neutral water after fertilization. In land-locked sockeye salmon, *Onchorhynchus nerka*, the embryos of mature female fish reared at pH 5.0 had rapidly decreasing eyeing rates after one week (Kitamura and Ikuta, 2000). They concluded that sub lethal acid stress at pH 5 may induce failure of immune and reproductive functions through the alteration of physiological mechanisms including the actions of endocrine factors. Oxygen is potentially as essential to energy production in animals as the nutrients derived from food intake (Kramer, 1987) and decreasing available O_2 in holding water for fish will lead to conservation of energy behaviourally and physiologically (Kramer, 1987). Egg production in fish represents a considerable energetic investment and depressed DO has the potential to limit fish reproductive output (Kramer, 1987). The low DO in the highest exposure concentrations resulted in the few number of eggs produced and the inability of eggs to hatch leading to reproductive failure in fish at these oxygen concentrations. Some authors have indicated that under true hypoxic conditions (DO<2.0 mg L^{-1}), fertility and hatching success was decreased in common carp *Cyprinus carpio* and atlantic croaker *Micropogonias undulatus* (Wu *et al*., 2003; Thomas *et al*., 2006). Wang *et al*. (2008) reported that hypoxia (DO 1.0±0.2 mg L^{-1}) did not prevent gonad development in or oocyte formation in common carp (*C. carpio*), but retarded final oocyte maturation and prevented spawning. Oocytes developed to the post-vitellogenic stage but final oocyte maturation and subsequent ovulation was impaired. They concluded that disruption in development was related to a decrease in serum Luteinizing Hormone (LH) that regulates final oocyte maturation.

Fish expend a lot of energy for growth and reproduction and the weight loss observed with increasing concentration of effluent may have a negative impact on fish fecundity and fertility (Begum and Vijayaraghavan, 1995). This in turn can undermine the quality and quantity of eggs produced by a reproducing adult. Lahnsteiner and Patarnello (2005) and Bonnet *et al*. (2007) are of the opinion that the representative criterion of the quality of a batch of eggs produced during spawning is the proportion of those eggs which successfully complete development into the next distinct biological stage of its species. For this study, significant decreases ($p<0.05$) in number of hatched eggs and percentage viablilty of eggs with increase in concentration was observed. A number of authors have indicated that mill effluents can affect fish reproduction in multiple ways with the most notable and consistent responses being decreased egg production (Ankley *et al*., 2001; Rickwood *et al*., 2006a; 2006b; Robinson, 1994; Kovacs *et al*., 1995; Borton *et al*., 2000; 2003; Hewitt *et al*., 2008). Ankley *et al*. (2001) reported a significant decrease in fecundity of the fathead minnow (*Pimephales promelas*) exposed to multiple point discharges from eleven Canadian industry effluents and concluded that egg production was negatively affected at concentrations of effluent lower than approximately half the effluent concentrations that negatively impacted in vitro steroid production indicating that variations in egg production may be a cheaper and early indicator of fish response to environmental stressors. The highest concentration of 13.20% in this study prevented egg viability as there were no hatchlings at this concentration. This indicates reproductive failure and a negative reproductive end point at concentrations that did not affect fish survival. Rickwood *et al*. (2006a) exposed mature adult fathead minnow to bleached kraft pulp mill effluents and reported that egg production was completely halted by one of the effluents used in the 11-mill survey from Jackfish bay in which effluent concentration did not exceed 40%. On-site exposures were subsequently used to test the effects of various process streams within the mill effluent before secondary treatment and the combined alkaline stream caused decreased spawning events and decreased egg production (Rickwood *et al*., 2006a).

The correlation of egg quality with somatic parameter showed a significant ($p<0.05$) positive relationship with the final body weight of fish and the number of surviving F_1 generation fries after 42 days. This indicates that the body weight of a female fish brooder has significant implications for egg viability and subsequent offspring survival as indicated by the higher weight of eggs and % survival of offsprings in control fish compared with exposure concentrations. The survivorship of the F_1 generation fries after 42-days of hatching is a very significant index of egg quality because it is a measure of the quality of the endogenous reserves contained in the yolk sac and the intrinsic survival potential of the larva (Gimenez *et al*., 2006). Such observation gives credence to the hypothesis of transfer of contaminants from parent to eggs. The extent to which the body weight of the brood-stock influenced the total number of eggs hatched and the survivorship of the F_1 generation fries is reflected by the coefficient of determination (R^2). The high

percentage (67 and 80%) obtained for number of hatched eggs and fries after 42 days respectively can be explained by the change in body weight of the exposed female brood stock. Similar results describing the relationship between parent fish and egg production of other fish species (Gerking, 1978; Springate and Bromage, 1985; Marsh, 1986; Hutchings, 1991) and *C. gariepinus* (Sule and Adikwu, 2004) have been reported. According to Sule and Adikwu larger broodstocks produced larger and heavier eggs which in turn produced heavier fry with higher survival rates. They further stated that this could be as a result of larger egg yolk reserve in larger eggs.

The ability of heavy metals to cause deleterious effects on fish reproduction and gamete development have been reported (Kime, 1995). Delistraty and Stone (2007) observed that low concentrations of lead negatively impacted fish health and reproduction. In this study, lead (Pb) concentrations in the industrial effluent were reported to be above acceptable limits for effluents discharged into surface waters NESREA, 2009. This observation calls for concern considering previous studies confirming the reproductive toxicity of lead to fish (Thomas and Trant, 1989; Thomas, 1990).

CONCLUSION

This study has demonstrated that exposure of *C. gariepinus* female brood stock to food and beverage industry effluent resulted in reproductive impairment in fish in lower exposures and reproductive failure in the highest exposure concentration. This was subsequently reflected in the significantly low survival rates of the F_1 generation fries of eggs from parent fish indicating poor species fitness and larval survival. Since reproductive success is a key factor in determining species survival, it is suggested that studies like this should complement other indices of reproductive success, for example, biochemical and physiological markers to ensure effective management of local fauna and preservation of species diversity in this and similar rivers receiving industrial effluents.

REFERENCES

Adeogun, A.O. and A.V. Chukwuka, 2012. Effect of textile factory effluent on otolith and somatic growth parameters in *Clarias gariepinus*. Zoologist, 9: 70-77.

Adeogun, A.O., 2004. Effects of Methanolic Extracts of Raphia hookeri (Mann and Wendl, 1804) on Life Stages of Clarias gariepinus (Burchell, 1862). Ph. D Theses, University of Ibadan, Ibadan, Nigeria.

Aluko, P.O. and M.H. Ali, 2001. Production of eight types of fast growing intergeneric hybrids from four clariid species. J. Aquac. Tropics., 16: 139-147.

Ankley, G.T., K.M. Jensen, M.D. Kahl, J.J. Korte and E.A. Makynen, 2001. Description and evaluation of a short-term reproduction test with the fathead minnow (*Pimephales promeleas*). Environ. Toxicol. Chem., 20: 1276-1290.

Begum, G. And S. Vijayaraghavan, 1995. Chronic effects of dimethoate on the reproductive potential of the fresh-water teleost, *Clarias batrachus.* Pestic. Sci., 44: 233-236. DOI: 10.1002/ps.2780440305

Bell, J.G. and J.R. Sargent, 2003. Arachidonic acid in aquaculture feeds: Current status and future opportunities. Aquaculture, 218: 491-499. DOI: 10.1016/S0044-8486(02)00370-8

Bonnet, E., A. Fostier and J. Bobe, 2007. Microarray-based analysis of fish egg quality after natural or controlled ovulation. BMC Genomics, 8: 55-55. DOI: 10.1186/1471-2164-8-55

Borton, D.L., W.R. Streblow, P.A. Van Veld, T.J. Hall and T.M. Bousquet, 2003. Comparison of potential biomarkers to reproduction during fathead minnow (*Pimephales promeleas)* life-cycle tests with Kraft mill effluents. Proceedings of the 3rd International Conference on the Environmental Fate and Effects of Pulp and Paper Mill Effluents, Rotorua, New Zealand, Nov. 9-13, SETAC, Pensacola, FL, USA., pp: 459-470.

Borton, D.L., W.R. Streblow, T.M. Bousquet and D.L. Cook, 2000. Fathead minnow (*Pimephales promeleas)* reproduction during multigeneration life-cycle tests with kraft mill effluents. Proceedings of the 4th International Conference on Environmental Impacts of the Pulp and Paper Industry Report 417, (EIPPIR' 00), Finnish Environment Institute, Helsinki, pp: 152-157.

Chukwu, L.O and O.S. Lawal, 2010. Joint Action Response and Haematological Profile of *Macrobrachium vollenhoevenii* (Herklots, 1857) exposed to binary mixtures of spent lubricant oil and dispersant. Res. J. Environ. Sci., 4: 115-126.

Chukwu, L.O. and B.O. Okhumale, 2009. Mode of joint action response to binary mixtures of three refined petroleum products by Nile tilapia *Oreochromis niloticus* fingerlings. Sci. Res. Essay, 4: 806-811.

Clesceri, L.S., 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Ass., Washington, ISBN-10: 0875532357, pp: 1220.

Delistraty, D. and A. Stone, 2007. Dioxins, metals and fish toxicity in ash residue from space heaters burning used motor oil. Chemosphere, 68: 907-914. DOI: 10.1016/j.chemosphere.2007.01.070

Dunham, A.E., B.W. Grant and K.L. Overall, 1989. Interfaces between biophysical and physiological ecology and the population ecology of terrestrial vertebrate populations. Physiol. Zool., 62: 335-355.

Evans, A.D. and M. Nipper, 2007. Toxicity of phenanthrene and lindane mixtures to marine invertebrates. Environ. Toxicol., 22: 495-501. DOI: 10.1002/tox.20279

Fakayode, S.O., 2005. Impact assessment of industrial effluent on water quality of the receiving Alaro river in Ibadan, Nigeria. J. Environ. Assessment Manage., 10: 1-13.

Finney, D.J., 2009. Probit Analysis. 1st Edn., Cambridge University Press, Cambridge, ISBN-10: 9780521135900, pp: 272.

Gerking, S.D., 1978. Ecology of Freshwater Fish Production. 1st Edn., Wiley, New York, ISBN-10: 0470993626, pp: 520.

Gimenez, G., A. Estévez, F. Lahnsteiner, B. Zecevic and J.G. Bell *et al*., 2006. Egg quality criteria in common dentex (*Dentex dentex*). Aquaculture, 260: 232-243. DOI: 10.1016/j.aquaculture.2006.06.028

Hewitt, L.M., T.G. Kovacs, M.G. Dube, D.L. MacLatchy and P.H. Martel *et al*., 2008. Altered reproduction in fish exposed to pulp and paper mill effluents: Roles of individual compounds and mill operating conditions. Environ. Toxicol. Chem., 27: 682-697. DOI: 10.1897/07-195.1

Hopkins, W.A., B.P. Staub, J.W. Snodgrass, B.E. Taylor and A.E. DeBiase *et al*., 2004. Responses of benthic fish exposed to contaminants in outdoor microcosms—examining the ecological relevance of previous laboratory toxicity tests. Aquat. Toxicol., 68: 1-12. DOI: 10.1016/j.aquatox.2004.01.021

Hugla, J.L. and J.P. Thome, 1999. Effects of polychlorinated biphenyls on liver ultrastructure, hepatic monooxygenases, and reproductive success in the barbel. Ecotoxical. Environ. Saf., 42: 265-273. DOI: 10.1006/eesa.1998.1761

Huisman, E.A. and C.J.J. Richter, 1987. Reproduction, growth, health control and aquacultural potential of the African catfish, *Clarias gariepinus* (Burchell 1822). Aquaculture, 63: 1-14. DOI: 10.1016/0044-8486(87)90057-3

Hutchings, J.A., 1991. Fitness consequences of variation in egg size and food abundance in brook trout, *Salvelinus frontinallis.* Evolution, 45: 1162-1168.

Ikuta, K. and S. Kitamura, 1995. Effects of low pH exposure of adult salmonids on gametogenesis and embryo development. Water Air Soil Pollu., 85: 327-332. DOI: 10.1007/BF00476850

Jensen, A.L., 1996. Beverton and Holt life history invariants result from optimal trade-off of reproduction and survival. Canadian J. Fisheries, Aquatic. Sci., 53: 820-822.

Jezierska, B., K. Lugowska, M. Witeska and P. Sarnowski, 2000. Malformations of newly hatched common carp larvae. Elect. J. Polish Agric. Univ., 3: 12-19.

Karasov, W.H., 1986. Energetics, physiology and vertebrate ecology. Trends Ecol. Evol., 1: 101-104. DOI: 10.1016/0169-5347(86)90034-0

Kime, D.E., 1995. The effects of pollution on reproduction in fish. Rev. Fish Biol. Fish, 5: 52-96. DOI: 10.1007/BF01103366

Kitamura, S. and K. Ikuta, 2000. Acidification severely suppresses spawning of hime salmon (land-locked sockeye salmon, *Oncorhynchus nerka*). Aquat. Toxicol., 51: 107-113. DOI: 10.1016/S0166-445X(00)00097-7

Kjorsvik, E., A. Mangor-Jensen and I. Holmefjord, 1990. Egg quality in fishes. Adv. Mar. Biol., 26: 71-113. DOI: 10.1016/S0065-2881(08)60199-6

Kovacs, T.G., J.S. Gibbons, L.A. Tremblay, B.I.O. Connor and P.V. Martel *et al*., 1995. The effects of a secondary-treated bleached kraft mill effluent on aquatic organisms as assessed by short-term and long-term laboratory tests. Ecotxicol. Environ. Saf., 31: 7-22. DOI: 10.1006/eesa.1995.1038

Kramer, D.L., 1987. Dissolved oxygen and fish behavior. Environ. Biol. Fishes, 18: 81-92. DOI: 10.1007/BF00002597

Lahnsteiner, F. and P. Patarnello, 2005. The shape of the lipid vesicle is a potential marker for egg quality determination in the gilthead seabream, Sparus aurata, and in the sharpsnout seabream, Diplodus puntazzo. Aquaculture, 246: 423-435. DOI: 10.1016/j.aquaculture.2005.01.018

Little, E.E., J.F. Fairchild and A.J. DeLoany, 1993. Behavioural methods for assessing impacts of contaminants on early life stage fishes. Am. Fisheries Soc. Symp., 14: 67-76.

Lye, C.M., C.L.J. Frid, M.E. Gill and D. McCormick, 1997. Abnormalities in the reproductive health of flounder *Platichthys flesus* exposed to effluent from a sewage treatment works. Marine Poll. Bull., 34: 34-41. DOI: 10.1016/S0025-326X(96)00061-6

Maeland, A., I. Ronnestad and R. Waagbo, 2003. Folate in eggs and developing larvae of Atlantic halibut, *Hippoglossus hippoglossus*. Aquac. Nutr., 9: 185-188. DOI: 10.1046/j.1365-2095.2003.00242.x

Marsh, E., 1986. Effects of egg size on offspring fitness and maternal fecundity in the orange-throat darter, *Etheostoma spectabide* (Pisces: Percidae). Coepia, 1986: 18-30.

Matthiessen, P. and J.W.N. Logan, 1984. Low concentration effects of endosulfan insecticide on reproductive behaviour in the tropical cichlid fish *Sarotherodon mossambicus*. bulletin of environmental contamination. Toxicology, 33: 575-583. DOI: 10.1007/BF01625587

McCue, M.D., 2010. Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. Comparative Biochem. Physiol., 156: 1-18. DOI: 10.1016/j.cbpa.2010.01.002

Murthy, A.S., 1986. Toxicity of Pesticides to Fish. 1st Edn., CRC Press, Florida, ISBN-10: 0849360587, pp: 160.

Nagy, K.A., I.A. Girard and T.K. Brown, 1999. Energetics of free-ranging mammals, reptiles, and birds. Annu. Rev. Nutr., 19: 247-277. DOI: 10.1146/annurev.nutr.19.1.247

Pathan, T.S., D.L. Sonawane and Y.K. Khillare, 2009. Toxicity and Behavioural Changes in Freshwater Fish *Rasbora daniconius* exposed to paper mill effluent. Bot. Res. Int., 2: 263-266.

Reish, D.L. and P.S. Oshida, 1986. Manual of methods in aquatic environment research. FAO Fisheries Technical. Study, 247: 1-65.

Rickwood, C.J., M.G. Dube, L.M. Hewitt, T.G. Kovacs and J.L. Parrott *et al*., 2006b. Use of paired fathead minnow (*Pimephales promeleas*) reproductive test. Part 1: Assessing biological effects of final bleached kraft pulp mill effluent using a mobile bioassay trailer syst. Environ. Toxicol. Chem., 25: 1836-1846. PMID: 16833146

Rickwood, C.J., M.G. Dube, M. Hewitt, T.G .Kovacs and D.L. Maclatchy, 2006a. Use of paired fathead minnow (*Pimephales promeleas)* reproductive test. Part 2: Source identification of biological effects at a bleached kraft pulp mill. Environ. Toxicol. Chem., 25: 1847-1856.

Robinson, R.D., 1994. Evaluation and development of laboratory protocol is for estimating reproductive inmpacts of pulp mill effluent on fish. PhD Theses. University of Guelph, Guelph, Ontario, Canada.

Scott, W.B. and J. Crossman, 1985. Freshwater Fishes of Canada. 1st Edn., Fisheries Research Board of Canada, Ottawa, ISBN-10: 0660102390, pp: 996.

Shatunovskii, M.T., N.V. Akimova and G.I. Ruban, 1996. Response of reproductive systems of fishes to anthropogenic impacts. J. Ichthyol., 36: 242-256.

Shields, R.J., N.P. Brown and N.R. Bromage, 1997. Blastomere morphology as a predictive measure of fish egg viability. Aquaculture, 155: 1-12. DOI: 10.1016/S0044-8486(97)00105-1

Sol, S.Y., L.L. Johnson, T.K. Collier, M.M. Krahn and U. Varanasi, 1995. Contaminant effects on reproductive output in north Pacific flatfish. Proceedings of the International Symposium on North Pacific Flatfish, Alaska Sea Grant Collection Program, (NPFASGCP' 95), Fairbanks, Alaska, pp: 547-560.

Springate, J.R.C. and N.R. Bromage, 1985. Effects of egg size on early growth and survival in rainbow trout (*Salmo gairdneri* Richardson). Aquaculture, 47: 163-172. DOI: 10.1016/0044-8486(85)90062-6

Srivastava, S., P. Prabhakar, B. Singh and B.C. Srivastava, 2007. Toxicity and behaviourof the fish *Labeo rohita* and *Channa punctatus* exposed to pulp and paper mill effluent. J. Ecotoxicol. Environ. Monit., 17: 241-244.

Sule, O.D. and I.A. Adikwu, 2004. Effect of Broodstock size on egg and larval size and survival of larvae of the African catfish, *Clarias gariepinus* under laboratory conditions. J. Aquatic. Sci., 19: 1-4.

Thomas, P. and J.M. Trant, 1989. Evidence that 17α,20β,21-trihydroxy-4-pregnen-3-one is a maturation-inducing steroid in spotted seatrout. Fish Physiol. Biochem., 7: 185-191. DOI: 10.1007/BF00004706

Thomas, P., 1990. Teleost model for studying the effects of chemicals on female reproductive endocrine function. J. Exper. Zool., 4: 126-128. DOI: 10.1002/jez.1402560421

Thomas, P., M.S. Rahman, J.A. Krumer and S. Lawson, 2006. Reproductive endocrine dysfunction in Atlantic croaker exposed to hypoxia. Marine Environ. Res., 62: S249-S252. DOI: 10.1016/j.marenvres.2006.04.031

Thorsen, A., E.A. Trippel and Y. Lambert, 2003. Experimental methods to monitor the production and quality of eggs of captive marine fish. J. Northwest Atl. Fish. Sci., 33: 55-70.

Tveiten, H., M. Jobling and I. Andreassen, 2004. Influence of egg lipids and fatty acids on egg viability and their utilization during embryonic development of spotted wolfish *Anahichas minor* Olafsen. Aquac. Res., 35: 152-161.

Vanerker, A.P., S. Satyanarayan and D.M. Dharmadhikari, 2004. Toxicity of herbal pharrnaceutical wastewater on fish *Lebistes reticulata* (Peter). J. Environ. Sci. Health. Part B, 39: 115-123. PMID: 15022745

Wang, S., S.S.F. Yuen, D.J. Randall, C.Y. Hung and T.K.N. Tsui *et al*., 2008. Hypoxia inhibits fish spawning via LH-dependent final Oocyte maturation. Comparative Biochem. Physiol.-Part C: Toxicol. Pharm., 148: 363-369. DOI: 10.1016/j.cbpc.2008.03.014

Whittow, G.C., 2000. Sturkie's Avian Physiology. 5th Edn., Academic Press, San Diego, ISBN-10: 9780127476056, pp: 685.

Wu, R.S.S., B.S. Zhou, D.J. Randall, N.Y.S. Woo and P.K.S. Lam, 2003. Aquatic hypoxia is an endocrine disruptor and impairs fish reproduction. Environ. Sci. Technol., 37: 1137-1141. DOI: 10.1021/es0258327

Wu, S.M., M.J. Shih and Y.C. Ho, 2007. Toxicological stress response and cadmium distribution in hybrid tilapia (*Oreochromis* sp.) upon cadmium exposure. Comp. Biochem. Physiol., Part C Pharmacol. Toxicol., 145: 218-226. DOI: 10.1016/j.cbpc.2006.12.003