Gonad histology in post fingerling of *Tilapia guineensis* exposed to Parateq

Histología de las gónodas en alevines de *Tilapia guineensis* expuestas a Parateq

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ABSTRACT

The study investigated the effect of Parateq on gonad morphology and changes in gonadosomatic index of post fingerlings of *Tilapia guineensis* exposed to sublethal Parateq concentrations for 12 weeks. Initial short- term static toxicity tests were run to determine 96 hr LC_{50} of Parateq in *T. guineensis* which was 5.47%. The Parateq concentrations used were 0.32%, 0.63%, 1.25 % and 2.5% vol/vol parateq/ water. The histological changes noted in the gonads of the exposed fish were inhibition of maturation in oocytes or delay in spermatogenesis which resulted in lack of spawning. In contrary, the four stages of spermatogenesis or oocytogenesis were present in the control and spawning occurred. The increasing degeneration of maturing eggs resulted in complete absence of matured egg in the female gonads of fish exposed to the highest concentration (2.5%) of the drilling fluid. The gonadal somatic index values were recorded in a decreasing order toward the higher tested concentrations. The gonadal somatic index values ranged from 2.34 to 1.25% in the female and from 0.32 to 0.09% in male, whereas in the control, it was 2.85% for female and 0.41% for male. The results revealed that discharge of drilling fluid such as parateq into the environment can lead to impairment in the reproductive success of aquatic organisms in the Niger Delta.

Key words: Gonads, *Tilapia guineensis*, parateq, drilling fluids, gonadal somatic index (GIS)

RESUMEN

El estudio investigó el efecto del Parateq sobre la morfología de las gónadas y los cambios en el índice gonadosomático de alevines de *Tilapia guineensis* expuestos a concentraciones subletales de Parateq durante 12 semanas. Las pruebas iniciales de toxicidad estática a corto plazo se reralizaron para determinar la LC_{50} a las 96 hr del Parateq en *T. guineensis* la cual fue 5,47%. Las concentraciones de Parateq usadas fueron 0,32, 0,63, 1,25 y 2,5% las cuales correspondieron a 6,25; 12,5; 25 y 50% de la LC_{50} a las 96 hr, respectivamente. Los cambios histológicos observados en las gónadas de los peces expuestos fueron: la inhibición de la maduración de los oocitos o el retraso en la espermatogénesis, la cual se tradujo en la falta de desove. Por el contrario, las cuatro etapas de la espermatogénesis o oocitogénesis estuvieron presentes en el control y se produjo el desove. El incremento de la degeneración de los huevos maduros dió como resultado la ausencia total de huevos madurados en las gónodas femeninas de los peces expuestos a la concentración más alta (2,5%) del fluido de perforación. Los valores del índice gonadosomático se registraron en orden decreciente hacia las mayores concentraciones probadas. Los valores del índice gonadosomático oscilaron entre 2,34 a 1,25% en las gónodas femeninas y de 0,32 a 0,09% en las masculinas, mientras en el control, fue 2,85% para las femeninas y 0,41% para las gónadas masculinas. Los mayores valores se obtuvieron en las gónadas femeninas comparados con las masculinas. Los resultados revelaron que Parateq tiene efectos deletéreos sobre los procesos reproductivos los cuales pueden conducir a un deterioro en el éxito reproductivo de *T. guineensis*, que afecte la supervivencia futura de la pesca en el delta del Níger.

Palabras clave: Gónadas, *Tilapia guineensis*, parateq, fluidos de perforación, índice gonadosomático

INTRODUCTION

The Niger Delta is the largest wetland in Africa and among the most productive ecosystems in the West Africa sub region and Delta is home to many important plant and animal species that the inhabitants rely on for food and livelihood. The wetland is characterized by oil activities (exploration and exploitation). The attendant waste generated from the activities and occasional spills that are discharged into the adjacent environment (OGP, 2006). One of these discharges arising from crude oil related activities are drilling wastes (fluids) that contain several toxic substances such as chromate, biocides,

organic polymers, hydrocarbons, heavy metals and trace elements that have the tendency to bioaccumulation and interfere with normal biological activities of organisms including man (Neff, 2002; PAS, 1995; Rushing *et al.,* 1991). Drilling fluids exposed to water may disperse or sink which will locally kill benthic organisms by smothering them or by inhibiting physiological activities (Patin, 1999; Cranford *et al.,* 1998; Bowmer *et al.,* 1996; Okpokwasili and Odokuma, 1996; Jones *et al.,* 1991). Other aquatic resources located at the top of the food chain have been reported to suffer severe physiological and reproductive setback that may eventually lead to death through direct or indirect contact via their gills, body surface and ingestion of contaminated food (Van Dyk, 2003; Stottl *et al.*, 1981).

Information on drilling mud in Nigeria is limited and often not availabe to the public. However, Soegianto *et al*. (2008) reported 96 hr LC_{50} of drilling waste between 30740 and 78271 mg L^{-1} for post larvae of tiger prawn *Penaeus monodon*. Similarly, 48 hr LC_{50} > 2000 mg L^{-1} for *Acartia tonsa* and 72 hr EC50 >1000 mg L^{-1} for *Corophuim volutor* for Parateq was reported by Baker-Hughes (2002). In 2005, 28159 tonnes of non aqueous drilling fluid associated with the drill cuttings were discharged into the environment (OGP, 2006). This quantity cumulatively may have various consequencies on the environmental integrity and biota and may be responsible for the growing complaint of low fish yield by the fisher folks. Recently, the steady declining yield of fin and non fin fishes has generated lots of concern attributed to oil industrial activities including drilling waste discharges (Kinigoma, 2001; Wills 2000; Patin, 1999). In the Niger delta region, efforts to the effect of oil activities on environment and biota had been tailored mostly on the effect of crude oil spills on water quality (IPS, 1989, 1990; RPI, 1985), phytoplankton (Chindah and Braide, 2001; NDES, 2000), periphyton (Chindah, 1998; Pudo and Fubara, 1988), benthos (Ekweozor *et al.,* 1987; Ekweozor and Snowden 1985), gastropod (Chindah *et al.,* 2000; Dambo, 1992), crustacean (Chindah *et a.l*, 2004), vegetation (Osuji and Ezebuiro, 2006: Obot *et al.,* 1992) and fish (NDES, 2000; IPS, 1989 & 1990; Powell, 1987).

Despite the huge drilling activities and the attendant drilling waste generated and discharged, little is known on the sublethal effect of drilling fluid such as Parateq that is commonly used in wetlands areas of the Niger Delta region. It is on the basis of this gap in knowledge that this study was undertaken in order to evaluate the possible effect of the drilling fluid on the development (reproductive) of the most common and widely distributed fish species in the region.

MATERIALS AND METHODS

The Parateq is a synthetic based fluid obtained from Baker Hughes Nigeria Limited made up of mosaic of complex chemical compounds and including heavy metals (Table 1). It is commonly used for drilling operations worldwide. Post fingerlings (7.21 - 7.25cm / >10.5g) of *T. guineensis* used were collected from the African Regional Aquaculture centre (ARAC), Buguma, Rivers State Nigeria.

The fish were transported in the early hours of the day to the laboratory in air bags with the pond water from the fish farm to avoid heat exertion.

In the laboratory, the fish were transferred immediately to the holding tanks [120 x 120 x 120cm]. The holding tanks were aerated, cleaned and the water renewed regularly (Reish and Oshida, 1986). Fish were fed twice daily with NIOMR feed (35% protein). During the acclimatization period, the

Table 1. Physical and chemical characteristics of Parateq

Parameter	Concentration	
Water	26.20	%
Base Fluid	73.80	%
Organophilic lignite(carbongel 11)	12.00	ppd
Organophilic clay (omniplex)	2.16	ppd
Lime	3.00	ppd
$CaCl_2$	32.78	ppd
Barite	105.26	ppd
Polyaminated fatty acid (omnimul)	8.62	ppd
pH	6.76	
Total Solid	587	mg/g
Total organic carbon	1.65	mg/ g
Chloride	0.63	mg/ g
Nitrate	1.60	mg/g
Total hydrocarbon	41.00	mg/g
Lead	2.16	ppm
Manganese	2.05	ppm
Zinc	5.82	ppm
Cadmium	0.00	ppm
Chromium	0.09	ppm
Barium	0.004	ppm

fish were gradually subjected to the dilution water until they could survive in the uncontaminated dilution water without showing signs of stress, such as discolouration or unusual behaviour. At the end of the acclimatization period, all the fish that were disease free, without any signs of stress, or damage were used for the experiment. Mortality during the holding period was less than one percent of the whole population.

The bioassay was conducted in ten 500 litre capacity [120 x 120 x 120cm] concrete tanks for five treatments using 2 replicates per treatment for post fingerling. The tests were conducted in the laboratory under room temperature using static renewal bioassay (Ca1/EPA, 2004).

Initial 96 hr short lethality test was carried out for the post fingerlings exposed to 0%, 2%, 4%, 8% and 10% of Parateq in water to determine the median lethal concentration (LC_{50}) (concentration of drilling fluid in water that will kill 50% of the fish population in 96 hours) as in Ca1/EPA,(2004). The LC_{50} was calculated based on the probit analysis through which five sublethal concentrations 2.5 %, 1.25 %, 0.63 %, 0.32 % and control were obtained in a volume to volume ratio (Reish and Oshida, 1986; Vincent-Akpu, 2001). Note, 1% = 1000ml/L.

A group of ten fish was randomly exposed to the different concentrations of the drilling fluids. Healthy fish were assigned to the aquaria and screened with a mosquito net to prevent fish escape. Exposure lasted for 12 weeks, during which freshly prepared test solutions were made weekly as the water is changed and tanks cleaned (Reisha and Oshida, 1986). The fish were fed with NIOMR feed at 4% of their weight twice daily. Water parameters (Temperature, pH, DO and alkalinity) of the test solution were monitored weekly throughout the duration of the experiment (APHA, 1998). At the end of the 12 weeks, four fish were sacrificed by a sharp blow to the head, weighted and the total length recorded. The gonads were excised from the fish and weighed. The gonodal somatic index (GIS) was calculated from gonad weight x 100 / body weight.

Care was taken not to squeeze any of the tissues and processed by methods given by Golder (1997) and Wester *et al.* (2003). The tissues were placed in a tissue cassette and fixed immediately in 10% neutral formalin in nalgene container for 36hr to avoid post–mortem changes.

The samples were washed in running tap and dehydrated in a graded series of industrial Methylated spirit (30, 50, 70, 80, 90 and 100 %) for specific time periods. The samples were then transferred to xylene for 5 minutes, until transparent (clear) and later transferred to 60^{o}C oven.

The samples were infiltrated and imbedded in paraffin wax blocks. After cooling, the imbedded samples were sectioned (5µm thick) using a wax microtome. The sample sections were stretched with an albumin and distilled water solution, mounted on glass microscopic slides and air dried. The dried sections were stained with Haemotoxylin and Eosin (H&E) staining techniques. Stained sections were then mounted with cover slide using entellan. Each slide was reviewed microscopically without any knowledge of its individual treatment and a histological report prepared. Photomicrographs were taken to illustrate some of the tissue pathology recorded.

The median lethal concentration (LC_{50}) and median lethal time(LT_{50}) were calculated using probit analysis. The significant differences among the treatments were assessed using analysis of variance (ANOVA) and were considered to be significant if $pP<0.05$ while T-test was used to determine mean difference between the physicochemical parameters.

RESULTS

The level of physicochemical parameters determined during the experiment at the various concentrations did not vary significantly ($p < 0.05$, $n = 4$) from those of the control (27.3 ± 0.04 °C; 4.26 ± 0.42 mg L^{-1}; 22.31 ± 0.48 mg L^{-1}; 7.01 ± 0.09 for temperature, dissolved oxygen, alkalinity and pH respectively).

In the short term lethality test, mortality increased with increased in concentration. Mortality in % was transformed to probit (5 corresponds to 50% mortality). While the time and concentrations were transformed into logarithrim(log). This gave 24, 48, 72 and 96 hr LC_{50} of 11.56%, 9.24%, 7.24% and 5.47% respectively for Parateq (Figure 1). The LT_{50} were 102.15 hr for 2%, 93.63 hr for 4%, 65.84 hr for 8% and 55.51 hr for 10% (Figure 2).

The mean gonadal somatic index (GIS) of post fingerlings exposed to different concentrations of Parateq is presented in Table 2. The GSI values

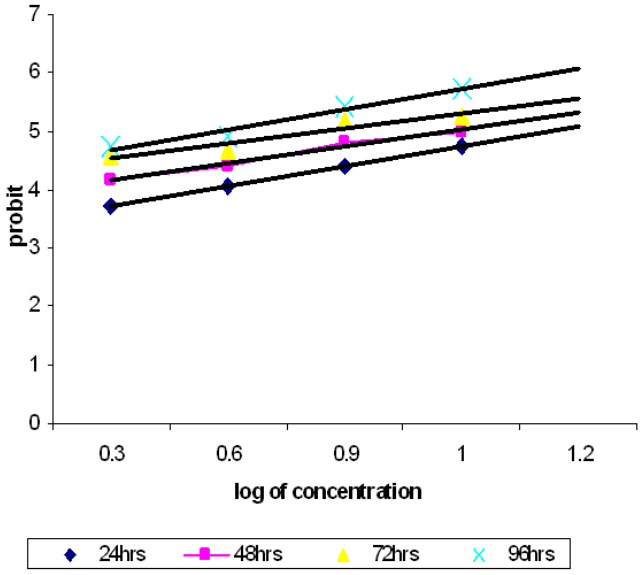

Figure 1. Plot of mortality in probit against log of concentration for *Tilapia guineensis* post fingerling exposed to Parateq.

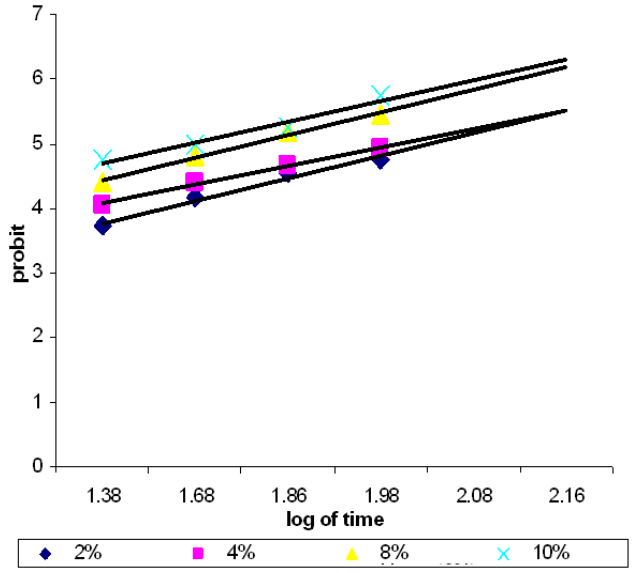

Figure 2. Plot of mortality in probit against log of time for *T. guineensis* post fingerlings exposed to Parateq.

Table 2. Effects of Parateq on gonadal somatic index of the post fingerling of *Tilapia guineensis*

Concentrations (%)	Female	Male
0	2.85	0.41
0.31	2.34	0.32
0.63	1.97	0.21
1.25	1.75 *	0.15
2.50	1.25 *	0.09 *

* significant difference with the controls (P< 0.05, n = 8)

decreases as the concentration of Parateq increases with exception of the control which had 2.85 and 0.41% for female and male gonads respectively. The analysis of variance showed that there was a significant difference at p< 0.05 in GIS values at the higher concentrations and the control as F- cal is greater than the critical F value both for female (F =100.97 >P= $6.38_{0.05}$) and male (F- = 31.55 > P= $6.38_{0.05}$) fishes.

Gross examination of the gonads gave no indication of swelling or discolouration. There was no discernible difference in the size of the left and right lobes of the gonads.

Four developmental stages were distinguished as primary oogonia, secondary oogonia, primary oocyte and secondary oocyte with the characteristically prominent zona pellucida in female gonads while spermatogonia, spermatocyte, spermatides and spermatozoan were found in the male gonads. Successful spawning occurred in the control weeks before the sampling (Plate 1). The female gonads in the control consist of lamellae filled with ova in various stages of development and the testis contains cluster of numerous spermatogenic cells (cysts) at various developmental stages in mature seminiferous tubule. The fibrous seminiferous tubule are intact and the tubules numerous. However, most of the cells in the seminiferous tubule were at the last stage of development mainly spermatocytes and few spermatids.

The histological changes observed in the exposed female gonads were inhibition of maturation in oocytes coupled with increasing number of atretic follicle. Parateq affected the gonads in a dose dependent manner. The severity of pathological changes became more intense as the concentration increases while there was complete absence of matured eggs in the female gonads of fish exposed to the highest concentration of Parateq. In ovaries of treated fish exposed to 0.32% Parateq, no discernible difference between treatment and control could be seen. More frequency of immature egg cells was observed in 0.63% while gonad in 1.25% Parateq contained many immature follicles accompanied by a slight decrease of early vitellogenic and increase in relative number of oogonia. At maximum concentration (2.5%) of Parateq, gonad development was inhibited which was reflected in decreased oocyte growth and high incidence of atresic follicle resulting in complete fusion of two follicles (Plate 1A). No spawning occurred in all fish exposed to Parateq.

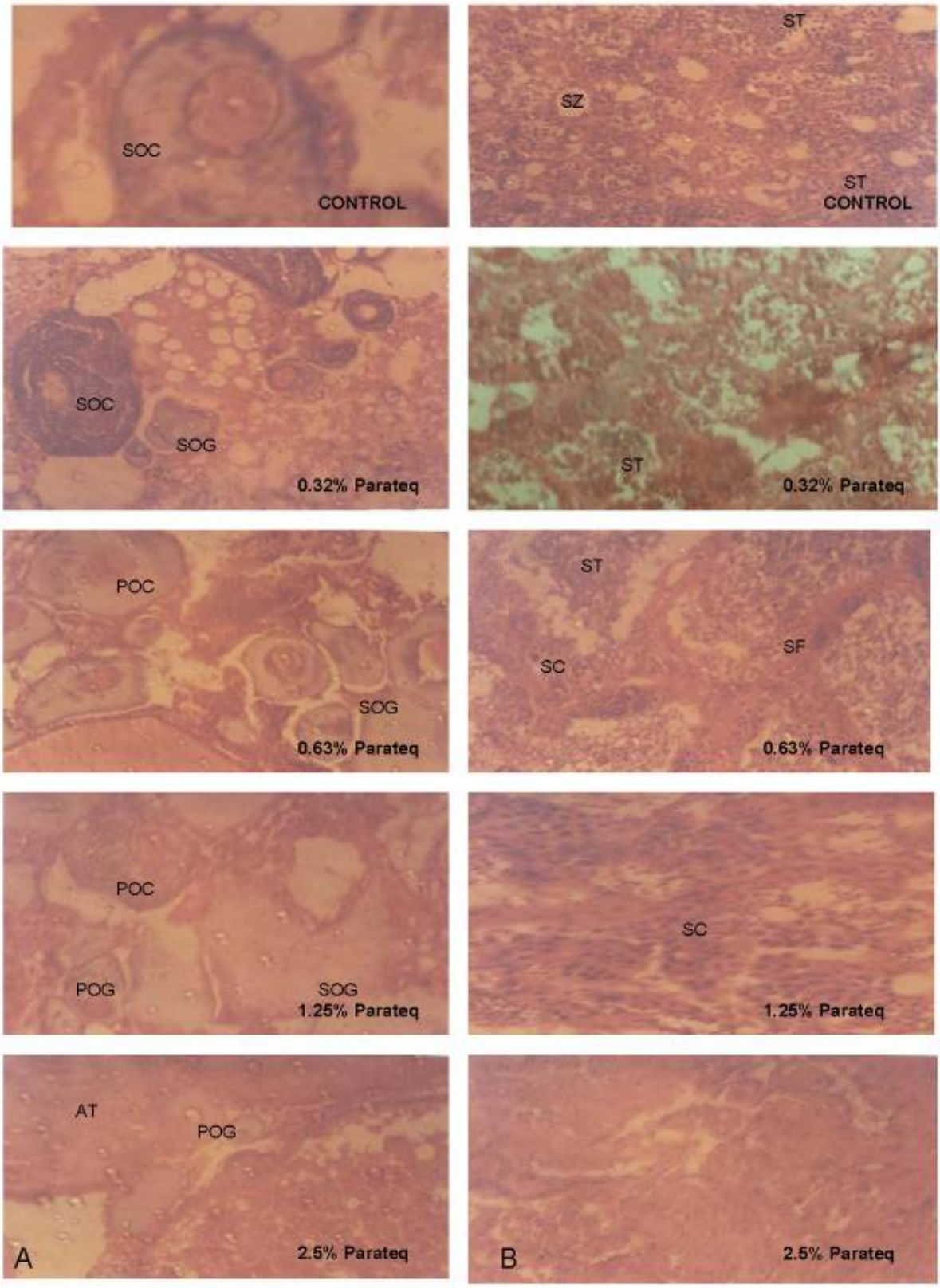

Plate 1. Changes in female and male gonads of T. guineensis post fingerling exposed to different concentration of Parateq. A – Female gonads and B - Male gonads; POG – Primary oogonia; SOG – Secondary oogonia; POC – Primary oocyte; SOC – Secondary oocyte; AT – Artesia; SA – spermatogonia; SC – Spermatocyte; ST – Spermatides; SZ – Spermatozoa, SF – Seminiferous tubule.

Parateq was observed to have induced a dose-dependent inhibition of spermatogenesis in testes of male fish. The gonads of the exposed fish at the higher concentrations were clearly distinguishable from the control gonads. The second lowest exposure concentration testes possessed reduced tubules with fewer spermatocytic stages. This effect increased with increasing concentration and at 0.63% and 1.25%, spermatocytic stages decreased and spermatogenesis appeared to be inhibited. The testes were characterised by enlarged semiferous tubule filled with spermatid and enlarged spermatocytes with a much greater number of mature sperm contained with in and the relative lack of germinal epithelium and primary and secondary spermtocyte. Post fingerlings of *T. guineensis* exposed to varying concentrations of Parateq had multiple lesions which is characterised by degeneration of germ cells and depletion of the numbers of seminiferous tubules. There was tendency to decrease in frequency of progressed stages (spermatozoan and spermatides) and increased presence of early developmental stages was observed as the concentration increases (Plate 1B).

In the fish exposed to the highest concentration of Parateq, inhibition of spermatogenesis was observed which was characterised by absent of matured sperm and atrophy of the seminiferous tubule.

DISCUSSION

The values used for sublethal testing are very much dependent on the acute toxicity tests performed, so that extrapolation using LC_{50} values needs to be done with caution. The percentage mortality, which increased progressively with increase in concentration of drilling fluid over time of exposure, is in agreement with previous findings (OGP, 2003; Bowmer *et al*., 1996). However, the acute toxicity was relatively low. This is probably because the test was not renewed daily during the 96hr bioassay period. Neff *et al.* (1981) noted that if aqueous mud fraction was renewed daily, its toxicity will increase several-fold, demonstrating that the toxic components may be lost from solution by volatilization.

The $48LC_{50}$ values for Parateq (9.24% = 92.4ml/L) obtained in this study was higher compared to what was obtained in Baker Hughes (2002) for the same drilling fluid which was 48hr LC_{50} > 2000 mg L^{-1} for *A. tonsa* and 72 hr EC50 >1000 mg L^{-1} for *C. volutor*.

Using a conventional toxicity rating classification system as a method for ranking and comparing relative toxicities of drilling fluids (Swan *et al*., 1994). Parateq with 96 hr LC_{50} of 0.54% or 5400 ml/L can be said to be slightly toxic since it lies within 1000 - 10000 mg L^{-1}.

Aquatic pollution is therefore less related to acute toxicity than to sublethal and long-term effects which are difficult to detect. Early toxic effects of pollution may however be on cellular or tissue level before significant changes can be identified in behaviour or external appearance (Martinez, *et al.,* 2004; Terio, 2004; Van Dyk, 2003).

Sublethal exposure to the drilling fluid resulted in noticeable effect on reproduction of *T. guineensis*. Four main developmental stages of oogenesis and four stages of spermatogenesis as modified by Stottl *et al* (1981) were identified. The developmental stages were characterized by the abundance of the stages of oogenesis which are primary oogonia, secondary oogonia, primary oocyte and secondary oocyte and spermatogonia, spermatocyte, spermatides and spermatozoan were found in spermatogenesis.

The reproductive success of the gonads exposed to Parateq was affected as shown by a decrease in relative weight (GSI) and a decrease frequency of mature oocytes or spermatocyte. This is similar to delay maturation and inpaired reproductive success observed by Bowmer *et al* (1996) when *Cardium edule* was exposed to drill cuttings using a long term model ecosystem bioassay. Similarly, Bhuiyan *et al* (2001) observed ovarian damage such as complete blockage and dissolution of ovigerous lamella in *Channa punctatus* exposed to sumithion.

The concentration dependent decrease in frequency of matured oocyte or spermatocyte in the different concentrations was due to inhibition of spermatogenesis or oogenesis. Disturbed oocyte development, or at least a delay in the final maturation, was revealed by the large proportion of unovulated yolk egg especially in the ovaries of the most severe exposure.

Vuorinen *et al* (2003) attributed this delay to stress-induced increase in cortisol concentration which in turn suppresses gonadotrophic hormone-(GTH) - Stimulated testosterone and 17β-estradiol production in peak vitellogenic follicles in *Coregonus*

albula L. This was supported by Wester *et al* (2003) in the study of the effects of hormone in Zebra fish. In spite of the fact that the effect of parateq on hormone was not investigated in this study, however the propensity observed in male *T. guineensis*, with delay in spermatogenesis was seen as implying that there is tendency toward a lower gonadal somatic index (GSI) in the exposed group with the observed retardation in spermatogenesis. Reduced androgen production might be behind the retarded spermatogenesis (Wester *et al,* 2003). Spermatogonia apparently did not under a further differentiation to spermatogenic cysts and spermatids. This possibly indicates a cessation of milt production revealed by the accumulation of large spermatocyte in the lumen of enlarged semiferous tubules and lack of intermediate stages. However, the possibility has to be concidered that the stand still in milt production is not pathological but rather the response of males to the stop of spawning activities in females. It is interesting to note that the histological response of testes to parateq is much like that observed in previous studies with 3-benzylidene camphor by Kunz *et al* (2006). A 21 days exposre to 3-benzylidene inhibited testical development, but showed less degeneration on fat head minnow (*Pimephales promelas*).

Spawning occurred in the control 3 weeks before sampling, which can explain the lack of many spermatozoa and secondary oocyte. Cranford *et al* (1998) observed that fertilization success of the sperm and egg of haddock, sea scallop and lobster were not significantly affected when exposed to water based drilling fluids concentration below 100 mg L^{-1}. In contrast, spawning did not occur in the post fingerlings exposed to various concentrations of Parateq even in the lowest concentration of 0.32%, indicating that reproductive process was hindered at one stage or the other.

The effect of the drilling fluid on GSI of *T. guineensis* was influenced by the level of gonadal activities as indicated by comparison of fish with or without the treatment. The decrease of GSI values observed in the drilling fluid-treated fish was associated with the concentration of the drilling fluid and pathological changes as shown by a decrease in the stages of gonadal maturation and increased frequency of histological changes.

The results of the present study revealed that Parateq has deleterious effects on the reproductive processes which could lead to impairment in the reproductive success of *T. guineensis*. Reproductive process in fishes involve changes in weight and structure of gonads, using the gonadal somatic index (expression of gonad weight as a percentage of the body weight) and histological changes in the gonads, can provide insight to any abnormality in the fish health. The inhibition of spermatogenesis or oogenesis coupled with high incidence of atresic follicle and lack of spawning observed in this study indicates its usefulness as indicator of physiological disturbances (Wester, *et al* 2003). Histological response of the fish gonads to environmental stress has shown to be a biomarker indicative tool to assist in the bio-monitoring process of aquatic ecosystems (Byuiyan *et al,* 2001). Therefore, with the extensive exploration drilling and production that occur in the Niger Delta, their ecological impacts must always be kept in mind.

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