The lack of endocrine disrupting effects in catfish (*Clarias gariepinus*) from a DDT sprayed area

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Abstract

The exposure and response of the catfish, *Clarias gariepinus*, was studied in male specimens collected in the vicinity of a DDT spraying programme to control malaria. Two sites were situated in the DDT sprayed areas and one site upstream from exposed areas, used as a reference site. The collected specimens were analysed for DDT bioaccumulation and the extent of associated effects. The concentration of all DDT metabolites including p,p'- and o,p'- forms of DDT, DDE and DDD, were measured in the adipose tissue, whilst the effects were measured using a range of biomarkers. This included assessing the effectiveness of plasma calcium, magnesium, zinc and alkali-labile phosphates (ALPs) as indirect measures of vitellogenin (VTG). Gonad condition was determined by calculating the gonadosomatic index (GSI) for each individual and comparing it with the gonad mass that were adjusted with Analysis of Covariance (ANCOVA). The presence of intersex in gonads was identified and the overall body condition determined using the condition factor (CF). Overall, none of the biomarkers showed significant change in the presence of high levels of DDT nor lindane, dieldrin and endosulfan II. Subtle responses in the plasma concentrations of calcium, ALP and gonad condition were evident in the catfish where DDT concentrations were highest, whilst no effects related to intersex and body condition were evident. Overall this study highlighted the tolerance of *C. gariepinus* to DDT contamination, the practical implications of using biomarkers in developing countries, and the need for further research into developing biomarkers for much needed biomonitoring programmes in areas where malarial control programmes continue to use DDT.

Keywords: DDT; Biomonitoring; Catfish; Biomarkers; Vitellogenin; Intersex

1 Introduction

With the increased awareness of hazardous environmental effects of persistent organic pesticides, there is a drive to reduce their usage and to promote less resistant chemicals (Falandysz, 1994). This was the case with the broad-spectrum pesticide dichlorodiphenyltrichloroethane (DDT), which is highly persistent (due to its high partitioning coefficient and resistance to degradation) and toxic to biological functioning (Vouk and Sheehan, 1983; Falandysz, 1994; Kime, 1998; Huang et al., 2003; Vasseur and Cossu-Leguille, 2006). It exists in the p,p'-DDT and o,p'-DDT forms, and when metabolised is degraded to dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) isomers for the two respective forms (these will all commonly be referred to as DDT in this paper, unless otherwise specified) (ATSDR, 2002). A global ban on all forms of DDT, was introduced at the UN's Stockholm Convention on Persistent Organic Pollutants (POPs), due to its environmental and social impacts (Bouwman, 2004). However, in some developing countries suffering from the malaria epidemic, an exemption was made possible, as DDT is relatively cost effective, easy to produce, and highly effective in the control of the malarial vector (mosquitos) compared to current alternatives (Wells and Leonard, 2006).

In South Africa, DDT spraying in malaria control programmes has been relatively consistent throughout the past 60 years (Tren and Bate, 2004; Bornman et al., 2009). Increased pressure on the South African government to ban DDT over the years, has led to a few periods where DDT spraying was stopped and replaced with pyrethroid alternatives such as deltamethrin (Mabaso et al., 2004). The implementation of pyrethroids, however, proved to be unsuccessful in controlling malaria effectively and, therefore, the use of DDT was re-implemented (Tren and Bate, 2004). Following the UN's multilateral agreement on POPs, South Africa applied for an exemption on the DDT ban and received authorisation on the premise that alternatives be incorporated where and when possible. Currently, DDT is utilised in malaria control programmes in the eastern malaria belt of South Africa, including the Limpopo, KwaZulu Natal and Mpumalanga Provinces (Bornman et al., 2009). It is sprayed through indoor residual spraying (IRS), using technical grade DDT that consists of 65-80% p,p'-DDT (active ingredient), with the remainder constituting o,p'-DDT and p,p'-DDD (Department of Health (SA), 2002; Tren and Bate, 2004).

Despite the usage of DDT in these malarial control programmes, there is still a scarcity of data regarding the specific environmental and social impacts of DDT IRS programmes, with even fewer data available on the concentrations and effects of DDT in the surrounding aquatic ecosystems. The present study was initiated to assess the sub-lethal effects induced by chronic exposure in aquatic organisms in a DDT sprayed area, using the well distributed and indigenous catfish species, *Clarias gariepinus*. Although relatively few ecotoxicological studies have utilised *C. gariepinus*, the use of catfish have proven to be a sufficient indicator in such studies

(Heath and Claassen, 1999; Bornman et al., 2007; Crafford and Avenant-Oldewage, 2010). They are relatively large, sedentary, bottom-dwelling omnivores, which scavenge and feed on a wide variety of prey (Skelton, 2001). This feeding behaviour increases their risk of exposure to organic pollutants, since these contaminants generally have a low solubility in water and tend to concentrate within the lipid-rich food sources (Kime, 1998; Connell, 1999). This would subsequently allow catfish to better reflect the current contamination status of DDT and other POPs and increase their susceptibility to health effects.

In terms DDTs health effects on fish, the most prominent responses, following chronic exposure, are associated with endocrine disruption (Iwaniuk et al., 2006). This can be via altering hormone secretion, interfering with hormone-receptor interactions, or modifying the metabolism of circulating hormones, which all can ultimately influence entire reproductive, nervous, behavioural and immune systems (Rodrigues et al., 2007). As it is not possible to assess all these effects, a selected number of biomarkers are used to identify the extent of effects commonly associated with DDT (Amiard et al., 2000).

Biomarkers were selected based on their relative sensitivity, reliability, repeatability, cost effectiveness and ability to identify endocrine disruptive effects at a variety of levels of biological complexity. This included the assessment of the vitellogenin (VTG) levels using alkali-labile phosphate (ALP), calcium (Ca), magnesium (Mg) and zinc (Zn) in the plasma. Although studies have shown the induction of VTG in *C. gariepinus* in response to EDC's (Braathen et al., 2009), no known studies have utilised ALP, Ca and Mg as indirect measures in this species. Studies using *Anguilla anguilla* and *Misgurnus anguillicaudatus*, however, showed that ALP, Ca, Mg and Zn are all present on the VTG molecule in high abundances and can, therefore, be linked to VTG concentrations (Verslycke et al., 2002; Versonnen, et al., 2004; Lv et al., 2006).

In order to identify higher levels of effects, the gonads were assessed for negative alterations in the gonad mass and cellular morphology, using gonad-somatic index (GSI), interpreted with analysis of covariance (ANCOVA) manipulated gonad mass and histologically assessed for intersex (female oocytes in male gonads) (Kime, 1998; Jobling and Tyler, 2003). The commonly utilised biomarker, GSI, calculates the percentage of gonad mass to body mass. It can be used to estimate the degree of effects on reproduction, based on the assumption that a reduction in relative gonad mass can occur in response to increased contaminant exposure (Schweer, 2002). It has successfully been used in *C. gariepinus,* however, is largely influenced by environmental conditions (DeGraaf and Janssen, 1996; Cavaco et al., 1997; Yalcin et al., 2001). To remove this strong influence, Packard and Boardman (1999) recommended an alternative, using a more statistically sound methodology involving ANCOVA. Since this methodology has seldom been utilised, both the GSI and ANCOVA methodologies will be evaluated and compared in this study.

Lastly, the CF was utilised as a general indicator of organismal health, which would prove advantageous in quickly identifying the extent of DDT effects (Anene, 2005). This index is based on the fact that there is usually depletion in the energy resources available in fish, as these resources are utilised to cope with the increased stresses posed by a toxicant.

The overall results from the biomarker responses and DDT bioaccumulation were used to (1) identify the current status of fish in the Luvuvhu River system in the DDT sprayed areas compared to a reference area, (2) identify a cause-effect response in *C. gariepinus*, and (3) identify the suitability of the suite of biomarkers from monitoring in DDT sprayed areas in developing countries.

2 Materials and Methods

2.1 Study area

The Luvuvhu River is situated within the DDT sprayed area of the Limpopo Province in the northern part of South Africa (Heath, 1999). It originates in the Soutpansberg Mountains and converges with the Limpopo River in the Kruger National Park at Crook's Corner. Three sites were selected in the Luvuvhu River, based on the position of the DDT IRS sprayed area. A reference site was situated beyond the DDT sprayed area at Albasini Dam (1R) and two sites within the DDT sprayed area at Nandoni Dam (2E) and Xikundu Weir (3E) (Figure 1).

2.2 Field Procedures

Male *C. gariepinus* were specifically selected as many endocrine disruptive effects are predominantly influenced by the sex of fish (Phillips and Rainbow, 1993; Kime, 1998). The objective was to sample 10 specimens (identified with Skelton (2001)) at each site using three 100mm mesh size gill nets, in two seasons. One in the dry season/low flow (LF) 2007 and one in the wet season/ high flow (HF) 2008, when there was increased IRS-spraying. A permit was obtained from the Limpopo Province conservation authorities. Despite all efforts, a full data set was only caught at 3E (Table 1). At 2E, only 3 and 4 fish were sampled in the LF and HF, respectively. This resulted in a reduced confidence in the results obtained from this site. Fortunately, larger data sets were available for sites representing a reference area and DDT-IRS sprayed area (3E), which would assist in increasing the confidence in the conclusions relating to DDT effects.

For each fish sampled, the total length (cm) and total wet mass (kg) were recorded (Table 1). Blood samples were then taken in vacutainers coated with EDTA and aprotinin from the caudal aorta, for biomarker analysis. The blood was kept on ice until centrifuged at 3 600rpm for 10 minutes at 4°C and the resulting plasma stored at -20°C. Fish were sacrificed ethically by severing vertebrae (AVMA, 2001). The gonads were removed, weighed

(to assess condition) and macroscopically assessed to estimate the gonad maturity. As indicated in Table 1, fish were characterised as mature and developing fish using the gonad development index described in Heath (1999). Gonads were prepared for histological analysis as described by Barnhoorn et al (2004). Then, adipose and muscle tissue samples were taken for DDT and metal analysis, respectively. The adipose tissue was stored in aluminium foil at -20°C until further EDC analysis and muscle at -20°C until further metal analysis as described indicated in section 2.3 and 2.4. Lastly, pectoral fins were removed for age determination and prepared according to Staples (1970). Sections of approximately 1mm were cut perpendicular to the basal recess and placed in ethanol before the number of rings was counted. The age estimates are represented in Table 1.

At each of the sites the physico-chemical water quality parameters (temperature, dissolved oxygen, conductivity, pH) were measured *in situ* using a Cyberscan DO100 meter, pHScan meter and Cyberscan CON400). Monitoring showed no levels exceeding the South African water quality guidelines (DWAF, 1996) and therefore variables were not further discussed in this paper. Water and sediment samples were also taken to screen for EDC contamination during this study. Samples were collected in triplicate in glass containers and stored under dark conditions at 4°C until further analysis. The results of the screen for organic contaminants were documented in Barnhoorn et al. (2010) and therefore, will not be detailed in this paper, whilst the results for the metal EDCs were included.

2.3 Chemical analysis

In this study, the concentrations of o,p'- and p,p'-DDT and its metabolites were assessed in the adipose tissue of *C. gariepinus* using gas chromatography spectrophotometer (GS-MS). The adipose was specifically selected as it provides an ideal tissue as organic contaminants, such as DDT, are highly lipophilic and tends to bioaccumulate in the adipose and subsequently reflect overall exposure over time (Parson, 1971; Heath and Claassen, 1999). In preparation for the GS-MS, the samples were extracted using solid phase C18 cartridges (Waters-Microsep) and a florisil cartridge (Barnhoorn et al., 2009; Bordet et al., 2002). The samples were eluted with 98:2 (v/v) and 85:15 (v/v) petroleum ether-diethyl ether. The resulting fractions were then combined and dried using nitrogen and reconstituted in methanol. DDT residues were analysed on a Agilent 7890A GC-MS with a 5975C mass spectrometer and Equity 1701 fused silica capillary column (Supelco), by an ISO 17025 Accredited Laboratory. The specific detection of OCs was done using selective ion monitoring (SIM) mode, as the detection limits were too low for a full spectrum analysis.

To screen for metal EDCs in the water and sediment, concentrations of cadmium, copper, lead, zinc, mercury and nickel were measured using inductive coupled mass spectrophotometer (ICP-MS), as described in

Barnhoorn et al. (2010). All samples were acid digested using a Milestone Ethos microwave. The samples were diluted in 1% nitric acid and the metals were determined using a Varian UltraMass 700 ICP-MS. Indium was used as an internal standard to correct for interferences from high-dissolved solids and concentrations validated with standard reference material (DOLT-3). All recoveries were very similar to DOLT-3 standards eg. Cd, Cu, Pb and Zn standards of 19.4, 31.2, 0.32 and 86.6 ug/g were met with recoveries of 18.81, 28.54, 0.51 and 70.73, respectively.

2.4 Biomarkers

The protein-bound phosphate, measured as ALP, was extracted from 10 µl of plasma according to the procedure described by Brasfield et al. (2002). Extracts were then assayed spectrophotometrically on the universal microplate reader from Biotek Instruments, Inc, using a modified method derived by Stanton (1968). The metals Ca, Mg and Zn were measured on the ICP-MS from 500 µl of prepared plasma as stipulated in Section 2.3.

The gonad condition was measured using the GSI index. The GSI was calculated as the percentage of gonad mass (g) to total body mass according to Schweer (2002). The gonad condition was also assessed by manipulating the gonad mass data using ANCOVA. The gonad mass (g) was plotted against fish body mass (kg) for each site of each season. Linear regression lines were then fit (best fit) to each data set. The sample mean deviated from best fit mean was then measured (sum of squares), which was used to determine a common slope between all the groups. This was in turn utilised to calculate an adjusted mean without confounding effects of body size for each site of each season and tested for significant differences using ANOVA (Packard and Boardman, 1999).

For the intersex determination, histological slides of the gonads were prepared as described in Barnhoorn et al. (2004). The slides were then examined for intersex using light microscopy with a range of magnifications between 20x and 100x.

The fish condition (CF) was calculated using the formula $K = W/(aL^b)$ according to Hagenaars et al. (2008). Where W is the total body mass (g) and L is the total length (cm). The parameters a and b were determined using the best fit values from the length-mass relationship ($W = aL^b$) of total number of fish within the study.

2.5 Statistics

The contaminant and biomarker data were statistically analysed for significant variations between reference and exposed sites as well as between the two seasons. Using SPSSv19, the normality (Shapiro-Wilks) and

homogeneity of variance (Levene's test) were tested prior to the statistical evaluation of the data sets. One way analysis of variance (ANOVA) was assessed and if significant, a post-hoc comparison was applied to test significance of each data set (Scheffe test for homogenous data or Dunnett's T3 test for non-homogenous data) (Zar, 1996). In the case where data sets did not meet assumptions of normality, the Kruskal-Wallis ANOVA were tested to find significant differences, as suggested by Wang and Riffel (2011). Relationships between the various biomarkers, inherent influencing factors and contaminants in the adipose tissue were identified using Pearson's correlation in SPSSv19. Water and sediment could not be correlated as too few readings were measured below detection limits. For all analyses, the significance level was noted when $p<0.05$. All graphs were drawn in Graphpad v.4 software.

3 Results

3.1 DDT contamination in fish, water and sediment

The average DDT concentrations in the adipose tissue are indicated in Table 2. Concentrations were the highest in the p,p'-DDE form, with levels ranging from 2.53 mg/kg to 34.47 mg/kg. These were significantly positively correlated with age ($r=0.54$) and maturity ($r=0.42$). In both seasons, a concentration gradient of 1R<2E<3E was evident, but did not differ significantly ($p<0.5$). The concentrations were above the Canadian guidelines of 0.014 mg/kg diet wet weight (CCME, 1999a) stipulated for the total concentration of DDT (i.e. the sum of all metabolites) allowed, with 3E, situated in the DDT sprayed areas, having the highest unacceptable concentrations present. No South African guidelines were available.

The organic pollutants that were measured in the water and sediment in this study were documented and discussed in Barnhoorn et al., (2010), Marchand et al. (2010) and Brink (2010). DDT concentrations were only detected above detection levels of 0.05 µg/l in water and 0.05 µg/kg (adjusted to 1% Total Organic Carbon (TOC)) in sediment, in the HF. In the water, the DDT levels were 0.60 µg/l at 1R and 1.80 µg/l at 3E, whilst in the sediment, the concentrations were 3.42 µg/kg and 11.17 µg/kg at 2E and 3E, respectively. Of the metals that were analysed, only Zn was present at levels above recommended levels (DWAF, 1996) in the water, with concentration of 3.61 µg/l in the HF. No EDC metals were present at levels of concern in the sediment.

3.2 Plasma phosphate and metal (calcium, magnesium and zinc) determination

The spatial and temporal variations are illustrated in Figure 2. Significant differences ($p<0.05$) were only observed with the plasma phosphate levels, where concentrations in the HF were significantly increased at 1R and 2E. When correlations were analysed between the plasma biomarkers and DDT metabolites in adipose tissue, significant negative relationships were observed with Mg, Ca and Zn concentrations in the presence of

DDT contamination (Table 2). The Ca levels were shown to be significantly influenced by maturity and gonad weight, which resulted in the significant negative correlations with GSI (r=-0.47). Regardless of this, levels of Ca from the total and mature data set showed the same temporal and spatial trend.

Correlations between the plasma biomarkers revealed that ALP and Ca had a significant positive correlation (r=0.33). In both biomarkers there was an increase in concentrations in the HF as compared to the LF and a spatial trend of 1R>3E in both seasons (Figure 2). Further significant relationships were observed between Mg and Zn (r=0.63) and Mg and GSI (r=0.39).

3.3 Gonadosomatic Index and ANCOVA adjusted gonads (alternative to GSI)

The gonad condition was represented in Figure 3 and showed no significant spatial and temporal differences in either index. The GSI indicated that the lowest gonad condition was observed in HF period for all sites, particularly at 3E, where contamination was greatest, and was significantly correlated with gonad maturity (r=0.66). This indicated that GSI is influenced by the natural reproductive stage of the fish, which can lead to incorrect conclusions about contamination effects (Billard and Khan, 2003). In this study, data sets were not separated into different maturity groups, due to the small sample size, but were considered when interpreting results of both endpoints. For the ANCOVA adjusted gonads, the spatial and temporal tendencies differed to that of the GSI results, with the gonad condition lowest in the LF. This could have been influenced by the higher percentage of developing fish present that have an inherently low gonad mass within the LF data set.

3.4 Intersex

Intersex was not observed in *C. gariepinus* testes either macroscopically or histologically. No visible structural changes occurred on the gonads and upon the histological evaluation there was no indication of oocytes present within any of the testicular tissue sampled. These histological sections were, therefore, not graphically represented.

3.5 Condition factor

In Figure 4, the mean CF values showed very little variations and only ranged from 0.91% to 1.07%. Although no significant spatial and temporal differences ($p<0.05$) were found, the condition factors in *C. gariepinus* from the two dams (1R and 2E) were higher as compared to those CF values in *C. gariepinus* at 3E, for all seasons. Furthermore, no real seasonal trends were observed at any of the sites. When the values were correlated with the DDT loads and other influencing factors, no relationships were found (Table 2).

4 Discussion

DDT spraying in the Luvuvhu River has been relatively continuous for the past 60 years. Despite the controversy of the use of DDT as a vector control, very little is known on the ecotoxicology of DDT on the receiving water courses. This is particularly concerning since the Luvuvhu River ecosystem is home to numerous sensitive aquatic species (Fouche et al., 2005; Brink, 2010). It was evident from the bioaccumulation data, that there is indeed a high risk of effects from DDT contamination on the aquatic ecosystems within areas where DDT is sprayed as well as surrounding areas. DDT and its metabolites were present in the water, sediment and fish samples at all sites. The concentrations in the water and sediment fluctuated between seasons, but the bioaccumulation data in the adipose tissue of *C. gariepinus* showed consistently high levels of all DDT metabolites. Sites situated in the DDT spraying zones, including the Nandoni Dam (2E) and Xikundu Weir (3E), were especially contaminated. Not only were there levels of DDT concentrations well above recommended guidelines, there was also toxic concentrations of other EDC's (Kime, 1998; CCME, 1999a,b; ASQG, 2000; AWQG, 2000). The additional screen of organic pollutants documented by Barnhoorn et al. (2010) indicated various contaminants that were above the detection levels of 0.05 µg/l in the water and 0.05 µg/kg adjusted to 1% TOC in the sediment. This included PCB153 (HF: 3E (2.80)), dieldrin (HF: 1R (1.60), 2E (2.40), 3E (3.50)) and lindane (HF: 1R (3.10), 2E (1.10), 3E (9.40)) in the water and heptachlor epoxide (LF: 2E(0.36), 3E(0.08)), endosulfan II (3E-HF: 0.56) and endrin aldehyde (3E-HF: 0.09) in the sediment.

In previous studies where fish were exposed to these concentrations of EDCs, a sub-lethal response was almost always induced (Holden, 1973; Kime, 1998; Vasseur and Cossu-Leguille, 2006). The present findings, in contrast, showed that exposure to DDT as well as other EDCs, resulted in few observable responses in male *C. gariepinus*. No significant relationships were identified, although, subtle changes were observed with the plasma biomarkers, Ca and ALP at a sub-cellular level.

The two plasma biomarkers were utilised as indirect measures of VTG circulating in the blood, based on numerous fish studies indicating a significant correlation between VTG and Ca, phosphate, Mg and Zn, respectively (Verslycke et al., 2002; Versonnen et al., 2004; Lv et al., 2006). In the presence of EDC's, high levels of the female protein VTG can unnaturally accumulate in male and juvenile fish (Tsai and Wang, 2000; Guerreiro *et al*., 2002; Gillespie and de Peyster, 2004). Evidence to support the induction of VTG in *C. gariepinus* in the presence of EDC's was recently shown in a laboratory study exposing males to both natural oestrogen (17alpha-estradiol [E2]) and sythentic EE2 (Braathen et al., 2009). Although a field study by Mdegela et al. (2010) showed that VTG was not induced in *C. gariepinus* specimens from sewage ponds with a potential of EDC contamination, no direct evidence of EDC contamination was shown. In the current study this induction of VTG in

C. gariepinus could not be conclusively verified, since VTG was not directly analysed. However, there was an indication that Ca and ALP levels did elevate in response to increased DDT, Zn, PCBs, lindane, endosulfane and endrine levels in water, sediment and adipose tissue in the HF. The fact that there was only a response evident when the EDC levels increased in the HF from the LF and not when EDCs increased in the DDT sprayed areas in each season, suggested that Ca and ALP were generally only sensitive to large variations in contaminants. These results were in agreement with Lv et al. (2006) who also only observed increased Ca concentrations at higher exposure levels of estradiol, indicative of an EDC chemical. Therefore, suggesting that plasma Ca and ALP could possibly be utilised as non-target sub-cellular biomarkers in contaminated areas. Despite this, the reliability of Ca and ALP as biomarkers needs to be further researched, due to the lack of any significant positive correlations with EDC contamination. The utilisation of the ALP assay was also shown to be insensitive in the present study. Using fish exposed to 17α – ethinylestradiol both Verslycke et al. (2002) and Versonnen et al. (2004) showed that plasma ALP and Ca assays have the same sensitivity to oestrogen effects. According to many authors this is largely because each Ca ion is bound to the phosphate groups on the VTG molecule (Gosh and Thomas, 1995; Fuentes et al., 2007). The concentrations in the present study contradicted this, with Ca measuring more than a 100 times greater than the ALP, despite the same trends observed with Ca and previously successful use of this protocol (Verslycke et al., 2002). This together with the fact that the ALP protocol is quite elaborate and requires extensive manipulations compared to the relatively simple measurements of Ca on the ICP-MS, indicates that Ca would be a better biomarker for use in cost effectively monitoring DDT contamination in developing areas.

In the instance of the gonad condition, there was no significant variation observed in either biomarker that would indicate the adverse effects of DDT. In consideration of the GSI biomarker, the overall levels were lower than expected at all of the sites. In the LF (measured in October 2007) the low GSI concentrations corresponded with the naturally low spawning capacity between July and November at all sites (Yalcin et al., 2001). However, in the HF the GSI values should have been much higher, as February to April is the peak period for *C. gariepinus* spawning in the southern hemisphere. According to De Graaf and Janssen (1996) the GSI during these periods are generally measured between 6 and 10%, about 10 to 30 times greater than the GSI values during the HF period. Similar low GSI values were observed in *C. gariepinus* sampled from two dams in the Rietvlei Nature Reserve, which was shown to be extensively contaminated with effluent from upstream anthropogenic activities (Barnhoorn et al., 2004). These results suggest that gonad conditions are extremely reduced at all three sites in the Luvuvhu River during the spawning season, possibly due to the greater EDC contamination in the HF. Having said this, it should be noted that the extent of the overall reduced gonad condition could have been exaggerated, as comparisons with laboratory *C. gariepinus* showed an average GSI value of 0.67% in mature control fish (Brink, 2010).

GSI also indicated that there was a reduced condition in fish from Xikundu (3E) that corresponded to the higher DDT and general EDC concentrations at this site. No such trend was observed in the ANCOVA manipulated data, with different temporal and spatial variations. These variations were, however, not significant and could not be related to the contamination observed in this system. This was in contrast to the strong emphasis on the utilisation of ANCOVA adjusted gonads instead of GSI, by Packard and Boardman (1999). A possible reason for this could be that the gonad mass was generally small and there was no significant ($p<0.05$) differences between the various sites and seasons. The influence of the natural reproductive stage of the fish, could have also played a role in influencing the interpretation of the gonad condition. In both biomarkers, the gonad condition was strongly influenced by the number of developing and mature fish present (Cavaco et al., 1997). In the LF there were more specimens classified as developing (with inherently low gonad mass) than mature specimens, which probably resulted in the under estimation of the gonad condition. In consideration of this it would be expected that the spatial and temporal trends in the LF were not accurately represented. Whilst in the HF at 1R and 3E, the maturities was evenly distributed and, therefore, were probably more accurate indicators of spatial trends. Due to the limited number of replicates in this study the gonads were not split between developing and mature individuals.

The overall lack of significant responses to contamination was attributed to the ability of *C. gariepinus* to prevent effects at higher levels of biological organisation. It is possible that the effects from DDT uptake is reduced in *C. gariepinus* due to their ability to metabolise, transform, sequester and/or eliminate contaminants more effectively. Although no studies have previously identified this, the high presence of DDE in the adipose tissue indicates that a large portion is metabolised and sequestered, which would render the contaminants relatively inert in the biological phase (Connell et al., 1999). Although, this would not guarantee permanent mitigation and adverse condition may lead to the utilisation of the lipid reserves and hence the release of the EDCs back into the blood stream.

These efficient mechanisms to handle DDT toxicity seemed to be rather species specific, especially when analysing the presence of intersex. In the present study the histological analysis of the gonads showed that there were no ovarian cells observed in the testicular tissue of male *C. gariepinus* in the presence of DDT. However, in concurrent studies conducted by Marchand et al. (2008) and Barnhoorn et al. (2010), gonadal intersexuality was observed in samples from the Mosambique Talapia (*Oreochromis mossambicus*) that were exposed to the same environmental conditions in the Luvuvhu River system. These results indicate that *C. gariepinus* was more resistant to endocrine disruption than *O. mossambicus*. This lack of effect in *C. gariepinus* was also shown in the laboratory studies done by Bornman et al. (2010), where catfish had no intersex after 21 days of exposure to low

concentrations of p,p'-DDT. Whilst, in the presence of high concentrations of contaminant, such as nonylphenol, *C. gariepinus* testes exhibited extensive intersex (Barnhorn et al., 2004).

The utilisation of the biomarkers, Mg, Zn and CF, were fairly ineffective in the current study, under the environmental conditions in DDT sprayed areas. In the instance of Mg and Zn, these sub-cellular biomarkers proved to be insensitive in male fish in the presence of organic contaminants. Like Ca and ALP these plasma metals were used as indirect indicators of VTG stimulation in males. Relatively few reports were found that assessed their adequacy in the presence of oestrogen mimicking chemicals, particularly as indirect biomarkers of VTG. Most of which had contradicting results, with some showing them as reliable indicators (Bjornsson and Haux, 1985; Lv et al., 2006), whilst others demonstrating their insensitivities (Blaise et al., 1999). The results of this study are similar to the latter publications as there were no positive correlations observed with DDT contamination or with Ca and ALP concentrations. The proposed reason for the insensitivity of these metals is that their respective amounts that bind to the VTG molecules are generally smaller. Zn was reported as binding to the VTG molecule at half the concentration of Ca, whilst Mg was reported as being bound to the VTG molecules at concentrations ten times lower than that of Ca (Gosh and Thomas, 1995; Montorzi et al., 1995; Anderson et al., 1998). Therefore, it can be concluded that these essential metals in the plasma could not be used as indicators of contamination and that the spatial and temporal variations observed in Figure 2 were only indicative of natural fluctuations, due to changes in uptake from water, sediment or food (Phillips and Rainbow, 1993).

Regarding CF, this biomarker was utilised as a general indicator of fish health that correlates the fish's body mass to its length and is in fact often used in aquaculture, to monitor feeding intensity, age and growth rates (Anene, 2005). Within an ecotoxicological context, the CF is based on the fact that there is usually depletion in the energy resources available, as these resources are utilised to cope with the increased stresses posed by a toxicant. An astounding number of ecotoxiciological based studies, including those measuring DDT, have incorporated this index as it is particularly popular due to its simplistic and cost effective nature (Hagenaars et al., 2008). In this study, the *C. gariepinus* did not seem to be a major indicator of DDT contamination. There was slightly lower health conditions evident at 3E during this study that may have been caused by the increased EDC contamination observed at this site. Despite this finding, there was still no significant correlation between the general CF and EDC concentrations in the water, sediment or adipose tissue. This is predominantly due to the large influence of environmental variations on fish body conditions. Since CF is related to changes in the body mass and length, fluctuations according to food availability, habitat quality, breeding activity or even age (Filbert and Hawkins, 1995) are expected. Although, in the present study, neither age nor breeding activity could be related to the CF through Pearson's correlation. The food availability and habitat quality may have resulted in

some of the spatial fluctuations in the CF. At the two predominantly lentic sites, 2E and to a lesser extent 1R catfish had higher CF than those from the weir 3E, suggesting that perhaps the lake habitats were more conducive to better catfish condition. In fact, according to Davies and Day (1998) rapid growth in dams is a common occurrence in fish species such as catfish, as the physico-chemical features provide conditions for greater food quantities.

5 Conclusion

Overall, high levels of DDT along with lindane, dieldrin, endosulfane II, PCB153 and Zn were present in the receiving water systems in the DDT IRS-sprayed areas. *C. gariepinus* was shown to be relatively tolerant to various doses of contaminant exposure. Risks of effects in this species were associated with increased concentrations in fish habitats and/or if fish are stressed and sequestered DDT in the adipose tissue become available. Subtle responses were only observed when fish where exposed to higher levels of contaminants in the summer season, which was when DDT IRS and pesticide spraying was most predominant. The lack of effects at higher level of organisation highlight the need for the inclusion of sub-cellular biomarkers that are indicative of the first phase of effects into a suite of biomarkers with various levels of EDC effects, which is utilised to monitor aquatic ecosystems in a DDT IRS-sprayed areas. The use of Ca, GSI, ANCOVA gonads, intersex and CF is particularly advantageous in developing countries where resources are often limited as were found to be relatively cost effective, reliable, scientifically sound and easily measurable. Furthermore, further studies should focus on more sensitive fish species from the Cyprinidae or Cichlidae families.

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7 References

Amiard, J.C., Caquet, T., Lagadic, L., 2000. Biomarkers as tools for environmental quality assessment, in: Lagadic, L., Caquet, T., Amiard, J.C., Ramade F. (Eds.), Use of biomarkers for environmental quality assessment. A.A. Balkema, Rotterdam.

Anderson, T.A., Levitt, D.G., Banaszak, L.J., 1998. The structural basis of lipid interactions in lipovitellin, a soluble lipoprotein. Structure. 6, 895–909.

Anene, A., 2005. Condition factor of four cichlid species of a man-made lake in Imo State, southeastern Nigeria. Turk. J. Fish Aquat. Sc. 5, 43-47.

ATSDR (Agency for toxic substances and disease registry), 2002. Toxicological profile for DDT, DDE, DDD. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Obtained from official ATSDR website: http://www.atsdr.cdc.gov/ToxProfiles. Retrieved 9/12/2011.

AVMA (American Veterinary Medical Association), 2001. AVMA panel on euthanasia, USA.

AWQG (Australian water quality guidelines), 2000. Obtained from official Australian governmental website http://www.environment.gov.au/water/policy-programs/nwqms/index.html#quality. Retrieved 1/2/2008.

Barnhoorn, I.E.J, Bornman, M.S., Pieterse, G.M., van Vuren, J.H.J., 2004. Histological evidence of intersex in feral sharptooth catfish (*Clarias gariepinus*) from an oestrogen-polluted water source in Gauteng, South Africa. Environ. Toxicol. 19, 603–608.

Barnhoorn, I.E.J, Bornman, Jansen van Rensburg, C., Bouwman, H., 2009. DDT residues in water, sediment, domestic and indigenous biota from a currently DDT-sprayed area. Chemosphere.77(9), 1236-1241.

Barnhoorn, I.E.J., Van Dyk, J.C., Pieterse, G.M., Bornman, M.S., 2010. Intersex in feral indigenous freshwater *Oreochromis mossambicus*, from various parts in the Luvuvhu River, Limpopo Province, South Africa. Ecotox. Environ. Safe. 73(7), 1537-1542.

Billard, S.M., Khan, R.A., 2003. Chronic stress in cunner, *Tautogolabrus adspersus*, exposed to municipal and industrial effluent. Ecotox. Environ. Safe. 55, 9-18.

Bjornsson, B.T., Haux, C., 1985. Distribution of calcium, magnesium and inorganic phosphate in plasma of estradiol-17 β treated rainbow trout. J. Comp. Physiol. 155, 347–352.

Blaise, C., Gagne, F., Pellerin, J., Hansen, P.D., 1999. Determination of vitellogenin-like properties in *Mya arenaria* hemolymph (Saguenay Fjord, Canada): a potential biomarker for endocrine disruption. Environ. Toxicol. 14, 455-465.

Bordet, F., Inthavong, D., Fremy, J.M. 2002. Interlaboratory study of a multiresidue gas chromatographic method for determination of organochlorine and pyrethroid pesticides and polychlorobiphenyls in milk, fish, eggs, and beef fat. J. AOAC Int. 85(6), 1398–1409.

Bornman, M.S., Van Vuren, J.H.J, Bouwman, H., De Jager, T.C., Genthe, B.B., Barnhoorn, I.E.J., 2007. Endocrine disruptive activity and the potential health risk in the Rietvlei Nature Reserve. Water Research Commission (WRC), Pretoria. Report No. 1505/1/07.

Bornman, R., De Jager, C., Worku, Z., Farias, P., Reif, S., 2009. DDT and urogenital malformations in newborn boys in a malarial area. BJU Int. 106, 405–411.

Bornman, M.S., Van Vuren, J.H.J, Barnhoorn, I.E.J., Aneck-Hahn, N., De Jager, C.J., Genthe, B., Pieterse, G.M., Van Dyk, J.C., 2010. Environmental exposure and health risk assessment in an area where ongoing DDT spraying occurs. Water Research Commission (WRC), Pretoria. Report No. K5/1674.

Bouwman H. 2004. South Africa and the Stockholm convention on persistent organic pollutants. S. Afr. J. Sci. 100, 323-328.

Braathen, M., Mdegela, R.H., Correia, D., Rundberget, T., Myburgh, J., Botha, C., Skaare, J.U., Sandvic, M. 2009. Vitellogenin in African sharptooth catfish (*Clarias gariepinus*): purification, characterization, and ELISA development. J. Toxicol. Environ. Health 72(A), 173-183.

Brasfield, S.M., Weber, L.P., Talent, L.G., Janz, D.M., 2002. Dose-response and time course relationships for vitellogenin induction in male western fence lizards (*Sceloporus occidentalis*) exposed to ethinylestradiol. Environ. Toxicol. Chem. 21(7), 1410-1416.

Brink, K.A., 2010. Effects of DDT on aquatic organisms in the Luvuvhu River. PhD unpublished thesis. University of Johannesburg, Johannesburg, pp.45-78.

Canadian Council of Ministers of the Environment (CCME). 1999b. Canadian sediment quality guidelines for the protection of aquatic life. Summary Tables. In: Canadian environmental quality guidelines, Canadian Council of Ministers of the Environment, 1999. Winnipeg.

Cavaco, J.E.B, Lambert, J.G.D, Schulz, R.W., Goos, H.J.T., 1997. Pubertal development of male African catfish, *Clarias gariepinus*. *In vitro* steroidogenesis by testis and interregnal tissue and plasma levels of sexual steroids. Fish Physiol. Biochem. 16, 129–139.

CCME (Canadian Council of Ministers of the Environment). 1999a. Canadian tissue residue guidelines for the protection of wildlife consumers of aquatic biota: DDT (total). In: Canadian environmental quality guidelines, Canadian Council of Ministers of the Environment, 1999. Winnipeg.

Connell, D., Lam, P., Richardson, B., Wu, R., 1999. Introduction to ecotoxicology. Blackwell Science Ltd., Cornwall, pp. 50-77.

Crafford, D., Avenant-Oldewage, A., 2010. Bioaccumulation of non-essential trace metals in tissues and organs of *Clarias gariepinus* (Sharptooth catfish) from the Vaal River system – strontium, aluminium, lead and nickel. Water S. Afr. 36(5), 622-640.

Davies, B., Day, J.A., 1998. Vanishing waters. UCT Press, Cape Town, pp. 242-284.

De Graaf, J., Janssen, G., 1996. Handbook on the artificial reproduction and pond rearing of the African catfish *C. gariepinus* in sub-saharan Africa. FAO fisheries Technical Paper 362, Rome.

Department of Health, South Africa. 2002. Obtained from official governmental website: www.doh.gov.za. Retrieved on1 April 2009.

DWAF (Department of Water Affairs). 1996. South African water quality guidelines. Volume 7: Aquatic Ecosystems. DWAF, Pretoria.

Falandysz, J., 1994. Polychlorinated biphenyl concentrations in cod-liver: evidence of a steady state condition of these compounds in the Baltic area oils and levels noted in Atlantic oils. Arch. Environ. Contam. Toxicol. 27, 266-271.

Filbert, R.B., Hawkins, C.P., 1995. Variation in condition of rainbow trout in relation to food, temperature, and individual length in the Green River, Utah. Trans. Am. Fish. Soc. 124, 824-835.

Fouche, P.S.O, Foord, S.H., Potgieter, N., van der Waal, B.C.W., van Ree, T. 2005. Towards an understanding of factors affecting the biotic integrity of rivers in the Limpopo province: niche partitioning, habitat preference and microbiological status in rheophilic biotopes of the Luvuvhu and Mutale Rivers. Water Research Commission (WRC), Pretoria. Report No. 1197/1/05.

Fuentes, J., Guerreiro, P.M., Modesto, T., Rotllant, J., Canario, A.V.M. Power, D.M., 2007. A PTH/PTHrP receptor antagonist blocks the hypercalcemic response to estradiol – 17. β. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, 956-960.

Gillespie, D.K, de Peyster, A. 2004. Plasma calcium as a surrogate measure for vitellogenin in fathead minnows (*Pimephales promelas*). Ecotox. Environ. Safe. 58, 90-95.

Gosh, P., Thomas, P., 1995. Binding of metals to red drum vitellogenin and incorporation into oocytes. Mar. Environ. Res. 39, 165–168.

Guerreiro, P.M., Fuentes, J, Canario, A.V.M., Power, D.M., 2002. Calcium balance in sea bream (*Sparus aurata*): the effect of oestradiol-17 β. J. Endocrinol. 173, 377–385.

Hagenaars, A., Knapen, D, Meyer, I.J., van der Ven, K., Hoff. P., De Coen, W., 2008. Toxicity evaluation of perluoroocatane sulfonate (PFOS) in the liver of common carp (*Cyprinus carpio*). Aquat. Toxicol. 88, 155–163.

Heath, R.G.M., 1999. A catchment-based assessment of the metal and pesticide levels of fish from the Crocodile River, Mpumalanga. Unpublished Phd Thesis. Rand Afrikaans University, Johannesburg.

Heath, R.G.M., Claassen, M., 1999. An overview of the pesticide and metal levels present in populations of the larger indigenous fish species selected in South African rivers. Water Research Commission (WRC), Pretoria. Report no: 428/1/99.

Holden, A.V., 1973. Effects of pesticides on fish, in: Edwards, C.A. (Ed)., Environmental pollution by pesticides. Plenum Publishing Company, London, pp. 213-254.

Huang, Y., Twidwell, D.L., Elrod, J,C., 2003. Occurrence and effects of endocrine disrupting chemicals in the environment. Pract. Periodical of Haz., Toxic, and Radioactive Waste Mgmt. 7, 241-252.

Iwaniuk, A.N., Koperski, D.T., Cheng, K.M., Elliott, J.E., Smith, L.K., Wilson, L.K., Wylie, D.R.W., 2006. The effects of environmental exposure of DDT on the brain of a songbird: changes in structures associated with mating and song. Behav. Brain Res. 173, 1-10.

Jobling, S., Tyler, C.R., 2003. Endocrine disruption in wild freshwater fish. Pure Appl. Chem. 75, 2219-2234.

Kime, D.E., 1998. Endocrine disruption in fish. Kluwer academic publishers, Dordrecht, pp. 1-378.

Lv, X., Shao, J., Song, M., Zhou, Q., Jiang, G., 2006. Vitellogenic effects of 17 β-estradiol in male Chinese loach (*Misgurnus anguillicaudatus*). Comp. Biochem. Physiol. 143(C), 127–133.

Mabaso, M.L.H., Sharp, B., Lengeler, C., 2004. Historical review of malarial control in southern Africa with emphasis on the use of indoor residual house-spraying. Trop. Med. Int. Health. 9(8), 846-856.

Marchand, M.J., Pieterse, G.M., Barnhoorn, I.E.J., 2008. Preliminary results on sperm motility and testicular histology of two feral fish species, *Oreochromis mossambicus* and *Clarias gariepinus*, from a currently DDT-sprayed area, South Africa. J. Appl. Ichthyol. 24(4), 423–429.

Marchand, M.J., Pieterse, G.M., Barnhoorn, I.E.J., 2010. Sperm motility and testicular histology as reproductive indicators of fish health of two feral fish species from a currently DDT sprayed area, South Africa. Chemosphere. 26, 707-714.

Mdegela, R.H., Braathen, M., Mosha, R.D., SKaare, J.U., Sandvik, M., 2010. Assessment of pollution in sewage ponds using biomarker responses in wild African sharptooth catfish (Clarias gariepinus) in Tanzania. Ecotoxicology. 19, 722–734.

Milestone ETHOS microwave. Cookbook digestion. Guide obtained with microwave, REV. 03_04.

Montorzi, M., Falchuk, K.H., Vallee, B.L., 1995. Vitellogenin and lipovitellin: zinc proteins of *Xenopus laevis* oocytes. Biochemistry. 34, 10851–10858.

Packard, G.C., Boardman, T.J., 1999. The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? Comp. Biochem. Physiol. 122, 37–44.

Parsons, A.M. 1971. Pesticide residues in fats and other lipids. Prog. Chem. Fats Other Lipids. 11, 243-293.

Phillips, D.J.H., Rainbow, P.S., 1993. Biomonitoring of trace aquatic contaminants. Chapman and Hall, Oxford, pp. 179-345.

Rodrigues, E.M., Medesani, D.A., Fingerman, M., 2007. Endocrine disruption in crustaceans due to pollutants: a review. Comp. Biochem. Phys. 146(A), 661-671.

Schweer, G., 2002. Draft detailed review paper on a fish two-generation toxicity test. EPA, Battelle. Report No. 68-W-01-02.

Skelton, P., 2001. A complete guide to the freshwater fishes of southern Africa. Struik publishers, Cape Town, pp. 229.

Stanton, M.G., 1968. Colorimetric determination of inorganic phosphate in the presence of biological material and adenosine triphosphate. Anal Biochem. 22, 27-34.

Staples, D.J., 1970. Methods of ageing red gurnard (Teleosti: Triglidae) by fin rays and otoliths. N.Z. J. Mar. Freshwat. Res. 5(1), 70-79.

Tren, R., Bate, R., 2004. South Africa's War against Malaria: Lessons for the Developing World. Cato Institute publishers. Pol. Anal. 513.

Tsai, C.L., Wang, L.H., 2000. Sex differences in the responses of serum calcium concentrations to temperature and estrogens in Tilapia, *Oreochromis mossambicus*. Zool. Stud. 39(1), 55–60.

Vasseur, P., Cossu-Leguille, C., 2006. Linking molecular interactions to consequent effects of persistent organic pollutants (POPs) upon populations. Chemosphere. 62, 1033-1042.

Verslycke, T., Vandenbergh, G.F., Versonnen, B., Arijs, K., Janssen, C.R., 2002. Induction of vitellogenesis in 17α-ethinylestradiol-exposed rainbow trout (*Oncorhynchus mykiss*): a method comparison. Comp. Biochem. Physiol. 132(C), 483-492.

Versonnen, B.J., Goemans, G., Belpaire, C., Janssen, C.R., 2004. Vitellogenin content in european eel (*Anguilla anguilla*) in Flanders, Belgium. Environ. Pollut. 128, 363–371.

Vouk, V.B., Sheehan, P.J., 1983. Methods for assessing the effects of chemicals on reproductive functions. John Wiley and Sons. New York.

Wang, M., Riffel, M. 2011. Making the right conclusions based on wrong results and small sample sizes: interpretation of statistical tests in ecotoxicology. Ecotox. Environ. Safe. 74(4), 684-692.

Wells, M., Leonard, L., 2006. DDT contamination in South Africa. Unpublished report, Groundwork consultancy, South Africa.

Yalcin, K., Solak, K., Akyurt, U., 2001. Certain reproductive characteristics of the catfish (*C. gariepinus*, Burchell, 1822) living in the river Asi, Turkey. Turk. J. Zool. 25, 453–460.

Zar, J.H., 1996. Biostatistical analysis. Prentice-hall, New Jersey.

Tables

Table 1. Number of male *C. gariepinus* specimens collected, with the biometry and gonad maturity mean (min – max) at each site.

	Low Flow (October 2007)			*High Flow (February 2008)*		
	1R-LF	*2E-LF*	*3E-LF*	*1R-HF*	*2E-HF*	*3E-HF*
# specimens	6	3	10	7	4	10
Mass (kg)	1.62 (0.7 - 3.6)	2.53 (2.0 - 3.1)	1.66 (1.4 - 2.2)	1.39 (0.6 - 3.4)	2.85 (1.7 -4.0)	1.40 (0.9-2.0)
Length (cm)	55.35 (42–70)	63.00 (56–68)	57.67 (55-66)	51.25 (42–68)	65.25 (59–72)	55.75 (52–61)
Age (years)	4.00 (2–8)	3.33 (2–5)	5.50 (4–8)	3.50 (2–6)	6.00 (4–8)	6.00 (4–10)
Maturity (%)						
Developing	*80*	*67*	*100*	*50*	*25*	*50*
Mature	*20*	*33*	*0*	*50*	*75*	*50*

Table 2. The mean (± SE) o,p'- and p,p'-DDT and metabolite concentrations in *C. gariepinus* adipose tissue.

	1R-LF	*2E-LF*	*3E-LF*	*1R-HF*	*2E-HF*	*3E-HF*
o,p'-DDT	0.09 (0.04)	0.18 (0.07)	2.25 (0.31)	0.07 (0.02)	0.09 (0.06)	0.42 (0.65)
p,p'-DDT	0.22 (0.23)	1.37 (1.05)	18.79 (2.35)	0.13 (0.15)	0.66 (1.86)	4.54 (10.48)
o,p'-DDE	<0.05	<0.05	0.09 (0.05)	0.06 (0.10)	0.33 (0.89)	0.28 (0.36)
p,p'-DDE	2.85 (4.69)	12.02 (8.79)	37.53 (6.38)	2.53 (2.17)	16.57 (0.47)	34.47 (65.01)
o,p'-DDD	<0.05	0.16 (0.07)	0.53 (0.19)	0.04 (0.14)	0.47 (1.38)	0.56 (0.78)
p,p'-DDD	0.26 (0.23)	2.45 (1.43)	8.05 (1.66)	0.40 (0.49)	3.50 (6.70)	8.20 (9.47)

Table 3. Pearson's correlation between biomarkers and DDT metabolites and other influencing factors.

	CF	**GSI[a]**	**GSI[b]**	**Mg**	**Ca[a]**	**Ca[b]**	**Zn**	**ALP**
DDT metabolites								
o,p'-DDT	-0.05	0.08	-0.29	-0.11	**-0.46[c]**	-0.37	-0.21	-0.30
p,p'-DDT	-0.12	0.07	-0.31	-0.18	**-0.43[c]**	-0.32	-0.30	-0.31
p,p '-DDE	-0.13	-0.01	-0.21	-0.41	0.26	0.31	-0.41	0.13
p,p'-DDE	-0.23	0.06	-0.42	-0.27	-0.09	0.09	**-0.44[c]**	-0.20
o,p '-DDD	-0.16	0.02	-0.30	-0.41	0.04	0.12	**-0.43[c]**	-0.02
p,p '-DDD	-0.20	0.02	-0.40	**-0.43[c]**	-0.06	0.06	**-0.54[c]**	-0.11
Biotic fluctuations								
Age	0.05	-0.02	-0.16	0.12	0.11	0.23	-0.09	0.06
Body length	0.28	0.11	-0.09	-0.03	-0.08	-0.14	-0.02	0.1
Body weight	0.34	0.03	-0.08	-0.04	-0.02	-0.12	0.03	0.12
Gonad weight	0.02	**0.87[c]**	**0.81[c]**	0.3	**-0.43[c]**	-0.30	0.28	-0.22
Maturity	-0.27	**0.66[c]**	0.00	0.16	**-0.52[c]**	0.00	0.18	-0.34

a, total specimens; b, mature specimens; c, significant correlations ($p<0.05$)

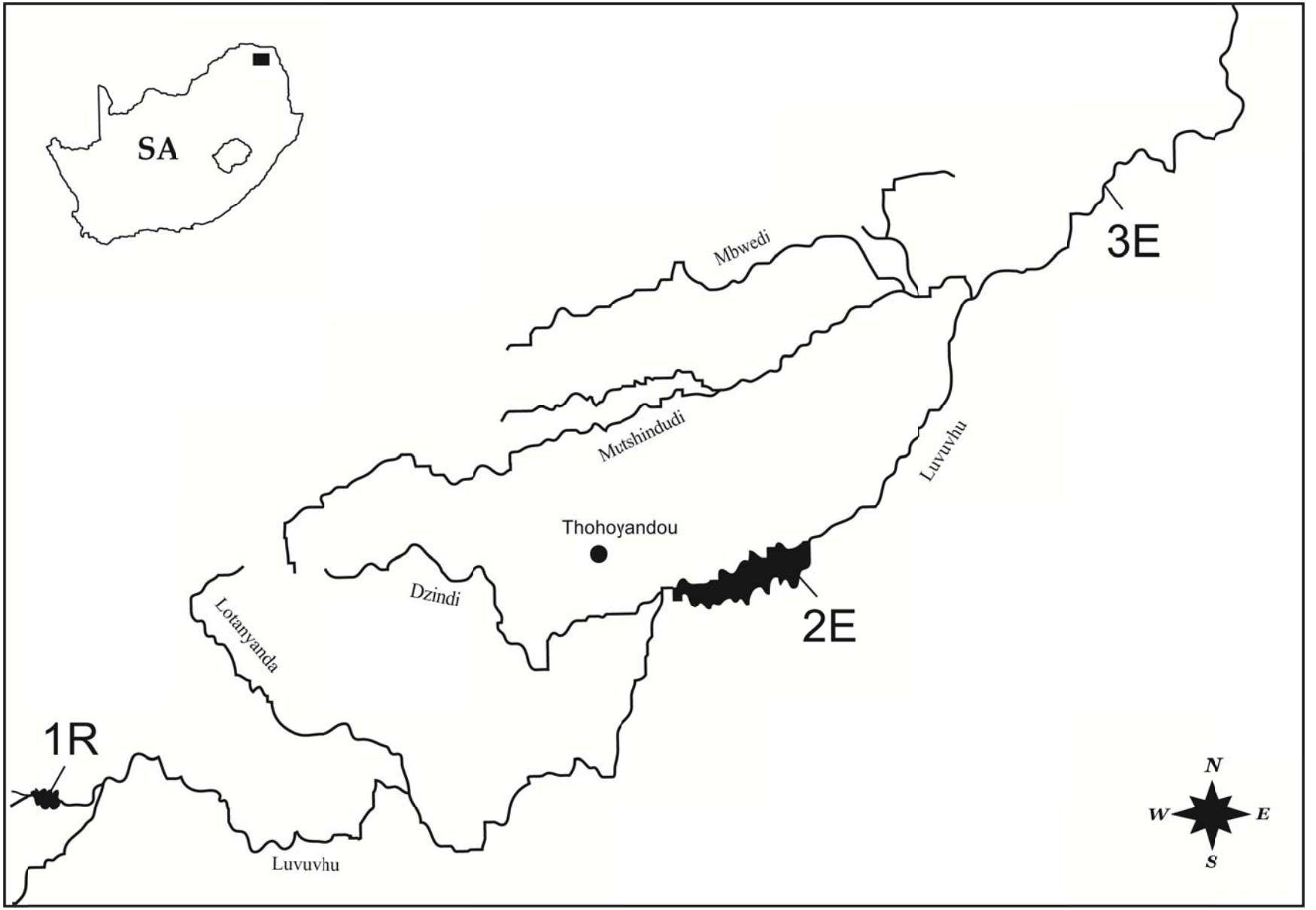

Figure 1. Locality of study sites in the Luvuvhu River catchment. Site 1R: Albasini Dam, Site 2E: Nandoni Dam and Site 3E: Xikundu Weir

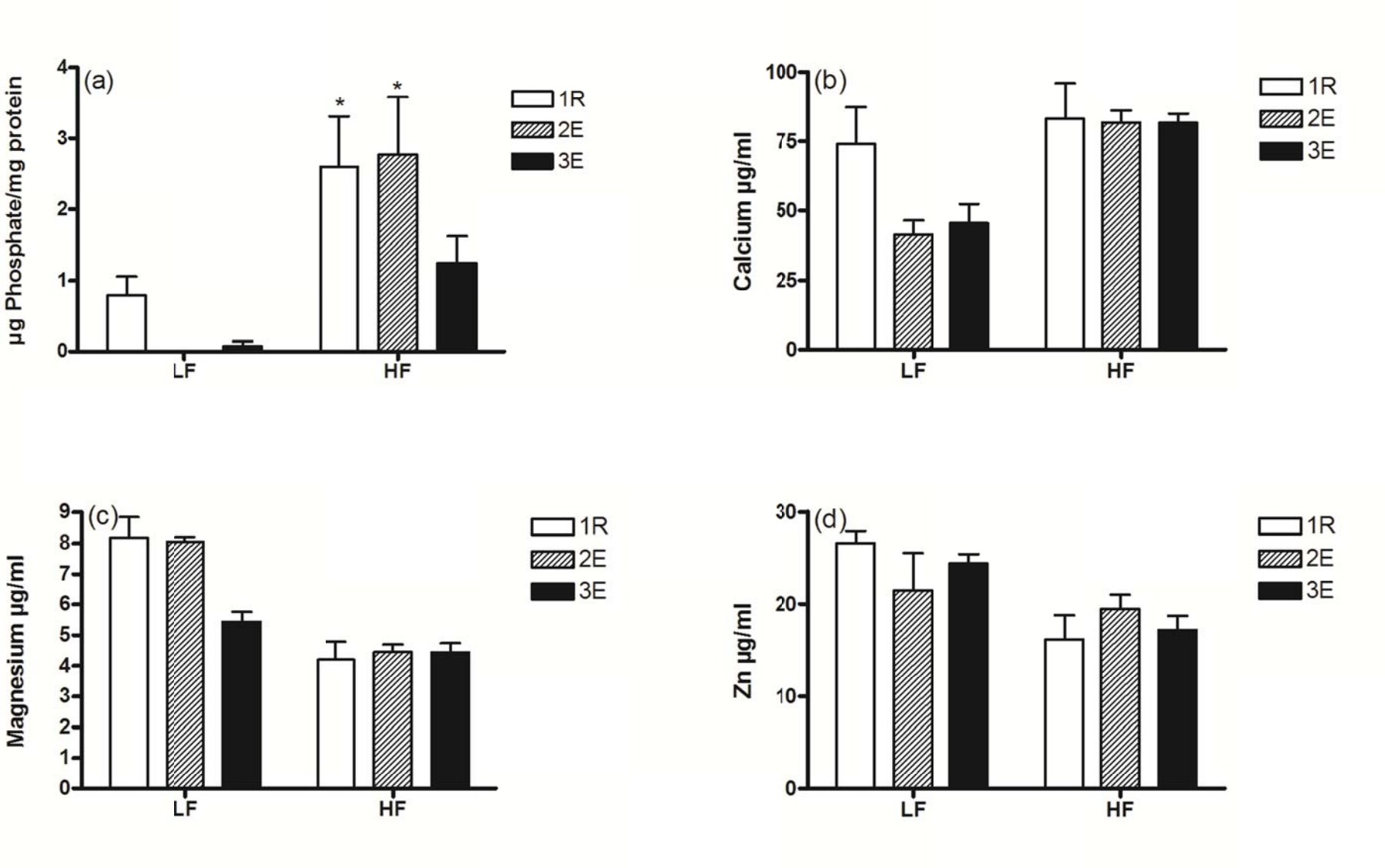

Figure 2. The mean (± SE) alkali-labile phosphate (a), calcium (b), magnesium (c) and zinc (d). Asterisks indicates significant differences from other sites ($p<0.05$).

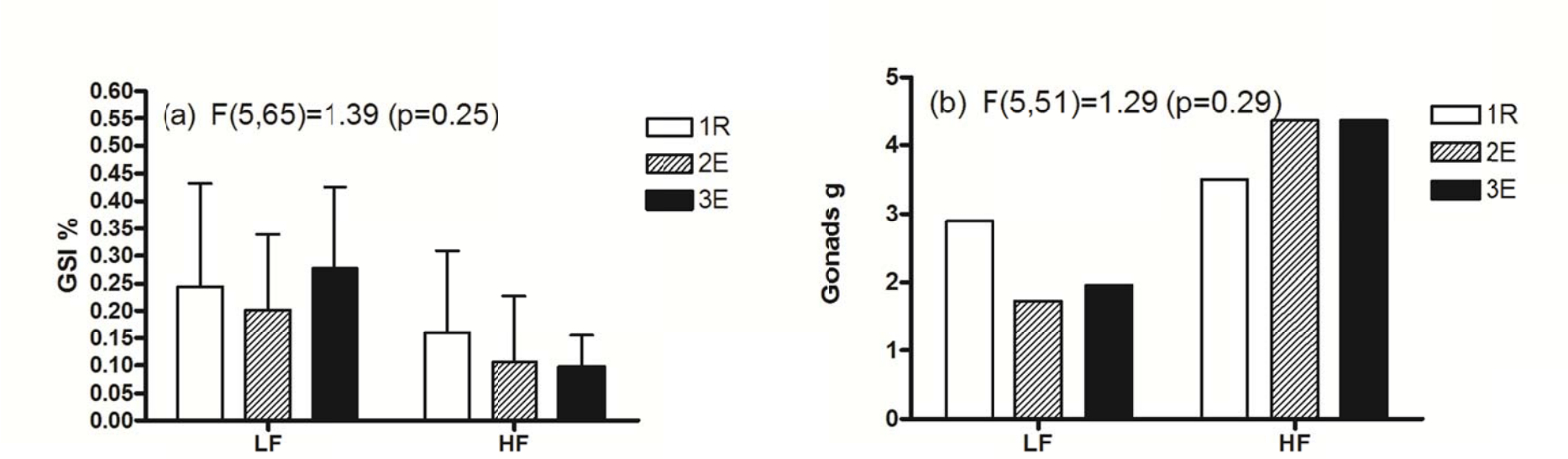

Figure 3. The mean (± SE) gonad somatic index (GSI) (a) and ANCOVA adjusted gonad means (b). The statistical analysis indicates that none of the groupings were significant ($p<0.05$) and F-ratio were low in all cases.

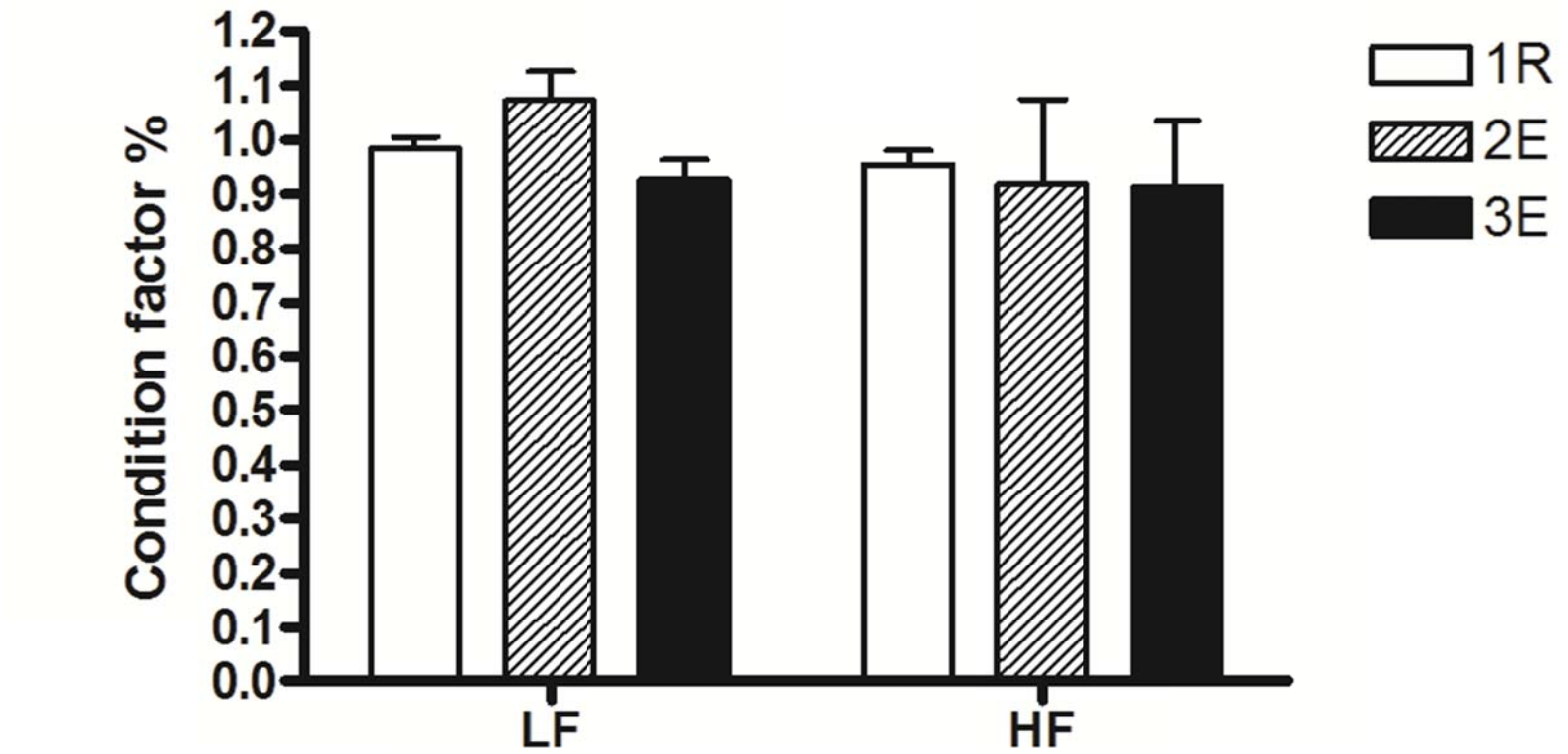

Figure 4. The mean (± SE) condition factor depicting spatial and temporal trends. No significant ($p<0.05$) differences observed.