

# Behavioural and physiological assessment of juveniles of African catfish (*Clarias gariepinus*) experimentally challenged with paraffin oil

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## Research Article

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# Abstract

The incidence of oil spills that finds their way into the aquatic environment constitutes a serious threat to the welfare of fish species and other aquatic biotas. However, the behavioural and stress response of *C. gariepinus* to paraffin oil (PO) has not been investigated under laboratory conditions to understand the welfare implication of this pollutant on fish species in petroleum polluted aquatic environments. Ninety-six juveniles of *C. gariepinus* were randomly allocated to 8fish/tank and subjected to four sub-lethal concentrations T1-0.4ml/L, T2-0.8ml/L, T3-1.6ml/L and T4-0.00 ml/L (control) of paraffin oil in triplicates. The fish were observed every 12-hours using the focal sampling technique for seven days. Behavioural traits observed were swimming (Non-active (NA), erratic (ER), Very-active (VA)), feeding (feed intake (FI), duration of feeding (DF)), aggressive (agonistic (AGO), number of bruises and scars (BS)) and respiratory (air gulping, (AG) mucus secretion (MS), Opercula rate (OR)) pattern. Glucose in blood samples were assessed for physiological changes. Data were subjected to Kruskal-Wallis tests. The result showed that concentrations of PO affected ( $P<0.05$ ) the duration of NA, ER and VA behaviour in the fish species; there was no significant ( $P<0.05$ ) difference between NA, ER and VA behaviour displayed by the *C. gariepinus* in T2 and T3. Feed responses during the experimental period were affected ( $P<0.05$ ) by the different concentrations of PO, but FI and DF were similar ( $P>0.05$ ) between T1 and T2. The concentration of PO increased AGO and the number of BS with the highest AGO and BS in T3. *C. gariepinus* in T2 and T3 displayed a similar ( $P>0.05$ ) pattern of AG, MS and OR. The least frequency of AG and MS during the experiment was found in T1. The OR was affected ( $P<0.05$ ) by PO exposure, and the highest OR was recorded in T3. Physiologically, exposure to PO increased ( $P<0.05$ ) glucose level in blood samples of the fish species with a higher glucose value in T3. The study concluded that higher and continuous exposure to concentrations of paraffin oil could alter behavioural and physiological responses, which might impair the welfare of *C. gariepinus* in their habitat. It is imperative to be cautious about the quantity of PO products that get into our aquatic environments.

## 1.0 Introduction

*Clarias gariepinus*, commonly known as African catfish, is considered a hardy fish species probably because they can tolerate adverse water quality conditions due to the presence of accessory breathing organs (Fawole et al., 2020). This breathing organ, among other attributes, further qualifies African catfish as the most cultured fish species (Adewunmi and Olaleye, 2010), with a low mortality rate during their culture period. In Africa, *Clarias gariepinus* is the most cultivated fish species. Its consumption cuts across several cultural, religious, and social beliefs, leading to an increase in the daily demand for African catfish (Akpaniteaku et al., 2005).

Nigeria is Africa's largest oil and gas producer and a major exporter of crude oil and petroleum products to other countries (Egborge, 2000; Ayman, 2014; George et al., 2014). The economic sector of Nigeria is highly dependent on the oil sector; it provides 65% of government budgetary revenues and 95% of foreign exchange earnings (World and bank, 2004).

Paraffin oil, popularly known as kerosene, represents one of the crude oil refinery products; it is widely used in many households as a source of cooking energy (Yekini, 2011). Beyond this, kerosene is being used in the aviation industry as an energy source (Glenn et al., 2011). It is important to note that paraffin oil is immiscible in water, and it floats on the water's surface at any slight spill or accidental discharge (Garry et al., 2007).

Incessant spillage of crude oil and related products occurs on land, water, and air (Ivon et al., 2021). Accidental discharge of these products eventually enters the aquatic environment through erosion and leaching (Absalom et al., 2009). Interestingly, the higher rate of urbanization and industrialization has led to an increase in the domestic and industrial use of these petroleum products. A rise in the spill of these crude oil products has been documented by Garry et al. (2007) and Yekini (2011). Unfortunately, these oil spills that always found their way into most freshwater and marine water outlets contain chemical constituents that alter the aquatic ecosystem and inflict serious health damage to the inhabiting fish species (Ayman, 2014). Nrior et al. (2019), in their toxicological bioassay of paraffin oil in tri-aquatic ecosystems, affirmed that a lethal concentration of 18.8% of PO has a higher toxicity strength in freshwater than brackish and marine water ecosystems.

Oil spills in water bodies can cause fish to be suffocated by preventing oxygen from dissolving in it; it can also cause difficulty in respiration, interference with the functioning of various organs, abnormal behaviour, and the death of the fish (Gabriel et al., 2007; Almeda et al., 2013; Nwani et al., 2013). Fish living in a polluted environment might ignore food and thus keep absorbing their bodies, reaching severe and mortality levels over a continuous period of exposure (Isaac et al., 2017; Melefa et al., 2020). Consumption of fish species from the polluted aquatic environment might cause exposure to a high concentration of oil components which may cause severe food poisoning and pneumonitis (Zala and Penn, 2004; Ayman, 2014).

Several studies have been conducted on the effect of leaked petroleum products on the aquatic environment (Egborge, 2000; Aisien et al., 2006; Absalom et al., 2009). Interestingly, Eriegha et al. (2019) studied the effect of water-soluble fractions of crude oil on the histopathology of *C. gariepinus* while the sub-lethal toxicity of kerosene on *C. gariepinus* by Ivon et al. (2021) reported a safe limit of 8.0ml/L of paraffin oil used in this study. However, to date, there is a dearth of information on the behavioural and physiological response of *C. gariepinus* exposed to paraffin oil, which necessitated this study.

## 2.0 Materials And Methods

### 2.1 Experimental site

The experiment was conducted at the wet laboratory of the Department of Aquaculture and Fisheries Management, Federal University of Agriculture Abeokuta. The area lies on latitude 7<sup>0</sup>13'N and longitude 3<sup>0</sup>26'E in the tropical rain forest vegetation with a mean annual temperature of 28.6<sup>0</sup>C.

## 2.2 EXPERIMENTAL FISH AND PARAFFIN OIL

A total of 120 juveniles of African catfish (*Clarias gariepinus*) with an average size of  $78.6 \pm 4.73$ g were obtained from the FUNAAB Hatchery complex. The fish were transported in 50L plastic buckets to the wet laboratory at 07:00h of the day. They were acclimatized for two weeks in a rectangular fibre tank (6m x 4m x 3m) during which they were fed with Coppens (3mm, crude protein = 45% and crude lipid = 12%, 4,300 kcal of digestible energy kg) fish feed at 3% body weight twice per day (8:00h and 17:00h). The fish were checked daily to ascertain their health status and the water quality parameters were maintained as follows: pH ( $6.70 \pm 0.56$ ), temperature ( $28.8 \pm 1.1^\circ\text{C}$ ) and dissolved oxygen ( $6.36 \pm 0.9$ mg/l) throughout the acclimatization period.

Paraffin oil (kerosene) used for this experiment was purchased from a filling station, transported in a 50L black plastic keg, and stored under laboratory concentrations.

## 2.3 Experimental procedure

12 white plastic (high-density polyethylene) tanks with dimensions 1.7m x 1.2m x 1.0m were used for this experiment. The tanks were washed and cleaned with disinfectants (Hyperox) before the start of the experiment. The  $LC_{50}$  value or safe limit of 8.0ml/L for paraffin oil reported by Ivon et al. (2021) was used to extrapolate three sub-lethal concentrations, at 5%, 10% and 20% of paraffin oil used in this study. A control group exposed to no concentration (0.0ml/L) of paraffin oil constituted the fourth group. This corresponds to T1 – 0.4ml/L, T2 – 0.8ml/L, T3 – 1.6ml/L and T4 – 0.0ml/L of paraffin oil. The stock solutions of the paraffin oil were prepared for the experiment using the serial dilution method described in Melefa et al. (2020). Ninety-six juveniles of *C. gariepinus* were selected from the acclimatization tank and randomly allocated into four groups. The 24 fish in each group was stocked at 8fish/tank in triplicates. Each group was randomly subjected to T1 - T4 sub-lethal concentrations of paraffin oil for seven days. The test solution was renewed every 24hours through the inlet to maintain the concentration of paraffin oil in the stock solution for each treatment. The fish were fed sparingly twice per day (8:00h and 17:00h) at 3% body weight with Coppens fish feed.

## 2.4 BEHAVIOURAL RESPONSES

The *Clarias gariepinus* exposed to the different sub-lethal concentrations of paraffin oil were observed for their behavioural responses at 24-hours intervals immediately after the renewal of the stock solution in each treatment during the experimental period, as described in Isaac et al. (2017). Each treatment was observed for 10minutes every 12 hours by one investigator to eliminate bias using the focal sampling technique. The behavioural observation was recorded as a group in each treatment since individual marking and monitoring might cause additional stress to the fish species used for the experiment. The frequency of each behaviour displayed by the specimen per tank and treatment were documented using codes, recording forms and abbreviations to facilitate the process. The duration of some behavioural

traits was determined using a laboratory stopwatch (Model 504, China). The swimming, feeding, aggressive and respiratory behaviour monitored in each treatment was adapted from Catarina et al. (2012) and Zworykin (2018), as detailed in Table 1.

Table 1  
Description of behaviour in the experiment

<b>Behavioural parameter</b>	<b>Description</b>
<b>Swimming</b>	
Non-active swimming	Staying on a spot/lying motionless at the floor of the tank
Erratic swimming	Unpredicted wandering behaviour
Very-active swimming	The continuous and healthy movement of the fish in the culture tanks
<b>Feeding</b>	
Feed intake	The quantity of pellets consumed per food ration
Duration of feeding	Time spent to consume food ration
<b>Aggressive</b>	
Agonistic traits	Chases, attacks, body bites and striving to jump out of culture tanks
Bruises and scars	Tender injury on skin/mark left after healing of an injury
<b>Respiratory</b>	
Air gulping	Fish breaks to the surface of the water and swallow atmospheric air
Mucus secretion	Slippery aqueous secretion from gill surfaces and skin
Opercular rate	Number of opercular movements in response to respiration per minute

## 2.5 COLLECTION OF BLOOD SAMPLES FOR GLUCOSE ANALYSIS

Two fish from each tank and treatment were randomly taken with a scoop net, and anaesthetized with MS-222 in a 10L bucket of water before blood collection for glucose analysis. The blood was extracted between 7:00 h and 8:00 h from the caudal vein using a 2.5ml heparinized syringe with 22Gx1½" according to the method of Di Marco et al. (2007). The whole blood withdrawal process took less than five minutes to prevent discomfort. Collected blood was gently stored with ethylenediamine tetraacetic

acid as the anticoagulant (20mM EDTA) and centrifuged at 3500rpm for 10mins to obtain serum samples used for glucose analysis. The glucose level in the serum samples was analyzed at the central biotechnology laboratory of the Federal University of Agriculture, Abeokuta, using the spectrophotometry method as described by Dacie and Lewis (2011) and recorded per treatment.

## 2.6 STATISTICAL ANALYSIS

All statistical analyses were done following the routines in SAS (2002). Obtained data on swimming, feeding, aggressive, respiratory-related behaviour and glucose level were checked for normality using Shapiro-Wilk tests making use of PROC UNIVARIATE. The data were not normally distributed even after transformation. The data were subjected to Kruskal-Wallis, a non-parametric test using PROC NPAR1WAY.

## 3.0 Results

### 3.1 Behavioural response

The behavioural response of the juveniles of *C. gariepinus* to different concentrations of paraffin oil is presented in Figures 1-4. Concentrations of paraffin oil affected the swimming-related behaviour displayed by the fish species in each group. The duration of non-active swimming ( $\chi^2=13.49$ , df =3, P=0.01), erratic swimming ( $\chi^2=2.51$ , df =3, P=0.04) and very-active ( $\chi^2=3.82$ , df =3, P=0.03) swimming behaviour varied with concentrations of paraffin oil. The group of fish species exposed to the highest concentration of paraffin oil had the highest non-active swimming period (Fig 1A), elevated period of erratic swimming (Fig 1B) and least very-active swimming behaviour (Fig 1C). On the contrary, the group of fish exposed to zero concentration of paraffin oil displayed a high very-active swimming behaviour with little or no erratic swimming traits during the experimental period (Fig 1).

Feeding behaviour displayed by *C. gariepinus* at different concentrations of paraffin oil is shown in Fig 2. The different concentrations of paraffin oil significantly affected the feed intake ( $\chi^2=3.19$ , df =3, P=0.04) and the duration of consuming the given feed ( $\chi^2=4.30$ , df =3, P=0.02). The least quantity of feed intake was observed in T3, which has the highest concentration of paraffin oil (Fig 2A), and the highest duration of feed consumption was recorded in T3 (Fig 2B). There was no significant difference between feed intake and feeding duration of fish species in T1 and T2. *C. gariepinus* in T4 consumed the highest quantity of feed within the shortest time.

Aggressive related behaviour displayed by juveniles of *C. gariepinus* is indicated in Figure 3. There was a significant effect of various concentrations of paraffin oil on the frequency of agonistic ( $\chi^2=2.91$ , df =3, P=0.01) behaviour displayed by the group of fish species in each treatment and the recorded number of bruises and scars ( $\chi^2=1.87$ , df =3, P=0.03). The highest agonistic behaviour (Fig 3A) and the corresponding number of bruises and scars (Fig 3B) were displayed by the group of *C. gariepinus*

exposed to the highest concentration of paraffin oil (T3). Although there was no significant difference between the agonistic behaviour displayed by fish species in Treatment 2 and 3; and recorded bruises and scars in treatments 1 and 2. On the other hand, fish species in T4 exhibited low agonistic behaviour with a corresponding lower number of bruises and scars.

The effect of paraffin oil on respiratory-related behaviour displayed by *C. gariepinus* at the different concentration levels is presented in Fig 4. The highest rate of air gulping (Fig 4A) mucus secretion (Fig 4B) and opercular rate (Fig 4C) was recorded in treatment exposed to the highest concentration of paraffin oil (T3). The concentration of paraffin oil affected the frequency of air gulping ( $\chi^2=1.15$ , df =3, P=0.00), secretion of aqueous mucus ( $\chi^2=0.93$ , df =3, P=0.03) and operculum rate ( $\chi^2=1.01$ , df =3, P=0.01) displayed by the fish species. Operculum rate increased with an increase in the concentration of the pollutant, and the effect of paraffin oil was higher in T3 (Fig 4C). However, the group of fish species exposed to zero concentration of paraffin oil displayed little or no respiratory-related behaviour during the experimental period.

## 3.2 Glucose response

The glucose level of *C. gariepinus* exposed to various concentrations of paraffin oil is presented in Figure 5. The concentration of paraffin oil influenced ( $\chi^2=6.53$ , df =3, P=0.04) the glucose level of the fish species. The highest glucose value was found in *C. gariepinus* exposed to the highest concentration of paraffin oil (T3). In contrast, the least was found in fish species exposed to 0.0mg/L concentration of paraffin oil (Fig 5).

## 4.0 Discussion

This study evaluated the effect of paraffin oil exposure on the behavioural and physiological response of juveniles of *C. gariepinus* under laboratory conditions. This study showed that the varying concentration of paraffin oil influenced the behavioural attributes of *C. gariepinus* under laboratory conditions. Fish species exposed to the highest concentration of paraffin oil displayed a high rate of non-active and erratic swimming styles with little or no very-active swimming behaviour. These observed altered swimming styles in the *C. gariepinus* exposed to paraffin oil could be a way of communicating that there is distress in their rearing enclosure. However, it could be a resultant effect of the changes in the physiological condition of the fish species (Di Marco et al., 2007). On the contrary, *C. gariepinus*, exposed to zero pollutants, swam actively and displayed no erratic swimming style. The recorded swimming behaviour in this study corroborates the findings of Aderolu et al. (2010) and (Ayoku et al., 2020), who studied the effect of petroleum-based chemicals and pesticides on the behaviour of *C. gariepinus*. Moreover, Bridget et al. (2020) stated that all chemical substances influenced the swimming behaviour of *C. gariepinus*.

The various concentrations of paraffin oil affected the feeding-related behaviour of the cultured *C. gariepinus*. The amount of feed consumed reduced with an increase in the concentration of paraffin oil.

In contrast, a corresponding increase in feeding duration was recorded at an increased concentration of paraffin oil. Neglecting feed and an additional reduction in feed intake could be an adaptive mechanism to their unfavourable rearing environment. This low response to feed might cause loss of weight, emaciation and death of the fish species at prolonged exposure to this pollutant. Reduced feed intake recorded in this study corroborates the findings of Isaac et al. (2017) and Melefa et al. (2020), who documented a decline in feed intake of catfish exposed to Glyphosate and Clotrimazole, respectively. However, this finding contradicts the assertion of Ayotunde et al. (2011), who reported a similar feeding pattern between adults of *C. gariepinus* exposed to *Carica papaya* and those groups not exposed to the same pollutant.

Aggressive act is an underlying indicator of uncomfortable feelings in an environment. Observed agonistic behaviour and the number of bruises and scars reported in this study portray these aquatic creatures' sentience nature (Sneddon et al., 2018). These displa

yed behavioural traits could communicate a threat to welfare and health in their rearing medium. These aggressive acts led to a visible attack by the fish species with an increase in the number of bruises and scars. These observed behaviour are similar to results documented by Dogan and Can (2011) and Nwani et al. (2014) in their study.

Respiratory distress in fish species within their aquatic environment occurs when certain strange fluids or other forms of toxicant penetrate their air sacs and prevent the utilization of dissolved oxygen in the water for respiration. Continuous exposure to this pollutant could make breathing difficult and cause mortality (Catarina et al., 2012) due to a higher toxicity strength of paraffin oil in the freshwater ecosystem (Nrior et al., 2019). Increased air gulping, secretion of aqueous mucus and the opercular rate displayed by *C. gariepinus* in this study could be categorized as a defence mechanism to neutralize the effect of the pollutant (Nwani et al., 2013; Somdare et al., 2015) in their respiratory tract. This respiratory behaviour displayed by the juveniles of *C. gariepinus* in this study signals an impairment in the gaseous exchange between respiratory surfaces (Nwani et al., 2014). The observed secreted mucus could be a way of clearing the gill region of accumulated mucus or debris to restore normal breathing habits. At the same time, the increased opercular rate could be a preventive mechanism. It involves pumping a large volume of water through the buccal cavity due to the reduced concentration of dissolved oxygen in paraffin contaminated water in their rearing enclosure. Meanwhile, Garry et al. (2007) and Yekini (2011) categorized kerosene as a combustible hydrocarbon with a complex mixture of toxic compounds which pose harmful effects on humans and other animals. Similar respiratory disorders were documented in fish species exposed to different pollutants (Gbadebo et al., 2009; George et al., 2014; Eriegha et al., 2019).



Nwani et al. (2014) documented the important role of glucose in the life of all living organisms, including fish species. Analysis of blood samples in this study elicited the increased glucose level, which increased with an increase in the concentration of paraffin oil. The observed glucose at higher concentrations could be a response to the stress and anxiety caused by the pollutant. The stress response to this pollutant led to a high mobilization of glucose through the biochemical pathway through which carbohydrate is broken down to provide an immediate energy source for the metabolic demand (Marcel et al., 2009). Continuous exposure of these fish species to contaminants even at higher concentrations could cause high glucose levels to leak into the blood and result in hyperglycemia (Marcel et al., 2009). Similar results were observed in Nile tilapia and African catfish exposed to water-soluble fractions of kerosene and crude oil (Absalom et al., 2009; Eriegha et al., 2019). In addition, Muyiwa et al. (2020) attributed the increased glucose level at higher concentrations of *Adenium obseum* to a defence mechanism of the fish to tolerate the toxicity of the pollutant.

## Conclusion

Paraffin oil is toxic to juveniles of *C. gariepinus*. Exposure of this fish species to sub-lethal concentrations of paraffin oil under laboratory conditions impaired the welfare of the fish, with significant changes in their behaviour and glucose level. Exposure of fish to a high level and continuous concentration of paraffin oil in petroleum polluted aquatic environment could increase fish mortality, and persistent exposure may gradually drive fish into extinction over time.

## Declarations

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### Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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### AUTHOR CONTRIBUTIONS

Ojelade, Oluwaseun Christianah and Iyasere Oluwaseun Serah contributed to the study conception and design. The preparation of materials and data collection were performed by Ojelade Oluwaseun

Christianah, Abdulraheem Ikililu, Lucas Jumoke, Ishola Ibukun, Akinde, Oluwole and Sotunde Oluwamuyiwa. Statistical analysis was done by Durosaro Samuel while the first draft of manuscript was written by Ojelade Oluwaseun Christianah, all authors commented on the previous versions of the manuscript and approved the final manuscript.

### **Data Availability Statement**

The set of data generated during the study are available from the corresponding author upon reasonable request

### **Ethics Statement**

The experiment was carried out in line with the Animal Care and Use Committee guidelines of the Federal University of Agriculture, Abeokuta, Nigeria. The used *C. gariepinus* in this study were properly taken care of to prevent all forms of discomfort. The approval number assigned to this experiment is FUNAAB/AEWC/2020/0024.

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# Figures

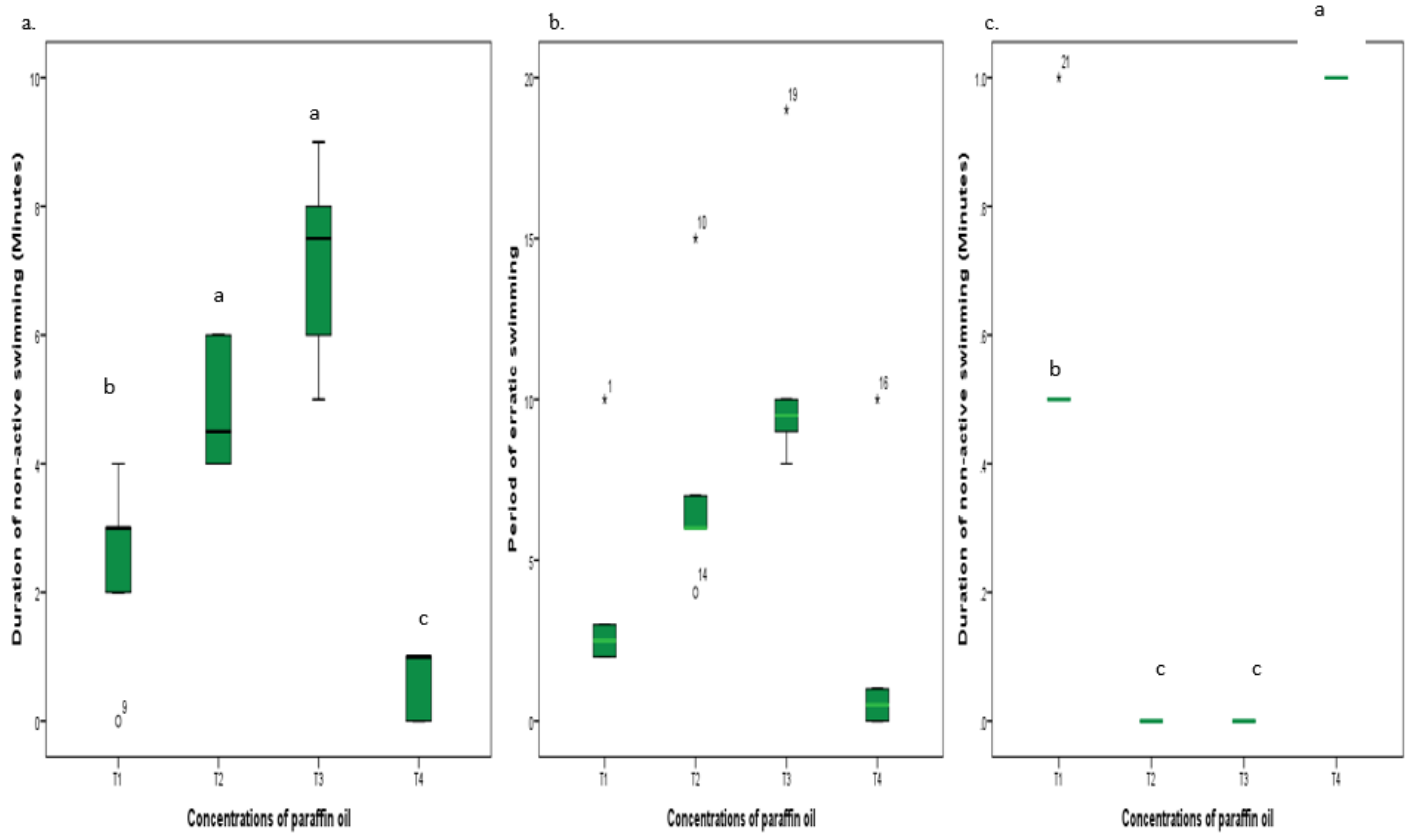
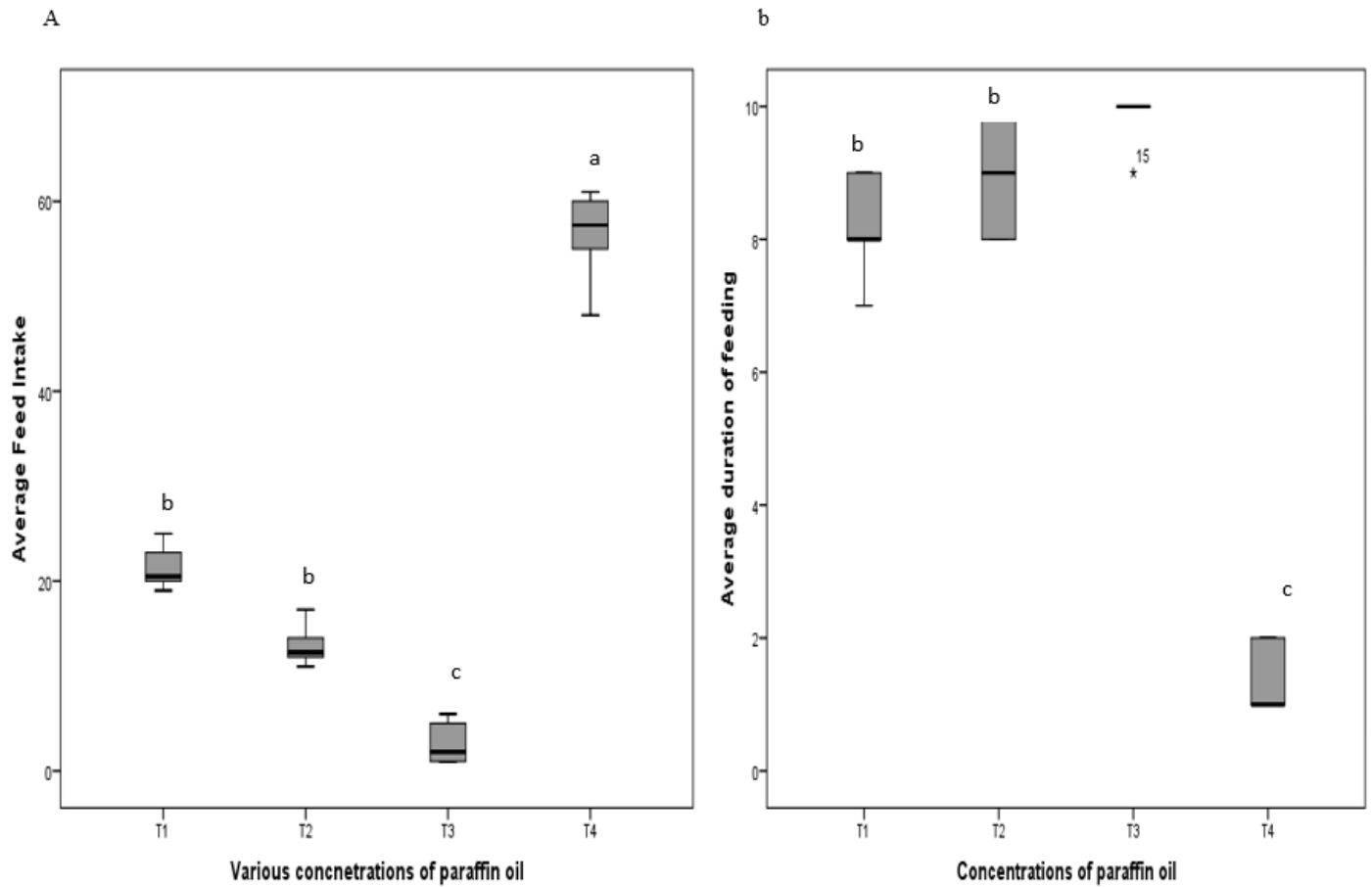


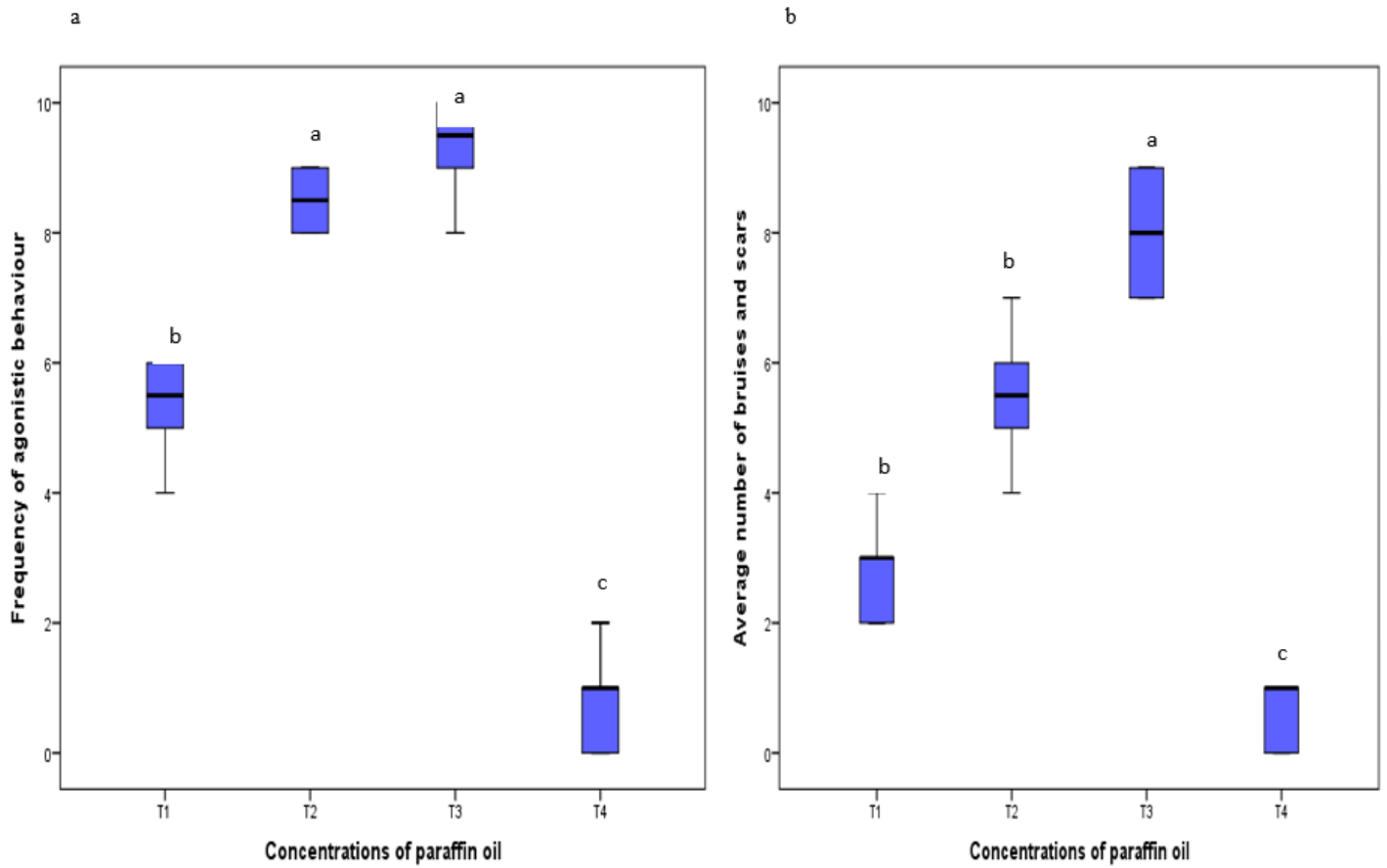
Figure 1

Effect of different concentrations of paraffin oil on the duration of a.) non-active swimming, b.) erratic swimming style, and c.) very-active swimming behaviour in juveniles of *C. gariepinus*. <sup>abc</sup> means with the same letter are not significantly different



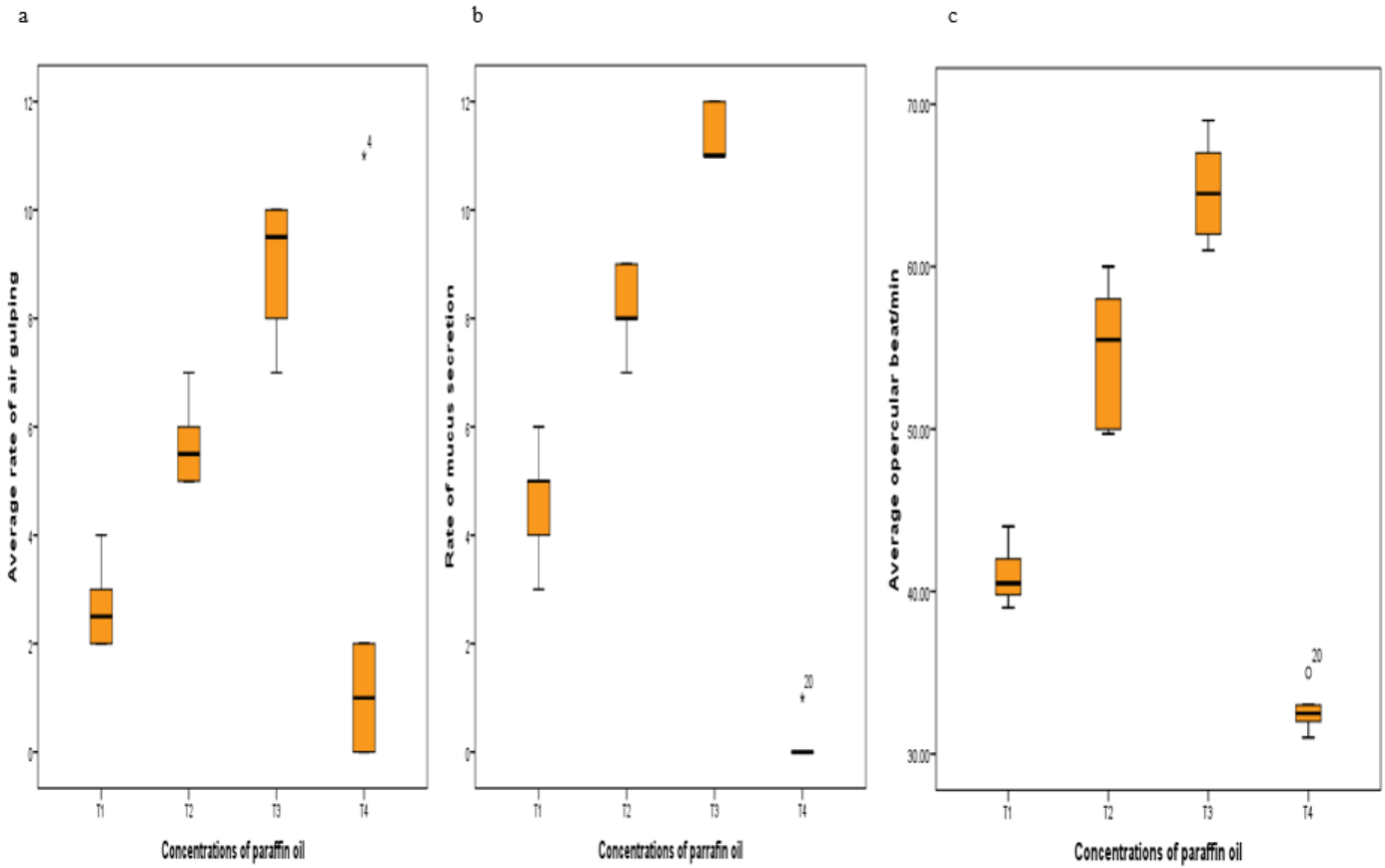
**Figure 2**

The effect of various concentrations of paraffin oil on average a.) feed consumed and b.) duration of feeding in juveniles of *C. gariepinus*, <sup>abc</sup> means with the same letter are not significantly different.



**Figure 3**

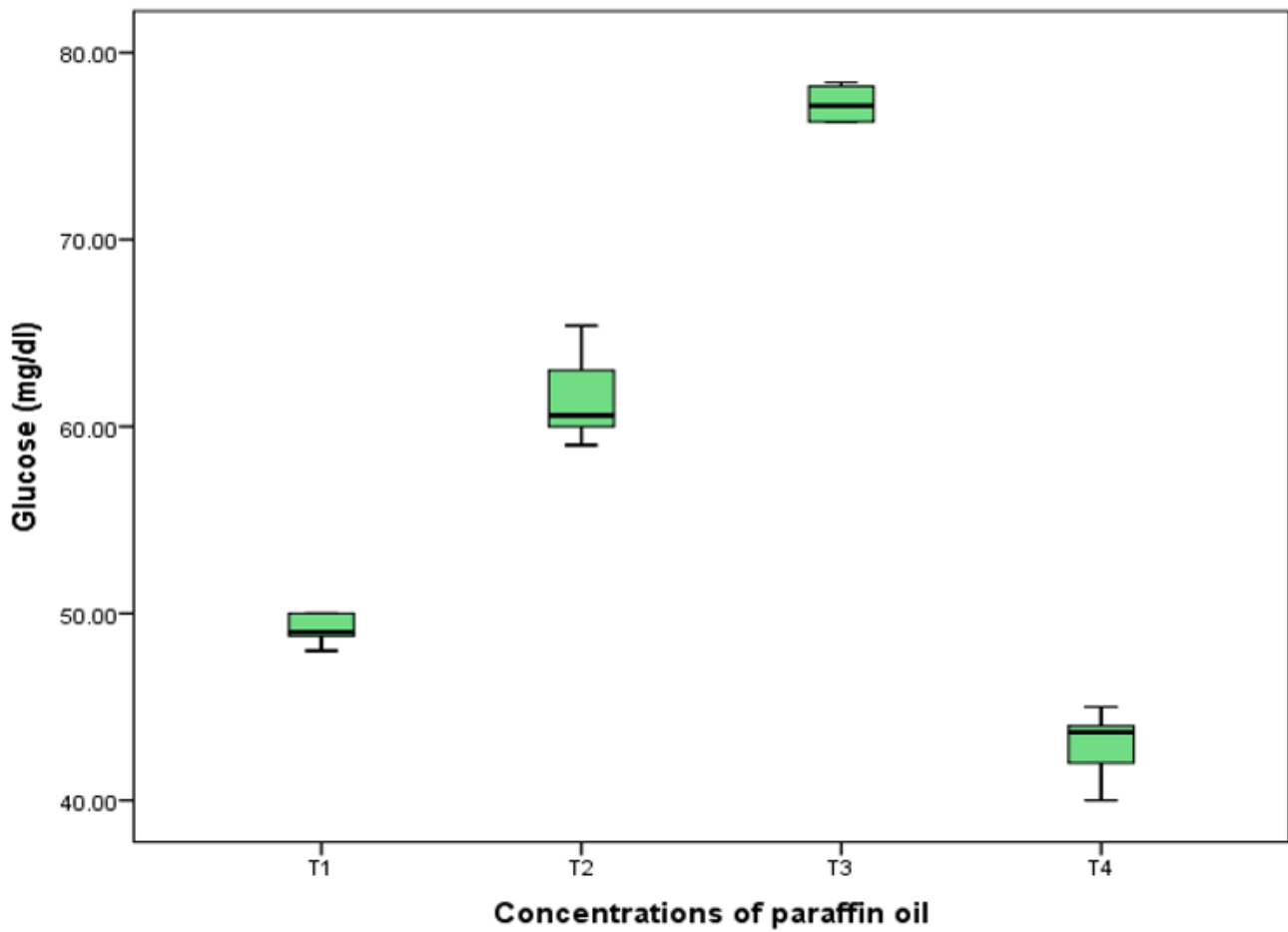
The average number of a.) agonistic behaviour and b.) bruises and scars displayed by juveniles of *C. gariepinus* at different concentrations of paraffin oil, <sup>abc</sup> means with the same letter are not significantly different



**Figure 4**

The effect of varying concentrations of paraffin oil on rate of a.) gulping atmospheric air b.) secretion of mucus and c.) opercular beat/min by juveniles of *C. gariepinus*.





**Figure 5**

The glucose (mg/dl) level of juveniles of *C. gariepinus* exposed to various concentrations of paraffin oil under laboratory conditions